MicroRNAs to boost
the productivity of
Chinese hamster ovary producer cells

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I hereby assure that I performed the present study independently without further help or other materials than stated.

Michaela Strotbek

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Abbreviations

2-ME	2-mercaptoethanol
°C	Degree Celsius
%	Percent (per hundred)
aa	Amino acid
Ab	Antibody
ADCC	Antibody dependent cell mediated cytotoxicity
Ago	Argonaute protein
AP	Alkaline phosphatase
APS	Ammonium persulfate
Asn	Asparagine
ATP	Adenosine triphosphate
BCA	Bicinchoninic acid
Bcl-2	B-cell lymphoma 2
BHK cells	Baby hamster kidney cells
BI	Boehringer-Ingelheim
BiP	Binding protein
bp	Base pairs
BSA	Bovine serum albumin
caspase	Cysteine-aspartic protease
cDNA	Complementary deoxyribonucleic acid
C. elegans	*Caenorhabditis elegans*
cel-miR	*Caenorhabditis elegans* microRNA
CERT	Ceramide transfer protein
CGE	Capillar gel electrophoresis
cgr-miR	*Cricetulus griseus* microRNA
CHO	Chinese hamster ovary
CHO-DG44	DHFR deficient Chinese hamster ovary cell clone
CHO-DXB11	DHFR deficient Chinese hamster ovary cell clone
CHO-K1	Chinese hamster ovary cell line
CO$_2$	Carbon dioxide
ConCERT	CERT overexpressing CHO cells
CPS I	carbamoyl phosphate synthetase I
Cq	Quantification cylce
DGCR8	DiGeorge critical region 8
DHFR	Dihydrofolat reductase
DMEM	Dulbecco's modified Eagle's medium
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleoside triphosphate
E. coli	*Escherichia coli*
EDTA	Ethylene di-amine tetra acedic acid
e.g.	For example (example given)
ELISA	Enzyme-linked immunosorbent assay
emGFP	Emerald green fluorescent protein
ER	Endoplasmatic reticulum
ES cells	Embryonic stem cells
Exp-5	Exportin-5

FAM	6-FAM (6-carboxyfluorescein)
FCS	Fetal calf serum
F.I.	Fluorescence intensity
g	Gram
g	Gravitational acceleration
G1-phase	Gap 1 phase
G418	Geneticin
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
Glc	Glucose
GlcNAc	N-Acetylglucosamine
GOI	Gene of interest
GS	Glutamine synthetase
GTP	Guanosintriphosphate
GW-bodies	"Glycine Tryptophan" bodies
h	Hour
HC	Heavy chain
HEK293	Human embryonic kidney cells 293
HeLa	Henrietta Lacks
HRP	Horseradish peroxidase
HSA	Human serum albumin
hsa-miR	*Homo sapiens* microRNA
HT	Hypoxanthine, thymidine
IgG	Immunoglobulin G
IL-8	Interleukin-8
IL-17F	Interleukin-17F
IPA	Ingenuity pathway analyses
IVC	Integral of viable cells
k	Kilo
kDa	Kilo Dalton
L	Liter
LB	Lysogeny broth
LC	Light chain
M	Molar
mA	Milliampere
MAPK	Mitogen-activated protein kinase
mg	Milligram (10^{-3} g)
min	Minute
mL	Milliliter (10^{-3} l)
mM	Millimolar (10^{-3} M)
mmu-miR	*Mus musculus* microRNA
mRNA	Messenger ribonucleic acid
miR-	Mature miRNA
mir-	microRNA precursor or gene name
miRISC	microRNA induced silencing complex
miRNA	microRNA (ribonucleic acid)
MSX	Methionine sulfoxamine
mTOR	Mammalian target of rapamycin
MTX	Methotrexat
MW	Molecular weight
ng	Nanogram (10^{-9} g)
NS0 cells	Murine myeloma cells
n.s.	Not significant

nt	Nucleotide
OD	Optical density
Opti-MEM	Opti-MEM I Reduced Serum Media
ORF	Open reading frame
OTC	Ornithine transcarbamoylase catalyze
p-bodies	Processing bodies
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline with Tween-20
PCR	Polymerase chain reaction
PDI	Protein disulphide isomerase
PMSF	Phenylmethanesulfonyl fluoride
Poly-A	Polyadenylation
Pre-miRNA	Precursor microRNA
Pri-miRNA	Primary microRNA
qRT-PCR	Quantitative real time polymerase chain reaction
RISC	RNA induced silencing complex
RLU	Relative luminescence units
RNA	Ribonucleic acid
RNAi	RNA interference
RNA pol II	RNA polymerase II
RPMI 1640	Roswell Park Memorial Institute medium 1640
s	Second
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM	Standard error of the mean
Ser	Serine
siRNA	Short interfering RNA
SM proteins	Sec1/munc18-like proteins
Tandem	Two in combination
TCA	Tricarbolic acid cycle
TEMED	N,N,N′,N′-tetramethylethan-1,2-diamin
Thr	Threonine
TMB	3,3', 5, 5'-Tetramethylbenzidine
tPA	Tissue plasminogen activator
TRIS	Tris(hydroxymethyl)aminomethane
U	Units
UPR	Unfolded protein response
UTR	Untranslated region
V	Volt
v/v	Volume/volume
w/v	weight/volume
Xbp-1	X-box binding protein 1
µg	Microgram (10^{-6} g)
µL	Microliter (10^{-6} l)
µM	Micromolar (10^{-6} M)

Summary

MicroRNAs (miRNAs) are short non-coding RNAs that post-transcriptionally regulate the expression of different target genes and, thus, potentially offer the opportunity to engineer networks of genes in order to achieve complex phenotypic changes in mammalian cells. The goal of this thesis was to explore whether this feature of miRNAs could be exploited as a strategy to improve therapeutic protein production processes by increasing the viable cell densities and/or productivity of mammalian producer cells. To identify miRNAs that increase the productivity of producer cells, a genome-wide functional miRNA screen was established and performed in Chinese hamster ovary (CHO) cells stably expressing an IgG1 that were grown in suspension in chemically defined medium. Using this approach nineteen human miRNAs were identified that significantly improved IgG titers in the primary screen. Almost half of these miRNAs could be validated to significantly increase the IgG concentrations and/or specific productivity when transiently introduced into CHO producer cells in a secondary screen. The increased titers of recombinant human serum albumin analyzed as a second secreted model protein pointed to product independent effects of most of the validated miRNAs. In addition, two of the validated miRNAs, hsa-miR-557 and *hsa-miR-1978*, also increased the secretion of an endogenously as well as a transiently expressed model protein in human cells, indicating that these miRNAs manipulate the cellular machinery by a universal mechanism. The strongest impact on the specific productivity of CHO cells was observed by a dead entry and thus artificial miRNA (*hsa-miR-1978*), which may represent a promising molecular tool for future synthetic biology approaches aimed at optimizing producer cells.

For further studies, the two miRNAs hsa-miR-557 and hsa-miR-1287, positively impacting the viable cell density and specific productivity, respectively, were selected. Transient experiments supported the idea of combining these two miRNAs to further boost cellular productivity. Using a vector-based expression system, CHO pools stably overexpressing these human miRNAs were successfully generated allowing the study of miRNA-engineered producer cells under industrially relevant culture conditions such as fed-batch cultivation. Importantly, three independent cell pools stably coexpressing miR-557 and miR-1287 gave rise to significantly increased IgG titers by 30% in fed-batch cultures whilst product quality was conserved, proving

the transferability of the transient results to a stable setting. Taken together, these results demonstrate that miRNA-based cell line engineering is an attractive approach toward the genetic optimization of CHO host cells for industrial applications.

Zusammenfassung

MicroRNAs (miRNAs) sind kurze nichtcodierende RNAs, die die Expression verschiedener Zielgene posttranskriptional regulieren und somit potentiell die Möglichkeit bieten, Netzwerke von Genen zu beeinflussen, um komplexe phänotypische Veränderungen in Säugerzellen zu erreichen. Das Ziel dieser Doktorarbeit war zu untersuchen, ob diese Eigenschaft von miRNAs als Strategie genutzt werden kann, um Prozesse zur Produktion von therapeutischen Proteinen durch Erhöhung der vitalen Zelldichten und/oder der Produktivität von Säugerzellen zu verbessern. Um miRNAs zu identifizieren, die in der Lage sind, die Produktivität von Produktionszellen zu steigern, wurde ein genomweiter funktionaler miRNA Screen etabliert und in Chinesischen Hamster Ovarien (CHO) Zellen, die stabil einen IgG Antikörper sezernieren und in Suspension in chemisch definiertem Medium wachsen, durchgeführt. Mit Hilfe dieses Ansatzes konnten neunzehn humane miRNAs in einem primären Screen identifiziert werden, die signifikant die IgG Konzentrationen steigerten. Nahezu die Hälfte dieser miRNAs konnte in einem sekundären Screen bestätigt werden, indem signifikant erhöhte IgG Konzentrationen beziehungsweise spezifische Produktivität nach transienter Transfektion der Produktionszellen nachgewiesen wurden. Die erhöhten Titer des rekombinanten humanen Serumalbumins, welches als zweites sezerniertes Modellprodukt analysiert wurde, wiesen auf produktunabhängige Effekte der meisten validierten miRNAs hin. Darüber hinaus steigerten zwei der validierten miRNAs, nämlich hsa-miR-557 und *hsa-miR-1978,* sowohl die Sekretion eines endogenen als auch eines transient exprimierten Modellproduktes in humanen Zellen, was auf die Manipulation eines universellen Mechanismus hindeutet. Der stärkste Einfluss auf die spezifische Produktivität von CHO Zellen wurde durch eine miRNA hervorgerufen, die als nicht mehr gültiger Datenbankeintrag aufgeführt ist und somit eine artifizielle RNA darstellt. Diese „miRNA" könnte damit in zukünftigen synthetischen Biologie-Ansätzen als ein molekulares Werkzeug zur Optimierung von Produktionszellen dienen.

Für weiterführende Studien wurden zwei miRNAs, die einen positiven Einfluss auf die vitale Zelldichte (hsa-miR-557) bzw. die spezifische Produktivität (hsa-miR-1287) hatten, ausgewählt. Die Ergebnisse transienter Studien unterstützten die Idee, eine Kombination dieser zwei miRNAs zu verwenden, um die zelluläre Produktivität weiter

zu steigern. Unter Verwendung eines Vektor-basierten Expressionssystems wurden erfolgreich CHO Pools generiert, die diese miRNAs stabil überexprimierten. Dies erlaubte die Untersuchung von miRNA-manipulierten Produktionszellen unter industriell relevanten Kulturbedingungen wie es die Fed-Batch Kultivierung darstellt. Von besonderer Bedeutung ist, dass für drei unabhängige Zellpools, die stabil miR-557 und miR-1287 koexprimierten, in Fed-Batch Kultivierungen eine signifikante Steigerung der IgG-Titer um 30% gemessen wurde, während die Produktqualität erhalten blieb. Dies beweist die Übertragbarkeit der transienten Ergebnisse auf einen stabilen Ansatz. Zusammenfassend zeigen die Ergebnisse dieser Arbeit auf, dass die miRNA-basierte Zelllinienentwicklung ein attraktiver Ansatz zur genetischen Optimierung von CHO Wirtszellen für die industrielle Anwendung ist.

1. Introduction

Biopharmaceuticals as therapeutics for the therapy of a broad range of diseases are delivered by the pharmaceutical biotech industry. Many of these complex molecules cannot be chemically synthesized but need to be produced in different organisms grown in biotechnological production processes. Bacterial organisms, mainly *E. coli,* can be used to synthesize small functional proteins with high yields, for example single-chain variable fragment (scFv) formats (Kipriyanov et al., 1995). Due to high doubling rates of bacterial organisms, biomass accumulates fast and delivers high amounts of product. A prominent example for a biotherapeutic produced in *E. coli* is insulin, a peptide hormone involved in the carbohydrate metabolism and released by the beta-cells of the islets of Langerhans (Leader et al., 2008). However, more complex proteins with higher molecular size and posttranslational modifications need to be produced in mammalian cells to obtain functional molecules with correct folding, disulphide bond formation and glycosylation. For the development of biopharmaceuticals the human cell line HEK293 (<u>h</u>uman <u>e</u>mbryonic <u>ki</u>dney) is often applied, since these cells are easily transfectable and small amounts for first experiments are available fast without time consuming screening of high producer clones.

For clinical studies of promising therapeutic candidates higher amounts of product produced under stricter safety criteria are required. Industry in the biotech field mainly works with BHK (baby hamster kidney) cells, mouse myeloma derived NS0 cells or, most frequently, CHO (Chinese hamster ovary) cells. Till 2004, 27 out of 38 mammalian derived proteins in the USA were produced in CHO cells (Birch and Racher, 2006). The preference of CHO cells is largely ascribable to their ability to mediate appropriate protein folding and human-like post-translational modification (Wurm, 2004). Moreover the experience and effort to improve CHO cells and their cultivation processes of more than two decades characterizes them as a worthy production host.

1.1. Chinese hamster ovary (CHO) cells

Chinese hamster ovary cells are derived from the Chinese hamster (*Cricetulus griseus*) that was first established as a laboratory animal in 1919 (Yerganian, 1958). The low chromosome number of 22 made them predestined for research of radiation cytogenetics.
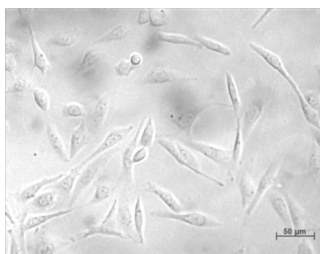
Figure 1: Chinese hamster (*Cricetulus griseus*) ovary derived cells.
Chinese hamster ovary (CHO) cells were isolated from the ovaries of this animal in 1957 and grown in cell culture. The CHO cells used to date are derived from these isolated cells. An example of adherent CHO-K1 cells is shown (bright field picture, scale bar represents 50 µm).

In the year 1957 T. Puck isolated cells from the ovaries of the Chinese hamster and grew these cells successfully in culture (Puck et al., 1958). The CHO cell line was born (see Figure 1). Later, 44 pathogenic viruses were tested for their danger of infection risk and the majority of them were not replicated in CHO cells which is an important issue concerning safety criteria of producer cells for therapeutic proteins applied in humans (Wiebe, 1989). CHO cells were the production host of the therapeutic tPA (tissue plasminogen activator), which was the first recombinant therapeutic protein produced in mammalian cells and approved for clinical use in 1987 (Kaufman et al., 1985). Further examples of biopharmaceuticals produced by CHO cells are listed in Table 1.

Table 1: Selected biologics produced in CHO cells. (Modified from Datta et al., 2013)

Trade name	Generic name	Product category	Biological importance	Manufacturer, FDA approval year
Zaltrap	Ziv-aflibercept	Recombinant fusion protein	Colon cancer drug	Sanofi Aventis US and Regeneron Pharmaceuticals 2010
Eylea	Aflibercept	Recombinant fusion protein	Wet (neovascular) age-related macular degeneration (AMD)	Regeneron Pharmaceuticals 2010
Actemra	Tocilizumab	Antibodies	Treatment of rheumatoid arthritis (RA)	Genentech 2010
Prolia	Denosumab	Antibodies	Osteoporosis in post menstrual women	Amgen 2010
Recothrom	Thrombin	Alpha blood factors, anticoagulants and thrombolytics	Coagulation Factor	ZymoGenetics, Bayer 2008
Arcalyst	Rilonacept	Recombinant fusion protein	Cryopyrin-Associated Periodic Syndromes	Regeneron Pharmaceuticals 2008
Xyntha	Factor VIII	Blood factors	Hemophilia A	Wyeth 2008
Herceptin	Trastuzumab	Antibodies	A single agent for treatment of HER2-overexpressing node-negative and node-positive breast cancer	Genetech 2008

To be continued

Continued

VectibixTM	Panitumumab	Antibodies	Antineoplastic, metastatic colorectal cancer	Amgen 2006
MYOZYME1	Glucosidase alfa	Enzymes	Enzyme Replacement Therapy, Pompe disease	Genzyme 2006
Orencia	Abatacept	Others	Treatment of adults with moderate to severe rheumatoid arthritis	Bristol-Meyers Squibb 2005
Naglazyme	Galsulfase	Enzymes	Mucopolysaccharidosis VI	BioMarin Pharmaceuticals 2005
Luveris	Lutropin alpha	Hormones	Luteinizing hormone for treatment of infertility	Merck Serono 2004
Avastin	Bevacizumab	Antibodies	Treatment of first-line metastatic colon or rectum cancer	Genentech 2004
Aldurazyme	Laronidase	Enzymes	Mucopolysaccharidosis I	Genzyme 2003
Amevive	Alefacept	Immunosuppressive dimeric fusion protein	Chronic plaque psoriasis	Biogen Idec 2003
Advate	Factor VIII	Blood factors, anticoagulants and thrombolytics	Hemophilia A	Baxter 2003
Xolair	Omalizumab	Antibodies	Asthma treatment	Genetech 2003
Raptiva	Efalizumab	Antibodies	Treatment of plaque psoriasis	Serono, Genentech 2003
Rebif	Interferon beta-1a	Interferons	Glycosylated interferon beta-1a for treatment of multiple sclerosis	Serono 2002
Humira	Adalimumab	Antibodies	Human IgG1 monoclonal antibody	Abbott 2002
Zevalin	Ibritumomab tiuxetan	Antibodies	Therapeutic radiopharmaceutical for treatment of non-Hodgkin's lymphoma	IDEC Pharmaceuticals 2002
Aranesp	Darbepoetin alfa	EPO and colony-stimulating factors	2nd generation recombinant form of erythropoetin for treatment of anemia	Amgen 2001
MabCampath	Alemtuzumab	Antibodies	Treatment of chronic lymphocytic leukaemia	Genzyme Corp. 2001
ReFacto	Factor VIII	Blood factors, anticoagulants and thrombolytics	Hemophilia A	Wyeth 2000
Thyrogen	Thyrotropin alfa	Hormones	Thyroid cancer	Genzyme 1998
Enbrel	Etanercep	Antibodies	A tumor necrosis factor antagonist	Immunex, 1998
Follistim	Follitropin beta	Hormones	Follicle stimulating hormone for treatment of infertility	NV Organon 1997
Benefix	Factor IX	Blood factors, anticoagulants and thrombolytics	Hemophilia B	Wyeth, Genetics Institute 1997
Gonal-F	Follitropin alfa	Hormones	Follicle stimulating hormone for treatment of anovulation and superovulation	Merck Serono 1997
Rituxan	Rituximab	Antibodies	Treatment of patients suffering from B-cell non-Hodgkins lymphoma	Genentech and IDEC Pharmaceuticals 1997
Avonex	Interferon beta-1a	Interferons	Glycosylated interferon beta-1 for treatment of multiple sclerosis	Biogen Idec 1996
Cerezyme	Imiglucerase	Enzymes	Beta-glucocerebrosidase	Genzyme 1994
Bioclate	rh Factor VIII	Blood factors, anticoagulants and thrombolytics	Hemophilia A	Aventis Behring 1993
Pulmozyme	Dornase alfa	recombinant human deoxyribonuclease I	Cystic fibrosis	Genentech 1993
Recombinate	Factor VIII	Blood factors, anticoagulants and thrombolytics	Hemophilia A	Baxter 1992
Procrit	Epoetin alfa	EPO and colony-stimulating factors	Erythropoetin	Orthobiotech 1990
Epogen	Epoetin alfa	EPO and colony-stimulating factors	Erythropoietin	Amgen 1989
Activase	Alteplase	Blood factors, anticoagulants and thrombolytics	Tissue-plasminogen activator (t-PA) for treatment of acute myocardial infarction	Genentech 1987

1.1.1. Bioprocesses

The definition of a bioprocess is the use of living organisms to synthesize or modify a product. It is divided into two main steps: the upstream and downstream processing. The upstream processing describes the generation of a suitable production host, growth of this host in a bioreactor to accumulate a product and the harvest of this product. The purification and formulation of the product is done during the downstream process. The production host system is chosen depending on the product. In such a bioprocess cells are inoculated and subsequent cell growth in standard production culture systems can then be described by four phases: lag, log, stationary and decline phase. The lag phase, in the beginning of a process, describes the time cells need to adapt until they start growing. In the log phase exponential growth of cells occurs, while the cell density reaches its maximum in the stationary phase. Finally, in the decline phase due to cell death cell density decreases (see Figure 2). The typical production process using CHO cells was dramatically improved over the years, including viable cell concentration and obtained amounts of product. Thereby, product yields can be in the range of g/L. In addition, the cultivation time could be prolonged from about one week up to more than two weeks (see Figure 2).
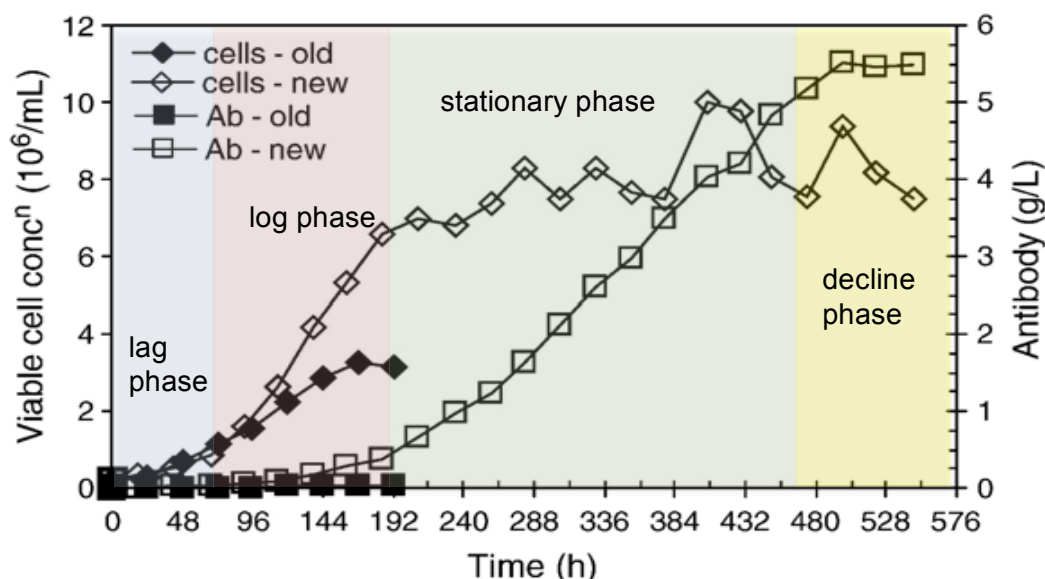
Figure 2: CHO cell growth and productivity.
Typical growth of suspension CHO cells in an old process (filled symbols) compared to improved newer process data (open symbols). Shown are viable cell densities (cells) and antibody concentrations (Ab). For the new process cell growth phases are indicated; Lag phase (blue), log phase (red), stationary phase (green) and the decline phase (yellow) (modified from (Birch and Racher, 2006).

Cultivation of cells is performed in bioreactors. The bioreactor is supplied with air and oxygen transfer is usually helped by agitation, which is also needed to mix nutrients and to keep the fermentation homogeneous. During the bioprocess different parameters are commonly monitored and partially regulated. Examples are oxygen, CO_2, stirring speed, sparging rate, pH, nutrient concentrations (e.g. glucose, glutamine), concentration of byproducts (e.g. glutamate, lactate), temperature, viability of cells and cell growth. Currently, different strategies for cell cultivation in bioreactors are applied. A fed-batch cultivation is based on feeding of nutrients that are essential for cells that are grown in a bioreactor. A batch cultivation is a similar process but no further nutrients are added, which limits the cultivation time due to limited nutrients. In comparison to batch and fed-batch in which the product is harvested at the end of the process, a perfusion process is defined by throughout skimming of the product over the time period of cultivation accompanied by medium inflow allowing a much longer cultivation time (see Figure 3).
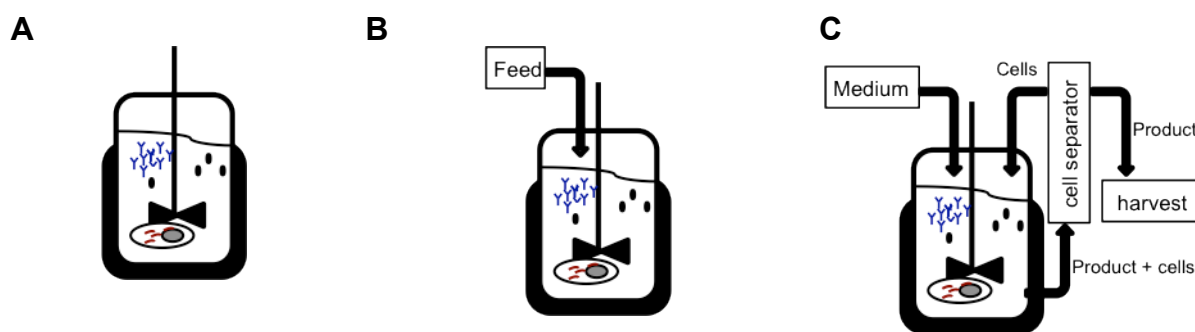
Figure 3: Bioprocesses.
Schematic diagram of a bioreactor with heating jacket and stirrer. Black dots indicate sparging to ensure oxygen supply. Cells are grown in culture medium and release their product (Y) into the medium. (A) Batch process. (B) Fed-Batch process. Concentrated feed is added to prolong nutrient supply and culture time. (C) Perfusion process. Medium is constantly added and simultaneously supernatant containing product is harvested. The challenge of this system is to keep the cells in the reactor (indicated by cell separator).

Over the last two decades significant improvements could be achieved in recombinant protein productivity of CHO cells. In 1987 a typical CHO batch process lasted seven days and resulted in cell densities of about 2×10^6 cells/mL and a specific productivity of 10 pg/cell/day. This increased to cell densities of more than 10×10^6 cells/mL and up to almost tenfold higher specific productivities obtained in fed-batch processes (Wurm, 2004).

1.1.2. CHO cells as producer host cells

CHO cells show a high efficiency in large-scale protein production. One reason for this is their adjusted ability to be grown in suspension cultures of defined chemical media without serum or without other proteins derived from animals. Moreover, protein free media were developed for growth of CHO cells (Zang et al., 1995). With these strategies high cell numbers could be achieved, which is one major goal of the biotechnological industry where high viable cell numbers and specific productivity are needed for high yields of product.

To generate a CHO cell line producing biologics a standard workflow is usually followed as shown in Figure 4. For expression of an exogenous protein an expression vector is transfected into the cells. Obligatory elements of such an expression vector are a bacterial selection marker (for example ampicillin or kanamycin resistance) and an origin of replication to amplify the shuttle DNA, for example in *E. coli*. The DNA encoding the desired protein product is cloned into this expression vector. The transcription of the gene of interest (GOI) is driven by a promotor. Furthermore, a mammalian selection marker (for example, neomycin, puromycin or blasticidine resistance) is expressed by this vector. The selection marker can be driven either by the GOI promotor or by a separate weak promotor. Due to selection pressure applied by the addition of appropriate antibiotics to the culture medium, most of the cells die and only the ones expressing the selection marker survive and are able to grow. For transfection of cells with recombinant DNA a variety of methods including calcium phosphate precipitation, cationic lipid transfection, electroporation and nucleofection are well established (Graham and van der Eb, 1973; Kim and Eberwine, 2010). Transfected DNA randomly integrates into the host genome, a rare event that not necessarily results in optimal expression of the transgene due to silencing or other epigenetic effects (Wurm, 2004). Integration of the expression vector in the heterochromatin may result in silencing or low level expression of the transgene. Euchromatin integration, however, is not a guarantee for an optimal transgene expression as other local factors play a role and transgene expression might be silenced by histone deacetylation or DNA methylation (Kwaks and Otte, 2006). To prevent these issues there is also the possibility of a site-specific integration if the vector and the host genome contain the necessary elements. Optimally, the integration site is screened to be an expression hotspot (Campbell et al., 2010; Kim and Eberwine, 2010). Subsequently, amplification of the transcript can

be triggered by the DHFR amplification system in DHFR deficient CHO cells (CHO-DG44, CHO-DXB11; (Urlaub et al., 1986). DHFR is an enzyme termed dihydrofolate reductase and catalyzes the reaction of folic acid into tetrafolic acid. This molecule serves as coenzyme for the generation of anabolic intermediates like hypoxanthine, thymidine and glycine, which are essential for the generation of purines and certain amino acids. Loss of DHFR can be substituted by providing hypoxanthine, thymidine (HT) and glycine in the cell culture medium. Removal of HT serves as a selection pressure for stable CHO-DG44 or CHO-DXB11 cell lines transfected with plasmids encoding for the GOI and in addition for DHFR. Thus, only cells expressing the plasmid encoded DHFR can survive. Furthermore, methotrexate (MTX) was established to amplify the expression of the exogenous genes. MTX is a folic acid analogue and competes with the natural substrate of DHFR. To synthesize tetrahydrofolate cells have to overexpress DHFR with increasing amounts of MTX in order to survive. This is mostly accompanied by an amplification of the expression of the GOI, the so called DHFR amplification system. Alternatively, the GS (glutamine synthetase) system can be used to enhance productivity. Although CHO cells are auxotroph for glutamine because they endogenously express GS, high amounts of MSX (Methionine Sulfoximine) block the endogenous CHO GS and are therefore lethal for the cells. The transfected enzyme converts glutamate and ammonium into glutamine, thereby enabling cell survival. Similar to the DHFR system, GS expression can be combined with GOI expression (Kuystermans et al., 2007).

Screening of single clones is based on properties like optimal production of the recombinant protein, cell growth, stress resistance, stability concerning the transgene expression and behavior in culture conditions that are comparable to production scales. This screening is necessary because single cell clones differ dramatically in above-mentioned characteristics dependent on the integration site of the transgene. On the one hand the general genomic instability of CHO cells is responsible for the variance of single clones. On the other hand this feature also leads to particular good producers. A high amount of clones, commonly several hundred, have to be screened in order to find the rare clones that combine all listed criteria of a good production cell line. As a last step of recombinant cell line generation, a master cell bank of the chosen cell clone is generated by cryoconservation (Jayapal et al., 2007).
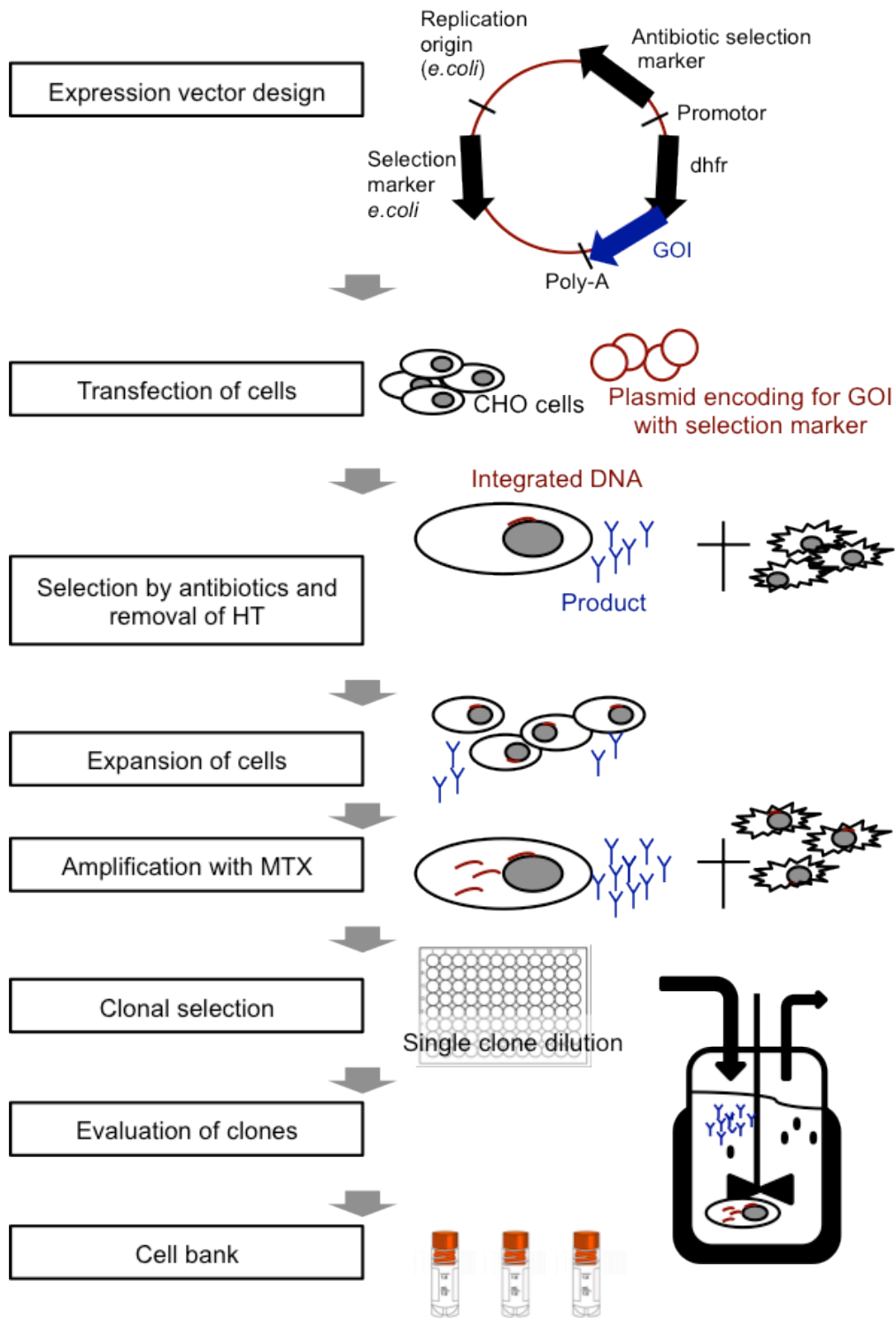
Figure 4: Workflow for the generation of a stable production cell line.
Expression plasmid encoding gene of interest (GOI) and selection marker as well as elements to produce DNA in bacteria (e.g. *E. coli*). Transfection of vector (red) into CHO cells and selection via antibiotic resistance by adding respective antibiotics and DHFR deficiency by removal of hypoxanthine and thymidine (HT). Expansion of surviving cells that also mainly secrete the product that is exemplarily shown as an antibody (Y). Amplification of transcript expression by increasing amounts of methotrexate (MTX). Clonal selection by, e.g., single clone dilution and expansion of single clones. Assess productivity of single clones and behavior in culture process. Screening for best and stable clone. Cryoconservation of chosen clone(s) for cell banking.

Research related to all these steps of recombinant cell line generation is ongoing in order to optimize the production of therapeutic proteins. One approach was to shorten the time of the whole procedure. As an example of successful optimization the overexpression of the cytokine interleukin 17F (IL-17F) in a producer cell line by Contie and colleagues can be mentioned (Contie et al., 2013). The authors described an improved recovery of cells after single clone selection and, consequently, shortening of the procedure by 30 days (Contie et al., 2013). For the biotechnological industry a shorter generation time of the production host means less costs and is thus an important engineering target. Further engineering of CHO producer host cells has been performed and is presented in the following chapters.

1.1.3.   Genetic engineering of CHO cells

Genetic engineering of CHO cells enables them to express an exogenously introduced product. For example, it was shown that codon optimization as well as the choice of the signal peptide that mediates the secretion of the product affected productivity (Kober et al., 2013; Lim et al., 2004). Furthermore, promotor elements were optimized considering facts such as stable and high expression of the product coding mRNA (Makrides, 1999).

Genetic engineering also includes the specific insertion of transgenic enzymes into the genome of CHO host cells. Thereby specific metabolic pathways, like the glycosylation of proteins (see chapter 1.1.4) can be altered and adapted to achieve higher amounts of product.

1.1.4.   Glycosylation engineering of CHO cells

About half of proteins that are synthesized by mammalian cells are estimated to be glycosylated (Apweiler et al., 1999). Glycosylation is a posttranslational modification that involves attachment of oligosaccharides to a protein onto asparagine (Asn) and serine/threonine (Ser/Thr) residues called N-glycosylation and O-glycosylation, respectively. N-glycosylation takes place in the ER where the membrane bound oligosaccharyl transferase complex transfers a preformed 14-mer oligosaccharide onto the side chain of the amino acid asparagine surrounded by the consensus sequence Asn-X-Ser/Thr, where X can be any amino acid except proline. However, it

was reported that only about 65% of the asparagine residues within such a consensus sequence were glycosylated (Butler and Meneses-Acosta, 2012). Subsequently, the core glycan tree is trimmed by ER resident glucosidases and mannosidases. In the Golgi complex, different enzymes mediate O-glycosylation. Due to inconsistent trimming of the glycan chains a variance of glycan structures occurs. This generates glycoproteins consisting of an invariant peptide sequence with variant glycan structures attached (see Figure 5). These variances differ in site occupancy and structure of glycans, called macroheterogenity and microheterogenity, respectively.
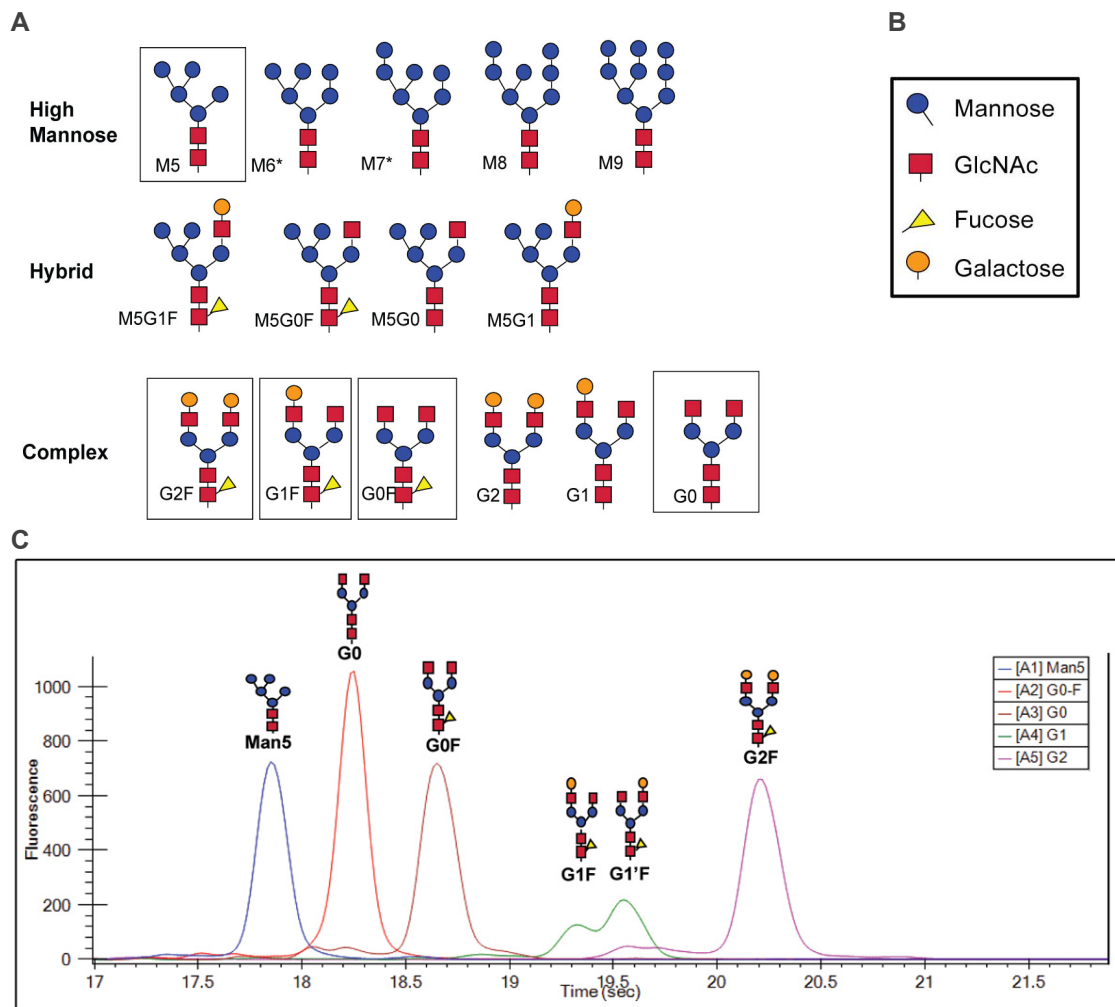
Figure 5: Glycosylation of IgGs (modified from (Jefferis, 2005).
(A) N-Glycans of an IgG. The emphasized structures can be measured by a chromatography based method. (B) Legend of sugars (GlcNAc: N-Acetylglucosamine). (C) Example of a result of a capillar gel electrophoresis (CGE) analysis of IgG N-glycans.

Glycosylation can affect biological properties including bioactivity, solubility, receptor recognition, secretion, *in vivo* clearance and antigenicity (Narhi et al., 1991; Storring, 1992; Takeuchi et al., 1988; Wasley et al., 1991). It was found that glycosylation decreased over the time of a culture process (Curling et al., 1990). This might be caused by diminished nutrient supply of mainly glucose and glutamine on the one hand and accumulation of interfering byproducts on the other hand (Hayter et al., 1992; Jenkins and Curling, 1994; Nyberg et al., 1999). Other parameters of the culture conditions like pH or temperature can also affect glycosylation. This was triggered by two major mechanisms: The time a protein remained in the respective organelle - the ER or Golgi - and the availability of precursors for the glycosylation. If the pH of the medium was not in an optimum range of pH 7.6-8, the activity of the transfer enzymes in the ER and Golgi were lowered and thereby glycosylation can be incomplete (Borys et al., 1993). However, to counter this effect the temperature can be decreased resulting in the prolonged presence of the nascent peptide chain in the ER. This increased the occupancy of glycosylation sides during ER import as shown for the recombinant product tPA (Andersen et al., 2000).

Sialylation is the last step of the glycosylation in the Golgi in which sialic acid is transferred to available galactose of the glycan structure. Sialylation of glycans is important for therapeutic proteins because proteins with this modification of glycans are protected from clearance by asialoglyoprotein receptors in the liver and thus have an increased circulatory half-life resulting in enhanced therapeutic efficacy (Weiss and Ashwell, 1989). Expression of 2,3-sialyl transferase and beta-1,4-galactosyltransferase resulted in more than 90% fully sialylated glycans and this was accompanied with a decrease in heterogeneity and improved pharmacokinetics (Weikert et al., 1999).

Another aspect that influences therapeutic efficacy of antibodies is the activation of the immune system by the antibody, which can be crucial for the therapeutic effectiveness. This is mediated by the antibody dependent-cell-mediated cytotoxicity (ADCC). The absence of fucose at glycans of IgG antibodies promotes ADCC. Hence, the deletion of the FUT8 gene encoding for the alpha1,6-fucosyltransferase in CHO (Malphettes et al., 2010) or a mutant CHO cell line (LEC 13, Professor P. Stanley, Albert Einstein Collage of Medicine) were employed that did not add fucose to the primary N-acetylglucosamine resulting in the production of therapeutic

antibodies with unfucosylated glycans (Shields et al., 2002). Furthermore, the absence of fucose led to an improved half-life because it enhanced the attachment to Fc receptors and a more effective recycling of the protein (Okazaki et al., 2004; Shields et al., 2002; Shinkawa et al., 2003). Of note, mouse cells can express glycan structures that are highly immunogenic in humans (Jenkins et al., 1996).

Glycosylation can differ between batches dependent on culture conditions, medium supplementation and the production host. Due to the impact of glycosylation on the biological function of the product, the quality control of this product parameter of each batch is important.

1.1.5. Engineering of secretion

Secretory proteins expressed in mammalian cells are synthesized at the membrane of the endoplasmatic reticulum (ER) where N-glycosylation takes place during their import into the ER. ER chaperones bind the nascent peptide chain to support proper folding of the protein and glycosylation enzymes modify the initially attached core of the sugar tree. Subsequently, properly folded proteins are transported to the Golgi apparatus where glycosylation is completed. The mature protein then reaches the plasma membrane and is released from the cell into the surrounding media by exocytosis.

Synthesis of high amounts of secretory protein can lead to cell stress due to an overload of the ER with nascent proteins. To overcome this cell stress, upregulation of a set of proteins including e.g. chaperons is induced in a process called unfolded protein response (UPR). Part of the UPR is Xbp-1, a transcription factor that is alternatively spliced when unfolded or malfolded proteins in the ER accumulate. The spliced form functions as transcriptional activator and ER chaperone genes are expressed. Indeed, the production capacity of CHO producer cells was markedly increased upon overexpression of the spliced form of Xbp-1 (Becker et al., 2008; Tigges and Fussenegger, 2006). Additionally, the overexpression of different ER chaperones and foldases turned out to be a successful strategy to engineer producer cells, however, in a product-specific manner (Borth et al., 2005; Dorner et al., 1992; Hwang et al., 2003; Mohan et al., 2007).

Of note, in several studies it was observed that the mRNA level of highly expressed proteins did not correlate with the secreted product, pointing to a translational or posttranslational bottleneck in productivity (Barnes et al., 2004; Fann et al., 1999; Ku et al., 2008). To overcome a bottleneck during protein secretion at the Golgi the stable expression of the lipid transfer protein CERT was applied. CERT functions at the level of the Golgi complex to ensure constitutive vesicular trafficking to the plasma membrane and could further augment the specific productivity of CHO-DG44 cells stably secreting a therapeutic antibody (Florin et al., 2009). Furthermore, the overexpression of the SM (Sec1/Munc18-like) proteins Sly1 and munc18c, essential coagonists of SNARE proteins in vesicle fusion, resulted in increased reporter protein secretion in CHO cells (Peng and Fussenegger, 2009). The combination of engineering approaches to improve the different limiting steps of protein secretion was also investigated in that study by combining the overexpression of Sly1 and munc18c with Xbp-1 (Peng and Fussenegger, 2009).

1.1.6. Metabolism engineering

Mammalian cells are very sensitive to stress caused by e.g., toxic byproducts, shear stress or nutrient depletion. In mammalian cell culture byproduct accumulation can inhibit cell growth and productivity or even induces cell death. Engineering of metabolism genes can be applied to overcome the environmental stress of cells. Accumulation of the toxic byproducts ammonium or lactate could be prevented by expression or knockdown of specific metabolic enzymes (Abston and Miller, 2005; Irani et al., 2002; Park et al., 2000; Zhou et al., 2011) or expression of a nutrient transporter (Wlaschin and Hu, 2007). To abolish ammonia accumulation glutamine synthetase was overexpressed. Thus, cells could be grown in the absence of glutamine in the medium and glutamate was utilized for glutamine synthesis. Two benefits could be observed: Firstly, the production of ammonia was greatly decreased. Secondly, the expression of recombinant protein was increased by 18% (Zhang et al., 2006). Another approach to influence ammonia production during a culture process dealt with the two liver enzymes carbamoyl phosphate synthetase I and ornithine transcarbamoylase involved in the first and second steps of the urea cycle. Indeed, when introduced into CHO cells less ammonia accumulation or even increased growth rates could be shown (Park et al., 2000).

1.1.7.   CHO cell engineering of cell cycle, growth and viability

One major aim of engineering CHO cells is to increase viable cell density, viability and culture longevity since this correlates with volumetric productivity. High proliferation rates are typically obtained with producer cells. Thereby high cell densities can be obtained in a short time period. However, in later process stages this can be detrimental because the high cell densities can exceed the support limits of the systems causing cell death and degradation of the product. To prolong the production phase a cell cycle arrest particularly in G1 phase, by mild hypothermia and nutrient control, or overexpression of transgenes that mediate the arrest have been applied. For this purpose the so-called biphasic process is often used. In the beginning cells are grown to high densities followed by induction of cell cycle arrest to maintain cells in G1 phase. Cells kept at the end of G1 phase are metabolically more active and have an increased cell size (Bi et al., 2004; Carvalhal et al., 2003). Even more important is the fact that genes involved in ribosome biogenesis and protein translation are highly expressed in G1 phase (al-Rubeai and Emery, 1990; al-Rubeai et al., 1992; Fussenegger et al., 1998; Moore et al., 1997), which is advantageous for translation of the product protein and thereby volumetric and specific productivity (Hendrick et al., 2001; Palermo et al., 1991).

Transgene overexpression of p21 and p27 was studied in 1997 and the following years to achieve cell cycle arrest (Fussenegger et al., 1997; Fussenegger et al., 1998; Mazur et al., 1998). p21 and p27 are cyclin dependent kinase inhibitors and negatively regulate cell cycle progression. Their inducible expression successfully increased productivity and G1 phase arrest of CHO cells. In general, combined expression of different transgenes often results in more distinct phenotypes than the targeting of one specific mechanism in the cell by overexpression of one "engineering transgene". It is thus conceivable to apply an engineering approach that has more global effects in the cell by e.g. using a metabolic sensor of the cell as a tool. A prominent example is mammalian target of rapamycin (mTOR). mTOR is well described to coordinate growth factor signals as well as energy status or amino acid availability. mTOR regulates transcriptional activity as a main player of different signaling pathways, e.g. the MAP kinase pathway (Wang and Proud, 2006). The observed phenotype mediated by mTOR overexpression in CHO cells combined increased cell growth and proliferation with higher robustness to sub-optimal culture conditions such as oxygen and growth factor limitations. Furthermore, enhanced

specific productivity of CHO-derived cell lines could be observed in mTOR engineered cells (Dreesen and Fussenegger, 2011).

A well-established chemical factor to achieve cell cycle arrest is sodium butyrate. Treatment with sodium butyrate arrests cells in G1 phase (Hendrick et al., 2001; Kim and Lee, 2002; Kruh, 1982) resulting in the accumulation of 67% of HeLa cells and 54% of CHO cells in the G1 phase (Hendrick et al., 2001; Xue and Rao, 1981). Furthermore, it inhibits histone deacetylases (HDACs) (Kruh, 1982; Pikaart et al., 1998). This can lead to improved transcription of the product, since HDAC-mediated silencing of transcription can be prevented. Unfortunately, sodium butyrate has the disadvantage to cause apoptosis (Kim and Lee 2002). This could be compensated by the combined expression of the anti-apoptotic protein Bcl-2 together with butyrate treatment leading to decreased butyrate induced apoptosis and increased recombinant product levels (Kim and Lee, 2000).

Furthermore, apoptosis inducing effectors can directly be engineered. RNA interference (RNAi) technology was used to suppress pro-apoptotic proteins such as Bax and Bak (Lim et al., 2006). RNAi enables translational inhibition of proteins by introducing short interfering RNAs (siRNA) into cells. These siRNAs are short noncoding RNAs with 21-23 bp length derived from cleaved long dsRNAs (Tuschl, 2001). Their function is to specifically silence gene expression, which is mediated by their complementary sequence to the mRNA of the respective target protein. By interaction of the siRNA associated RISC (RNA induced silencing complex) with the target mRNA, translation is diminished and in the case of Bax and Bak, pro-apoptotic proteins were downregulated (Lim et al., 2006). As a result viable cell densities were increased in the fed-batch culture of suspension CHO cells. Extreme conditions e.g. nutrient depletion or high osmolarity were used to investigate prolonged survival. Interferon gamma used as a model product had 35% increased productivity with these engineered cells (Lim et al., 2006).

Another class of short non-coding RNAs are microRNAs that will be described in the following chapters 1.2 and 1.3.

1.2. MicroRNAs

MicroRNAs or miRNAs are short 18-24 nucleotide long noncoding RNAs that are involved in the posttranscriptional regulation of protein expression. They were first discovered in 1993 in the worm *C. elegans,* where the gene lin-4 was described as the origin of a noncoding RNA that interacted with the transcript of lin-14 thereby repressing its expression (Lee et al., 1993). Another miRNA studied in *C. elegans* was termed let-7. Both *C. elegans* miRNAs were involved in developmental timing (Olsen and Ambros, 1999; Reinhart et al., 2000).

Within a few years this protein expression regulation system was also described in plants (Jones-Rhoades et al., 2006). Later many other miRNAs were found, also in mammalian cells (Landgraf et al., 2007).

1.2.1. MicroRNA biogenesis

miRNAs are encoded in the genome of worms, plants, animals and even viruses. It is estimated that about 1% of the genome encodes for miRNAs. miRNAs were found to be encoded in exonic or intronic regions of noncoding transcripts or intronic in protein coding transcripts. About 50% of miRNAs are found in clusters, in which more than one miRNA is transcribed as one polycistronic transcript of primary miRNAs (pri-miRNA). Expression can be triggered either by their own promotor or, for intronic miRNAs, by the promotor of the respective gene.

The biogenesis of miRNAs is schematically shown in Figure 6. miRNAs have 5-´cap structures as well as poly-A tails and are mainly RNA polymerase II transcribed in contrast to most small RNAs that are transcribed by RNA polymerase III. A pri-miRNA typically forms hairpin structures and is processed in the nucleus by the microprocessor complex consisting of DROSHA, a RNAse III enzyme, and DGCR8 (DiGeorge critical region 8) (Kim, 2005). This step results in 60-80 nt long hairpin stem-loop structures with an 3' two nucleotide overhang and a characteristic 22 nt stem called precursor miRNA (pre-miRNA).

Alternatively, pre-miRNAs can be generated by so called mirtrons during the splicing process of mRNA, provided that the miRNA is encoded within the intron of the mRNA

(Yang and Lai, 2011). The pre-miRNA is subsequently exported to the cytoplasm by exportin-5, a nucleus pore that specifically exports pre-miRNA to the cytoplasm in a RAN-GTP dependent manner.

In the cytoplasm further maturation of the pre-miRNA is mediated by another RNase III enzyme called DICER. DICER removes the loop of the hairpin. Thereby generating an approximately 22 nt long double stranded RNA. After unwinding of the double strand by a helicase, the now single stranded RNA, the mature miRNA, is loaded onto the RISC complex referred to miRISC (miRNA induced silencing complex). The Ago protein (Argonaut protein), a component of the miRISC can cleave the miRNA-mRNA duplex.

In many cases only one RNA strand of the duplex is loaded onto the RISC complex and is termed the guide strand. Selection of the guide strand depends on the thermostability of the base pairing of the miRNA duplex with the strand harboring the weaker base pairing at the 5' end being predominantly loaded. The other strand, the passenger strand or miRNA* strand, diminishes fast (Kim, 2005).

Interestingly, if both strands are loaded onto the RISC complex, they can have different functions by targeting different mRNAs. It was even shown that the two strands derived from one hairpin could have inverse biological effects although it is not fully understood how this is regulated by the cell (Jiang et al., 2010).
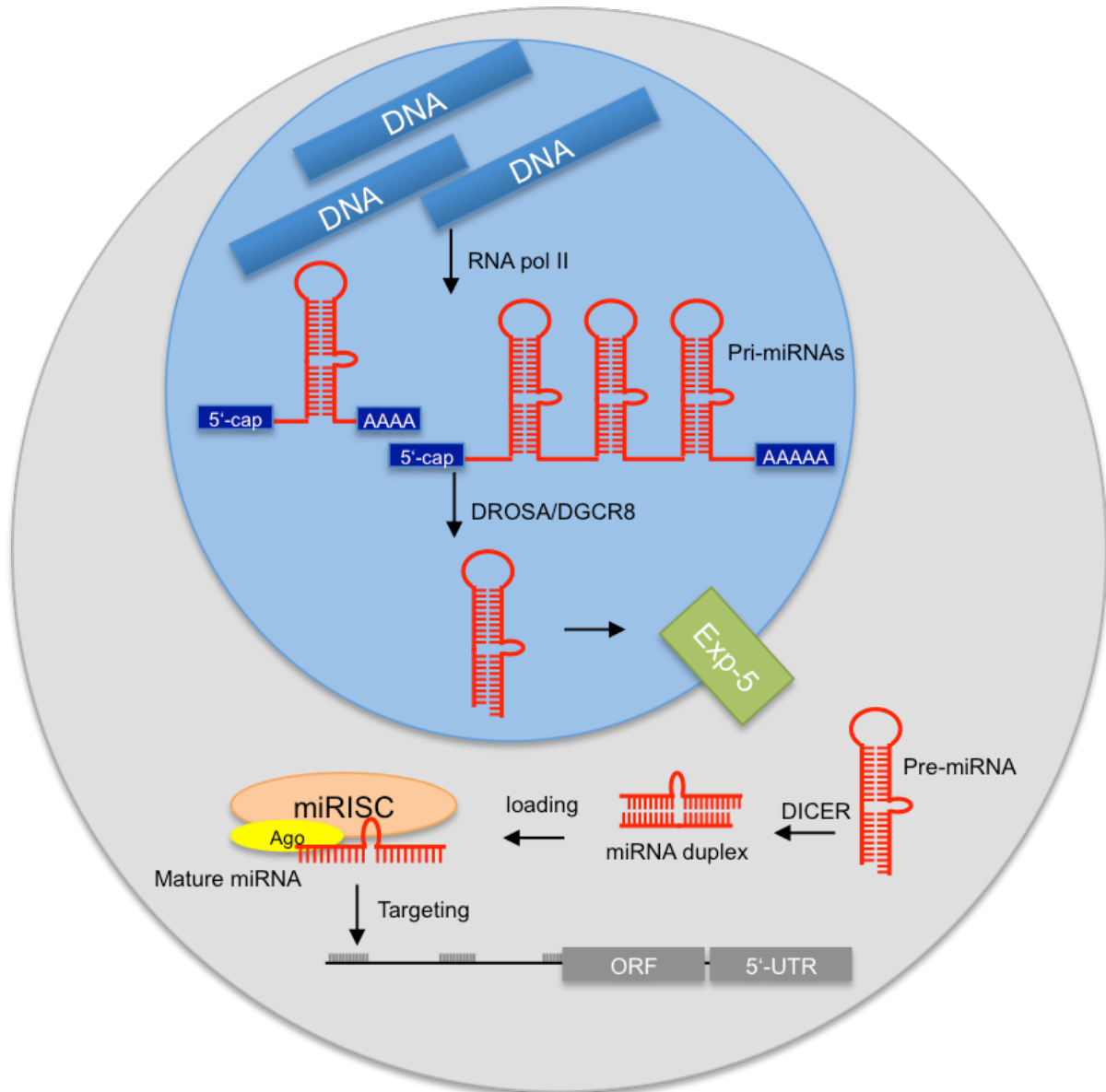
Figure 6: Biogenesis of microRNAs (modified from Sun et al., 2010).
miRNAs are encoded in the genome either singly or in combination, consequently, being transcribed as clusters. Primary miRNAs (pri-miRNA) form typical hairpin structures. The first processing step is mediated by DROSHA/DGCR8 in the nucleus. This results in a precursor miRNA (pre-miRNA) with a hairpin of about 70 nt in length and a two nucleotide 3' overhang. These structural features are recognized by the nuclear export pore exportin-5 (exp-5, green) and the pre-miRNA is transported into the cytoplasm in a RAN-GTP dependent manner. DICER cuts off the loop of the hairpin as a second processing step. One strand of the miRNA-miRNA* duplex is loaded into the RISC (orange) associated with Ago (yellow). Translational inhibition of the target mRNA is mediated by imperfect base pairing with the 3'UTR (untranslated region). ORF (open reading frame)

1.2.2.   MicroRNA nomenclature

In 2001 the term microRNA was established together with a universally applicable new nomenclature of miRNAs organized by the mirbase (see chapter 1.2.4). The nomenclature of miRNAs consists of a prefix of three letters encoding for the species, followed by "–miR" and a number (e.g. hsa-miR-557, whereby "hsa" encodes a human miRNA: _homo sapiens_). The naming of the previously described "let-miRNAs" (see chapter 1.2) does not fit to this nomenclature but these early identified miRNAs kept their names. The names of homologous miRNAs contain the same number, whereas single nucleotide polymorphisms are indicated by a, b, c etc. (e.g. hsa-miR-20a and hsa-miR-20b). Identical mature miRNA sequences derived from hairpins that are encoded on different loci are indicated by attached -1, -2 etc. The gene name or hairpin of the precursor is termed with "mir-xx" whereas the mature sequence is called "miR-xx". miRNAs derived from different arms of the stem of the hairpin have the suffix "-3p" or "-5p" (e.g. hsa-miR-125b-3p which is the mature miRNA derived from the 3' arm of the stem of the hairpin). If one strand of the stem is abundant whereas the other one is not, the abundant one is the guide strand and the non abundant is the passenger strand or miRNA* (star). miRNA with the same seed region (nucleotides 2-8 of the 5' end of the mature miRNA) are summarized as miRNA families (Griffiths-Jones et al., 2006).

1.2.3.   MicroRNA target recognition

miRNAs posttranscriptionally regulate protein expression. The sequence of the mature miRNA mediates the specific targeting of the RISC loaded miRNA. It was shown that the binding sites for miRNAs are mainly located in the 3'-UTR (untranslated region) of mRNAs. Imperfect base pairing of the miRNA to the target mRNA induces the translational inhibition or degradation of that mRNA, whereas perfect matches mediate mainly mRNA degradation. Due to the short sequence of a miRNA and the fact that the sequence is usually not 100% complementary to the target mRNA, one miRNA can target mRNAs of many different proteins. miRNAs contain two major regions: Nucleotides 2-8 of the miRNA belong to the seed sequence. The seed region has to be complementary to the target mRNA for functional miRNA targeting, but this is not completely sufficient. The interaction between the seed sequence of the miRNA and the target mRNA gets stabilized by

supplementary pairing of typically nucleotide 13-16 of the miRNAs, belonging to the complementary sequence. If a mismatch in the seed pairing is present, the 3' miRNA pairing is described to be crucial and called compensatory pairing (see Figure 7B and C). While slightly imperfect pairing generally does not hinder function of the miRNA, bulges caused by mismatches between miRNA and mRNA within the region where Ago associates with the miRNA are not tolerated.

One mRNA can often be targeted by more than one miRNA and therefore can provide more than one binding site. These multiple target sites can either be for one specific miRNA or also for different miRNAs (Friedman et al., 2009) (see Figure7A). Translational silencing was described to be more efficient if more than one miRNA binding site is present on a mRNA. Furthermore, the proximity of these sites seemed to enhance miRNA-mediated effects. The existence of multiple target sites allows fine-tuning of protein levels in the cells by translational inhibition or mRNA destabilization (see chapter 1.2.5). Many miRNA sequences as well as their target sequences are conserved between the different species or have homologues in distant species. These evolutionally conserved target sites were found to mediate stronger effects than non-conserved sites. More than 60% of protein-coding mRNAs carry conserved consensus sites for the regulation by miRNAs (Friedman et al., 2009).

A
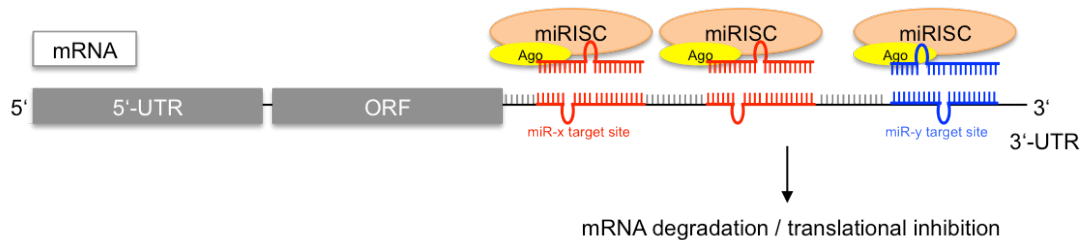
B
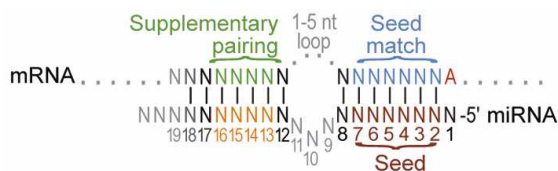
C
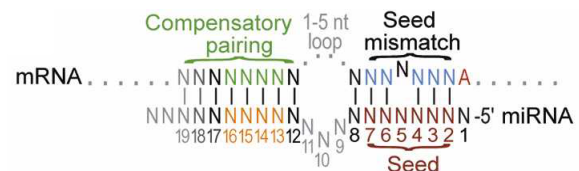
Figure 7: Targeting and base pairing between microRNA and target mRNA.
(A) RISC loaded miRNA targets mRNA by imperfect base pairing to 3'UTR of mRNA. mRNAs with several miRNA target sites for the same (two red sequences) and different miRNAs (indicated by the red and blue sequence). (B) Important for miRNA function is a perfect match of 5' miRNA nucleotides 2-8 termed seed (brown). Additional pairing typically of nucleotides 13-16 stabilizes the interaction and is a supplementary pairing (yellow). Nucleotides in between can loop out. (C) In the rare case that a mismatch in the seed occurs the pairing of the miRNA´s 3' region is crucial and compensates the insufficient seed pairing (compensatory pairing, yellow, modified from (Friedman et al., 2009; Sun et al., 2010).

1.2.4.   MicroRNA databases and target prediction

Information on miRNAs is available through databases, with mirbase (www.mirbase.org) collecting the published miRNAs and ensuring a general nomenclature (see chapter 1.2.2). Currently, 21,264 entries representing hairpins that express 25,141 mature miRNAs of 193 species are listed (release 19, Aug. 2012). To date, 2,042 mature miRNAs for humans are available (Griffiths-Jones, 2004; Griffiths-Jones et al., 2006; Griffiths-Jones et al., 2008; Kozomara and Griffiths-Jones, 2011). Tarbase (Sethupathy et al., 2006) and miRecords (Xiao et al., 2009) are databases that collect miRNA-mRNA interaction data categorized into predicted or experimentally validated miRNA-mRNA pairs. The validated data are obtained from published literature and for prediction several online tools are available (see Table 2). Commonly used ones are TargetscanS, PicTar, miRanda, RNAhybrid, PITA, mirWIP, mirTarget2 (miRDB) and DIANA-microT (Sun et al., 2010). The target prediction algorithms consider different features. A huge impact is provided by the base pairing between the miRNA seed sequence and mRNA but also conservation and availability of target sites play a role and are considered. Table 2 lists a selection of target prediction algorithms and their respective prediction criteria. Although improvement of these algorithms is an ongoing process the results obtained by these prediction analyses are characterized by a high predictive sensitivity but low predictive specificity. For this reason a large number of putative miRNA target interactions with high false positive rate are generated (Sun et al., 2010).

Table 2: Target prediction tools and their algorithms (modified from Sun et al., 2010).

Tool	Most important criteria for prediction
TargetscanS	Seed match, sequence complementarity, minimal free energy of miRNA/target duplex, sequence preferences of target sites
PicTar	Seed match, sequence complementarity, minimal free energy of miRNA/target duplex and combinatorial miRNA-target interactions
miRanda	Seed match, sequence complementarity, minimal free energy of miRNA/target duplex
RNAhybrid	Seed match, sequence complementarity, minimal free energy of miRNA/target duplex and statistical significance of miRNA-target interactions
PITA	Seed match, sequence complementarity, minimal free energy of miRNA/target duplex and target-site accessibility
mirWIP	Seed match, sequence complementarity, minimal free energy of miRNA/target duplex, statistical significance of miRNA-target interactions and target-site accessibility
mirTarget2 (miRDB)	Based on machine-learning technique; a computational model was trained by a variety of features concerning miRNA-target interactions
DIANA-microT	Seed match, sequence complementarity, minimal free energy of miRNA/target duplex and sequence preferences of target sites

1.2.5.   MicroRNA function and regulation

The main function of miRNAs is the regulation of protein expression at the level of mRNAs. This can be achieved by mRNA degradation, translational inhibition mediated by deadenylation, or inhibition of translation initiation or elongation (Fabian et al., 2010). A study that compared mRNA levels of newly synthesized proteins before and after miRNA overexpression showed that in most cases the decreased protein level was mediated by both, translational inhibition as well as mRNA level decrease (Selbach et al., 2008). The miRNA mediated instability of mRNAs can furthermore be caused by cleavage. This was particularly described to occur in the case of miRNAs with perfect base pairing to their target, which was mainly observed in plants (Voinnet, 2009). The impact of miRNA regulation on the level of a single protein was relatively small and rarely exceeded fourfold decrease. Therefore, the often very strong regulatory effects caused by miRNAs were mediated by the sum of small changes within the expression level of many proteins, which was reflected in the widespread presence of conserved miRNA target sequences (see chapter 1.2.3).

Regulation of protein levels by miRNAs is essential for early embryogenesis and development. This was demonstrated by genetic ablation of DICER in mice. DICER deficient cells could not generate mature functional miRNAs (see chapter 1.2.1), which was lethal for mice in early embryonic phase (Bernstein et al., 2003). The coordination of a low expression of one miRNA and a high expression of another miRNA can have a synergistic effect, since different miRNA can affect the same transcription factor by different modes. For example, miR-21 inhibited expression of the transcription factor Oct4 but has a low expression in ES (embryonic stem) cells, whereas highly expressed miR-290 inhibited Oct4 inhibitors, thereby supporting the self-renewal potential of ES cells (Singh et al., 2008). miRNA expression does not necessarily have to be tissue specific as several miRNAs are expressed ubiquitously. However, expression of certain miRNAs can influence differentiation characteristics of cells. For example, expression of miR-1 was muscle specific whereas miR-124 was mainly expressed in brain tissue. Delivering miR-124 into a cell culture shifted the expression profile towards that of neuronal cells, whereas delivering miR-1 shifted the protein profile towards that of muscle cells (Lim et al., 2005). This highlights the importance of miRNA regulation. Moreover, there are miRNAs that are expressed only temporally, some of which were shown to play an important role in

the development of certain tissues or developmental timing of organisms (Lee and Ambros, 2001).

The abundance of miRNAs, which are regulators of a huge pool of genes by translational inhibition of protein expression, needs to be tightly regulated. It could be shown that mature miRNA levels can differ dramatically in a tissue or stage specific manner although precursor levels stayed constant (Ruegger and Grosshans, 2012). In addition, the level of several miRNAs, which are involved in cell cycle regulation and are expressed as one cluster in their primary miRNA state, can differ in their level as mature miRNAs (Ruegger and Grosshans, 2012). This strongly hints to the existence of regulation mechanisms during biogenesis and maturation of miRNAs in which the loop of the hairpin plays a role. The loop regions are strongly conserved between species for 14% of pri-miRNAs, implying that the conserved loops are a target for regulation (Michlewski et al., 2008). Some factors that regulate miRNA maturation have already been identified: SMAD, p53, p68/p70, hnRNP A1 and KSRP promote the maturation of miRNAs, whereas NF90 and lin-28 have an inhibitory function (Ruegger and Grosshans, 2012; Trabucchi et al., 2009). Additional regulation of miRNA function is provided by compartmentalization and sequestration of mRNAs in distinct areas in the cell termed p-bodies (processing bodies) or GW-bodies. These p-bodies are enriched in proteins that mediate inhibition of mRNA translation, namely deadenylation, decapping and degradation of mRNAs. Also the accumulation in stress granules was reported (Fabian et al., 2010). Dysregulated miRNA levels have been described to be the reason for several human diseases. One of the best-studied function of miRNAs in diseases is their role in tumorigenesis and other cancer associated processes as epithelial to mesenchymal transition (EMT), metastasis or invasion. miRNAs can play a role as tumor suppressors or oncogenes, the latter called oncomiRs. The members of the miR-17-92 cluster, consisting of 6 miRNAs are important examples of oncomiRs. The oncogene c-myc and the oncomiR cluster miR-17-92 regulate each other and mediate apoptosis protective mechanisms (Concepcion et al., 2012).

Importantly, miRNAs also affect processes relevant to biotechnological engineering of cells such as apoptosis, stress resistance and cell cycle regulation or proliferation, secretion of proteins and vesicular transport (Mendell and Olson, 2012; Poy et al., 2007; Zhang et al., 2010).

1.3. MicroRNAs and CHO cells

The first miRNA described in CHO cells was miR-21. It was identified in a biphasic cultivation process. Expression profiles were generated using bioarrays to detect human, rat and mouse miRNAs. 26 differentially expressed miRNAs were detected after temperature shift. Sequencing confirmed the existence of cgr-miR-21 (cgr for the species _Cricetulus griseus_) with a homologous sequence to miR-21 (Gammell et al., 2007). Four years later the transcriptome of CHO cells was published (Becker et al., 2011) and also the genome was sequenced and published (Xu et al., 2011). With these data 387 miRNAs could be annotated in CHO cells (Hackl et al., 2011). Currently, the number of official hamster specific mature miRNAs in the mirbase is 307, which is only 1/6 of the amount of known human miRNAs. Thus, the assumption that this list might be incomplete is obvious.

1.3.1. MicroRNA expression studies in CHO cells

To date, studies performed in CHO cells to identify miRNAs in order to engineer these cells were based on miRNA expression profiling (Barron et al., 2011; Druz et al., 2011; Gammell et al., 2007; Hammond et al., 2012; Hernandez Bort et al., 2012; Lin et al., 2011). For example, one day after temperature shift ten deregulated miRNAs were identified (Barron et al., 2011). Next, parental CHO cells were compared to recombinant protein producing cells using either microarrays based on human, mouse and rat miRNAs or on human and rodent homology (Hammond et al., 2012; Lin et al., 2011). Two miRNAs, miR-221 and miR-222 were downregulated in the producer cells. Interestingly, two miRNAs of the let-7 family were affected by MTX amplification (Lin et al., 2011) and Hammond showed that 80% of the detected miRNAs were differentially expressed due to recombinant protein production (Hammond et al., 2012). In contrast to these array-based profiles, another study succeeded in detecting miRNAs in hamster cells cultivated under different conditions by Illumina sequencing. In total, 350 miRNAs and miRNAs* were identified by comparing sequence homology to other species (Johnson et al., 2011). Recently, miRNAs and mRNAs were profiled in different stages of a CHO culture. More than 100 miRNAs were differentially regulated relative to the start of a glutamine free batch culture (Hernandez Bort et al., 2012). Cell clones with low and high growth rates were compared resulting in 51 miRNAs with expression correlating to growth

performance (Clarke et al., 2012). Finally, using mouse and rat miRNA arrays, differentially regulated miRNAs after nutrient depletion of CHO cells were identified at a time when apoptosis was induced. Strikingly, regulation of mmu-miR-466h was connected to the downregulation of five anti-apoptotic genes (Druz et al., 2011).

1.3.2. Engineering of CHO cells with microRNAs

As detailed above miRNAs could be identified that were differentially regulated in the late stage of a glutamine free suspension culture (Hernandez Bort et al., 2012), in recombinant protein expressing cells compared with parental cells (Hammond et al., 2012; Lin et al., 2011), in cells with different growth rates (Clarke et al., 2012), in cells undergoing apoptosis upon nutrient depletion (Druz et al., 2011) and in cells shifted to lower temperature (Barron et al., 2011; Gammell et al., 2007). However, so far only a few studies have made attempts to engineer CHO cells by manipulating miRNA levels. The transient inhibition of mmu-miR-466h partially rescued the decrease in viability after nutrient depletion of a CHO cell culture (Druz et al., 2011). In another study, overexpression of miR-7 dramatically decreased viable CHO cell density (Barron et al., 2011), however, attempts to decrease miR-7 levels were not successful. Finally, growth rates of CHO cells slightly increased after transient vector-based expression of miR-17 and to a minor extent also of miR-221. Of note, 4 out of 5 validated targets of miR-17 were downregulated at the mRNA level after miR-17 expression, indicating that functionally processed mature miRNAs were expressed with this strategy (Jadhav et al., 2012).

1.4. Aim of the thesis

MicroRNAs (miRNAs) are short non-coding RNAs that post-transcriptionally regulate the expression of different target genes and, thus, potentially offer the opportunity to engineer networks of genes in order to achieve complex phenotypic changes in mammalian cells. The hypothesis was that this feature of miRNAs could be exploited as a strategy to improve therapeutic protein production processes that use mammalian cells as a host. Chinese hamster ovary (CHO) cells are the most common mammalian cell line employed for the manufacture of therapeutic proteins. Although engineering approaches aimed at increasing the viable cell density or specific productivity of CHO cells have been reported, in many cases, these were not tested in an industrially relevant setting, namely in stable cell lines grown in suspension under serum-free conditions. In this thesis, a genome-wide functional miRNA screen was to be established and performed in a high producer CHO cell line stably expressing an IgG1 (CHO-IgG1), using the antibody productivity as a readout. The aim was to then design a vector-based expression system to stably express the productivity enhancing miRNAs identified by the screening in the CHO-IgG1 producer cells. This should enable the analysis of the performance of these cells in fed-batch cultures, which closely mimic the production process, to address whether miR-engineering holds promise for the genetic optimization of CHO cells for production purposes.

2. Materials and Methods

2.1. Materials

2.1.1. Instruments

Table 3: Instruments and company

Instrument	Company
Automated cell counter TC10	Biorad, Munich, Germany
CEDEX cell quantification system	Roche, Mannheim, Germany
CellObserver: HS CellObserver inverse microscope, AxioCam HR 12 bit camera, Plan Apochromat 20×/0.8 M27 objective lense	Carl Zeiss MicroImaging GmbH, Jena, Germany
Centrifuge Heraeus Fresco 17	ThermoFisher Scientific, Waltham, MA, USA
Centrifuge Rotana 460R	Hettich, Tuttlingen, Germany
Cytomics FC-500	Beckman Coulter, Krefeld, Germany
Electroblotter: Semi Dry Blotter PEGASUS	Phase, Lübeck, Germany
Electrophoresis: Mini-PROTEAN 3	Biorad, Munich, Germany
FACSDiVa option – FACSVantage SE	BD Bioscience, Heidelberg, Germany
Film Developing Machine Agfa Curic 60	Agfa, Düsseldorf, Germany
Gel Documentation Camera Felix 2000, Dark hood DH-50, transilluminator UST-20M-8R	Biostep, Jahnsdorf, Germany
YSI-1500	YSI Life Science, Langenfeld, Germany
LabChip GXII capillary gel electrophoresis (CGE)	Caliper Life Sciences, Hopkinton, USA
Microplate reader	TECAN INFINITE, Männedorf / Switzerland
Minitron incubator	Infors, Einsbach, Germany
Multiskan FC reader	ThermoFisher Scientific, Waltham, MA, USA
NanoDrop spectrometer	ThermoFisher Scientific, Waltham, MA, USA
Nanophotometer	Implen, Munich, Germany
Nucleofector™ II Device, 96-well Shuttle™ Device	Lonza, Verviers, Belgium
Incubator	Varolab, Giesen, Germany
pH meter FiveEasy	Mettler Toledo, Giessen, Germany
Power Supply EPS-300	Amersham Pharmica Biotech, Freiburg, Germany
Quantitative PCR Cfx96	Biorad, Munich, Germany
Sterile bench Flow V	Varolab, Giessen, Germany
Thermocycler TC-512	Biostep GmbH, Jahnsdorf, Germany
Vortexer444-1372	VWR International, Bruchsal, Germany
Water Bath WNB 10	Memmert, Schwabach, Germany
Water Purification Milli-Q Reference	Millipore, Schwalbach, Germany

2.1.2. Consumables

Table 4: Consumables and company

Consumable	Company
50kDa Amicon centrifugal filter units	Millipore, Schwalbach, Germany
Blotting Paper, 3MM	Whatmann, Schleicher Schuell, Dassel, Germany
Biodyne® Nylon Transfer Membrane	PALL, Ann Arbor, MI, USA
Cell culture flasks, plates and dishes	Greiner, Frickenhausen, Germany
Counting chamber (disposable)	Biorad, Munich, Germany
Counting chamber (disposable)	Roche, Mannheim, Germany
ELISA plates	Greiner, Frickenhausen, Germany
Medical X-ray film	CEA, Strangnas, Sweden
Protein A HP spin trap columns	GE Healthcare, Dornstadt, Germany
Shake flasks 125 mL	Corning, Wiesbaden, Germany
qPCR plates MLL-9651	Biorad, Munich, Germany

2.1.3. Chemicals

Table 5: Chemicals and company

Chemical	Company
3, 3', 5, 5'-Tetramethylbenzidine (TMB)	BD Bioscience, Heidelberg, Germany
4-nitrophenyl phosphate disodium salt hexahydrate (4-NPP)	Sigma-Aldrich, Taufkirchen, Germany
Acetic acid	Carl Roth GmBH & Co., Karlsruhe, Germany
Acid sulphur (H_2SO_4)	Carl Roth GmBH & Co., Karlsruhe, Germany
Acrylamide (Rotiphorese Gel 30)	Carl Roth GmBH & Co., Karlsruhe, Germany
Agar	Carl Roth GmBH & Co., Karlsruhe, Germany
Agarose	Carl Roth GmBH & Co., Karlsruhe, Germany
Ammonium persulfate (APS)	Carl Roth GmBH & Co., Karlsruhe, Germany
β-Mercaptoethanol (β-ME)	Sigma-Aldrich, Taufkirchen, Germany
Blocking reagent	Roche Diagnostics, Basel, Switzerland
Bovine Serum Albumin (BSA)	Sigma-Aldrich, Taufkirchen, Germany
Bromphenol blue	Serva, Heidelberg, Germany
Coomassie brilliant blue R250	Carl-Roth GmBH & Co., Karlsruhe, Germany
Complete protease inhibitor cocktail	Roche, Mannheim, Germany
Crystal violet	Merck, Darmstadt, Germany
Dimethyl sulfoxide (DMSO)	Carl-Roth GmBH & Co., Karlsruhe, Germany
Dithiothreitol (DTT)	Carl-Roth GmBH & Co., Karlsruhe, Germany
DNAorange	Sigma-Aldrich, Taufkirchen, Germany
Ethylendiamintetraessigsäure (EDTA)	Carl-Roth GmbH & Co., Karlsruhe, Germany
Ethanol	VWR, Darmstadt, Germany
Ethidium bromide	Roche Diagnostics, Basel, Switzerland
Gene Ruler 1 kb Ladder	MBI Fermentas, St. Leon-Rot, Germany
Glycerol	Carl-Roth GmbH & Co., Karlsruhe, Germany
Glycine	Carl-Roth GmbH & Co., Karlsruhe, Germany
HRP SuperSignal®West substrate pico	Pierce Biotechnology, Rockford, USA
HRP SuperSignal®West substrate dura	Pierce Biotechnology, Rockford, USA
Human albumin (#T844.2)	Carl-Roth GmbH & Co., Karlsruhe, Germany
Isopropanol	Carl Roth GmbH & Co., Karlsruhe, Germany
KCl	Carl-Roth GmbH & Co., Karlsruhe, Germany
KH_2PO_4	Carl Roth GmbH & Co., Karlsruhe, Germany
Methanol	Carl-Roth GmbH & Co., Karlsruhe, Germany
Magnesium chloride ($MgCl_2$)	Carl-Roth GmbH & Co., Karlsruhe, Germany
"PageRuler" prestained protein ladder	MBI Fermentas, St. Leon-Rot, Germany
Peptone	Carl Roth GmbH & Co., Karlsruhe, Germany
Phenylmethylsulphonyl fluoride (PMSF)	Sigma-Aldrich, Taufkirchen, Germany
NaF	Carl Roth GmbH & Co., Karlsruhe, Germany
Sodium azide	Sigma-Aldrich, Taufkirchen, Germany
Sodium chloride	Carl Roth GmbH, Karlsruhe, Germany
Sodium dodecyl sulfate (SDS)	Carl Roth GmbH & Co., Karlsruhe, Germany
Sodium hydroxide (NaOH)	Carl Roth GmbH & Co., Karlsruhe, Germany
Na_2HPO_4	Carl Roth GmBH & Co., Karlsruhe, Germany
Sodium orthovanadate Na_3VO_4	Sigma-Aldrich, Taufkirchen, Germany
Tetramethyldiethyldiamine (TEMED)	Carl Roth GmbH & Co., Karlsruhe, Germany
Thiozolylblue tetrazolium bromide (MTT)	Sigma-Aldrich, Taufkirchen, Germany
Thimerosal	Carl Roth GmbH & Co., Karlsruhe, Germany
Tris-(hydroxymethyl)-aminomethane (Tris)	Carl Roth GmbH & Co., Karlsruhe, Germany
Triton X-100	Carl Roth GmbH & Co., Karlsruhe, Germany
Tween-20	Carl Roth GmbH & Co., Karlsruhe, Germany
Tween-80	Carl Roth GmbH & Co., Karlsruhe, Germany
Yeast extract	Carl Roth GmbH & Co., Karlsruhe, Germany
$ZnCl_2$	Carl Roth GmbH & Co., Karlsruhe, Germany

2.1.4. Buffers and solutions

Table 6: Buffers and solutions with their recipe

Buffer	Recipe
Blocking solution (Western blot)	0.5% (v/v) blocking reagent, 0.05% (v/v) Tween-20, 0.01% (v/v) thimerosal in PBS
Blotting buffer	200 mM glycine, 25 mM Tris, 20% (v/v) methanol
Coomassie stain solution	40% (v/v) methanol, 10% (v/v) acetic acid, 0.1% (w/v) coomassie brilliant blue
Destain solution	10 % (v/v) acetic acid 40 % (v/v) methanol in H_2O
Laemmli protein sample buffer (5x)	312.5 mM Tris, pH 6.8, 25% (v/v) glycerol, 10% (w/v) SDS, 0.05 % (w/v) bromophenol blue 25 % β-mercaptoethanol (for reducing, without for non-reducing)
Lysis buffer (TEB)	50 mM Tris pH 7.5, 150 mM NaCl, 10 mM NaF, 0.1 M Glycerol, 1 mM EDTA, 1% Triton X-100 1mM Na_3VO_4, 0.5 mM PMSF, Complete protease inhibitor cocktail
PBS (Phosphate Buffered Saline)	140 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4
PBST	0.05 % (v/v) Tween-20 in PBS
SDS-PAGE running buffer	25 mM Tris pH 8.8, 192 mM glycine, 0.1% SDS
Substrate buffer	0.1 M glycine 1 mM $ZnCl_2$ 1 mM $MgCl_2$, pH 10.4 with NaOH
TAE (Tris-Acetat-EDTA), pH 8,0	40 mM Tris-acetate, 1 mM EDTA, pH 8.3

2.1.5. Cell lines, reagents, cell culture and bacterial medium

Table 7: Bacteria medium and plates

Medium	Components
LB medium	1 % peptone, 0.5 % yeast extract, 1 % NaCl
LBspec medium	LB medium (50 µg/mL spectinomycin)
LBspec agar plates	LB-Medium with 1.3 % agar, autoclave (50 µg/mL spectinomycin)

Table 8: Cell lines

Cell line	Characteristics	Tissue	Source
CHO-DG44 (CHO-IgG1*)	Stably secreting an IgG1, single cell clone, suspension cells, serum free adapted	Chinese hamster ovary	Boehringer-Ingelheim
CHO-DG44 (conCERT*)	Stably expressing human CERT, single cell clone, suspension cells, serum free adapted	Chinese hamster ovary	Boehringer-Ingelheim
CHO-DG44 (CHO-HSA*)	Stably secreting HSA, cell pool, suspension, serum free adapted	Chinese hamster ovary	Boehringer-Ingelheim
CHO-K1	Adherent	Chinese hamster ovary	Boehringer-Ingelheim
HeLa	Adherent	Human cervix adenocarcinoma	ATCC
MDA-MB468	Adherent, endogenously expressing IgG1 target	Human mammary adenocarcinoma	CLS

*kindly provided by Boehringer-Ingelheim (Florin et al., 2009)

Table 9: Cell culture reagents and antibiotics

Reagent	Company
10 x Trypsin EDTA	Life Technologies, Darmstadt, Germany
BI CHO suspension medium, BI CHO production medium, BI CHO feed medium	Boehringer-Ingelheim, Biberach/Riss, Germany
Blasticidin S	Life Technologies, Darmstadt, Germany
DMEM, RPMI 1640, OptiMEM	Life Technologies, Darmstadt, Germany
Doxycycline, HCl	Merck Biosciences, Darmstadt, Germany
FCS (fetal calf serum)	PAA, Laboratories, Pasching, Austria
G418	Life Technologies, Darmstadt, Germany
Glucose (sterile)	Sigma-Aldrich, Taufkirchen, Germany
TransIT-HeLaMONSTER®	Mirus Bio, Madison, USA
Methothrexat	Sigma-Aldrich, Taufkirchen, Germany
Lipofectamine™ 2000	Life Technologies, Darmstadt, Germany
Oligofectamine	Life Technologies, Darmstadt, Germany
Plus reagent	Life Technologies, Darmstadt, Germany
Puromycin	Calbiochem, San Francisco, USA
RNAiMAX	Life Technologies, Darmstadt, Germany
Spectinomycin	Sigma-Aldrich, Taufkirchen, Germany
TransIT-293 Transfection reagent	Mirus Bio, Madison, USA
Trypan blue	Sigma-Aldrich, Taufkirchen, Germany

2.1.6. siRNAs, miRNAs, DNA oligos, Primer and Plasmids

The microRNA library (CS-001010 mimic microRNA library, lot #09167) was purchased from Dharmacon and detailed information is attached in the appendix (see 6.2.1). The miRNAs for further analysis were obtained from ThermoFisher Scientific (see Table 10). All siRNAs (see Table 13) were purchased from MWG Eurofins (Ebersberg, Germany). Sequencing primers (see Table 12) were included in the BLOCK-iT™ Pol II miR RNAi Expression Vector Kit (see Table 16) and qRT-PCR primer and probes were included in the Taqman® microRNA Assays (see Table 11) (both purchased from Life Technologies). DNA oligonucleotides (see Table 14) that were used for vector cloning were obtained from Biomers (Ulm, Germany).

Table 10: MicroRNAs and detail information

microRNAs	Sequence	MIMAT	Order number
hsa-miR-23b*	UGGGUUCCUGGCAUGCUGAUUU	0004587	C-301170-01
hsa-miR-125a-3p	ACAGGUGAGGUUCUUGGGAGCC	0004602	C-301060-01
hsa-miR-149	UCUGGCUCCGUGUCUUCACUCCC	0000450	C-300631-07
hsa-miR-183	UAUGGCACUGGUAGAAUUCACU	0000261	C-300559-07
hsa-miR-185*	AGGGGCUGGCUUUCCUCUGGUC	0004611	C-301192-01
hsa-miR-193b*	CGGGGUUUUGAGGGCGAGAUGA	0004767	C-301093-01
hsa-miR-365*	AGGGACUUUCAGGGGCAGCUGU	0022833	C-301299-00
hsa-miR-450b-3p	UUGGGAUCAUUUUGCAUCCAUA	0004910	C-301222-01
hsa-miR-557	GUUUGCACGGGUGGGCCUUGUCU	0003221	C-300875-01
hsa-miR-612	GCUGGGCAGGGCUUCUGAGCUCCUU	0003280	C-300937-01
hsa-miR-644	AGUGUGGCUUUCUUAGAGC	0003314	C-300971-01
hsa-miR-885-3p	AGGCAGCGGGGUGUAGUGGAUA	0004948	C-301244-01
hsa-miR-892a	CACUGUGUCCUUUCUGCGUAG	0004907	C-301217-01
hsa-miR-1271	CUUGGCACCUAGCAAGCACUCA	0005796	C-301312-00
hsa-miR-1275	GUGGGGGAGAGGCUGUC	0005929	C-301407-00
hsa-miR-1285	UCUGGGCAACAAAGUGAGACCU	0005876	C-301339-00
hsa-miR-1287	UGCUGGAUCAGUGGUUCGAGUC	0005878	C-301341-00
hsa-miR-1293	UGGGUGGUCUGGAGAUUUGUGC	0005883	C-301347-00
hsa-miR-1978 ([+])	GGUUUGGUCCUAGCCUUUCUA	0009988	Custom order
Mimic miRNA control #1[++]	UCACAACCUCCUAGAAAGAGUAGA	0000039	CN-001000-01
Nontargeting control miRNA Dy547	n.a.	--	CP-004500-01

[+] no official miRNA, [++] based on cel-miR-67; both blast against *C. griseus*: only hits with score <40)

Table 11: qPCR primers

miRNA	Primer assay ID for qPCR (TM: Taqman) and revers transcription (RT)
hsa-miR-557	TM001525, RT001525
hsa-miR-1287	TM002828, RT002828
hsa-miR-1978	Custom order: ID CSPACO7
RNU6B	TM001093, RT001093

Table 12: Sequencing primers

Sequencing primer	Sequence
EmGFP forward primer	5'-GGCATGGACGAGCTGTACAA-3'
miRNA reverse primer	5'-CTCTAGATCAACCACTTTGT-3'
miRNA forward primer	5'-TCCCAAGCTGGCTAGTTAAG-3'

Table 13: siRNAs

siRNAs	Sequence
siLacZ-FAM	5'-FAM-GCGGCUGCCGGAAUUUACCdTdT-3'
siHSA	5'-AUUCCAGAAUGCGCUAUUAdTdT-3'
siCERT854	5'-GAACAGAGGAAGCAUAUAAdTdT-3'
siCERT1128	5'-CCACAUGACUUACUCAUUAdTdT-3'
siLC	5'-AGGCCAAAGUACAGUGGAAdTdT-3'

Table 14: DNA oligonucleotides

miRNA	DNA oligo sequence
hsa-miR-557 forward	5´-TGCTGGTTTGCACGGGTGGGCCTTGTCTGTTTTGGCCACTGACTGACAGACAAGGCCCACGTGCAAAC-3'
hsa-miR-557 reverse	5'-CCTGGTTTGCACGTGGGCCTTGTCTGTCAGTCAGTGGCCAAAACAGACAAGGCCCACCCGTGCAAACC-3'
hsa-miR-1287 forward	5'-TGCTGTGCTGGATCAGTGGTTCGAGTCGTTTTGGCCACTGACTGACGACTCGAACCACATCCAGCA-3'
hsa-miR1287 reverse	5´-CCTGTGCTGGATGTGGTTCGAGTCGTCAGTCAGTGGCCAAAACGACTCGAACCACTGATCCAGCAC-3'
hsa-miR1978 forward	5´- TGCTGGGTTTGGTCCTAGCCTTTCTAGTTTTGGCCACTGACTGACTAGAAAGGCTAACCAAACC-3'
hsa-miR1978 reverse	5´- CCTGGGTTTGGTTAGCCTTTCTAGTCAGTCAGTGGCCAAAACTAGAAAGGCTAGGACCAAACCC-3'
Neg. control miRNA*	5'-TGCTGGAAATGTACTGCGCGTGGAGACGTTTTGGCCACTGACTGACGTCTCCACGCAGTACATTT-3'
LacZ**	5'-TGCTGAAATCGCTGATTTGTGTAGTCGTTTTGGCCACTGACTGACGACTACACATCAGCGATTT-3'

*Blast against *cricetulus griseus*: no match

**Blast against *cricetulus griseus*: only hits with score <40

2.1.7. Antibodies

Table 15: Antibodies

Antibody	Conjugated	Specie	Dilution	Company
Anti-human Fc fragment (#109-005-008)	-	goat	1:500 (ELISA)	Dianova, Hamburg, Germany
Anti-human kappa light chain (#A3813)	AP	goat	1:5000 (ELISA)	Sigma-Aldrich
Anti-Flag	-	mouse	1:1000 (WB)	Sigma-Aldrich
Anti-GAPDH	-	rabbit	1:2500 (WB)	Sigma-Aldrich
Anti-human IgG1	PE	goat	1: 500 (staining)	Sigma-Aldrich
Anti-mouse	HRP	sheep	1:10 000 (WB)	GE Healthcare
Anti-rabbit	HRP	donkey	1:10 000 (WB)	GE Healthcare
IgG isotype control (#027102)	-	human	-	Life Technologies

2.1.8. Kits and enzymes

Table 16: Kits

Kit	Company
BLOCK-iT™ Pol II miR RNAi Expression Vector Kit (K4936-00)	Life Technologies
Cell Line Nucleofector® Kit V and SG Cell Line 96-well Nucleofector™ (#V4SC3096)	Lonza
CyQUANT® Direct Cell Proliferation Assay	Life Technologies
DyNAmo ColorFlash Probe qPCR Kit	ThermoFisher Scientific
Human Albumin ELISA Quantitation Set (#E80-129)	Bethyl Labs, Montgomery, TX, USA
Interleukin 8 human ELISA set #31330089 (Streptavidinpoly-HRP, #31334248)	ImmunoTools, Friesoythe, Germany
mirVana™ miRNA Isolation Kit	Life Technologies
Pierce™ BCA Protein Assay Kit	ThermoFisher Scientific
ProfilerPro Glycan Profiling Kit Ver 2	Caliper Life Sciences
PureLink® HiPure Plasmid Midiprep Kit	Life Technologies
PureLink® Quick Gel Extraction Kit	Life Technologies
PureLink® PCR Purification Kit	Life Technologies
TaqMan® MicroRNA Reverse Transcription Kit	Life Technologies

Table 17: Enzymes

Enzyme	Company
T4 DNA ligase (EL0335)	Fermentas (ThermoFisher Scientific)
*BamH*I	Fermentas (ThermoFisher Scientific)
*Bgl*II	Fermentas (ThermoFisher Scientific)
*Xho*I	Fermentas (ThermoFisher Scientific)
*Dra*I	Fermentas (ThermoFisher Scientific)

2.2. Methods

2.2.1. Cell culture of adherent cells

MDA-MB468 and HeLa cells were grown at 37°C and 5% CO_2 in humidified atmosphere in an incubator in DMEM/F-12 and RPMI1640 medium, respectively, supplemented with 10% fetal calf serum (FCS). Cells were subcultured every 3 to 4 days when cell confluence was reached. In brief, cells were washed with PBS and incubated with trypsin/EDTA at 37°C. Enzyme reaction was stopped by addition of culture medium. Cells were pelleted by centrifugation at 250x*g* for 5 min at RT and resuspended in fresh culture medium. Cells were used for further experiments and 1/3 - 1/10 of the cell suspension was delivered into new culture flask and provided with fresh medium for routine cell culture.

2.2.2. Cell culture of suspension cells

Suspension cultures of human serum albumin or monoclonal antibody producing CHO-DG44 and stable transfectants thereof were incubated in a BI proprietary chemically defined, serum-free media (BI CHO suspension medium) supplemented with 400 nM Methotrexat (MTX) and 500 µg/mL G418 for selection. Seed stock cultures were sub-cultivated every 2–3 days with seeding densities of 3×10^5–2×10^5 cells/mL respectively. Cells were grown in T-flasks in humidified incubators at 37°C and 5% CO_2. The cell concentration and viability was determined by trypan-blue exclusion using a counting chamber.

2.2.3. Fed-batch cultivation

3×10^5 cells/mL cells were seeded into 125 mL shake flasks in 30 mL of BI-proprietary production medium (BI CHO production medium) without antibiotics or MTX. The cultures were agitated at 120 rpm at 37°C and 5% CO_2 in a minitron incubator. On day 3, CO_2 was reduced to 2%. BI-proprietary feed solution (BI CHO feed medium) was added daily and pH was adjusted to pH 7.0 using $NaCO_3$. Glucose concentration was measured daily with YSI-1500 and adjusted to 4 g/L with sterile glucose if concentration was below 2 g/L. Cell densities and viability were determined by trypan-blue exclusion using an automated cell counter TC10.

Cumulative specific productivity was calculated as product concentration at the given day divided by the "integral of viable cells" (IVC) until that time point.

2.2.4. Transient transfection of RNAs in CHO-DG44 cells and microscopic analysis

CHO-DG44 cells were transfected with oligofectamine (4 µL) using 100 nM miR-Dy547, siRNA or buffer for mock transfection according to the manufacturer's instructions at a cell density of 7.5 x 10^4 c/1mL.

ConCERT cells (1 x 10^6 cells) were transfected with 100 nM siRNA using nucleofector Kit V program U-23.

Transfected cells were seeded in 12 well plates. The next day cells were washed with PBS and resuspended in medium without phenol red. Draq5 was added in a dilution 1:1000. Pictures of the living cells were taken with an HS CellObserver inverse microscope equipped with AxioCam HR 12 bit camera and with Plan Apochromat 20×/0.8 M27 objective lenses.

2.2.5. CyQuant assay

Viable CHO-DG44 cell numbers were determined using the CyQuant cell proliferation assay on day 4 post transfection according to manufacturer's instructions. Measurement was performed with a microplate reader TECAN INFINITE with ex/em 480/535 nm.

2.2.6. Transient expression of human miRNAs in CHO-DG44 cells - miRNA screen

CHO-DG44 cells stably secreting an IgG1 antibody were cultivated in BI proprietary chemically defined, serum-free medium supplemented with 500 µg/mL G418 and 400 nM MTX and were passaged every 2 or 3 days with a seeding density of 3 × 10^5 cells/mL or 2 × 10^5 cells/mL.

Cells were transfected with 1 µM RNA with a microRNA library via nucleofection one day after passaging (4 × 10^5 cells/sample) in SG Cell Line 96-well Nucleofector™ Kit using the Amaxa 96-well Shuttle Device and program 96-DT-133 according to the manufacturer´s instructions. Then 1/8 each of transfected cells were seeded into four

96-well U-bottom plates. One day after transfection the volume of the medium was doubled by addition of fresh medium without antibiotics or MTX. Supernatants were collected on days 1-4 post transfection by centrifugation of one 96-well plate each day (290xg, 5 minutes). Supernatants were transferred into a fresh 96-well plate and stored at -20°C. A non-targeting siRNA coupled to FAM (siLacZ-FAM) and cells that were transfected without RNA (mock) were used as negative controls. Transfection efficiency was determined by flow cytometry analysis of siLacZ-FAM transfected cells. As a positive control, a siRNA targeting the light chain of the IgG1 antibody (siLC) was used.

2.2.7. Statistical analysis

Antibody concentrations were normalized to the mean of antibody concentrations of all miRNA samples per plate. P-value was calculated with Student´s t-test (two-tailed, unpaired) using the duplicate antibody concentrations. The control group for Student's t-test consisted of antibody concentrations of all negative controls (siLacZ and mock transfected cells).

2.2.8. Validation screen

CHO-DG44 cells either stably secreting an IgG1 or human serum albumin (HSA) were transfected via nucleofection in quadruplicates as described above and seeded into 12-well plates. Cell densities and viability were determined by trypan blue exclusion using a CEDEX cell quantification system. Product concentrations in the supernatant were measured by ELISA according to chapter 0 for IgG1 and 2.2.17 for HSA. Specific productivity was calculated as described in the fed-batch cultivation chapter. siLacZ-FAM and siRNAs targeting the product siLC and siHSA served as controls. Statistical analysis was performed on product concentrations of day 1-4 using a two-way ANOVA followed by a Bonferroni post-test.

2.2.9. MicroRNA combination

CHO-DG44 cells stably secreting an IgG1 were transfected with two different miRNAs simultaneously as described in the validation screen. To this end, hsa-miR-557 and hsa-miR-1287 were transfected at a concentration of 0.5 µM. In case of the

single transfections, amount of total RNA was adjusted to 1 µM by adding mimic miRNA negative control #1. Analysis was done as described in the validation screen. As control the mimic miRNA negative control #1 was transfected.

2.2.10. Transient transfection of HeLa cells

HeLa cells were reverse transfected with RNAiMAX. Firstly, RNAiMAX was diluted 1:10 in 50 µL Optimem and RNA was added. After incubation for 15 min at RT 4 x 10^4 cells were added in 250 µL RPMI1640 medium containing 10% FCS. Transfected cells were cultivated at 37°C, 5% CO^2 in 12-well plates. Final concentration of RNA was 10 nM.

To measure the secretion of ssHRP cells were additionally transfected with 50 ng of a plasmid encoding ssHRP-Flag two days post-transfection (kindly provided by V. Malhotra, (von Blume et al., 2012) with TransIT-HeLaMONSTER®. The next day, medium was replaced by medium without phenol red and supernatant was collected after 4h and 6h. HRP amount was measured by incubation of 20 µL supernatant with 100 µL substrate HRP SuperSignal®West in a white 96-well plate for 15-30 min at RT. Luminescence signal was measured with a microplate reader TECAN INFINITE.

To assess IL-8 secretion, the medium was exchanged 48 h after transfection and supernatant was collected after 6 h and 24 h. IL-8 in the supernatant was analyzed by ELISA analysis according to chapter 2.2.18.

2.2.11. Transient DNA transfection in CHO-DG44 cells

CHO-DG44 cells stably secreting an IgG1 were transfected with pcDNA6.2-GW/emGFP-miRNA vectors by nucleofection. 0.5 µg plasmid DNA were mixed with 4 × 10^5 cells and nucleofected with the conditions described in chapter 2.2.6.

2.2.12. Stable overexpression of microRNAs

CHO-DG44 cells stably secreting an IgG1 were transfected with pcDNA6.2-GW/emGFP-miRNA vectors using Lipofectamine 2000 and Plus reagent. Cells were selected with 10 µg/mL Blasticidin S. miRNA positive populations were obtained by fluorescent activated sorting of GFP positive cells. miRNA overexpression was verified by qRT-PCR analysis (see chapter 2.2.15).

2.2.13. Cloning of microRNA expression plasmids

To stably express miRNAs the BLOCK-iT™ Pol II miR RNAi Expression Vector Kit (pcDNA6.2-GW/emGFP-miRNA) was used. For that purpose, DNA oligonucleotides encoding hsa-miR-557, hsa-miR-1287 and *hsa-miR-1978* were designed as described in the manual (sequences see Table 14). Hairpin structure was analyzed using mfold (online software, (Zuker, 2003). DNA strands were hybridized and ligated into the 3'-UTR of emerald GFP reporter protein as described by the manufacturer. To generate a vector containing two different miRNAs in tandem the chaining technique was applied (see chapter 2.2.14). To generate appropriate controls the negative control miRNA supplied by the kit and the siLacZ were used. Sequence integrity was confirmed by sequencing (GATC) with the primer provided by the kit (see Table 12) emGFP forward and miRNA reverse.

2.2.14. Chaining of microRNAs in expression plasmids

BLOCK-iT™ Pol II miR RNAi Expression Vector system can be used to express more than one miRNA or one miRNA with multiple copies. Therefore the method chaining was used to clone these constructs as described in the manual. In brief, the miRNA cassette was excised with the enzymes *BamH*I and *Xho*I and vector containing already one miRNA was opened with the enzymes *Bgl*II and *Xho*I. DNA was mixed with orange loading buffer and was separated in a 1% agarose gel prepared with TAE buffer. Bands were visualized with ethidium bromide and bands of appropriate size were excised from the gel. Size was verified with a DNA ladder. The DNA was eluted with the PureLink® Quick Gel Extraction Kit. The DNA insert was ligated into the vector using the T4 DNA ligase according to manufacturer's instructions. Competent *E. coli* (BLOCK-iT™ Pol II miR RNAi Expression Vector Kit) were transformed with the DNA and plated on agar plates containing spectinomycin (see Table 7). Colonies were picked and the DNA was extracted with a PureLink® HiPure Plasmid Midiprep Kit, positive clones were identified by digestion with *BamH*I and *Bgl*II, and verified by sequencing with the sequencing primer (see Table 12) provided by the kit. The GFP cassette was removed from the generated constructs as described in the manual. Briefly, D*ra*I digest was used to excise the emGFP cassette. The DNA was separated in a 0.6% agarose gel and bands of the vector size were excised from the gel. The DNA was extracted and religated using the T4 DNA ligase

according to manufacturer's instructions. The following steps were performed as described above. Control digest was done with *Dra*I and sequencing with miRNA reverse and forward primer (see Table 12).

2.2.15. RNA extraction and quantitative real-time PCR analysis

For determination of gene expression analysis by quantitative real-time PCR total RNA was extracted from 2×10^5-2×10^6 CHO cells using mirVANA^{TM} miRNA Isolation Kit according to the manufacturer´s protocol. The concentrations of RNA samples were quantified using a Nanophotometer at OD 260/280 nm. Total RNA (10 ng) was reverse transcribed into cDNA using Taqman® microRNA Assay and TaqMan® MicroRNA Reverse Transcription Kit according to the manufacturer´s instructions in a thermocycler. Quantitative PCR was performed with Taqman® microRNA Assays and DyNAmo ColorFlash Probe qPCR Kit using a Cfx96 device. RNU6B (U6 small nuclear 2) was used as reference. Calculation was done with the single threshold method and $2^{-\Delta Cq}$ values were calculated (Biorad CFX manager software 2.1). Thermo protocols see Table 18.

Table 18: Thermo protocols of RT and qPCR

Reverse transcription			qPCR		
30 min	16 °C		10 min	95 °C	
30 min	42 °C		15 sec	95 °C	Repeat 40
5 min	85 °C		60 sec	60 °C	cycles

2.2.16. Determination of recombinant antibody (IgG1) concentration

To assess recombinant antibody production in transfected cells, supernatants were collected from cell cultures. The product concentration was analyzed by enzyme linked immunosorbent assay (ELISA). In brief, ELISA plates were coated using a goat anti-human Fc fragment diluted 1:500 in coating buffer (0.1 M sodium carbonate buffer, pH 9.5) by incubation at 4°C overnight. Each washing step was done three times with washing buffer (0.15% Tween-20 in PBS). Plates were blocked for 1 h at room temperature with PBS supplemented with 1% human albumin. Samples and standard were diluted in assay diluent (PBS supplemented with 0.5% human albumin and 0.01% Tween-80). Purified IgG1 antibody was used as a standard. After washing of the plate, diluted supernatants and standards were added and incubated for 1.5 h

at room temperature. Plates were washed as described. The detection was performed with an anti-human kappa light chain alkaline phosphate conjugated antibody diluted 1:5000 in assay diluent. After 1 h incubation at room temperature and washing of the plates, 4-NPP solved in substrate buffer (0.1 M glycine, 1 mM $ZnCl_2$, 1 mM $MgCl_2$, pH 10.4) was used as a substrate. Enzyme reaction was stopped with 3 M NaOH after 15-20 min and the absorbance was measured at 405 nm (reference 492 nm) with a multiskan FC reader.

2.2.17. Human Albumin ELISA

Albumin concentrations in cell culture supernatants were analyzed by Human Albumin ELISA Quantitation Set according to manufacturer's instructions. As a coating antibody goat anti-human albumin and as a detection antibody HRP-conjugated goat anti-human albumin were used. TMB was added as a substrate and enzyme reaction was stopped with 1 M H_2SO_4. The absorbance was measured at 450 nm with a multiskan FC reader.

2.2.18. Interleukin-8 (IL-8) ELISA

IL-8 release into the medium was determined by ELISA. In brief, ELISA plates were coated using an anti-IL-8 antibody diluted 1:100 in coating buffer (0.1 M sodiumcarbonat buffer, pH 9.5) by incubation at 4°C overnight. Each washing step was done 5 times with PBS. Plates were blocked for 1 h at room temperature with PBS supplemented with 3% albumin. Samples and standard were diluted in RPMI1640 medium supplemented with 10% FCS. The standard was provided by the kit. After washing of the plate with PBS with 0.005% Tween-20, diluted supernatants and standards were added and incubated for 1 h at room temperature. Plates were washed as described. Subsequently, biotinylated anti-human IL-8 was incubated for 1 h at RT diluted 1:100 in PBS 0.05% Tween-20. Detection was performed with poly-streptavidin conjugated with horseradish peroxidase diluted 1:15,000 in PBS with 1% albumin and 0.05% Tween-20. After 1 h incubation at room temperature and washing of the plate, the TMB substrate was added. The enzyme reaction was stopped with 1 M H_2SO_4 after 15-20 min and the absorbance was measured at 450 nm with a multiskan FC reader.

2.2.19. Antibody purification

The cell culture supernatant obtained from a fed-batch cultivation with CHO-DG44 cells stably secreting an IgG1 was concentrated using 50 kDa Amicon centrifugal filter units. The concentrate containing the antibody was purified with Protein A HP spin trap columns according to the manufacturer's instruction. The antibody elution buffer comprises 0.1M glycine-HCl pH 2.7 and was neutralized with 1M Tris-HCl to pH 7. The buffer was exchanged to PBS using the same filter units as before. Protein concentration was determined photometrically by measuring absorbance at 280 nm using a NanoDrop spectrometer.

2.2.20. Flow cytometry staining

MDA-MB468 cells were stained with purified IgG1 antibodies obtained from a fed-batch cultivation of parental cells or miRNA overexpressing cells. Therefore, 5×10^5 MDA-MB468 cells were incubated with 10 ng purified IgG1 antibody for 1 h on ice in staining buffer (PBS containing 2% FCS and 0.02% sodium azide). Cells were washed two times with staining buffer and incubated with a goat anti-human IgG1 coupled to PE for 45 min on ice. Cells were washed two times with staining buffer and analyzed by flow cytometry. As a negative control unstained cells and cells that were incubated with a purified human IgG isotype control were used.

2.2.21. Cell lysis, SDS-PAGE and Western Blot (WB)

siRNA transfected cells were harvested two days after transfection by centrifugation for 5 min at 250x*g*. Cells were lysed by incubation for 10 min on ice in TEB cell lysis buffer. After centrifugation for 15 min at 16,000x*g* at 4°C to remove the cell debris, protein concentration was assessed by BCA assay according to manufacturer's instructions. In brief, 2 µL cell lysate or BSA standard were incubated with a bicinchoic acid containing solution at 37°C. Peptide bonds of proteins reduce Cu^{2+} ions into Cu^+ which chelates bicinchonic acid resulting in a purple color that can be measured at 550 nm with a multiscan FC reader. Protein amount could be calculated with the BSA standard curve and samples were prepared with 5x Laemmli buffer that negatively charges the proteins and boiled for 10 min at 95 °C. SDS-polyacrylamide gels were prepared as shown in Table 19. 25 µg protein was loaded and proteins

were separated by their size by applying an electric field of 80 to 100 V to the gels after adding 1x SDS running buffer. Separated proteins were immobilized on nitrocellulose membranes by semi-dry blotting. Protein transfer was performed with 1.5 mA/1 cm^2, 9 V for 2 h and membranes were blocked with 0.5% blocking solution for 30 min at RT to prevent unspecific binding of primary antibodies. These were diluted as shown in Table 15 in blocking solution and incubated overnight at 4°C. Membranes were washed three times for 10 min with PBST and incubated with the HRP coupled secondary antibody for 1 h at RT. After washing with PBST membranes were incubated with ECL substrate for 5 min at RT and exposed to the x-ray films for different exposure times to visualize proteins.

Table 19: SDS-Gel recipes

	Separation gel			Stacking gel
	6 %	10 %	12 %	5 %
H$_2$O	7.9 ml	5.9 ml	4.9 mL	3.4 ml
Acrylamide	3.0 ml	5.0 ml	6.0 mL	830 µl
1.5 M TRIS (pH 8.8)	3.8 ml	3.8 ml	3.8 mL	-
1 M TRIS (pH 6.8)	-	-	-	630 µl
10 % SDS	150 µL	150 µL	150 µL	50 µl
10 % APS	150 µL	150 µL	150 µL	50 µl
TEMED	12 µL	6 µl	6 µl	5 µl

2.2.22. SDS-PAGE

To assess antibody integrity the supernatant containing 2.5 µg antibody was boiled in sample buffer and subjected to a 12 % TRIS-Glycine gel under reducing conditions or to a 6 % TRIS-Glycine gel under non-reducing conditions without heating as described in 2.2.21. Protein bands were stained with Coomassie brilliant blue.

2.2.23. Analysis of the glycosylation pattern

The antibody glycosylation pattern was analyzed with the ProfilerPro Glycan Profiling Kit Ver 2 on a CGE instrument according to the manufacturer's protocol. Antibodies were deglycosylated using PNGase F. The released glycans were labeled with a fluorescent dye in a hydrazide reaction. Samples then underwent microchip based separation by CGE. Electropherograms were analyzed by the LabChip GX software package to identify and quantify the individual sugar structures. All values were normed to 100% total sugar structures per sample and comprise the N-linked bi-

antennary high mannose (Man5) and the complex structures in the presence (A2FG0; A2FG1; A2FG2) and absence (A2G0) of fucose.

2.2.24. Target prediction

TargetScan 6.2 (Friedman et al., 2009; Garcia et al., 2011; Grimson et al., 2007; Lewis et al., 2005) was used with the setting for only conserved target sites, Diana microT-CDS (Maragkakis et al., 2009; Papadopoulos et al., 2009) with strict threshold and bioinformatics tool MirTarget2 on miRDB (Wang, 2008; Wang and El Naqa, 2008) with default settings. The genes predicted by all three software tools to be specific for hsa-miR-557 or hsa-miR-1287 were used for core analysis by Ingenuity pathway analysis.

3. Results

In order to identify microRNAs that influence producer cells in a positive manner the idea was to perform a genome-wide functional screen in Chinese hamster ovary cells that stably secrete a therapeutic antibody as a model product (CHO-IgG1). To do so, a transient transfection of a human mimic miRNA library was carried out, thereby artificially overexpressing human miRNAs in hamster cells. Therefore, the first challenge was to optimize a protocol to achieve an excellent transfection efficiency of CHO-IgG1 cells.

3.1. Mimic miRNA library screen

3.1.1. Optimization of transient transfection to establish a miRNA screen protocol in CHO cells

To optimize the transfection efficiency of CHO-DG44 cells a non-targeting miRNA transfection control labeled with Dy547 was transfected and cells were analyzed by fluorescence microscopy. Cells that were positive for the dye could be counted and transfection efficiency was calculated. For this purpose the number of fluorescence positive cells was divided by the total cell number. With this method transfection was optimized and a protocol was developed resulting in 90-100% fluorescence positive cells using the transfection method lipofection with the reagent Oligofectamine (see Figure 8, center panel). This method works with positively charged lipid-based reagents that complex negatively charged nucleic acids e.g. RNA and delivers them to the cell by interaction with the plasma membrane. In a second approach cells were transfected by nucleofection. The transfection method nucleofection applies a short electrical field to the cells and cell membranes are temporarily permeable for the RNA to diffuse into the cell. The dye distribution differed depending on the transfection method used. Oligofectamine seemed to result in lipoplexes attached to the cell membrane whereas after nucleofection the dye was equally distributed in the cytoplasm (see Figure 8A, right panel). This led to the assumption that lipoplexes formed by Oligofectamine might not enter the cell. To verify this hypothesis, CHO cells stably overexpressing human Flag-tagged CERT (Ceramide transfer protein) were used and transfected with siRNAs targeting human CERT using lipofection and

nucleofection. siRNAs have a similar structure compared to miRNAs and they are supposed to behave similarly concerning transfection. Indeed, only after nucleofection a clear reduction of the CERT protein level was detected as analyzed by Western Blot with Flag specific antibodies. By contrast, CERT protein reduction was not detectable after lipofection with Oligofectamine (see Figure 8B). This confirmed the assumption that Oligofectamine is not effective for CHO-IgG1 cell transfection. Hence, the strategy was to optimize the nucleofection protocol for the screen.
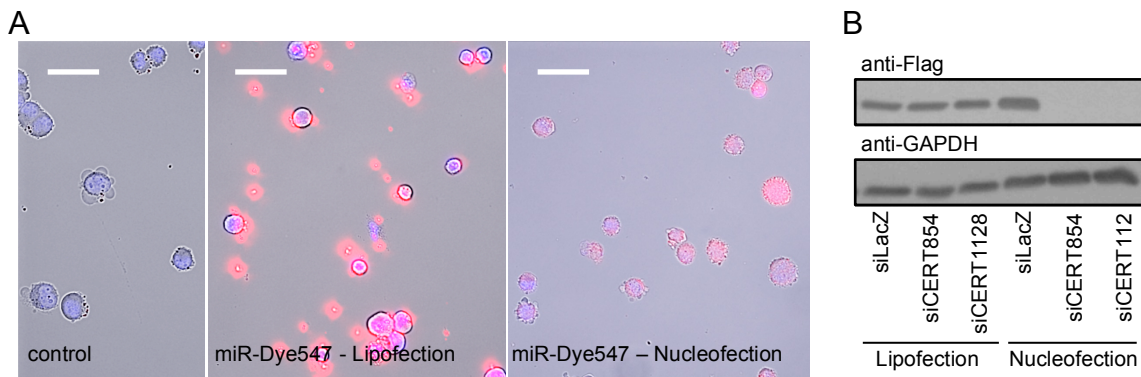
Figure 8: Transfection of CHO-DG44 cells via different methods
(A) CHO-DG44 were transfected with a non-targeting control miRNA labeled with Dy547 (miR-Dy547) shown in red using either lipofection with Oligofectamine (center panel) or nucleofection with program U23 (right panel). Mock transfected cells were used as a control (left panel). Nuclei were stained with Draq5 (blue). Microscopic analysis was performed with viable unfixed cells. Overlays of fluorescence and bright field images are shown. Scale bar represents 50 µm (B) Transfection efficiency was monitored by transfecting CHO-DG44 cells stably overexpressing human Flag-tagged CERT with two different siRNAs both targeting human CERT (siCERT854 and siCERT1128) using either lipofection (Oligofectamine) or nucleofection (program U23). Control cells were transfected with non-targeting siRNA (siLacZ). Cell lysates were analyzed by Western blot and CERT was detected with anti-Flag antibody. As a loading control GAPDH was detected.

Next, the nucleofection protocol had to be adapted to the 96-well format. To monitor transfection efficiency, a non-targeting siRNA labeled with a FAM dye (siLacZ-FAM) was used, enabling the analysis of cells by flow cytometry. As a second control a siRNA targeting the mRNA of the light chain of the IgG1 antibody (siLC) was chosen. This approach had the advantage that successful transfection and functionality of this siRNA could be controlled by the detection of the antibody produced by the cell. Different nucleofection programs were tested using the nucleofection Kit SG recommended for CHO cells regarding viability and transfection efficiency, which was monitored by flow cytometry of siLacZ-FAM transfected cells. Program 96-DT-133 resulted in close to 100% transfection efficiency and more than 80% viable cells after transfection. The protocol turned out to be robust. In three independent experiments

with eight replicates each, a reproducible transfection efficiency was observed as shown in Figure 9A where one representative example of siLacZ-FAM transfected CHO-IgG1 is depicted.

Furthermore the IgG1 antibody concentrations in the supernatant of siLC transfected cells were monitored in the same setting and a significant decrease was observed compared to mock and siLacZ control whereas cell densities after 4 days were similar (see Figure 9B and C). The antibody concentration accumulated over 4 days and the effect of siLC diminished at day 4 due to intracellular dilution of the siRNA caused by cell division. This protocol and the established controls complied with all criteria important for the screen, namely reproducible transfection efficiency with satisfying viability and increasing antibody concentrations over 4 days. As a readout for the screen, ELISA analysis to determine the antibody concentrations in the supernatant was planned.
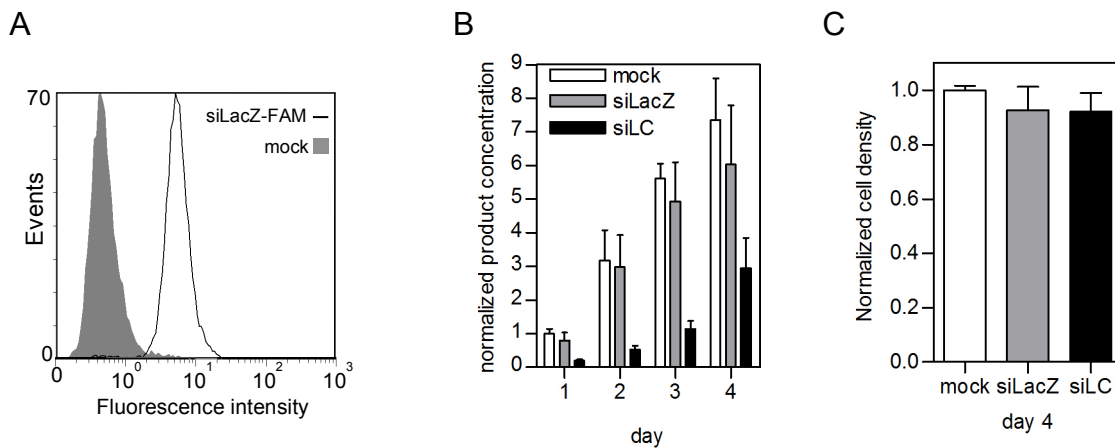
Figure 9: Transfection controls to monitor transfection efficiency
(A) Transfection efficiency was analyzed by transfecting CHO-DG44 stably secreting an IgG1 with a non-targeting control siRNA labeled with FAM (siLacZ-FAM) and flow cytometry analysis (black line). Control cells were mock transfected (grey filled peak). Shown is one representative result. (B) CHO-DG44 cells stably secreting an IgG1 antibody were transfected with a siRNA targeting the light chain of the antibody (siLC) (black bars) or siLacZ-FAM (grey bars). Mock transfected cells served as a control (white bars). Shown is the antibody concentration (IgG1) assessed over 4 days normalized to the antibody concentration of the mock control on day 1. (C) Mock transfected cells (white bar), siLacZ (grey bar) and siLC (black bar) transfected cells were analyzed by CyQuant assay 4 days after transfection. Values are normalized to a mock control. (B) and (C) show the means of the results of 3 independent experiments with 8-fold replicates (Error bars are SEM).

3.1.2. Mimic miRNA library screen in CHO-DG44 producer cells: screen quality and screen results

To investigate the impact of miRNAs on the productivity of an industrially relevant producer cell line, a genome wide screen was performed in CHO-DG44 stably secreting an IgG1 antibody (CHO-IgG1). Cells were transiently transfected with a human mimic miRNA library (see appendix 6.2.1) consisting of 879 miRNAs in a 96-well format (detailed screen workflow see Figure 10A). The transfection controls siLacZ-FAM and siLC described above were also used during the screen to verify optimal transfection efficiency. Moreover, a mock control was performed. All controls were included in duplicates on each plate during the screen. First, the antibody concentration obtained in the supernatant was measured over four days by ELISA analysis without considering cell growth. For each plate the transfection efficiency was monitored by flow cytometry analysis of the siLacZ-FAM transfected cells and ELISA analysis of supernatants after siLC transfection. Two independent experiments were performed. This resulted in 7,032 data points for the miRNA transfected cells and 528 data points for the negative and positive controls (raw data see appendix 6.2.2). To assess the quality of the screen, antibody concentrations of the biological replicate #1 were plotted against the ones of replicate #2, resulting in a correlation coefficient of 0.87 (Figure 10B). In this plot, one data point represents the antibody concentration in the supernatant after transfection with one specific miRNA. Consequently, changes in antibody concentrations in the supernatant are directly linked to a specific miRNA. Furthermore, the fold change was calculated by dividing each measured antibody concentration value by the mean antibody concentration of all miRNA samples of the respective screen plate on the respective day (see Figure 10C). Thereby, differences between single plates were equalized and they could directly be compared. Most miRNAs had no effect on the antibody concentrations as shown in Figure 10C, in which most data points converged around 1. In addition, most data points were within the variance range of about 20% (see Figure 10C, marked by grey dotted lines), which is in line with pre-experiments performed during establishment of the screening procedure (data not shown). Thus, to identify miRNAs that increased the antibody concentration in the supernatant the cut-off was set to 1.4 with the second criterion that both replicates are >1.3 increased (highlighted by crosses in Figure 10C) which was true for all that were >1.4. In addition, the *p*-value of the two biological replicates was calculated (see Table 20 and appendix 6.2.2).
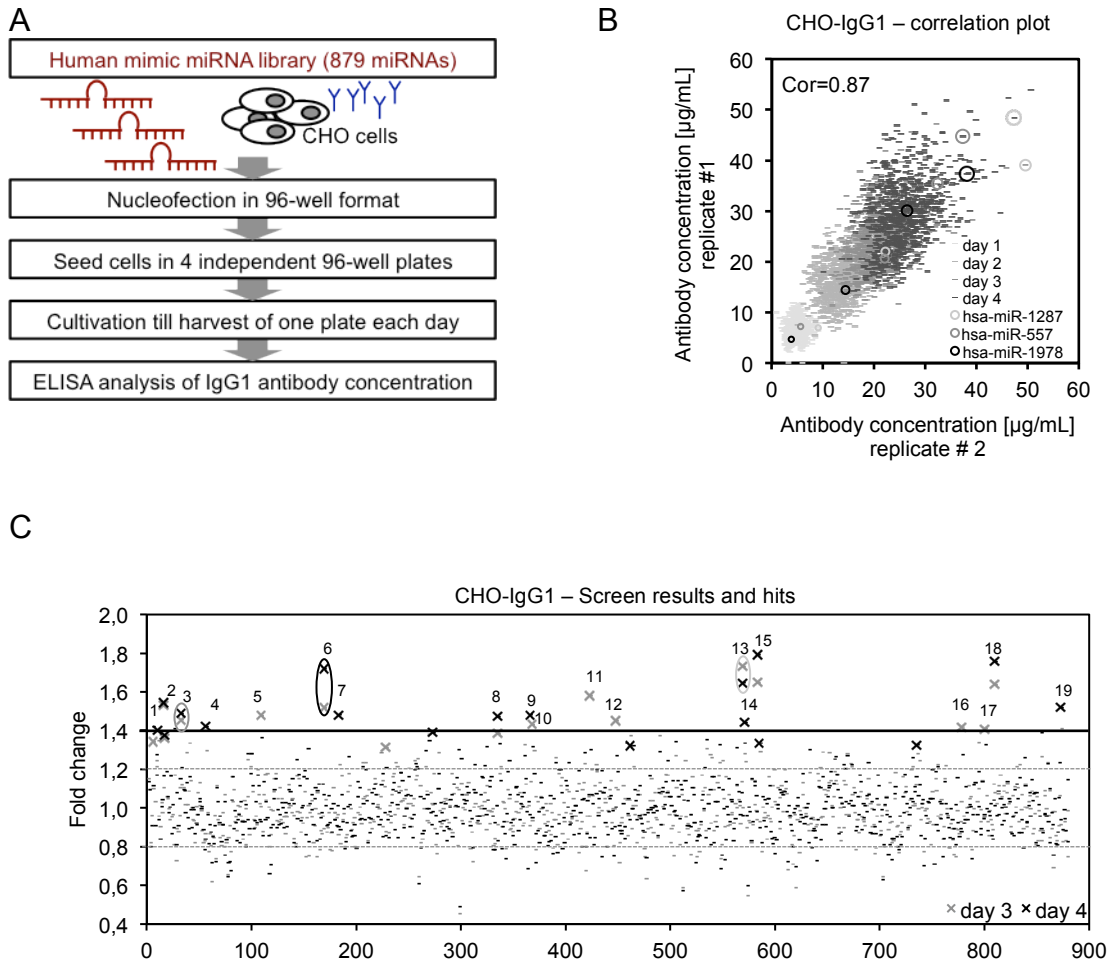
Figure 10: MicroRNA screen in IgG1-producing CHO cells
(A) Workflow of the screen procedure. CHO-DG44 cells secreting a human IgG1 antibody were transiently transfected with a human mimic miRNA library consisting of 879 human miRNAs. Antibody concentrations in the supernatant of the transfected cells were determined by ELISA on day 1 – 4 post transfection. (B) Correlation plot of antibody concentrations measured on day 1 to 4. Biological replicate #1 of the screen plotted against replicate #2. Data points of three specific miRNAs, hsa-miR-557 (light grey), hsa-miR-1287 (dark grey) and *hsa-miR-1978* (black) are circled (days are indicated by the circle size). (C) Fold change of the antibody concentration based on the mean of the respective plate (grey dotted lines: variance region) is shown for day 3 (grey dots) and day 4 (black dots), crosses indicate data points for which both duplicates were increased > 1.3 fold and scored as hits if the mean was increased >1.4 fold (black line). (1: hsa-miR-193b*, 2: hsa-miR-185*, 3: hsa-miR-557, 4: hsa-miR-149, 5: hsa-miR-1275, 6: *hsa-miR-1978*, 7: hsa-miR-450b-3p, 8: hsa-miR-1271, 9: hsa-miR-644, 10: hsa-miR-1285, 11: hsa-miR-885-3p, 12: hsa-miR-1293, 13: hsa-miR-1287, 14: hsa-miR-892a, 15: hsa-miR-612, 16: hsa-miR-23b*, 17: hsa-miR-125a-3p, 18: hsa-miR-365*, 19: hsa-miR-183).

In sum, fifteen miRNAs induced a significant decrease below 0.7 fold of antibody concentration. More importantly, nineteen miRNAs resulted in an increase of antibody concentration above the threshold of 1.4 fold change (see Figure 10C) and eighteen of them induced a significant product increase. These nineteen miRNA hits are summarized in Table 20.

Table 20: microRNA screen hits.
Fold increase on day 3 and 4 with *p*-values, significant *p* < 0.05 (^+p > 0.05)

No.	MicroRNAs	Fold increase		*p*-value	
		Day 3	Day 4	Day 3	Day 4
1	hsa-miR-193b*	1,396	1,442	3,3E-03	1,7E-02
2	hsa-miR-185*	1,583	1,590	1,8E-05	1,1E-03
3	hsa-miR-557	1,501	1,529	2,3E-04	3,7E-03
4	hsa-miR-149	1,344	1,464	1,1E-02	1,2E-02
5	hsa-miR-1275	1,494	1,377	2,1E-02	4,3E-01
6	*hsa-miR-1978*	1,556	1,744	7,8E-02	3,1E-02
7^+	hsa-miR-450b-3p	1,258	1,503	8,5E-01$^+$	3,3E-01$^+$
8	hsa-miR-1271	1,415	1,520	2,6E-04	4,0E-04
9	hsa-miR-644	1,164	1,529	1,1E-01	3,4E-04
10	hsa-miR-1285	1,464	1,357	6,3E-05	1,2E-02
11	hsa-miR-885-3p	1,608	1,323	2,1E-05	4,3E-02
12	hsa-miR-1293	1,477	1,294	1,2E-03	8,2E-02
13	hsa-miR-1287	1,785	1,687	4,9E-11	1,1E-05
14	hsa-miR-892a	1,273	1,480	4,5E-03	1,8E-03
15	hsa-miR-612	1,699	1,837	1,2E-09	1,6E-07
16	hsa-miR-23b*	1,443	1,291	5,1E-05	1,3E-02
17	hsa-miR-125a-3p	1,432	1,339	7,4E-05	4,9E-03
18	hsa-miR-365*	1,653	1,805	1,4E-07	3,1E-06
19	hsa-miR-183	1,421	1,560	3,6E-04	1,1E-03

3.1.3. Validation of the microRNA screen hits in CHO-IgG1

To validate the nineteen miRNAs as screen hits (see Table 20), CHO-IgG1 cells were transfected with each of these miRNAs in four independent experiments in quadruplicate in a 12-well format. Furthermore, cell densities were determined to characterize the effects caused by transient miRNA expression in more detail. Antibody concentrations and cell densities were measured over four days. Figure 11 shows the results of the antibody concentrations measured on day 3 and 4 after transfection (Figure 11A) and both, the specific productivity (Figure 11C) as well as the cell densities (Figure 11B) obtained on day 3. Indeed, transfection of twelve miRNAs (hsa-miR-23b*, hsa-miR-125a-3p, hsa-miR-183, hsa-miR-193b*, hsa-miR-356*, hsa-miR-450b-3p, hsa-miR-557, hsa-miR-612, hsa-miR-885-3p, hsa-miR-1275, hsa-miR-1287 and *hsa-miR-1978*) out of nineteen resulted in an IgG1 concentration that was increased compared to the control (see Figure 11A), although to a minor extent compared to the primary screen. Six of these miRNAs (hsa-miR-125a-3p, hsa-miR-193b*, hsa-miR-557, hsa-miR-612, hsa-miR-885-3p, hsa-miR-1287) significantly improved the antibody concentration on day 3 whereas three additional miRNAs significantly increased antibody titers at earlier time points (hsa-miR-450b-3p, hsa-miR-644 and hsa-miR-1275, data not shown). miRNA-induced effects were also observed with respect to specific productivity of CHO-IgG1 cells (Figure 11C). All

tested nineteen miRNAs increased the specific productivity more than 1.4 fold and for six of them (hsa-miR-193b*, hsa-miR-612, hsa-miR-644, hsa-miR-885-3p, hsa-miR-hsa-miR-1287 and *hsa-miR-1978*) this effect was statistically significant. Of note, most of the miRNAs had a negative influence on cell densities (Figure 11B). Cell density of miRNA-transfected cells was decreased on day 3 by about 30 to 50% compared to control transfected cells. Three miRNAs did not negatively impact CHO cell growth and of interest, one of them, hsa-miR-557, even had a positive effect on proliferation of CHO-IgG1 cells that was significant on day 4 (data not shown).

A
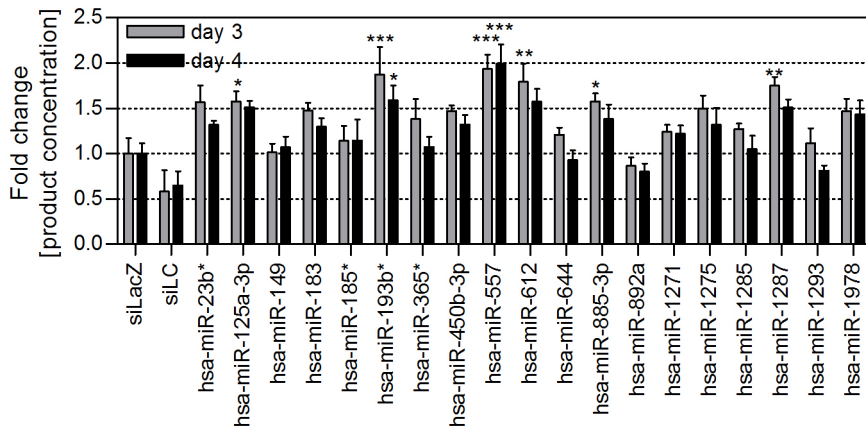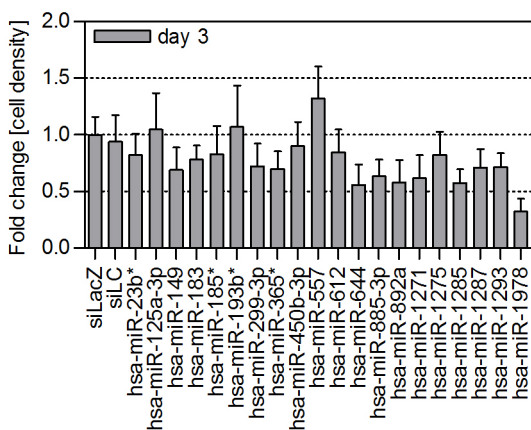
B   C
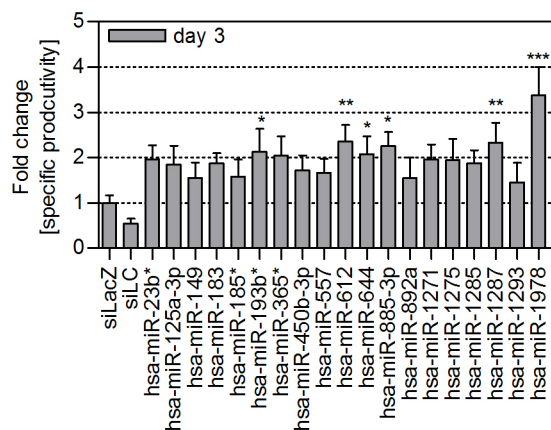
Figure 11: Validation of the microRNA screen in CHO-IgG1 cells
(A) CHO-IgG1 cells were transiently transfected with the indicated miRNAs. Product concentration in the supernatant (A), cell density (B), and specific productivity (C) were monitored over 4 days. Results were normalized to the control (siLacZ). As an additional control, a siRNA targeting the product (siLC=light chain) was used. Graphs show the mean of four independent experiments. Data were obtained on day 3 (grey bars) and day 4 (black bars) post transfection. Error bars represent SEM. Statistical analysis was done with two-way anova bonferroni posttest considering data of day 1 to 4. (*<0.05, **<0.01, ***<0.001).

3.1.4. Secondary screen of the microRNA screen hits in CHO-HSA cells

To exclude the possibility that miRNA mediated antibody concentration changes are specific for the product IgG1 or the CHO-IgG1 cell clone, a stable pool of CHO-DG44 cells secreting human serum albumin (CHO-HSA) was used as a second model cell line. CHO-HSA cells were transiently transfected with the selected 19 miRNAs using the same settings as for CHO-IgG1. Transfection efficiency was analyzed as described for the screen with siLacZ-FAM and a siRNA targeting human serum albumin (siHSA), resulting in a similar transfection efficiency (data not shown). HSA concentrations and cell densities were measured over four days. Results of day 3 and 4 after transfection for the product concentration, and those of day 3 for the specific productivity and cell densities are shown in Figure 12.
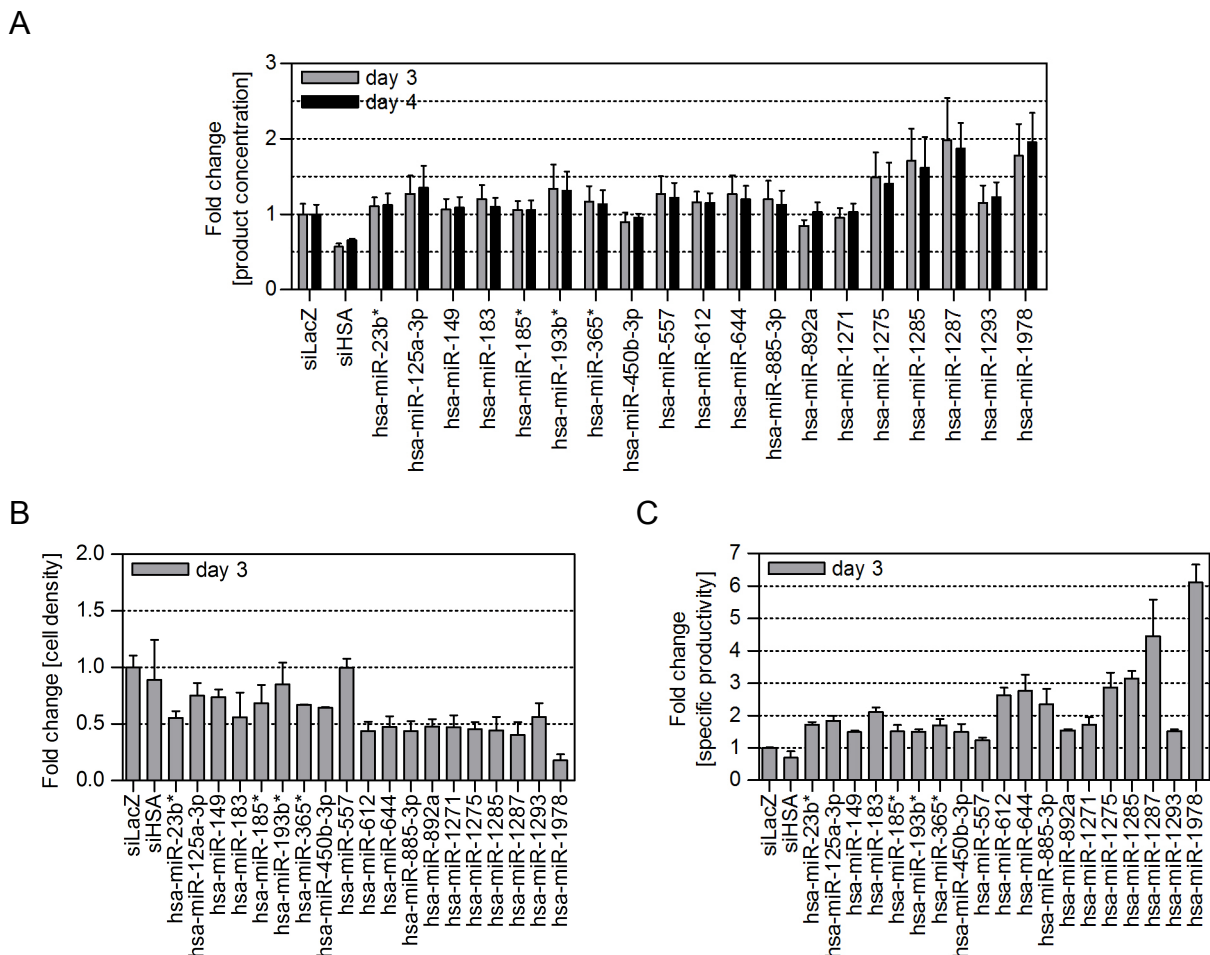
Figure 12: Secondary microRNA screen in CHO-HSA cells
(A) CHO-DG44 cells stably secreting HSA (human serum albumin, CHO-HSA) were transiently transfected with the indicated miRNAs. Product concentration in the supernatant (A), cell density (B), and specific productivity (C) were monitored over 4 days. Cells transfected with siLacZ served as a control. Results were normalized to the control (siLacZ). As an additional control a siRNA targeting the product (siHSA) was used. Graphs show the mean of the data of two independent experiments obtained on day 3 (grey bars) and day 4 (black bars) post transfection. Error bars represent SEM.

Ten miRNAs (see Figure 12A) led to a 20% increase of HSA concentrations in the supernatant, eight of which (hsa-miR-125a-3p, hsa-miR-183, hsa-miR-193b*, hsa-miR-557, hsa-miR-885-3p, hsa-miR-1275, hsa-miR-1287 and *hsa-miR-1978*) were confirmed in CHO-IgG1 to increase antibody titers, pointing to a product independent effect. In general, all miRNAs caused an increase in the specific productivity of CHO-HSA cells (see Figure 12C), which is consistent with the CHO-IgG1 results. Similarly, cell density was affected in a negative manner by most of the miRNAs with a decrease of about 50% (see Figure 12B), the only exception being hsa-miR-557.

I thus could confirm increased antibody concentrations mediated by twelve of the nineteen primary hits, six of which were validated as top hits (hsa-miR-125a-3p, hsa-miR-193b*, hsa-miR-557, hsa-miR-612, hsa-miR-885-3p and hsa-miR-1287), resulting in significantly increased product concentrations and further functioning in a product independent manner. Hsa-miR-1287 and hsa-miR-557 overexpression resulted in the highest product concentrations in both model cell lines. Furthermore, specific productivity was the highest after transfection of CHO-IgG1 and CHO-HSA cells with *hsa-miR-1978*.

3.1.5. Combination of microRNA screen hits in CHO-IgG1

Based on the specific effects on the specific productivity and cell growth, respectively, it was conceivable that the combination of *hsa-miR-1978*, hsa-miR-1287 and hsa-miR-557 could further boost the productivity of a producer cell. At first, CHO-IgG1 cells were transiently transfected with these three miRNAs singly or in combination and cell density, specific productivity, and product concentration for 4 days post-transfection were determined. Results of day 3 and 4 are shown in Figure 13. Transfection of hsa-miR-557 and hsa-miR-1287 had no effect on cell densities. *Hsa-miR-1978* decreased cell numbers by approximately 50%, which was also a milder effect compared to the decrease of cell densities observed during the validation screen (see Figure 11). This can be explained by the fact that only half of the miRNA amount applied in the validation screen was used for transfection. The product concentration of cells transfected with half of the miRNA amount also increased less strongly as in the validation experiment but was still increased compared to the control. Strikingly, the combination of two miRNAs enhanced cellular

productivity in all three cases compared to the respective single miRNAs, pointing to an additive effect.
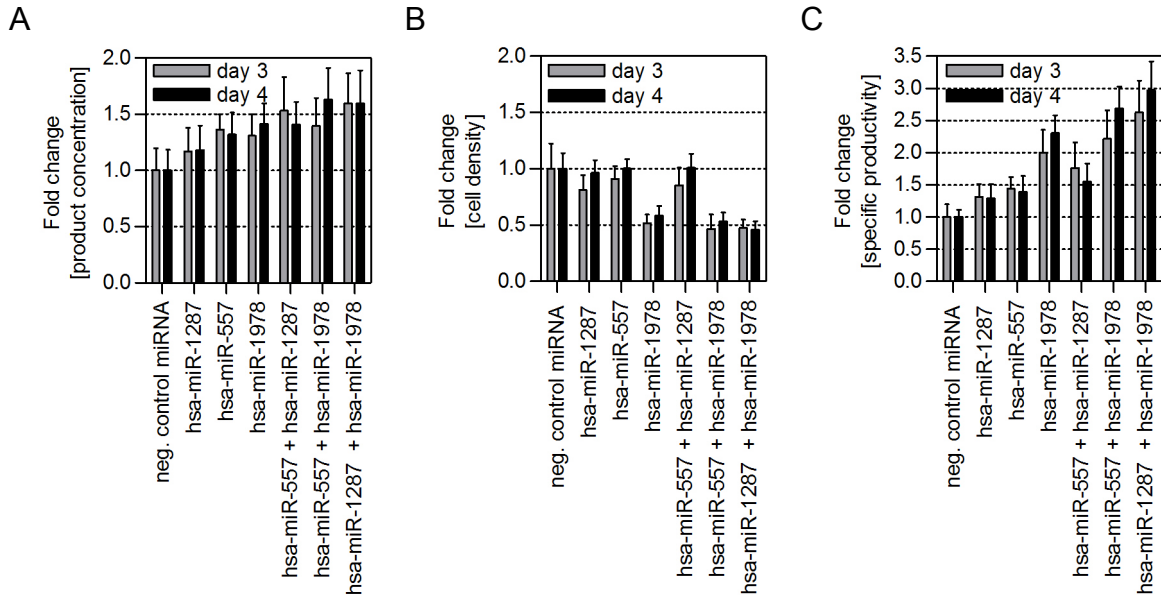
Figure 13: Transient combination of microRNAs in CHO-IgG1 cells
CHO-IgG1 cells were transfected with a negative control miRNA (neg. control miRNA), hsa-miR-557, hsa-miR-1287 and *hsa-miR-1978* or their combination. Shown are the results (fold change compared to a control miRNA) obtained on day 3 (grey bars) or day 4 (black bars) for product concentration (A), cell density (B) and specific productivity (C). Bars represent the mean of fife independent experiments; error bars represent SEM. Statistical analysis was done with two-way anova bonferroni posttest considering data of day 1 to 4 (**<0.01). (Significant specific productivity: 1978 + 1287-1978**, 1978 + 557-1978 n.s., 1287 + 557-1287 n.s., 557 + 557-1287 n.s.)

3.1.1. Concentration dependent effects of microRNAs

To get further insight whether the chosen miRNA concentration of 1 µM applied in the validation experiment or 0.5 µM which was used in the combination experiment was at the bottom limit to result in a detectable phenotype the amount of hsa-miR-557 was exemplarily titrated in CHO-IgG1 cells. Figure 14 shows the normalized results for the specific productivity on days 3 and 4 after hsa-miR-557 transfection. The results indicate that 0.5 and 1 µM miRNA are within an optimum concentration range and that specific productivity cannot be further improved by doubling the miRNA amount. However, it also seems that a concentration of at least 1 µM is required to maintain the miRNA induced effects up to day 4.
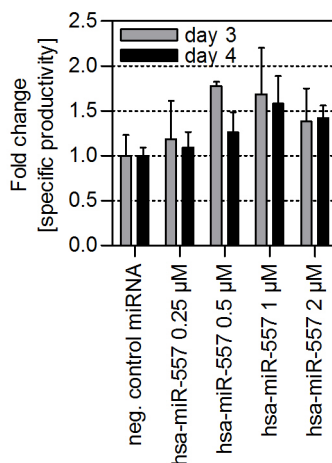
Figure 14: Titration of microRNA hsa-miR-557 in CHO-IgG1 cells
CHO-IgG1 cells were transfected with a negative control miRNA (neg. control miRNA) or hsa-miR-557 using different amounts of the miRNA as indicated. Shown are the results (fold change compared to control miRNA) obtained on day 3 (grey bars) or day 4 (black bars) for the specific productivity. Bars represent the mean of 2 independent experiments, error bars represent SEM.

In summary, the validation of the screen hits was successful, with six miRNAs identified as top hits. Many of the screen hits furthermore functioned in a product independent manner. Moreover, transient expression of a combination of 2 different miRNAs further boosted the performance of the model cell line CHO-IgG1. This could be demonstrated for three different miRNAs and their respective combinations. The data indicate that the transient miRNA expression leads to short effects with the maximum for most miRNAs at day 3 post transfection. To prolong the miRNA mediated phenotypic changes a vector based miRNA expression was establish to stably overexpress miRNAs.

3.2. Vector based microRNA overexpression in CHO-DG44 cells

3.2.1. Vector generation and transient microRNA expression verification

Recently, Jadhav *et al.* described an approach for the vector based miRNA expression in CHO cells. In brief, hairpin sequences of precursor miRNAs are cloned into the BLOCK-IT POLII miR expression system. The mature miRNA is embedded in a given miRNA backbone derived from the mouse miRNA-155 including an optimized loop, and 3'- and 5'-miR-flanking regions. Using this vector, the authors demonstrated the transient expression of mature functional hamster specific miRNAs.

To stably express the human miRNAs hsa-miR-557, hsa-miR-1287 and *hsa-miR-1978* or their combinations in CHO cells the respective mature human miRNA sequences were thus cloned (see Table 14) into the BLOCK-IT POLII miR vector. Figure 15 visualizes the generated constructs. To express two miRNAs in combination the coding sequences were cloned in tandem in the order detailed in Figure 15. In addition, a control vector encoding a negative control miRNA (pcDNA6.2-GW7emGFP-neg. control miR) was also generated.
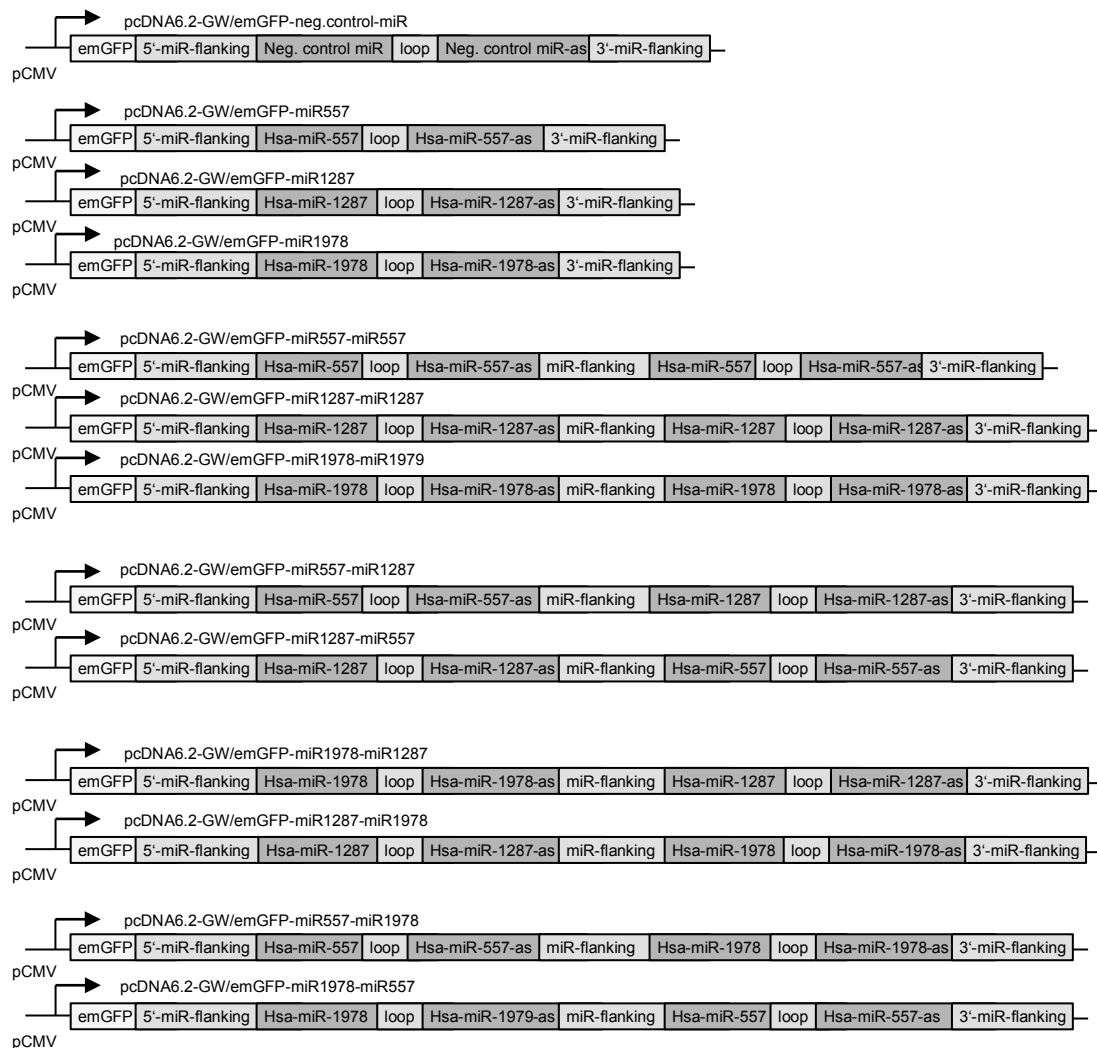
Figure 15: MicroRNA expression vectors
Scheme showing the constructs used to express miRNAs along with a GFP reporter. The expression vectors contain one miRNA cassette either encoding a negative control miRNA (pcDNA6.2-GW/emGFP-neg. control-miR), the miRNA hsa-miR-557, hsa-miR-1287 or *hsa-miR-1978* (pDNA6.2-GW7emGFP-miR557, pcDNA6.2-GW/emGFP-miR1287, pcDNA6.2-GW/emGFP-*miR1978*) or two miRNA cassettes in tandem as indicated. Flanking region and loop are based on mouse miRNA mmu-miR-155, "-as" indicates modified antisense sequence.

To verify that these expression constructs express mature miRNAs, CHO-IgG1 cells were transiently transfected with the indicated plasmids and quantitative RT-PCR analysis was performed. As a positive control the respective mature miRNAs were transfected. Indeed, a signal was obtained for cells transfected with the hsa-miR-557, hsa-miR-1287 and *hsa-miR-1978* encoding plasmids (see Figure 16), whereas in the negative controls a signal was hardly detectable. Of note, in case of *hsa-miR-1978* only the tandem constructs but not the single cassette-containing construct led to a *hsa-miR-1978* signal. It can thus be concluded that CHO-IgG1 cells successfully transcribed and processed the hsa-miR-557, hsa-miR-1287 and *hsa-miR-1978* from the plasmids. Higher miRNA levels for all three miRNAs were detected when vectors containing two miRNA cassettes were transfected compared to the respective transfection of vectors with single miRNA-encoding cassettes. Noteworthy, hsa-miR-557, hsa-miR-1287 and *hsa-miR-1978* were detected in cells transfected with a vector containing both miRNAs in tandem, with different expression levels of the individual miRNAs depending on the order of the combination. Therefore, the order of the miRNAs seems to have an impact on the expression level. For the generation of stable CHO-IgG1 cell pools, the constructs resulting in the higher expression levels of the two individual miRNAs were chosen: miR557-miR1287, miR557-miR1978 and miR1978-miR1287. Of note, the expression level of vector-based miRNAs was still about 100 fold lower compared to those of obtained by the transfection of mature miRNAs.
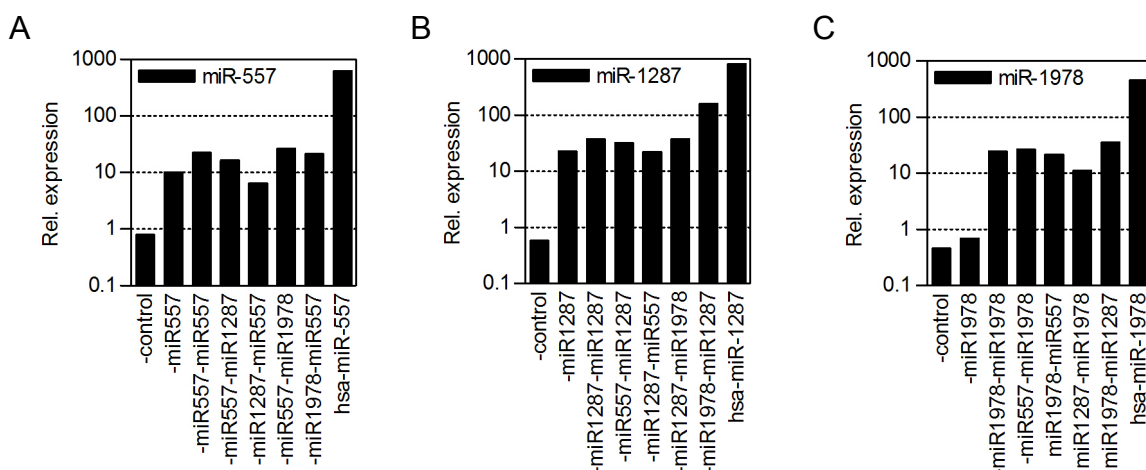
Figure 16: Transient vector based microRNA expression in CHO cells
CHO-IgG1 cells were transiently transfected with the miRNA expression vectors as indicated. As controls a control vector (pcDNA6.2-GW/emGFP-lacZ) or the respective mature miRNA (hsa-miR-557, hsa-miR1287 or *hsa-miR-1978*) were used. Two days after transfection (for vector transfection) or one day after transfection (for mature miRNA transfection), RNA was extracted and levels of the mature miRNAs hsa-miR-557 (A), hsa-miR-1287 (B) and *hsa-miR-1978* (C) were measured by qRT-PCR analysis. Relative expression was calculated by normalization to RNU6B using the ΔCq method.

3.2.2. Stable microRNA overexpression in CHO-DG44 cells

Next, stable CHO-IgG1 cell pools were generated using the single miRNA and miRNA combination expression constructs or pools expressing a miRNA negative control by selection with Blasticidin S. The stable pools were sorted for GFP positive cells to enrich for miRNA expressing cells. Moreover, this strategy allows to prolong the time of stable miRNA expression because the miRNA negative cell clones are removed and thus cannot overgrow the miRNA positive cells due to some growth benefits. Indeed, similar mean GFP fluorescence intensities were observed after 50 days in culture and no increase of a GFP negative peak was detected (see Figure 17).
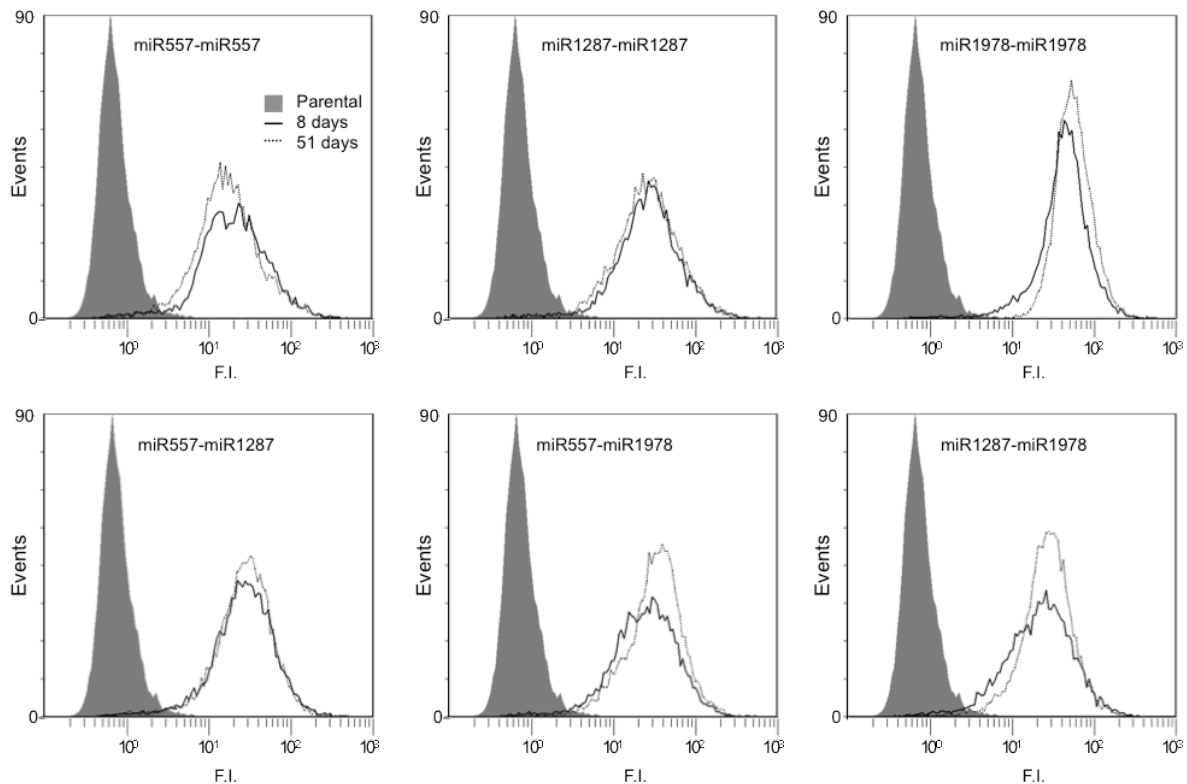
Figure 17: Stability of stable vector based microRNA expression in CHO cells
CHO-IgG1 cells were stably transfected with the miRNA expression vectors as indicated and sorted for GFP positivity. Cells were cultured for 8 and 51 days and analyzed by flow cytometry. As a control parental cells were used (grey filled peak). (F.I. = fluorescence intensity of the GFP signal)

The stable cell pools were then analyzed for miRNA expression by qRT-PCR. As a positive control, CHO-IgG1 cells transiently transfected with the mature miRNAs were used. Indeed, expression of the mature miRNAs hsa-miR-557 and hsa-miR-1287 could be detected in the stable pools (Figures 18A and 18B). Of note, miRNA levels in the stable cell lines were comparable to transient vector based expression

(Figure 16), indicating that in the case of hsa-miR-557 and hsa-miR-1287 the cells did not compensate long-term miRNA expression. There was no clear overexpression of *hsa-miR-1978* in general and especially in the combination miR1978-miR1278 in the stable cells (data not shown). The stable expression of miR1978-miR-1978 was more than tenfold lower than the miRNA expression of hsa-miR-557 or hsa-miR-1287. To test whether the low *hsa-miR-1978* expression was due to the preferential expression of GFP, respective plasmids without the GFP cassette were generated. However, transient transfection of these plasmids resulted in even lower miRNA expression levels than the transfection of plasmids with GFP. Therefore, stable pools with the original GFP-containing miR1287-miR1978 and miR1978-miR557 vectors were generated to check whether this order was advantageous in the stable condition. Indeed, the *hsa-miR-1978* expression level of stably expressed miR1287-miR1978 was approximately twofold higher than that of miR1978-miR1287, but miR1978-miR557 resulted in a twofold lower *hsa-miR-1978* expression than miR557-miR1978. In summary, the highest stable *hsa-miR-1978* expression levels were achieved with the constructs indicated in Figure 18C. Although the miRNA levels were only marginally increased these pools were used for further analyses because low expression levels might be sufficient to mediate phenotypic changes.
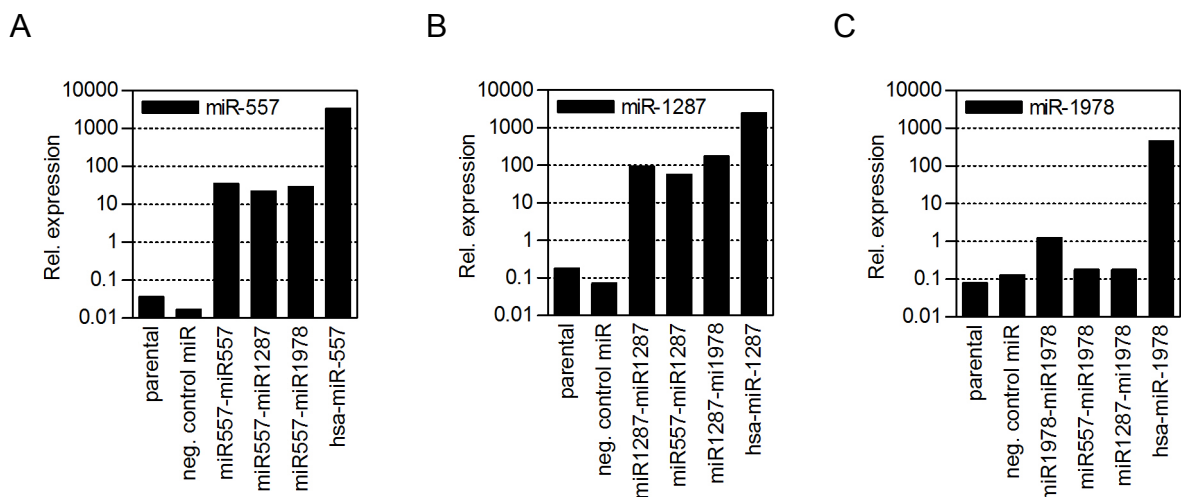
Figure 18: Stable microRNA expression in CHO cells
CHO-IgG1 cells were stably transfected with the miRNA expression vectors as indicated. As controls parental cells, a negative control miRNA expressing vector (pcDNA6.2-GW/emGFP-neg. control miR) or the respective mature miRNAs (hsa-miR-557, hsa-miR-1287 or *hsa-miR-1978*) were used. RNA was extracted and levels of the mature miRNAs hsa-miR-557 (A), hsa-miR-1287 (B) and *hsa-miR-1978* (C) were measured by qRT-PCR analysis. Relative expression (rel. expression) was calculated by normalization to RNU6B using the ΔCq method.

3.3. Phenotype of stable microRNA overexpressing CHO-IgG1 cells

3.3.1. Productivity of stable microRNA overexpressing CHO-IgG1 cells

To address whether the CHO-IgG1 pools stably overexpressing the miRNAs or their combinations demonstrate improved productivity, these cells were investigated in fed-batch cultures. miRNA overexpression was monitored by qRT-PCR (see Figure 18). Stable miRNA overexpressing CHO-IgG1 pools as well as the parental cells (CHO-IgG1) that served as a control were cultivated in shake flasks in duplicates with daily feeding to mimic conditions of fed-batch cultivation. Samples were taken from day 3 on to determine product concentrations in the supernatant, cell growth, viability, and specific productivity. The mean specific productivity was calculated and normalized to that of the control cells (see Figure 19A).
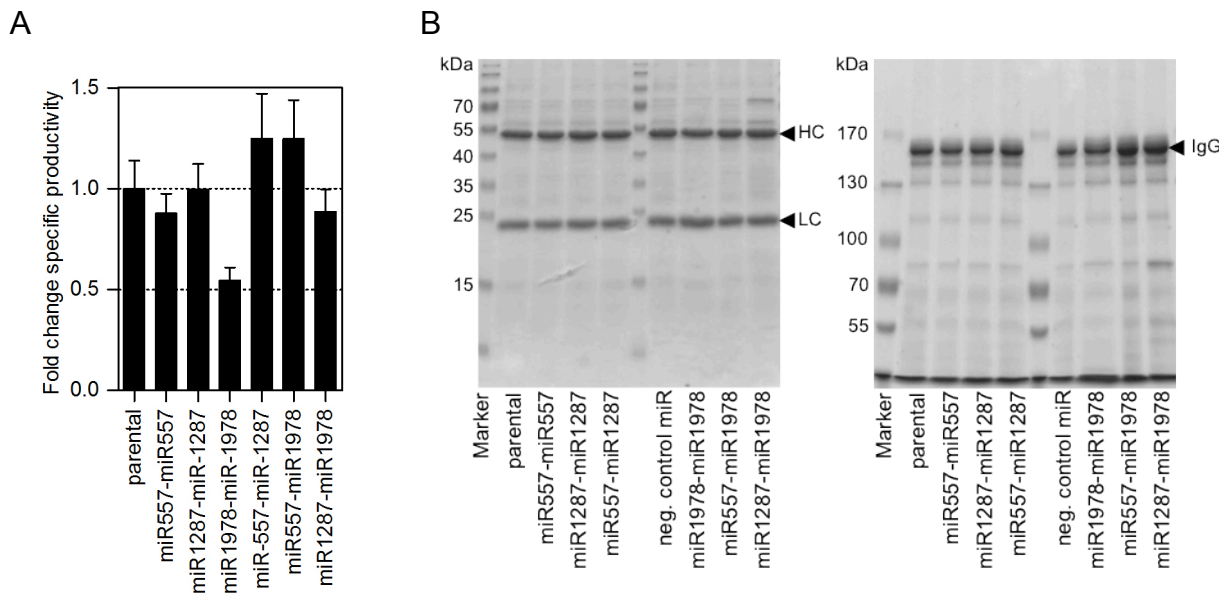
Figure 19: Fed-batch with CHO cells stably overexpressing microRNA(s)
(A) The indicated stable CHO-IgG1 cell pools were grown in fed-batch cultures. Parental cells were used as a negative control. The mean specific productivity over 7 days was calculated and normalized to the control. Error bars represent SEM. Two independent experiments were done. (B) Antibody from the indicated cell supernatant was analyzed by Coomassie blue staining after separation by reducing (left) and non-reducing (right) SDS-PAGE. Arrows indicate the antibody specific protein band(s). (HC = heavy chain, LC = light chain)

The overexpression of the single miRNAs hsa-miR-557 and hsa-miR-1287 had no positive impact on the mean specific productivity whereas *hsa-miR-1978* negatively impacted CHO-IgG1 productivity during fed-batch culture, indicating that small levels of this miRNA are sufficient to mediate a function. Noteworthy, cells expressing the

miRNA combinations miR557-miR1287 and miR557-miR1978 showed a slight increase in specific productivity. Supernatants obtained during this experiment were analyzed by reducing and non-reducing SDS-PAGE to assess whether miRNA overexpression had an impact on antibody integrity in general. In all samples, the gel showed the expected protein bands, indicating that the produced IgG1 antibodies were intact, independent of the stable miRNA overexpression (see Figure 19B). Assessment of product quality is described in more detail in the following chapter.

3.3.2. Product quality of IgG1 produced in stable microRNA overexpressing CHO-IgG1 cells

To exclude altered product quality of antibodies produced in miRNA overexpressing producer cells during fed-batch cultivation, the IgG1 antibody was purified from the supernatant of the stable cell pools and binding to the IgG1 antigen expressed on the surface of MDA-MB468 cells was analyzed using flow cytometry. Of note, an antibody concentration was chosen that did not saturate the binding capacity of the antigen. As a control, antibody purified from the parental CHO-IgG1 cells was used. As shown in Figure 20A, binding of the antibody to the cells was efficient and similar in all samples, indicating that the stable expression of miRNAs does not affect the binding properties of the IgG1 antibody. The isotype human IgG control did not bind to the MDA-MB468 cells proving specificity of the signal.

Moreover, the glycosylation pattern of the purified antibody was analyzed because correct glycosylation is important for the antibody function (Jefferis, 2005). Again, purified antibody from parental CHO-IgG1 cells served as a positive control. The glycan chains were enzymatically released from the antibody and analyzed with microchip-based separation by CGE. Likewise, the glycosylation of the antibody purified from CHO-IgG1 stably expressing miRNAs was comparable to the pattern observed in IgG1 produced in parental cells (see Figure 20B).
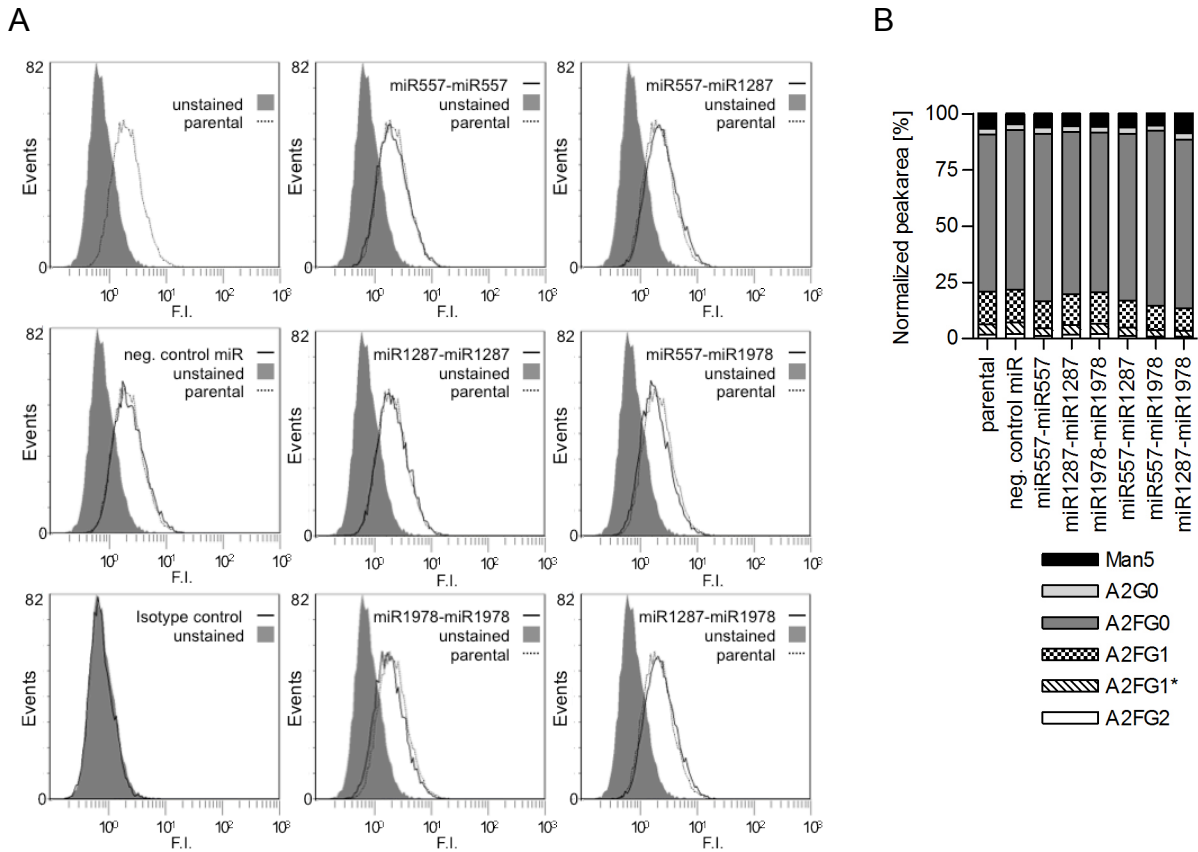
Figure 20: IgG1 antibody produced in CHO cells stably overexpressing microRNAs
(A) IgG1 antibody produced in CHO cells stably overexpressing the different miRNAs or their combination were purified. As controls, IgG1 produced in parental CHO cells (dotted line) or in CHO cells overexpressing a negative control miRNA were used. Antibody purified from the indicated cell supernatant was incubated with MDA-MB468 cells and antigen binding was analyzed by flow cytometry (F.I. = fluorescence intensity). Unstained cells were used as a negative control (grey peak) as well as isotype control stained cells. (B) Glycans were released from the purified antibody produced in the indicated supernatants and analyzed by microchip based CGE separation. Shown is the relative amount of the glycans.

3.3.3. Verification of microRNA induced phenotype in CHO-IgG1 stably expressing the microRNA combination miR557-miR1287

Based on all the previous results, the impact of stable expression of miR557-miR1287 was verified because this combination induced an improved specific productivity compared to the respective controls. To exclude clonal artifacts three independent pools of CHO-IgG1 cells expressing the miR557-miR1287 combination were generated. In addition, four independent pools of CHO-IgG1 cells expressing the negative control miRNA were generated. These pools were sorted for GFP as described and miRNA overexpression was monitored by qRT-PCR (Figure 21B). Stable miRNA overexpressing CHO-IgG1 pools as well as the parental cells (CHO-IgG1) were cultivated in shake flasks in duplicates in fed-batch cultivation as

described above. Samples were taken from day 3 on to determine product concentrations in the supernatant, cell growth, viability, and specific productivity (see Figure 21A).
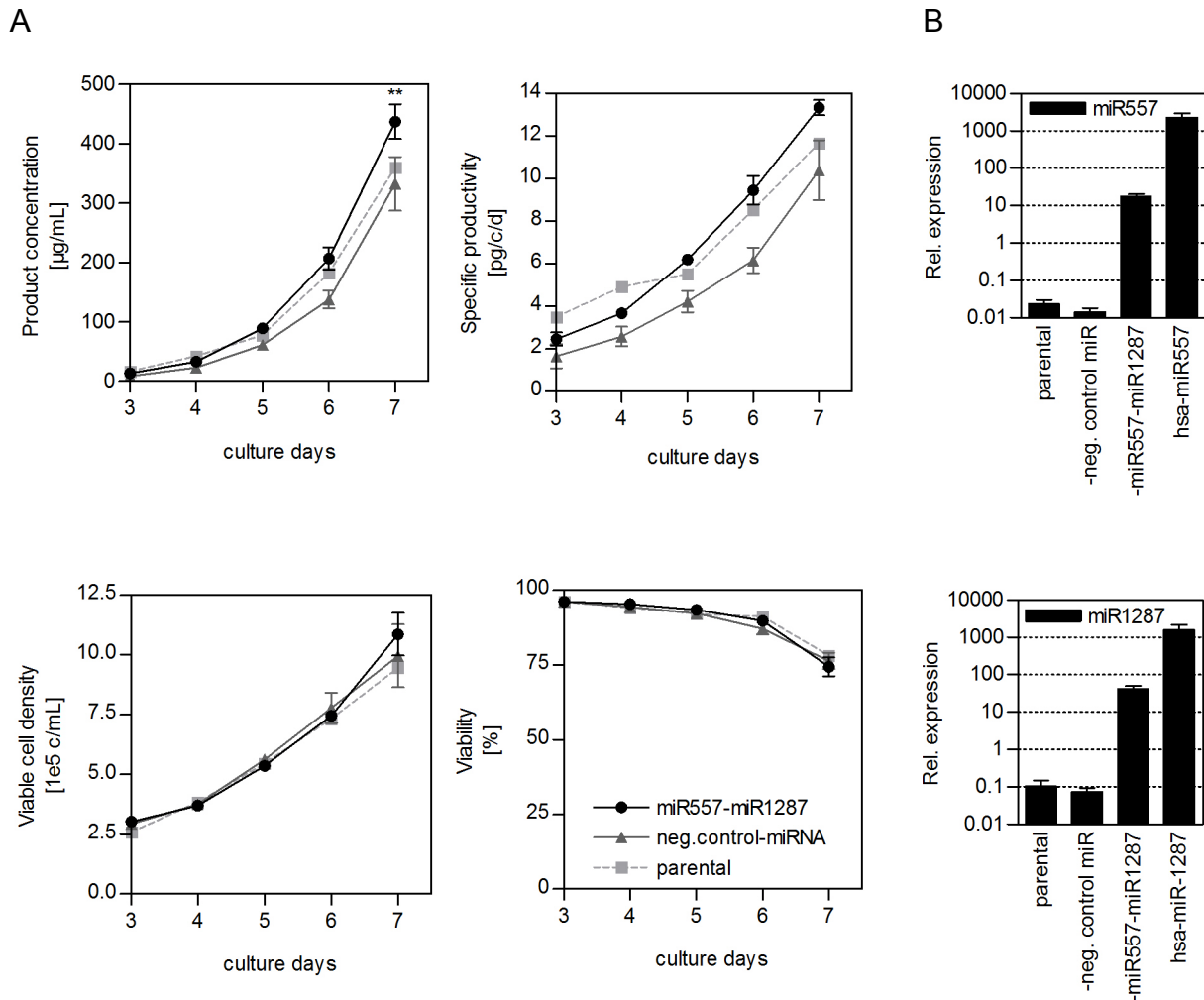
Figure 21: CHO-IgG1 cells stably overexpressing the microRNA combination miR557-miR1287
CHO-IgG1 cells stably expressing two different miRNAs (pcDNA6.2-GW/emGFP-miR557-miR1287) (black circle), a negative control miRNA (pcDNA6.2-GW/emGFP-neg. control miR) (dark grey triangle) and CHO-IgG1 cells (parental) (light grey square) were subjected to fed-batch analysis (A). Shown is the product concentration in the supernatant, the cell density, cell viability and specific productivity. The experiment was performed with three independent stable miR557-miR1287 CHO-IgG1 pools and four independent negative control miRNA pools and shown is the mean of the pools. Three independent experiments were performed and shown is one representative experiment. Statistical analysis was done using two-way anova bonferroni posttest (**<0.01, error bars represent SEM). (B) miRNA overexpression was verified by qRT-PCR analysis. Shown is the mean of the independent pools. Parental cells and cells transiently transfected with the mature miRNAs hsa-miR-557 or hsa-miR-1287 served as controls. Error bars represent SEM. Values were normalized to the RNU6B control calculated by ΔCq method.

The negative control miRNA expressing CHO-IgG1 cells were similar to the parental cells with respect to cell growth and viability. However, a slight decrease of antibody

concentration and specific productivity compared to parental cells was observed. This could be due to the additional burdening of the cells caused by miRNA expression and selection pressure. Strikingly, miR557-miR1287 overexpression resulted in a significant increase in antibody concentration of about 1.3 fold compared to the negative control and the parental cells. Growth and viability of miR557-miR1287 cells were similar to parental and negative control miRNA expressing cells with a marginal tendency to accelerated cell growth. This could be due to the hsa-miR-557 effect observed in the transient experiments (see Figure 11). Finally, specific productivity was also improved compared to the negative control miRNA expressing cells and the parental cells.

In sum, a screen using a miRNA library was established that resulted in six miRNA candidates with the potential to improve productivity of an industrially relevant producer cell line. Specifically, I could show that the stable expression of hsa-miR-557 together with hsa-miR-1287 significantly improved the specific productivity and product concentration in fed-batch culture without affecting product quality in a negative manner.

3.4. Potential mechanism of microRNA mediated increase in productivity

3.4.1. MicroRNA target prediction of hsa-miR-557 and hsa-miR-1287

Target prediction software tools can predict possible targets of human miRNAs in human cells. *Hsa-miR-1978* is not an official miRNA and therefore it is not included in common target prediction tools and target prediction cannot be performed with *hsa-miR-1978*. The genome data for the target prediction of target mRNAs are derived from human cells because to my knowledge there are no target prediction software tools available using hamster genome data. To identify possible targets of hsa-miR-557 and hsa-miR-1287 target prediction analysis was performed. miRNAs have the potential to target and thereby regulate the expression of many different genes. Several target prediction algorithms were developed to identify possible miRNA target genes by considering mainly complementary base pairing of the seed sequence of miRNAs to mRNAs. Therefore, three different target prediction algorithms were applied for hsa-miR-557. TargetScan 6.2 predicted 515 genes,

miRDB 399 genes and microT-CDS 1295 genes (Figure 22A). The same algorithms were also used for hsa-miR-1287. Here TargetScan 6.2 suggested 162 genes, miRDB 150 genes and microT-CDS 1149 genes (Figure 22B). Next, the overlap between potential targets predicted by these algorithms was determined. As shown in Figure 22A and B, this resulted in 111 and 24 genes for hsa-miR-557 and hsa-miR-1287, respectively.
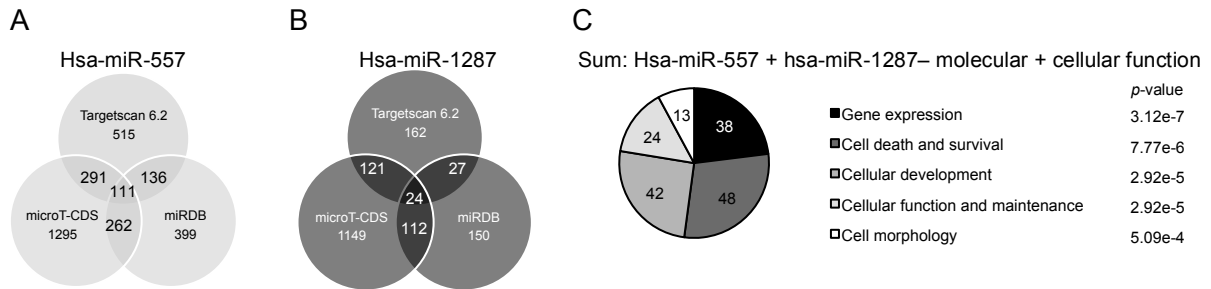
Figure 22: MicroRNA target prediction *in silico*
Venn diagrams show the number of predicted genes and the overlap of the target genes of hsa-miR-557 (A) and hsa-miR-1287 (B) predicted by three different human miRNA target prediction software tools. (C) The hsa-miR-557-specific target genes (111 genes) plus the hsa-miR-1287-specific genes (24 genes) were used for Ingenuity core analysis and molecular and cellular functions associated with predicted genes are summarized. Numbers in the pie chart correspond to the number of genes associated with the respective functions. The analysis was done for the genes predicted for hsa-miR-557 and hsa-miR-1287.

Subsequently, these genes were analyzed using Ingenuity pathway analysis (IPA). The results of this analysis are shown as molecular and cellular functions connected with these genes (Figure 22C). Most predicted genes were assigned to cell death and survival whereas association to gene expression was most significant. In addition, some genes were involved in cellular development, cellular function and maintenance or cell morphology. Of note, not all genes of the 111 and 24 were included in the 5 categories of molecular and cellular functions. The *p*-values indicate a statistically significant, non-random association.

3.4.2. Conservation of biological effects of microRNAs in human cells

In the screen human miRNAs in hamster cells were used and therefore it was interesting whether the human miRNAs miR-557, miR-1287 and *miR-1978* might have similar functions in human cells. Therefore, the three miRNAs hsa-miR-557,

hsa-miR-1287 and *hsa-miR-1978* were transfected in human HeLa cells and two readouts to measure product secretion were chosen. Firstly, endogenous IL-8 secreted into the cell culture supernatant was measured as a model product (see Figure 23A). Secondly, horseradish peroxidase (HRP) was transiently expressed in HeLa cells and the secreted HRP amount was determined with an enzymatic assay resulting in a luminescence signal (see Figure 23B). Cells transfected with a negative control miRNA served as a control. IL-8 concentration and RLU (relative luminescence units) were normalized to the control. *Hsa-miR-1978* and hsa-miR-557 increased the levels of both proteins in the supernatant whereas hsa-miR-1287 did not alter the IL-8 and HRP amount in the described setting (data not shown).
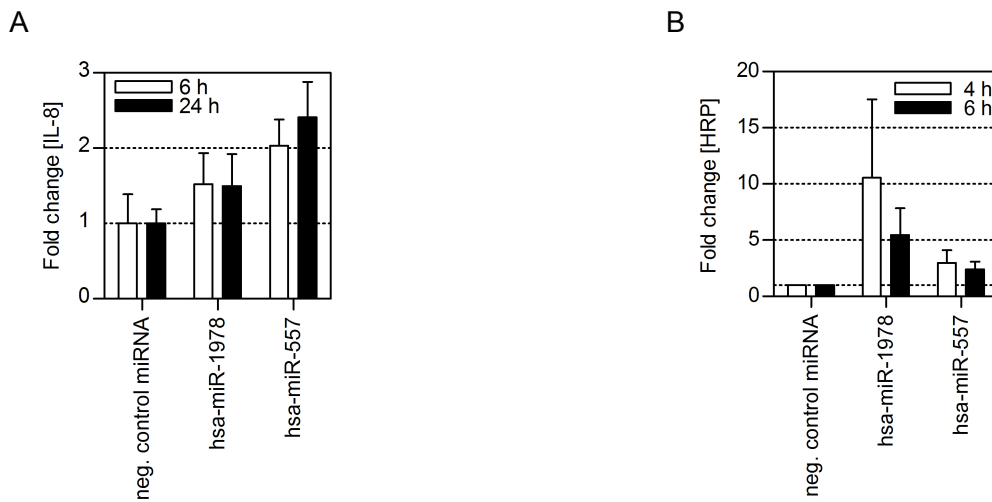
Figure 23: Conservation of mechanism – microRNAs in human cells
Hela cells were transiently transfected with the indicated human miRNAs. (A) Two days post transfection, the medium was changed and endogenous IL-8 secretion was measured after 6 h (white bars) and 24 h (black bars). Values were normalized to those of the negative control miRNA. (B) Transiently expressed HRP accumulated in the supernatant for 4 h (white bars) and 6 h (black bars) was measured by an enzymatic assay and luminescence units were normalized to the negative control miRNA. (A) and (B) show the mean values of 3 independent experiments, error bars represent SEM.

This indicates that hsa-miR-557 and *hsa-miR-1978* might regulate conserved mRNA targets in hamster cells. In general, further work has to be done to identify the mechanisms underlying the action of these miRNAs that result in the hyperproductive phenotype.

4. Discussion

In the past years miRNAs have been discussed as an attractive tool for cell line engineering approaches of mammalian host cells (Muller et al., 2008). However, despite intense sequencing efforts regarding the genome (Cao et al., 2012; Hammond et al., 2011; Xu et al., 2011), proteome (Baycin-Hizal et al., 2012), transcriptome (Becker et al., 2011; Birzele et al., 2010; Clarke et al., 2011), and various miRNA profiling studies performed in CHO cells (Hackl et al., 2012; Hackl et al., 2011; Hammond et al., 2012; Johnson et al., 2011), so far there have only been a few examples linking the expression of a specific miRNA to a desired cellular phenotype (Clarke et al., 2012; Druz et al., 2011).

For example, in response to nutrient depletion, mmu-miR-466h was found to be strongly upregulated, implicating a function for this miRNA in the apoptotic response. Accordingly, the transient inhibition of miR-466h enhanced the expression of several anti-apoptotic genes, leading to increased cell viability and decreased caspase activation in nutrient-depleted medium (Druz et al., 2012). In the same vein, miR-7 was found to be downregulated in response to low temperature, suggesting a role for miR-7 in cell cycle regulation. However, inhibition of endogenous miR-7 using an antagomir was not efficient and hence failed to have an effect on cell growth (Barron et al., 2011).

So far, only one study performed a miRNA-based phenotypic screening in CHO cells comparing miRNA profiles of fast growing CHO cell clones to ones with slow growth rates (Clarke et al., 2012). However, to the best of my knowledge a miRNA mediated hyper-proliferating CHO cell has not been published. This highlights the difficulty of selecting specific miRNAs for the genetic manipulation of host cells merely based on expression data.

4.1. MicroRNA screening design

Herein, a functional screen aiming at the identification of miRNAs that impact the productivity of an IgG secreting CHO producer cell line is described. The approach presented in this thesis differs from the strategies applied so far (Barron et al., 2011; Druz et al., 2011; Gammell et al., 2007; Hammond et al., 2012; Hernandez Bort et al., 2012; Lin et al., 2011). Namely, in this study miRNAs were transiently introduced into CHO cells and then phenotypic screening was carried out, whereas in previous studies the reverse strategy was applied. This ensured a direct link between miRNA overexpression and phenotype. Furthermore, because human miRNAs were introduced into a foreign host, the effects on cellular productivity may not necessarily stem from the modulation of normal endogenous pathways but may be due to the regulation of artificial hamster mRNA targets.

miRNAs operate by translational inhibition of newly synthesized proteins in the cell (Fabian et al., 2010), meaning that the protein level of their targets decreases after miRNA overexpression (Selbach et al., 2008). When transiently expressed, the time at which miRNA-mediated effects can be detected is dependent on the half-life of the target protein (Pratt et al., 2002) and the nature of the miRNA (Fabian et al., 2010). In addition, miRNA levels in the cell decrease with each cell division, causing dilution of the cytosolic miRNA level. Taken together, these two aspects provided a small time window in which the product titers might be affected and thus miRNA-induced product changes were expected to be rather small. It was hence fundamentally important to optimize the transfection efficiency of the cells for the screen. A low transfection efficiency would have made the detection of differences in the secreted product amount even more difficult.

Another challenge was to find an appropriate positive control for the screen due to the lack of studies describing a miRNA with a clear measurable phenotype in CHO producer cells at the time the project was started. As an alternative, a siRNA specific for the product was designed. This siRNA provided a robust control because of its strong effect on the measured product concentration but it did not substitute for a miRNA control that increased the product level.

Due to the lack of a commercially available hamster miRNA library, a human miRNA library was used in the screen. At the time the project was initiated only one hamster miRNA had been published (Gammell et al., 2007). Recently, next-generation sequencing of small RNAs isolated from CHO cells enabled the annotation of 387 mature hamster miRNAs, revealing a high degree of conservation between different species (Hackl et al., 2011). The human miRNAs expressed in CHO cells are thus likely to function by utilizing the endogenous machinery to regulate physiologically relevant targets.

In this thesis, almost 900 miRNAs were analyzed for their functional impact on the product titer of CHO producer cells. Although the annotation of hamster miRNAs might not be completed yet (personal communication M. Hackl, University of Natural Resources and Life Sciences, Vienna), it is very likely that some of these human miRNAs are not endogenously expressed in CHO cells. Indeed, nine of the nineteen screen hits are currently not represented in the hamster miRNAs listed in mirbase. Of note, it was reported that *hsa-miR-1978* shows an overlap with a tRNA sequence and thus this miRNA was removed from the mirbase and referred to as dead entry (www.mirbase.org). Nevertheless, this RNA fragment is termed miRNA in the whole thesis for simplicity reasons. For the screen, a miRNA library was chosen that comprises sequences that correspond to mature double-stranded miRNAs and thus mimic the expression of miRNAs (Wang, 2011), but the use of an inhibitory library would also have been possible. Human inhibitory libraries consist of RNA molecules that basepair with human endogenous miRNAs and inhibit their function (Ebert et al., 2007). Such an inhibitor can *per se* only be functional provided the endogenous miRNA target is available. Because the number of annotated hamster miRNAs is much smaller compared to human miRNAs (www.mirbase.org), a subset of human miRNA inhibitors may not have targets in hamster cells. In addition, members of the same miRNA family are predicted to interact with the same target mRNAs by prediction tools that focus on seed pairing (Lewis et al., 2005) and therefore have redundant functional roles (Abbott et al., 2005; Obad et al., 2011). It is very likely that the inhibition of only one family member may not be sufficient to modulate the expression of such targets (e.g. (Abbott et al., 2005; Bao et al., 2012). As the inhibitory approach seemed to be less promising the screen was performed with the mimic library.

4.2. MicroRNA screen results

Several miRNA screens have been published using cell migration, cell viability or AP (alkaline phosphatase) activity as a marker of osteogenic differentiation as a readout (Nakano et al., 2013; Schoolmeesters et al., 2009; Yang et al., 2013). The method to analyze these screen results depends on the readout and there are also different possibilities e.g. normalization to control or calculation of the z-score, which is a method that considers the standard deviation (Schoolmeesters et al., 2009). The literature that discusses the analyses of RNAi screen results suggests as a first step a quality control of the results and, subsequently, normalization of the data to define screen hits (Birmingham et al., 2009). Consequently, the results were first visualized in the plate format by color-codes (data not shown). This visualization helps to quickly identify artifacts that manifest themselves in spatial patterns, e.g. edge effects (Boutros et al., 2006). Moreover, analysis of the antibody concentrations was done by normalization with the mean values of each plate. Thereby, the relative signal per plate was obtained, eliminating or equalizing plate-to-plate differences (Boutros et al., 2006). This method was based on the assumption that on average the miRNAs have no effect on productivity. I also considered normalizing the antibody concentrations with the median of the respective plate because this value is less sensitive towards outliers (Malo et al., 2006). Furthermore, it could not be excluded that miRNAs with a positive or negative effect on productivity were clustered in the library plates, shifting the mean more strongly than the median (Malo et al., 2006). Consequently, both strategies to analyze the data were adopted and compared: The cut-off was set above and below the assay variability ratio (Zhang et al., 2008), respectively, to define screen hits, which were similarly increased using median-normalization and mean-normalization. Alternatively, results can be normalized to the negative controls instead of using the sample values for normalization. The negative controls were represented by four wells per screen plate and due to the small number these values were susceptible to variance. Therefore the conclusion can be made that the normalization to a higher sample number is more meaningful than to the small number of controls (Birmingham et al., 2009).

In sum, eighteen miRNAs significantly increased productivity in the primary screen, eight of which could be validated to have a significant positive impact on CHO-IgG1 antibody titer and/or specific productivity in the secondary screen, demonstrating the

reproducibility of the screen results and further proving that the 96-well format faithfully reflects the conditions of larger cultivation scales. Positive enough, most of the miRNAs also increased the productivity of a CHO cell pool stably secreting HSA, indicating that the action of the identified miRNAs is both clone and product independent. miRNAs affecting cellular programs in mammalian cells that might be relevant for CHO cell engineering were collected in a recent review (Muller et al., 2008) and, interestingly, there is no overlap with the miRNAs identified here, highlighting that the screen provides new interesting candidates for producer cell optimization.

The literature addressing potential functional roles of the eight validated miRNAs is limited. Most reports described an involvement of miRNAs in cancer. For example, the overexpression of hsa-miR-125a-3p reduced proliferation and migration of HEK293T cells (Ninio-Many et al., 2013). Dysregulation of miRNAs in cancer is shown for hsa-miR-557 (Chen et al., 2013; Katayama et al., 2012), hsa-miR-612 (Jian et al., 2011), hsa-miR-885-3p (Guled et al., 2009) and hsa-miR-1287 (Yao et al., 2013). Hsa-miR-644 was identified as a circulating marker miRNA in bladder cancer, whereas little is known about hsa-miR-193* because the sense strand is mainly biologically active (e.g. Lenarduzzi et al., 2013; Nakano et al., 2013). In this line, only one study connects hsa-miR-193b* overexpression with resistance to a specific chemotherapeutic used for cancer therapy (Ziliak et al., 2012). Of note, only for a few miRNAs out of the validated hits specific targets were identified. For example, a recent publication demonstrated that hsa-miR-125a-3p regulates the tyrosine kinase Fyn, which has a pivotal role in cell adhesion, proliferation, migration and survival, and whose overexpression is associated with several types of cancer (Ninio-Many et al., 2013). Hsa-miR-612 directly regulates Akt2 through which epithelial-mesenchymal transition (EMT) and metastasis were inhibited (Tao et al., 2013). Overexpression of both miRNAs, hsa-miR-612 and hsa-miR-125a-3p had a negative impact on cell proliferation (Ninio-Many et al., 2013; Tao et al., 2013), which, if conserved in hamster cells, would not explain the improved productivity observed in this study. Taken together, the published literature mainly related to human cells does not shed light onto the mechanism underlying the miRNA mediated improvement of productivity in CHO cells.

In general, the positive hits could not be grouped into functional miRNA families and were not found to belong to miRNA clusters. It is thus conceivable that the miRNAs affect different mechanisms and pathways in the cell. This is especially obvious for the three miRNAs hsa-miR-557, hsa-miR-1287 and *hsa-miR-1978* because they show distinct phenotypes. Specifically, hsa-miR-557 increased cell density, whereas h*sa-miR-1978* mediated a strong growth arrest and hsa-miR-1287 influenced specific productivity. The combination of miRNAs could thus have an additive or synergistic impact on product yields. This idea of combining miRNA is physiologically relevant as miRNAs were shown to act combinatorially (Couts et al., 2013) and are furthermore often expressed in clusters to act together to induce a specific phenotype (Olive et al., 2010). Of note, two miRNAs out of the screen hits, namely hsa-miR-1285 and hsa-miR-612 are an exception because they contain the same seed region. In the case of hsa-miR-1285 targeting of p53 is described. Surprisingly, hsa-miR-612 does not seem to regulate this protein (Tian et al., 2010). This suggests that miRNAs with the same seed region do not necessarily target the same transcripts but can have distinct targets.

Additionally, five of the 15 miRNAs that reduced the antibody titer below 0.7 fold are members of two miRNA families (miR-302 and miR-518*), suggesting that these miRNAs suppress the productivity of cells via common underlying mechanisms. Recently, it was published that the miRNA cluster miR-302-367 suppresses cell growth by blocking G1/S cell cycle transition in cervical carcinoma cells with direct downregulation of cyclin D1 and Akt (Cai et al., 2013). This could explain the decreased IgG titer after transfection of miR-302 family members and suggests a conserved mechanism in hamster cells. Furthermore, an upregulation of p21 and p27 was shown (Cai et al., 2013). Inducible p21 and p27 expression was successfully applied in the context of biphasic processes (Fussenegger et al., 1998). Accordingly, inducible expression of miR-302 family members could thus be investigated in a biphasic process to mediate growth arrest in G1 and prolong culture time and productivity. As an engineering tool miRNA expression would be advantageous compared to protein expression because the cell would keep its whole protein synthesis capacity for the product.

Interestingly, two out of the 19 product increasing miRNA hits and six out of the 15 product decreasing miRNA hits were miRNA*. These are the miRNA strands that are quickly degraded in the human background and are therefore described to be non-functional. One of the positive miRNA* hits, hsa-miR-23b*, as well as hsa-miR-125a-3p were described to be upregulated in fast growing CHO cells (Clarke et al., 2012), indicating that CHO cells regulate the expression of these particular miRNAs. However, proliferation of CHO-K1 was not successfully boosted by overexpression of miR-23b* (Sanchez, 2013). Likewise, an increased cell number could not be detected upon hsa-miR-23b* or hsa-miR-125a-3p transfection in the validation screen. The mechanism by which these miRNAs increased productivity thus remains elusive.

While the miRNA concentrations used in the primary screen and validation experiments negatively impacted cell growth, reducing the miRNA concentration by half was sufficient to have negligible effects on cell growth. This was especially evident in the case of hsa-miR-1287 and *hsa-miR-1978.* High miRNA concentrations are therefore not necessarily advantageous for the engineering of producer cells, instead, the constant delivery of a moderate miRNA amount may be required. Vector-based expression of the miRNA of interest driven from an optimized promoter may provide a solution, as this also allows for the stable integration of the miRNA sequence information into the host genome.

4.3. Vector based microRNA expression

Vector-based miRNA expression systems allow for the generation of cell lines with stable and thereby prolonged miRNA expression. First a commercially available miRNA expression vector pCMV-miRNA was used. The vector contains the precursor miRNA sequence plus about 300 bp upstream and downstream flanking regions corresponding to the genomic background of the respective human miRNA (www.origene.com). Unfortunately, miRNA expression could not be detected after stable cell line generation although the transient expression was promising (data not shown). An explanation could be that long-term expression requires appropriate flanking regions of the precursor to ensure the proper processing of the miRNA into the mature form (Chen et al., 2004; Zeng and Cullen, 2005). This is in line with the literature suggesting that the precursor structure as well as the flanking sequences

play a role during miRNA biogenesis (Chen et al., 2004; Starega-Roslan et al., 2011; Zeng and Cullen, 2005).

The alternative strategy was thus to use an expression vector containing verified flanking regions and an optimized hairpin loop for miRNA processing (Lagos-Quintana et al., 2002). The cloned sequence is more artificial because the precursor sequence differs from the endogenously expressed one. The mature miRNA sequence is unmodified whereas the antisense strand sequence is modified to ensure that the desired strand of the miRNA hairpin stem is loaded into the RISC and is thereby functional. The introduction of more than one miRNA encoding cassette into an expression vector dramatically increased miRNA levels (Sun et al., 2006). In line with this, increased mature miRNA levels were observed when two copies of miR-557 and miR-1287, respectively, were cloned into the pcDNA6.2-GW/emGFP-miRNA vector. Moreover, using this strategy, two different miRNA expression cassettes could be cloned in tandem. By qRT-PCR, no endogenous expression of the miRNAs miR-557 and miR-1287 was detected in CHO cells under basal conditions. This is in a line with a previous report, which did not identify the corresponding hamster miRNAs (Hackl et al., 2012).

Different expression levels of the tested miRNAs were observed depending on their position in the tandem expression constructs. Generally, neither the first position nor the second position was advantageous. It is thus conceivable that processing of the hairpins is dependent on the order of the miRNA sequence due to secondary structure formation, as observed for the splicing efficiency of mRNAs (Rogic et al., 2008). By contrast, another publication claimed no effect of secondary structure variants on the processing efficiency of miRNA precursors (Diederichs and Haber, 2006). However, in this study, both in the transient and stable setting, one specific order of the miRNAs cloned in tandem resulted in higher expression levels. This was not the case for *hsa-miR-1978,* which will be discussed in more detail in the following section.

Selection for stable cells always results in a subpopulation of single clones that manage to express the selection marker but not the transgene. To reduce the number of this transgene-negative population in the stable pools, GFP positive cells were sorted. The probability that GFP and miRNA expression correlate is very high

because the miRNA and GFP encoding mRNA are expressed as one transcript. In the case of hsa-miR-557 and hsa-miR-1287 the data indicated that this approach was successful because a decrease in the GFP signal was not detectable after several weeks in culture and a GFP negative population did not appear. However, it was not possible to detect *hsa-miR-1978* in these stable pools. An explanation could be that the initial expression of functional *hsa-miR-1978* mediated a strong growth delay, which would be in line with the observations made in the transient setting of the validation screen. It is very likely that this growth arrest of the cell subpool expressing *hsa-miR-1978* led to the overgrowth of cells characterized by low *hsa-miR-1978* expression levels. The separation of these cell subpopulations might occur during the processing steps of the miRNA. This is in line with the high GFP intensity per cell detected in the stable pools. The transcript encoding for GFP plus the miRNA hairpin in the 3' UTR of GFP is either processed to generate a mature miRNA accompanied by polyA tag removal. Without the polyA tag translational initiation is inhibited and GFP expression is reduced (Sachs et al., 1997). Alternatively, the miRNA precursor hairpin is not processed and the mRNA is available for GFP translation. Presumably, a mixture of both mechanisms is commonly present in the cell (personal communication Life Technology). Consequently, the less miRNA gets processed, the more GFP would be generated by the cell. The theory is thus that during cultivation cell clones with low miRNA processing efficiency were enriched, which was, however, not investigated in more detail.

Stable expression of *hsa-miR-1978* would have been very interesting for an engineering approach because the transient effects of this miRNA on the cellular productivity were enormously high. To study the consequences of stable *hsa-miR-1978* expression a screening for *hsa-miR-1978* positive single clones could be carried out. Nevertheless, the difficulties in generating cells with stable *hsa-miR-1978* expression imply the functionality of the expressed miRNA, arising from its growth inhibitory effects seen in the transient studies. As the direct targets of *hsa-miR-1978,* hsa-miR-1287 and hsa-miR-557 were unknown, functionality of the miRNAs in CHO cells could only be assessed indirectly through the analysis of cell productivity.

4.4. Stable microRNA expression and phenotype

As a proof of principle for the use of miR-engineered cells in industrial applications, stable cell pools expressing the miRNAs of interest were generated and their performance was analyzed in fed batch cultures. It turned out that the single miRNA expression was not sufficient to improve the productivity of CHO-IgG1 cells. This could be dependent on the miRNA level as in the combination approaches the overall transgene-miRNA expression levels were higher. Several independently generated stable pools of miR557-miR1287 expressing cells were generated and further investigated. The productivity of these cell pools reflects the mean productivity of numerous clones. It is thus conceivable that a subset of clones isolated from these populations will present with even higher productivity. The improved performance of stably expressing miR557-miR1287 CHO pools in fed-batch cultures provides support for the initial hypothesis that productivity-enhancing miRNAs identified in a transient screen can be applied by stable expression to improve the productivity of CHO producer cells in an industrially relevant setting.

Furthermore, the growth rate of cells stably expressing human miR-557 and miR-1287 was similar to that of the parental and vector control cells, indicating that ectopic miRNA expression does not impose a metabolic burden on the cells. Although the levels of stably expressed miR-557 and miR-1287 were 100-fold lower compared to transient transfections, the performance of these cells was improved in fed batch cultures. This indicates that relatively modest miRNA overexpression may be sufficient to confer alterations of cellular characteristics when present over a longer period of time.

A next step could be the isolation of single clones from the miR557-miR1287 overexpressing pools. It would be interesting to investigate clones with different miRNA overexpression levels regarding their productivity to determine the most favorable miRNA level in the stable condition. In addition, a fed-batch process that lasts longer than 7 days could be performed to investigate the maximum product titers that can be achieved with cell pools overexpressing the miRNA combination.

This first functional miRNA screen in CHO producer cells was performed in a model cell line that has already been selected based on its good productivity. The results

demonstrate that cellular productivity is not at its limit but can be boosted even further. It was previously shown that the stable expression of the lipid transfer protein CERT, which functions at the level of the Golgi complex to ensure constitutive vesicular trafficking to the plasma membrane, can further augment the specific productivity of CHO cells secreting an IgG1 or HSA (Florin et al., 2009). This indicates that this CHO producer cell line may have a bottleneck at the level of protein secretion. It is possible that the screen hit miRNAs act in part by alleviating this bottleneck, through the modulation of protein synthesis and transport, thereby increasing the specific productivity of cells. To investigate this question in more detail a target prediction analysis was performed.

4.5. Potential molecular mechanism underlying the microRNA mediated phenotype

It would be interesting to figure out the mechanism responsible for the miRNA mediated phenotype change. Although there are a few reports on alterations of miR-557 (Chen et al., 2013; Katayama et al., 2012; Mosakhani et al., 2012) and miR-1287 (Guo et al., 2012; Wang et al., 2012) expression levels in different cancer types, no specific targets have been identified to date. *Hsa-miR-1978* was removed from the mirbase due to overlap with a tRNA sequence. This means that *hsa-miR-1978* was falsely annotated as a miRNA and this was corrected by eliminating the annotation from the mirbase. Nevertheless, *hsa-miR-1978* was included in the library, and thus screened, and intriguingly gave rise to the strongest elevation of specific productivity. That has two main consequences: first, there are no research data available concerning biological functions of *hsa-miR-1978* because it is not a miRNA. Second, using the *hsa-miR-1978* RNA sequence is strictly speaking not a miRNA engineering but rather a synthetic biology approach (Lynch and Gill, 2012). Irrespective of its origin, the *hsa-miR-1978* RNA sequence may function in a miRNA-like fashion by translational silencing of specific mRNA targets.

To gain more insight into the mechanism of action, it was first investigated if the three miRNAs hsa-miR-557, hsa-miR-1287 and *hsa-miR-1978* might have conserved functions in human cells. Indeed, in the human cell line HeLa the miRNAs *hsa-miR-1978* and hsa-miR-557 enhanced the amount of secreted model proteins, pointing to

a conserved mechanism between human and hamster cells. Of note, both miRNAs did not affect cell proliferation of HeLa cells. A reason for this could be the different culture conditions: HeLa cells were grown under adherent culture conditions and in the presence of serum whereas CHO producer cells were grown in suspension in chemically defined media. Hsa-miR-1287 did not improve secreted product levels in human cells in the described setting. Although miRNA sequences are highly conserved between species, the expression of miRNAs can be different even between tissues (Lagos-Quintana et al., 2002) and also their function can differ between tissues (Schier and Giraldez, 2006). This could be explained by the fact that the function of miRNA is dependent on e.g. target availability (Sood et al., 2006) or by the pattern of expression of other miRNAs because target sequences of different miRNAs on mRNAs can be overlapping and thereby competitive.

Secondly, I was interested in theoretically predicted targets. Target prediction algorithms have been developed that give rise to a high number of theoretical targets with a high false positive rate (Sun et al., 2010). The results of the target prediction analysis cannot substitute for wet lab data but can help to develop a possible idea that can be followed up in experimental work. Due to lack of target prediction analysis tools based on hamster genome data, target prediction was performed with algorithms based on the mRNA sequences expressed by human cells. Here, the combined targets of hsa-miR-1287 and hsa-miR-557 were especially interesting due to the positive impact on productivity of CHO cells stably expressing these miRNAs.

To identify potential pathways associated with the predicted miRNA targets, Ingenuity pathway analysis was performed. The results suggested an involvement of hsa-miR-557 and hsa-miR-1287 in anti-apoptotic mechanisms. However, several issues have to be considered: Firstly, the number of predicted targets of hsa-miR-557 was threefold higher than that of hsa-miR-1287, shifting the emphasis of the analysis to the former miRNA. Secondly, hsa-miR-1287 mediated effects on secretion were not conserved in human cells in our experimental setting. Thus, predicted targets of this miRNA might not be relevant for the effect observed in CHO producer cells. Consequently, hsa-miR-1287 might target hamster genes that cannot be identified *in silico*. Finally, in the experimental fed-batch format, cell death events were not relevant because the cells were harvested before entering stationary phase. Thus, to

address whether stable expression of the two miRNAs positively affects cell viability, a fed-batch culture, which lasts until the beginning of the decline phase has to be performed.

However, an appropriate and more reliable tool would use the hamster genome for target predictions, which is not yet available. Even if wet lab data were generated a hamster prediction tool would be helpful to distinguish mRNAs that contain a target sequence for the respective miRNA and therefore are more probable to be direct targets rather than indirectly regulated mRNAs. A hamster based target prediction tool could thus help to find primary targets and select them from secondarily regulated genes. Therefore, to elucidate the molecular mechanism by which hsa-miR-557, hsa-miR-1287 and *hsa-miR-1978* impact on cell productivity, analysis of transcriptome changes will be required. However, due to the promiscuous nature of miRNAs, it is most likely that the downregulation of a single target mRNA is not sufficient to account for the enhanced viable cell number and/or specific productivity. It is rather the simultaneous modulation of several targets that act in concert (Abston and Miller, 2005; Serva et al., 2012) to support a hyperproductivity phenotype of cells.

Finally, even if the molecular mechanism by which the miRNAs act remains to be elucidated, the miRNAs can be used as tools to engineer biotechnologically relevant producer cells as long as the product quality is secured. So far there has been no indication that the product quality was affected by miRNA overexpression. Furthermore, based on the protocol established here, further screens can now be tailored to identify candidate miRNAs that positively impact additional parameters relevant to the bioprocess, such as stress tolerance or metabolic disorders of cells. The fact that different miRNAs can be encoded in tandem or more copies on a single vector, analogous to the clustered genomic organization of miRNAs, provides an unlimited tool-box that can be designed to meet the needs of the particular bioprocess.

In summary, my data support the notion that the optimization of mammalian host cells based on miR-engineering is a promising strategy toward the more efficient and affordable production of recombinant proteins for future therapeutic applications.

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6. Supplements

6.1. MicroRNA expression vector

attB1 | EmGFP | 5' miR flanking region ACGA | CAGG 3' miR flanking region | attB2

pcDNA™6.2-GW/ EmGFP-miR 5699 bp

P CMV, TK pA, f1 ori, SV40 ori, EM7, Blasticidin, SV40 pA, pUC ori, Spectinomycin

6.2. Mimic microRNA library

6.2.1. MicroRNA library details

Plate	Mature Sanger ID	PreCursor Accession	Mature Sequence
	Dharmacon miRIDIAN® microRNA Library - Human Mimic		
	CS-001010 Lot 09167		
1	hsa-miR-561	MI0003567	CAAAGUUUAAGAUCCUUGAAGU
1	hsa-miR-615-5p	MI0003628	GGGGGUCCCCGGUGCUCGGAUC
1	hsa-miR-589	MI0003599	UGAGAACCACGUCUGCUCUGAG
1	hsa-miR-128	MI0000447	UCACAGUGAACCGGUCUCUUU
1	hsa-miR-557	MI0003563	GUUUGCACGGGUGGGCCUUGUCU
1	hsa-miR-500	MI0003184	UAAUCCUUGCUACCUGGGUGAGA
1	hsa-miR-1225-3p	MI0006311	UGAGCCCCUGUGCCGCCCCCAG
1	hsa-miR-1305	MI0006372	UUUUCAACUCUAAUGGGAGAGA
1	hsa-miR-595	MI0003607	GAAGUGUGCCGUGGUGUGUCU
1	hsa-miR-559	MI0003565	UAAAGUAAAUAUGCACCAAAA
1	hsa-miR-891a	MI0005524	UGCAACGAACCUGAGCCACUGA
1	hsa-miR-193b*	MI0003137	CGGGGUUUUGAGGGCGAGAUGA
1	hsa-miR-374a*	MI0000782	CUUAUCAGAUUGUAUUGUAAUU
1	hsa-miR-92a-1*	MI0000093	AGGUUGGGAUCGGUUGCAAUGCU
1	hsa-miR-146a*	MI0000477	CCUCUGAAAUUCAGUUCUUCAG
1	hsa-miR-34a	MI0000268	UGGCAGUGUCUUAGCUGGUUGU
1	hsa-miR-19a*	MI0000073	AGUUUUGCAUAGUUGCACUACA
1	hsa-miR-486-5p	MI0002470	UCCUGUACUGAGCUGCCCCGAG
1	hsa-miR-30b*	MI0000441	CUGGGAGGUGGAUGUUUACUUC
1	hsa-miR-9	MI0000466	UCUUUGGUUAUCUAGCUGUAUGA
1	hsa-miR-606	MI0003619	AAACUACUGAAAAUCAAAGAU
1	hsa-miR-548b-5p	MI0003596	AAAAGUAAAUUGGGGUUUUGGCC
1	hsa-miR-887	MI0005562	GUGAACGGGCGCCAUCCCGAGG
1	hsa-miR-373	MI0000781	GAAGUGCUUCGAUUUUGGGGUGU
1	hsa-miR-363*	MI0000764	CGGGUGGAUCACGAUGCAAUUU
1	hsa-miR-2113	MI0003939	AUUUGUGCUUGGCUCUGUCAC
1	hsa-miR-1299	MI0006359	UUCUGGAAUCUGUGGUGAGGGA
1	hsa-miR-450a	MI0001652	UUUUUGCGAUGUGUUCCUAAUAU
1	hsa-miR-921	MI0005713	CUAGUGAGGGACAGAACCAGGAAUC
1	hsa-miR-33a	MI0000091	GUGCAUUGUAGUUGCAUUGCA
1	hsa-miR-220b	MI0005529	CCACCACCGUGUCUGACACUU
1	hsa-miR-518d-5p	MI0003171	CUCUAGAGGGAAGCAUUUUACCUUG
1	hsa-miR-924	MI0005716	AGAGUCUUGUGAUGUCUUGC
1	hsa-miR-876-5p	MI0005542	UGGAUUUCUUUGUGAAUCACCA
1	hsa-miR-518f	MI0003154	GAAAGCGCUUCUCUUUUAGAGG
1	hsa-miR-154	MI0000480	UAGGUUAUCCGUGUUGCCUUCG
1	hsa-miR-146b-5p	MI0003129	UGAGAACUGAAUUCCAUAGGCU
1	hsa-miR-1236	MI0006326	CCUCUUCCCCUUGUCUCUCCAG
1	hsa-miR-487b	MI0003530	AAUCGUACAGGGUCAUCCACUU
1	hsa-miR-339-5p	MI0000815	UCCCUGUCCUCCAGGAGCUCACG
1	hsa-miR-302d*	MI0000774	ACUUUAACAUGGAGGCACUUGC
1	hsa-miR-621	MI0003635	GGCUAGCAACAGCGCUUACCU
1	hsa-miR-32*	MI0000090	CAAUUUAGUGUGUGUGAUAUUU
1	hsa-miR-200c*	MI0000650	CGUCUUACCCAGCAGUGUUUGG
1	hsa-miR-193b	MI0003137	AACUGGCCCUCAAAGUCCCGCU
1	hsa-miR-190	MI0000486	UGAUAUGUUUGAUAUAUUAGGU
1	hsa-miR-1973	MI0009983	ACCGUGCAAAGGUAGCAUA
1	hsa-miR-518f*	MI0003154	CUCUAGAGGGAAGCACUUUCUC
1	hsa-miR-135a	MI0000452	UAUGGCUUUUUAUUCCUAUGUGA
1	hsa-miR-1255b	MI0006435	CGGAUGAGCAAAGAAAGUGGUU
1	hsa-miR-299-3p	MI0000744	UAUGUGGGAUGGUAAACCGCUU
1	hsa-miR-15a	MI0000069	UAGCAGCACAUAAUGGUUUGUG
1	hsa-miR-488*	MI0003123	CCCAGAUAAUGGCACUCUCAA
1	hsa-miR-106b*	MI0000734	CCGCACUGUGGGUACUUGCUGC
1	hsa-miR-16-2*	MI0000115	CCAAUAUUACUGUGCUGCUUUA
1	hsa-miR-671-3p	MI0003760	UCCGGUUCUCAGGGCUCCACC
1	hsa-miR-556-3p	MI0003562	AUAUUACCAUUAGCUCAUCUUU
1	hsa-miR-99a*	MI0000101	CAAGCUCGCUUCUAUGGGUCUG
1	hsa-miR-645	MI0003660	UCUAGGCUGGUACUGCUGA
1	hsa-miR-802	MI0003906	CAGUAACAAAGAUUCAACCUUGU
1	hsa-miR-29c*	MI0000735	UGACCGAUUUCUCCUGGUGUUC
1	hsa-miR-548c-3p	MI0003630	CAAAAAUCUCAAUUACUUUUGC
1	hsa-miR-15a*	MI0000069	CAGGCCAUAUUGUGCUGCCUCA
1	hsa-miR-193a-3p	MI0000487	AACUGGCCUACAAAGUCCCAGU
1	hsa-miR-545	MI0003516	UCAGCAAACAUUUAUUGUGUGC
1	hsa-miR-181a-2*	MI0000269	ACCACUGACCGUUGACUGUACC
1	hsa-miR-369-3p	MI0000777	AAUAAUACAUGGUUGAUCUUU
1	hsa-miR-935	MI0005757	CCAGUUACCGCUUCCGCUACCGC
1	hsa-miR-1306	MI0006443	ACGUUGGCUCUGGUGGUG
1	hsa-miR-302c	MI0000773	UAAGUGCUUCCAUGUUUCAGUGG
1	hsa-miR-298	MI0005523	AGCAGAAGCAGGGAGGUUCUCCCA
1	hsa-miR-185*	MI0000482	AGGGGCUGGCUUUCCUCUGGUC
1	hsa-miR-1324	MI0006657	CCAGACAGAAUUCUAUGCACUUUC
1	hsa-miR-188-5p	MI0000484	CAUCCCUUGCAUGGUGGAGGG
1	hsa-miR-1268	MI0006405	CGGGCGUGGUGGUGGGGG
1	hsa-miR-655	MI0003677	AUAAUACAUGGUUAACCUCUUU
1	hsa-miR-149	MI0000478	UCUGGCUCCGUGUCUUCACUCCC
1	hsa-miR-668	MI0003761	UGUCACUCGGCUCGGCCCACUAC
1	hsa-miR-208b	MI0005570	AUAAGACGAACAAAAGGUUUGU
1	hsa-miR-541	MI0005539	UGGUGGGCACAGAAUCUGGACU
2	hsa-miR-30a	MI0000088	UGUAAACAUCCUCGACUGGAAG
2	hsa-miR-1468	MI0003782	CUCCGUUUGCCUGUUUCGCUG
2	hsa-let-7e*	MI0000066	CUAUACGGCCUCCUAGCUUUCC
2	hsa-miR-875-5p	MI0005541	UAUACCUCAGUUUUAUCAGGUG
2	hsa-miR-548p	MI0006420	UAGCAAAAACUGCAGUUACUUU
2	hsa-miR-302a*	MI0000738	ACUUAAACGUGGAUGUACUUGCU
2	hsa-miR-1825	MI0008193	UCCAGUGCCCUCCUCUCC
2	hsa-miR-516b-3p	MI0003180	UGCUUCCUUUCAGAGGGU
2	hsa-miR-370	MI0000778	GCCUGCUGGGGUGGAACCUGGU
2	hsa-miR-124	MI0000443	UAAGGCACGCGGUGAAUGCC
2	hsa-miR-382	MI0000790	GAAGUUGUUCGUGGUGGAUUCG
2	hsa-miR-10b	MI0000267	UACCCUGUAGAACCGAAUUUGUG
2	hsa-miR-1250	MI0006385	ACGGUGCUGGAUGUGGCCUUU
2	hsa-miR-519e*	MI0003145	UUCUCCAAAAGGGAGCACUUUC
2	hsa-miR-337-3p	MI0000806	CUCCUAUAUGAUGCCUUUCUUC
2	hsa-miR-1913	MI0008334	UCUGCCCCCUCCGCUGCUGCCA
2	hsa-miR-194*	MI0000732	CCAGUGGGGCUGCUGUUAUCUGG
2	hsa-miR-98	MI0000100	UGAGGUAGUAAGUUGUAUUGUU
2	hsa-miR-553	MI0003558	AAAACGGUGAGAUUUUGUUUU
2	hsa-miR-583	MI0003583	CAAAGAGGAAGGUCCCAUUAC
2	hsa-miR-640	MI0003655	AUGAUCCAGGAACCUGCCUCU
2	hsa-miR-33b*	MI0003646	CAGUGCCUCGGCAGUGCAGCCC
2	hsa-miR-886-3p	MI0005527	CGCGGGUGCUUACUGACCCUU
2	hsa-miR-548c-5p	MI0003630	AAAAGUAAAUGCGGUUUUUGCC
2	hsa-miR-379*	MI0000787	UAUGUAACAUGGUCCACUAACU
2	hsa-miR-376a	MI0000784	AUCAUAGAGGAAAAUCCACGU
2	hsa-miR-518a-3p	MI0003170	GAAAGCGCUUCCCUUUGCUGGA
2	hsa-miR-138-1*	MI0000476	GCUACUUCACAACACCAGGGCC
2	hsa-miR-662	MI0003670	UCCCACGUUGUGGCCCAGCAG
2	hsa-miR-18a	MI0000072	UAAGGUGCAUCUAGUGCAGAUAG
2	hsa-miR-663b	MI0006336	GGUGGCCCGGCCGUGCCUGAGG
2	hsa-miR-599	MI0003611	GUUGUGUCAGUUUAUCAAAC
2	hsa-miR-622	MI0003636	ACAGUCUGCUGAGGUUGGAGC
2	hsa-miR-630	MI0003644	AGUAUUCUGUACCAGGGAAGGU
2	hsa-miR-302d	MI0000774	UAAGUGCUUCCAUGUUUGAGUGU
2	hsa-miR-30e*	MI0000749	CUUUCAGUCGGAUGUUUACAGC
2	hsa-miR-130b	MI0000748	CAGUGCAAUGAUGAAAGGGCAU
2	hsa-miR-554	MI0003559	GCUAGUCCUGACUCAGCCAGU
2	hsa-miR-616*	MI0003629	ACUCAAAACCCUUCAGUGACUU
2	hsa-miR-132	MI0000449	UAACAGUCUACAGCCAUGGUCG
2	hsa-miR-34c-5p	MI0000743	AGGCAGUGUAGUUAGCUGAUUGC
2	hsa-miR-323-3p	MI0000807	CACAUUACACGGUCGACCUCU
2	hsa-miR-130b*	MI0000748	ACUCUUUCCCUGUUGCACUAC
2	hsa-miR-1275	MI0006415	GUGGGGGAGAGGCUGUC
2	hsa-miR-1280	MI0006437	UCCCACCGCUGCCACCC
2	hsa-miR-337-5p	MI0000806	GAACGGCUUCAUACAGGAGUU
2	hsa-miR-181c	MI0000271	AACAUUCAACCUGUCGGUGAGU
2	hsa-let-7g*	MI0000433	CUGUACAGGCCACUGCCUUGC

2	hsa-miR-542-5p	MI0003686	UCGGGGAUCAUCAUGUCACGAGA
2	hsa-miR-214*	MI0000290	UGCCUGUCUACACUUGCUGUGC
2	hsa-miR-106b	MI0000734	UAAAGUGCUGACAGUGCAGAU
2	hsa-miR-153	MI0000463	UUGCAUAGUCACAAAAGUGAUC
2	hsa-miR-20a	MI0000076	UAAAGUGCUUAUAGUGCAGGUAG
2	hsa-miR-648	MI0003663	AAGUGUGCAGGGCACUGGU
2	hsa-miR-220a	MI0000297	CCACACCGUAUCUGACACUUU
2	hsa-miR-548a-3p	MI0003593	CAAAACUGGCAAUUACUUUUGC
2	hsa-miR-151-5p	MI0000809	UCGAGGAGCUCACAGUCUAGU
2	hsa-miR-149*	MI0000478	AGGGAGGGACGGGGGCUGUGC
2	hsa-miR-296-3p	MI0000747	GAGGGUUGGGUGGAGGCUCUCC
2	hsa-miR-455-5p	MI0003513	UAUGUGCCUUUGGACUACAUCG
2	hsa-miR-551b*	MI0003575	GAAAUCAAGCGUGGGUGAGACC
2	hsa-miR-296-5p	MI0000747	AGGGCCCCCCCUCAAUCCUGU
2	hsa-miR-1323	MI0003786	UCAAAACGUGAGGGGCAAUUUCU
2	hsa-miR-604	MI0003617	AGGCUGCGGAAUUCAGGAC
2	hsa-miR-551a	MI0003556	GCGACCCACUCUUGGUUUCCA
2	hsa-miR-563	MI0003569	AGGUUGACAUACGUUUCCC
2	hsa-miR-1915	MI0008336	CCCCAGGGCGACGCGGCGGG
2	hsa-miR-181d	MI0003139	AACAUUCAUUGUUGUCGGUGGGU
2	hsa-miR-411	MI0003675	UAGUAGACCGUAUAGCGUACG
2	hsa-miR-708*	MI0005543	CAACUAGACUGUGAGCUUCUAG
2	hsa-miR-320c	MI0003778	AAAAGCUGGGUUGAGAGGGU
2	hsa-miR-92b*	MI0003560	AGGGACGGGACGCGGUGCAGUG
2	hsa-miR-409-3p	MI0001735	GAAUGUUGCUCGGUGAACCCCU
2	hsa-miR-1262	MI0006397	AUGGGUGAAUUUGUAGAAGGAU
2	hsa-miR-1976	MI0009986	CCUCCUGCCCUCCUUGCUGU
2	hsa-miR-18a*	MI0000072	ACUGCCCUAAGUGCUCCUUCUGG
2	hsa-miR-126*	MI0000471	CAUUAUUACUUUUGGUACGCG
2	hsa-miR-1290	MI0006352	UGGAUUUUUGGAUCAGGGA
2	hsa-miR-30e	MI0000749	UGUAAACAUCCUUGACUGGAAG
2	hsa-miR-507	MI0003194	UUUUGCACCUUUUGGAGUGAA
3	hsa-miR-218	MI0000294	UUGUGCUUGAUCUAACCAUGU
3	hsa-miR-1978	MI0009988	GGUUUGGUCCUAGCCUUUCUA
3	hsa-miR-99b*	MI0000746	CAAGCUCGUGUCUGUGGGUCCG
3	hsa-miR-1	MI0000651	UGGAAUGUAAAGAAGUAUGUAU
3	hsa-miR-384	MI0001145	AUUCCUAGAAAUUGUUCAUA
3	hsa-miR-20b*	MI0001519	ACUGUAGUAUGGGCACUUCCAG
3	hsa-miR-380*	MI0000788	UGGUUGACCAUAGAACAUGCGC
3	hsa-miR-658	MI0003682	GGCGGAGGGAAGUAGGUCCGUUGGU
3	hsa-miR-136	MI0000475	ACUCCAUUUGUUUUGAUGAUGGA
3	hsa-miR-1224-3p	MI0003764	CCCCACCUCCUCCUUCUCCUCAG
3	hsa-miR-1231	MI0006321	GUGUCUGGGCGGACAGCUGC
3	hsa-miR-539	MI0003514	GGAGAAAUUAUCCUUGGUGUGU
3	hsa-miR-525-3p	MI0003152	GAAGGCGCUUCCCUUUAGAGCG
3	hsa-miR-210	MI0000286	CUGUGCGUGUGACAGCGGCUGA
3	hsa-miR-517c	MI0003174	AUCGUGCAUCCUUUUAGAGUGU
3	hsa-miR-135b	MI0000810	UAUGGCUUUUCAUUCCUAUGUGA
3	hsa-miR-549	MI0003679	UGACAACUAUGGAUGAGCUCU
3	hsa-miR-638	MI0003653	AGGGAUCGCGGGCGGGUGGGCCU
3	hsa-miR-383	MI0000791	AGAUCAGAAGGUGAUUGUGGCU
3	hsa-miR-155*	MI0000681	CUCCUACAUAUUAGCAUUAACA
3	hsa-miR-1286	MI0006348	UGCAGGACCAAGAUGAGCCCU
3	hsa-miR-222	MI0000299	AGCUACAUCUGGCUACUGGGU
3	hsa-miR-548f	MI0006374	AAAAACUGUAAAACUUU
3	hsa-miR-633	MI0003648	CUAAUAGUAUCUACCACAAUAAA
3	hsa-miR-1298	MI0003938	UUCAUUCGGCUGUCCAGAUGUA
3	hsa-miR-575	MI0003582	GAGCCAGUUGGACAGGAGC
3	hsa-miR-1538	MI0007259	CGGCCCGGGCUGCUGCGUGUUCCU
3	hsa-miR-496	MI0003136	UGAGUAUUACAUGGCCAAUCUC
3	hsa-miR-518a-5p	MI0003170	CUGCAAAGGGAAGCCCUUUC
3	hsa-miR-509-5p	MI0003196	UACUGCAGACAGGUGGCAAUCA
3	hsa-miR-517a	MI0003161	AUCGUGCAUCCCUUUAGAGUGU
3	hsa-miR-2054	MI0010488	CUGUAAUAUAAAAUUUAAUUUAUU
3	hsa-miR-26b	MI0000084	UUCAAGUAAUUCAGGAUAGGU
3	hsa-miR-381	MI0000789	UAUACAAGGGCAAGCUCUCUGU
3	hsa-miR-532-5p	MI0003205	CAUGCCUUGAGUGUAGGACCGU
3	hsa-miR-34b	MI0000742	CAAUCACUAACUCCACUGCCAU
3	hsa-miR-148a*	MI0000253	AAAGUUCUGAGACACUCCGACU
3	hsa-miR-664	MI0006442	UAUUCAUUUAUCCCCAGCCUACA
3	hsa-miR-518c*	MI0003159	UCUCUGGAGGGAAGCACUUUCUG
3	hsa-miR-522	MI0003175	AAAAUGGUUCCCUUUAGAGUGU
3	hsa-miR-19b-2*	MI0000075	AGUUUUGCAGGUUUGCAUUUCA
3	hsa-miR-1254	MI0006388	AGCCUGGAAGCUGGAGCCUGCAGU
3	hsa-miR-1539	MI0007260	UCCUGCGCGUCCCAGAUGCCC
3	hsa-miR-17	MI0000071	CAAAGUGCUUACAGUGCAGGUAG
3	hsa-miR-625*	MI0003639	GACUAUAGAACUUUCCCCCUCA
3	hsa-miR-342-5p	MI0000805	AGGGGUGCUAUCUGUGAUUGA
3	hsa-miR-99a	MI0000101	AACCCGUAGAUCCGAUCUUGUG
3	hsa-miR-15b*	MI0000438	CGAAUCAUUAUUUGCUGCUCUA
3	hsa-miR-19a	MI0000073	UGUGCAAAUCUAUGCAAAACUGA
3	hsa-miR-362-3p	MI0000762	AACACACCUAUUCAAGGAUUCA
3	hsa-miR-216b	MI0005569	AAAUCUCUGCAGGCAAAUGUGA
3	hsa-miR-320a	MI0000542	AAAAGCUGGGUUGAGAGGGCGA
3	hsa-miR-374b	MI0005566	AUAUAAUACAACCUGCUAAGUG
3	hsa-let-7d	MI0000065	AGAGGUAGUAGGUUGCAUAGUU
3	hsa-miR-1204	MI0006337	UCGUGGCCUGGUCUCCAUUAU
3	hsa-miR-661	MI0003669	UGCCUGGGUCUCUGGCCUGCGCGU
3	hsa-miR-1228*	MI0006318	GUGGGCGGGGGCAGGUGUGUG
3	hsa-miR-363	MI0000764	AAUUGCACGGUAUCCAUCUGUA
3	hsa-miR-331-5p	MI0000812	CUAGGUAUGGUCCCAGGGAUCC
3	hsa-miR-548b-3p	MI0003596	CAAGAACCUCAGGUGCUUUUGU
3	hsa-miR-708	MI0005543	AAGGAGCUUACAAUCUAGCUGGG
3	hsa-miR-412	MI0002464	ACUUCACCUGGUCCACUAGCCGU
3	hsa-miR-450b-3p	MI0005531	UUGGGAUCAUUUUGCAUCCAUA
3	hsa-miR-1228	MI0006318	UCACACCUGCCUCGCCCCCC
3	hsa-miR-302b	MI0000772	UAAGUGCUUCCAUGUUUUAGUAG
3	hsa-miR-568	MI0003574	AUGUAUAAAUGUAUACACAC
3	hsa-miR-941	MI0005763	CACCCGGCUGUGUGCACAUGUGC
3	hsa-miR-541*	MI0005539	AAAGGAUUCUGCUGUCGGUCCCACU
3	hsa-miR-376a*	MI0000784	GUAGAUUCUCCUUCUAUGAGUA
3	hsa-miR-409-5p	MI0001735	AGGUUACCCGAGCAACUUUGCAU
3	hsa-miR-556-5p	MI0003562	GAUGAGCUCAUUGUAAUAUGAG
3	hsa-miR-433	MI0001723	AUCAUGAUGGGCUCCUCGGUGU
3	hsa-miR-519c-3p	MI0003148	AAAGUGCAUCUUUUUAGAGGAU
3	hsa-miR-1972	MI0009982	UCAGGCCAGGCACAGUGGCUCA
3	hsa-miR-154*	MI0000480	AAUCAUACACGGUUGACCUAUU
3	hsa-miR-593	MI0003605	UGUCUCUGCUGGGGUUUCU
3	hsa-miR-624	MI0003638	CACAAGGUAUUGGUAUCACCU
3	hsa-miR-525-5p	MI0003152	CUCCAGAGGGAUGCACUUUCU
3	hsa-miR-429	MI0001641	UAAUACUGUCUGGUAAAACCGU
3	hsa-miR-548d-3p	MI0003668	CAAAAACCACAGUUUCUUUUGC
4	hsa-miR-21	MI0000077	UAGCUUAUCAGACUGAUGUUGA
4	hsa-miR-501-5p	MI0003185	AAUCCUUUGUCCCUGGGUGAGA

4	hsa-miR-140-3p	MI0000456	UACCACAGGGUAGAACCACGG
4	hsa-miR-548o	MI0006402	CCAAAACUGCAGUUACUUUUGC
4	hsa-miR-675	MI0005416	UGGUGCGGAGAGGGCCCACAGUG
4	hsa-miR-1260	MI0006394	AUCCCACCUCUGCCACCA
4	hsa-miR-145	MI0000461	GUCCAGUUUUCCCAGGAAUCCCU
4	hsa-miR-1256	MI0006390	AGGCAUUGACUUCUCACUAGCU
4	hsa-miR-502-5p	MI0003186	AUCCUUGCUAUCUGGGUGCUA
4	hsa-miR-1308	MI0006441	GCAUGGGUGGUUCAGUGG
4	hsa-miR-1237	MI0006327	UCCUUCUGCUCCGUCCCCCAG
4	hsa-miR-1253	MI0006387	AGAGAAGAAGAUCAGCCUGCA
4	hsa-miR-326	MI0000808	CCUCUGGGCCCUUCCUCCAG
4	hsa-miR-635	MI0003650	ACUUGGGCACUGAAACAAUGUCC
4	hsa-miR-1297	MI0006358	UUCAAGUAAUUCAGGUG
4	hsa-miR-592	MI0003604	UUGUGUCAAUAUGCGAUGAUGU
4	hsa-miR-625	MI0003639	AGGGGGAAAGUUCUAUAGUCC
4	hsa-miR-518e*	MI0003169	CUCUAGAGGGAAGCGCUUUCUG
4	hsa-miR-371-5p	MI0000779	ACUCAAACUGUGGGGGCACU
4	hsa-miR-20b	MI0001519	CAAAGUGCUCAUAGUGCAGGUAG
4	hsa-miR-375	MI0000783	UUUGUUCGUUCGGCUCGCGUGA
4	hsa-miR-620	MI0003634	AUGGAGAUAGAUAUAGAAAU
4	hsa-miR-1276	MI0006416	UAAAGAGCCCUGUGGAGACA
4	hsa-miR-1238	MI0006328	CUUCCUCGUCUGUCUGCCCC
4	hsa-miR-506	MI0003193	UAAGGCACCCUUCUGAGUAGA
4	hsa-miR-877	MI0005561	GUAGAGGAGAUGGCGCAGGG
4	hsa-miR-200c	MI0000650	UAAUACUGCCGGGUAAUGAUGGA
4	hsa-miR-377*	MI0000785	AGAGGUUGCCCUUGGUGAAUUC
4	hsa-miR-520g	MI0003166	ACAAAGUGCUUCCCUUUAGAGUGU
4	hsa-miR-653	MI0003674	GUGUUGAAACAAUCUCUACUG
4	hsa-miR-15b	MI0000438	UAGCAGCACAUCAUGGUUUACA
4	hsa-miR-96*	MI0000098	AAUCAUGUGCAGUGCCAAUAUG
4	hsa-miR-629*	MI0003643	GUUCUCCCAACGUAAGCCCAGC
4	hsa-miR-335*	MI0000816	UUUUUCAUUAUUGCUCCUGACC
4	hsa-miR-636	MI0003651	UGUGCUUGCUCGUCCCGCCCGCA
4	hsa-miR-24	MI0000080	UGGCUCAGUUCAGCAGGAACAG
4	hsa-miR-425	MI0001448	AAUGACACGAUCACUCCCGUUGA
4	hsa-miR-1207-5p	MI0006340	UGGCAGGGAGGCUGGGAGGGG
4	hsa-miR-192	MI0000234	CUGACCUAUGAAUUGACAGCC
4	hsa-miR-410	MI0002465	AAUAUAACACAGAUGGCCUGU
4	hsa-miR-548g	MI0006395	AAAACUGUAAUUACUUUUGUAC
4	hsa-miR-493	MI0003132	UGAAGGUCUACUGUGUGCCAGG
4	hsa-miR-1274a	MI0006410	GUCCCUGUUCAGGCGCCA
4	hsa-miR-637	MI0003652	ACUGGGGGCUUUCGGGCUCUGCGU
4	hsa-miR-555	MI0003561	AGGGUAAGCUGAACCUCUGAU
4	hsa-miR-503	MI0003188	UAGCAGCGGGAACAGUUCUGCAG
4	hsa-miR-125b-1*	MI0000446	ACGGGUUAGGCUCUUGGGAGCU
4	hsa-miR-548n	MI0006399	CAAAAGUAAUUGUGGAUUUUGU
4	hsa-miR-196a	MI0000238	UAGGUAGUUUCAUGUUGUUGGG
4	hsa-miR-923	MI0005715	GUCAGCGGAGGAAAAGAAACU
4	hsa-miR-490-3p	MI0003125	CAACCUGGAGGACUCCAUGCUG
4	hsa-miR-100	MI0000102	AACCCGUAGAUCCGAACUUGUG
4	hsa-miR-1248	MI0006383	ACCUUCUUGUAUAAGCACUGUGCUAAA
4	hsa-let-7c	MI0000064	UGAGGUAGUAGGUUGUAUGGUU
4	hsa-miR-340*	MI0000802	UCCGUCUCAGUUACUUUAUAGC
4	hsa-miR-10a	MI0000266	UACCCUGUAGAUCCGAAUUUGUG
4	hsa-miR-1201	MI0006338	AGCCUGAUUAAAACACUGCAUUGGA
4	hsa-miR-939	MI0005761	UGGGGAGCUGAGGCUCUGGGGGUG
4	hsa-miR-520a-3p	MI0003149	AAAGUGCUUCCCUUUGGACUGU
4	hsa-miR-23a	MI0000079	AUCACAUUGCCAGGGAUUUCC
4	hsa-miR-24-1*	MI0000080	UGCCUACUGAGCUGAUAUCAGU
4	hsa-miR-505*	MI0003190	GGGAGCCAGGAAGUAUUGAUGU
4	hsa-miR-187	MI0000274	UCGUGUCUUGUGUUGCAGCCGG
4	hsa-miR-365	MI0000767	UAAUGCCCCUAAAAAUCCUUAU
4	hsa-miR-615-3p	MI0003628	UCCGAGCCUGGGUCUCCCUCUU
4	hsa-miR-495	MI0003135	AAACAAACAUGGUGCACUUCUU
4	hsa-miR-485-5p	MI0002469	AGAGGCUGGCCGUGAUGAAUUC
4	hsa-miR-32	MI0000090	UAUUGCACAUUACUAAGUUGCA
4	hsa-miR-7-1*	MI0000263	CAACAAAUCACAGUCUGCCAUA
4	hsa-miR-1470	MI0007075	GCCCUCCGCCCGUGCACCCCG
4	hsa-miR-876-3p	MI0005542	UGGUGGUUUACAAAGUAAUUCA
4	hsa-miR-626	MI0003640	AGCUGUCUGAAAAUGUCUU
4	hsa-miR-367	MI0000775	AAUUGCACUUUAGCAAUGGUGA
4	hsa-miR-1977	MI0009987	GAUUAGGGUGGUGUAAGCUGUUAA
4	hsa-miR-24-2*	MI0000081	UGCCUACUGAGCUGAAACACAG
4	hsa-let-7a*	MI0000060	CUAUACAAUCUACUGUCUUUC
4	hsa-miR-148b	MI0000811	UCAGUGCAUCACAGAACUUUGU
4	hsa-miR-665	MI0005563	ACCAGGAGGCUGAGGCCCU
4	hsa-miR-136*	MI0000475	CAUCAUCGUCUCAAAUGAGUCU
5	hsa-miR-512-3p	MI0003140	AAGUGCUGUCAUAGCUGAGGUC
5	hsa-miR-146b-3p	MI0003129	UGCCCUGUGGACUCAGUUCUGG
5	hsa-miR-320b	MI0003776	AAAAGCUGGGUUGAGAGGGCAA
5	hsa-miR-34c-3p	MI0000743	AAUCACUAACCACACGGCCAGG
5	hsa-miR-656	MI0003678	AAUAUUAUACAGUCAACCUCU
5	hsa-miR-431	MI0001721	UGUCUUGCAGGCCGUCAUGCA
5	hsa-miR-611	MI0003624	GCGAGGACCCCUCGGGGUCUGAC
5	hsa-miR-145*	MI0000461	GGAUUCCUGGAAAUACUGUUCU
5	hsa-miR-526b*	MI0003150	GAAAGUGCUUCCUUUUAGAGGC
5	hsa-miR-623	MI0003637	AUCCCUUGCAGGGGCUGUUGGGU
5	hsa-miR-650	MI0003665	AGGAGGCAGCGCUCUCAGGAC
5	hsa-miR-194	MI0000488	UGUAACAGCAACUCCAUGUGGA
5	hsa-miR-448	MI0001637	UUGCAUAUGUAGGAUGUCCCAU
5	hsa-miR-431*	MI0001721	CAGGUCGUCUUGCAGGGCUUCU
5	hsa-miR-29c	MI0000735	UAGCACCAUUUGAAAUCGGUUA
5	hsa-miR-580	MI0003587	UUGAGAAUGAUGAAUCAUUAGG
5	hsa-miR-340	MI0000802	UUAUAAAGCAAUGAGACUGAUU
5	hsa-miR-325	MI0000824	CCUAGUAGGUGUCCAGUAAGUGU
5	hsa-miR-940	MI0005762	AAGGCAGGGCCCCCGCUCCCC
5	hsa-miR-1295	MI0006357	UUAGGCCGCAGAUCUGGGUGA
5	hsa-miR-144	MI0000460	UACAGUAUAGAUGAUGUACU
5	hsa-miR-497	MI0003138	CAGCAGCACACUGUGGUUUGU
5	hsa-miR-886-5p	MI0005527	CGGGUCGGAGUUAGCUCAAGCGG
5	hsa-miR-664*	MI0006442	ACUGGCUAGGGAAAAUGAUUGGAU
5	hsa-miR-497*	MI0003138	CAAACCACACUGUGGUGUUAGA
5	hsa-miR-197	MI0000239	UUCACCACCUUCUCCACCCAGC
5	hsa-miR-224	MI0000301	CAAGUCACUAGUGGUUCCGUU
5	hsa-miR-485-3p	MI0002469	GUCAUACACGGCUCUCCUCUCU
5	hsa-miR-331-3p	MI0000812	GCCCCUGGGCCUAUCCUAGAA
5	hsa-miR-1224-5p	MI0003764	GUGAGGACUCGGGAGGUGG
5	hsa-miR-1263	MI0006398	AUGGUACCCUGGCAUACUGAGU
5	hsa-miR-199a-5p	MI0000242	CCCAGUGUUCAGACUACCUGUUC
5	hsa-miR-105	MI0000111	UCAAAUGCUCAGACUCCUGUGGU
5	hsa-miR-651	MI0003666	UUUAGGAUAAGCUUGACUGAAUG
5	hsa-miR-27a*	MI0000085	AGGGCUUAGCUGCUUGUGAGCA
5	hsa-miR-1303	MI0006370	UUUAGAGACGGGGUCUUGCUCU

5	hsa-miR-367*	MI0000775	ACUGUUGCUAAUAUGCAACUCU	6	hsa-miR-371-3p	MI0000779	AAGUGCCGCCAUCUUUUUGAGUGU
5	hsa-miR-508-5p	MI0003195	UACUCCAGAGGGCGUCACUCAUG	6	hsa-miR-1292	MI0006433	UGGGAACGGGUUCCGGCAGACGCUG
5	hsa-miR-768-5p	MI0005117	GUUGGAGGAUGAAAGUACGGAGUGAU	6	hsa-miR-593*	MI0003005	AGGCACCAGCCAGGCAUUGCUCAGC
5	hsa-miR-125b	MI0000446	UCCCUGAGACCCUAACUUGUGA	6	hsa-miR-1291	MI0006353	UGGCCCUGACUGAAGACCAGCAGU
5	hsa-miR-217	MI0000293	UACUGCAUCAGGAACUGAUUGGA	6	hsa-miR-125b-2*	MI0000470	UCACAAGUCAGGCUCUUGGGAC
5	hsa-miR-509-3-5p	MI0005717	UACUGCAGACGUGGCAAUCAUG	6	hsa-miR-1293	MI0006355	UGGGUGGUCGGGAGAUUUGUGC
5	hsa-miR-147b	MI0005544	GUGUGCGGAAAUGCUUCUGCUA	6	hsa-let-7d*	MI0000065	CUAUACGACCUGCUGCCUUUCU
5	hsa-miR-618	MI0003632	AAACUCUACUUGUCCUUUCUGAGU	6	hsa-miR-492	MI0003131	AGGACCUGCGGGACAAGAUUCUU
5	hsa-miR-423-3p	MI0001445	AGCUCGGUCUGAGGCCCCUCAGU	6	hsa-miR-499-3p	MI0003183	AACAUCACAGCAAGUCUGUGCU
5	hsa-miR-502-3p	MI0003186	AAUGCACCUGGGCAAGGAUUCA	6	hsa-miR-504	MI0003189	AGACCCUGGUCAGACCUCUGGU
5	hsa-miR-659	MI0003683	CUUGGUUCAGGGAGGGUCCCCA	7	hsa-miR-488	MI0003123	UUGAAAGGCUAUUUCUUGGUC
5	hsa-miR-195	MI0000489	UAGCAGCACAGAAAUAUUGGC	7	hsa-miR-191*	MI0000465	GCUGCGCUUGGAUUUCGUCCCC
5	hsa-miR-888	MI0005537	UACUCAAAAAGCUGUCAGUCA	7	hsa-miR-1304	MI0006371	UUUGAGGCUACAGUGAGAUGUG
5	hsa-miR-379	MI0000787	UGGUAGACUAUGGAACGUAGG	7	hsa-miR-1279	MI0006426	UCAUAUUGCUUCUUUCU
5	hsa-miR-513c	MI0006649	UUCUCAAGGAGGUGUCGUUUAU	7	hsa-miR-920	MI0005712	GGGGAGCUGUGGAAGCAGUA
5	hsa-miR-1227	MI0006316	CGUGCCACCCUUUUCCCCAG	7	hsa-miR-550	MI0003600	AGUGCCUGAGGGAGUAAGAGCCC
5	hsa-miR-519b-3p	MI0003151	AAAGUGCAUCCUUUUAGAGGUU	7	hsa-miR-205*	MI0000285	GAUUUCAGUGGAGUGAAGUUC
5	hsa-miR-548i	MI0006421	AAAAGUAAUUGCGGAUUUUGCC	7	hsa-miR-23b	MI0000439	AUCACAUUGCCAGGGAUUACC
5	hsa-miR-610	MI0003623	UGAGCUAAAUGUGUGCUGGGA	7	hsa-miR-202	MI0003130	AGAGGUAUAGGGCAUGGGAA
5	hsa-miR-644	MI0003659	AGUGUGGCUUUCUUAGAGC	7	hsa-miR-187*	MI0000274	GGCUACAACACAGGACCCGGGC
5	hsa-miR-499-5p	MI0003183	UUAAGACUUGCAGUGAUGUUU	7	hsa-miR-132*	MI0000449	ACCGUGGCUUUCGAUUGUUACU
5	hsa-miR-33a*	MI0000091	CAAUGUUUCCACAGUGCAUCAC	7	hsa-miR-135b*	MI0000810	AUGUAGGGCUAAAAGCCAUGGG
5	hsa-miR-589*	MI0003599	UCAGAACAAAUGCCGGUUCCCAGA	7	hsa-miR-342-3p	MI0000805	UCUCACACAGAAAUCGCACCCGU
5	hsa-miR-376b	MI0002466	AUCAUAGAGGAAAAUCCAUGUU	7	hsa-miR-2110	MI0010629	UUGGGGAAACGGCCGCUGAGUG
5	hsa-miR-1283	MI0003832	UCUACAAAGGAAAGCGCUUUCU	7	hsa-let-7a-2*	MI0000061	CUGUACAGCCUCCUAGCUUUCC
5	hsa-miR-1271	MI0003814	CUUGGCACCUAGCAAGCACUCA	7	hsa-miR-657	MI0003681	GGCAGGUUCUCACCCUCUCUAGG
5	hsa-miR-483-3p	MI0002467	UCACUCCUCUCCUCCCGUCUU	7	hsa-miR-124*	MI0000443	CGUGUUCACAGCGGACCUUGAU
5	hsa-miR-518c	MI0003159	CAAAGCGCUUCUCUUUAGAGUGU	7	hsa-miR-524-5p	MI0003160	CUACAAAGGGAAGCACUUUCUC
5	hsa-miR-1289	MI0006350	UGGAGUCCAGGAAUCUGCAUUUU	7	hsa-miR-127-3p	MI0000472	UCGGAUCCGUCUGAGCUUGGCU
5	hsa-miR-1827	MI0008195	UGAGGCAGUAGAUUGAAU	7	hsa-miR-21*	MI0000077	CAACACCAGUCGAUGGGCUGU
5	hsa-miR-22*	MI0000078	AGUUCUUCAGUGGCAAGCUUUA	7	hsa-miR-29b-1*	MI0000105	GCUGGUUUCAUAUGGUGGUUUAGA
5	hsa-miR-944	MI0005769	AAAUUAUUGUACAUCGGAUGAG	7	hsa-miR-99b	MI0000746	CACCCGUAGAACCGACCUUGCG
5	hsa-miR-758	MI0003757	UUUGUGACCUGGUCCACUAACC	7	hsa-miR-1914*	MI0008335	GGAGGGGUCCCGCACUGGGAGG
5	hsa-miR-602	MI0003615	GACACGGGCGACAGCUGCGGCCC	7	hsa-miR-597	MI0003609	UGUGUCACUCGAUGACCACUGU
5	hsa-miR-519d	MI0003162	CAAAGUGCCUCCCUUUUAGAGUG	7	hsa-miR-100*	MI0000102	CAAGCUUGUAUCUAUAGGUAUG
5	hsa-miR-586	MI0003594	UAUGCAUUGUAUUUUUAGGUCC	7	hsa-miR-891b	MI0005534	UGCAACUUACCUGAGUCAUUGA
5	hsa-miR-424	MI0001446	CAGCAGCAAUUCAUGUUUUGAA	7	hsa-miR-609	MI0003622	AGGGUGUUUCUCUCAUCUCU
5	hsa-miR-1184	MI0006277	CCUGCAGCGACUUGAUGGCUUCC	7	hsa-miR-516b	MI0003172	AUCUGGAGGUAAGAAGCACUUU
5	hsa-miR-1251	MI0006386	ACUCUAGCUGCCAAAGGCGCU	7	hsa-miR-542-3p	MI0003686	UGUGACAGAUUGAUAACUGAAA
5	hsa-miR-1285	MI0006346	UCUGGGCAACAAAGUGAGACCU	7	hsa-miR-1911	MI0008332	UGAGUACCGCCAUGUCUGGUUUG
5	hsa-miR-196a*	MI0000279	CGGCAACAAGAAACUGCCUGAG	7	hsa-miR-186	MI0000483	CAAAGAAUUCUCCUUUUGGGCU
5	hsa-miR-770-5p	MI0005118	UCCAGUACCACGGUGUCAGGGCCA	7	hsa-miR-103	MI0000109	AGCAGCAUUGUACAGGGCUAUGA
5	hsa-miR-223	MI0000300	UGUCAGUUUGUCAAAUACCCCA	7	hsa-miR-1182	MI0006275	GAGGGUCUUGGGAGGGAUGUGAC
5	hsa-miR-587	MI0003595	UUUCCAUAGGUGAUGAGUCAC	7	hsa-miR-152	MI0000462	UCAGUGCAUGACAGAACUUGG
6	hsa-miR-520d-5p	MI0003164	CUACAAAGGGAAGCCCUUUC	7	hsa-miR-432	MI0003133	UCUUGGAGUAGGUCAUUGGGUGG
6	hsa-miR-1267	MI0006404	CCUGUUGAAGUGUAAUCCCCA	7	hsa-miR-199a-3p	MI0000242	ACAGUAGUCUGCACAUUGGUUA
6	hsa-miR-1270	MI0006407	CUGGAGAUAUGGAAGAGCUGUGU	7	hsa-miR-491-5p	MI0003126	AGUGGGGAACCCUUCCAUGAGG
6	hsa-miR-513b	MI0006648	UUCACAAGGAGGUGUCAUUUAU	7	hsa-miR-29b-2*	MI0000107	CUGGUUUCACAUGGUGGCUUAG
6	hsa-miR-378	MI0000786	ACUGGACUUGGAGUCAGAAGG	7	hsa-let-7f-2*	MI0000068	CUAUACAGUCUACUGUCUUUCC
6	hsa-miR-452*	MI0001733	CUCAUCUGCAAAGAAGUAAGUG	7	hsa-miR-302c*	MI0000773	UUUAACAUGGGGGUACCUGCUG
6	hsa-miR-324-3p	MI0000813	ACUGCCCCAGGUGCUGCUGG	7	hsa-miR-1245	MI0006380	AAGUGAUCUAAAGGCCUACAU
6	hsa-miR-182	MI0000272	UUUGGCAAUGGUAGAACUCACACU	7	hsa-miR-579	MI0003586	UUCAUUUGGUAUAAACCGCGAUU
6	hsa-miR-1915*	MI0008336	ACCUUGCCUUGCUGCCCGGGCC	7	hsa-miR-765	MI0005116	UGGAGGAGAAGGAAGGUGAUG
6	hsa-miR-101	MI0000103	UACAGUACUGUGAUAACUGAA	7	hsa-miR-1269	MI0006406	CUGGACUGAGCCGUGCUACUGG
6	hsa-miR-96	MI0000098	UUUGGCACUAGCACAUUUUUGCU	7	hsa-miR-182*	MI0000272	UGGUUCUAGACUUGCCAACUA
6	hsa-miR-185	MI0000482	UGGAGAGAAAGGCAGUUCCUGA	7	hsa-miR-1180	MI0006273	UUUCCGGCUCGCGUGGGUGUGU
6	hsa-miR-302e	MI0006417	UAAGUGCUUCCAUGCUU	7	hsa-miR-369-5p	MI0000777	AGAUCGACCGUGUUAUAUUCGC
6	hsa-miR-1914	MI0008335	CCCUGUGCCCGGCCCACUUCUG	7	hsa-miR-654-3p	MI0003676	UAUGUCUGCUGACCAUCACCUU
6	hsa-miR-1178	MI0006271	UUGCUCACUGUUCUUCCCUAG	7	hsa-miR-105*	MI0000111	ACGGAUGUUUGAGCAUGUGCUA
6	hsa-miR-744	MI0005559	UGCGGGGCUAGGGCUAACAGCA	7	hsa-miR-1246	MI0006381	AAUGGAUUUUUGGAGCAGG
6	hsa-miR-1274b	MI0006427	UCCCUGUUCGGGCGCCA	7	hsa-miR-122	MI0000442	UGGAGUGUGACAAUGGUGUUUG
6	hsa-miR-647	MI0003662	GUGGCUGCACUCACUUCCUUC	7	hsa-miR-200b*	MI0000342	CAUCUUACUGGGCAGCAUUGGA
6	hsa-miR-562	MI0003568	AAAGUAGCUGUACCAUUUGC	7	hsa-miR-566	MI0003572	GGGCGCCUGUGAUCCCAAC
6	hsa-miR-551b	MI0003575	GCGACCCAUACUUGGUUUCAG	7	hsa-miR-7-2*	MI0000264	CAACAAAUCCCAGUCUACCUAA
6	hsa-miR-202*	MI0003130	UUCCUAUGCAUAUACUUCUUUG	7	hsa-miR-1229	MI0006319	CUCUCACCACUGCCCUCCCACAG
6	hsa-miR-582-5p	MI0003589	UUACAGUUGUUCAACCAGUUACU	7	hsa-miR-632	MI0003647	GUGUCUGCUUCCUGUGGGA
6	hsa-miR-520h	MI0003175	ACAAAGUGCUUCCCUUUAGAGU	7	hsa-miR-330-5p	MI0000803	UCUCUGGGCCUGUGUCUUAGGC
6	hsa-miR-19b	MI0000074	UGUGCAAAUCCAUGCAAAACUGA	7	hsa-miR-614	MI0003627	GAACGCCUGUUCUUGCCAGGUGG
6	hsa-let-7b	MI0000063	UGAGGUAGUAGGUUGUGUGGUU	7	hsa-miR-518e	MI0003169	AAAGCGCUUCCCUUCAGAGUG
6	hsa-miR-1909*	MI0008330	UGAGUGCCGGUGCCUGCCCUG	7	hsa-miR-432*	MI0003133	CUGGAUGGCUCCUCCAUGUCU
6	hsa-miR-508-3p	MI0003195	UGAUUGUAGCCUUUUGGAGUAGA	7	hsa-miR-513a-5p	MI0003191	UUCACAGGGAGGUGUCAU
6	hsa-miR-578	MI0003585	CUUCUUGUGCUCUAGGAUUGU	7	hsa-miR-574-3p	MI0003581	CACGCUCAUGCACACACCCACA
6	hsa-miR-631	MI0003645	AGACCUGGCCCAGACCUCAGC	7	hsa-miR-548l	MI0006361	AAAAGUAUUUGCGGGUUUUGUC
6	hsa-miR-1200	MI0006332	CUCCUGAGCCAUUCUGAGCCUC	7	hsa-miR-103-as	MI0007261	UCAUAGCCCUGUACAAUGCUGCU
6	hsa-miR-141*	MI0000457	CAUCUUCCAGUACAGUGUUGGA	7	hsa-miR-1259	MI0006393	AUAUAUGAUGACUUAGCUUUU
6	hsa-miR-769-5p	MI0003834	UGAGACCUCUGGGUUCUGAGCU	7	hsa-miR-93	MI0000095	CAAAGUGCUGUUCGUGCAGGUAG
6	hsa-miR-151-3p	MI0000809	CUAGACUGAAGCUCCUUGAGG	7	hsa-miR-1281	MI0006428	UCGCCUCCUCCUCUCCC
6	hsa-miR-31	MI0000089	AGGCAAGAUGCUGGCAUAGCU	7	hsa-miR-596	MI0003608	AAGCCUGCCCGGCUCCUCGGG
6	hsa-miR-654-5p	MI0003676	UGGUGGGCCGCAGAACAUGUGC	7	hsa-miR-101*	MI0000103	CAGUUAUCACAGUGCUGAUGCU
6	hsa-miR-129-3p	MI0000473	AAGCCCUUACCCCAAAAAGCAU	7	hsa-miR-200b	MI0000342	UAAUACUGCCUGGUAAUGAUGA
6	hsa-miR-218-2*	MI0000295	CAUGGUUCUGUCAAGCACCGCG	7	hsa-miR-526b	MI0003150	CUCUUGAGGGAAGCACUUUCUGU
6	hsa-miR-141	MI0000457	UAACACUGUCUGGUAAAGAUGG	7	hsa-miR-29a*	MI0000087	ACUGAUUUCUUUUGGUGUUCAG
6	hsa-miR-297	MI0005775	AUGUAUGUGUGCAUGUGCAUG	7	hsa-miR-1975	MI0009985	CCCCCACAACCGCGCUUGACUAGCU
6	hsa-let-7b*	MI0000063	CUAACACACCUGGAGUUCCC	7	hsa-miR-1208	MI0006341	UCACUGUUCAGACAGGCGGA
6	hsa-miR-1284	MI0006431	UCUAUACAGACCCUGGCUUUUC	7	hsa-miR-373*	MI0000781	ACUCAAAAUGGGGGCGCUUUCC
6	hsa-miR-20a*	MI0000076	ACUGCAUUAUGAGCACUUAAAG	7	hsa-miR-509-3p	MI0003196	UGAUUGGUACGUCUGUGGGUAG
6	hsa-miR-490-5p	MI0003125	CCAUGGAUCUCCAGGUGGGU	7	hsa-miR-140-5p	MI0000456	CAGUGGUUUUACCCUAUGGUAG
6	hsa-miR-484	MI0002468	UCAGGCUCAGUCCCCUCCCGAU	7	hsa-miR-641	MI0003656	AAAGACAUAGGAUAGAGUCACCUC
6	hsa-miR-617	MI0003631	AGACUUCCCAUUUGAAGGUGGC	7	hsa-miR-224*	MI0000301	AAAAUGGUGCCCUAGUGACUACA
6	hsa-miR-494	MI0003134	UGAAACAUACACGGGAAACCUC	7	hsa-miR-1910	MI0008331	CCAGUCCUGUGCCUGCCGCCU
6	hsa-miR-513a-3p	MI0003191	UAAAUUUCACCUUUCUGAGAAGG	8	hsa-miR-377	MI0000785	AUCACACAAAGGCAACUUUUGU
6	hsa-miR-486-3p	MI0002470	CGGGGCAGCUCAGUACAGGAU	8	hsa-miR-1287	MI0006349	UGCUGGAUCAGUGGUUCGAGUC
6	hsa-miR-521	MI0003176	AACGCACUUCCCUUUAGAGUGU	8	hsa-miR-30c-1*	MI0000736	CUGGGAGAGGGUUGUUUACUCC
6	hsa-miR-1277	MI0006419	UACGUAGAUAUAUAUGUAUUUU	8	hsa-miR-422a	MI0001444	ACUGGACUUAGGGUCAGAAGGC
6	hsa-miR-107	MI0000114	AGCAGCAUUGUACAGGGCUAUCA	8	hsa-miR-451	MI0001729	AAACCGUUACCAUUACUGAGUU
6	hsa-miR-215	MI0000291	AUGACCUAUGAAUUGACAGAC	8	hsa-miR-205	MI0000285	UCCUUCAUUCCACCGGAGUCUG
6	hsa-miR-29a	MI0000087	UAGCACCAUCUGAAAUCGGUUA	8	hsa-miR-767-5p	MI0003763	UGCACCAUGGUUGUCUGAGCAUG
6	hsa-miR-92b	MI0003560	UAUUGCACUCGUCCCGGCCUCC	8	hsa-miR-188-3p	MI0000484	CUCCCACAUGCAGGGUUUGCA
6	hsa-miR-649	MI0003664	AAACCUGUGUUGUUCAAGAGUC	8	hsa-miR-643	MI0003658	ACUUGUAUGCUAGCUCAGGUAG
6	hsa-miR-302b*	MI0000772	ACUUUAACAUGGAAGUGCUUUC	8	hsa-miR-346	MI0000826	UGUCUGCCCGCAUGCCUGCCUCU
6	hsa-miR-1226	MI0006313	UCACCAGCCCUGUGUUCCCUAG	8	hsa-miR-1294	MI0006356	UGUGAGGUUGGCAUUGUUGUCU
6	hsa-miR-92a-2*	MI0000094	GGGUGGGGAUUUGUUGCAUUAC	8	hsa-miR-608	MI0003621	AGGGGUGGUGUUGGGACAGCUCCGU
6	hsa-miR-150*	MI0000479	CUGGUACAGGCCUGGGGGACAG	8	hsa-miR-374b*	MI0005566	CUUAGCAGGUUGUAUUAUCAUU
6	hsa-miR-1205	MI0006338	UCUGCAGGGUUUGCUUUGAG	8	hsa-miR-27b*	MI0000440	AGAGCUUAGCUGAUUGGUGAAC
6	hsa-miR-106a	MI0000113	AAAAGUGCUUACAGUGCAGGUAG	8	hsa-miR-634	MI0003649	AACCAGCACCCCAACUUUGGAC
6	hsa-miR-122*	MI0000442	AACGCCAUUAUCACACUAAAUA	8	hsa-miR-1203	MI0006335	CCCGGAGCCAGGAUGCAGCUC
6	hsa-miR-885-3p	MI0005560	AGGCAGCGGGGUGUAGUGGAUA	8	hsa-miR-936	MI0005758	ACAGUAGAGGGAGGAAUCGCAG
6	hsa-miR-143	MI0000459	UGAGAUGAAGCACUGUAGCUC	8	hsa-miR-130a*	MI0000448	UUCACAUUGUGCUACUGUCUGC
6	hsa-miR-532-3p	MI0003205	CCUCCCACACCCAAGGCUUGCA	8	hsa-miR-639	MI0003654	AUCGCUGCGGUUGCGAGCGCUGU
6	hsa-miR-26b*	MI0000084	CCUGUUCUCCAUUACUUGGCUC	8	hsa-miR-937	MI0005759	AUCCGCGCUCUGACUCUCUGCC
6	hsa-miR-30d	MI0000255	UGUAAACAUCCCCGACUGGAAG	8	hsa-miR-1307	MI0006444	ACUCGGCGUGGCGUCGGUCGUG
6	hsa-miR-518b	MI0003156	CAAAGCGCUCCCUUUAGAGGU	8	hsa-miR-892a	MI0005528	CACUGUGUCCUUUCUGCGUAG
6	hsa-miR-493*	MI0003132	UUGUACAUGGUAGGCUUUCAUU	8	hsa-miR-200a	MI0000737	UAACACUGUCUGGUAACGAUGU
6	hsa-miR-1197	MI0006656	UAGGACACAUGGUCUACUUCU	8	hsa-miR-523	MI0003153	GAACGCGCUUCCCUAUAGAGGGU

8	hsa-miR-1265	MI0006401	CAGGAUGUGGUCAAGUGUUGUU
8	hsa-miR-18b*	MI0001518	UGCCCUAAAUGCCCCUUCUGGC
8	hsa-miR-372	MI0000780	AAAGUGCUGCGACAUUUGAGCGU
8	hsa-miR-222*	MI0000299	CUCAGUAGCCAGUGUAGAUCCU
8	hsa-miR-103-2*	MI0000108	AGCUUCUUUACAGUGCUGCCUUG
8	hsa-miR-30d*	MI0000255	CUUUCAGUCAGAUGUUUGCUGC
8	hsa-miR-361-3p	MI0000760	UCCCCCAGGUGUGAUUCUGAUUU
8	hsa-miR-548h	MI0006411	AAAAGUAAUCGCGGUUUUUGUC
8	hsa-miR-138	MI0000476	AGCUGGUGUUGUGAAUCAGGCCG
8	hsa-miR-548a-5p	MI0003612	AAAAGUAAUUGCGAGUUUUACC
8	hsa-miR-591	MI0003603	AGACCAUGGGUUCUCAUUGU
8	hsa-miR-548j	MI0006345	AAAAGUAAUUGCGGUCUUUGGU
8	hsa-miR-548m	MI0006400	CAAAGGUAUUUGUGGGUUUU
8	hsa-miR-220c	MI0005536	ACACAGGGCUGUUGUGAAGACU
8	hsa-miR-548k	MI0006354	AAAAGUACUUGCGGAUUUUGCU
8	hsa-miR-663	MI0003672	AGGCGGGGCGCCGCGGGACCGC
8	hsa-miR-299-5p	MI0000744	UGGUUUACCGUCCCACAUACAU
8	hsa-miR-134	MI0000474	UGUGACUGGUUGACCAGAGGGG
8	hsa-miR-545*	MI0003516	UCAGUAAAUGUUUAUUAGAUGA
8	hsa-miR-200a*	MI0000737	CAUCUUACCGGACAGUGCUGGA
8	hsa-miR-875-3p	MI0005541	CCUGGAAACACUGAGGUUGUG
8	hsa-miR-769-3p	MI0003834	CUGGGAUCUCCGGGGUCUUGGUU
8	hsa-miR-129*	MI0000252	AAGCCCUUACCCCAAAAAGUAU
8	hsa-miR-1257	MI0006391	AGUGAAUGAUGGGUUCUGACC
8	hsa-miR-1244	MI0006379	AAGUAGUUGGUUUGUAGAGGGU
8	hsa-miR-143*	MI0000459	GGUGCAGUGCUGCAUCUCUGGU
8	hsa-miR-196b	MI0001150	UAGGUAGUUUCCUGUUGUUGGG
8	hsa-miR-22	MI0000078	AAGCUGCCAGUUGAAGAACUGU
8	hsa-miR-203	MI0000283	GUGAAAUGUUUAGGACCACUAG
8	hsa-miR-29b	MI0000105	UAGCACCAUUUGAAAUCAGUGU
8	hsa-miR-588	MI0003597	UUGGCCACAAUGGGUUAGAAC
8	hsa-miR-362-5p	MI0000762	AAUCCUUGGAACCUAGGUGUGAGU
8	hsa-miR-628-3p	MI0003642	UCUAGUAAGAGUGGCAGUCGA
8	hsa-miR-517b	MI0003165	UCGUGCAUCCCUUUAGAGUGUU
8	hsa-miR-605	MI0003618	UAAAUCCCAUGGUGCCUUCUCCU
8	hsa-miR-26a-1*	MI0000083	CCUAUUCUUGGUUACUUGCACG
8	hsa-miR-558	MI0003564	UGAGCUGCUGUACCAAAAU
8	hsa-miR-660	MI0003684	UACCCAUUGCAUAUCGGAGUUG
8	hsa-miR-612	MI0003625	GCUGGGCAGGGCUUCUGAGCUCCUU
8	hsa-miR-155	MI0000681	UUAAUGCUAAUCGUGAUAGGGGU
8	hsa-miR-219-1-3p	MI0000296	AGAGUUGAGUCUGGACGUCCCG
8	hsa-miR-1911*	MI0008332	CACCAGGCAUUGUGGUCUCC
8	hsa-miR-191	MI0000465	CAACGGAAUCCCAAAAGCAGCUG
8	hsa-miR-454*	MI0003820	ACCCUAUCAAUAUUGUCUCUGC
8	hsa-miR-520c-3p	MI0003158	AAAGUGCUUCCUUUUAGAGGGU
8	hsa-miR-125a-5p	MI0000469	UCCCUGAGACCCUUUAACCUGUGA
8	hsa-miR-449b*	MI0003673	CAGCCACAACUACCCUGCCACU
8	hsa-miR-501-3p	MI0003185	AAUGCACCCGGGCAAGGAUUCU
8	hsa-miR-767-3p	MI0003763	UCUGCUCAUACCCCAUGGUUUCU
8	hsa-miR-452	MI0001733	AACUGUUUGCAGAGGAAACUGA
8	hsa-miR-208a	MI0000251	AUAAGACGAGCAAAAAGCUUGU
8	hsa-let-7a	MI0000060	UGAGGUAGUAGGUUGUAUAGUU
8	hsa-miR-514	MI0003198	AUUGACACUUCUGUGAGUAGA
8	hsa-miR-221	MI0000298	AGCUACAUUGUCUGCUGGGUUUC
8	hsa-miR-877*	MI0005561	UCCCUCUUCCUCCCUCCCUGA
8	hsa-miR-339-3p	MI0000815	UGAGCGCCUCGACGACAGAGCCG
9	hsa-miR-598	MI0003610	UACGUCAUCGUUGUCAUCGUCA
9	hsa-miR-181b	MI0000270	AACAUUCAUUGCUGUCGGUGGGU
9	hsa-miR-1249	MI0006384	ACGCCCUUCCCCCCCCUUCUUCA
9	hsa-let-7f	MI0000067	UGAGGUAGUAGAUUGUAUAGUU
9	hsa-miR-1185	MI0003844	AGAGGAUACCCUUUGUAUGUU
9	hsa-miR-142-3p	MI0000458	UGUAGUGUUUCCUACUUUAUGGA
9	hsa-miR-425*	MI0001448	AUCGGGAAUGUCGUGUCCGCCC
9	hsa-miR-216a	MI0000292	UAAUCUCAGCUGGCAACUGUGA
9	hsa-miR-576-5p	MI0003583	AUUCUAAUUUCUCCACGGUUUU
9	hsa-miR-214	MI0000290	ACAGCAGGCACAGACAGGCAGU
9	hsa-miR-582-3p	MI0003589	UAACUGGUUGAACAACUGAACC
9	hsa-miR-1207-3p	MI0006340	UCAGCUGGCCCUCAUUUC
9	hsa-miR-329	MI0001725	AACACACCUGGUUAACCUCUUU
9	hsa-miR-30c-2*	MI0000254	CUGGGAGAAGGCUGUUUACUCU
9	hsa-miR-17*	MI0000071	ACUGCAGUGAAGGCACUUGUAG
9	hsa-miR-25	MI0000082	CAUUGCACUUGUCUCGGUCUGA
9	hsa-miR-1282	MI0006429	UCGUUUGCCUUUUUCUGCUU
9	hsa-miR-195*	MI0000489	CCAAUAUUGGCUGUGCUGCUCC
9	hsa-miR-571	MI0003578	UGAGUUGGCCAUCUGAGUGAG
9	hsa-miR-1979	MI0009989	CUCCCACUGCUUCACUUGACUA
9	hsa-let-7g	MI0000433	UGAGGUAGUAGUUUGUACAGUU
9	hsa-miR-624*	MI0003638	UAGUACCAGUACCUUGUGUUCA
9	hsa-miR-7	MI0000263	UGGAAGACUAGUGAUUUUGUUGU
9	hsa-miR-374a	MI0000782	UUAUAAUACAACCUGAUAAGUG
9	hsa-miR-1302	MI0006362	UUGGGACAUACUUAUGCUAAA
9	hsa-miR-627	MI0003641	GUGAGUCUCUAAGAAAAGAGGA
9	hsa-miR-328	MI0000804	CUGGCCCUCUCUGCCCUUCCGU
9	hsa-miR-1322	MI0006653	GAUGAUGCUGCUGAUGCUG
9	hsa-miR-590-3p	MI0003602	UAAUUUUAUGUAUAAGCUAGU
9	hsa-miR-302f	MI0006418	UAAUUGCUUCCAUGUUU
9	hsa-miR-301a	MI0000745	CAGUGCAAUAGUAUUGUCAAAGC
9	hsa-miR-196b*	MI0001150	UCGACAGCACGACACUGCCUUC
9	hsa-miR-31*	MI0000089	UGCUAUGCCAACAUAUUGCCAU
9	hsa-miR-1258	MI0006392	AGUUAGGAUUAGGUCGUGGAA
9	hsa-miR-223*	MI0000300	CGUGUAUUUGACAAGCUGAGUU
9	hsa-miR-301b	MI0005568	CAGUGCAAUGAUAUUGUCAAAGC
9	hsa-miR-106a*	MI0000113	CUGCAAUGUAAGCACUUCUUAC
9	hsa-miR-671-5p	MI0003760	AGGAAGCCCUGGAGGGGCUGGAG
9	hsa-miR-184	MI0000481	UGGACGGAGAACUGAUAAGGGU
9	hsa-miR-34a*	MI0000268	CAAUCAGCAAGUAUACUGCCCU
9	hsa-let-7i*	MI0000434	CUGCGCAAGCUACUGCCUUGCU
9	hsa-miR-28-3p	MI0000086	CACUAGAUUGUGAGCUCCUGGA
9	hsa-miR-577	MI0003544	UAGAUAAAAUAUUGGUACCUG
9	hsa-miR-137	MI0000454	UUAUUGCUUAAGAAUACGCGUAG
9	hsa-miR-519e	MI0003145	AAGUGCCUCCUUUUAGAGUGUU
9	hsa-miR-768-3p	MI0005117	UCACAAUGCUGACACUCAAACUGCGUGAC
9	hsa-miR-801	MI0005202	GAUUGCUUGCGUGCGGAAUCGAC
9	hsa-miR-892b	MI0005538	CACUGGCUCCUUUCUGGGUAGA
9	hsa-miR-18b	MI0001518	UAAGGUGCAUCUAGUGCAGUUAG
9	hsa-miR-27a	MI0000085	UUCACAGUGGCUAAGUUCCGC
9	hsa-let-7c*	MI0000064	UAGAGUUACACCCUGGGAGUUA
9	hsa-miR-646	MI0003661	AAGCAGCUGCCUCUGAGGC
9	hsa-miR-376c	MI0000776	AACAUAGAGGAAAUUCCACGU
9	hsa-miR-219-2-3p	MI0000740	AGAAUUGUGGCUGGACAUCUGU
9	hsa-miR-206	MI0000490	UGGAAUGUAAGGAAGUGUGUGG
9	hsa-miR-1234	MI0006324	UCGGCCUGACCACCCACCCCAC
9	hsa-miR-345	MI0000825	GCUGACUCCUAGUCCAGGGCUC
9	hsa-miR-16-1*	MI0000070	CCAGUAUUAACUGUGCUGCUGA

9	hsa-miR-744*	MI0005559	CUGUUGCCACUAACCUCAACCU
9	hsa-miR-1247	MI0006382	ACCCGUCCCGUUCGUCCCCGGA
9	hsa-miR-1288	MI0006432	UGGACUGCCUGAUCGACAGGAG
9	hsa-miR-126	MI0000471	UCGUACCGUGAGUAAUAAUGCG
9	hsa-miR-30b	MI0000441	UGUAAACAUCCUACACUCAGCU
9	hsa-miR-515-5p	MI0003144	UUCUCCAAAAGAAAGCACUUUCUG
9	hsa-miR-338-3p	MI0000814	UCCAGCAUCAGUGAUUUUGUUG
9	hsa-miR-516a-5p	MI0003180	UUCUCGAGGAAAGAAGCACUUUC
9	hsa-miR-569	MI0003576	AGUUAAUGAAUCCUGGAAAGU
9	hsa-miR-221*	MI0000298	ACCUGGCAUACAAUGUAGAUUU
9	hsa-miR-30a*	MI0000088	CUUUCAGUCGGAUGUUUGCAGC
9	hsa-miR-1261	MI0006396	AUGGAUAAGGCUUUGGCUU
9	hsa-miR-720	MI0006654	UCUCGCUGGGGCCUCCA
9	hsa-miR-642	MI0003657	GUCCCUCUCCAAAUGUGUCUUG
9	hsa-miR-1255a	MI0006389	AGGAUGAGCAAAGAAAGUAGAUU
9	hsa-miR-518d-3p	MI0003171	CAAAGCGCUUCCCUUUGGAGC
9	hsa-miR-601	MI0003614	UGGUCUAGGAUUGUUGGAGGAG
9	hsa-miR-23a*	MI0000079	GGGGUUCCUGGGGAUGGGAUUU
9	hsa-miR-548e	MI0006344	AAAAACUGAGACUACUUUUUGCA
9	hsa-miR-552	MI0003557	AACAGGUGACUGGUUAGACAA
9	hsa-miR-380	MI0000788	UAUGUAAUAUGGUCCACAUCUU
9	hsa-miR-1537	MI0007258	AAAACCGUCUAGUUACAGUUGU
10	hsa-miR-760	MI0005567	CGGCUCUGGGUCUGUGGGGA
10	hsa-miR-550*	MI0003600	UGUCUUACUCCCUCAGGCACAU
10	hsa-miR-1321	MI0006652	CAGGGAGGUGAAUGUGAU
10	hsa-miR-1206	MI0006339	UGUUCAUGUAGAUGUUUAAGC
10	hsa-miR-198	MI0000240	GGUCCAGAGGGGAGAUAGGUUC
10	hsa-miR-943	MI0005768	CUGACUGUUGCCGUCCUCCAG
10	hsa-miR-520a-5p	MI0003149	CUCCAGAGGGAAGUACUUUCU
10	hsa-miR-133a	MI0000450	UUUGGUCCCCUUCAACCAGCUG
10	hsa-miR-424*	MI0001446	CAAAACGUGAGGCGCUGCUAU
10	hsa-miR-1826	MI0008194	AUUGAUCAUCGACACUUCGAACGCAAU
10	hsa-miR-212	MI0000288	UAACAGUCUCCAGUCACGGCC
10	hsa-miR-1225-5p	MI0006311	GUGGGUACGGCCCAGUGGGGGG
10	hsa-miR-616	MI0003629	AGUCAUUGGAGGGUUUGAGCAG
10	hsa-miR-181a	MI0000289	AACAUUCAACGCUGUCGGUGAGU
10	hsa-miR-933	MI0005755	UGUGCGCAGGGAGACCUCUCCC
10	hsa-miR-2052	MI0010486	UGUUUUGAUAACAGUAAUGU
10	hsa-miR-543	MI0005565	AAACAUUCGCGGUGCACUUCUU
10	hsa-miR-23b*	MI0000439	UGGGUUCCUGGCAUGCUGAUUU
10	hsa-miR-524-3p	MI0003160	GAAGGCGCUUCCCUUUGGAGU
10	hsa-miR-572	MI0003579	GUCCGCUCGGCGGUGGCCCA
10	hsa-miR-26a	MI0000083	UUCAAGUAAUCCAGGAUAGGCU
10	hsa-miR-193a-5p	MI0000487	UGGGUCUUUGCGGGCGAGAUGA
10	hsa-miR-1469	MI0007074	CUCGGCGCGGGGCGCGGGCUCC
10	hsa-miR-450b-5p	MI0005531	UUUUGCAAUAUGUUCCUGAAUA
10	hsa-miR-510	MI0003197	UACUCAGGAGAGUGGCAACUAC
10	hsa-miR-26a-2*	MI0000750	CCUAUUCUUGAUUACUUGUUUC
10	hsa-miR-1909	MI0008330	CGCAGGGGCCGGGUGCUCACCG
10	hsa-miR-186*	MI0000483	GCCCAAAGGUGAAUUUUUUGGG
10	hsa-miR-133b	MI0000822	UUUGGUCCCCUUCAACCAGCUA
10	hsa-miR-511	MI0003127	GUGUCUUUUGCUCUGCAGUCA
10	hsa-let-7i	MI0000434	UGAGGUAGUAGUUUGUGCUGUU
10	hsa-miR-181a*	MI0000289	ACCAUCGACCGUUGAUUGUACC
10	hsa-miR-613	MI0003626	AGGAAUGUUCCUUCUUUGCC
10	hsa-miR-148a	MI0000253	UCAGUGCACUACAGAACUUUGU
10	hsa-miR-934	MI0005756	UGUCUACUACUGGAGACACUGG
10	hsa-miR-320d	MI0008190	AAAAGCUGGGUUGAGAGGA
10	hsa-miR-411*	MI0003675	UAUGUAACACGGUCCACUAACC
10	hsa-miR-544	MI0003515	AUUCUGCAUUUUUAGCAAGUUC
10	hsa-miR-942	MI0005767	UCUUCUCUGUUUUGGCCAUGUG
10	hsa-miR-607	MI0003620	GUUCAAAUCCAGAUCUAUAAC
10	hsa-miR-92a	MI0000093	UAUUGCACUUGUCCCGGCCUGU
10	hsa-miR-922	MI0005714	GCAGCAGAGAAUAGGACUACGUC
10	hsa-miR-520f	MI0003146	AAGUGCUUCCUUUUAGAGGGUU
10	hsa-miR-93*	MI0000095	ACUGCUGAGCUAGCACUUCCCG
10	hsa-miR-183*	MI0000273	GUGAAUUACCGAAGGGCCAUAA
10	hsa-miR-515-3p	MI0003144	GAGUGCCUUCUUUUGGGAGCGUU
10	hsa-miR-1974	MI0009984	UGGUUGUAGUCCGUGCGAGAAUA
10	hsa-miR-127-5p	MI0000472	CUGAAGCUCAGAGGGCUCUGAU
10	hsa-miR-204	MI0000284	UUCCCUUUGUCAUCCUAUGCCU
10	hsa-miR-512-5p	MI0003140	CACUCAGCCUUGAGGGCACUUUC
10	hsa-miR-520e	MI0003143	AAAGUGCUUCCUUUUUGAGGG
10	hsa-miR-890	MI0005533	UACUUGGAAAGGCAUCAGUUG
10	hsa-miR-449b	MI0003673	AGGCAGUGUAUUGUUAGCUGGC
10	hsa-miR-1471	MI0007076	GCCCGCGUGUGGAGCCAGGUGU
10	hsa-miR-181c*	MI0000271	AACCAUCGACCGUUGAGUGGAC
10	hsa-miR-192*	MI0000234	CUGCCAAUUCCAUAGGUCACAG
10	hsa-miR-500*	MI0003184	AUGCACCUGGGCAAGGAUUCUG
10	hsa-miR-33b	MI0003646	GUGCAUUGCUGUUGCAUUGC
10	hsa-miR-585	MI0003592	UGGGCGUAUCUGUAUGCUA
10	hsa-miR-34b*	MI0000742	UAGGCAGUGUCAUUAGCUGAUUG
10	hsa-miR-139-5p	MI0000261	UCUACAGUGCACGUGUCUCCAG
10	hsa-miR-564	MI0003570	AGGCACGGUGUCAGCAGGC
10	hsa-miR-873	MI0005564	GCAGGAACUUGUGAGUCUCCU
10	hsa-miR-1264	MI0003758	CAAGUCUUAUUUGAGCACCUGUU
10	hsa-miR-1273	MI0006409	GGGCGACAAAGCAAGACUCUUUCUU
10	hsa-miR-570	MI0003577	CGAAAACAGCAAUUACCUUUGC
10	hsa-miR-874	MI0005532	CUGCCCUGGCCCGAGGGACCGA
10	hsa-miR-454	MI0003820	UAGUGCAAUAUUGCUUAUAGGGU
10	hsa-miR-148b*	MI0000811	AAGUUCUGUUAUACACUCAGGC
10	hsa-miR-517*	MI0003161	CCUCUAGAUGGAAGCACUGUCU
10	hsa-miR-519a	MI0003178	AAAGUGCAUCCUUUUAGAGUGU
10	hsa-miR-1243	MI0006373	AACUGGAUCAAUUAUAGGAGUG
10	hsa-miR-1179	MI0006272	AAGCAUUCUUUCAUUGGUUGG
10	hsa-miR-629	MI0003643	UGGGUUUACGUUGGGAGAACU
10	hsa-miR-1226*	MI0006313	GUGAGGGCAUGCAGGCCUGGAUGGGG
10	hsa-miR-95	MI0000097	UUCAACGGGUAUUUAUUGAGCA
10	hsa-miR-548d-5p	MI0003668	AAAAGUAAUUGUGGUUUUGGCC
10	hsa-miR-573	MI0003580	CUGAAGUGAUGUGUAACUGAUCAG
10	hsa-miR-576-3p	MI0003583	AAGAUGUGGAAAAAUUGGAAUC
10	hsa-miR-125a-3p	MI0000469	ACAGGUGAGGUUCUUGGGAGCC
11	hsa-miR-199b-5p	MI0000282	CCCAGUGUUUAGACUAUCUGUUC
11	hsa-miR-584	MI0003591	UUAUGGUUUGCCUGGGACUGAG
11	hsa-miR-590-5p	MI0003602	GAGCUUAUUCAUAAAAGUGCAG
11	hsa-miR-520d-3p	MI0003164	AAAGUGCUUCUCUUUGGUGGGU
11	hsa-miR-1296	MI0003780	UUAGGGCCCUGGCUCCAUCUCC
11	hsa-miR-16	MI0000070	UAGCAGCACGUAAAUAUUGGCG
11	hsa-miR-28-5p	MI0000086	AAGGAGCUCACAGUCUAUUGAG
11	hsa-miR-487a	MI0002471	AAUCAUACAGGGACAUCCAGUU
11	hsa-miR-378*	MI0000786	CUCCUGACUCCAGGUCCUGUGU
11	hsa-miR-183	MI0000273	UAUGGCACUGGUAGAAUUCACU
11	hsa-miR-219-5p	MI0000296	UGAUUGUCCAAACGCAAUUCU
11	hsa-miR-365*	MI0000769	AGGGACUUUCAGGGGCAGCUGU

11	hsa-miR-628-5p	MI0003642	AUGCUGACAUAUUUACUAGAGG
11	hsa-miR-619	MI0003633	GACCUGGACAUGUUUGUGCCCAGU
11	hsa-miR-146a	MI0000477	UGAGAACUGAAUUCCAUGGGUU
11	hsa-miR-1300	MI0006360	UUGAGAAGGAGGCUGCUG
11	hsa-miR-144*	MI0000460	GGAUAUCAUCAUAUACUGUAAG
11	hsa-miR-483-5p	MI0002467	AAGACGGGAGGAAAGAAGGGAG
11	hsa-miR-1233	MI0006323	UGAGCCCUGUCCUCCCGCAG
11	hsa-miR-1912	MI0008333	UACCCAGAGCAUGCAGUGUGAA
11	hsa-miR-1202	MI0006334	GUGCCAGCUGCAGUGGGGGAG
11	hsa-miR-10a*	MI0000266	CAAAUUCGUAUCUAGGGGAAUA
11	hsa-miR-600	MI0003613	ACUUACAGACAAGAGCCUUGCUC
11	hsa-miR-138-2*	MI0000455	GCUAUUUCACGACACCAGGGUU
11	hsa-miR-603	MI0003616	CACACACUGCAAUUACUUUUGC
11	hsa-miR-567	MI0003573	AGUAUGUUCUUCCAGGACAGAAC
11	hsa-miR-30c	MI0000736	UGUAAACAUCCUACACUCUCAGC
11	hsa-miR-1266	MI0006403	CCUCAGGGCUGUAGAACAGGGCU
11	hsa-miR-218-1*	MI0000294	AUGGUUCCGUCAAGCACCAUGG
11	hsa-miR-335	MI0000816	UCAAGAGCAAUAACGAAAAAUGU
11	hsa-miR-1301	MI0003815	UUGCAGCUGCCUGGGAGUGACUUC
11	hsa-let-7e	MI0000066	UGAGGUAGGAGGUUGUAUAGUU
11	hsa-miR-520b	MI0003155	AAAGUGCUUCCUUUUUAGAGGG
11	hsa-miR-498	MI0003142	UUUCAAGCCAGGGGGCGUUUUUC
11	hsa-miR-27b	MI0000440	UUCACAGUGGCUAAGUUCUGC
11	hsa-miR-211	MI0000287	UUCCCUUUGUCAUCCUUCGCCU
11	hsa-miR-1272	MI0006408	GAUGAUGAAUGCAGCAAAUUCUGAAA
11	hsa-miR-130a	MI0000448	CAGUGCAAUGUUAAAAGGGCAU
11	hsa-miR-885-5p	MI0005560	UCCAUUACACUACCCUGCCUCU
11	hsa-miR-300	MI0005525	UAUACAAGGGCAGACUCUCUCU
11	hsa-miR-330-3p	MI0000803	GCAAAGCACACGGCCUGCAGAGA
11	hsa-miR-19b-1*	MI0000074	AGUUUUGCAGGUUUGCAUCCAGC
11	hsa-miR-2053	MI0010487	GUGUUAAUUAAACCUCUAUUUAC
11	hsa-miR-505	MI0003190	CGUCAACACUUGCUGGUUUCCU
11	hsa-miR-1908	MI0008329	CGGCGGGGACGGCGAUUGGUC
11	hsa-miR-323-5p	MI0000807	AGGUGGUCCGUGGCGCGUUCGC
11	hsa-miR-489	MI0003124	GUGACAUCACAUAUACGGCAGC
11	hsa-let-7f-1*	MI0000067	CUAUACAAUCUAUUGCCUUCCC

11	hsa-miR-324-5p	MI0000813	CGCAUCCCCUAGGGCAUUGGUGU
11	hsa-miR-338-5p	MI0000814	AACAAUAUCCUGGUGCUGAGUG
11	hsa-miR-455-3p	MI0003513	GCAGUCCAUGGGCAUAUACAC
11	hsa-miR-1181	MI0006274	CCGUCGCCGCCACCCGAGCCG
11	hsa-miR-574-5p	MI0003581	UGAGUGUGUGUGUGUGAGUGUGU
11	hsa-miR-766	MI0003836	ACUCCAGCCCCACAGCCUCAGC
11	hsa-miR-938	MI0005760	UGCCCUUAAAGGUGAACCCAGU
11	hsa-miR-147	MI0000262	GUGUGUGGAAAUGCUUCUGC
11	hsa-miR-423-5p	MI0001445	UGAGGGGCAGAGAGCGAGACUUU
11	hsa-miR-1252	MI0006434	AGAAGGAAAUUGAAUCAUUUA
11	hsa-miR-1278	MI0006425	UAGUACGUGCAUAUCAUCUAU
11	hsa-miR-652	MI0003667	AAUGGCGCCACUAGGGUUGUG
11	hsa-miR-675*	MI0005416	CUGUAUGCCCUCACCGCUCA
11	hsa-miR-361-5p	MI0000760	UUAUCAGAAUCUCCAGGGGUAC
11	hsa-miR-135a*	MI0000452	UAUAGGGAUUGGAGCCGUGGCG
11	hsa-miR-150	MI0000479	UCUCCCAACCCUUGUACCAGUG
11	hsa-miR-142-5p	MI0000458	CAUAAAGUAGAAAGCACUACU
11	hsa-miR-889	MI0005540	UUAAUAUCGGACAACCAUUGU
11	hsa-miR-421	MI0003685	AUCAACAGACAUUAAUUGGGCGC
11	hsa-miR-888*	MI0005537	GACUGACACCUCUUUGGGUGAA
11	hsa-miR-10b*	MI0000267	ACAGAUUCGAUUCUAGGGGAAU
11	hsa-miR-302a	MI0000738	UAAGUGCUUCCAUGUUUUGGUGA
11	hsa-miR-25*	MI0000082	AGGCGGAGACUUGGGCAAUUG
11	hsa-miR-139-3p	MI0000261	GGAGACGCGGGCCCUGUUGGAGU
11	hsa-miR-491-3p	MI0003126	CUUAUGCAAGAUUCCCUUCUAC
11	hsa-miR-581	MI0003588	UCUUGUGUUCUCUAGAUCAGU
11	hsa-miR-1183	MI0006276	CACUGUAGGUGAUGGUGAGAGUGGGCA
11	hsa-miR-190b	MI0005545	UGAUAUGUUUGAUAUUGGGUU
11	hsa-miR-449a	MI0001648	UGGCAGUGUAUUGUUAGCUGGU
11	hsa-miR-9*	MI0000466	AUAAAGCUAGAUAACCGAAAGU
11	hsa-miR-129-5p	MI0000252	CUUUUUGCGGUCUGGGCUUGC

6.2.2. Screen result raw data: miRNA samples

miRNA	Plate	Antibody concentrations [µg/mL]								p-value	
		Day 1 #1	Day 1 #2	Day 2 #1	Day 2 #2	Day 3 #1	Day 3 #2	Day 4 #1	Day 4 #2	day 3	day 4
hsa-miR-561	1	5,303	8,498	15,134	20,351	22,903	31,255	27,607	35,338	0,183	0,496
hsa-miR-891a	1	4,989	10,735	15,031	21,289	20,241	31,592	27,902	32,401	0,363	0,714
hsa-miR-606	1	4,787	4,433	10,852	10,898	18,222	20,019	21,258	22,869	0,127	0,117
hsa-miR-220b	1	5,070	9,517	13,215	18,219	20,657	24,320	24,439	25,485	0,755	0,379
hsa-miR-302d*	1	5,048	9,896	15,211	19,900	23,338	28,216	28,440	33,010	0,381	0,614
hsa-miR-299-3p	1	5,109	10,593	15,468	22,064	27,740	35,305	40,063	37,741	0,004	0,015
hsa-miR-29c*	1	4,436	9,003	14,646	21,099	22,005	26,545	23,123	27,036	0,738	0,394
hsa-miR-298	1	5,044	8,100	16,477	18,744	26,842	31,330	37,199	32,429	0,040	0,139
hsa-miR-615-5p	1	4,572	8,997	13,642	21,194	22,304	35,287	28,570	36,140	0,058	0,373
hsa-miR-193b*	1	4,295	10,768	14,459	17,191	26,188	37,505	33,999	43,550	0,003	0,017
hsa-miR-548b-5p	1	0,902	9,987	6,742	17,792	19,094	26,101	23,220	28,831	0,786	0,532
hsa-miR-518d-5p	1	4,586	9,020	11,316	14,542	19,125	23,413	21,960	24,294	0,452	0,188
hsa-miR-621	1	4,835	9,960	11,579	17,961	22,825	25,171	25,788	29,815	0,814	0,843
hsa-miR-15a	1	5,146	9,292	11,105	25,651	22,300	22,990	29,903	27,212	0,798	0,987
hsa-miR-548c-3p	1	5,535	9,309	13,578	19,287	21,556	30,724	26,181	38,789	0,319	0,360
hsa-miR-185*	1	5,766	11,862	18,750	25,894	32,221	39,626	34,088	51,822	0,000	0,001
hsa-miR-589	1	5,610	10,055	17,106	21,240	29,036	34,747	35,873	39,918	0,003	0,028
hsa-miR-374a*	1	5,039	10,533	15,262	21,144	22,296	27,259	28,416	37,201	0,606	0,318
hsa-miR-887	1	5,183	11,478	15,113	18,114	23,385	25,603	29,157	28,777	0,678	0,935
hsa-miR-924	1	5,078	10,105	14,800	17,186	20,921	25,707	25,127	28,840	0,990	0,693
hsa-miR-32*	1	5,633	11,265	14,373	17,848	21,057	22,826	25,534	29,951	0,609	0,832
hsa-miR-488*	1	5,215	11,610	17,178	18,227	26,326	28,060	28,986	35,531	0,165	0,385
hsa-miR-15a*	1	5,254	9,851	14,878	15,533	20,998	23,617	21,479	26,396	0,705	0,261
hsa-miR-1324	1	5,374	9,052	14,617	16,569	21,780	23,429	24,306	32,066	0,787	0,916
hsa-miR-128	1	5,546	7,600	15,692	16,654	16,314	21,832	20,015	29,924	0,125	0,383
hsa-miR-92a-1*	1	4,763	10,438	17,555	23,900	26,279	28,257	27,861	51,104	0,157	0,014
hsa-miR-373	1	4,015	10,459	9,362	15,005	12,631	22,041	15,692	29,751	0,034	0,164
hsa-miR-876-5p	1	4,572	9,752	17,085	18,450	18,481	23,617	24,392	35,579	0,406	0,746
hsa-miR-200c*	1	5,788	9,526	17,089	16,287	18,915	22,196	21,930	28,294	0,312	0,400
hsa-miR-106b*	1	4,990	10,498	15,684	17,588	18,287	27,202	23,371	35,031	0,828	0,891
hsa-miR-193a-3p	1	5,770	7,312	16,824	17,079	23,694	28,027	27,531	36,275	0,364	0,434
hsa-miR-188-5p	1	5,536	11,442	15,802	20,585	21,263	31,855	23,313	44,496	0,255	0,222
hsa-miR-557*	1	5,625	7,152	22,117	20,616	32,372	35,436	37,312	44,737	0,000	0,004
hsa-miR-146a*	1	5,842	8,785	16,871	19,797	23,175	28,636	25,859	34,820	0,357	0,682
hsa-miR-363*	1	4,639	7,720	15,791	16,168	23,062	28,191	26,185	34,239	0,411	0,704
hsa-miR-518f	1	5,396	9,884	16,352	14,136	21,724	25,900	24,158	29,642	0,867	0,678
hsa-miR-193b	1	5,520	9,020	22,049	16,530	28,718	31,280	31,842	29,951	0,018	0,585
hsa-miR-16-2*	1	5,438	9,000	19,167	14,871	23,197	24,122	25,463	26,685	0,910	0,539
hsa-miR-545	1	5,457	8,252	16,572	15,491	22,621	28,438	24,804	24,243	0,432	0,324
hsa-miR-1268	1	5,566	9,508	18,754	23,462	27,060	35,864	26,964	37,338	0,005	0,401
hsa-miR-500	1	5,802	10,258	20,197	20,302	17,148	37,852	29,067	25,054	0,165	0,707
hsa-miR-34a	1	6,051	11,742	17,274	17,480	20,546	26,464	22,906	26,676	0,955	0,357
hsa-miR-2113	1	6,093	9,737	17,198	15,715	20,193	23,798	27,966	26,886	0,623	0,773
hsa-miR-154	1	5,800	11,652	17,329	16,079	21,343	22,118	27,165	27,115	0,556	0,720
hsa-miR-190	1	5,885	9,666	16,574	15,097	18,396	19,935	22,052	25,900	0,131	0,265
hsa-miR-671-3p	1	6,439	10,187	18,492	13,786	22,755	24,225	24,800	25,909	0,959	0,431
hsa-miR-181a-2*	1	5,916	9,303	18,951	19,477	19,595	25,811	24,824	25,123	0,815	0,380
hsa-miR-655	1	5,935	9,079	17,911	17,316	23,158	27,283	25,222	27,991	0,498	0,627
hsa-miR-1225-3p	1	5,885	8,904	19,459	15,774	20,834	32,886	27,873	29,670	0,216	0,972
hsa-miR-19a*	1	6,015	9,590	18,025	15,863	22,857	24,328	25,935	31,067	0,930	0,976
hsa-miR-1299	1	5,899	9,581	21,460	14,206	24,478	21,040	30,154	24,183	0,830	0,726
hsa-miR-146b-5p	1	5,402	9,473	17,152	11,242	21,075	19,754	26,964	28,347	0,287	0,815
hsa-miR-1973	1	5,267	8,826	16,352	12,218	19,107	19,038	23,951	24,823	0,122	0,309
hsa-miR-556-3p	1	5,434	9,922	14,354	11,583	19,317	19,324	21,666	21,160	0,145	0,085
hsa-miR-369-3p	1	5,262	9,318	15,224	10,541	18,185	22,600	22,748	23,389	0,286	0,183
hsa-miR-149	1	5,026	11,092	15,166	17,105	25,808	35,409	33,397	45,493	0,011	0,012
hsa-miR-1305	1	6,061	7,864	16,980	20,986	18,744	27,308	25,273	28,080	0,908	0,639
hsa-miR-486-5p	1	5,603	8,701	11,920	18,280	17,388	18,776	20,841	24,175	0,058	0,143
hsa-miR-450a	1	5,573	8,605	13,370	18,280	18,365	22,243	25,764	24,149	0,271	0,378
hsa-miR-1236	1	5,644	10,670	13,546	14,800	18,035	19,527	24,412	23,582	0,099	0,267
hsa-miR-518f*	1	4,707	6,283	8,431	11,555	14,700	13,852	19,618	19,882	0,001	0,035
hsa-miR-99a*	1	5,614	10,421	13,123	18,731	17,279	23,068	23,348	23,355	0,253	0,206
hsa-miR-935	1	4,798	9,693	12,724	17,411	18,874	20,718	21,876	23,011	0,199	0,139
hsa-miR-668	1	4,365	10,527	16,319	20,634	21,207	23,688	21,830	28,553	0,743	0,411
hsa-miR-595	1	4,511	7,669	12,475	14,339	14,637	20,056	20,648	18,799	0,032	0,034
hsa-miR-30b*	1	5,092	10,708	18,892	21,407	24,650	27,561	28,461	32,280	0,319	0,675
hsa-miR-921	1	4,681	10,350	17,281	17,940	22,741	20,680	27,076	29,588	0,552	0,943
hsa-miR-487b	1	4,834	9,590	15,877	16,317	21,998	20,307	25,899	24,806	0,425	0,431
hsa-miR-135a	1	4,834	8,669	17,513	13,864	19,644	19,633	24,871	27,937	0,179	0,593
hsa-miR-645	1	1,533	8,852	13,792	16,933	17,899	19,693	22,887	22,345	0,101	0,150
hsa-miR-1306	1	4,787	8,739	14,125	15,931	20,605	25,131	26,121	23,861	0,861	0,382
hsa-miR-208b	1	5,087	7,397	15,134	13,839	19,361	20,421	22,252	28,445	0,211	0,432
hsa-miR-559	1	5,467	10,276	16,128	21,416	18,881	31,974	26,169	30,956	0,465	0,988

hsa-miR-9	1	4,818	9,834	14,255	16,920	17,381	19,958	20,038	21,610	0,092	0,063
hsa-miR-33a	1	4,193	7,683	12,946	18,005	16,844	21,755	22,217	23,717	0,145	0,175
hsa-miR-339-5p	1	4,318	6,910	11,468	11,147	14,779	15,927	18,276	17,069	0,005	0,010
hsa-miR-1255b	1	4,572	7,680	10,533	10,140	20,438	18,627	19,498	19,938	0,168	0,034
hsa-miR-802	1	-0,237	7,654	10,842	13,307	19,520	23,100	19,906	19,673	0,460	0,036
hsa-miR-302c	1	4,597	6,726	10,479	12,847	18,022	17,905	18,096	19,100	0,053	0,017
hsa-miR-541	1	4,809	8,339	13,341	20,047	20,671	34,531	24,146	33,585	0,138	0,955
hsa-miR-30a	2	7,847	3,822	17,756	10,105	24,086	17,895	30,344	24,619	0,395	0,783
hsa-miR-382	2	6,268	4,115	14,489	10,946	24,631	19,627	25,643	16,709	0,659	0,077
hsa-miR-640	2	6,307	4,043	16,807	10,190	22,457	15,563	25,048	21,297	0,120	0,192
hsa-miR-663b	2	6,754	3,468	15,948	12,057	21,068	17,534	24,513	19,201	0,144	0,106
hsa-miR-34c-5p	2	6,390	4,274	14,456	11,441	20,669	15,214	19,637	19,362	0,053	0,030
hsa-miR-106b	2	6,568	4,011	13,577	10,346	23,088	18,050	21,095	22,698	0,316	0,108
hsa-miR-551b*	2	6,842	3,962	14,125	14,846	21,380	17,842	23,249	22,141	0,177	0,156
hsa-miR-320c	2	5,956	0,047	14,046	0,051	24,292	16,775	23,947	14,996	0,312	0,031
hsa-miR-1468	2	6,002	3,115	16,468	9,301	23,199	15,804	26,603	21,985	0,168	0,299
hsa-miR-10b	2	6,192	3,468	15,001	10,459	20,979	16,832	23,363	20,576	0,110	0,112
hsa-miR-33b*	2	5,540	3,235	12,976	9,223	19,878	14,287	23,525	17,370	0,026	0,052
hsa-miR-599	2	6,218	3,369	15,507	10,308	24,161	17,968	24,275	19,569	0,410	0,110
hsa-miR-323-3p	2	5,894	2,447	15,032	7,675	20,149	13,064	23,093	14,916	0,017	0,023
hsa-miR-153	2	5,888	3,018	15,114	9,635	23,694	16,931	30,525	18,050	0,275	0,304
hsa-miR-296-5p	2	6,482	3,818	16,339	11,655	22,512	20,114	24,184	20,272	0,460	0,126
hsa-miR-92b*	2	6,238	3,756	17,813	13,045	28,947	21,730	33,626	26,920	0,475	0,693
hsa-let-7e*	2	5,286	4,074	14,041	10,100	18,572	16,210	21,551	20,272	0,033	0,066
hsa-miR-1250	2	6,493	4,923	14,577	14,912	24,518	24,302	27,952	27,453	0,700	0,824
hsa-miR-886-3p	2	6,015	4,800	17,237	14,750	25,530	25,761	30,318	29,856	0,405	0,725
hsa-miR-622	2	6,551	4,523	15,515	14,974	24,074	19,792	26,553	25,782	0,608	0,554
hsa-miR-130b*	2	5,685	4,029	15,979	11,332	21,003	17,026	21,930	20,027	0,119	0,068
hsa-miR-20a	2	5,602	4,465	14,336	10,456	19,726	16,581	24,422	19,719	0,062	0,118
hsa-miR-1323	2	5,864	4,497	16,397	11,880	19,943	18,641	28,419	20,553	0,143	0,322
hsa-miR-409-3p	2	6,369	4,921	14,570	11,270	19,773	17,065	23,123	20,389	0,076	0,101
hsa-miR-875-5p	2	7,011	4,921	17,130	13,353	24,719	23,454	28,484	26,442	0,789	0,779
hsa-miR-519e*	2	6,074	4,171	14,932	13,244	20,048	22,670	25,955	26,300	0,470	0,548
hsa-miR-548c-5p	2	5,501	4,266	14,892	13,725	22,463	18,922	24,863	19,581	0,336	0,126
hsa-miR-630	2	5,772	4,167	16,047	12,858	23,471	17,423	22,488	21,143	0,296	0,104
hsa-miR-1275	2	6,101	5,483	20,163	17,766	32,326	27,336	32,351	31,506	0,021	0,427
hsa-miR-648	2	6,061	3,687	16,233	7,171	24,764	11,625	24,544	16,171	0,072	0,050
hsa-miR-604	2	6,242	4,307	14,846	14,363	24,305	19,080	27,598	25,801	0,549	0,643
hsa-miR-1262	2	5,781	4,590	16,664	13,827	28,002	19,782	20,222	23,667	0,846	0,110
hsa-miR-548p	2	5,675	4,886	16,079	13,364	20,420	21,193	25,568	22,997	0,356	0,297
hsa-miR-337-3p	2	4,653	4,691	16,045	14,699	23,014	24,168	23,034	21,697	0,930	0,134
hsa-miR-379*	2	4,888	3,939	15,541	12,613	21,916	18,695	21,187	19,104	0,271	0,044
hsa-miR-302d	2	4,262	3,832	13,273	9,447	16,767	15,397	16,697	14,445	0,010	0,002
hsa-miR-1280	2	4,440	4,284	13,906	11,421	18,819	16,032	16,592	16,502	0,034	0,004
hsa-miR-220a	2	5,160	4,411	15,249	13,944	21,765	18,255	21,106	21,078	0,228	0,072
hsa-miR-551a	2	5,233	4,703	15,399	14,721	21,506	18,406	20,848	22,009	0,220	0,086
hsa-miR-1976	2	5,090	4,776	16,000	13,634	22,518	21,014	24,032	25,046	0,565	0,326
hsa-miR-302a*	2	6,688	4,970	14,577	14,172	24,061	22,371	27,688	23,815	0,961	0,490
hsa-miR-1913	2	6,405	4,792	14,613	15,650	25,010	22,408	28,230	25,954	0,896	0,712
hsa-miR-376a	2	6,112	4,407	13,531	11,134	22,341	15,679	23,850	18,699	0,120	0,080
hsa-miR-30e*	2	5,686	4,669	13,832	16,351	23,943	18,646	24,574	22,081	0,458	0,204
hsa-miR-337-5p	2	5,310	4,264	12,841	11,208	20,190	17,342	25,444	17,915	0,099	0,098
hsa-miR-548a-3p	2	5,612	4,335	13,013	14,108	25,996	18,863	24,746	20,841	0,740	0,163
hsa-miR-563	2	5,857	4,064	11,887	11,506	22,971	15,420	24,697	18,773	0,138	0,101
hsa-miR-18a*	2	6,442	4,890	13,183	14,718	24,061	18,582	24,342	23,889	0,464	0,279
hsa-miR-1825	2	6,024	3,779	15,042	11,582	18,975	18,206	25,599	21,613	0,086	0,229
hsa-miR-194*	2	5,616	2,682	14,841	9,406	21,368	17,294	22,843	19,465	0,148	0,075
hsa-miR-518a-3p	2	6,086	3,783	15,753	11,767	19,288	15,818	29,276	19,535	0,038	0,315
hsa-miR-130b	2	5,697	4,788	15,522	12,549	20,949	17,721	25,774	20,336	0,148	0,183
hsa-miR-181c	2	5,017	4,117	14,429	12,940	22,414	16,421	24,458	18,292	0,158	0,084
hsa-miR-151-5p	2	6,455	5,007	16,418	13,816	24,167	20,962	28,334	23,402	0,776	0,508
hsa-miR-1915	2	5,921	4,737	16,566	14,234	21,699	18,749	28,399	20,207	0,258	0,302
hsa-miR-126*	2	6,986	4,179	16,108	13,697	22,996	18,036	27,875	23,291	0,306	0,465
hsa-miR-516-3p	2	4,819	4,361	13,027	13,766	20,237	18,932	24,851	23,951	0,173	0,310
hsa-miR-98	2	5,233	4,917	14,140	17,061	21,284	20,819	28,023	26,108	0,404	0,707
hsa-miR-138-1*	2	4,847	3,654	12,880	11,665	17,864	16,084	20,974	17,809	0,022	0,028
hsa-miR-554	2	5,810	4,319	13,005	11,553	19,949	18,695	25,637	22,395	0,146	0,269
hsa-let-7g*	2	5,116	3,917	10,308	11,519	18,578	14,513	19,726	17,966	0,015	0,020
hsa-miR-149*	2	5,737	4,449	13,852	16,537	24,688	22,287	30,411	28,301	0,960	0,861
hsa-miR-181d	2	6,491	4,064	12,622	12,477	21,965	17,765	25,128	19,529	0,209	0,133
hsa-miR-1290	2	5,670	4,439	13,391	14,383	23,174	23,104	28,269	26,777	0,939	0,790
hsa-miR-370	2	5,808	5,042	15,476	14,750	22,329	17,294	28,666	23,846	0,202	0,569
hsa-miR-553	2	5,596	5,251	12,131	15,263	19,773	15,679	22,814	21,166	0,044	0,113
hsa-miR-662	2	5,950	4,794	14,376	13,763	22,177	16,341	25,556	21,631	0,142	0,228
hsa-miR-616*	2	5,917	4,827	14,724	14,806	22,207	15,305	23,856	23,716	0,100	0,245
hsa-miR-542-5p	2	6,376	5,424	16,089	16,216	26,530	20,539	27,681	26,913	0,947	0,749
hsa-miR-296-3p	2	5,821	5,023	16,616	16,558	23,812	19,978	32,145	24,343	0,598	0,927
hsa-miR-411	2	4,942	5,234	12,950	13,620	16,789	13,376	20,437	19,975	0,003	0,045
hsa-miR-30e	2	4,778	5,641	13,462	17,934	20,272	18,055	25,221	28,479	0,131	0,669
hsa-miR-124	2	5,116	5,290	14,958	15,821	21,314	19,348	30,653	30,677	0,274	0,624
hsa-miR-583	2	3,919	3,886	11,536	10,215	14,234	15,846	20,114	18,756	0,003	0,029
hsa-miR-18a	2	5,050	4,313	14,798	13,703	19,120	19,938	25,456	22,747	0,167	0,278
hsa-miR-132	2	5,208	3,857	12,851	12,931	21,488	20,230	27,759	22,826	0,366	0,424
hsa-miR-214*	2	4,958	4,594	13,369	14,775	18,721	19,527	25,177	21,595	0,127	0,209
hsa-miR-455-5p	2	4,134	3,925	11,466	11,912	17,451	15,697	22,648	20,190	0,015	0,085
hsa-miR-708*	2	4,795	5,363	14,301	14,105	18,969	17,615	26,030	23,334	0,069	0,344
hsa-miR-507	2	4,474	4,179	15,504	13,924	19,440	20,296	28,908	23,322	0,208	0,547
hsa-miR-218	3	3,239	2,444	8,394	5,391	14,166	13,674	20,537	16,219	0,001	0,015
hsa-miR-1231	3	3,141	3,286	12,933	7,799	19,863	20,238	24,675	23,219	0,232	0,261
hsa-miR-1286	3	3,040	3,084	12,483	8,439	19,066	16,909	27,171	22,290	0,054	0,350
hsa-miR-517a	3	3,176	2,944	11,573	7,035	14,615	14,441	20,813	16,173	0,002	0,017
hsa-miR-19b-2*	3	3,047	3,593	12,112	8,732	16,886	15,874	22,834	18,927	0,013	0,065
hsa-miR-216b	3	3,199	2,819	11,585	8,121	16,490	15,598	24,006	18,956	0,009	0,089
hsa-miR-708	3	3,280	2,917	14,006	9,018	17,638	18,036	28,502	21,180	0,047	0,365
hsa-miR-556-5p	3	3,640	3,915	14,108	10,275	16,481	16,688	26,183	20,837	0,016	0,221
hsa-miR-1978	3	3,858	4,718	14,397	14,381	26,430	30,058	38,129	37,270	0,078	0,031
hsa-miR-539	3	3,276	4,567	11,220	10,833	18,435	20,459	23,984	20,720	0,158	0,134
hsa-miR-222	3	3,190	3,908	9,966	8,852	15,669	16,893	20,649	19,303	0,012	0,040
hsa-miR-2054	3	2,871	5,631	9,999	10,980	16,272	17,202	20,192	19,924	0,018	0,041
hsa-miR-1254	3	3,107	5,671	11,593	12,100	17,344	19,118	22,681	23,974	0,065	0,204
hsa-miR-320a	3	3,333	3,338	12,519	9,046	14,158	15,149	21,414	19,303	0,002	0,049
hsa-miR-412	3	3,443	4,022	12,645	10,878	14,895	17,680	22,508	21,365	0,012	0,110
hsa-miR-433	3	3,349	3,677	10,720	10,005	15,008	13,911	20,700	17,838	0,002	0,026
hsa-miR-99b*	3	3,557	5,081	11,922	12,990	19,695	21,575	21,844	22,916	0,325	0,135
hsa-miR-525-3p	3	4,084	3,464	13,013	9,509	20,216	15,949	22,245	19,100	0,059	0,058
hsa-miR-548f	3	3,822	3,833	12,676	8,502	20,056	16,077	20,339	20,806	0,058	0,055
hsa-miR-26b	3	3,732	3,632	12,339	9,559	18,485	16,845	21,875	18,138	0,041	0,040
hsa-miR-1539	3	3,921	3,526	15,642	10,150	18,821	17,001	25,923	18,891	0,051	0,138
hsa-miR-374b	3	3,721	3,343	13,281	8,167	16,743	15,275	23,114	16,829	0,009	0,040
hsa-miR-450b-3p	3	4,414	3,657	18,115	11,673	21,132	24,540	36,834	28,514	0,852	0,334
hsa-miR-519c-3p	3	3,713	3,173	11,778	8,126	15,203	15,128	21,927	17,317	0,004	0,033
hsa-miR-1	3	3,647	4,412	14,505	13,036	19,770	23,748	24,653	22,283	0,564	0,216
hsa-miR-210	3	3,250	3,501	10,087	9,361	18,417	16,516	20,030	20,794	0,035	0,050
hsa-miR-633	3	3,464	3,595	13,039	9,733	18,541	17,468	23,567	20,360	0,054	0,111
hsa-miR-381	3	3,512	2,189	12,033	6,331	16,405	13,176	20,111	14,824	0,002	0,009
hsa-miR-17	3	3,151	3,526	10,374	9,002	15,713	15,704	19,118	17,148	0,007	0,013
hsa-let-7d	3	3,273	3,030	13,309	8,296	16,900	15,102	25,192	15,802	0,009	0,054
hsa-miR-1228	3	3,118	4,005	13,992	12,069	19,326	21,032	22,692	21,526	0,251	0,119
hsa-miR-1972	3	3,951	4,457	14,551	12,704	20,604	23,255	30,295	25,513	0,606	0,862
hsa-miR-384	3	3,665	4,294	14,429	11,451	20,462	20,958	22,787	21,959	0,338	0,135
hsa-miR-517c	3	3,700	3,717	12,216	10,709	19,831	17,212	20,293	18,581	0,082	0,029
hsa-miR-1298	3	3,486	3,479	11,700	9,955	16,410	16,651	18,752	18,641	0,015	0,019
hsa-miR-532-5p	3	3,424	3,893	12,214	9,661	16,281	18,200	18,309	19,237	0,029	0,019
hsa-miR-625*	3	3,502	3,798	11,242	11,280	16,672	18,597	20,568	19,622	0,040	0,042

hsa-miR-1204	3	2,786	2,659	8,617	8,242	13,949	15,827	20,976	16,881	0,003	0,022
hsa-miR-302b	3	3,582	3,632	10,776	8,000	13,712	15,662	18,422	16,414	0,002	0,008
hsa-miR-154*	3	3,971	3,908	12,920	9,944	17,642	18,663	25,642	21,002	0,061	0,204
hsa-miR-20b*	3	4,324	4,648	15,003	9,752	18,637	20,748	26,183	23,732	0,186	0,378
hsa-miR-135b	3	3,780	3,662	11,045	8,923	16,708	20,833	24,038	24,358	0,100	0,288
hsa-miR-575	3	3,776	4,057	11,344	8,245	18,362	17,587	20,874	20,263	0,053	0,055
hsa-miR-34b	3	3,758	4,105	12,521	11,000	15,687	18,503	20,946	20,531	0,025	0,060
hsa-miR-342-5p	3	3,956	4,097	13,784	12,409	18,752	27,510	27,503	25,864	0,937	0,640
hsa-miR-661	3	3,728	3,439	13,420	12,144	17,339	21,724	23,162	21,829	0,169	0,142
hsa-miR-568	3	3,900	4,487	13,441	12,426	16,476	23,080	21,943	19,423	0,200	0,058
hsa-miR-593*	3	3,895	4,711	16,705	14,692	21,506	24,486	27,706	24,865	0,898	0,573
hsa-miR-380*	3	3,454	4,733	13,890	12,444	17,928	21,822	22,734	22,709	0,210	0,157
hsa-miR-549	3	3,337	2,966	12,907	6,864	18,174	12,438	20,941	15,455	0,005	0,014
hsa-miR-1538	3	3,291	5,131	12,992	11,257	19,025	21,696	21,693	20,148	0,279	0,066
hsa-miR-148a*	3	2,962	4,763	10,545	11,748	16,012	18,448	19,643	18,641	0,028	0,024
hsa-miR-99a	3	3,033	4,092	9,659	12,996	17,466	17,658	22,141	19,483	0,038	0,063
hsa-miR-1228*	3	3,055	4,587	10,569	13,235	14,986	18,410	23,732	23,491	0,018	0,229
hsa-miR-941	3	3,046	5,179	11,119	13,420	15,770	19,352	23,146	21,885	0,038	0,144
hsa-miR-624	3	3,381	5,147	11,235	13,206	15,744	18,896	24,991	23,396	0,031	0,287
hsa-miR-658	3	3,946	5,000	13,765	13,004	19,201	23,683	24,502	24,769	0,490	0,338
hsa-miR-638	3	3,990	5,962	13,346	12,606	20,533	21,329	24,980	21,928	0,380	0,215
hsa-miR-496	3	3,299	5,078	12,668	10,932	18,293	21,661	22,418	21,705	0,223	0,116
hsa-miR-664	3	3,582	4,575	13,880	12,374	16,078	21,937	24,610	17,591	0,119	0,074
hsa-miR-15b*	3	3,182	4,339	10,976	10,194	16,003	21,152	20,060	20,008	0,087	0,041
hsa-miR-363	3	3,426	4,472	11,655	11,348	18,844	20,589	20,156	21,076	0,189	0,056
hsa-miR-541*	3	3,563	4,892	12,966	13,083	21,612	26,363	24,670	24,447	0,817	0,328
hsa-miR-525-5p	3	3,521	4,369	11,244	10,577	16,837	16,780	18,801	18,575	0,019	0,018
hsa-miR-136	3	3,840	4,140	12,140	11,334	20,004	22,428	28,502	23,504	0,439	0,529
hsa-miR-383	3	3,781	4,633	12,894	10,972	20,542	20,708	23,888	19,160	0,323	0,090
hsa-miR-518a-5p	3	3,440	4,804	9,923	10,432	15,840	16,893	19,678	17,885	0,013	0,020
hsa-miR-518c*	3	3,981	5,200	12,995	12,100	23,476	25,423	26,661	26,348	0,690	0,610
hsa-miR-19a	3	3,732	4,799	10,261	11,765	16,766	19,218	22,487	18,938	0,054	0,059
hsa-miR-331-5p	3	3,695	4,872	9,430	9,891	15,008	17,549	19,959	17,457	0,012	0,019
hsa-miR-376a*	3	3,665	5,669	10,432	13,276	16,900	20,787	20,879	22,121	0,105	0,089
hsa-miR-429	3	3,318	5,101	8,930	12,400	16,667	17,811	21,662	20,397	0,028	0,070
hsa-miR-1224-3p	3	4,038	5,293	14,014	12,955	18,005	21,839	23,019	22,065	0,216	0,145
hsa-miR-155*	3	3,541	4,409	13,389	13,429	19,219	24,131	24,621	23,352	0,545	0,265
hsa-miR-509-5p	3	3,499	6,085	12,777	13,124	20,609	23,214	24,931	24,782	0,602	0,365
hsa-miR-522	3	3,536	5,682	12,354	12,896	18,151	17,844	18,702	20,208	0,054	0,029
hsa-miR-362-3p	3	3,949	5,702	13,688	13,832	20,927	25,172	24,811	22,935	0,913	0,254
hsa-miR-548b-3p	3	3,801	4,497	12,357	13,561	22,124	22,980	24,016	20,788	0,772	0,137
hsa-miR-409-5p	3	3,977	3,640	10,819	10,662	16,641	20,572	17,947	19,130	0,088	0,017
hsa-miR-548d-3p	3	3,822	3,759	11,613	11,719	19,746	21,655	23,684	23,720	0,337	0,237
hsa-miR-21	4	3,167	7,246	10,573	20,712	21,686	35,154	26,220	40,612	0,077	0,259
hsa-miR-1237	4	3,354	7,378	12,168	20,011	18,779	35,863	26,653	39,995	0,173	0,267
hsa-miR-375	4	2,978	7,088	12,790	22,585	21,377	32,195	26,245	37,618	0,224	0,432
hsa-miR-15b	4	2,496	6,432	9,029	15,789	16,458	31,976	20,050	30,609	0,763	0,432
hsa-miR-548g	4	2,819	6,833	9,632	17,994	20,229	34,807	21,489	32,485	0,148	0,696
hsa-miR-490-3p	4	3,248	8,615	11,283	20,478	22,587	33,914	22,068	38,866	0,085	0,665
hsa-miR-24-1*	4	3,150	6,718	9,980	21,547	19,099	33,463	21,407	35,346	0,306	0,953
hsa-miR-876-3p	4	3,405	9,176	12,880	22,552	19,656	34,132	21,950	39,274	0,217	0,641
hsa-miR-501-5p	4	3,197	6,398	11,215	20,663	24,627	37,828	27,050	37,967	0,007	0,356
hsa-miR-1253	4	2,965	6,343	8,579	17,434	19,495	29,570	19,730	31,607	0,673	0,482
hsa-miR-620	4	3,337	8,369	11,507	21,295	23,047	28,589	25,325	33,937	0,373	0,810
hsa-miR-96*	4	3,112	7,063	10,260	17,115	20,186	25,504	21,337	31,849	0,855	0,628
hsa-miR-493	4	2,873	6,862	7,311	15,942	18,524	35,263	20,337	26,851	0,223	0,229
hsa-miR-100	4	3,426	7,663	11,209	19,169	20,359	34,480	23,304	30,637	0,156	0,691
hsa-miR-505*	4	2,998	8,373	10,798	26,628	22,119	44,293	24,814	43,548	0,001	0,196
hsa-miR-626	4	3,932	8,632	13,010	27,177	22,856	36,829	25,212	45,205	0,025	0,128
hsa-miR-140-3p	4	4,054	8,505	12,091	25,871	22,196	39,175	26,781	41,926	0,013	0,178
hsa-miR-326	4	3,568	9,804	11,638	21,355	25,423	39,653	29,628	36,853	0,002	0,270
hsa-miR-1276	4	1,868	6,952	6,254	11,621	11,710	22,600	13,700	20,652	0,030	0,007
hsa-miR-629*	4	3,350	7,544	10,489	20,592	21,333	37,189	23,316	36,438	0,043	0,767
hsa-miR-1274a	4	2,373	8,220	8,393	17,503	15,906	27,080	17,642	27,183	0,510	0,140
hsa-miR-1248	4	2,109	6,351	8,664	17,190	15,348	29,106	18,614	30,646	0,693	0,343
hsa-miR-187	4	2,235	7,718	8,421	22,993	14,545	32,990	19,571	30,423	0,887	0,388
hsa-miR-367	4	3,324	7,054	11,296	20,402	17,997	35,024	22,653	36,947	0,277	0,781
hsa-miR-548o	4	4,372	7,659	14,141	22,618	27,262	34,306	28,112	45,483	0,009	0,057
hsa-miR-635	4	3,779	7,263	13,377	24,325	22,924	33,092	25,413	29,455	0,099	0,774
hsa-miR-1238	4	3,057	7,863	10,646	18,208	20,370	27,317	27,269	31,663	0,859	0,840
hsa-miR-335*	4	3,371	6,862	10,441	17,813	19,452	28,116	22,569	33,487	0,876	0,886
hsa-miR-637	4	3,568	8,543	15,292	22,651	30,040	40,622	31,996	43,185	0,000	0,035
hsa-let-7c	4	2,735	8,734	10,586	18,160	21,421	34,937	22,342	32,719	0,092	0,794
hsa-miR-365	4	3,116	8,704	9,992	22,706	19,323	34,683	19,571	32,438	0,205	0,534
hsa-miR-1977	4	3,175	7,101	10,183	20,169	19,056	34,524	21,807	33,702	0,233	0,836
hsa-miR-675	4	3,036	7,854	17,222	23,997	22,924	38,810	29,895	49,259	0,010	0,012
hsa-miR-1297	4	2,852	7,846	12,238	17,871	16,059	31,537	22,116	35,731	0,876	0,944
hsa-miR-506	4	3,057	6,905	10,616	15,731	13,379	30,496	22,199	31,793	0,627	0,697
hsa-miR-636	4	2,865	5,920	8,715	12,876	15,156	24,631	19,220	29,994	0,219	0,339
hsa-miR-555	4	3,384	6,453	14,329	21,197	22,074	35,198	24,504	37,674	0,065	0,559
hsa-miR-340*	4	3,367	8,479	13,363	21,695	25,180	42,063	26,742	39,482	0,001	0,288
hsa-miR-615-3p	4	3,350	8,424	11,632	19,622	18,939	32,757	17,675	36,146	0,382	0,688
hsa-miR-453	4	2,702	6,799	8,242	16,607	17,390	29,997	20,579	38,507	0,904	0,830
hsa-miR-1260	4	3,637	6,534	11,382	15,437	20,067	26,879	22,665	32,176	0,965	0,773
hsa-miR-592	4	3,260	7,114	10,804	20,196	21,124	29,894	24,220	27,036	0,440	0,471
hsa-miR-877	4	3,337	6,445	13,416	17,743	24,788	26,709	23,425	24,784	0,384	0,278
hsa-miR-24	4	3,175	6,364	12,686	16,126	22,018	32,049	25,362	31,066	0,191	0,921
hsa-miR-503	4	3,448	6,406	10,670	14,909	22,924	30,063	22,497	27,369	0,259	0,376
hsa-miR-10a	4	3,962	6,505	11,996	14,736	19,613	28,789	20,753	25,217	0,761	0,177
hsa-miR-495	4	3,521	5,839	13,324	18,706	19,667	27,761	21,337	28,629	0,896	0,383
hsa-miR-24-2*	4	3,602	6,671	13,108	20,745	20,348	32,392	20,475	37,108	0,287	0,969
hsa-miR-145	4	3,508	5,485	11,757	17,935	19,302	26,040	22,966	36,042	0,807	0,835
hsa-miR-625	4	4,002	7,871	14,221	24,008	25,841	34,277	26,666	32,522	0,018	0,816
hsa-miR-200c	4	3,836	7,467	11,320	15,317	19,077	25,451	20,986	26,204	0,695	0,229
hsa-miR-425	4	4,536	7,854	14,491	19,228	23,013	32,049	26,487	36,230	0,137	0,514
hsa-miR-125b-1*	4	3,953	7,391	12,738	16,845	20,555	31,618	22,557	30,385	0,332	0,606
hsa-miR-1201	4	3,719	7,033	11,092	17,786	18,524	31,237	19,175	39,454	0,589	0,873
hsa-miR-485-5p	4	3,940	7,229	11,763	22,541	20,023	38,560	24,503	41,335	0,045	0,311
hsa-let-7a*	4	4,045	8,254	10,622	20,930	15,408	26,805	21,360	36,023	0,427	0,988
hsa-miR-1256	4	5,556	5,639	16,342	20,625	25,539	29,423	29,815	43,223	0,137	0,064
hsa-miR-518e*	4	2,500	3,659	5,021	7,434	9,510	13,721	10,090	17,968	0,000	0,001
hsa-miR-377*	4	3,953	5,370	18,040	18,492	23,907	34,060	29,084	41,192	0,047	0,124
hsa-miR-1207-5p	4	4,487	7,680	15,195	17,248	20,262	27,539	22,151	38,847	0,843	0,660
hsa-miR-548n	4	4,581	6,509	13,154	16,221	21,919	29,157	24,220	25,780	0,432	0,383
hsa-miR-939	4	4,761	6,773	14,060	15,926	23,352	31,024	25,878	31,934	0,170	0,947
hsa-miR-32	4	3,784	6,739	11,507	17,647	18,197	34,234	21,419	36,768	0,322	0,912
hsa-miR-148b	4	4,465	7,318	13,966	19,881	20,099	36,224	23,268	35,223	0,098	0,883
hsa-miR-502-5p	4	4,496	6,206	14,783	15,652	22,991	31,947	25,765	36,523	0,143	0,549
hsa-miR-371-5p	4	4,146	7,778	16,101	16,644	23,725	34,386	22,414	39,445	0,045	0,589
hsa-miR-520g	4	4,146	6,658	11,345	13,557	20,883	26,248	21,713	34,303	0,938	0,883
hsa-miR-192	4	3,810	6,965	12,416	12,470	19,452	19,650	19,265	32,579	0,169	0,521
hsa-miR-196a	4	3,853	6,960	10,847	14,283	19,120	25,623	19,890	33,937	0,724	0,685
hsa-miR-520a-3p	4	3,546	5,382	8,823	11,672	14,744	22,833	16,198	29,446	0,104	0,171
hsa-miR-7-1*	4	3,628	6,505	11,061	17,333	21,003	30,768	21,114	42,441	0,366	0,465
hsa-miR-665	4	4,274	8,687	12,751	22,200	20,359	36,599	24,479	45,800	0,078	0,133
hsa-miR-1308	4	4,323	6,466	15,188	20,679	23,998	27,117	22,521	39,075	0,424	0,610
hsa-miR-20b	4	3,624	5,976	12,854	14,278	21,289	25,332	20,974	33,234	0,989	0,717
hsa-miR-653	4	3,637	7,318	12,155	13,082	19,678	27,384	19,378	34,209	0,948	0,665
hsa-miR-410	4	4,116	7,829	12,123	15,773	22,152	29,290	22,593	30,916	0,394	0,654
hsa-miR-923	4	3,654	7,625	13,350	16,385	18,971	28,560	19,639	35,317	0,882	0,786
hsa-miR-23a	4	4,279	6,939	14,268	16,829	22,498	27,880	21,725	39,549	0,507	0,638
hsa-miR-1470	4	4,067	7,041	14,647	18,765	20,632	31,756	22,139	45,138	0,314	0,249
hsa-miR-136*	4	4,248	7,939	14,777	21,334	20,175	29,297	22,761	44,008	0,620	0,271
hsa-miR-512-3p	5	7,426	6,586	18,334	21,568	25,561	24,696	28,258	31,106	0,518	0,800

hsa-miR-650	5	6,085	8,196	16,288	24,108	26,188	26,464	30,133	42,804	0,281	0,065
hsa-miR-144	5	6,001	6,919	14,782	19,702	21,786	21,558	28,564	28,945	0,542	0,976
hsa-miR-1263	5	6,509	7,021	17,129	23,220	26,829	27,458	33,365	45,375	0,170	0,012
hsa-miR-217	5	5,966	6,357	14,679	18,774	24,530	23,592	30,474	31,495	0,796	0,571
hsa-miR-513c	5	6,234	7,026	15,922	20,673	25,881	22,410	34,281	33,716	0,773	0,198
hsa-miR-1283	5	5,567	6,749	15,061	20,876	22,042	22,694	31,889	34,108	0,721	0,294
hsa-miR-519d	5	4,943	6,831	15,205	19,937	23,667	24,240	33,692	35,369	0,826	0,158
hsa-miR-146b-3p	5	5,270	6,772	14,697	20,421	21,664	26,838	27,984	29,076	0,744	0,981
hsa-miR-194	5	6,518	6,690	12,663	15,405	18,833	20,417	20,775	25,970	0,178	0,209
hsa-miR-497	5	6,803	6,628	12,988	15,476	20,158	19,641	27,602	27,450	0,212	0,791
hsa-miR-199a-5p	5	6,191	7,599	12,967	15,243	21,536	19,622	22,837	24,013	0,315	0,212
hsa-miR-509-3-5p	5	6,269	6,617	18,219	17,687	28,527	21,236	28,028	32,285	0,582	0,713
hsa-miR-1227	5	6,100	6,438	15,460	16,469	23,790	20,751	26,671	22,503	0,695	0,333
hsa-miR-1271	5	6,039	6,693	20,290	26,164	35,620	32,016	39,888	48,017	0,000	0,000
hsa-miR-586	5	5,896	7,392	19,066	21,563	31,691	26,004	40,938	38,141	0,049	0,010
hsa-miR-320b	5	7,411	9,210	15,292	25,978	27,049	31,876	33,280	34,716	0,029	0,198
hsa-miR-448	5	4,359	6,809	8,921	18,947	19,379	24,468	18,924	28,301	0,606	0,232
hsa-miR-886-5p	5	5,884	7,845	15,323	21,905	25,054	25,187	24,569	29,393	0,520	0,692
hsa-miR-105	5	6,964	7,781	16,693	23,914	25,550	24,642	26,905	28,469	0,526	0,821
hsa-miR-147b	5	6,867	6,542	15,498	17,366	25,343	23,193	26,209	25,395	0,739	0,497
hsa-miR-519b-3p	5	5,230	5,639	14,824	19,526	21,264	17,389	21,538	22,341	0,147	0,110
hsa-miR-483-3p	5	6,085	6,088	17,271	19,706	24,118	21,505	30,777	26,781	0,845	0,971
hsa-miR-424	5	6,015	7,246	15,295	23,992	27,519	25,997	35,345	35,494	0,217	0,104
hsa-miR-34c-3p	5	5,131	7,985	15,739	24,245	23,983	27,028	29,786	44,699	0,435	0,044
hsa-miR-431*	5	6,167	8,328	14,573	20,765	20,464	22,794	27,285	36,413	0,532	0,442
hsa-miR-664*	5	5,526	6,105	13,251	17,418	22,929	20,626	27,361	27,677	0,568	0,790
hsa-miR-651	5	5,727	5,598	12,991	17,262	22,544	21,822	27,164	28,883	0,671	0,885
hsa-miR-618	5	5,349	6,913	15,447	17,587	24,312	18,695	26,336	24,548	0,505	0,444
hsa-miR-548i	5	5,253	5,565	15,047	15,115	26,995	19,401	25,573	22,728	0,956	0,283
hsa-miR-518c	5	5,689	6,941	17,934	20,006	26,135	21,855	33,391	32,269	0,815	0,313
hsa-miR-1184	5	5,096	8,399	17,029	23,421	26,372	24,187	30,616	35,535	0,484	0,287
hsa-miR-656	5	7,014	8,216	17,479	25,207	28,892	29,739	29,184	34,584	0,032	0,435
hsa-miR-29c	5	4,755	7,263	14,326	19,400	24,136	23,625	24,075	28,523	0,847	0,576
hsa-miR-497*	5	5,308	6,606	15,195	20,761	29,024	23,799	29,260	26,482	0,270	0,856
hsa-miR-27a*	5	5,791	8,263	16,963	23,658	24,054	24,535	29,549	31,193	0,731	0,675
hsa-miR-423-3p	5	6,656	7,550	15,626	18,934	27,936	21,861	34,733	28,072	0,576	0,506
hsa-miR-610	5	5,549	8,111	14,944	19,801	24,324	20,221	31,339	28,492	0,696	0,757
hsa-miR-1289	5	5,808	6,876	16,212	19,041	23,854	21,170	35,213	28,760	0,761	0,421
hsa-miR-1251	5	7,258	8,160	19,782	25,029	31,831	32,789	39,579	35,827	0,002	0,031
hsa-miR-431	5	6,606	8,343	17,195	23,494	26,319	28,478	29,722	39,086	0,144	0,170
hsa-miR-580	5	6,530	8,055	17,046	19,386	23,860	25,146	27,279	31,233	0,675	0,880
hsa-miR-197	5	5,730	7,967	12,766	14,740	18,414	20,724	18,948	22,046	0,172	0,053
hsa-miR-1303	5	5,899	8,754	16,548	20,540	27,709	21,874	28,436	32,985	0,603	0,617
hsa-miR-502-3p	5	6,150	8,237	15,226	17,918	23,110	21,223	26,993	30,406	0,667	0,986
hsa-miR-644	5	6,045	7,544	18,787	19,395	28,641	26,920	38,728	49,726	0,110	0,000
hsa-miR-1827	5	5,639	9,198	15,619	17,051	21,973	23,585	27,691	28,907	0,836	0,937
hsa-miR-1285	5	5,966	9,412	22,005	24,582	34,002	35,769	38,642	39,779	0,000	0,012
hsa-miR-611	5	7,467	8,889	17,882	25,054	30,249	32,986	32,987	37,205	0,003	0,122
hsa-miR-340	5	5,645	7,553	15,974	20,609	27,441	24,394	22,669	29,192	0,352	0,518
hsa-miR-224	5	5,407	7,608	14,669	14,190	21,043	17,337	20,530	22,130	0,134	0,082
hsa-miR-367*	5	5,937	9,126	18,108	21,362	27,578	22,887	25,447	26,961	0,496	0,560
hsa-miR-659	5	5,642	8,319	13,394	19,634	24,671	23,286	30,082	28,186	0,819	0,903
hsa-miR-499-5p	5	5,604	8,796	15,915	15,814	22,748	20,836	28,066	20,349	0,572	0,291
hsa-miR-22*	5	6,170	7,078	16,506	16,956	23,983	27,608	34,642	32,799	0,376	0,222
hsa-miR-196a*	5	7,585	8,901	17,993	18,685	24,942	23,685	31,831	30,791	0,726	0,519
hsa-miR-145*	5	6,832	6,992	14,288	21,362	25,331	23,725	29,132	30,398	0,669	0,784
hsa-miR-325	5	5,730	8,650	13,240	21,130	24,371	22,754	21,384	29,649	0,938	0,457
hsa-miR-485-3p	5	6,539	7,458	11,938	18,023	25,260	19,680	24,757	26,140	0,751	0,445
hsa-miR-508-5p	5	6,033	6,296	12,892	16,832	29,281	21,045	26,702	25,285	0,516	0,526
hsa-miR-195	5	6,039	8,343	14,123	19,670	27,364	21,137	26,317	27,814	0,745	0,707
hsa-miR-33a*	5	6,199	7,126	14,899	15,945	26,912	18,624	25,108	22,813	0,835	0,263
hsa-miR-944	5	5,366	8,653	14,769	16,691	22,614	20,567	25,498	23,496	0,523	0,321
hsa-miR-770-5p	5	5,538	6,598	17,219	18,765	26,888	23,099	32,850	30,327	0,551	0,477
hsa-miR-526b*	5	5,296	7,120	13,620	19,225	20,603	21,374	21,927	27,783	0,392	0,366
hsa-miR-940	5	6,264	7,691	14,175	20,462	23,514	23,953	24,450	29,408	0,889	0,683
hsa-miR-331-3p	5	4,235	7,712	10,953	16,092	19,052	19,576	20,960	24,570	0,145	0,161
hsa-miR-768-5p	5	5,166	7,343	13,627	19,332	23,754	22,099	29,536	32,985	0,878	0,527
hsa-miR-888	5	5,744	7,585	13,579	16,427	24,700	21,203	27,145	23,496	0,885	0,427
hsa-miR-589*	5	5,404	8,529	16,586	22,074	34,675	26,988	37,210	36,128	0,008	0,055
hsa-miR-758	5	5,599	6,595	12,326	14,597	21,269	18,540	24,188	21,434	0,213	0,164
hsa-miR-223	5	6,483	3,965	18,547	12,381	28,802	18,637	30,680	23,804	0,895	0,740
hsa-miR-623	5	6,039	8,163	16,527	27,644	31,510	26,078	30,880	40,599	0,052	0,092
hsa-miR-1295	5	4,919	8,922	12,493	20,600	28,157	26,261	32,408	39,120	0,163	0,089
hsa-miR-1224-5p	5	5,878	7,749	14,958	18,596	27,405	28,918	32,454	31,741	0,083	0,404
hsa-miR-125b	5	5,575	7,761	13,897	17,249	19,973	22,205	23,521	23,489	0,412	0,219
hsa-miR-379	5	5,869	8,032	16,171	16,584	28,730	21,598	28,532	27,799	0,514	0,911
hsa-miR-376b	5	5,543	7,738	16,357	15,865	24,353	21,078	23,241	25,608	0,818	0,313
hsa-miR-602	5	4,668	8,096	18,909	18,199	24,606	23,006	31,636	36,245	0,868	0,204
hsa-miR-587	5	6,448	7,657	18,892	17,171	27,721	25,706	32,889	33,382	0,224	0,279
hsa-miR-520d-5p	5	3,654	5,417	9,839	14,326	16,668	21,142	23,600	33,805	0,110	0,986
hsa-miR-96	6	3,081	4,794	11,114	20,793	20,204	29,267	29,997	40,016	0,620	0,130
hsa-miR-202*	6	4,022	5,519	10,576	18,791	17,568	24,801	22,595	29,340	0,436	0,524
hsa-miR-141*	6	3,308	5,042	10,166	19,241	19,130	24,691	22,118	30,297	0,603	0,563
hsa-miR-1284	6	3,853	3,717	9,926	17,411	17,818	25,211	23,711	30,368	0,510	0,703
hsa-miR-107	6	3,353	4,512	9,768	16,769	20,628	26,786	24,104	31,947	0,897	0,885
hsa-miR-106a	6	2,957	4,027	7,818	16,146	18,843	24,396	20,715	28,354	0,532	0,328
hsa-miR-371-3p	6	2,740	3,630	9,855	15,691	20,366	22,314	27,031	29,908	0,466	0,970
hsa-miR-1267	6	3,436	4,979	9,246	17,889	19,005	24,193	24,116	33,835	0,527	0,934
hsa-miR-185	6	3,406	4,538	8,646	16,160	17,656	24,974	25,768	36,352	0,465	0,562
hsa-miR-582-5p	6	3,414	4,327	7,193	13,931	14,153	21,033	18,738	31,288	0,040	0,391
hsa-miR-769-5p	6	3,926	5,370	10,122	19,279	18,918	33,276	27,428	41,010	0,338	0,187
hsa-miR-20a*	6	4,072	5,718	9,615	16,412	16,355	24,432	23,735	31,711	0,290	0,829
hsa-miR-215	6	3,501	5,309	8,738	15,627	17,793	24,404	16,881	29,673	0,418	0,206
hsa-miR-122*	6	3,448	5,594	12,347	19,331	20,266	29,671	25,830	33,455	0,563	0,808
hsa-miR-1292	6	3,251	5,361	13,339	20,602	22,163	28,898	25,743	33,980	0,433	0,768
hsa-miR-1270	6	3,918	8,102	13,272	24,363	26,970	27,426	36,057	35,346	0,164	0,091
hsa-miR-302e	6	3,929	4,112	7,984	12,338	14,603	15,468	19,004	26,795	0,003	0,172
hsa-miR-520h	6	3,696	4,668	7,519	16,663	17,018	22,589	19,295	30,796	0,202	0,395
hsa-miR-151-3p	6	4,730	5,342	9,632	16,704	18,081	25,461	25,768	32,991	0,570	0,857
hsa-miR-490-5p	6	7,806	4,602	9,545	17,247	19,343	29,184	25,015	36,051	0,744	0,650
hsa-miR-29a	6	5,164	5,587	10,291	17,294	19,168	24,041	21,373	26,375	0,528	0,255
hsa-miR-885-3p	6	4,157	7,232	11,632	22,359	27,356	45,570	30,397	44,100	0,000	0,043
hsa-miR-593*	6	3,353	5,123	12,325	22,571	24,481	29,234	26,275	34,186	0,206	0,701
hsa-miR-513b	6	3,478	5,207	12,175	18,500	20,616	27,650	23,244	35,533	0,778	0,856
hsa-miR-1914	6	4,122	4,886	9,181	16,093	19,680	20,381	19,610	26,467	0,229	0,182
hsa-miR-19b	6	4,304	5,034	9,187	18,097	18,431	33,263	20,764	27,136	0,384	0,263
hsa-miR-31	6	4,597	5,210	11,334	20,547	23,920	31,394	25,978	35,100	0,124	0,648
hsa-miR-484	6	4,726	4,882	10,008	22,940	24,469	28,882	27,988	40,377	0,230	0,189
hsa-miR-92b	6	4,993	6,567	12,130	20,386	18,081	26,669	20,825	34,698	0,727	0,837
hsa-miR-143	6	4,741	7,696	11,114	21,560	21,639	22,865	22,460	38,330	0,690	0,677
hsa-miR-1291	6	3,562	6,680	12,786	21,459	24,892	25,128	25,583	36,531	0,546	0,563
hsa-miR-378	6	3,615	5,232	11,732	21,688	23,061	25,132	33,367	32,961	0,786	0,276
hsa-miR-1178	6	4,643	4,729	8,436	15,685	18,593	26,311	18,001	24,477	0,747	0,079
hsa-let-7b	6	3,357	5,853	11,252	23,560	23,223	24,581	24,251	37,878	0,841	0,564
hsa-miR-654-5p	6	3,104	5,449	9,724	18,845	18,843	29,400	27,180	35,668	0,784	0,504
hsa-miR-617	6	3,654	5,449	9,615	18,527	18,393	28,720	23,220	29,208	0,941	0,563
hsa-miR-649	6	3,520	6,303	7,893	14,644	15,442	26,496	19,053	26,623	0,398	0,168
hsa-miR-532-3p	6	2,804	6,445	7,995	16,937	16,743	28,902	21,788	26,915	0,852	0,306
hsa-miR-125b-2*	6	2,628	5,511	8,366	20,895	18,318	30,522	23,735	31,985	0,705	0,854
hsa-miR-452*	6	4,242	6,128	12,820	19,438	23,023	25,995	27,403	31,661	0,674	0,828
hsa-miR-744	6	3,730	6,068	10,406	21,682	23,297	27,874	22,057	39,769	0,419	0,592
hsa-miR-1909*	6	3,696	4,426	8,932	18,330	17,806	24,103	17,856	34,281	0,388	0,547
hsa-miR-129-3p	6	4,479	5,126	9,008	18,260	18,830	26,363	22,925	33,416	0,787	0,913
hsa-miR-494	6	3,608	4,835	5,995	15,677	16,143	20,203	15,765	27,976	0,063	0,111

miRNA											
hsa-miR-302b*	6	4,363	5,349	10,313	18,868	18,431	27,113	21,520	30,045	0,836	0,496
hsa-miR-26b*	6	3,262	5,011	11,445	20,364	19,755	31,169	23,895	33,327	0,454	0,997
hsa-miR-1293	6	4,037	6,868	14,373	24,008	29,046	36,267	32,572	39,284	0,001	0,082
hsa-miR-324-3p	6	4,192	5,941	13,278	20,282	21,851	27,515	31,150	35,768	0,630	0,247
hsa-miR-1274b	6	3,631	5,441	8,517	15,583	14,491	17,878	20,557	31,619	0,011	0,545
hsa-miR-508-3p	6	3,524	5,706	7,225	16,558	15,092	22,257	21,459	28,852	0,095	0,406
hsa-miR-218-2*	6	3,845	5,792	8,803	17,227	17,418	24,773	18,798	31,351	0,418	0,399
hsa-miR-513a-3p	6	4,593	4,673	9,073	19,814	15,993	28,050	18,847	32,275	0,639	0,468
hsa-miR-1226	6	3,175	5,187	8,646	23,097	19,605	31,829	22,791	36,138	0,404	0,843
hsa-miR-30d	6	2,777	5,008	9,041	19,740	16,168	25,303	22,424	28,688	0,351	0,462
hsa-let-7d*	6	3,391	5,896	10,894	18,031	23,385	22,918	21,154	26,411	0,942	0,246
hsa-miR-182	6	3,922	5,698	12,119	19,782	22,811	23,307	34,494	40,775	0,916	0,033
hsa-miR-647	6	5,602	5,443	10,133	19,499	16,881	21,722	21,178	34,961	0,145	0,895
hsa-miR-578	6	3,391	4,952	8,490	21,162	16,656	27,780	23,183	37,383	0,688	0,695
hsa-miR-141	6	4,107	6,106	8,398	18,565	17,093	31,313	21,581	32,271	0,765	0,685
hsa-miR-486-3p	6	4,227	6,429	10,406	25,844	22,238	41,808	31,741	43,654	0,004	0,033
hsa-miR-92a-2*	6	3,791	6,548	7,465	18,995	14,891	26,548	23,858	32,758	0,352	0,939
hsa-miR-518b	6	3,342	6,749	9,360	20,674	19,367	26,729	25,793	30,467	0,914	0,905
hsa-miR-492	6	3,239	0,157	11,699	23,617	22,948	32,476	36,299	37,449	0,121	0,050
hsa-miR-1915*	6	3,334	5,864	9,594	20,936	14,866	30,434	24,768	37,967	0,808	0,516
hsa-miR-562	6	3,524	4,748	9,964	18,130	17,468	29,467	23,784	35,781	0,967	0,783
hsa-miR-631	6	3,474	5,380	8,501	19,383	13,602	24,640	25,151	27,382	0,135	0,570
hsa-miR-297	6	3,707	3,964	8,754	18,287	18,256	32,266	25,768	35,190	0,503	0,658
hsa-miR-521	6	8,552	4,967	8,382	17,718	17,143	26,456	23,171	31,544	0,580	0,761
hsa-miR-150*	6	3,296	5,779	9,089	19,959	17,181	32,705	28,598	34,148	0,580	0,510
hsa-miR-493*	6	2,748	5,553	9,719	16,689	17,081	26,375	23,993	29,451	0,562	0,647
hsa-miR-499-3p	6	3,421	5,686	11,483	17,903	18,643	25,215	24,362	27,080	0,609	0,485
hsa-miR-101	6	2,584	4,684	7,770	19,145	15,092	23,757	22,228	27,620	0,162	0,375
hsa-miR-551b	6	3,179	4,515	7,615	19,325	14,065	22,173	19,731	30,662	0,063	0,414
hsa-miR-1200	6	2,766	5,169	6,349	18,091	11,345	23,780	24,276	30,442	0,043	0,761
hsa-let-7b*	6	2,588	5,238	6,143	15,703	11,697	17,351	20,059	21,425	0,002	0,060
hsa-miR-1277	6	3,228	5,146	8,490	19,797	16,143	24,978	24,830	31,292	0,319	0,892
hsa-miR-1205	6	3,566	7,636	7,172	17,681	17,493	24,754	25,904	28,668	0,424	0,747
hsa-miR-1197	6	3,017	8,242	8,533	20,731	18,680	28,882	30,535	34,983	0,878	0,322
hsa-miR-504	6	3,520	6,900	10,905	17,579	16,706	24,220	27,428	27,847	0,301	0,812
hsa-miR-488	7	3,047	5,733	10,683	15,364	19,805	26,912	22,125	31,426	0,998	0,658
hsa-miR-132*	7	4,472	7,035	11,765	13,081	16,905	25,360	24,772	28,564	0,427	0,638
hsa-miR-29b-1*	7	3,658	4,654	10,361	10,888	15,947	19,018	27,627	24,037	0,035	0,502
hsa-miR-186	7	3,571	5,821	11,104	10,632	19,193	24,285	24,217	29,703	0,560	0,689
hsa-miR-1245	7	3,992	4,500	10,456	10,688	18,101	19,830	25,529	28,128	0,114	0,665
hsa-miR-122	7	3,908	4,063	10,462	7,861	18,593	18,552	23,851	28,598	0,085	0,564
hsa-miR-513a-5p	7	4,000	3,503	9,396	9,001	16,749	16,824	21,381	24,320	0,019	0,167
hsa-miR-526b	7	4,050	4,171	12,341	10,267	22,439	21,071	30,374	26,604	0,563	0,974
hsa-miR-191*	7	2,632	8,361	12,516	20,729	20,734	35,620	26,809	42,553	0,095	0,156
hsa-miR-135b*	7	3,534	7,733	10,706	15,096	17,591	26,141	20,415	28,357	0,595	0,311
hsa-miR-99b	7	2,848	6,495	8,925	14,504	15,216	21,997	23,986	33,984	0,090	0,932
hsa-miR-103	7	3,675	7,668	10,760	14,622	17,248	23,515	28,621	30,106	0,286	0,859
hsa-miR-579	7	3,600	7,475	9,984	14,812	16,758	23,573	26,512	26,481	0,253	0,608
hsa-miR-200b*	7	3,750	7,032	11,400	14,096	18,475	24,175	26,979	27,105	0,465	0,703
hsa-miR-574-3p	7	3,350	7,119	10,283	18,321	22,211	24,693	29,662	30,793	0,970	0,700
hsa-miR-29a*	7	3,284	6,271	11,175	14,362	20,180	27,195	29,587	27,695	0,903	0,997
hsa-miR-1304	7	3,317	6,663	13,127	14,912	19,420	24,292	31,164	34,879	0,589	0,292
hsa-miR-342-3p	7	3,197	10,129	11,824	19,162	16,827	22,341	24,395	31,974	0,176	0,915
hsa-miR-1914*	7	3,763	5,748	12,001	14,174	16,387	16,048	27,297	29,088	0,011	0,917
hsa-miR-1182	7	4,355	9,004	13,960	17,775	15,255	20,887	22,923	31,070	0,059	0,696
hsa-miR-765	7	3,430	5,573	11,571	13,824	14,330	22,779	26,544	34,473	0,088	0,652
hsa-miR-566	7	2,956	5,832	10,498	12,377	12,614	17,957	25,035	27,213	0,004	0,547
hsa-miR-548l	7	3,550	5,293	10,599	12,784	16,514	22,484	23,642	28,996	0,167	0,580
hsa-miR-1975	7	3,688	5,892	14,511	16,116	20,773	30,898	29,910	41,985	0,376	0,084
hsa-miR-1279	7	2,864	6,654	10,134	16,787	18,416	26,630	21,856	29,424	0,767	0,475
hsa-miR-2110	7	2,777	8,616	10,128	17,859	17,748	25,184	25,593	35,360	0,498	0,659
hsa-miR-597	7	2,931	6,731	8,804	14,433	14,495	21,078	25,329	31,035	0,047	0,915
hsa-miR-152	7	3,130	6,501	8,804	12,946	14,457	18,331	23,100	27,196	0,013	0,404
hsa-miR-1269	7	3,267	6,743	9,130	15,291	16,387	22,455	25,825	33,953	0,158	0,763
hsa-miR-7-2*	7	3,975	8,424	7,518	14,206	18,180	25,081	23,131	35,589	0,536	0,862
hsa-miR-103-as	7	3,750	5,920	7,218	9,276	11,890	11,345	16,050	14,335	0,000	0,002
hsa-miR-1208	7	4,246	6,303	10,997	15,900	19,943	26,149	27,521	27,780	0,913	0,814
hsa-miR-920	7	4,789	9,990	14,728	17,330	18,023	33,278	29,254	31,545	0,424	0,670
hsa-let-7a-2*	7	3,230	7,023	9,408	12,302	14,962	24,125	18,833	25,120	0,175	0,113
hsa-miR-100*	7	3,725	7,426	10,528	15,067	15,021	21,306	25,256	22,931	0,064	0,277
hsa-miR-432	7	3,525	7,331	12,767	18,160	13,864	23,892	25,920	29,262	0,113	0,803
hsa-miR-182*	7	3,475	7,071	10,200	14,494	14,583	20,428	22,529	25,872	0,037	0,288
hsa-miR-1229	7	3,629	7,050	11,193	17,326	17,876	20,922	24,395	26,947	0,154	0,477
hsa-miR-1259	7	3,242	5,177	11,453	13,307	13,369	17,269	23,235	24,647	0,004	0,261
hsa-miR-373*	7	4,417	6,510	14,040	17,104	17,817	29,837	26,459	32,609	0,866	0,828
hsa-miR-550	7	3,608	8,294	12,259	13,909	17,935	27,083	29,490	27,235	0,764	0,949
hsa-miR-657	7	2,508	6,918	10,014	13,040	14,447	23,457	22,892	26,414	0,118	0,340
hsa-miR-891b	7	3,850	7,690	7,800	11,306	15,138	20,484	17,537	20,541	0,048	0,023
hsa-miR-199a-3p	7	4,008	6,492	8,245	9,805	14,563	15,190	21,618	22,653	0,003	0,121
hsa-miR-1180	7	3,683	5,986	8,470	11,523	13,689	22,434	19,607	25,934	0,061	0,162
hsa-miR-632	7	3,758	5,593	8,434	12,115	14,777	21,654	22,218	23,506	0,067	0,167
hsa-miR-93	7	3,039	6,078	6,996	10,734	14,311	16,721	22,218	23,366	0,005	0,162
hsa-miR-509-3p	7	2,740	5,131	10,641	12,733	16,094	20,632	24,217	25,037	0,073	0,337
hsa-miR-205*	7	3,758	6,906	10,623	15,107	21,883	24,920	25,529	29,913	0,985	0,828
hsa-miR-124*	7	3,625	8,042	10,629	16,586	18,573	25,434	27,217	33,996	0,628	0,998
hsa-miR-609	7	3,742	5,688	9,654	14,181	17,297	22,420	26,353	26,588	0,209	0,604
hsa-miR-491-5p	7	3,060	6,686	10,295	16,720	18,770	26,119	28,567	35,869	0,745	0,390
hsa-miR-369-5p	7	3,342	5,087	9,263	12,241	15,362	20,237	22,498	22,313	0,047	0,136
hsa-miR-330-5p	7	3,459	6,760	10,224	13,561	21,348	31,767	32,885	39,367	0,255	0,075
hsa-miR-1281	7	3,575	6,698	10,468	14,312	18,396	24,927	25,056	29,268	0,543	0,725
hsa-miR-140-5p	7	3,829	6,233	10,236	11,953	19,233	21,690	24,101	23,447	0,296	0,244
hsa-miR-23b	7	4,739	9,076	12,679	19,567	19,548	32,788	27,862	30,334	0,323	0,910
hsa-miR-524-5p	7	4,438	7,065	10,772	14,032	15,040	22,470	21,722	21,196	0,101	0,087
hsa-miR-516b	7	4,075	5,554	11,146	14,572	18,987	32,804	33,488	27,888	0,373	0,621
hsa-miR-29b-2*	7	3,958	6,431	9,022	14,326	17,876	26,623	30,169	31,533	0,693	0,593
hsa-miR-654-3p	7	4,551	5,963	10,379	13,262	14,816	20,944	22,591	20,894	0,051	0,100
hsa-miR-614	7	3,854	7,050	10,074	14,138	16,964	20,294	25,856	29,100	0,089	0,782
hsa-miR-596	7	3,867	7,044	9,894	11,090	17,199	21,469	23,579	24,380	0,148	0,265
hsa-miR-641	7	4,539	8,558	12,171	13,551	21,665	23,443	27,478	27,128	0,772	0,750
hsa-miR-202	7	3,838	7,727	12,837	14,769	20,091	27,098	30,018	28,466	0,930	0,882
hsa-miR-127-3p	7	4,250	9,677	13,741	17,692	21,011	29,011	34,367	36,118	0,551	0,114
hsa-miR-542-3p	7	4,530	5,526	9,948	10,082	13,108	16,585	20,374	15,779	0,003	0,013
hsa-let-7f-2*	7	4,313	6,248	9,606	10,283	14,885	17,716	21,505	18,441	0,012	0,040
hsa-miR-105*	7	4,409	6,850	10,200	15,451	21,100	29,374	27,990	30,135	0,499	0,917
hsa-miR-518e	7	4,313	7,413	10,427	13,157	19,095	23,067	27,723	25,103	0,412	0,594
hsa-miR-101*	7	4,058	5,972	8,294	11,243	18,583	17,139	22,363	16,237	0,048	0,027
hsa-miR-224*	7	4,050	6,746	10,623	15,644	20,565	26,245	25,350	18,657	0,984	0,115
hsa-miR-187*	7	3,309	6,710	10,807	12,129	17,346	23,406	25,140	28,524	0,285	0,666
hsa-miR-21*	7	3,080	6,769	9,834	12,695	17,395	18,816	27,926	23,129	0,059	0,457
hsa-miR-1911	7	4,564	5,943	12,218	14,369	19,105	23,906	30,277	30,964	0,505	0,631
hsa-miR-302c*	7	4,651	6,038	11,157	13,645	19,253	16,728	25,056	24,243	0,054	0,339
hsa-miR-1246	7	3,979	6,716	9,894	13,933	18,504	19,984	27,255	28,237	0,138	0,832
hsa-miR-432*	7	4,300	5,972	9,335	11,855	20,476	17,805	24,248	30,740	0,129	0,786
hsa-miR-200b	7	4,213	6,897	11,134	9,837	20,872	14,867	23,830	20,005	0,050	0,109
hsa-miR-1910	7	3,796	6,663	11,376	12,551	19,706	16,469	27,404	19,677	0,059	0,225
hsa-miR-377	8	7,173	6,552	17,182	18,738	25,046	27,150	27,182	27,901	0,319	0,794
hsa-miR-1294	8	7,358	5,996	16,502	17,665	33,347	23,814	40,126	36,287	0,064	0,023
hsa-miR-1307	8	8,087	5,554	17,146	14,069	27,689	23,454	35,746	29,404	0,422	0,345
hsa-miR-361-3p	8	6,941	5,927	16,289	13,605	28,442	24,981	33,079	29,997	0,225	0,484
hsa-miR-299-5p	8	6,777	5,749	17,080	16,407	26,684	24,882	32,407	27,635	0,378	0,738
hsa-miR-196b	8	6,524	7,205	15,785	17,109	24,356	22,643	27,423	28,905	0,957	0,911
hsa-miR-558	8	7,049	7,294	17,782	18,639	27,641	30,387	28,971	33,882	0,042	0,502
hsa-miR-449b*	8	6,345	6,377	14,745	14,452	25,747	25,405	25,167	30,879	0,419	0,885
hsa-miR-1287	8	9,089	7,022	22,236	22,008	49,568	39,102	47,426	48,438	0,000	0,000

hsa-miR-608	8	7,399	6,630	18,555	21,159	28,280	36,913	36,846	40,975	0,001	0,015
hsa-miR-892a	8	6,733	6,768	17,350	21,100	26,400	36,594	44,979	38,973	0,005	0,002
hsa-miR-548h	8	7,259	6,453	16,052	19,304	21,854	23,889	29,991	25,965	0,862	0,876
hsa-miR-134	8	5,353	4,986	10,689	12,958	13,877	14,074	18,312	16,657	0,001	0,009
hsa-miR-22	8	5,422	6,682	14,625	22,755	21,642	26,015	29,464	29,063	0,862	0,878
hsa-miR-660	8	5,291	6,744	12,416	16,210	22,768	24,439	26,720	30,550	0,926	0,999
hsa-miR-501-3p	8	4,721	4,575	11,617	10,699	23,433	18,458	22,084	26,563	0,385	0,302
hsa-miR-30c-1*	8	8,270	5,837	22,219	17,550	31,876	26,820	31,128	35,933	0,032	0,240
hsa-miR-374b*	8	8,234	6,242	19,718	18,000	22,718	23,806	25,516	28,679	0,975	0,713
hsa-miR-200a	8	6,648	6,292	17,851	19,911	24,620	27,299	38,219	27,878	0,345	0,293
hsa-miR-138	8	8,949	7,362	18,431	26,284	29,772	28,555	33,095	31,720	0,037	0,364
hsa-miR-545*	8	6,735	7,290	16,486	24,945	22,169	27,917	32,986	29,007	0,541	0,569
hsa-miR-203	8	6,821	7,063	17,105	23,865	25,164	23,008	25,553	31,700	0,789	1,000
hsa-miR-612	8	9,124	6,481	26,459	21,821	45,864	38,498	50,599	53,827	0,000	0,000
hsa-miR-767-3p	8	6,692	4,870	19,201	13,331	27,923	19,846	24,864	27,460	0,848	0,553
hsa-miR-422a	8	7,023	6,599	17,466	22,886	30,897	35,054	37,761	40,038	0,001	0,015
hsa-miR-27b*	8	5,296	5,157	14,061	19,131	19,265	20,387	19,362	28,668	0,202	0,272
hsa-miR-523	8	5,358	6,772	11,159	24,089	24,223	26,961	27,770	33,932	0,417	0,594
hsa-miR-548a-5p	8	8,003	7,216	16,035	23,775	26,093	22,336	27,473	26,969	0,754	0,735
hsa-miR-200a*	8	8,177	7,302	17,481	24,279	28,109	21,737	31,615	29,186	0,571	0,670
hsa-miR-29b	8	7,261	6,429	17,882	22,154	26,388	22,034	26,362	26,223	0,755	0,574
hsa-miR-155	8	7,435	6,392	18,661	18,219	24,518	18,443	28,369	31,360	0,500	0,766
hsa-miR-452	8	7,476	6,051	18,610	16,009	25,858	23,254	29,386	31,627	0,661	0,651
hsa-miR-451	8	7,240	6,006	19,656	18,897	32,193	27,343	30,354	30,851	0,022	0,634
hsa-miR-634	8	5,931	5,987	11,826	20,305	20,208	27,913	32,017	33,013	0,798	0,350
hsa-miR-1265	8	6,385	7,482	14,085	20,945	21,807	25,588	22,946	28,341	0,900	0,474
hsa-miR-591	8	9,397	7,826	18,604	23,042	24,967	26,355	23,257	25,915	0,402	0,332
hsa-miR-875-3p	8	7,893	6,912	19,598	22,108	29,248	27,861	24,424	23,176	0,061	0,247
hsa-miR-588	8	6,236	7,629	20,625	26,927	28,292	32,882	25,350	23,832	0,010	0,332
hsa-miR-219-1-3p	8	5,886	6,190	17,529	22,353	24,616	27,913	20,376	24,216	0,292	0,130
hsa-miR-208a	8	6,823	6,460	17,410	16,784	27,847	24,105	24,279	24,413	0,342	0,304
hsa-miR-205	8	6,973	6,858	17,227	19,825	30,764	25,931	30,255	29,106	0,073	0,800
hsa-miR-1203	8	6,172	5,736	17,763	21,735	27,592	24,392	29,202	28,944	0,339	0,915
hsa-miR-18b*	8	7,846	8,618	18,797	26,057	28,897	24,985	31,856	32,021	0,195	0,426
hsa-miR-548j	8	6,725	6,979	16,498	22,857	22,347	23,192	26,575	27,471	0,833	0,699
hsa-miR-769-3p	8	7,872	7,449	20,866	26,960	30,802	26,231	31,324	31,529	0,064	0,501
hsa-miR-362-5p	8	7,561	7,203	16,603	28,728	23,827	26,215	30,370	31,761	0,544	0,558
hsa-miR-1911*	8	7,696	6,614	18,138	21,461	31,236	23,579	31,248	32,134	0,147	0,461
hsa-let-7a	8	8,522	7,635	18,359	21,410	35,602	25,648	32,339	30,879	0,011	0,473
hsa-miR-767-5p	8	6,396	7,612	19,912	21,707	27,242	28,820	32,540	33,638	0,091	0,284
hsa-miR-936	8	6,039	7,983	14,751	26,090	29,211	25,712	25,942	31,250	0,138	0,994
hsa-miR-372	8	4,639	6,161	12,035	18,861	18,277	16,441	21,576	20,513	0,032	0,070
hsa-miR-548m	8	8,601	8,034	20,984	29,037	28,548	20,463	30,611	28,219	0,678	0,850
hsa-miR-129*	8	6,220	8,064	16,227	31,960	26,129	22,561	27,935	28,168	0,718	0,890
hsa-miR-628-3p	8	6,363	8,461	17,179	25,070	24,439	24,495	24,094	29,146	0,685	0,630
hsa-miR-191	8	7,196	6,601	18,792	14,887	21,160	20,352	23,511	28,125	0,347	0,500
hsa-miR-514	8	6,544	5,500	14,597	15,295	15,528	20,662	20,592	26,315	0,060	0,216
hsa-miR-188-3p	8	7,233	6,560	16,490	17,824	24,604	19,196	26,853	30,217	0,600	0,982
hsa-miR-130a*	8	7,747	6,426	18,403	25,370	24,474	24,902	26,355	31,667	0,627	0,927
hsa-miR-222*	8	6,476	6,239	11,935	13,372	16,754	13,428	21,489	19,108	0,003	0,047
hsa-miR-220c	8	6,912	6,943	16,896	22,679	24,423	23,610	28,159	31,069	0,808	0,812
hsa-miR-1257	8	6,544	5,598	16,105	23,856	26,865	24,656	27,516	33,994	0,382	0,610
hsa-miR-517b	8	4,089	6,742	11,244	21,174	17,995	21,171	19,180	29,702	0,174	0,319
hsa-miR-454*	8	5,660	6,314	15,080	16,339	24,647	25,819	22,731	31,444	0,494	0,713
hsa-miR-221	8	5,775	7,431	15,117	14,015	25,117	23,399	28,403	28,058	0,742	0,924
hsa-miR-643	8	7,104	7,632	17,138	17,749	23,761	30,487	32,065	36,600	0,176	0,172
hsa-miR-639	8	6,392	6,775	19,725	21,216	29,953	27,921	27,278	28,027	0,045	0,814
hsa-miR-103-2*	8	6,821	6,865	15,646	21,242	26,484	24,704	27,777	25,700	0,416	0,650
hsa-miR-548k	8	7,373	5,654	16,170	18,269	27,013	22,643	29,668	25,923	0,593	0,841
hsa-miR-1244	8	7,082	6,764	16,408	19,014	24,699	24,294	30,141	28,227	0,677	0,893
hsa-miR-605	8	6,722	5,883	17,346	17,362	24,687	22,049	29,484	29,981	0,995	0,790
hsa-miR-520c-3p	8	6,276	4,700	15,405	11,082	23,106	22,138	23,056	24,826	0,791	0,261
hsa-miR-877*	8	7,405	7,323	19,731	16,343	31,017	29,470	38,432	37,389	0,014	0,027
hsa-miR-346	8	7,658	7,709	15,933	18,927	22,935	23,928	27,420	27,733	0,976	0,800
hsa-miR-937	8	7,425	7,494	19,470	17,881	21,650	26,683	24,390	21,984	0,768	0,193
hsa-miR-30d*	8	6,799	6,606	16,712	17,005	24,955	23,634	29,625	21,888	0,731	0,492
hsa-miR-663	8	6,895	7,295	16,795	18,819	25,719	22,460	27,408	24,735	0,788	0,539
hsa-miR-143*	8	6,794	6,448	15,821	16,427	22,370	20,952	26,317	25,211	0,540	0,491
hsa-miR-26a-1*	8	6,151	6,366	15,149	15,622	22,695	23,340	22,327	27,175	0,904	0,353
hsa-miR-125a-5p	8	7,056	6,086	16,979	13,642	24,829	22,018	22,077	20,230	0,979	0,075
hsa-miR-339-3p	8	6,823	5,695	18,351	14,610	34,463	25,999	28,272	26,373	0,015	0,754
hsa-miR-598	9	3,584	9,603	10,952	25,115	20,724	31,659	23,956	40,031	0,314	0,428
hsa-miR-582-3p	9	2,528	9,100	9,223	21,129	21,776	33,672	24,122	39,535	0,124	0,450
hsa-miR-1979	9	2,981	8,531	9,587	17,299	19,230	24,029	19,709	27,834	0,534	0,247
hsa-miR-301a	9	2,894	6,478	11,796	16,084	18,429	25,144	22,835	28,815	0,573	0,502
hsa-let-7i*	9	3,310	8,335	14,108	22,894	24,185	34,742	26,085	40,505	0,032	0,271
hsa-let-7c*	9	3,398	6,886	14,646	16,809	21,590	28,077	25,417	30,759	0,593	0,897
hsa-miR-1288	9	3,355	7,448	11,560	18,218	20,434	28,351	24,328	34,472	0,708	0,854
hsa-miR-720	9	3,171	6,267	10,952	15,077	18,989	26,490	21,666	30,138	0,826	0,515
hsa-miR-181b	9	4,250	9,677	14,764	25,002	21,412	28,135	26,818	32,614	0,608	0,794
hsa-miR-1207-3p	9	4,124	10,585	11,970	21,717	19,773	32,411	23,819	39,954	0,335	0,443
hsa-miR-624*	9	4,697	10,272	14,932	23,159	29,341	28,252	27,813	35,117	0,050	0,497
hsa-miR-196b*	9	3,967	10,814	14,468	22,428	21,861	30,242	29,065	38,385	0,334	0,225
hsa-miR-28-3p	9	4,207	10,109	14,721	22,221	19,049	27,078	22,657	34,624	0,918	0,998
hsa-miR-646	9	4,940	10,415	13,538	18,448	18,900	24,467	24,516	32,162	0,547	0,945
hsa-miR-126	9	4,066	11,929	13,354	22,239	22,832	25,053	27,263	36,671	0,829	0,425
hsa-miR-642	9	4,496	11,923	17,322	25,804	23,680	30,218	27,762	44,232	0,196	0,085
hsa-miR-1249	9	4,239	9,007	12,474	23,086	24,300	27,321	30,823	40,875	0,373	0,087
hsa-miR-329	9	4,787	9,677	12,894	20,074	23,470	27,309	29,046	37,249	0,460	0,281
hsa-miR-7	9	6,231	7,388	16,976	15,191	25,090	27,797	26,045	36,162	0,263	0,555
hsa-miR-31*	9	5,005	7,808	14,131	15,362	19,602	20,873	21,124	28,007	0,260	0,331
hsa-miR-577	9	5,434	7,952	17,261	16,142	20,514	24,889	23,409	37,093	0,814	0,700
hsa-miR-376c	9	4,712	6,863	13,947	13,816	16,081	21,714	19,884	30,159	0,110	0,390
hsa-miR-30b	9	5,687	8,335	17,927	17,652	20,647	25,992	25,057	32,642	0,991	0,958
hsa-miR-1255a	9	4,706	7,335	14,477	15,680	18,802	21,796	22,721	34,877	0,270	0,967
hsa-let-7f	9	4,073	9,341	16,028	24,675	24,788	26,530	27,485	42,716	0,402	0,129
hsa-miR-30c-2*	9	4,796	9,660	11,691	22,094	25,311	25,008	28,838	39,588	0,511	0,185
hsa-miR-374a	9	4,950	7,688	15,859	17,344	19,954	23,727	23,733	35,284	0,585	0,834
hsa-miR-1258	9	4,915	10,500	18,121	21,486	25,049	33,113	27,854	45,261	0,042	0,065
hsa-miR-137	9	4,390	8,070	11,939	17,274	19,909	23,455	19,933	39,763	0,545	0,776
hsa-miR-219-2-3p	9	4,524	8,925	13,515	16,135	18,288	25,723	20,742	34,595	0,629	0,820
hsa-miR-515-5p	9	4,548	9,597	17,652	18,150	24,328	31,605	23,798	35,474	0,099	0,810
hsa-miR-518d-3p	9	4,596	11,125	15,456	20,335	23,569	31,647	24,426	46,909	0,129	0,106
hsa-miR-1185	9	4,923	9,423	14,342	20,359	19,346	29,802	23,363	34,573	0,663	0,935
hsa-miR-17*	9	4,784	11,095	13,312	21,086	22,656	27,350	25,676	31,273	0,550	0,971
hsa-miR-1302	9	4,366	9,514	13,774	19,683	20,083	24,175	25,148	36,390	0,658	0,610
hsa-miR-223*	9	3,871	9,216	13,505	16,001	15,032	20,690	20,767	29,590	0,050	0,410
hsa-miR-519e	9	3,734	8,740	12,859	22,090	19,376	23,243	20,732	31,071	0,460	0,516
hsa-miR-206	9	4,244	11,259	16,397	21,618	20,064	22,520	21,847	39,672	0,456	0,617
hsa-miR-338-3p	9	4,980	8,987	15,003	16,967	19,629	19,597	20,531	35,204	0,176	0,857
hsa-miR-601	9	4,952	10,380	16,140	23,841	23,514	24,024	23,697	44,255	0,879	0,215
hsa-miR-142-3p	9	4,997	9,114	15,076	20,058	23,739	25,070	28,510	35,634	0,701	0,410
hsa-miR-25	9	4,816	8,620	12,872	18,573	20,598	22,503	21,812	28,592	0,514	0,412
hsa-miR-627	9	4,636	8,931	11,945	20,621	21,346	20,889	24,589	33,374	0,418	0,932
hsa-miR-301b	9	3,885	8,321	11,693	21,454	19,388	22,569	24,527	28,687	0,390	0,627
hsa-miR-768-3p	9	4,442	8,897	13,021	21,216	18,997	22,026	25,723	31,106	0,304	0,959
hsa-miR-1234	9	4,701	8,782	13,641	21,813	22,032	25,257	29,367	29,706	0,915	0,827
hsa-miR-516a-5p	9	4,994	8,928	13,549	20,199	20,644	25,189	22,596	33,474	0,875	0,888
hsa-miR-23a*	9	5,446	7,909	17,151	23,721	24,508	27,495	28,767	41,625	0,337	0,121
hsa-miR-425*	9	4,032	9,603	15,852	22,066	19,901	29,227	25,530	35,168	0,664	0,681
hsa-miR-1282	9	3,731	8,161	13,388	21,522	19,640	27,066	21,741	27,600	0,999	0,343
hsa-miR-328	9	4,148	8,568	14,869	16,171	22,168	22,619	27,282	30,365	0,728	0,962
hsa-miR-106a*	9	4,980	8,208	13,335	16,600	18,795	22,239	24,578	23,156	0,306	0,253
hsa-miR-801	9	4,304	9,546	11,713	22,752	19,139	22,146	26,392	28,856	0,327	0,809

hsa-miR-345	9	4,814	9,417	12,012	17,377	18,400	15,815	25,909	23,970	0,025	0,376
hsa-miR-569	9	4,844	8,191	13,688	17,030	17,877	17,642	24,137	23,774	0,044	0,262
hsa-miR-548e	9	4,019	7,650	14,195	24,226	21,173	21,840	24,148	35,766	0,503	0,752
hsa-miR-216a	9	4,422	8,531	18,233	21,761	18,675	27,960	30,285	35,299	0,991	0,318
hsa-miR-195*	9	3,547	8,771	13,941	21,877	18,888	27,710	29,966	32,064	0,985	0,566
hsa-miR-1322	9	4,308	7,246	9,456	16,193	17,512	21,263	25,479	26,096	0,153	0,495
hsa-miR-671-5p	9	4,676	6,723	15,395	14,801	18,018	22,064	23,794	24,059	0,232	0,259
hsa-miR-892b	9	4,191	6,554	15,854	18,053	18,743	25,031	25,020	30,842	0,598	0,867
hsa-miR-16-1*	9	4,196	5,257	15,404	14,115	12,210	17,156	15,250	20,534	0,002	0,011
hsa-miR-221*	9	5,108	5,900	19,644	17,392	16,893	19,353	22,628	22,382	0,060	0,143
hsa-miR-552	9	4,804	5,736	20,095	19,536	23,534	21,687	23,657	34,393	0,788	0,924
hsa-miR-576-5p	9	4,621	8,908	14,481	20,991	21,000	27,228	29,125	29,460	0,783	0,873
hsa-miR-571	9	4,890	7,871	13,183	15,738	20,510	23,990	24,477	29,939	0,690	0,733
hsa-miR-590-3p	9	5,015	6,945	13,484	15,781	19,316	20,744	18,485	24,499	0,229	0,089
hsa-miR-184	9	4,308	7,599	18,089	22,360	22,640	28,633	23,582	30,434	0,410	0,698
hsa-miR-18b	9	4,407	7,308	14,474	19,748	18,118	24,057	17,196	29,891	0,415	0,229
hsa-miR-744*	9	5,245	6,562	12,253	15,003	18,918	17,954	16,346	25,848	0,077	0,075
hsa-miR-30a*	9	4,910	7,458	14,815	17,854	20,395	21,382	19,755	28,209	0,372	0,268
hsa-miR-380	9	4,465	6,699	14,231	18,008	19,599	17,590	18,326	30,138	0,087	0,297
hsa-miR-214	9	4,163	7,437	8,699	22,728	20,789	26,847	23,463	38,310	0,866	0,593
hsa-let-7g	9	4,203	9,238	15,048	21,933	24,720	28,809	24,101	39,832	0,218	0,432
hsa-miR-302f	9	5,333	6,016	12,612	16,721	18,956	21,927	21,869	26,056	0,293	0,264
hsa-miR-34a*	9	4,551	5,252	14,865	13,796	17,862	23,477	21,719	22,774	0,334	0,127
hsa-miR-27a	9	3,832	5,284	13,033	14,569	14,691	20,421	20,425	23,338	0,039	0,107
hsa-miR-1247	9	5,076	4,899	15,823	17,649	16,704	22,613	24,318	29,556	0,185	0,685
hsa-miR-1261	9	3,625	6,620	12,177	15,695	18,541	21,154	20,661	25,128	0,206	0,170
hsa-miR-1537	9	3,980	5,555	12,488	18,638	19,807	21,883	22,424	25,367	0,364	0,257
hsa-miR-760	10	4,764	8,338	16,562	19,370	27,129	30,189	31,968	32,869	0,056	0,362
hsa-miR-212	10	4,240	7,485	12,869	17,062	23,313	29,396	26,476	33,466	0,280	0,747
hsa-miR-26a	10	3,102	8,500	12,662	16,611	23,254	30,323	35,530	36,457	0,218	0,079
hsa-let-7i	10	3,855	7,688	13,022	16,665	21,907	33,707	35,290	31,166	0,117	0,270
hsa-miR-92a	10	3,944	6,612	9,900	13,640	22,064	26,617	32,134	30,488	0,720	0,519
hsa-miR-520e	10	4,389	6,109	11,825	12,637	19,744	24,322	27,580	27,604	0,634	0,803
hsa-miR-139-5p	10	4,280	6,054	11,631	11,442	20,429	24,593	30,990	28,491	0,761	0,789
hsa-miR-519a	10	4,168	7,315	11,683	13,290	23,843	27,693	31,233	30,194	0,382	0,616
hsa-miR-550*	10	5,343	9,026	18,578	21,482	27,038	32,299	33,743	30,431	0,024	0,406
hsa-miR-1225-5p	10	4,404	8,144	12,971	19,450	21,193	29,188	29,003	34,375	0,509	0,463
hsa-miR-193a-5p	10	5,616	6,653	17,835	19,065	28,882	30,859	36,994	40,376	0,020	0,017
hsa-miR-181a*	10	5,203	6,690	15,779	18,525	22,445	28,487	31,210	35,316	0,446	0,267
hsa-miR-922	10	4,654	7,882	14,959	17,826	21,623	28,889	31,490	36,688	0,493	0,192
hsa-miR-890	10	4,997	7,344	15,904	19,203	25,185	30,317	29,827	41,462	0,114	0,097
hsa-miR-564	10	5,502	7,700	19,760	22,411	30,733	32,441	41,338	40,051	0,003	0,005
hsa-miR-1243	10	4,684	7,946	14,300	16,381	22,932	26,138	33,115	32,257	0,667	0,330
hsa-miR-1321	10	4,512	10,140	17,928	20,068	26,305	35,826	38,747	30,050	0,007	0,170
hsa-miR-616	10	4,983	7,324	12,857	16,023	19,865	26,831	27,517	24,942	0,999	0,564
hsa-miR-1469	10	5,126	8,458	16,824	16,283	22,310	27,284	31,178	31,585	0,601	0,508
hsa-miR-613	10	4,491	8,087	15,656	18,301	22,571	28,264	31,540	32,610	0,456	0,407
hsa-miR-520f	10	4,294	5,131	10,704	12,803	16,363	22,360	20,384	22,802	0,152	0,093
hsa-miR-449b	10	4,569	7,371	13,553	15,733	19,275	30,253	23,403	28,236	0,615	0,500
hsa-miR-873	10	4,562	9,031	14,271	17,544	19,895	29,882	26,029	32,975	0,583	0,834
hsa-miR-1179	10	4,959	4,545	13,884	11,695	23,004	26,307	28,791	27,863	0,636	0,942
hsa-miR-1206	10	3,208	7,529	12,046	17,658	19,550	27,540	28,760	34,512	0,944	0,471
hsa-miR-181a	10	5,311	7,720	15,174	17,416	21,100	24,427	30,940	32,158	0,832	0,482
hsa-miR-450b-5p	10	4,880	6,143	15,140	14,294	24,133	21,091	33,008	31,404	0,789	0,390
hsa-miR-148a	10	3,439	6,571	11,076	15,209	18,234	22,013	29,973	34,232	0,244	0,404
hsa-miR-93*	10	5,212	6,740	17,888	19,390	25,889	27,409	34,894	38,642	0,232	0,053
hsa-miR-1471	10	5,348	6,906	15,370	16,513	21,418	23,556	30,862	33,383	0,754	0,401
hsa-miR-1264	10	5,177	5,392	13,435	13,557	16,236	18,788	21,810	21,824	0,036	0,104
hsa-miR-629	10	5,486	7,351	19,652	18,480	25,518	31,762	33,045	40,014	0,059	0,061
hsa-miR-198	10	5,384	8,062	16,730	19,982	28,477	24,698	30,237	25,702	0,242	0,874
hsa-miR-933	10	4,156	7,132	12,722	18,664	21,392	26,065	28,018	26,278	0,891	0,722
hsa-miR-510	10	4,920	5,988	16,325	18,622	31,938	27,933	44,751	29,528	0,019	0,047
hsa-miR-934	10	4,034	6,656	14,534	16,181	24,202	22,208	36,253	26,964	0,958	0,477
hsa-miR-183*	10	4,526	7,020	15,409	17,912	22,584	25,277	31,123	26,808	0,833	0,935
hsa-miR-181c*	10	6,902	7,476	18,007	17,952	25,260	26,015	37,638	25,178	0,406	0,509
hsa-miR-1273	10	4,142	5,699	12,349	14,409	19,061	22,496	26,817	19,149	0,352	0,178
hsa-miR-1226*	10	4,486	4,755	14,196	12,250	20,456	24,173	28,652	22,958	0,707	0,498
hsa-miR-943	10	6,096	7,309	19,386	19,150	26,647	30,131	34,042	35,689	0,070	0,136
hsa-miR-2052	10	5,725	6,853	15,760	20,224	27,658	32,677	28,607	34,292	0,015	0,498
hsa-miR-26a-2*	10	5,716	5,332	15,640	14,806	23,710	25,277	31,196	28,428	0,678	0,776
hsa-miR-320d	10	5,627	6,365	15,154	14,248	24,262	22,834	29,383	28,997	0,943	0,892
hsa-miR-515-3p	10	4,836	5,674	12,645	14,385	17,467	22,073	26,960	28,174	0,197	0,798
hsa-miR-192*	10	4,983	5,435	13,562	12,751	17,969	21,242	23,911	27,993	0,176	0,520
hsa-miR-570	10	5,520	5,313	15,779	13,822	20,850	24,671	29,329	31,848	0,831	0,637
hsa-miR-95	10	5,579	4,147	16,412	11,016	23,455	20,236	31,389	26,256	0,585	0,963
hsa-miR-520a-5p	10	5,217	6,738	14,739	16,266	18,293	23,183	24,194	27,458	0,346	0,501
hsa-miR-543	10	4,534	6,625	14,101	15,304	15,895	21,916	25,777	29,054	0,111	0,771
hsa-miR-1909	10	6,154	7,287	19,813	16,440	20,793	23,205	30,743	31,643	0,624	0,537
hsa-miR-411*	10	5,150	6,947	14,748	13,602	15,295	22,208	24,784	24,942	0,100	0,366
hsa-miR-1974	10	4,740	7,982	15,128	17,453	19,125	30,346	33,445	37,554	0,623	0,101
hsa-miR-500*	10	6,880	5,839	17,764	14,315	18,276	26,015	29,270	27,407	0,665	0,945
hsa-miR-874	10	4,864	6,298	13,756	15,646	17,467	25,841	25,477	26,852	0,543	0,554
hsa-miR-548d-5p	10	5,541	6,050	14,659	12,031	20,889	25,093	30,200	24,427	0,897	0,753
hsa-miR-133a	10	5,830	7,422	16,961	18,555	23,254	27,836	29,637	24,296	0,427	0,690
hsa-miR-23b*	10	5,698	7,873	21,475	22,157	31,796	38,460	41,617	36,688	0,000	0,013
hsa-miR-186*	10	4,997	5,940	15,603	16,374	22,189	25,221	28,535	27,959	0,897	0,927
hsa-miR-544	10	5,221	5,657	12,942	17,463	19,873	25,948	29,687	27,908	0,874	0,967
hsa-miR-127-5p	10	4,235	5,588	13,972	18,796	20,316	26,453	32,953	35,171	0,990	0,193
hsa-miR-33b	10	4,955	5,555	13,542	18,744	21,392	28,361	29,945	29,849	0,583	0,760
hsa-miR-454	10	3,825	5,013	10,797	17,557	17,268	24,843	26,542	31,218	0,410	0,951
hsa-miR-573	10	5,371	5,745	15,007	19,668	24,799	30,556	30,287	31,428	0,120	0,592
hsa-miR-424*	10	5,847	7,174	16,426	17,069	23,596	28,224	32,684	29,689	0,355	0,538
hsa-miR-524-3p	10	5,527	6,736	15,776	16,893	25,227	26,877	29,401	32,404	0,327	0,584
hsa-miR-133b	10	2,632	6,453	13,468	14,532	17,280	21,532	24,098	21,092	0,155	0,149
hsa-miR-942	10	4,696	6,303	14,114	17,286	21,048	22,447	29,049	30,356	0,561	0,796
hsa-miR-204	10	4,819	6,483	13,724	17,994	21,277	28,436	30,241	38,513	0,588	0,171
hsa-miR-585	10	5,041	6,703	16,118	19,576	24,803	31,486	31,370	38,217	0,086	0,142
hsa-miR-148b*	10	4,432	5,694	12,301	14,881	18,703	24,948	25,455	28,383	0,583	0,681
hsa-miR-576-3p	10	4,259	5,001	13,771	15,575	22,977	28,081	29,787	29,643	0,431	0,793
hsa-miR-1826	10	4,724	8,394	18,619	22,939	29,103	37,493	37,066	35,870	0,001	0,062
hsa-miR-572	10	5,240	6,781	14,314	20,068	24,004	30,620	31,306	30,592	0,155	0,576
hsa-miR-511	10	4,512	6,440	15,756	17,239	20,595	28,935	27,356	27,621	0,612	0,784
hsa-miR-607	10	4,897	7,125	13,837	17,143	19,305	27,103	26,524	26,395	0,958	0,602
hsa-miR-512-5p	10	5,396	7,340	15,923	19,315	24,396	32,417	33,552	36,263	0,072	0,133
hsa-miR-34b*	10	5,073	7,231	15,147	16,381	21,556	25,623	26,871	31,067	0,931	0,934
hsa-miR-517*	10	6,240	6,701	14,885	14,891	21,264	26,049	27,669	31,772	0,912	0,793
hsa-miR-125a-3p	10	4,698	8,569	17,524	19,882	31,968	37,677	43,642	37,609	0,000	0,005
hsa-miR-199b-5p	11	4,627	6,662	13,220	17,923	23,157	28,252	25,323	30,737	0,395	0,886
hsa-miR-219-5p	11	4,230	5,603	11,853	13,022	22,867	21,755	23,609	25,934	0,706	0,355
hsa-miR-1202	11	4,603	5,243	12,610	14,806	21,256	19,905	25,870	29,105	0,316	0,784
hsa-miR-1301	11	4,621	6,768	12,068	16,861	21,847	25,852	24,150	29,819	0,856	0,693
hsa-miR-330-3p	11	4,594	7,156	12,693	17,538	20,650	24,569	24,790	32,951	0,789	0,954
hsa-miR-455-3p	11	4,400	7,611	12,506	17,126	19,625	24,418	21,359	26,907	0,631	0,282
hsa-miR-675*	11	4,078	6,900	11,820	17,996	19,461	23,452	19,917	27,352	0,493	0,233
hsa-miR-25*	11	3,758	7,084	12,374	19,423	22,351	32,090	20,435	34,562	0,169	0,854
hsa-miR-584	11	4,310	7,215	11,972	16,773	24,043	27,836	25,649	31,391	0,349	0,980
hsa-miR-365*	11	4,953	8,061	17,917	24,894	32,254	47,165	46,263	52,377	0,000	0,000
hsa-miR-10a*	11	4,654	6,630	11,497	15,744	21,216	28,187	25,558	28,379	0,627	0,690
hsa-let-7e	11	4,901	8,942	11,972	18,557	23,650	28,127	28,229	32,749	0,359	0,655
hsa-miR-19b-1*	11	5,041	7,473	12,820	18,067	23,937	29,955	23,698	30,661	0,196	0,729
hsa-miR-1181	11	5,162	8,081	11,764	18,566	23,582	33,009	26,642	32,115	0,080	0,857
hsa-miR-361-5p	11	5,637	6,981	14,305	17,532	23,468	27,021	23,458	27,941	0,492	0,482
hsa-miR-139-3p	11	5,921	7,137	14,358	18,806	22,681	28,343	26,387	29,710	0,435	0,889
hsa-miR-590-5p	11	6,369	7,900	15,042	18,504	25,620	26,744	25,441	31,832	0,305	0,998

hsa-miR-628-5p	11	6,247	8,418	17,495	18,043	27,319	26,170	27,849	24,147	0,219	0,528
hsa-miR-600	11	5,137	6,782	16,276	15,986	22,129	24,643	26,150	30,213	0,990	0,915
hsa-miR-520b	11	8,726	8,646	22,720	18,200	26,389	25,445	26,718	27,657	0,352	0,729
hsa-miR-2053	11	5,783	8,101	15,667	18,297	24,999	29,392	30,708	32,921	0,166	0,444
hsa-miR-574-5p	11	6,271	7,008	15,305	16,012	22,661	26,525	28,014	30,702	0,652	0,860
hsa-miR-135a*	11	6,395	7,988	17,966	18,593	23,958	26,584	29,402	28,248	0,486	0,962
hsa-miR-491-3p	11	6,042	7,887	14,141	16,808	21,269	22,290	21,334	27,844	0,568	0,334
hsa-miR-520d-3p	11	5,238	6,159	16,135	14,139	22,621	18,996	25,099	23,406	0,358	0,294
hsa-miR-619	11	5,083	6,331	16,928	15,750	23,917	27,428	29,873	23,071	0,400	0,605
hsa-miR-138-2*	11	6,100	5,684	16,745	14,985	23,776	22,331	25,918	21,762	0,914	0,251
hsa-miR-498	11	6,296	7,881	18,388	19,277	26,051	27,380	33,808	27,137	0,223	0,658
hsa-miR-505	11	5,972	6,660	15,667	15,328	24,265	24,074	28,014	26,358	0,766	0,729
hsa-miR-766	11	6,397	7,202	18,333	18,492	27,192	31,200	31,998	30,754	0,036	0,508
hsa-miR-150	11	7,124	7,188	18,147	18,117	26,092	26,978	28,928	29,612	0,249	0,877
hsa-miR-581	11	5,845	6,883	15,768	16,544	23,262	21,242	24,955	23,727	0,690	0,303
hsa-miR-1296	11	6,819	8,357	19,643	19,429	27,401	31,500	36,318	27,912	0,029	0,405
hsa-miR-146a	11	4,924	5,006	13,947	11,908	24,727	22,921	25,046	23,672	0,863	0,306
hsa-miR-603	11	4,289	7,884	13,119	16,315	23,828	27,557	26,995	25,312	0,397	0,552
hsa-miR-27b	11	5,123	6,622	14,563	14,618	20,065	23,080	21,064	23,287	0,519	0,123
hsa-miR-1908	11	5,983	7,533	17,425	19,327	23,194	25,114	35,281	35,081	0,770	0,117
hsa-miR-938	11	6,758	7,335	17,807	20,450	23,978	30,317	28,020	34,761	0,173	0,508
hsa-miR-142-5p	11	4,652	6,343	13,982	16,858	20,514	23,008	23,505	28,430	0,564	0,523
hsa-miR-1183	11	6,040	8,299	13,127	17,143	22,125	26,180	24,605	27,685	0,771	0,551
hsa-miR-16	11	6,483	8,619	14,318	19,154	25,933	23,726	22,487	34,652	0,591	0,989
hsa-miR-1300	11	5,800	7,348	16,946	19,154	26,064	25,056	26,101	34,743	0,423	0,668
hsa-miR-567	11	6,123	6,684	17,006	14,854	24,610	23,374	24,705	29,031	0,815	0,672
hsa-miR-211	11	6,438	8,054	16,537	17,567	24,829	27,611	31,197	32,968	0,299	0,406
hsa-miR-323-5p	11	5,633	8,380	11,795	15,822	17,004	25,020	28,086	29,976	0,402	0,923
hsa-miR-147	11	5,403	7,796	11,523	15,767	17,898	25,440	29,761	30,283	0,546	0,737
hsa-miR-889	11	5,625	8,392	11,519	15,529	22,331	24,470	29,089	31,380	0,985	0,699
hsa-miR-190b	11	3,150	7,709	9,210	17,257	18,671	20,476	21,354	24,585	0,172	0,176
hsa-miR-28-5p	11	5,932	6,920	19,569	17,623	27,834	22,962	27,405	33,159	0,459	0,691
hsa-miR-144*	11	4,219	6,696	13,709	13,880	21,236	18,493	17,821	27,753	0,208	0,165
hsa-miR-30c	11	6,061	7,049	16,077	18,824	24,290	22,982	24,098	33,195	0,917	0,996
hsa-miR-1272	11	5,787	7,011	15,173	16,084	21,574	21,050	23,814	30,126	0,459	0,691
hsa-miR-489	11	5,568	6,448	11,576	14,456	19,905	20,140	21,854	28,310	0,228	0,396
hsa-miR-423-5p	11	5,454	7,171	13,521	19,169	27,997	33,082	39,356	38,305	0,011	0,016
hsa-miR-421	11	4,343	5,401	10,611	12,347	20,546	21,928	24,785	23,847	0,443	0,301
hsa-miR-449a	11	5,202	7,185	12,020	18,338	25,324	27,230	23,442	31,327	0,289	0,766
hsa-miR-487a	11	6,838	6,290	21,560	14,400	26,597	24,470	23,756	30,504	0,428	0,720
hsa-miR-483-5p	11	6,256	7,527	16,727	16,855	21,952	26,159	28,728	24,645	0,798	0,641
hsa-miR-1266	11	6,622	9,288	21,088	18,386	27,458	31,934	31,363	37,746	0,024	0,157
hsa-miR-130a	11	5,646	7,455	16,694	14,292	20,562	25,889	26,387	25,439	0,964	0,514
hsa-let-7f-1*	11	4,388	6,834	13,400	13,380	17,783	23,060	18,567	27,878	0,291	0,198
hsa-miR-1252	11	5,303	7,166	16,386	15,497	22,314	30,367	29,463	32,162	0,284	0,599
hsa-miR-888*	11	5,070	6,837	15,621	12,707	19,250	24,022	25,382	29,214	0,535	0,749
hsa-miR-9*	11	5,035	6,765	15,201	15,151	20,879	26,536	24,145	27,115	0,897	0,471
hsa-miR-378*	11	4,225	5,900	8,735	11,230	17,496	15,783	19,256	23,130	0,016	0,076
hsa-miR-1233	11	4,673	7,685	12,083	16,631	24,476	22,684	28,534	30,167	0,933	0,862
hsa-miR-218-1*	11	6,706	5,629	15,730	12,380	22,597	18,528	24,135	21,305	0,314	0,157
hsa-miR-885-5p	11	6,189	6,446	14,630	13,218	20,819	18,967	22,425	22,968	0,211	0,156
hsa-miR-324-5p	11	5,908	6,625	13,900	15,171	20,238	18,848	24,966	23,814	0,169	0,309
hsa-miR-1278	11	5,874	7,157	13,029	16,202	22,331	21,313	27,438	31,697	0,579	0,821
hsa-miR-10b*	11	6,373	7,511	16,481	17,161	25,889	25,224	33,691	35,530	0,423	0,152
hsa-miR-129-5p	11	5,326	7,436	14,934	15,986	22,202	21,770	24,573	28,043	0,620	0,577
hsa-miR-183	11	6,085	6,971	23,701	19,259	39,184	28,343	40,123	45,128	0,000	0,001
hsa-miR-1912	11	4,914	6,293	16,084	14,760	23,974	15,393	22,963	26,969	0,191	0,380
hsa-miR-335	11	5,152	6,629	15,959	16,498	22,758	19,731	25,125	30,533	0,445	0,848
hsa-miR-300	11	4,508	7,152	13,835	17,246	23,408	21,176	25,687	27,245	0,701	0,603
hsa-miR-338-5p	11	4,528	6,837	11,706	15,082	20,138	19,930	23,140	26,520	0,230	0,362
hsa-miR-652	11	4,098	7,278	13,620	18,336	25,251	22,183	29,112	35,245	0,894	0,395
hsa-miR-302a	11	4,623	6,593	12,433	13,646	19,433	16,378	19,957	26,458	0,051	0,196

6.2.3. Screen result raw data: positive and negative controls

		antibody concentration [µg/mL]							
		day1		day2		day3		day4	
Plate	sample	#1	#2	#1	#2	#1	#2	#1	#2
1	siLC-104	1,23	1,63	2,17	2,70	4,20	5,56	7,20	5,10
1	siLC-104	1,14	1,60	2,02	2,47	3,88	6,27	6,71	4,99
2	siLC-104	0,76	0,77	2,06	1,49	4,47	2,99	8,37	5,98
2	siLC-104	0,85	0,92	1,97	1,69	4,61	3,05	8,22	5,63
3	siLC-104	0,64	0,99	1,52	2,01	4,10	5,06	7,16	7,86
3	siLC-104	0,72	1,03	1,38	1,87	3,48	4,41	5,34	6,56
4	siLC-104	0,91	1,26	1,82	2,39	5,28	7,96	7,02	17,20
4	siLC-104	0,72	1,23	1,11	2,34	4,78	4,64	5,75	18,47
5	siLC-104	1,11	1,99	2,23	2,96	5,35	5,69	12,12	8,62
5	siLC-104	0,97	1,81	2,03	2,98	4,73	4,97	9,54	7,32
6	siLC-104	0,83	1,44	1,38	2,92	2,63	4,65	9,93	8,99
6	siLC-104	0,89	1,86	1,44	2,57	2,76	4,37	9,19	8,44
7	siLC-104	1,02	0,84	1,57	1,96	3,38	3,98	10,16	10,53
7	siLC-104	1,04	0,88	1,75	2,18	2,93	4,99	9,58	10,39
8	siLC-104	1,28	1,30	2,55	2,32	4,39	5,21	8,27	8,27
8	siLC-104	1,21	1,27	2,48	1,85	5,39	4,98	6,78	8,00
9	siLC-104	0,87	1,14	1,58	3,05	3,60	4,35	8,28	8,90
9	siLC-104	0,84	0,96	1,61	3,42	3,68	3,72	5,79	8,25
10	siLC-104	0,97	1,11	2,55	2,39	4,89	5,41	9,02	9,47
10	siLC-104	0,95	1,10	2,48	2,26	5,12	5,36	9,71	6,73
11	siLC-104	1,18	0,97	1,28	1,90	4,84	3,94	7,73	6,14
11	siLC-104	1,07	0,92	1,35	2,16	5,07	4,39	7,78	7,67

		antibody concentration [µg/mL]							
		day 1		day 2		day 3		day 4	
Platte	sample	#1	#2	#1	#2	#1	#2	#1	#2
1	mock	6,60	8,33	20,30	19,73	22,38	30,56	33,37	34,94
1	mock	6,17	9,36	17,11	16,48	20,89	25,77	29,75	23,71
1	siLacZ	5,54	8,87	17,05	15,73	19,70	28,73	27,37	19,77
1	siLacZ	5,50	9,55	15,38	14,93	20,09	22,80	28,91	21,60
1	mean of samples	5,06	9,36	15,32	17,29	21,23	25,65	25,57	29,56
1	median of samples	5,20	9,52	15,37	17,25	20,96	24,32	25,17	28,40
2	mock	6,14	5,72	16,02	17,81	26,94	26,13	32,97	30,88
2	mock	4,78	4,99	14,32	15,61	20,74	20,01	26,35	25,57
2	siLacZ	5,19	4,91	15,67	14,68	20,88	22,81	28,41	21,91
2	siLacZ	5,11	4,46	13,30	13,51	20,92	20,74	23,80	26,18
2	mean of samples	5,75	4,27	14,76	12,74	21,97	18,42	25,18	21,91
2	median of samples	5,82	4,31	14,82	13,14	21,94	18,05	24,96	21,60
3	mock	3,33	4,11	13,59	12,79	19,63	24,99	30,92	26,44
3	mock	2,82	3,77	10,53	11,28	17,07	19,96	21,09	21,95
3	siLacZ	2,92	4,80	12,94	12,15	17,30	20,89	19,70	21,77
3	siLacZ	2,91	4,01	10,63	10,63	16,68	19,99	19,02	21,75
3	mean of samples	3,54	4,19	12,30	10,79	17,89	19,34	23,09	20,87
3	median of samples	3,54	4,10	12,36	10,95	17,64	18,63	22,69	20,63
4	mock	4,77	6,53	17,86	22,34	26,48	32,67	28,57	48,24
4	mock	4,42	6,16	14,06	25,21	22,82	25,73	23,35	43,66
4	siLacZ	4,02	6,15	14,35	18,18	22,41	28,41	25,07	50,95
4	siLacZ	3,44	6,47	11,01	18,73	19,41	26,79	21,25	43,80
4	mean of samples	3,53	7,19	11,88	18,61	20,42	31,38	22,76	34,96
4	median of samples	3,51	7,08	11,70	18,18	20,35	31,69	22,46	35,27
5	mock	5,67	7,80	16,48	22,81	26,70	27,07	40,11	41,48
5	mock	5,04	8,02	14,41	21,80	23,34	25,65	29,16	32,59
5	siLacZ	4,58	7,54	14,09	18,23	23,32	23,72	32,32	30,49
5	siLacZ	5,40	7,54	14,50	20,44	24,03	24,21	29,34	34,35
5	mean of samples	5,93	7,49	15,58	19,58	25,31	23,53	28,87	30,64
5	median of samples	5,90	7,57	15,38	19,40	24,82	22,95	28,48	29,29
6	mock	3,09	10,72	10,78	19,20	19,08	30,35	32,24	30,89
6	mock	2,73	5,08	10,53	15,44	15,65	24,37	25,56	31,53
6	siLacZ	3,84	6,39	10,89	19,99	18,69	24,47	27,88	29,64
6	siLacZ	3,06	6,37	9,92	17,92	18,01	25,03	28,66	28,62
6	mean of samples	3,78	5,41	9,73	18,88	18,84	26,67	24,20	32,67
6	median of samples	3,56	5,35	9,62	18,82	18,41	26,34	23,76	32,27
7	mock	3,32	6,27	12,28	16,86	21,92	26,50	28,47	25,97
7	mock	3,79	6,23	11,39	13,44	19,13	21,39	27,07	23,45
7	siLacZ	3,96	5,12	11,00	14,73	22,48	22,25	31,40	22,19
7	siLacZ	3,51	5,22	11,67	13,48	22,41	20,83	31,96	31,11
7	mean of samples	3,72	6,68	10,62	13,89	17,50	22,85	25,37	27,80
7	median of sample	3,75	6,69	10,48	14,06	17,78	22,48	25,20	28,01
8	mock	7,28	6,78	16,09	18,96	26,01	34,06	30,64	34,34
8	mock	6,77	5,56	15,43	18,81	22,55	25,12	26,29	28,91
8	siLacZ	6,91	6,86	16,88	17,75	24,79	25,54	25,51	30,47
8	siLacZ	6,95	-0,05	14,40	15,86	20,05	22,35	23,83	24,50
8	mean of samples	6,92	6,64	16,96	19,83	26,10	24,96	28,69	29,58
8	median of samples	6,86	6,61	17,12	19,22	25,14	24,47	27,86	28,98
9	mock	3,84	6,50	12,36	19,34	22,09	25,69	25,46	31,68
9	mock	4,90	5,95	13,91	20,18	20,69	21,65	25,19	29,33
9	siLacZ	3,90	5,18	11,93	16,86	19,32	20,17	24,25	26,10
9	siLacZ	3,59	5,01	9,96	15,17	18,37	19,66	21,05	31,52
9	mean of samples	4,43	8,37	14,12	19,33	20,46	24,86	24,03	32,75
9	median of samples	4,48	8,53	14,03	19,61	19,93	24,68	24,11	32,39
10	mock	5,78	7,54	20,85	19,94	27,05	32,12	29,68	29,77
10	mock	4,97	6,82	17,26	19,61	24,14	29,91	31,37	26,82
10	siLacZ	5,28	6,17	17,11	14,92	24,53	28,78	31,44	26,96
10	siLacZ	4,63	6,04	12,41	16,54	21,80	31,33	27,61	29,01
10	mean of samples	4,91	6,81	14,96	16,31	22,41	27,13	30,58	30,78
10	median of samples	4,94	6,74	14,92	17,07	21,99	26,72	30,22	30,46
11	mock	6,82	8,10	19,82	21,05	28,57	23,47	31,28	31,27
11	mock	6,91	6,01	17,49	14,87	26,00	18,69	25,03	25,55
11	siLacZ	8,19	7,60	18,04	19,10	23,95	22,00	26,60	25,71
11	siLacZ	5,83	5,92	13,99	15,70	24,35	18,54	25,65	26,83
11	mean of samples	5,45	7,15	14,87	16,61	23,25	24,92	26,51	29,51
11	median of samples	5,45	7,15	14,56	16,81	23,16	24,64	25,56	29,11

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CV

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