# Dharmacon™ part of GE Healthcare



# Recent Tools for MicroRNA Functional Analysis

Aaron Chen, Ph.D. Field Application Scientist, Level Biotechnology Inc.



# **Mini-seminar topics**

# (1) Basic RNAi tools

~ 60 min (siRNA, miRNA and shRNA)

# (2) Long noncoding RNA

~ 30 min (siRNA and Solaris assay)

# (3) Gene over-expression

~ 30 min (ORF/cDNA clones)

# (4) miRNA workflow and application

~ 30min (miRNA )

# (5) CRISPR/Cas9 Genome Editing

~ 30min (CRISPR)





# Curriculum Vitae

#### EDUCATION AND POSITIONS HELD

- 1. Ph.D., Microbiology, National Taiwan University
- 2. Post-Doctoral Fellow, National Institute of Cancer Research, NHRI
- 3. Post-Doctoral Fellow, ABRC, Academia Sinica
- 4. Field Application Scientist, Level Biotechnology Inc

#### HONORS:

「生策會第八屆國家新創獎」-傑出學研技術

#### **PUBLICATIONS:**

#### A. Journals

1. Yu-Ching Teng, Cheng-Feng Lee, Ying-Shiuan Li, <u>Yi-Ren Chen</u>, Pei-Wen Hsiao, Meng-Yu Chan, Feng-Mao Lin, Hsien-Da Huang, Yen-Ting Chen, Yung-Ming Jeng, Qing Yan, Ming-Daw Tsai, and Li-Jung Juan. Histone Demethylase Retinoblastoma Binding Protein-2 Promotes Lung Tumorigenesis and Cancer Metastasis.

#### Cancer Res, 2013.

2. Chih-Hung Hsu, Kai-Lin Peng, Ming-Lun Kang, <u>Yi-Ren Chen</u>, Yu-Chih Yang, Chin-Hsien Tsai, Chi-Shen Chu, Yung-Ming Jeng, Yen-Ting Chen, Feng-Mao Lin, Hsien-Da Huang, Yun-Yuh Lu, Yu-Ching Teng, Shinn-Tsuen Lin, Ruo-Kai Lin, Fan-Mei Tang, Sung-Bau Lee, Huan Ming Hsu, Jyh-Cherng Yusend email, Pei-Wen Hsiaosend email, Li-Jung Juan. TET1 Suppresses Cancer Invasion by Activating the Tissue Inhibitors of Metalloproteinases. **Cell Reports, 2012.** 

3. Wu CC, Liu MT, Chang YT, Fang CY, Chou SP, Liao HW, Kuo KL, Hsu SL, <u>Chen YR</u>, Wang PW, Chen YL, Chuang HY, Lee CH, Chen M, Wayne Chang WS, Chen JY. Epstein-Barr Virus DNase (BGLF5) induces genomic instability in human epithelial cells. **Nucleic Acids Res, 2010.** 

4. <u>Chen YR</u>, Liu MT, Chang YT, Wu CC, Hu CY, Chen JY. Epstein-Barr virus latent membrane protein 1 represses DNA repair through the PI3K/Akt/FOXO3a pathway in human epithelial cells. **J Virol, 2008.** 



### **Aaron Chen**

5. Liu MT, Chang YT, Chuang YC, <u>Chen YR</u>, Lin CS, Chen JY. Epstein-Barr virus latent membrane protein 1 represses p53-mediated DNA repair and transcriptional activity. **Oncogene, 2005.** 

6. Liu MT, <u>Chen YR</u>, Chen SC, Hu CY, Lin CS, Chang YT, Wang WB, Chen JY. Epstein-Barr virus latent membrane protein 1 induces micronucleus formation, represses DNA repair and enhances sensitivity to DNA-

damaging agents in human epithelial cells. Oncogene, 2004.

#### B. Books

 Yi-Ren Chen, Pei-Wen Hsiao, Feng-Ming Lin, Guan-James Wu, and Chin-Hsien Tsai. Evidence-based Anticancer Herbal Medicine for Prostate Cancer. Evidence-based Anticancer Complementary and Alternative Medicine: Herbal Medicine for Various Cancers. Springer. 2011.





# Outline

- Background of miRNAs
- Workflow and Tools for miRNA researches
- Applications of miRNAs tools





# Outline

# Background of miRNAs

# Workflow and Tools for miRNA researches

# Applications of miRNAs tools





# Non-coding RNAs (ncRNA)





# **Classes of Non-coding RNAs**

### **1. Housekeeping RNA**

→ tRNA, rRNA, snoRNA, snRNA
 → Translation, ribosome mature, RNA splicing...etc

### 2. Small regulatory RNA

→ microRNA (21-24 nt) , piRNA (26-31 nt)

 $\rightarrow$  development and disease

# 3. Long noncoding RNA (IncRNA)

- → ≥200 nt
- → Most prevalent class of ncRNA
- → XIST, HOTAIR...
- $\rightarrow$  development and disease





De Sacco et. al. Int. Journal of Mol. Sci. (2012), 13.
 Taft et al., J Pathol 2010; 220: 126–139, Non-coding7RNAs: regulators of disease



# **Endogenous microRNA Pathway**



Your Gene Company part of GE Healthcare



# **Endogenous microRNA Pathway**







# Structure of transient microRNA duplex





# **Endogenous microRNA Pathway**



# **MicroRNA Biology**

- microRNAs genes represent 2-3% of genome (~2000 miRNAs identified in humans)
- Approximately 2/3 of human protein coding genes are regulated by microRNAs
- Each microRNA regulates ~200 target genes.
- Each gene can be regulated by multiple microRNAs.
- microRNAs are responsible for proliferation, apoptosis and differentiation
- microRNAs are critical in normal development and etiology of disease





# **Number of miRNA-related Publications**



- ※ 1993, First miRNA lin-4 in C. elegans
- % 2000, miRNA let-7 in C. elegans → in human
- $\% \hspace{0.2cm} 3900 \hspace{-0.2cm} \rightarrow \hspace{-0.2cm} 5000 \hspace{-0.2cm} \rightarrow \hspace{-0.2cm} 6500 \hspace{-0.2cm} \rightarrow \hspace{-0.2cm} 7500$

- \* Since then, miRNA has been reported:
- 1. Targeted most development related genes
- 2. Neuronal development and synapse formation.
- 3. Stem cells maintenance and differentiation.
- 4. Cancer as onco-miR.
- 5. Heart disease.
- 6. Viral infections process.



# Outline

# Background of miRNAs

Workflow and Tools for miRNA researches

# Applications of miRNAs tools





# Strategies for a successful miRNA research





# Strategies for a successful miRNA research







### http://www.microrna.org

microRNA.org - Targets and Expression Predicted microRNA targets & target downregulation scores. Experimentally observed expression patterns. August 2010 Release Last Update: 2010-11-01 [ release notes ]					
miRNA Target mRNA	miRNA Expression Downloads FAQ				
You are currently searching:	Display Options				
<ul> <li>Homo sapiens</li> <li>FOXO3</li> <li>AK301304 NM_201559</li> <li>AK303933</li> </ul>	View target sites of conserved miRNAs with good mirSVR scores  Selected miRNAs:				
miRNA Stats:	fou may add additional mixivAs to the box above.				
<ul> <li>Homo sapiens: 1100</li> <li>Mus musculus: 717</li> <li>Rattus norvegicus: 387</li> <li>Drosophila melanogaster: 186</li> <li>Caenorhabditis elegans: 233</li> </ul>	Image: State of the sector				
Query Target Sites:					
Displayed miRNAs ordered by sum of mirSVR scores:	FOXO3 forkhead box 03				
<sup>·</sup> hsa-miR-217 4207 4843	mik-223 1 Aggaucacugaggaagggggaagugggcaargcagacccucaracugacacaagacuacagagaaracccuuugc 75				
<ul> <li>nsa-miR-182 56 895</li> <li>hsa-miR-23a 4442</li> <li>hsa-miR-23b 4442</li> <li>hsa-miR-495 376 3383</li> <li>hsa-miR-381 4395</li> </ul>	mir-182 76 CAARUCUGCUCUCAGCARGUGGACAGUGAUACCGUUUACAGCUUUACAGCUUUGUGARUCCCACGCCAUUUUUCCU 150 mir-520c-3p mir-520b				
<ul> <li>hsa-miR-590-5p 1186</li> <li>hsa-miR-300 4395</li> <li>hsa-miR-340 2021</li> <li>hsa-miR-212 149</li> </ul>	miR-302e miR-302d miR-302c miR-302a miR-373				
<sup>-</sup> hsa-miR-223 28 <sup>-</sup> hsa-miR-132 151	miR-322 miR-302b miR-212 miR-302b 151 AACCCAGCAGAGACUGUUAAUGGCCCCUUACCCUGGGUGAAGCACUUACCCUUGGAACAGAACUCUAAAAAGUAU 225				





### genes.mit.edu/targetscan

TargetScanHuman         Prediction of microRNA targets       Release 6.2: June 2012					
Search for predicted microRNA targets in mammals	[Go to TargetScanMouse] [Go to TargetScanWorm] [Go to TargetScanFly] [Go to TargetScanFish]				
1. Select a species Human •					
AND					
2. Enter a human Entrez Gene symbol (e.g. "LIN28A")					
AND/OR					
3. Do one of the following:					
Select a broadly conserved* microRNA family miR-128/128ab					
Select a conserved* microRNA family Conserved microRNA families					
Select a poorly conserved microRNA family Poorly conserved microRNA families     Note that these families also include small RNAs that have been misclassified as miRNAs.					
Enter a microRNA name (e.g. "mmu-miR-1") e.g has-miR-128					
Submit Reset					
* broadly conserved = conserved across most vertebrates, usually to zebrafish conserved = conserved across most mammals, but usually not beyond placental mammals					





### http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/



Select a Genome Алу 👻	Enter miRNA id	
	Enter Gene Name e.g. AKT	1
	Enter EnsEMBL identifier	
	Select a GO class Function	
	Enter GO Term	
	ø by phrase	
	O AND OR	
Keyword Search		
	Search	





Hit information for ENST00000349310

Gene Name	AKT1							
Transcript	ENST00000349310 @							
Gene	ENSG00000142208 @							
Description	RAC-alpha serine/threonine-protein kinase (EC 2.7.11.1) (RAC-PK-alpha) (Protein kinase B) (PKB) (C-AKT). [Source:Uniprot/SWISSPROT;Acc:P31749]							
	Mouse over the targets for more information							
	100 200 300 400 500 600 700 800							
Alignment View [HTML] [Java]	H. Sapiens       ENSTOOG      GGC							
	Rfam ID Score Energy Base P Poisson P Org P Start End Alignment							
	hsa-miR-501-3p 17.3212 -23.25 7.223040e-03 7.197020e-03 7.197020e-03 76 97							
	CUAUCUCACGUCUGGUCCCAGA hsa-miR-504 17.2211 -29.76 2.168600e-02 2.145250e-02 2.145250e-02 570 591    : :  ::  :: :      :  GATGGGGGATGGGCCAGGGTTT							
	hsa-miR-502-3p 17.0209 -20.45 1.139990e-02 1.133520e-02 1.133520e-02 76 97 I:							





# Strategies for a successful miRNA research





# miRNA qPCR Detection Assay







# Strategies for a successful miRNA research







# Whole miRNA qPCR panels





# Strategies for a successful miRNA research







# **Endogenous microRNA Pathway**







# **Biological tools for studying microRNA**







# **Biological tools for studying microRNA**







# **Biological tools for studying microRNA**



Inhibit miRNA function

Overexpress miRNA level

van Rooij E et al. Circulation Research. 2008;103:919-928



# **Biological tools for studying miRNA**







# Structure of transient microRNA duplex





## **Endogenous microRNA Pathway**





Gene Company part of GE Healthcare

# Using miRNA mimics to investigate miRNA-regulated gene

#### microRNA gain of function experiments Control Experimental Control Gene A \*\*\* Gene B \*\*\* +++ Gene C endogenous +++ (+) miRNA X mimics miRNA X Gene D \*\*\* \*\*\* Gene E +++ Gene F +++ +++ Experimental

Gain-of-function phenotype





# **Design of miRNA mimics**

### miRBase Database

at University of Manchester

### http://www.mirbase.org/

mR8sse	
miRBase	MANCHESTER 1824
Home Search Browse Help Download Blog Submit	Search
How - Fourier Burling Polymon Luna - Fooma	
Latest miRBase blog posts       miRBase website "at risk", Thu 10th to Fri 18th Nov       By sam (November 8,	miRNA count: 18226 entries 2011) <u>Release 18</u> : November 2011
Due to server room refurbishment, the mRBase website may experience some instability between Thu 10th and Fri 18th November 2011. The plan is for just 30 minutes or so down time at either end of that period, but the website should be considered "at risk" throughout. Apologies for any inconvenience.	Search by miRNA name or keyword
mittage 1st released After a little more pain than usual, miRBase 18 is finally released. The database contains 18226 entries representing hairpin precursor miRNAs, expressing 21643 mature miRNA products, in 168 species. That represents 1488 new hairpin sequences and 1929 nove mature conducts. The full README file is available on the ETP site As provin sky discussed, we have (	Go Example
	Download published miRNA data
miRBase: the microRNA database	Download page   ETP site
miRBase provides the following services:	This site is featured in:
<ul> <li>The miRBase database is a searchable database of published miRNA sequences and annotation. Each entry in the miRBase Sequence database represents a predicted hairpin portion of a miRNA transcript (term mir in the database), with information on the location and sequence of the mature miRNA sequence (termed miR). Both hairpin and mature sequences are available for <u>searching</u> and <u>browsing</u>, and entries can be optimized by usang knowled in a database. All consistences and applications and sequences are available for <u>searching</u> and <u>browsing</u>, and entries can be optimized by usang knowled in the database.</li> </ul>	NetWatch - Science 303:1/41 (2004) hed Highlights, Web watch - Nature Reviews Genetics 5:244 (2004)
<ul> <li>The miRBase Registry, rovides miRNA gene hunters with unique names for novel miRNA genes prior to publication of results. Visit the help pages for more information about the naming service.</li> <li>The miRBase Targets database and pipeline has been retranded as microCosm, and is now hosted at the EBI. The microCosm resource continues to be maintained by the Enricht group, miRBase currently link TargetScan and Pictura, and aims to provide at more extensive target prediction agregation service in the future.</li> </ul>	s miRNAs to targets predicted by microCosm,
To receive email notification of data updates and feature changes please subscribe to the miRBase announcements mailing list. Any queries about the website or naming service should be directed at mirbase@manc	hester.ac.uk.
miRBase is hosted and maintained in the Faculty of Life Sciences at the University of Manchester with funding from the BBSRC, and was previously hosted and supported by the Wellcome Trust Sanger Institute.	
References	
If you make use of the data presented here, please cite the following articles in addition to the primary data sources: miRBase: integrating microRNA annotation and deep-sequencing data. Kozomara A, Griffiths-Jones S.	
NAR 2011 39(Database Issue):D152-D157 miRBase: tools for microRNA genomics.	
Griffiths-Jones S, Saini HK, van Dongen S, Enright AJ. NAR 2008 36(Database Issue):D154-D158	
mIRBase: microRNA sequences, targets and gene nomenclature. Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ. NAR. 2006 34(Database Issue):D140-D144	
The microRNA Registry. Griffiths-Jones S. NAR 2004 32(Database Issue):D109-D111	
The following publications provide guidelines on miRNA annotation:	
A uniform system for microRNA annotation. Ambros V, Bartel D, Bartel DP, Burge CB, Carrington JC, Chen X, Dreyfuss G, Eddy SR, Griffiths-Jones S, Marshall M, Matzke M, Ruvkun G, Tuschl T. RNA 2003 9(3):277-279	
Criteria for annotation of Dath MicroBNAs. Meyers BC, Axtell MJ, Bartel B, Bartel DP, Baulcombe D, Bowman JL, Cao X, Carrington JC, Chen X, Green PJ, Griffiths-Jones S, Jacobsen SE, Mallory AC, Martienssen RA, Poethig RS, Qi Y, Vaucheret H, Voinnet O Plant Cell. 2008 20(12):3186-3190	Watanabe Y, Weigel D, Zhu JK.



# Functionality of miRNA mimics (by target gene)





# Functionality of miRNA mimics (by Reporter)

Stemloop Structure	5'u u gagcguu          uucgcga 3'c u	uuc gga ggggccg            cuu cucuggc uuu	uag cu cacugu gay IIIIII II gugaca cu caa	u gaggu u       cuuua a c
5' mature microRNA	hsa-miR-128-1-5p	MIMAT0026477	15-CGGGGCCGUAGC	ACUGUCUGAGA-37
3' mature	nsa-mik-126-5p	MIMA10000424	50-UCACAGUGAAC	LGGUCUCUUU-70

- 1. Insert a <u>complementary target</u> <u>sequence of a microRNA 5p or</u> <u>3p in the 3' UTR of the Renilla</u> luciferase gene.
- 2. Firefly luciferase gene serves as a normalizing control.
- 3. Co-transfect this dual luciferase plasmid with mimic, inhibitor, or control molecules.




### Functionality of miRNA mimics (by Reporter)







### miRNA mimics design-1





### 2<sup>-</sup>O-Methyl-RNA

- show the same behavior as DNA
- protect against nuclease degradation
- efficient in blocking targeting RNA functions
- can be combined with phosphothioate oligonuclotides for further stabilization





### miRNA mimics design-2





### Active strand or Passenger strand ?



### Overexpress miRNAs 5P? or 3P? Loading

van Rooij E et al. Circulation Research. 2008;103:919-928



### miRNA mimics design-3





### miRNA mimics design : Functionality



Function for 11 human microRNAs was assayed in HeLa and HepG2 cells using a dual Luciferase assay (normalized to the control - no mimic). 10 nM mimic at 48 hours





### **Biological tools for studying miRNA**







# Using miRNA inhibitors to investigate miRNA-regulated gene





### miRNA inhibitors design





46

### **Potency and Longevity of Hairpin Inhibitors**





### **Biological tools for studying miRNA**







### microRNA-based scaffolds



Flanking regions

- Well known microRNA-based scaffold (ex. miR-30...)
- Contains Drosha and Dicer sites
- Maintain accurate processing
- Less disruptive use of more "natural" microRNA expression cassette
- High functionality
- Minimal activity of passenger strand



### Some background on Lenti-virus



- Family: Retroviridae
- Subfamily: Lentivirus
- Species: HIV type 1
- Integrates genome into <u>dividing</u> and <u>non-dividing cells</u>
- Mostly intronic integration
- Lentiviruses use host cell machinery to assemble proteins and encapsulate RNA genome
- Infective virions are released to repeat lifecycle





### Viral Packaging and sh-miRNA vector design



### Basic anatomy of a lentiviral miRNA plasmid





- <u>microRNA-based scaffold</u> for consistent processing of mature microRNA
- <u>Several promoters (7)</u> for efficient constitutive expression
- <u>Florescent proteins (2)</u> expression to visualize expression
- <u>RRE</u> enhances titer by increasing packaging efficiency of full-length viral genomes
- <u>WPRE</u> enhances the stability and translation of transcripts





### Importance Of Promoter Activity For Lentiviral miRNA Expression



OVCAR-8 (human ovarian adenocarcinoma)



Jurkat (human T cell leukemia)



A549 (human lung adenocarcinoma)









HEK293T (human embryonic kidney adenocarcinoma)

#### Several cell lines $\rightarrow$ MOI = 15 $\rightarrow$ 72 hours





### **Biological tools for studying miRNA**







### microRNA Sponges



Modified picture from Nature Reviews Drug Discovery 9, 775-789 (October 2010)



### Strategies for a successful miRNA research





### Strategies for a successful miRNA research





### microRNA phenotypic screening

#### Gain-of-function screen







### Phenotype ?

#### Loss-of-function screen





microRNA Inhibitor Library



Phenotype ?



## Outline

### Background of miRNAs

Workflow and Tools for miRNA researches

### Applications of miRNAs tools





### Strategies for a successful miRNA research







### Phenotype observation (ex. EMT in Cancer)



Gene Company part of GE Healthcare

### Model Cell Lines : MCF7 and MDA-MB-231



- F-Actin : cytoskeleton protein
- E-Cadherin : marker for epithelial cells
- Vimentin : marker for mesenchymal cells





### Strategies for a successful miRNA research







### miRNA website-1





### miRNA website-2

	Rfam ID	Score	Energy	Base P	Poisson P	Org P	Start	End	Alignment
niprot/SWISSPROT;Acc:P37275]	hsa-miR-148b*	17.2006	-22.8	1.782740e-02	1.766940e-02	1.766940e-02	1558	1581	CGGACUCACAUAUUGUCUUGAA
	hsa-miR-374a*	17.1061	-21.6	2.652490e-02	2.617620e-02	2.617620e-02	739	761	UUAAUGUUAU-GUUAGACUAUUC         : :           ACTAACAATGTTAATCTGATAAG
700 800	hsa-miR-125b-1*	16.7281	-28.26	1.094780e-02	1.088810e-02	1.088810e-02	895	916	CAGGGUUCUCGGAUUGGGC ::::!!!!:::!!!!!!! ::TTTCAAGGCTCTAACCCG
	hsa-miR-600	16.564	-23.35	3.200810e-02	3.150130e-02	3.150130e-02	859	882	UCGUUCCGAGAACA-GACAUUCA        :            AGCAAGACCTGTGTGCTGTAAGT
	hsa-miR-200c	16.564	-26.86	3.194860e-02	8.856540e-04	8.856540e-04	1299	1320	GGUAGUAAUGGGCCGUCAUAAU :       :!!!!!!!!!!! :TCTTCA-AACCTGGCAGTATTA
	hsa-miR-200c	16.4699	-16.71	3.471230e-02	8.856540e-04	8.856540e-04	1228	1250	UAGUAAUGGGCCGUCAUAAU   :     :        ATTTTACCTATCAGTATTA
	hsa-let-7f-2*	16.4445	-21.28	3.385890e-02	3.329210e-02	1.338350e-02	71	92	CCUUUCUGUCAUCUGACAUAU
	hsa-miR-200b	16.4445	-21.8	3.621550e-02	8.856540e-04	8.856540e-04	1300	1320	AGUAGUAAUGGUCCGUCAUAAU
	hsa-miR-586	16.35	-10.38	6.324010e-02	6.128190e-02	5.441890e-03	1905	1926	UGGAUUUUUAUGUUACGUA  :                ATCTCACAATAAAATGCAT -
	hsa-miR-429	16.35	-17.47	3.722480e-02	7.350480e-05	7.350480e-05	354	376	UGCCAAAAUGGUCU-GUCAUAAU  :      :            ATIGTTTTATCTTATCAGTATTA
	hsa-miR-200b	16.2555	-15.61	4.268900e-02	8.856540e-04	8.856540e-04	1229	1250	AGUAGUAAUGGUCCGUCAUAAU     :               TGATTTTTACCTATCAGTATTA
	hsa-miR-429	16.161	-17.23	4.388200e-02	7.350480e-05	7.350480e-05	1229	1250	AAAAUGGUCUGUCAUAAU

NST00000320985							
NSG00000148516							
inc finger E-box-binding hon	neobox 1 (Transcripti	ion factor 8) (NIL-:	2-A zinc finger prot	ein) (Negative regu	ulator of IL2). [Sour	ce:Uniprot/SWISSF	ROT;Acc:P3727
Mouse over the target	ts for more inform	nation					
U							
·			-				
400	000	202	400	500		700	000
100	200	300	400	500	600	700	800
	-						
H. Sapiens	ENUCG	-UUUUUCUA	GAAGGAAAAU	AA-AUUCUAA	UUGAUAAUGA	A	
P. Troglodytes	ENUCG	-UUUUUCUA	GAAGGAAAAU	AA-AUUCUAA	UUGAUAAUGA	A	
M. Mulatta	EN	-NNNNNNN	NNNNNNNN	NNNNNNNNN	NNNNNNNNN	NNNNNNNNN	NNNNNNNN
B. Taurus	ENUAG	-UUUUUCUA	GAAAAGAAAG		UUGGUAAUGA	AG	
C Familiaris	ENUAG		AAGGAAAAU	AACAIIICIIAA	UUGGUAAUGA	Δ	
C. Familiario	ENGAG	-IUCIUCIA	AACCAAA	IUCUAC	UUCCUANUCA	Λ	
	DIN GAG	-000000M	AAAGGAAA	OUCUAC	OUGGUARUGA	A	
M. Musculus	ENALCALIAC	THE REPORT OF TAXABLE PARTY OF TAXABLE P	TATICCAAAAA	7 7 7 7 7 7 7 7 7	LICA CUIA ALICA	A	
M. Musculus G. Gallus	ENAUGAUAG	UUUUUUUCUAI	JAUGGAAAAA	AAUAAA	UCAGUAAUGA	A	
G. Gallus	ENAUGAUAG	UUUUUUCUAI	JAUGGAAAAA	AAUAAUAA	UCAGUAAUGA	A	
M. Musculus G. Gallus	ENAUGAUAG 1	UUUUUUCUA	JAUGGAAAAA	AAUAAA	UCAGUAAUGA	A	
M. Musculus G. Gallus	ENAUGAUAG 1	UUUUUUCUA	JAUGGAAAAA	AAUAAA	UCAGUAAUGA	A	





### The miR-200 Family

- are regulators of EMT-MET
- miR-200A, -200B, -200C,141 and 429
- Target genes: ZEB1 & ZEB2, which are transcriptional repressors of E-cadherin
- is quite low expression in advanced cancer and metastasis (Lee, 2006; Mohammadizadeh, 2009; Park, 2008)
- Absence of E-cadherin from a tumor biopsy indicates a poor prognosis and possible metastasis





### Strategies for a successful miRNA research







### The expression of miR-200 Family in two cell lines



#### miRNA qPCR assay

	MCF7 / MDA-231	
	Fold Change	ANOVA P*-value
hsa-miR-141	19	6.66E-07
hsa-miR-200c	18	2.13E-08
hsa-miR-200a	8	3.97E-07
hsa-miR-200b	6	0.00001
hsa-miR-429	5	3.17E-06





### Strategies for a successful miRNA research





### **MDA-MB-231: Transition to Epithelial Phenotype**





Dharmacon™

part of GE Healthcare

**DRI** 

Gene Company

### **MCF-7: Transition to Mesenchymal Phenotype**





Dharmacon™

Gene Company part of GE Healthcare

ORIGENE

### The function of miR-200–Zeb1–E-cadherin axis

#### In vitro

The miR-200 family plays an essential role in tumor suppression by inhibiting epithelial-mesenchymal transition (EMT)





In vivo?


# miR-200–Zeb1–E-cadherin axis is deregulated in metastatic tumors.





Up : noninvasive tumor Down: highly invasive IC2 tumor



#### Genes Dev. 2009 23: 2152-2165



#### miR-200 Expression Inhibits Metastasis







Time (days)

### **RNAi innovation-Summary**



part of GE Healthcare

Your Gene Company

## Thank You for Your Attention

Aaron Chen, Ph.D. Field Application Scientist, Level Biotechnology Inc. aaronchen@mail.level.com.tw



