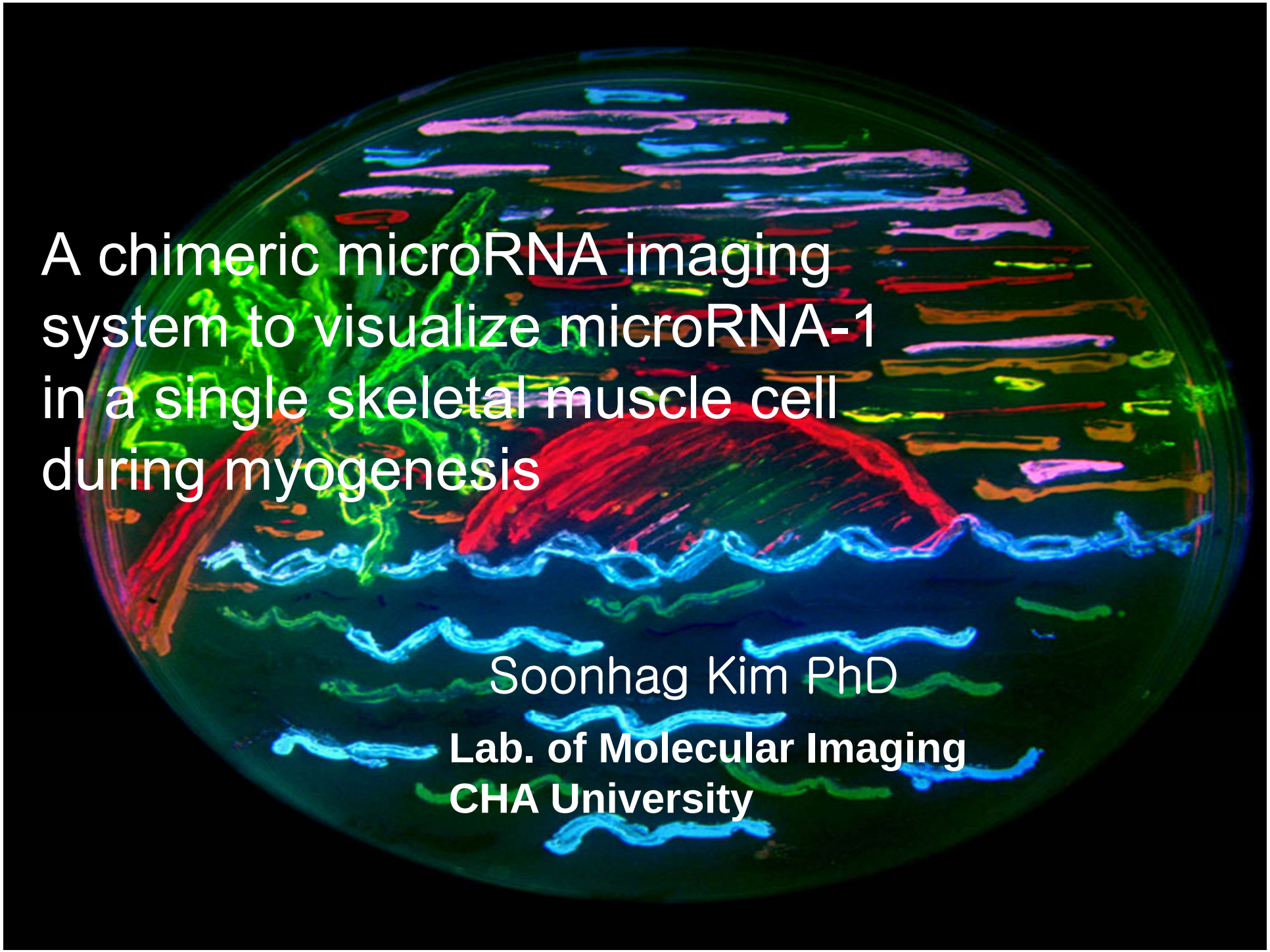


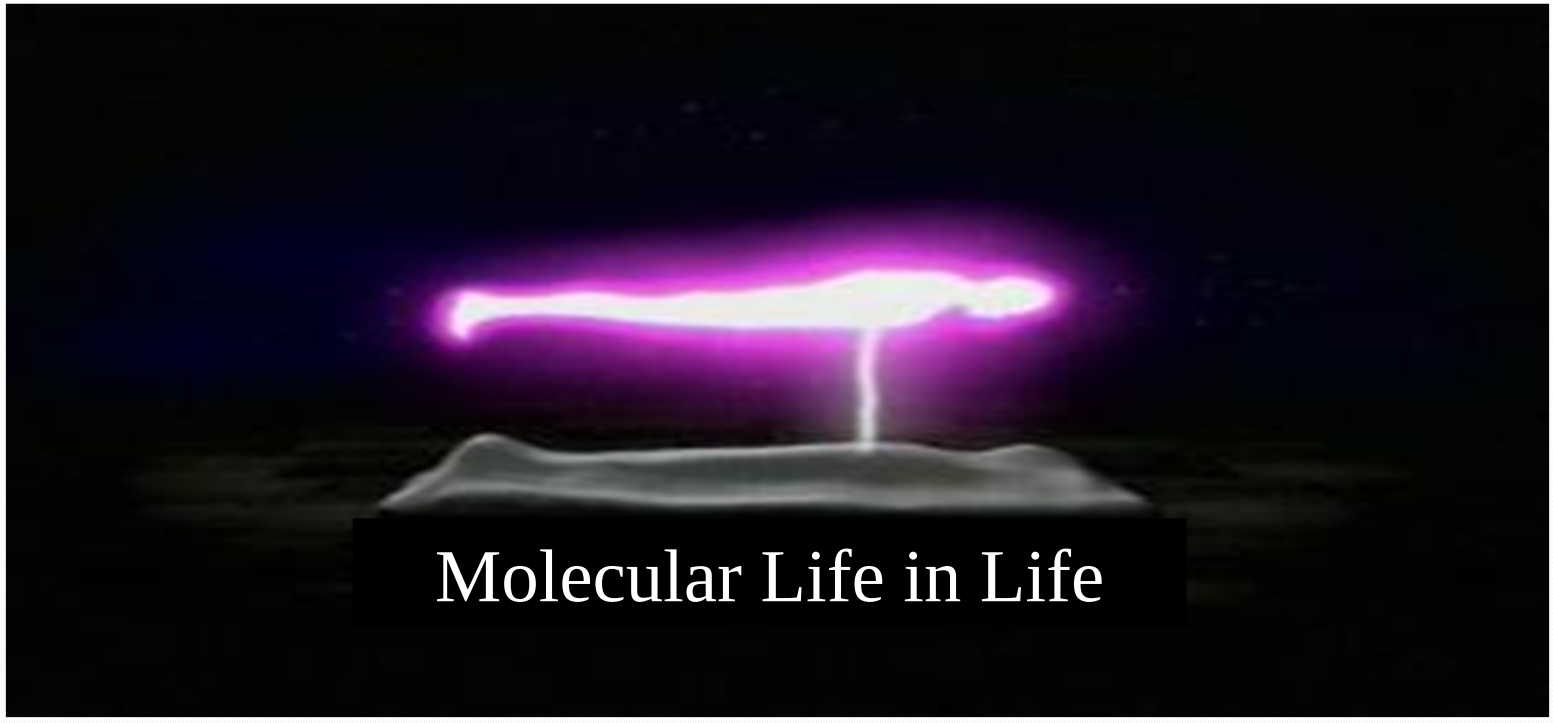
A chimeric microRNA imaging system to visualize microRNA-1 in a single skeletal muscle cell during myogenesis

Soonhag Kim PhD

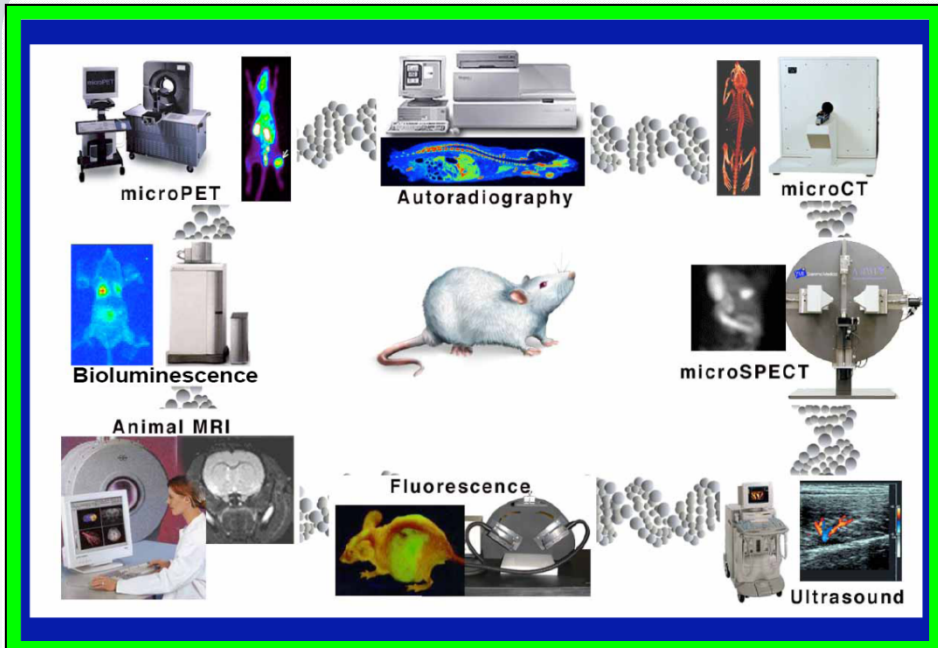
Lab. of Molecular Imaging
CHA University

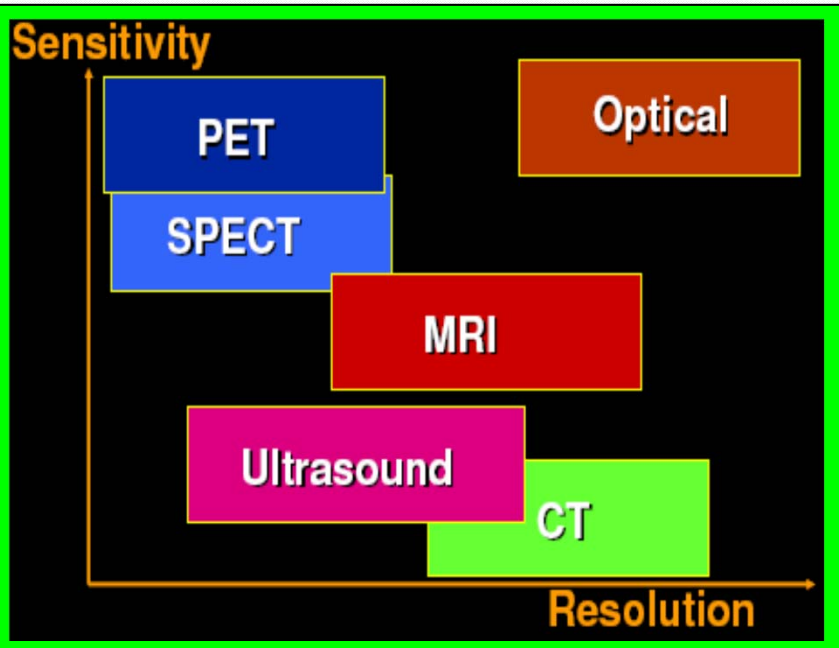
In vivo Molecular Imaging

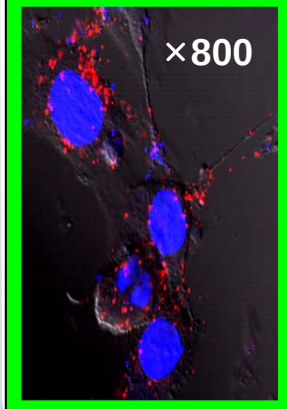
- **visualize and quantify** characterization of biological mechanisms at the cellular and molecular level without sacrificing the animal



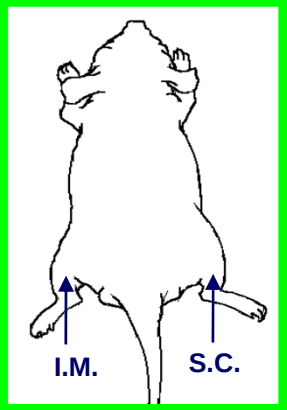
Molecular Imaging Modalities



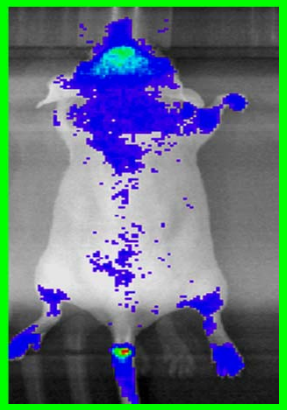




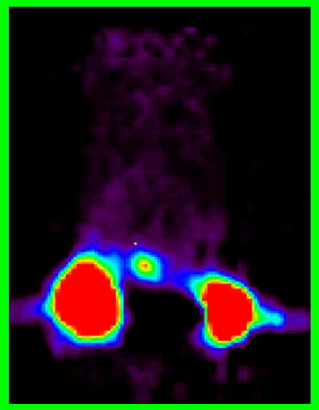
Confocal microscope



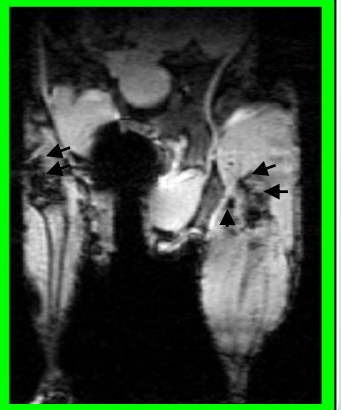
Bioluminescence



Fluorescence



microPET

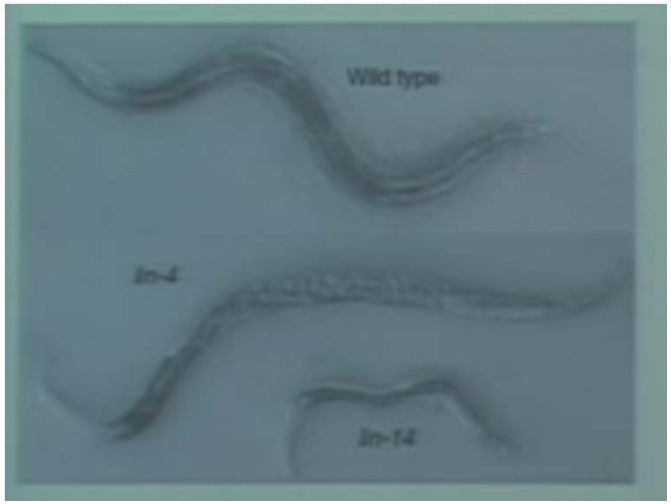


MRI

Hwang et al, 2009

microRNAs (miR)

- The regulation of developmental timing (line 14 gene) by Lin-4 in *C. elegans* (found by Ambros) *(Cell.1993; 75:843)*
- small non-coding RNAs
- 20~25 ribonucleotide
- Regulated by RNA polymerase II



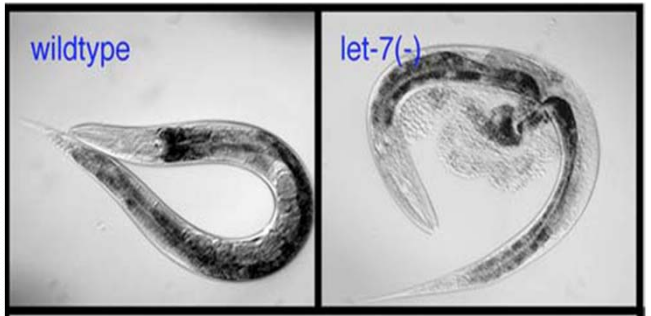
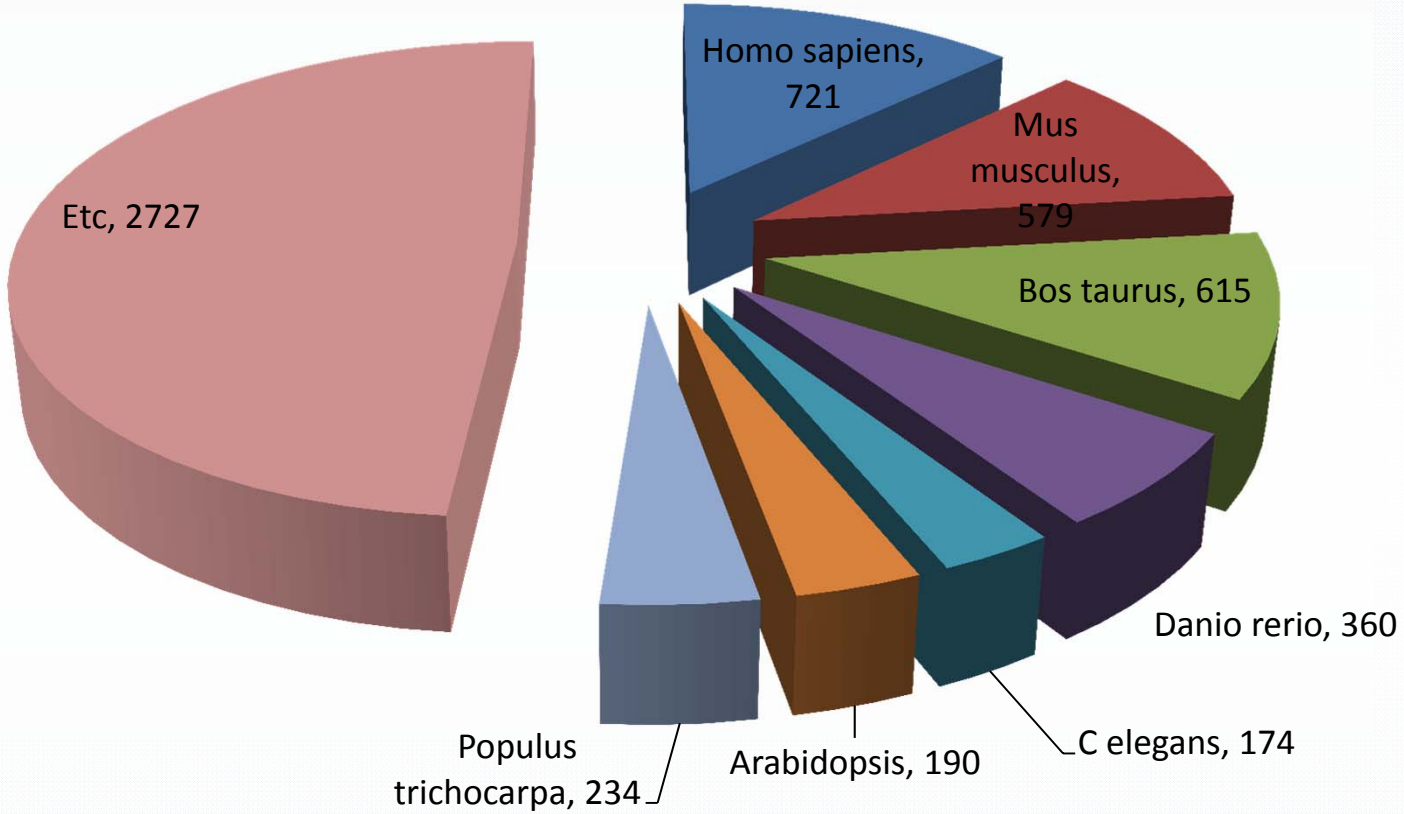


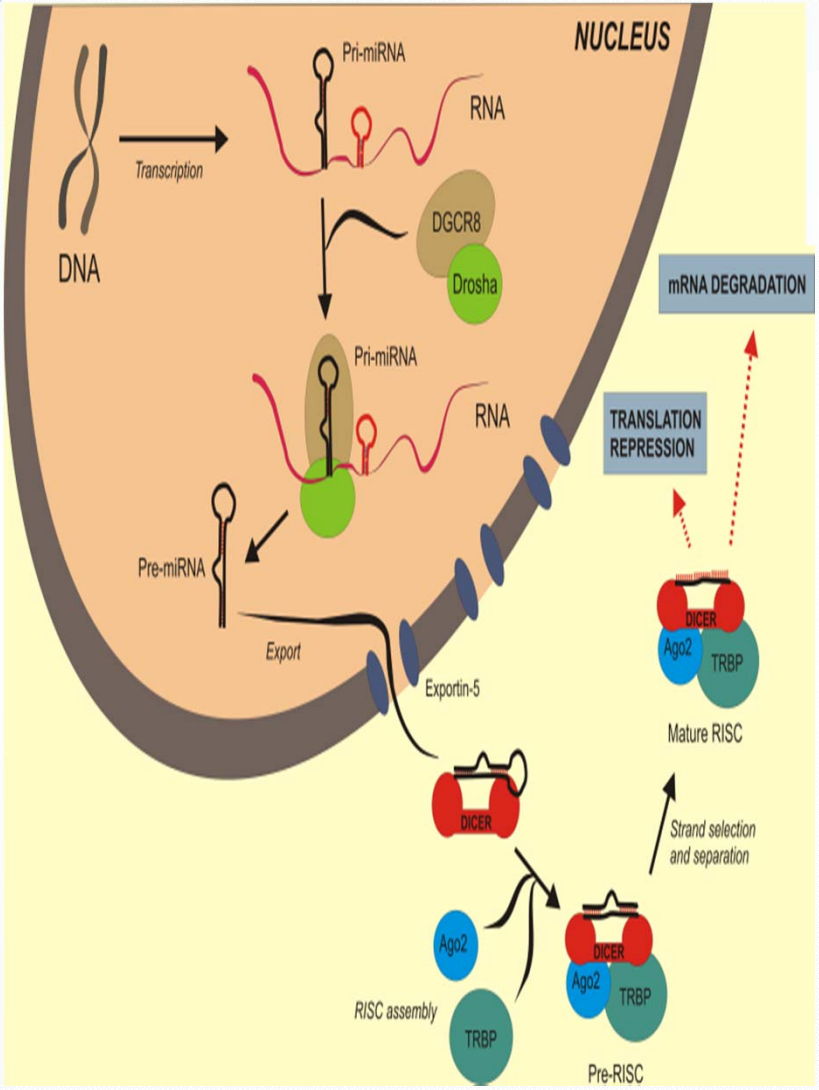
Figure 1. Mutation of the let-7 miRNA gene in the nematode C. elegans results in developmental abnormalities that often lead to rupturing of the worms.

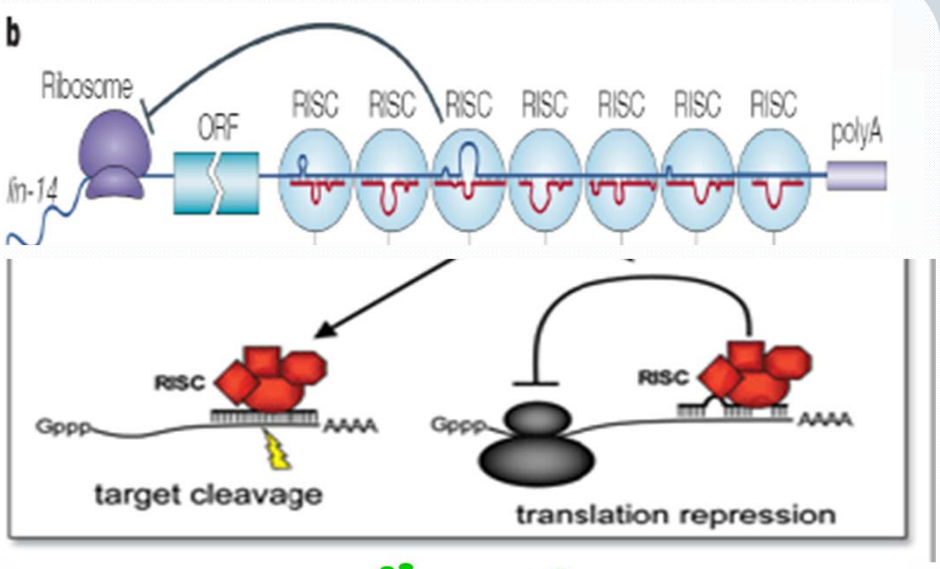
How many microRNAs?

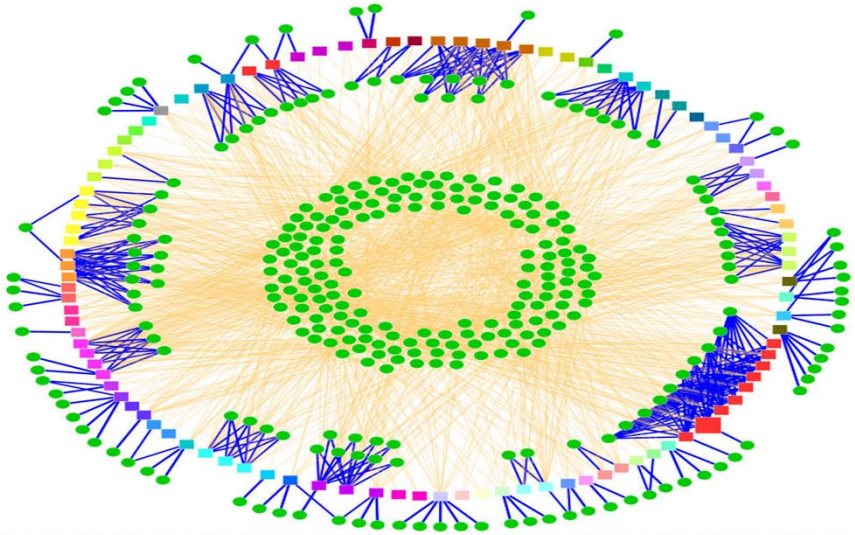


- miRBase release 14: http://www.mirbase.org

microRNA biogenesis and function



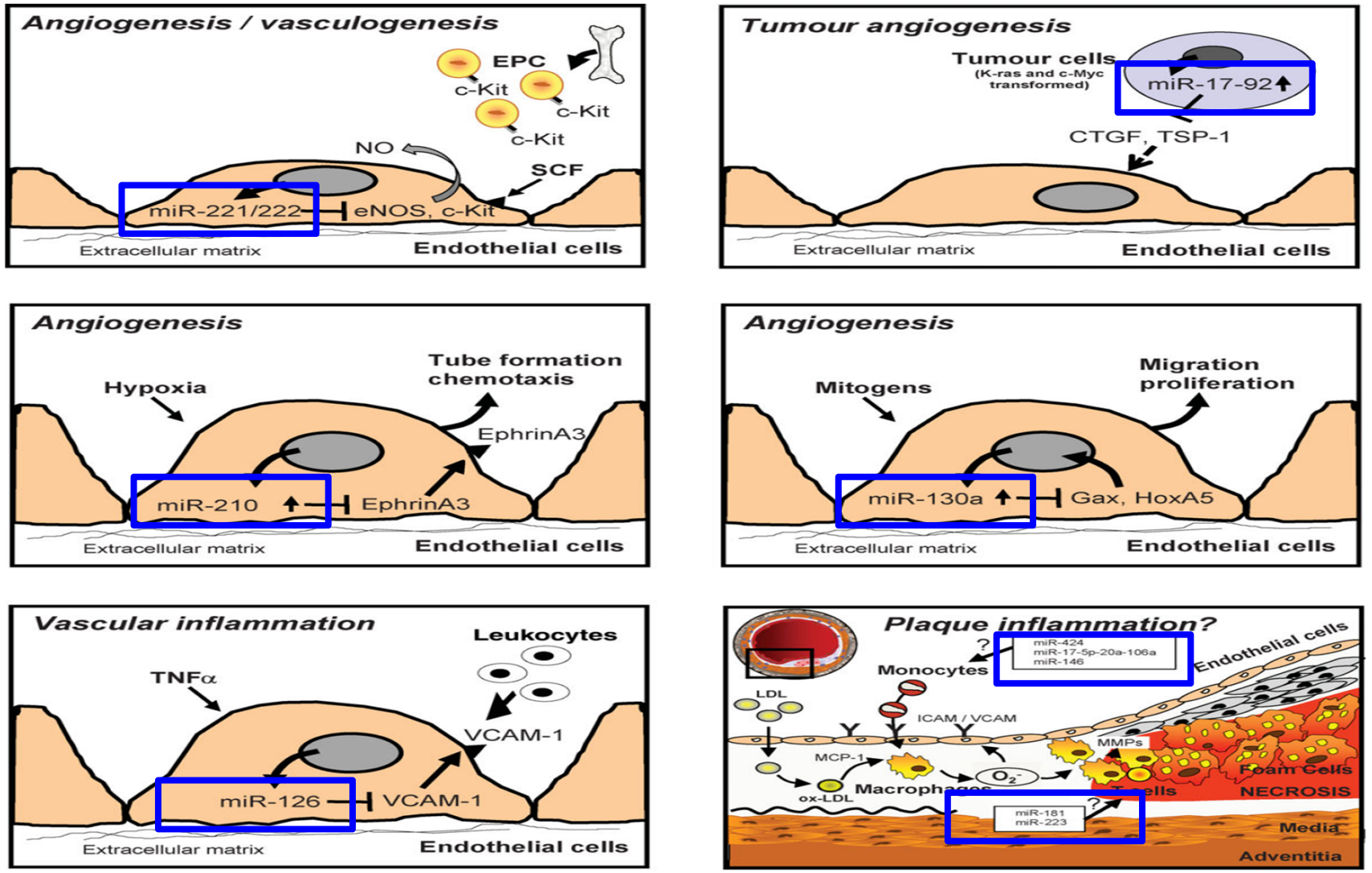




1,597 gene ( )/ 104 microRNA( )

Tissue-specific expression of microRNA

Table 1 | **Expression studies on mammalian microRNAs**



Nature, 2004, 5: 522

Abnormal expression of microRNA: Disease

Abnormal expression of microRNAs in cancer cells:

Down-regulation-deletion in Chronic Lymph. Leukemia	*miR-15 a miR-16*
Up-regulation in B cell lymphomas	*miR-155*
Up-regulation-amplifikation in B cell lymphomas	*miR- 17-92 klaster*
Down-regulation in lung cancer	*miR-26a a miR-99a, let-7*
Down-regulation in colon cancer	*miR-143-miR-145 klaster*
Up-regulation in Burkitt lymphoma	*miR-155*
Up-regulation in glioblastoma	*miR-221*

Other diseases

Parkinson , X-linked mental retardation	miR-224
Diabetes	miR-375
Cardiac hypertrophy in stress condition	miR-209, miR1-2
Defective Immnumity	miR-155

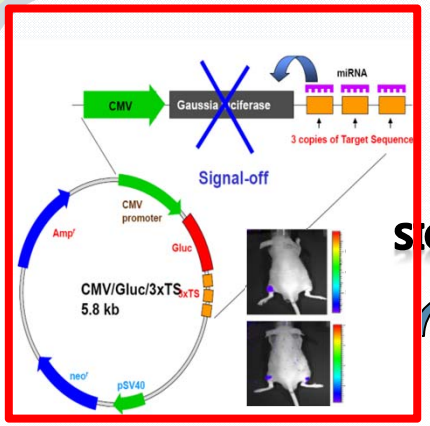
microRNA research trend

MARKET SEGMENT VENDOR	DISCOVERY AND CLONING	ISOLATION AND PURIFICATION	LABELING AND DETECTION	EXPRESSION PROFILING	FUNCTIONAL ANALYSIS
Ambion/ABI		×		×	×
Asuragen				×	
Cepheid					×
Exiqon				×	
Febit				×	
GenoSensor				×	
IDT	×				
Invitrogen				×	
LC Sciences				×	
Miltenyi				×	
Mirus			×		
Ordway Research Institute				×	
Promega					×
QIAGEN		×		×	
SBI	×			×	×
Stratagene/Agilent		×		×	
ThermoFisher					×
USB/Affymetrix			×		

By CHIP & RT-PCR

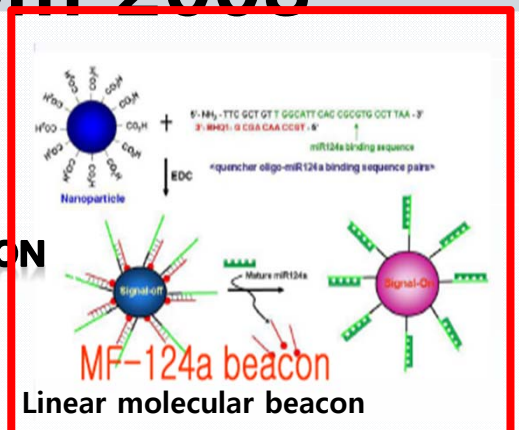
Source: Select Biosciences Industry Research

How have we evolved to image microRNAs from 2008



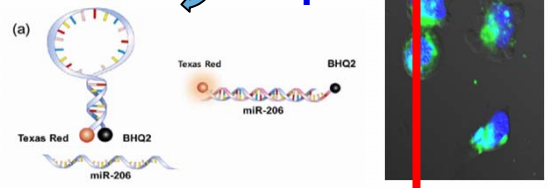
Reporter gene (signal-off)

SIGNAL-ON



Linear molecular beacon

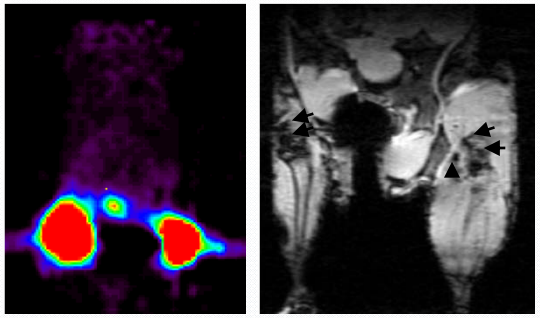
Simple



Stem-loop molecular beacon

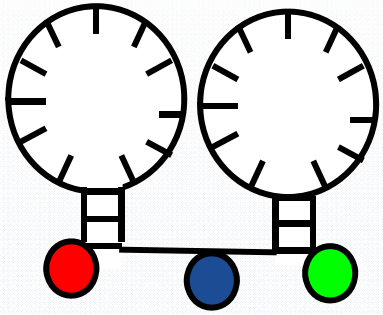
ALL-IN-ONE CASSETTE VECTOR

Multiple



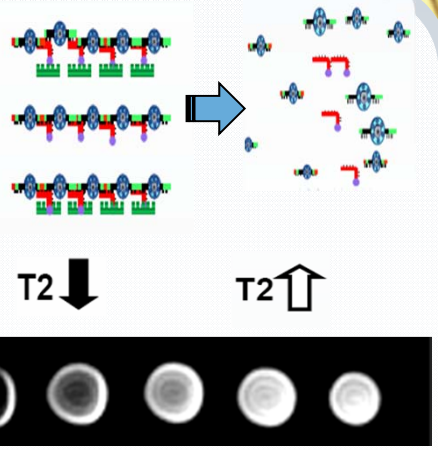
PET
Pre-differentiation

MRI
Post-differentiation



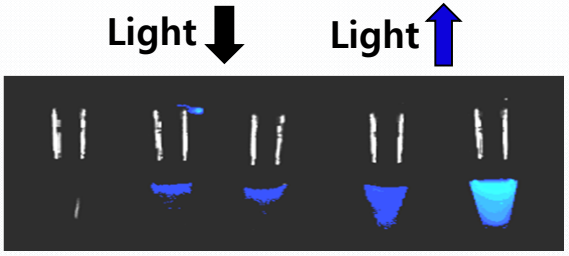
Dual molecular beacon

CLINICAL NANO-BIOSENSOR



Magnetic Resonance Imaging

Extension



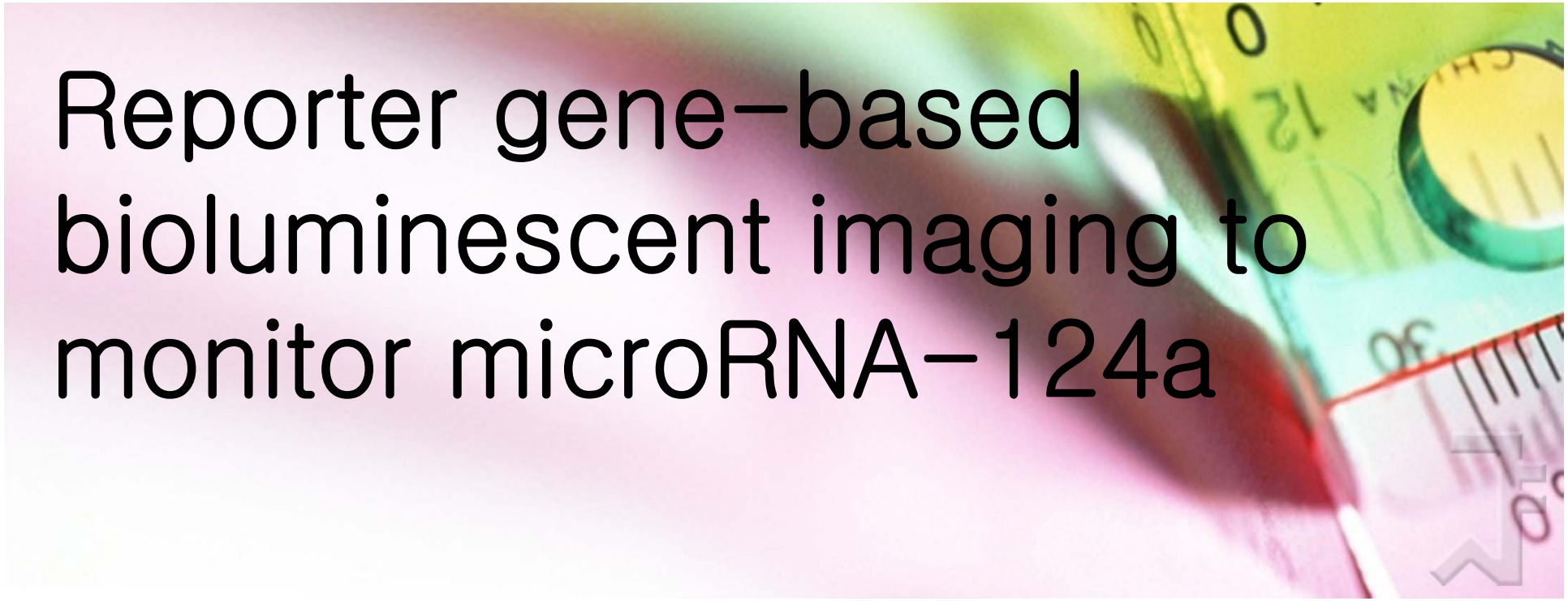
Quntum Dot-based optical Imaging

PROTOCOL

A reporter gene imaging system for monitoring microRNA biogenesis

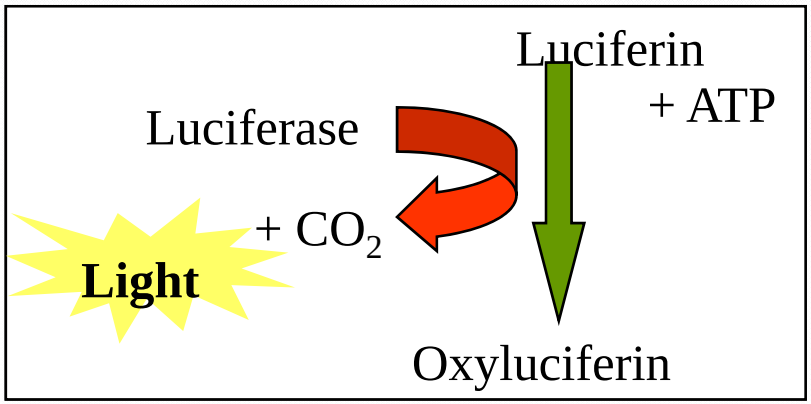
Hae Young Ko[1,2], Do Won Hwang[1], Dong Soo Lee[1,3] & Soonhag Kim[4]

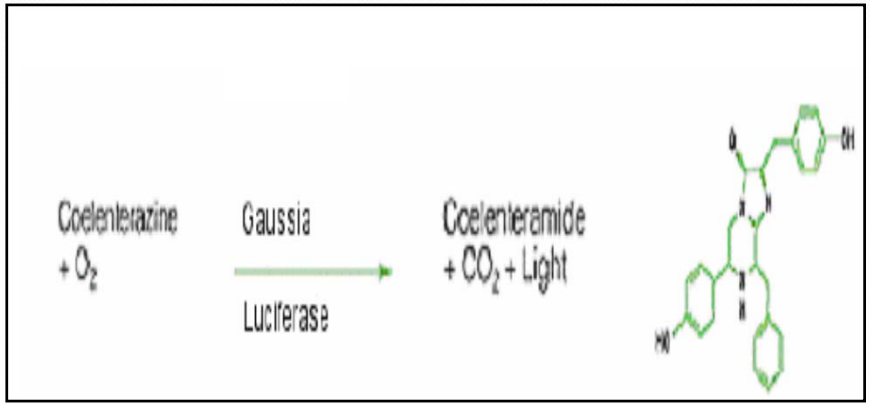
[1]Department of Nuclear Medicine, Seoul National University College of Medicine, Seoul, Korea. [2]Interdisciplinary Course of Radiation Applied Life Science, Seoul National University College of Medicine, Seoul, Korea. [3]Department of Nuclear Medicine and Department of Molecular Medicine and Biopharmaceutical Science, College of Medicine, Seoul, Korea. [4]Laboratory of Molecular Imaging, Department of Applied Bioscience, CHA Stem Cell Institute, CHA University, Seoul, Korea. Correspondence should be addressed to S.K. (kimsoonhag@empal.com) and D.S.L. (dsl@plaza.snu.ac.kr).



Reporter gene-based bioluminescent imaging to monitor microRNA-124a

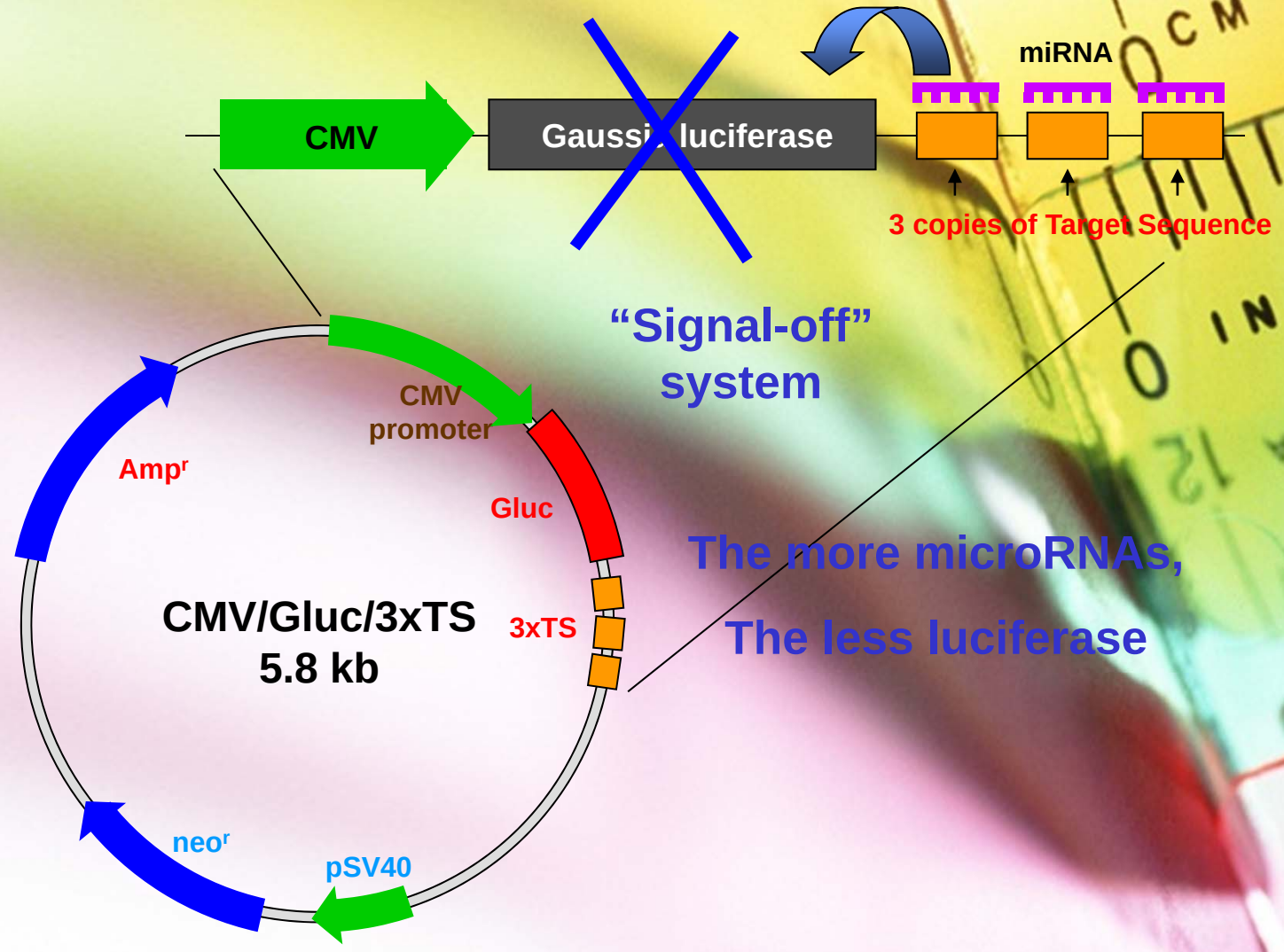
Bioluminescent proteins





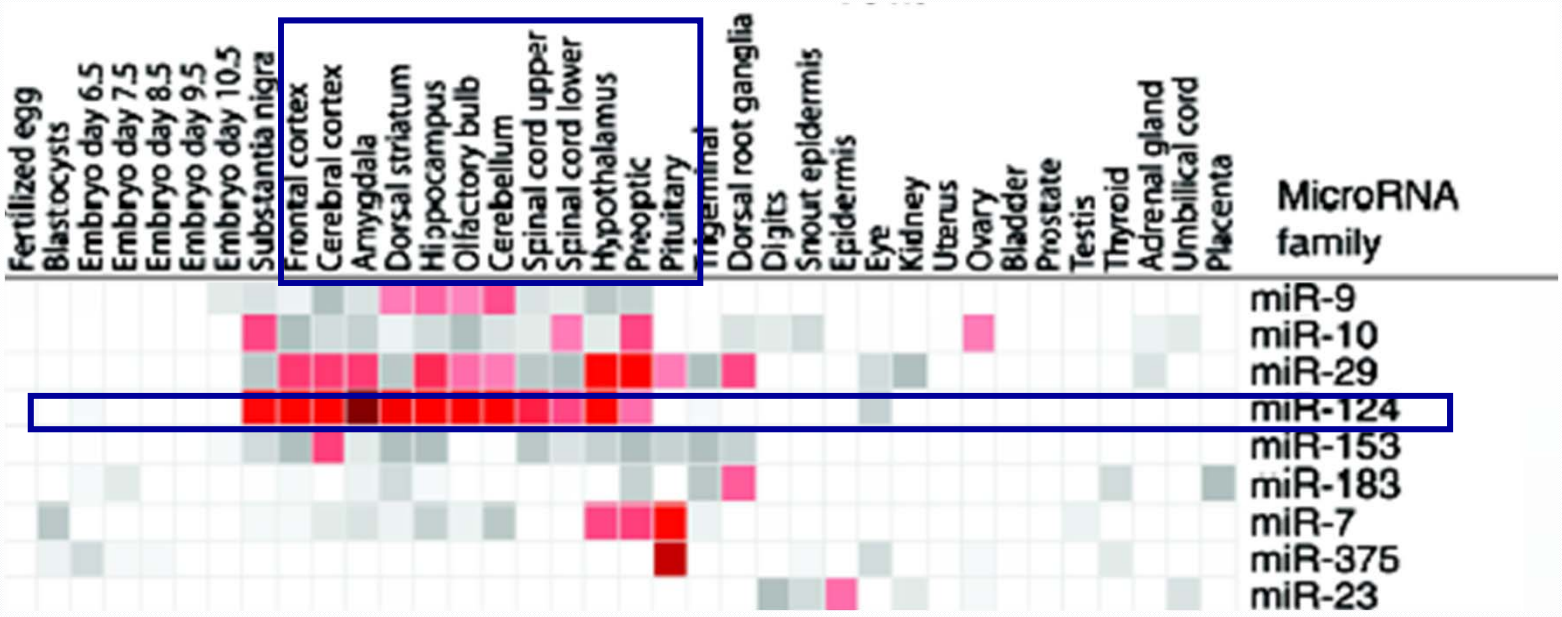
	substrate	Maximal emission wavelength
Firefly	Luciferin	560 nm
Renilla	coelenterazine	480 nm
Gaussia	coelenterazine	470 nm

Bioluminescent imaging reporter gene: mature microRNAs



microRNA124a (miR124a)

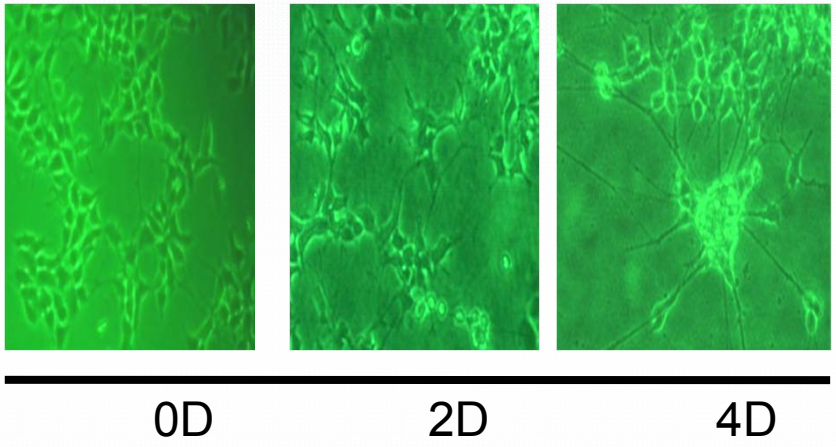
- Brain specific expression

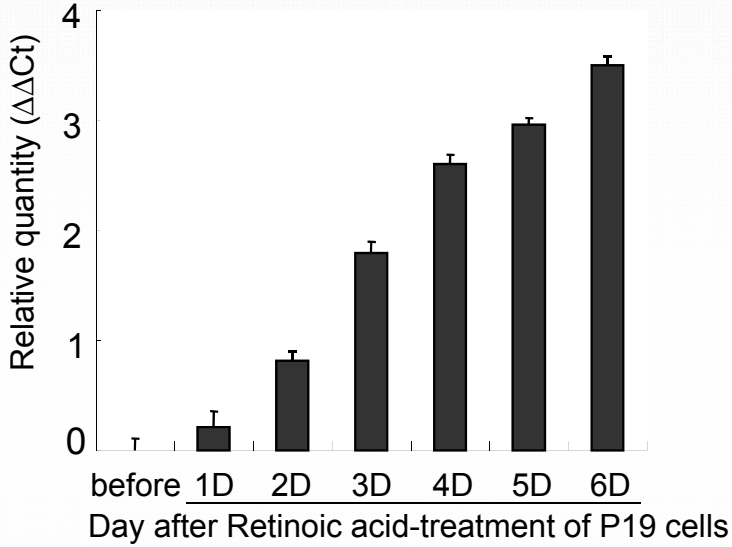


ANNA M. K. et al. (2006)

miR124a: gradually increased expression during neurogenesis

P19 cell (rat embryonal carcinoma cell line)



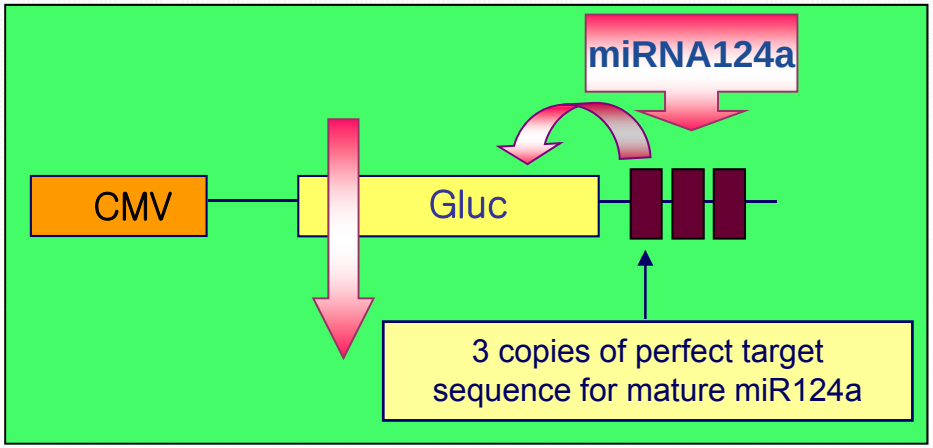


mature miR124a: Real-time PCR

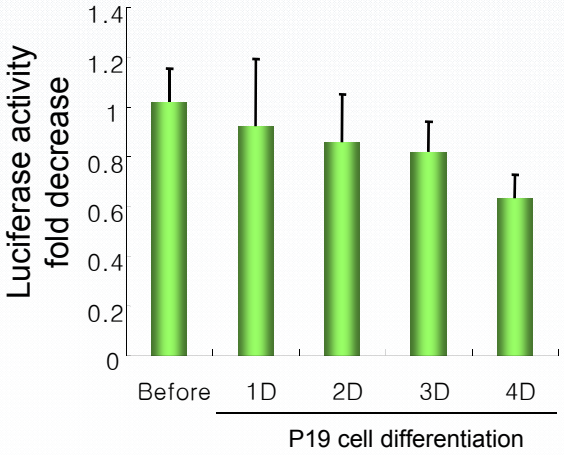
***Ko* et al. FEBS J (2009)**

Imaging of single-stranded mature miRNA 124a during neurogenesis

- **CMV/Gluc/3xPT_miR124a: miR124a reporter gene**



mature -miR124a: in vitro



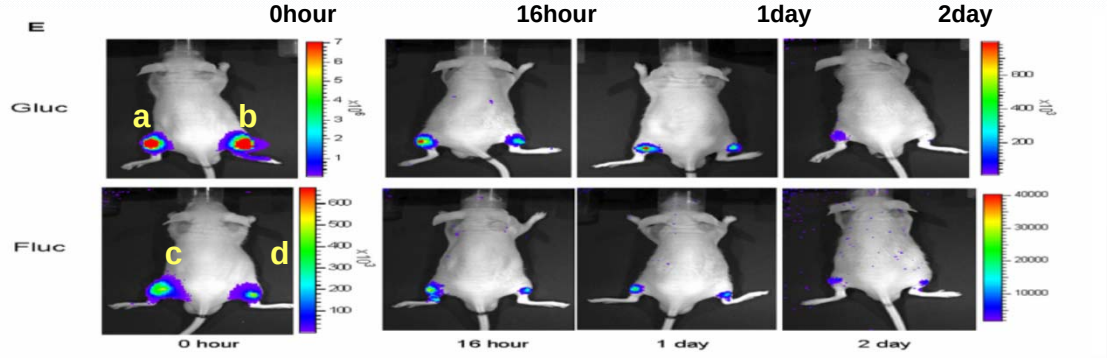
mature -miR124a: in vivo

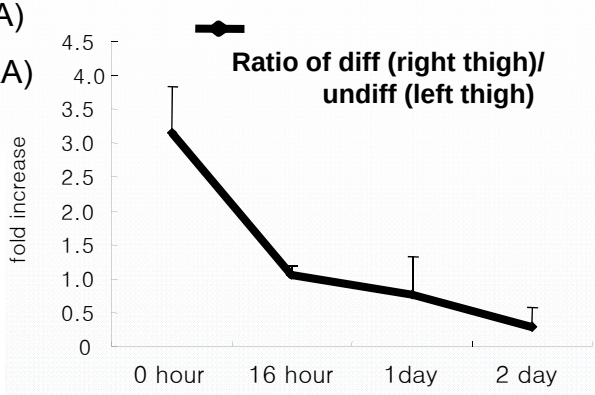
a. CMV/Gluc/3xPT_miR124a (-RA)

b.CMV/Gluc/3xPT_miR124a (+ RA)

c. CMV/Firefly luciferase (-RA)

d. CMV/Firefly luciferase (+ RA)





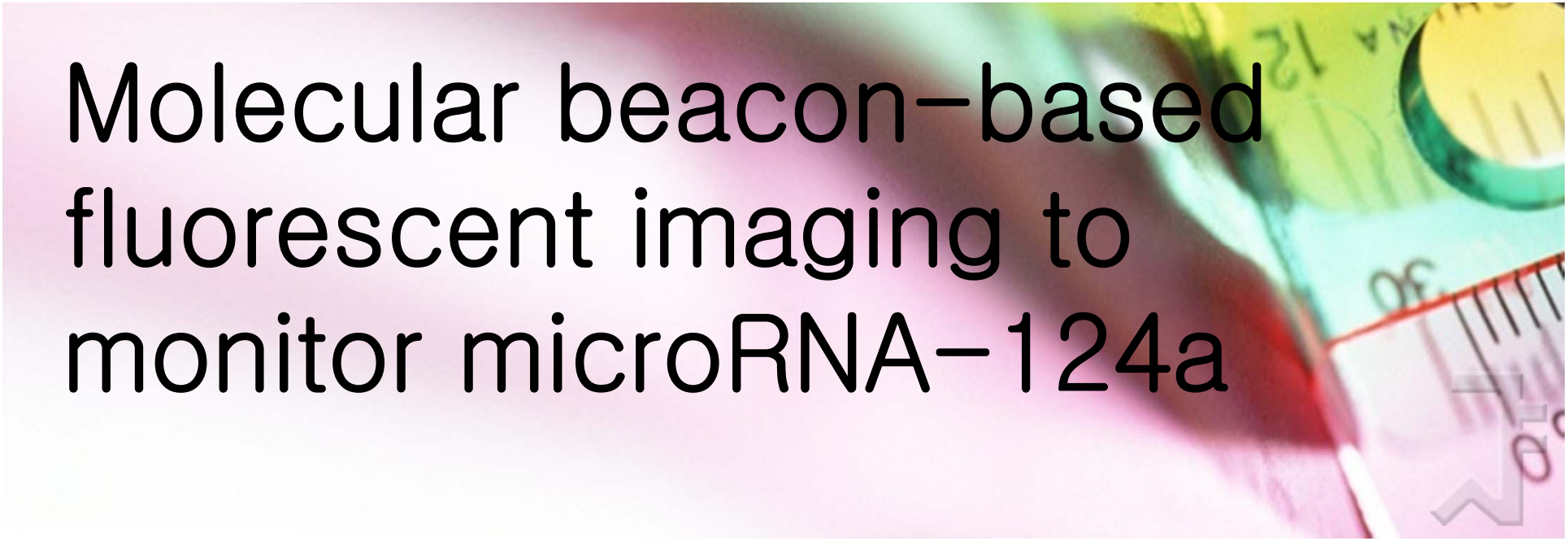
small NANO MICRO

Molecular beacons

Smart Magnetic Fluorescent Nanoparticle Imaging Probes to Monitor MicroRNAs

*Do Won Hwang, In Chan Song, Dong Soo Lee, and Soonhag Kim**

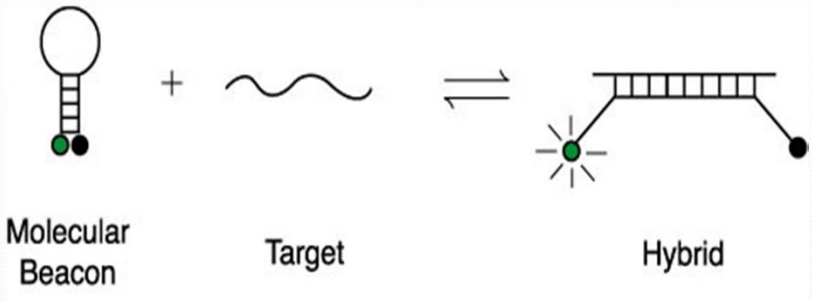
small 2010, 6, No. 1, 81–88

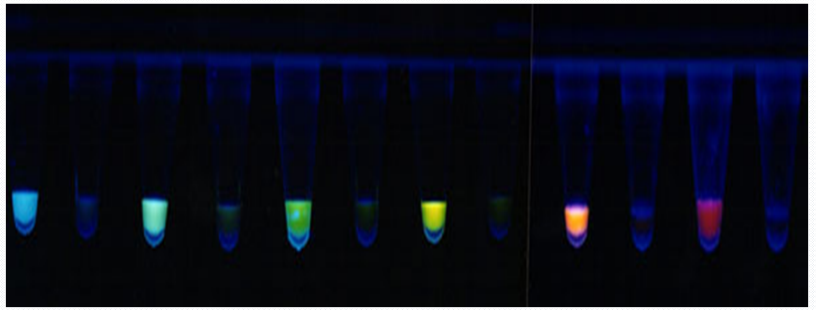


Molecular beacon-based fluorescent imaging to monitor microRNA-124a

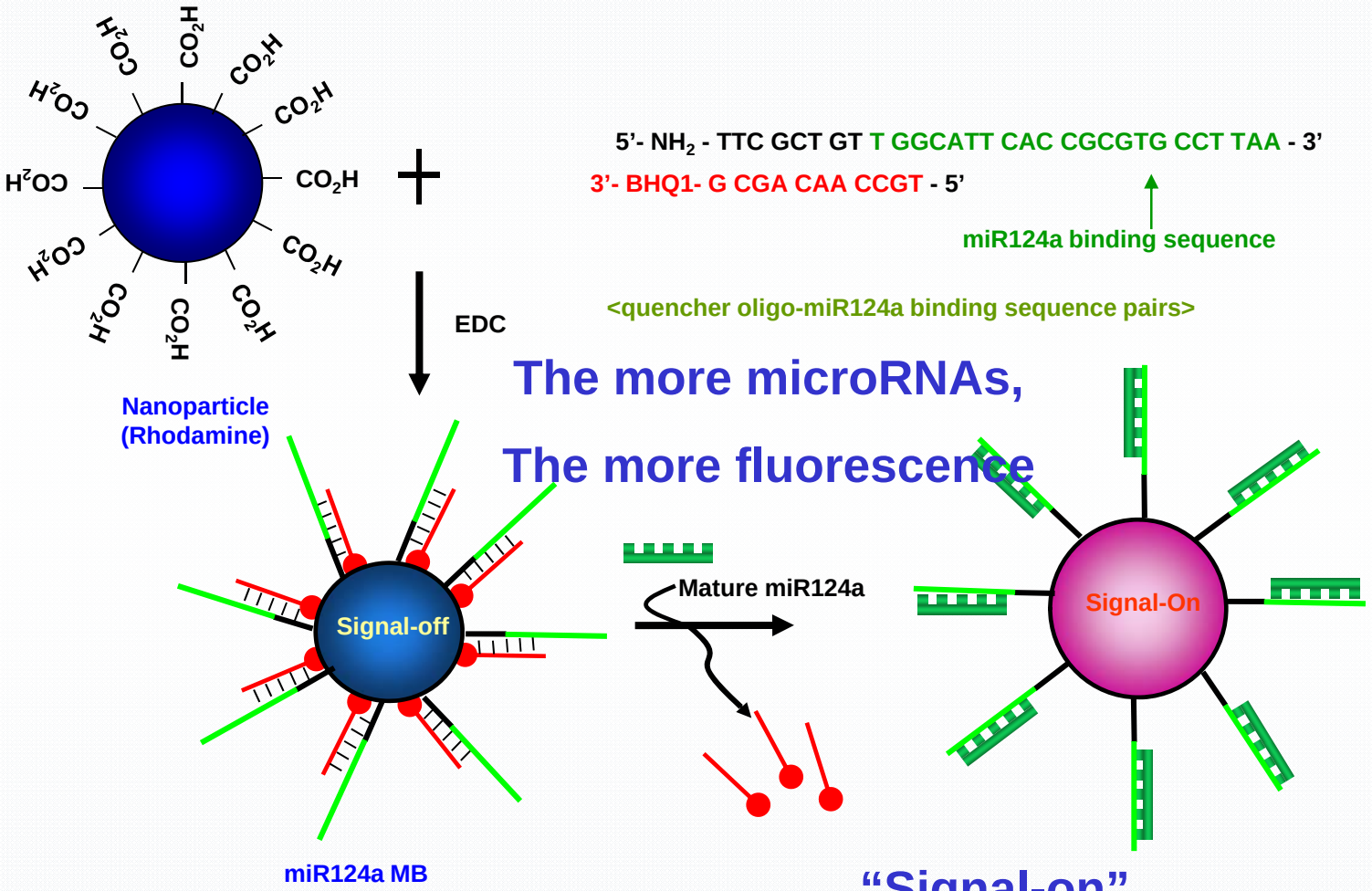
Molecular beacon (MB)

- oligonucleotide hybridization probes that contains a fluorephore and a non-fluoroscent quencher on each arm

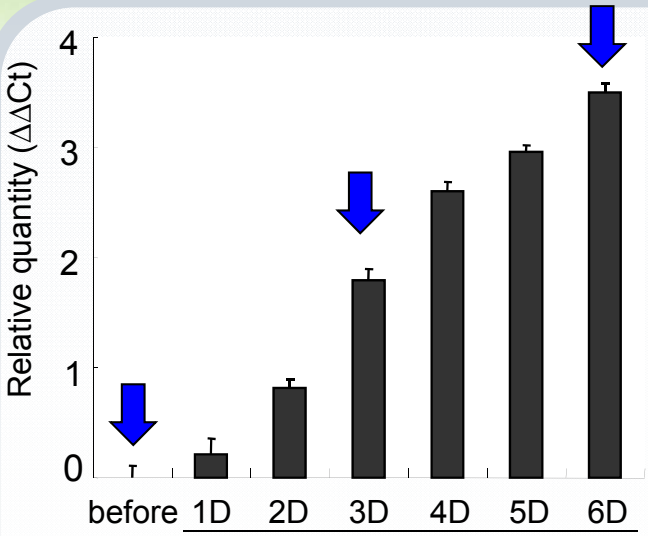




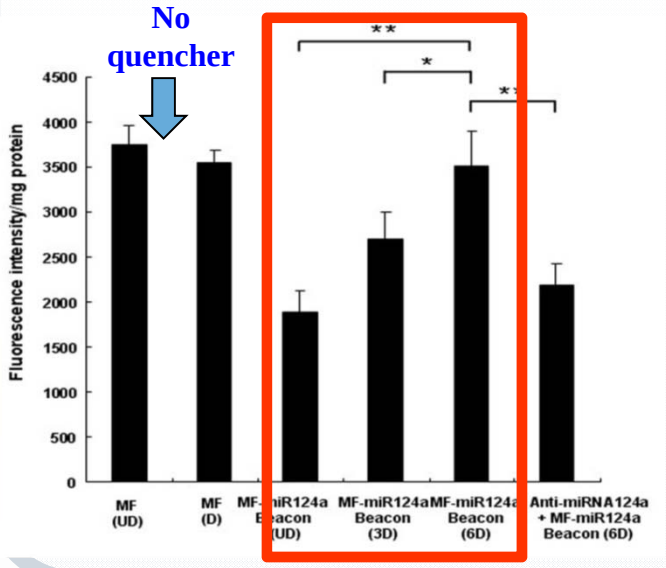
miR124a molecular beacon

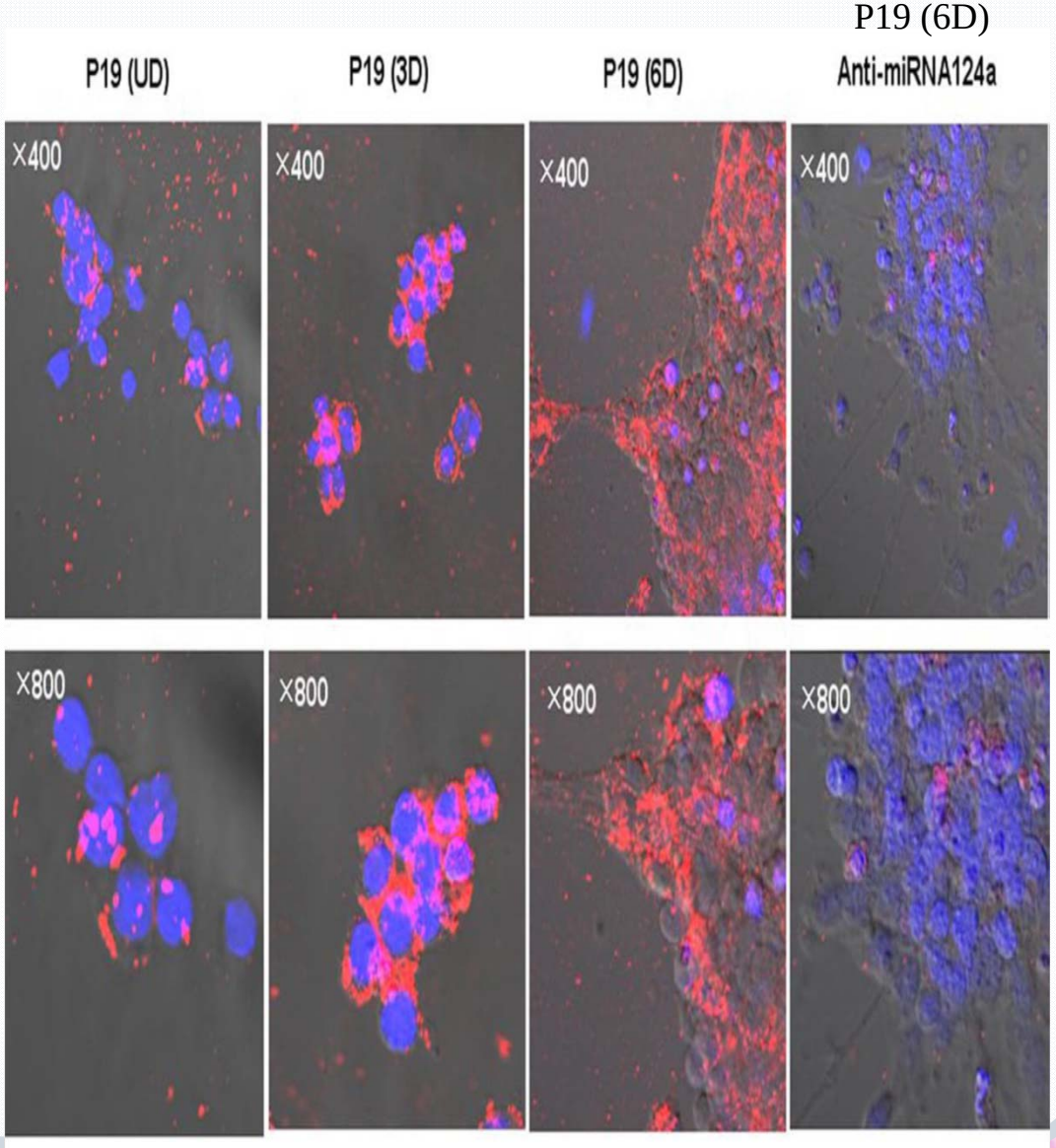


In vitro assay of miR124a molecular beacon in P19 cells

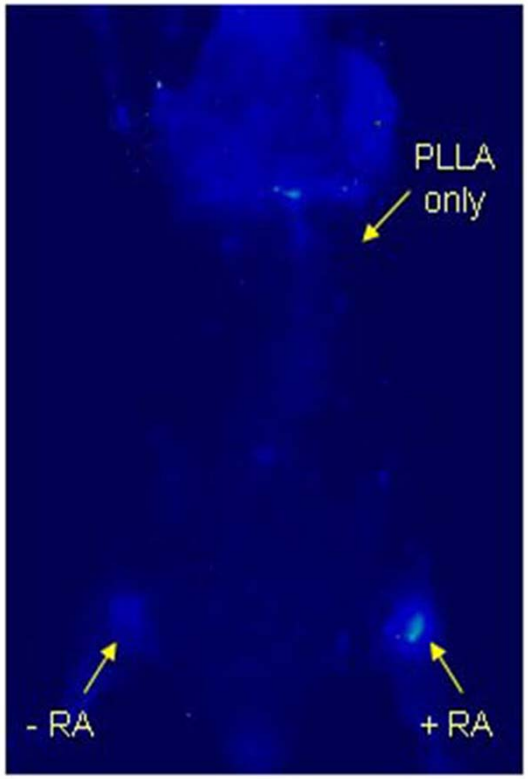


mature miR124a: Real-time PCR





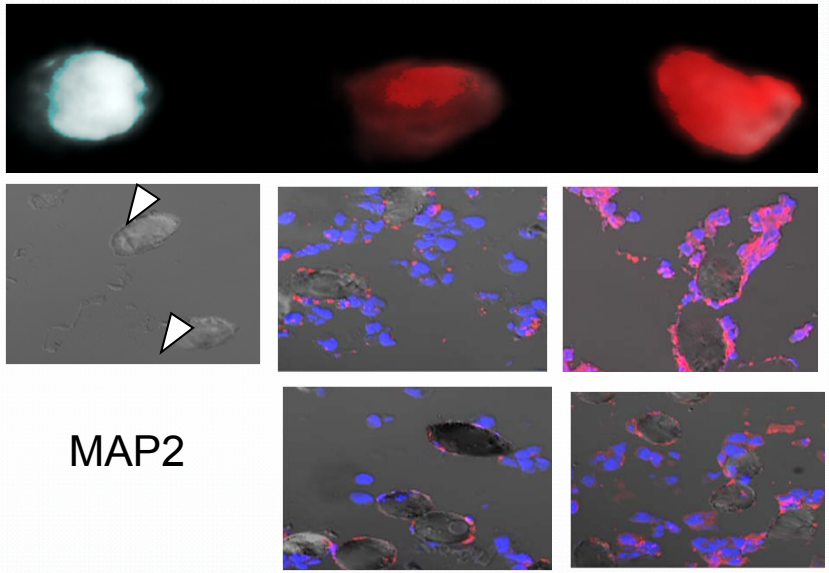
In vivo imaging of miR124a molecular beacon



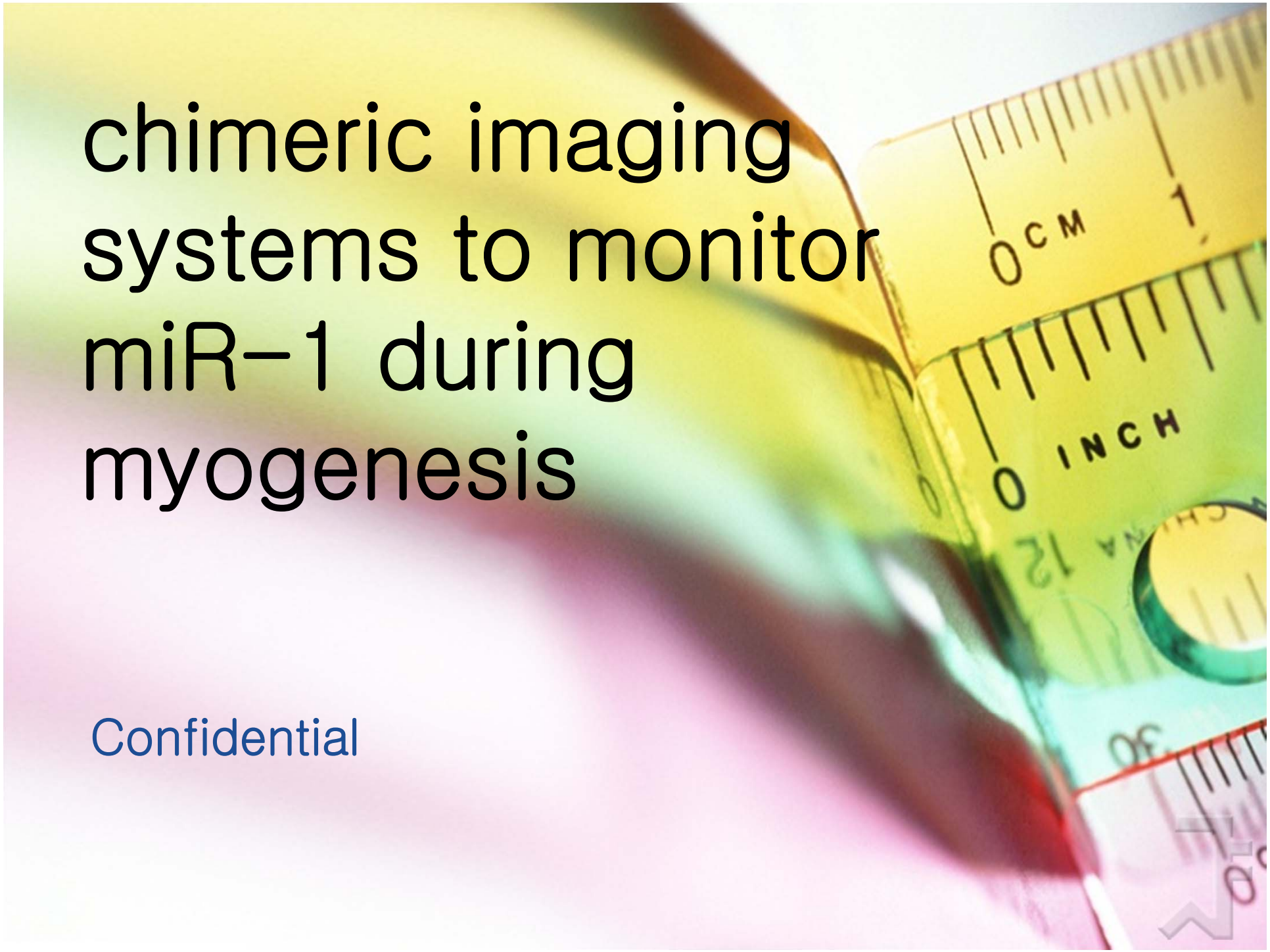
<Day 2>

RA(-)
Left thigh

RA(+)
Right thigh



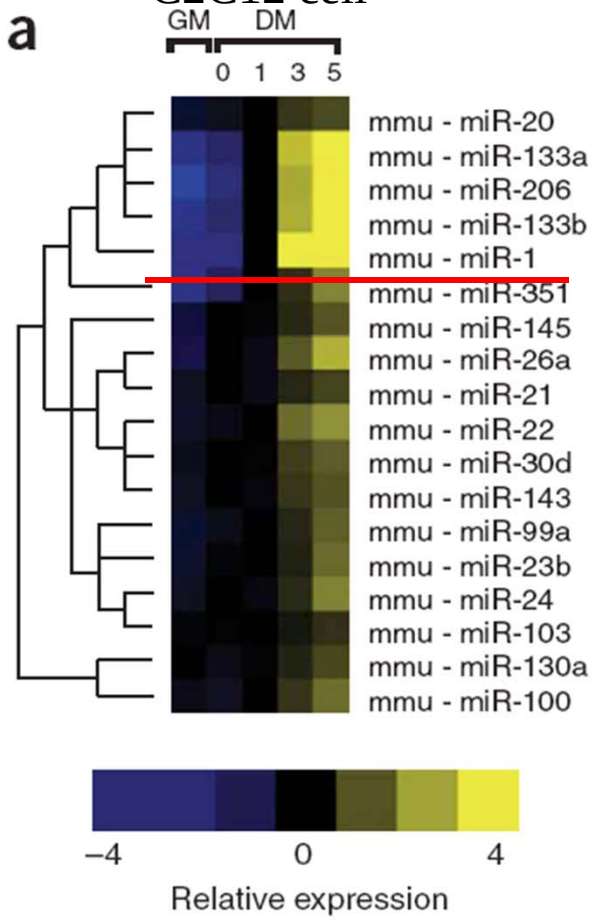
chimeric imaging systems to monitor miR-1 during myogenesis



Confidential

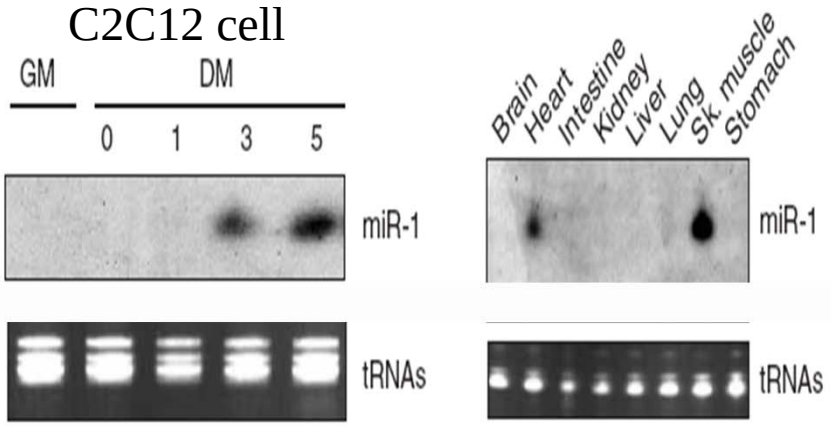
miR-1 promotes myogenesis

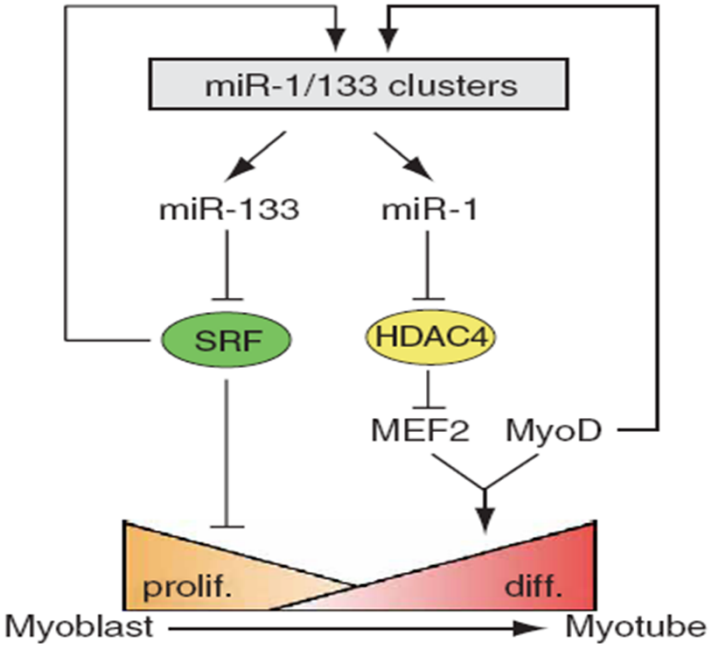
C2C12 cell



Nature genetics, 2006

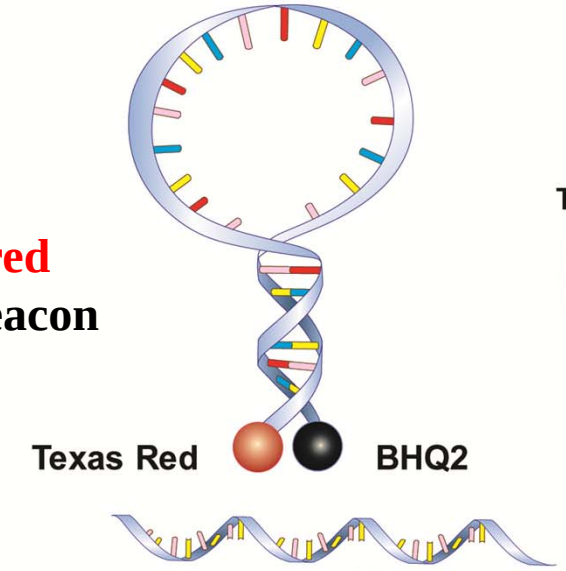
C2C12 cell



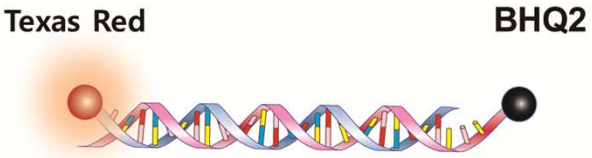


Alternative miRNA imaging systems

Stem loop-structured
miRNA Molecular beacon
"SIGNAL-ON"



miR-1

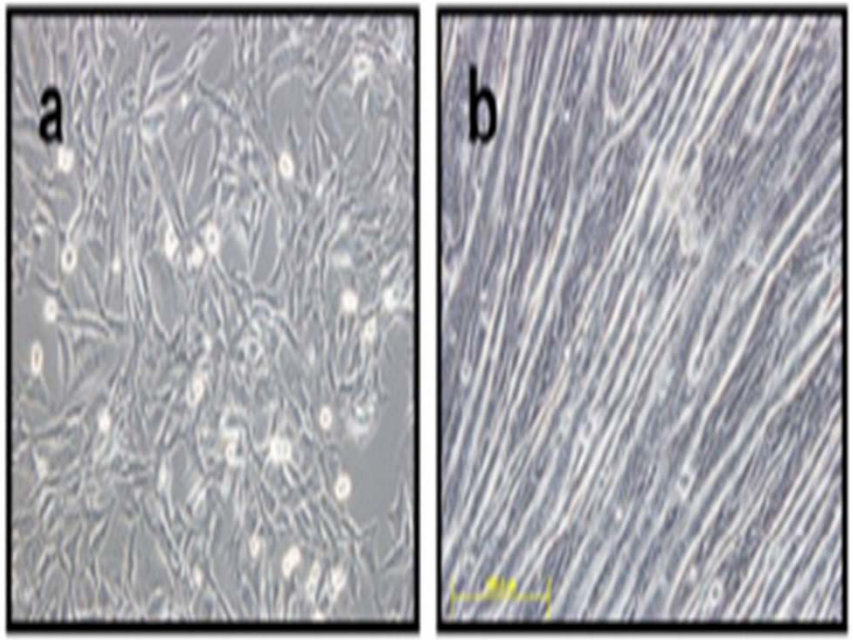


miR-1

Reporter gene- based
miRNA imaging vector
"SIGNAL-OFF"

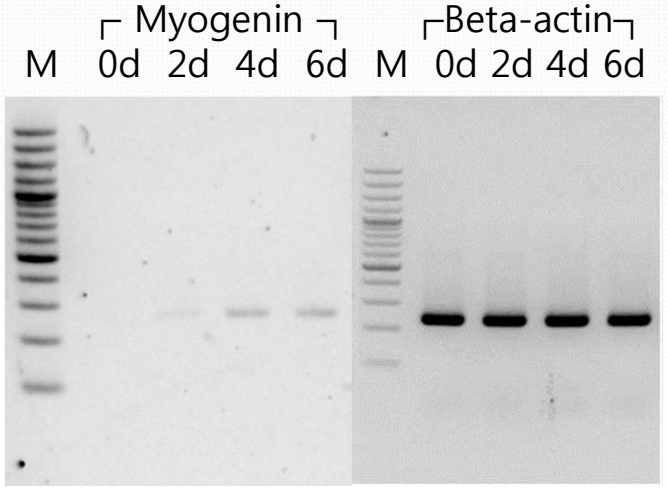
CMV/Gluc/mir1_3xPT

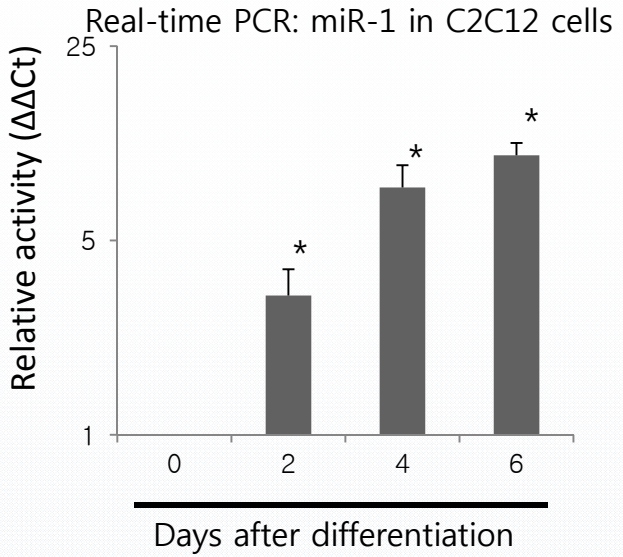
miR-1 is gradually increased during myogenesis



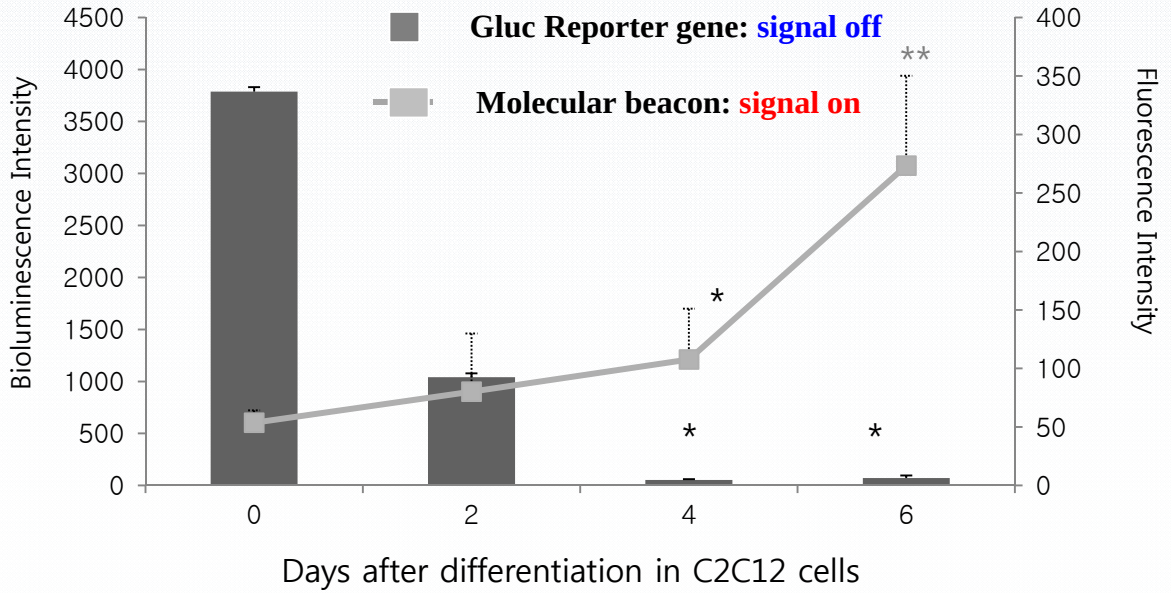
C2C12 in GM, Undifferentiated

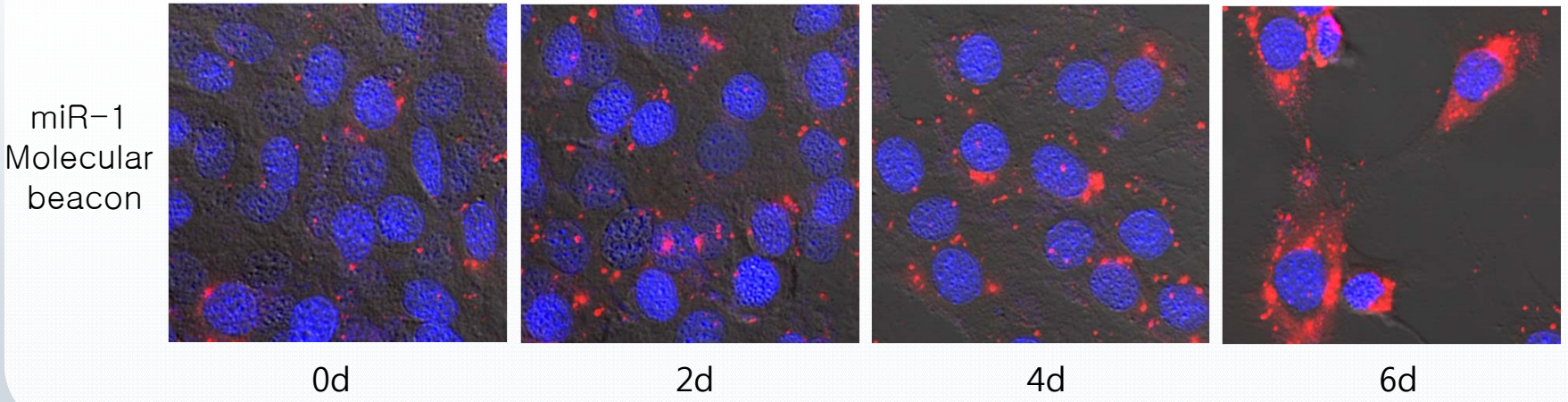
C2C12 in DM, differentiated



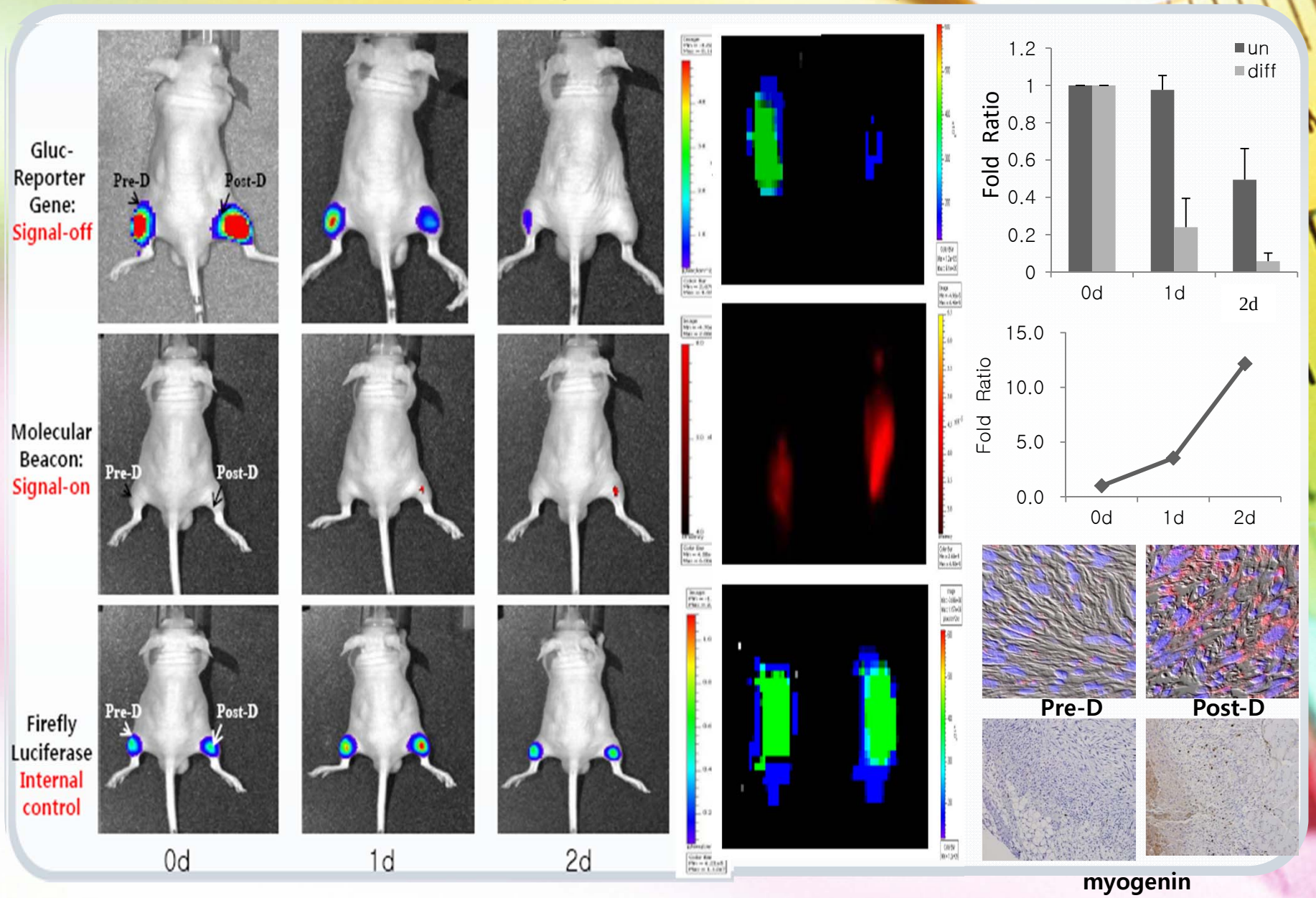


In vitro assay by co-transfection of reporter gene and molecular beacon





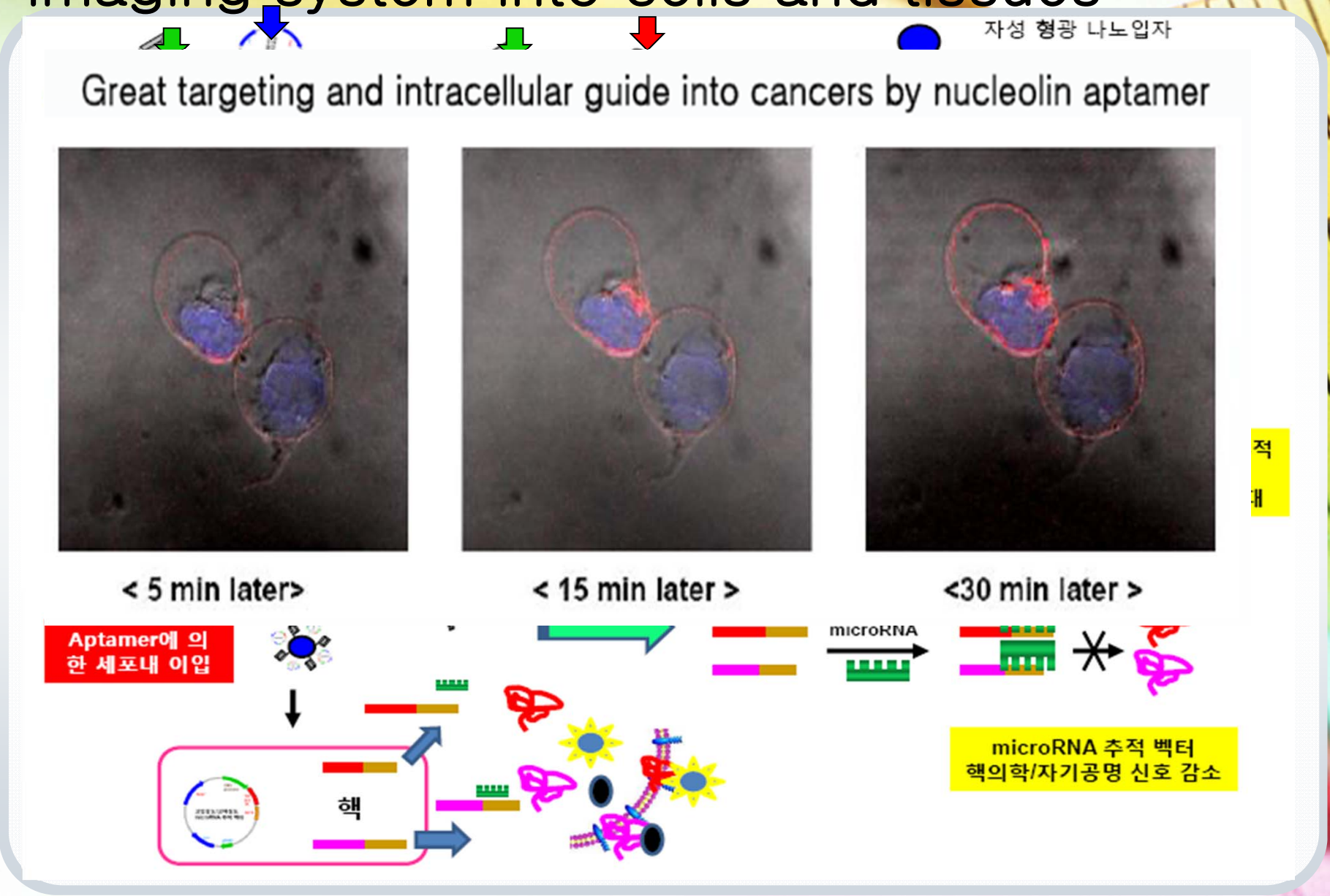
In vivo assay by co-transfection of reporter gene and molecular beacon



Conclusion

- The chimeric miRNA imaging system provides the complementary imaging information from the miRNA Molecular Beacon confirming that the dramatic signal decrease in miRNA reporter gene was caused by the increase in miRNA

On-going work: targeting delivery of miRNA imaging system into cells and tissues



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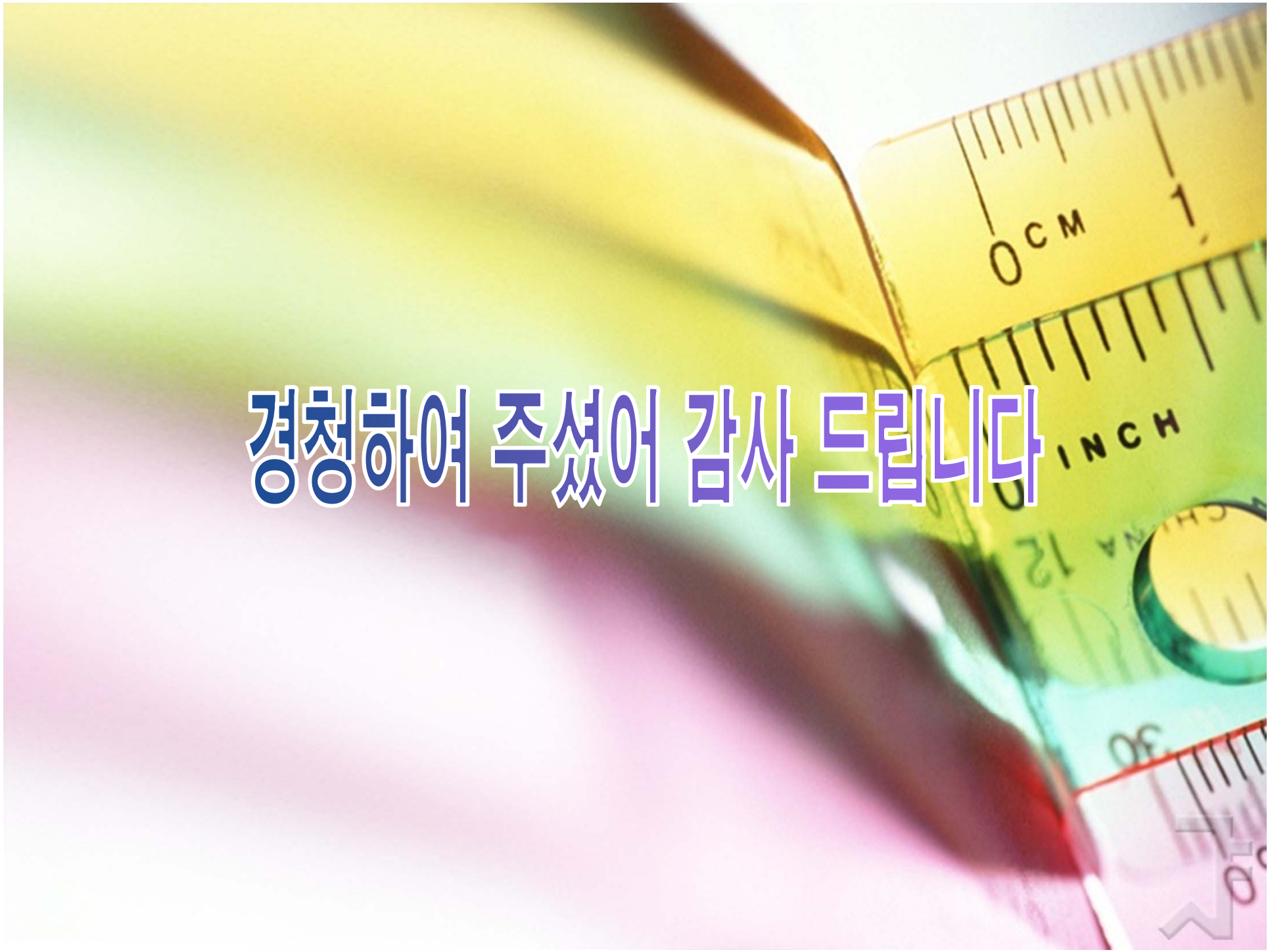
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