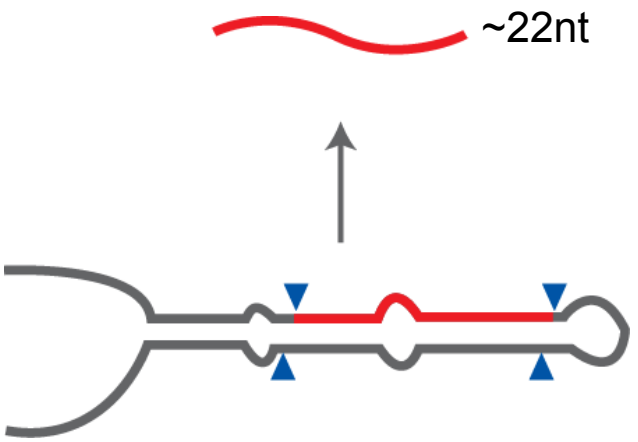
Basics of microRNA study in diabetes

Young-Kook Kim

What is microRNA?

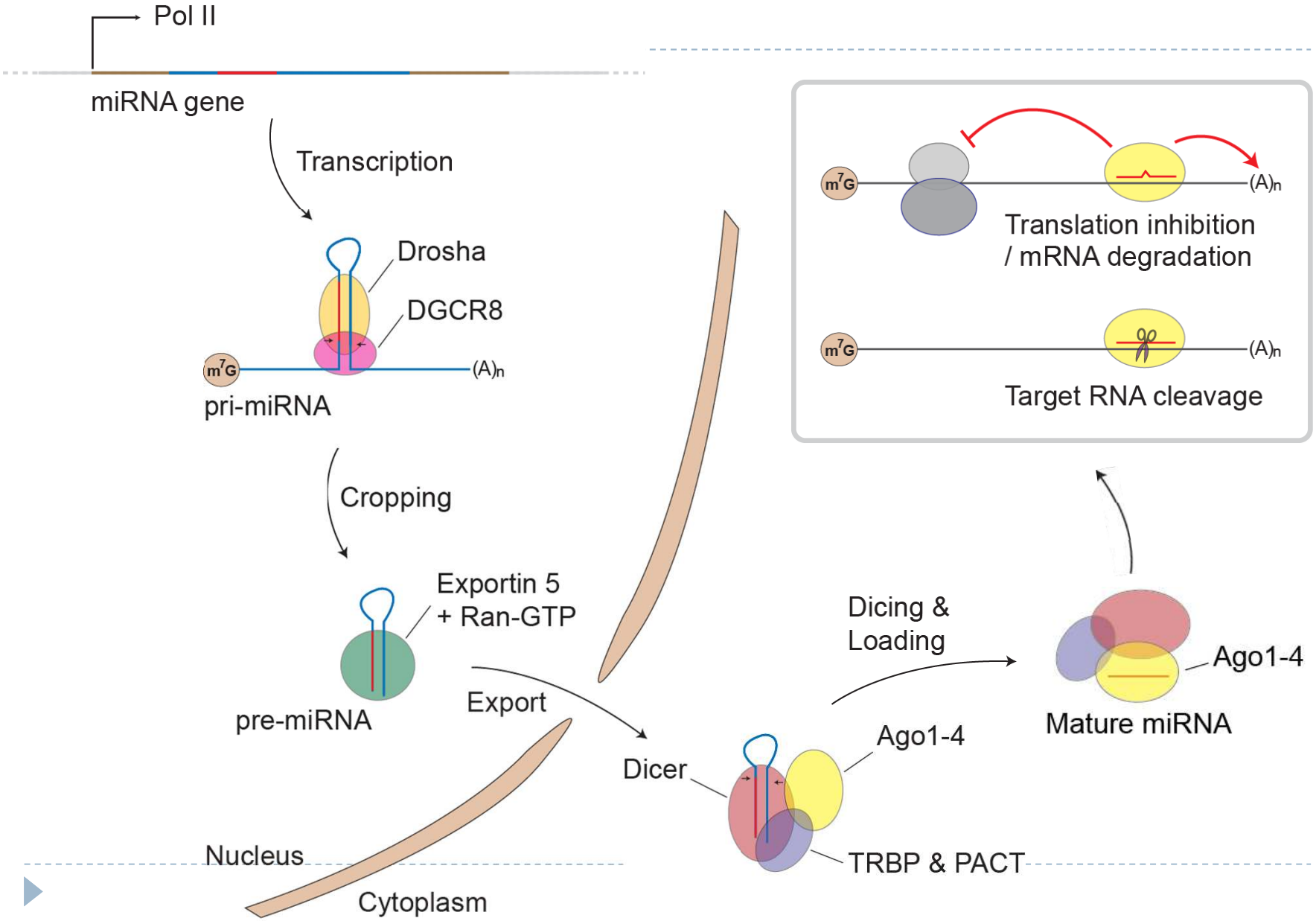


- ~22nt single-stranded RNAs
- Generated from endogenous stem-loop precursors

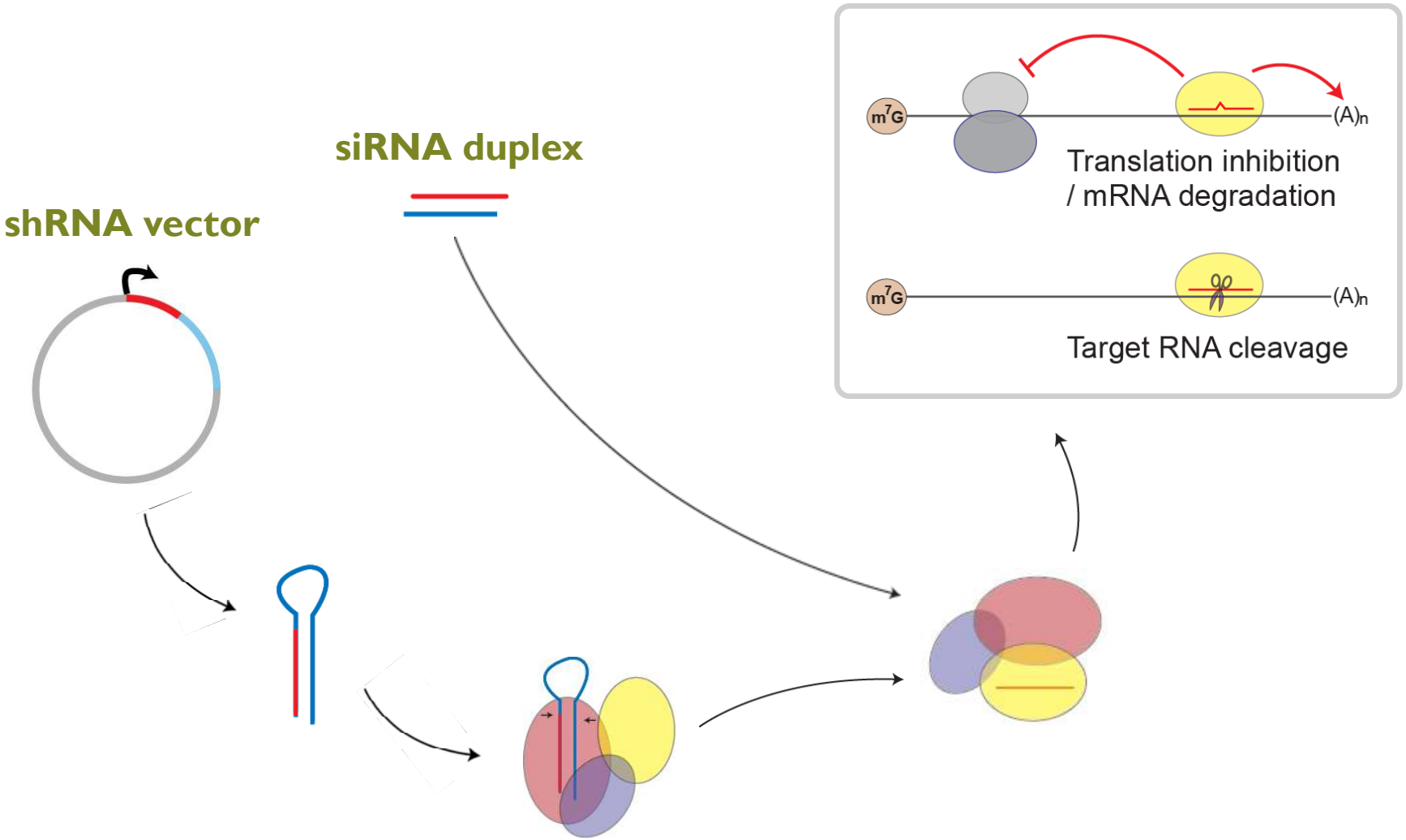
- Often conserved among species
- Abundant in animals and plants (100-1000 genes / species)

- Function in a variety of regulatory pathways
  (development, differentiation, proliferation, tumorigenesis, antiviral defense…)

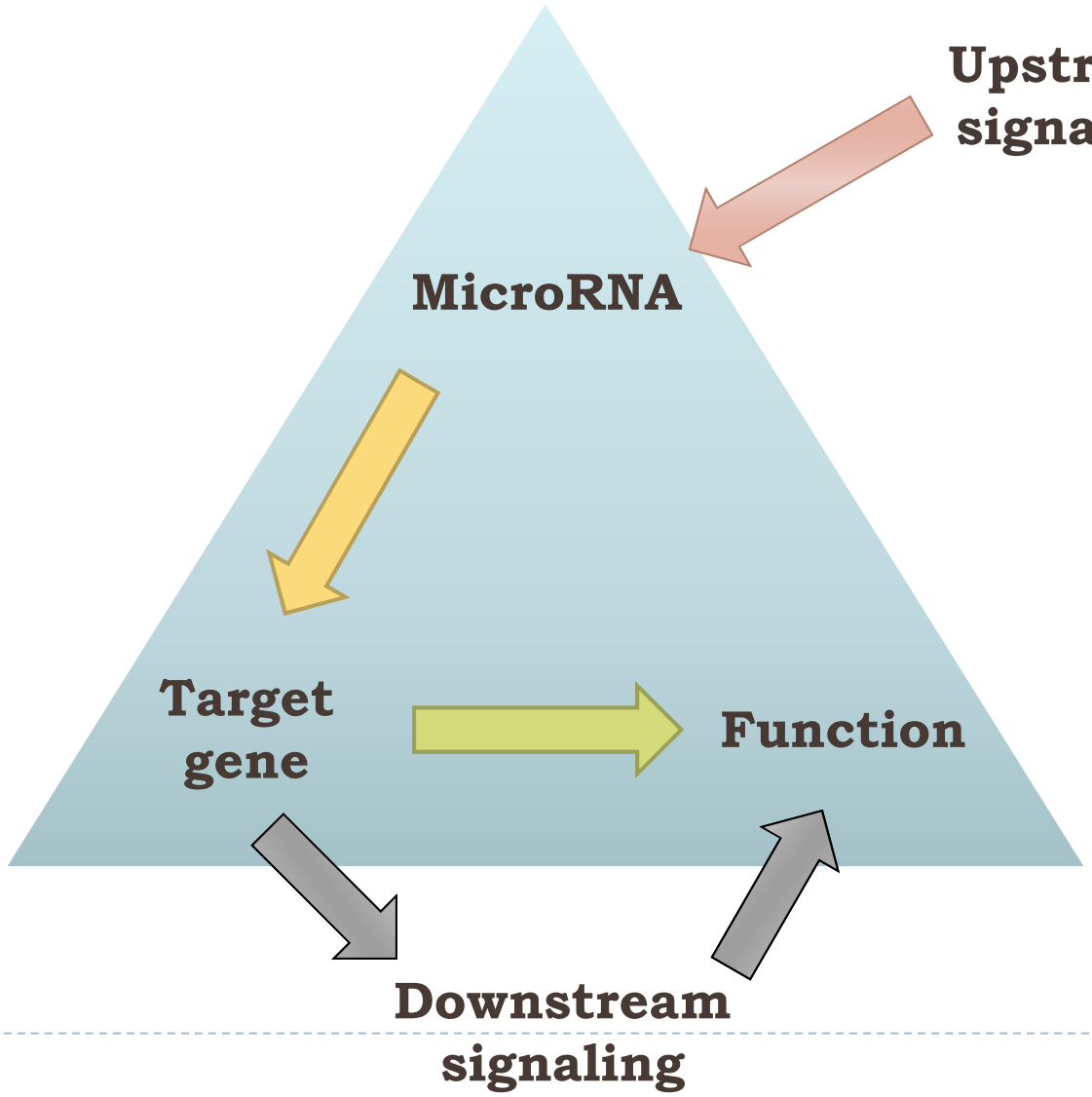
MicroRNA biogenesis in animal



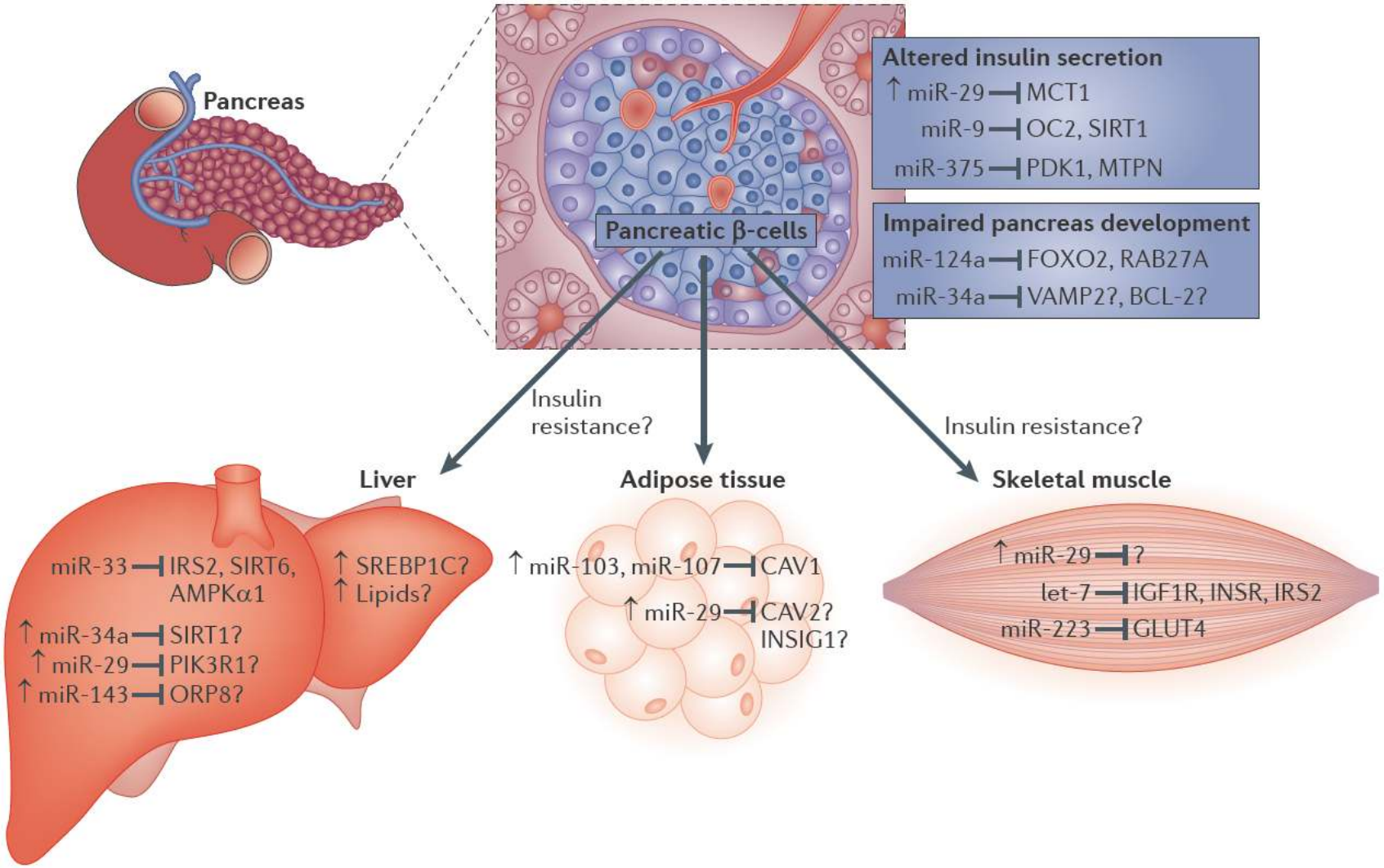
siRNA or shRNA to the pathway



MicroRNAs function by target regulation



MicroRNA regulation of insulin signaling and glucose homeostasis



Rottiers and Näär, NRMCB 13:239

MicroRNA-based

Expression profiling

Screening – Reporter, Mutagenesis

Functional study of microRNAs

Target search – bioinformatics

Target gene-based

Discovering microRNA in diabetes

- Type I diabetes
  - Nonobese mouse
  - Streptozotocin-treated mouse
- Type II diabetes
  - ob/ob mouse
  - db/db mouse
  - Goto-Kakizaki rat
- Patients
  - Blood sample
  - Tissue

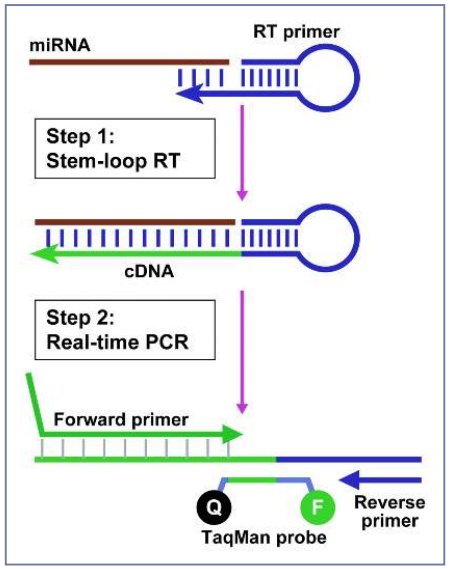
Animal models*	Characteristics	Tissue	Detection	miR changes†
Type 1 diabetes animal models				
NOD mice	Susceptibility to spontaneous development of T1D	Pancreatic islets	qPCR	**miR-21,-34a, -146a**
Streptozotocin-induced T1D mice	Destruction of B cell by repeated injection of streptozotocin	Liver	array	**miR-34a**
				miR-122
Type 2 diabetes animal models				
db/db mice	Leptin receptor gene deficiency	Pancreatic islets	qPCR	**miR-34a,-146a**
	Obese, hyperglycemic, hyperinsulinemic	Liver	array	**miR-19a, -183, -207, -212, -326, -328, -335, -409, 434-3P, 467**
				miR-33, -34c, -129-3P, -133a, -137, -142-5P, -144, -146, -384, -448
	Insulin resistant	Adipose tissue	qPCR	**miR-335**
ob/ob mice	Leptin gene deficiency	Liver	array	**miR-31, -34a, -103, -107, -194, 200a, -221, -335-5P**
	Obese, hyperglycemic, hyperinsulinemic			*miR-2, -29c, -122, -451*
			qPCR	**miR-335**
	Insulin resistant	Adipose tissue	array	**miR-146b, -221, -222**
				miR-30a-5P, -30c, -99b, -103, -107, -125b, -143, -148a, -422b
			qPCR	**miR-335**
BTBR-ob & B6-ob mice vs BTBR & B6 mice	Diabetes-susceptible BTBR mice or diabetes-resistant B6 mice were inbred with ob/ob mice	Pancreatic islets	array	**miR-34a, -34b, 132, -133a, -126-5P, -152, -185, -212**
				miR-7b, -31, -184, -204
		Liver	array	**miR-34a, -205**
	Obese			*miR-17-3P, -133a, -151, -201, -298, -328, -329, -330, -380-5p*
	Mild and transient (B6-ob) or severely (BTBR-ob) hyperglycemic	Adipose tissue	array	**miR-221, -222, -342**
				miR-1, -34b, -34c, -133b, -135a, -141, -200a, -200b, -200c, -215, -375, -429
GK rats	Nonobese Wistar substrain Develop T2D early in life	Pancreatic islets	qPCR	**miR-124a**
				miR-375
		Liver	Northern	**miR-29a,-29b,-29c**
			array	**miR-125a**
			array	**miR-103, -195**
		Adipose tissue	Northern	**miR-29a,-29b,-29c**
			array	**miR-125a**
			array	**miR-222, -27a**
		Muscle	array	**miR-29a,-29b,-29c,-150**

Guay et al, Translational Res 157:253

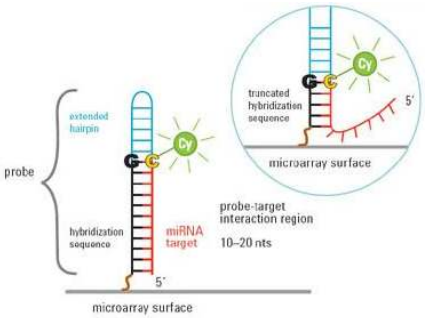
How to measure the level of microRNA?

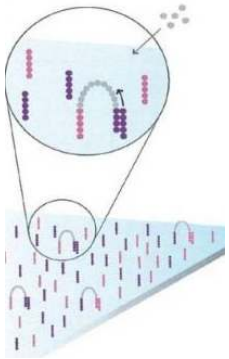
- Low throughput
  - Northern
  - Real Time PCR

- High throughput
  - Microarray
  - TaqMan® Real Time PCR Array
  - High Throughput Sequencing

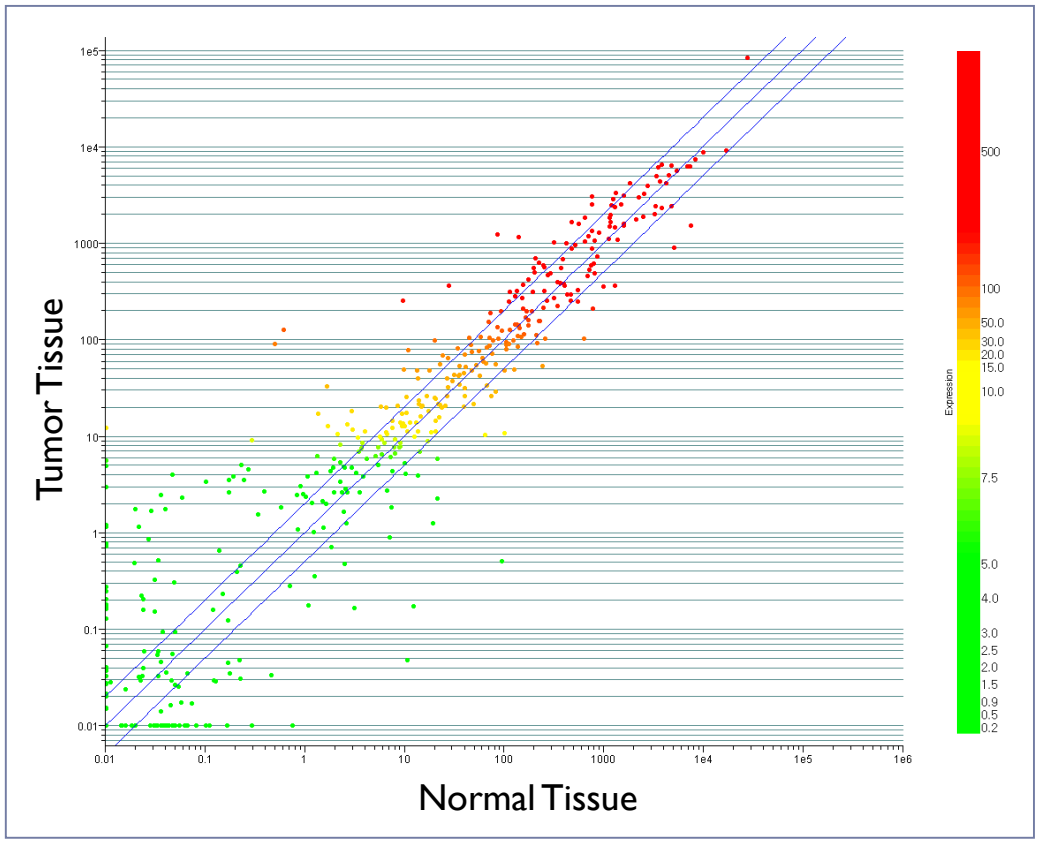


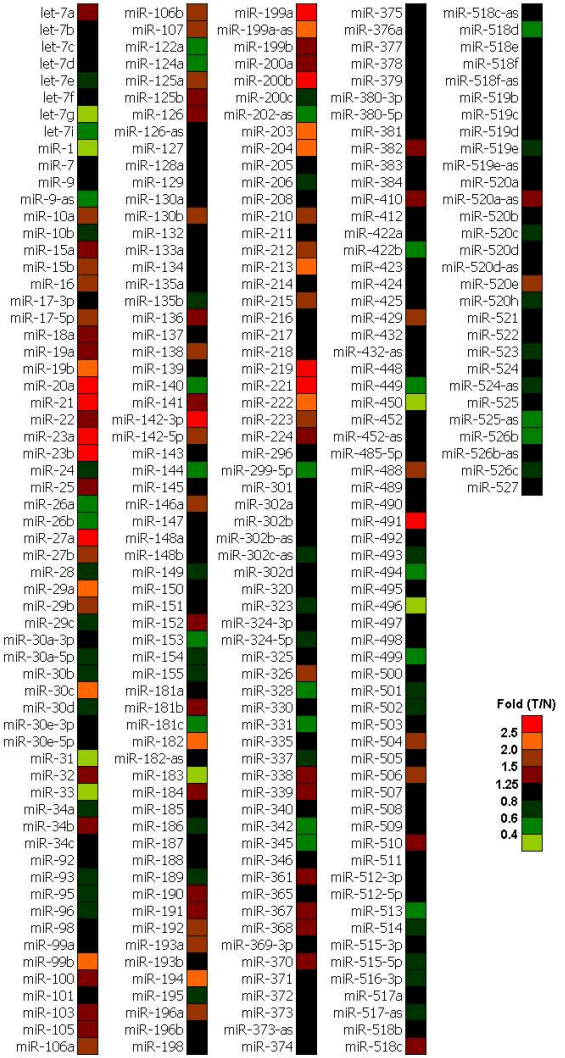
Chen et al, NAR 33:e179





Screening
- MicroRNAs increased in stomach cancer



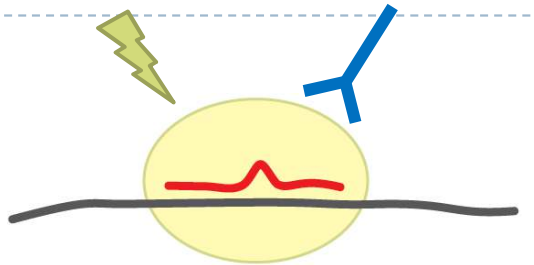


Testing with several companies
- **Agilent,** Affymetrix, Invitrogen…

with Jieun Yu in Han-Kwang Yang Lab

To find the target of microRNA

- Biochemical method is hard to set up
  → Ago-CLIP-Seq

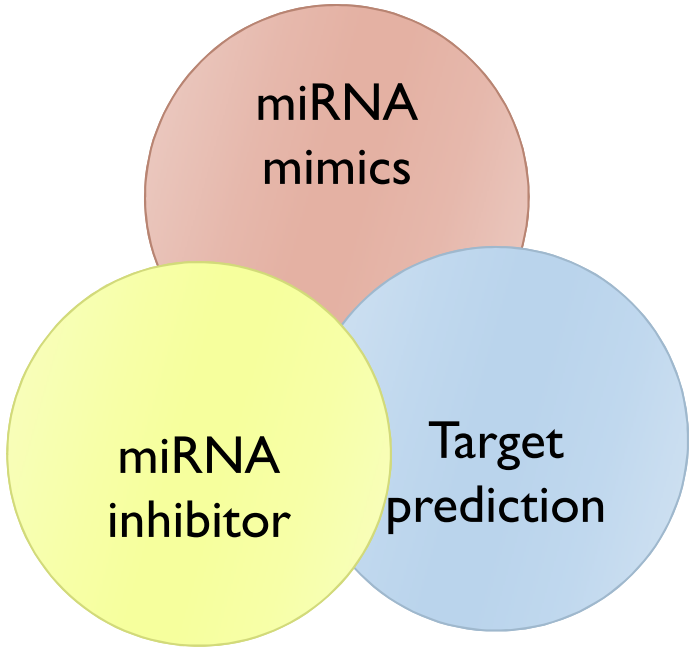


- MicroRNA over-expression / inhibition
  → Microarray or High-throughput sequencing
  → Hundreds of target candidates

- Target prediction program
  → Hundreds of target candidates

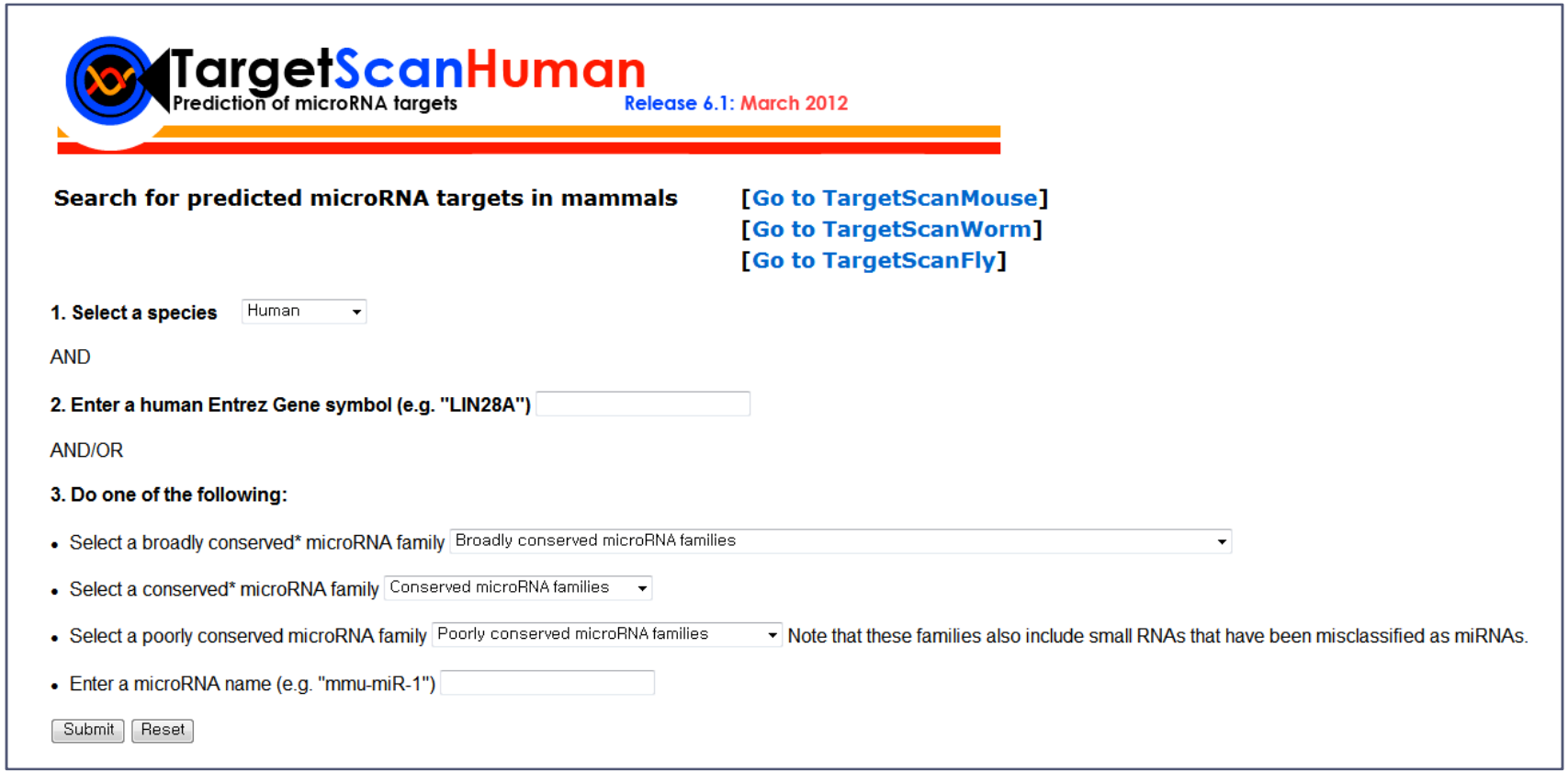
Target gene → prediction

- Target search through bioinformatics program
  - TargetScan
  - miRanda (miRBase)
  - PicTar
  - RNAHybrid
  - Diana-microT
  - miTarget
  - :
- Intersect with expression profiling data

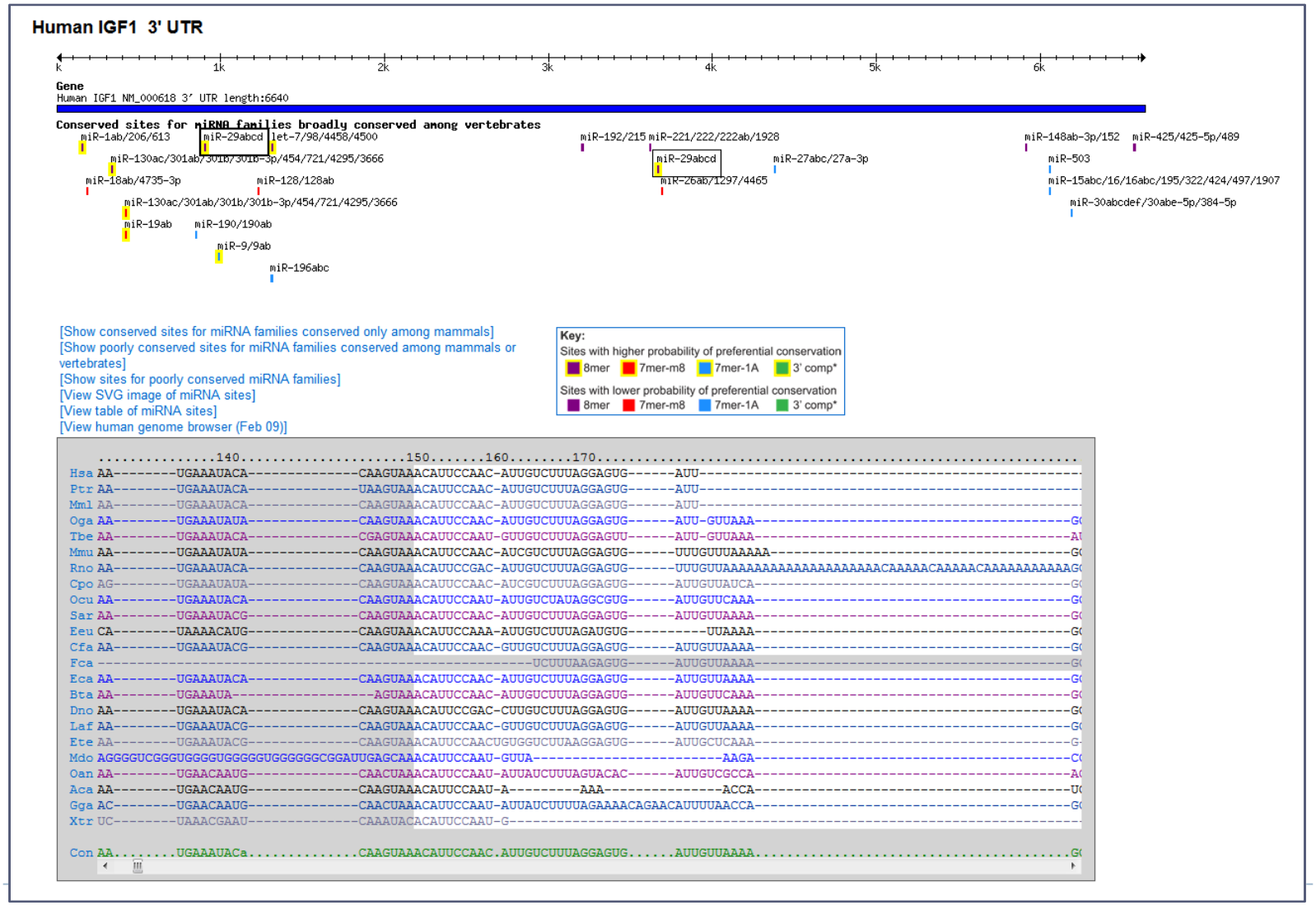


Target prediction algorithm - TargetScan

- www.targetscan.org

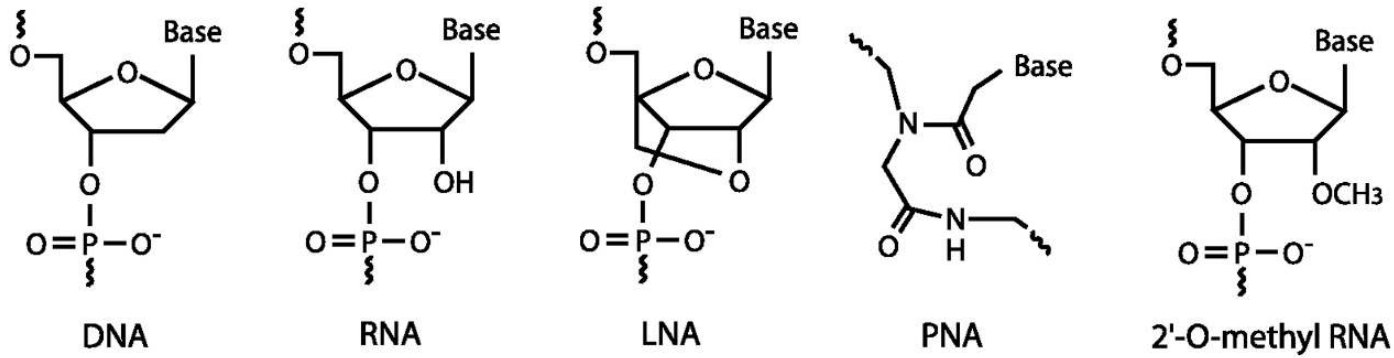


Target prediction algorithm - TargetScan



Target gene → verification

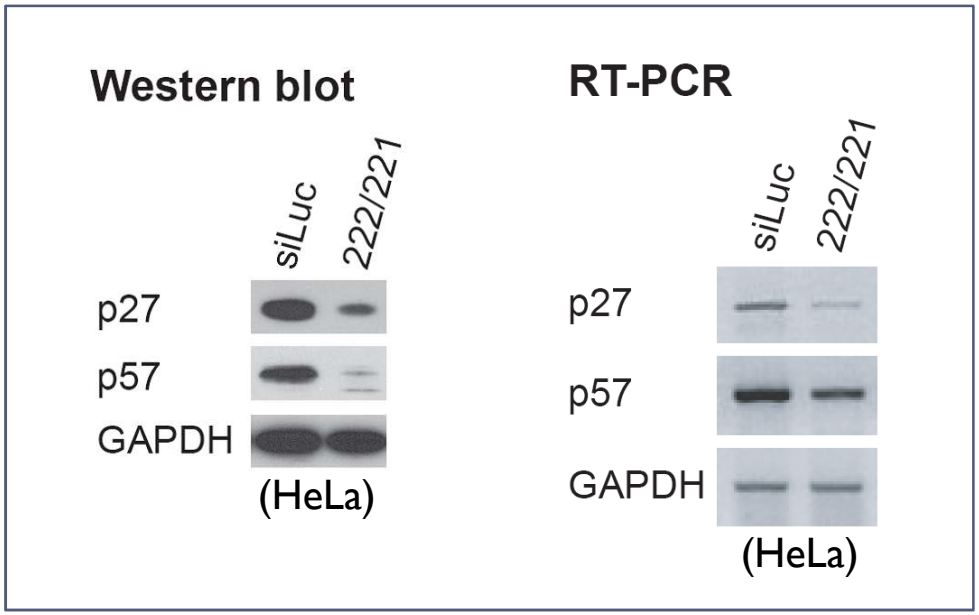
- Protein level? mRNA level?
- Which cell lines?
- What kind of (and how much) inhibitor?



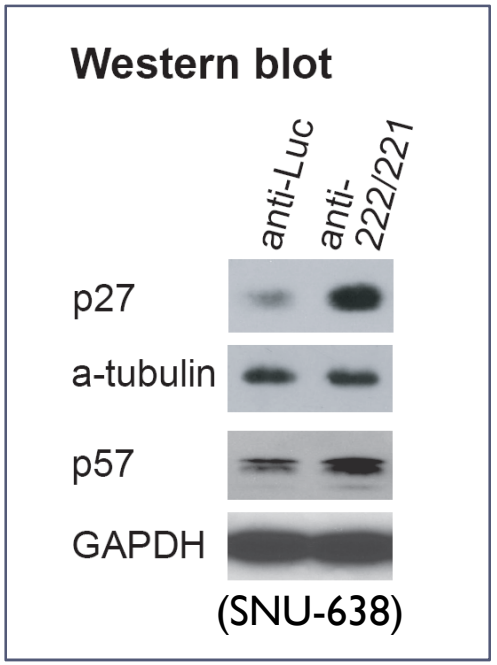
- 3’-UTR cloning and Luciferase assay

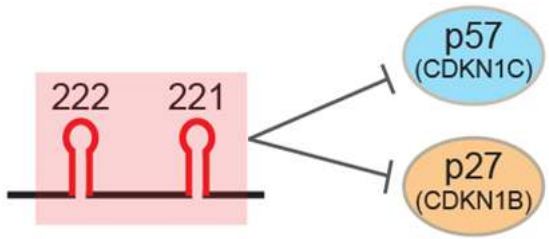
Verification
- MicroRNA overexpression or inhibition

miRNA mimic



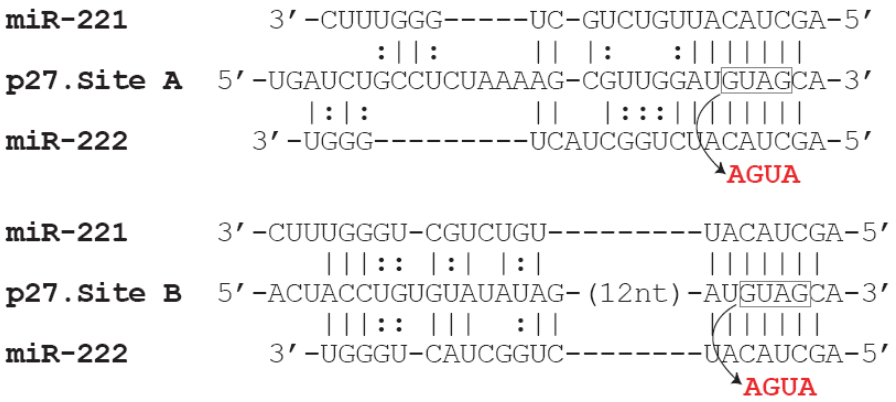
miRNA inhibitor

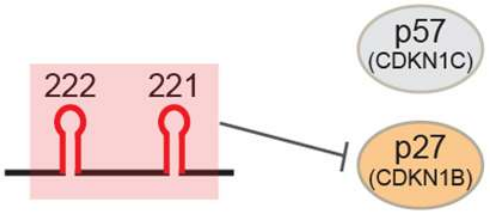


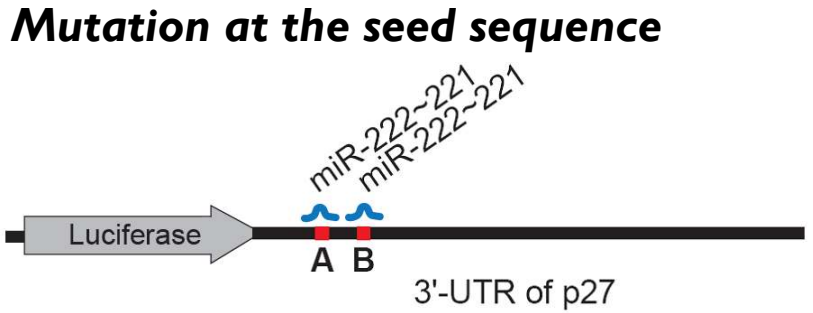


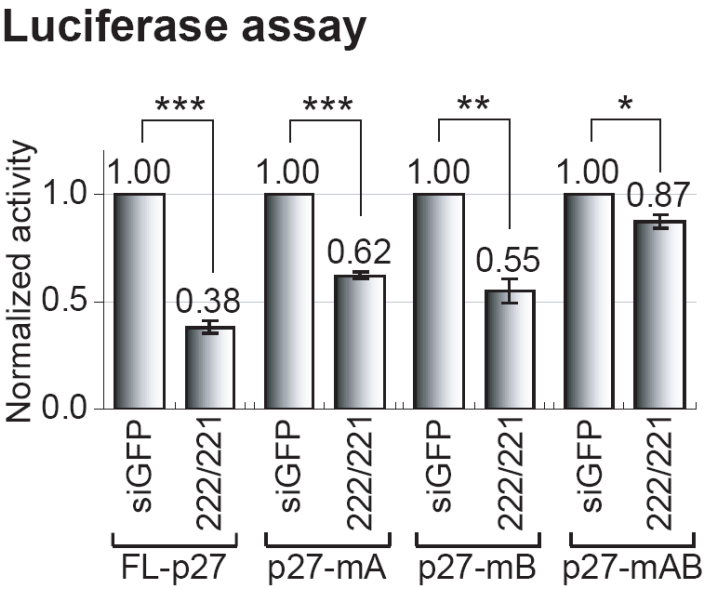
miR-222~221 induces both mRNA degradation and translational inhibition of p27 and p57.

Direct regulation of target gene → mutagenesis





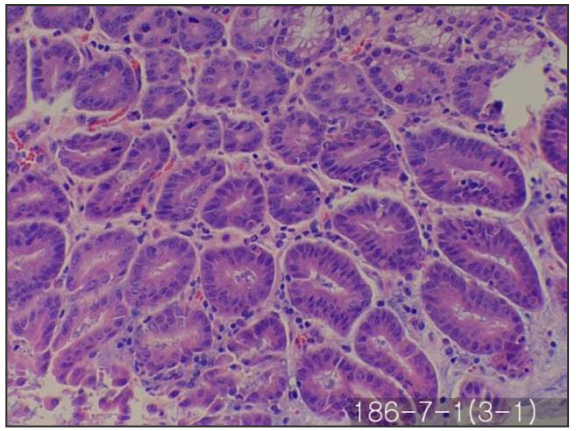




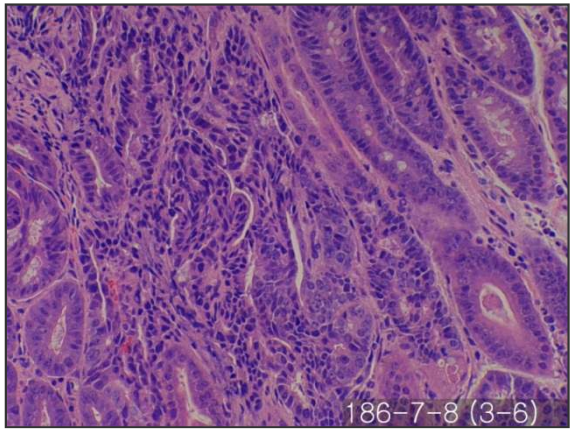
Physiological experiment

Apply to physiological system in each lab

(ex) miR-222-221 transgenic mouse



Stomach, adenoma



Duodenum, cancer

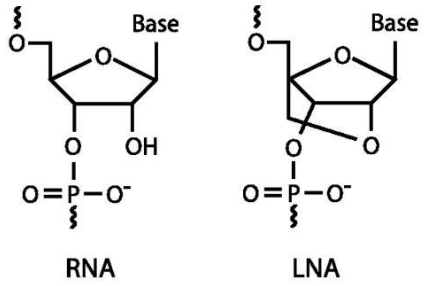
with HK Yang's & WH Kim's lab

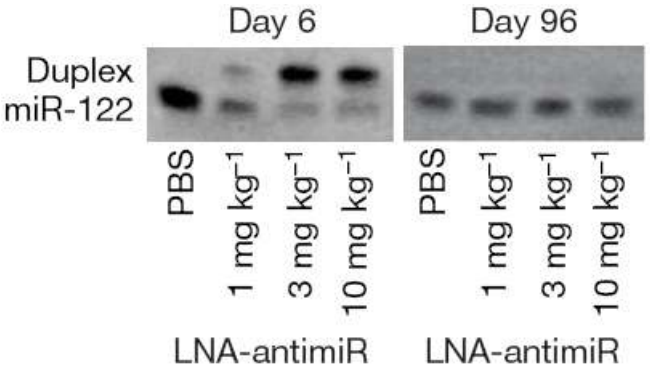
MicroRNAs in therapy

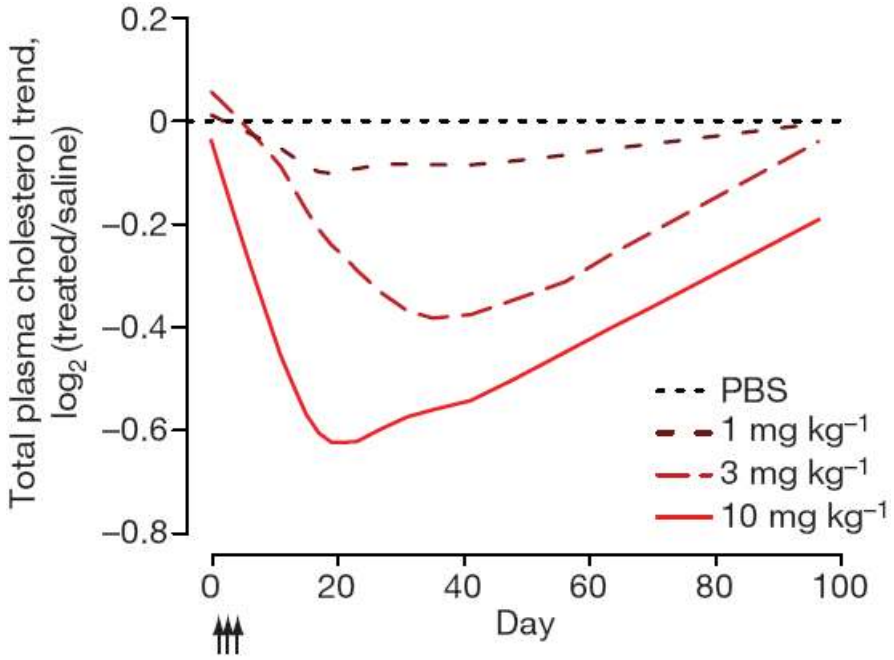
- miRNA inhibitor
  - (ex) miR-122
- Biomarker
  - Exosome vesicle
  - Ago-associated miRNAs
  - HDL-associated miRNAs
- Circulating miRNAs as endocrine signals

Silencing of miR-122 in non-human primates by LNA-antimiR

LNA as microRNA inhibitor

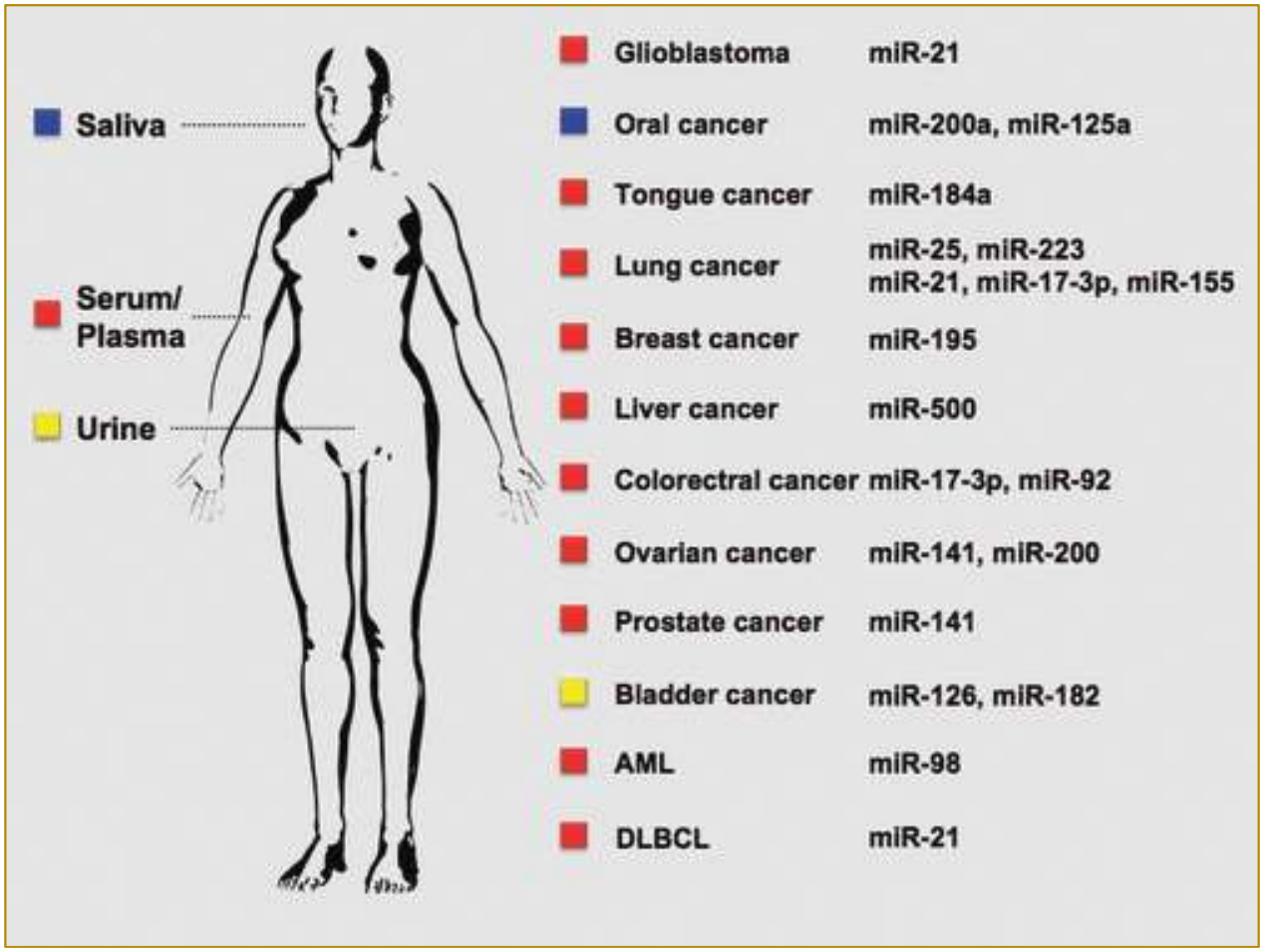






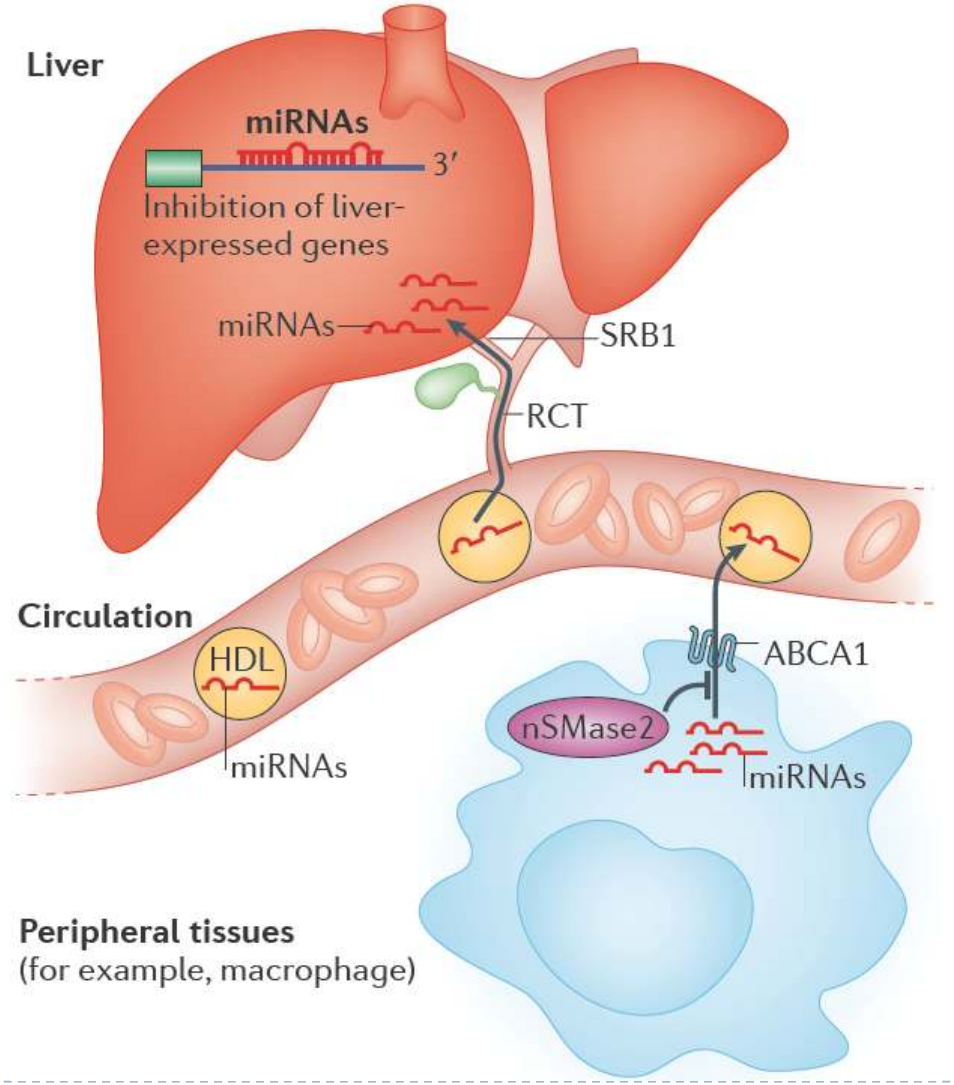
Elmen et al, Nature 452:896

Circulating microRNAs as potential biomarkers for diagnosis and prognosis



Kosaka et al, Cancer Sci 101:2087

Circulating microRNAs associated with HDL



Rottiers and Näär, NRMCB 13:239

MicroRNA study in the future

- More functional studies are required.
- More studies are required for clinical application.