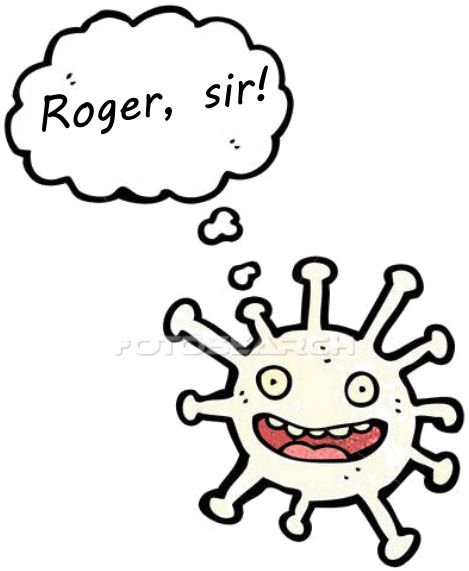
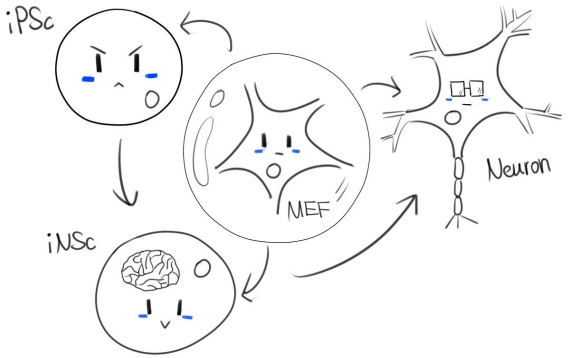
"Tet and Cre" dependent genetic logic circuit and its application in mammalian cells





Outline

- Introduction
- Project & Modeling
- Results & Summary
- Human practice & safety
- Acknowledgements

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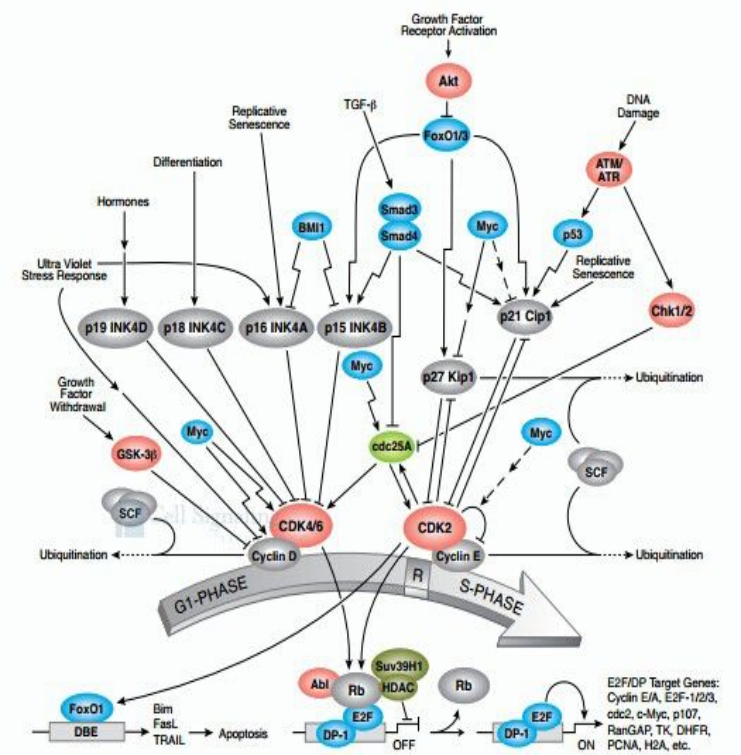
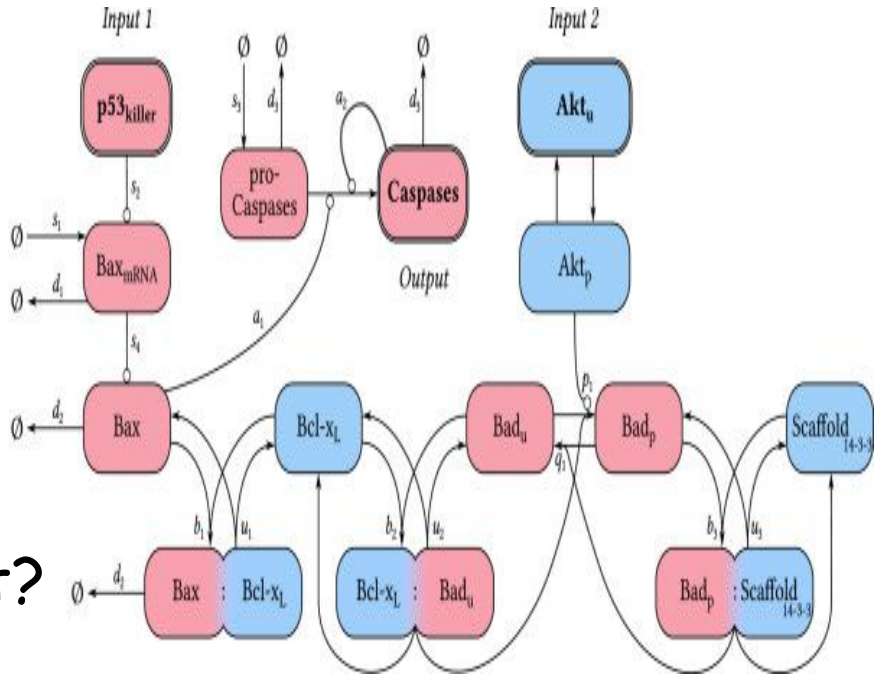
Introduction



What we hope is to construct genetic logic circuits like this.



BMC Syst Biol. 2013 Jul 24;7:67. doi: 10.1186/1752-0509-7-67.

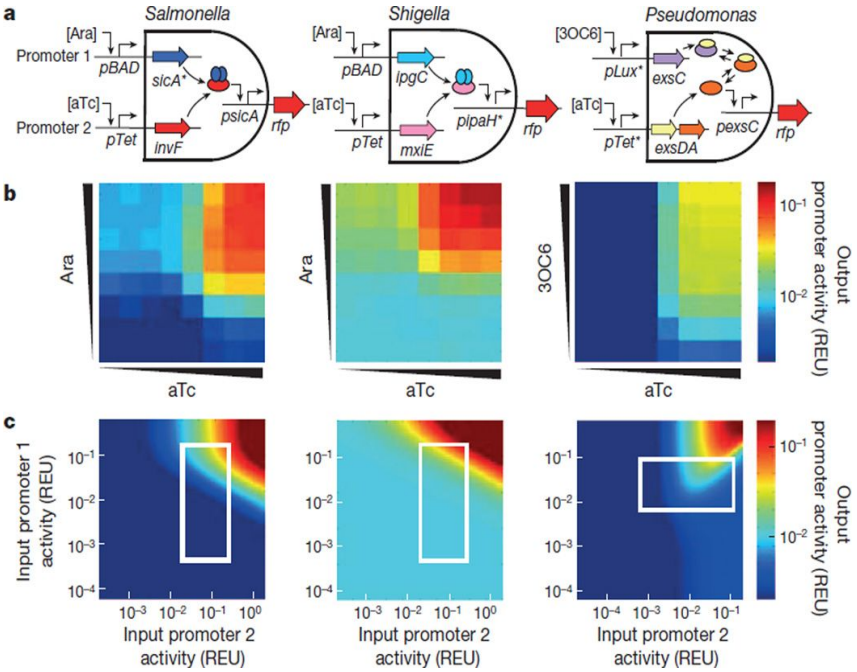
All cells!

Internal or from environment?

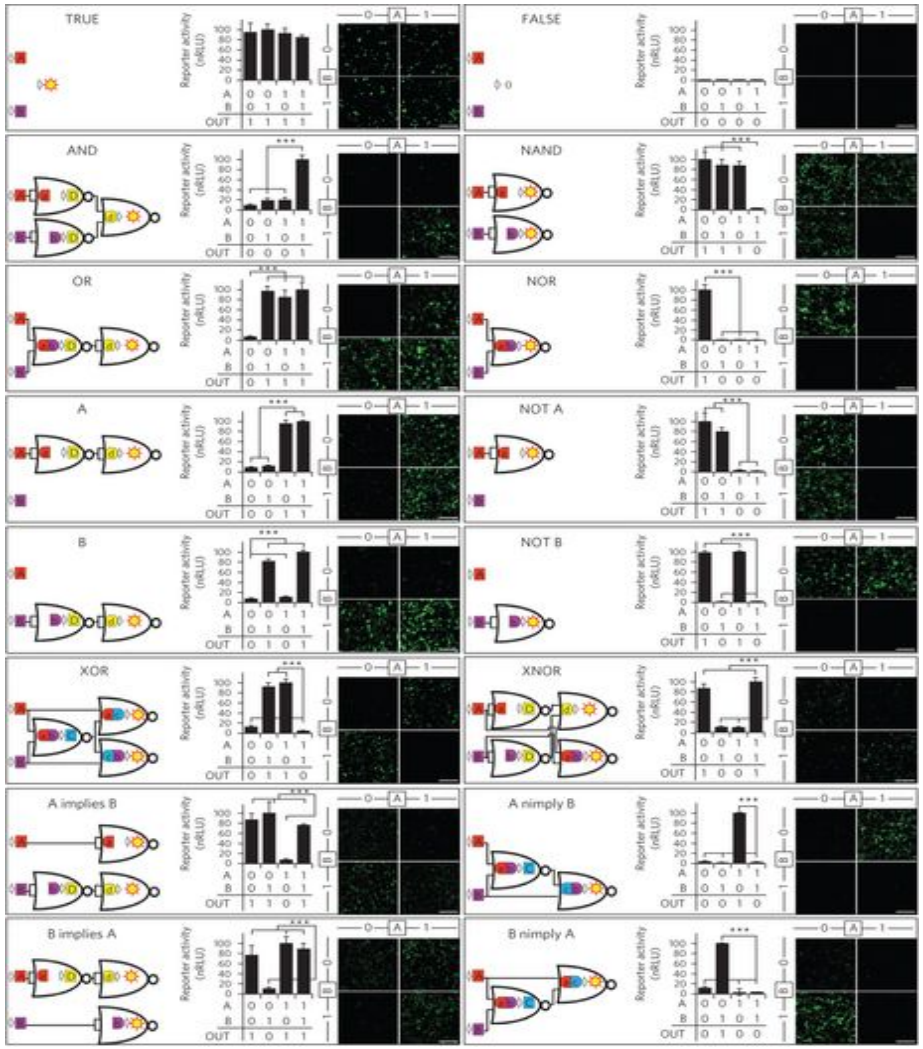
Produce appropriate cellular response

Introduction

Previous work in both prokaryotic and eukaryotic cells



Moon et al. Nature. 2012 Nov 8;491(7423):249-53.



Garber et al. Nat Chem Biol. 2014 Mar;10(3):203-8.

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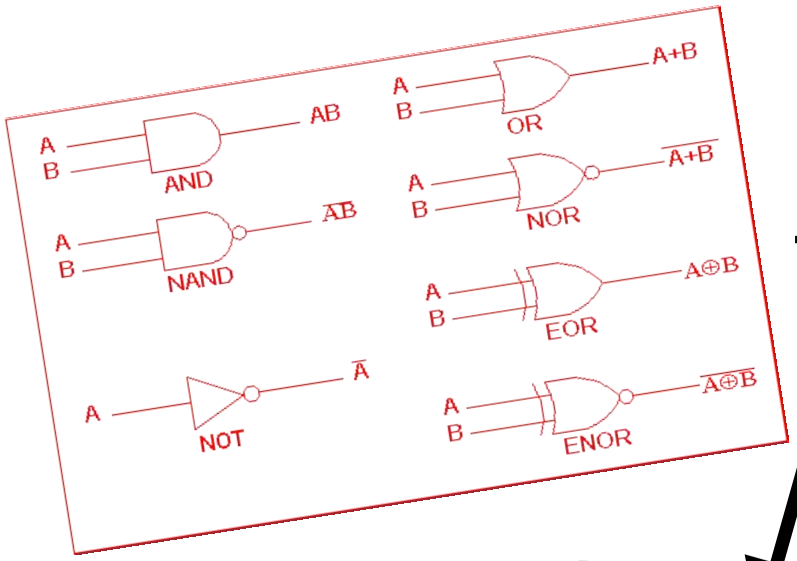
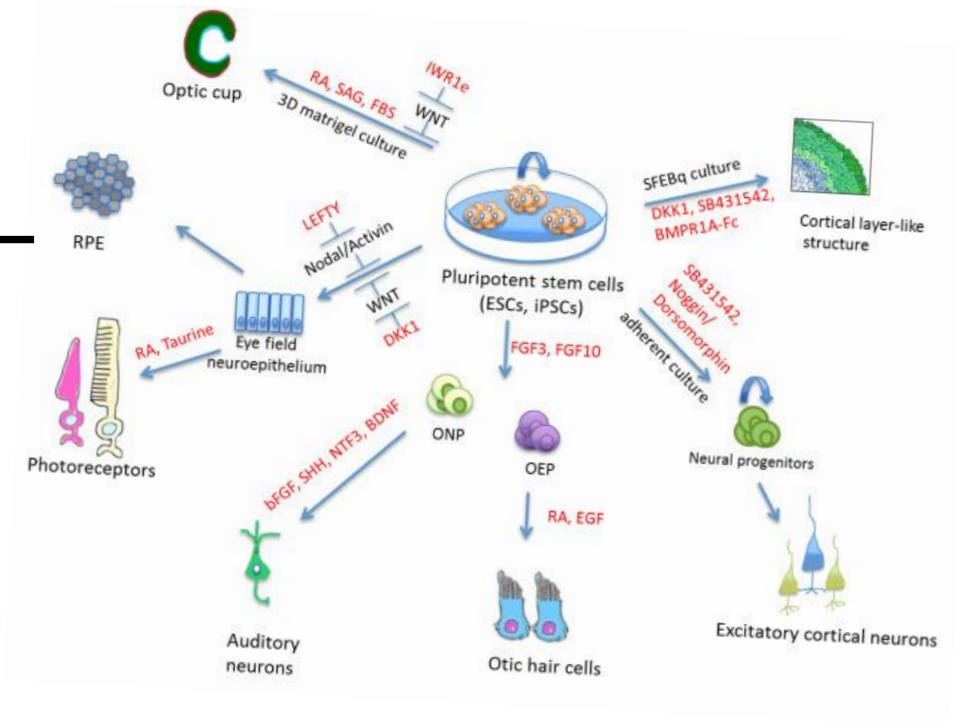
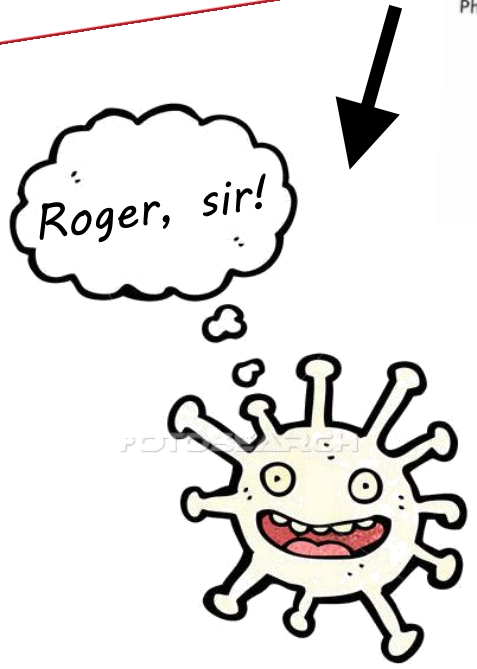
Project -- Brainstorming

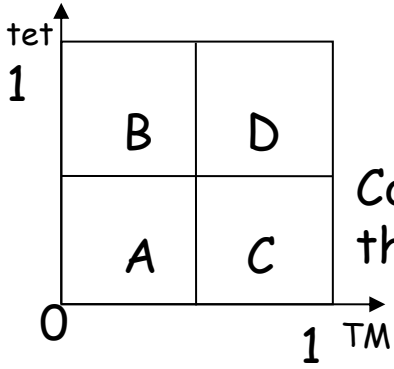


+

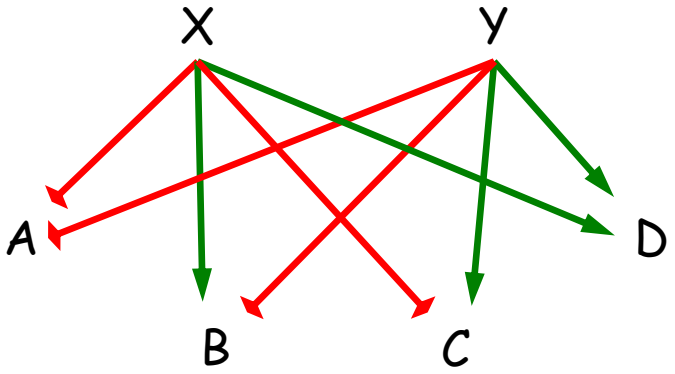




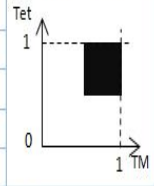
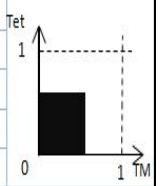
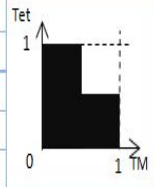
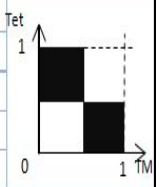
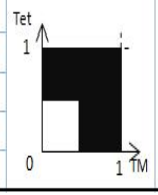
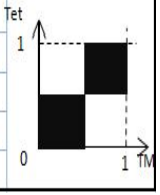
Project -- Motif searching



Consider a 2 input system, there will be 4 input states.



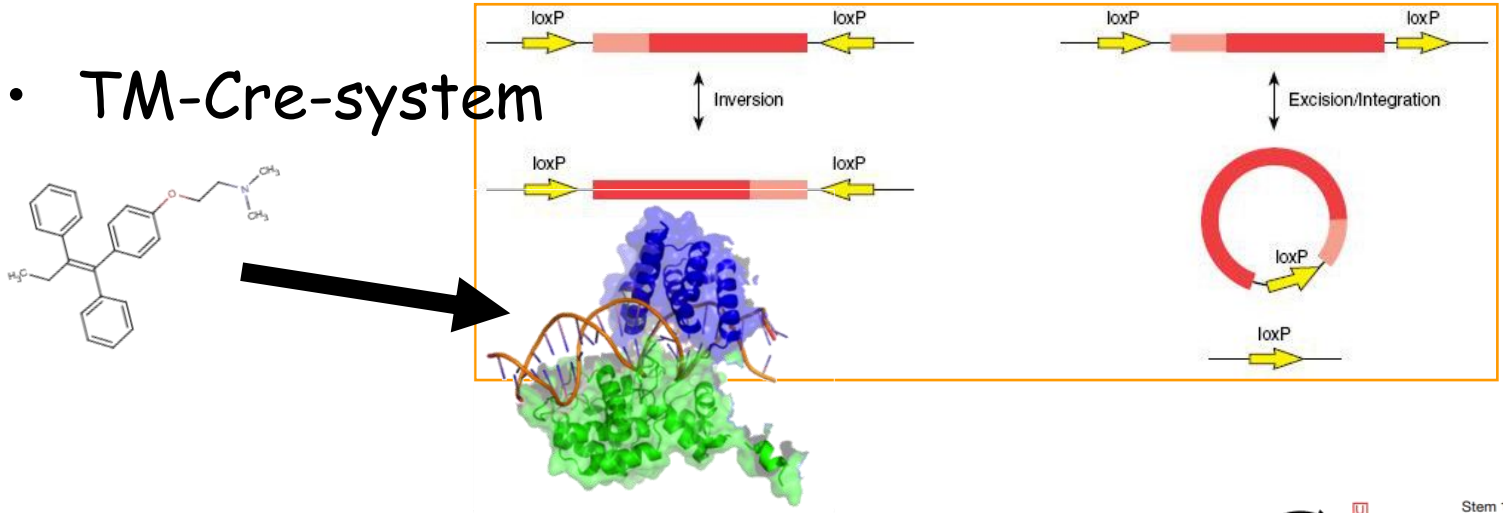
We choose Bi-Fan motif as the blueprint of our circuit design.

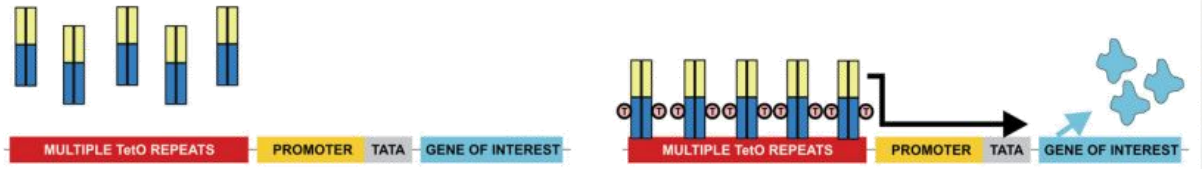
Gate	Truth Table			Predicted	Gate	Truth Table			Predicted
	Drug 1	Drug 2	Out			Drug 1	Drug 2	Out	
AND	0	0	0		NOR	0	0	0	
	1	0	0			1	0	1	
	0	1	0			0	1	1	
	1	1	1			1	1	1	
NAND	Drug 1	Drug 2	Out		XOR	Drug 1	Drug 2	Out	
	0	0	1			0	0	0	
	1	0	1			1	0	1	
	0	1	1			0	1	1	
	1	1	0			1	1	0	
OR	Drug 1	Drug 2	Out		XNOR	Drug 1	Drug 2	Out	
	0	0	0			0	0	1	
	1	0	1			1	0	0	
	0	1	1			0	1	0	
	1	1	1			1	1	1	

If output signals of A/B/C/D regions can be separated, theoretically we can construct any common logic gate!

Project -- Basic Elements

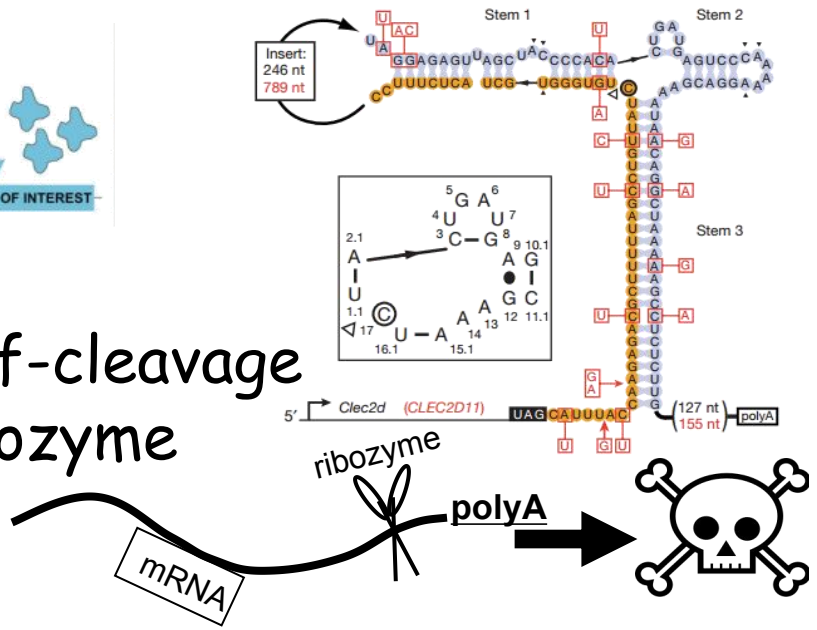
- TM-Cre-system



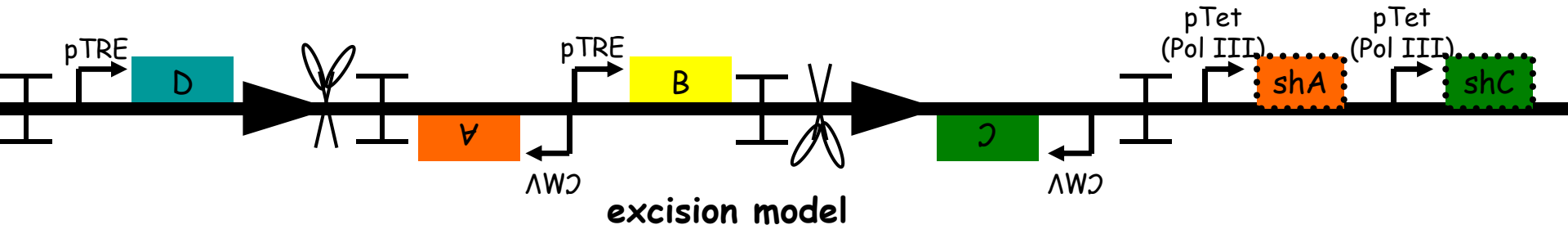


- Tet-on system

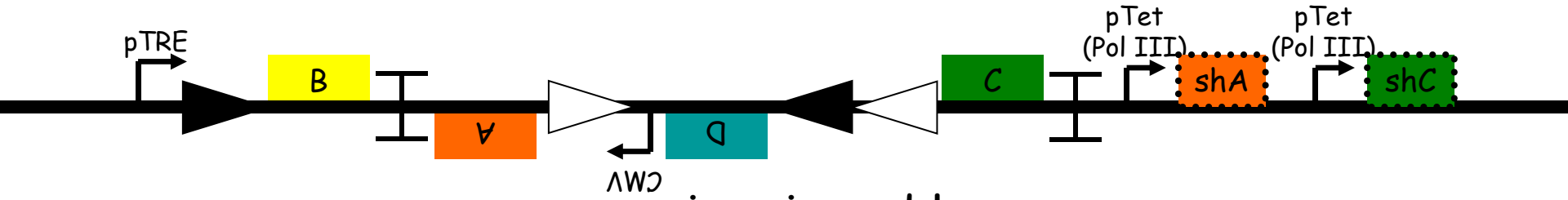
- Self-cleavage Ribozyme



Project -- Overview

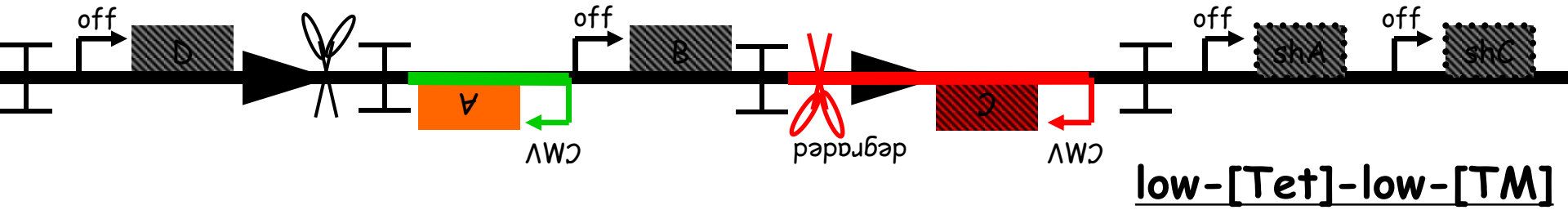


excision model



inversion model

Project -- Excision Model (0,0)



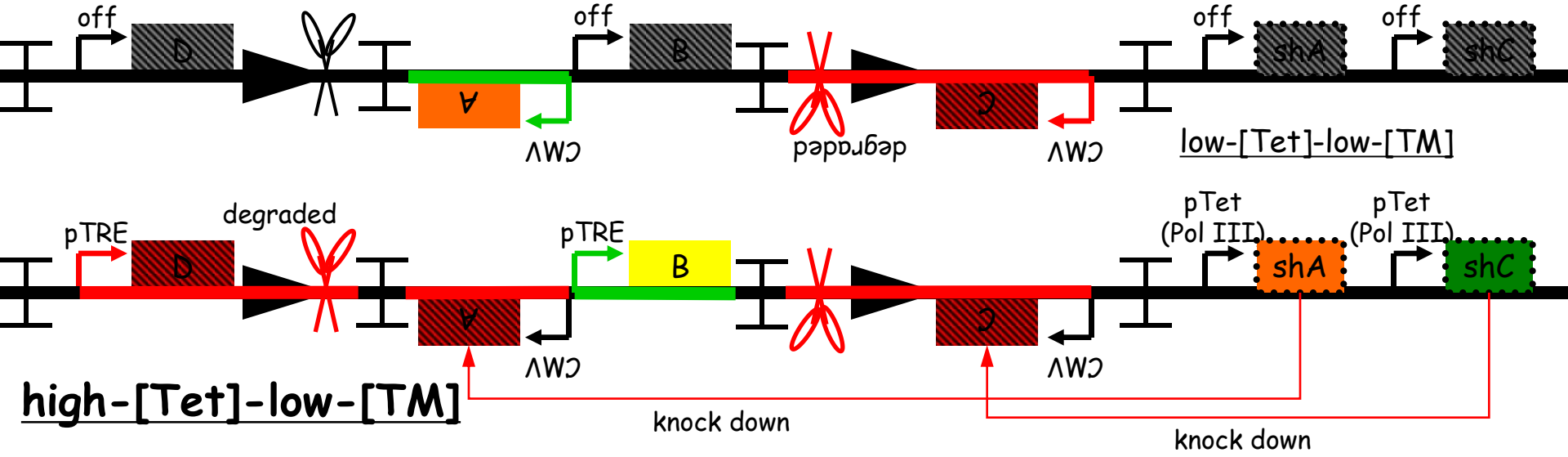
low-[Tet]-low-[TM]

- "off" --- no expression because no Tet (nor Dox)

Result: only A is expessed

- scissor ( ) --- self-cleavage ribozyme
- " " --- SV40pA, transcription termination signal
- triangle ( ) --- loxP site
- green line ( ) --- successfully expressed
- red line ( ) --- transcribed but undergo degradation

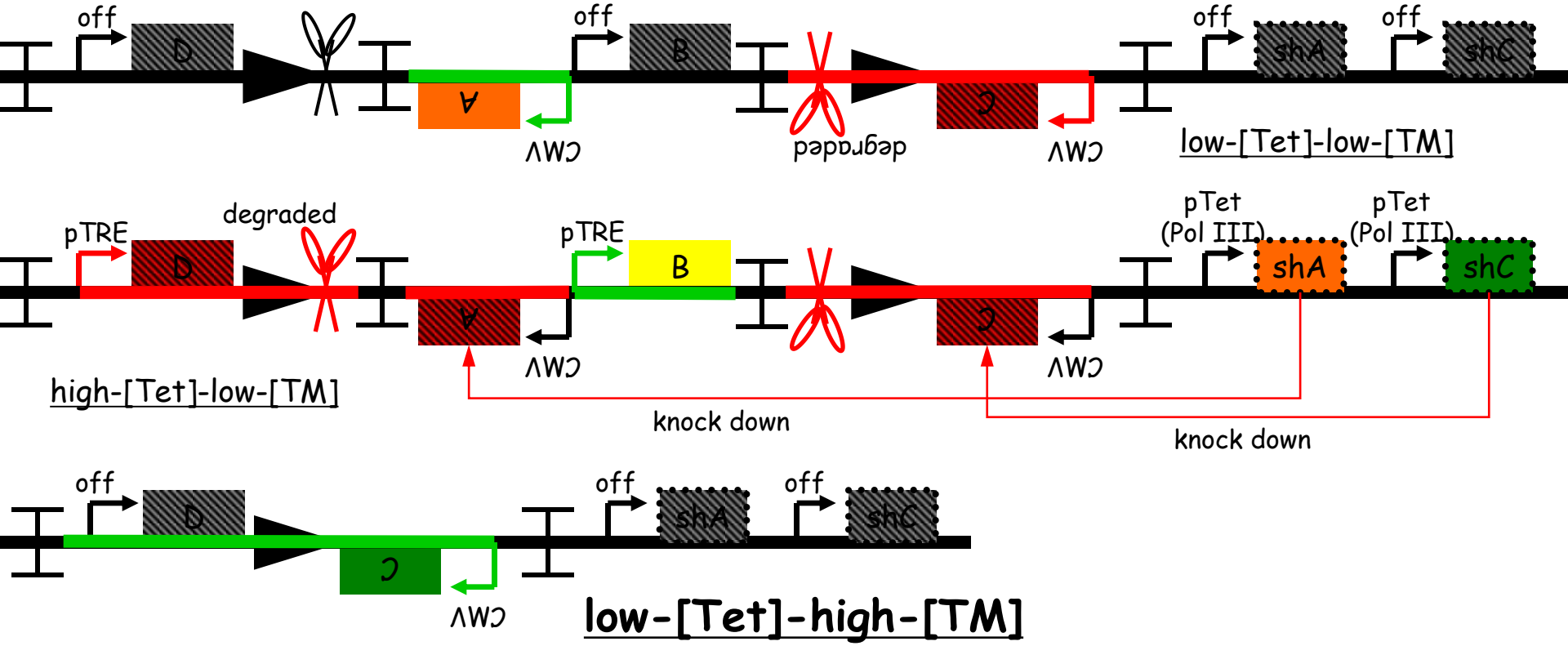
Project -- Excision Model (1,0)



- knock down --- shRNA undergo microRNA genesis and produce siRNA, knock down target gene
- scissor ( ) --- self-cleavage ribozyme
- green line ( ) --- successfully expressed
- red line ( ) --- transcribed but undergo degradation

Result: only B is expessed

Project -- Excision Model (0,1)

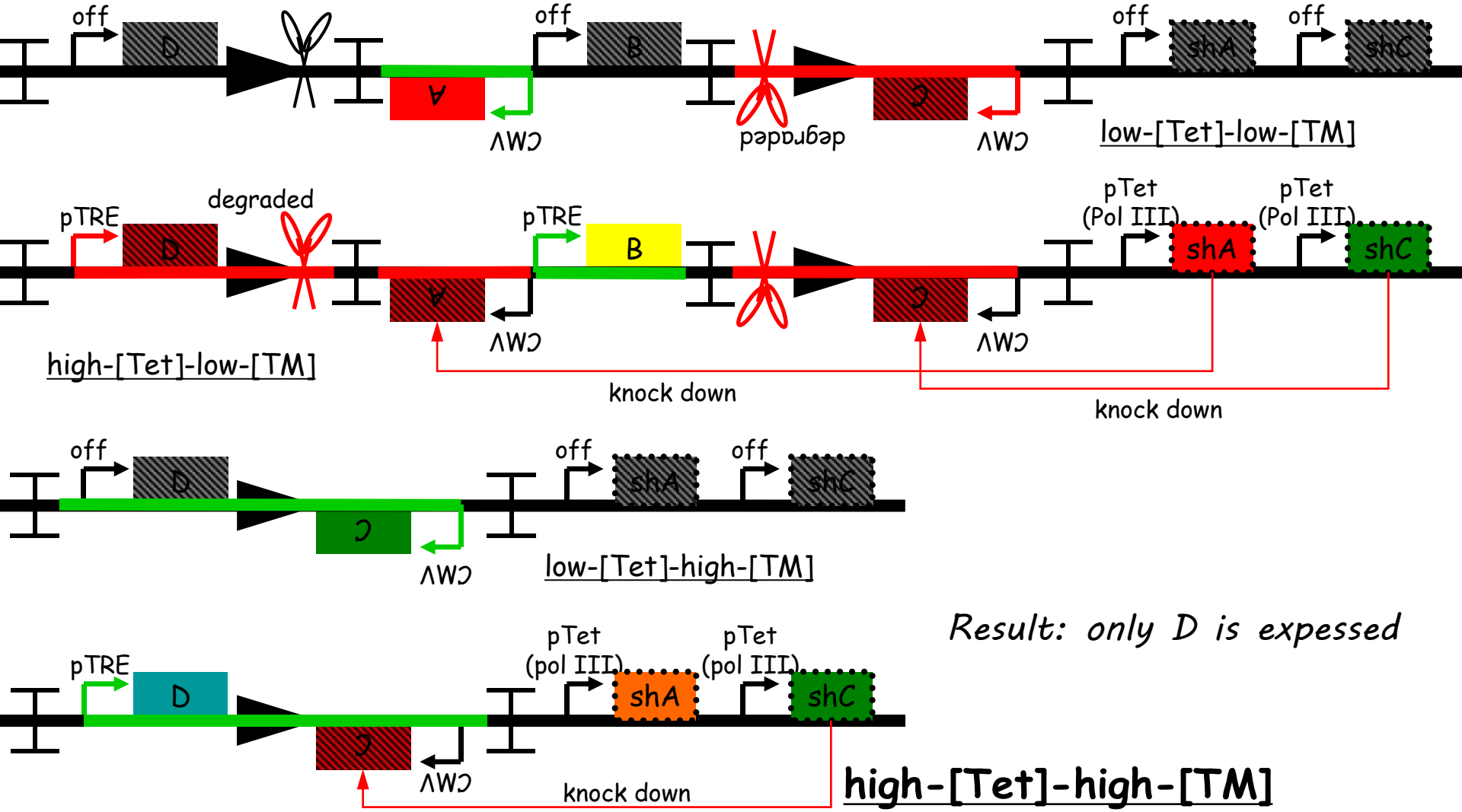


- triangle (▶) --- loxP site

Result: only C is expessed

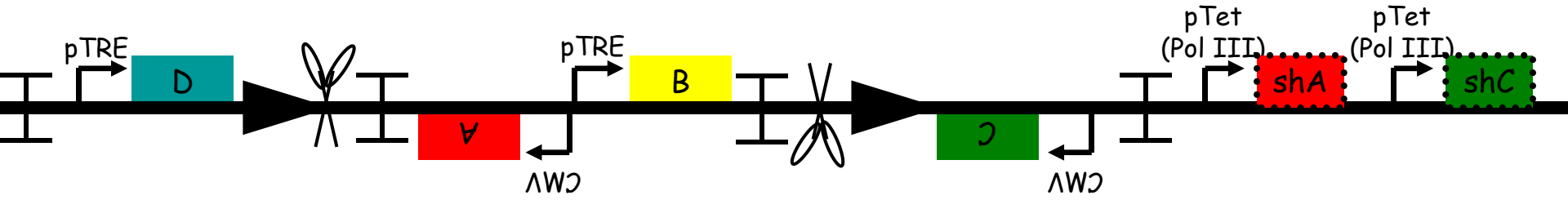
- sequence shorten?! --- TM activate Cre recombinase, excise sequence between loxP

Project -- Excision Model (1,1)

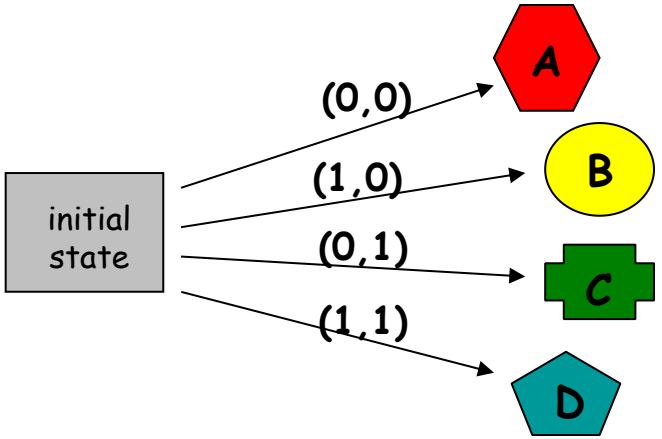


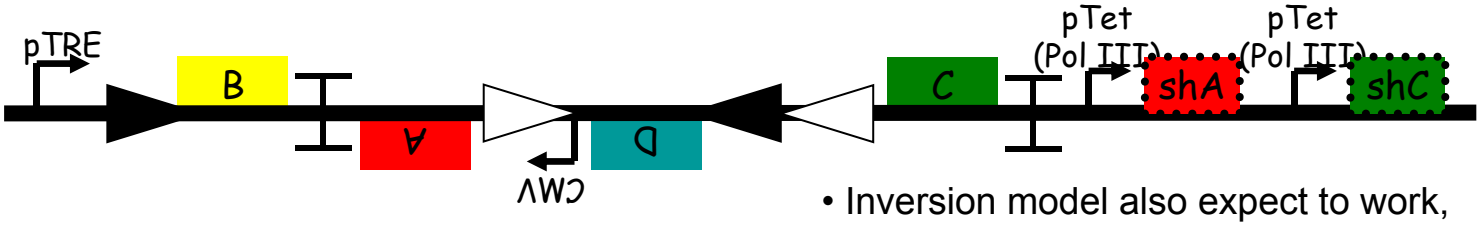
Result: only D is expessed

Project -- Design Conclution



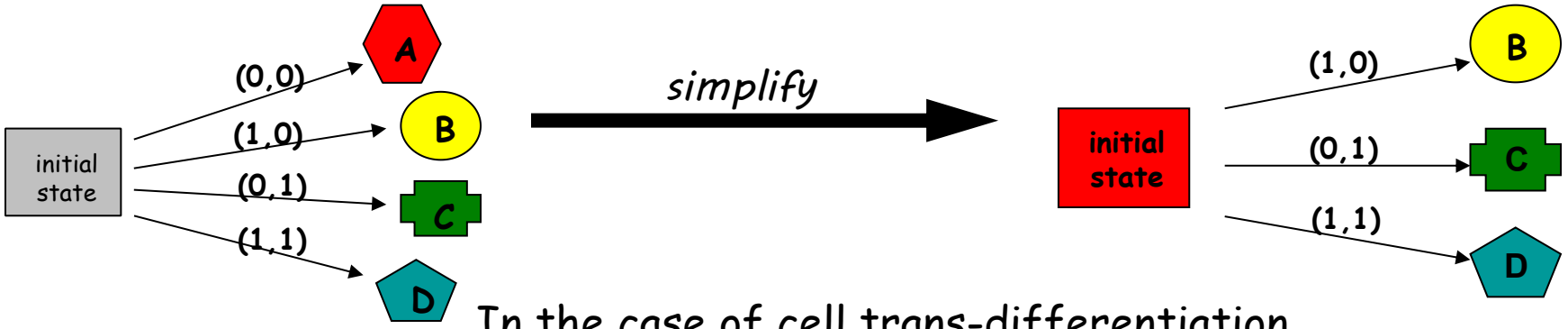
End up a 2-input-4-output system,
can be used as framework constructing
ANY kinds of logic gates!



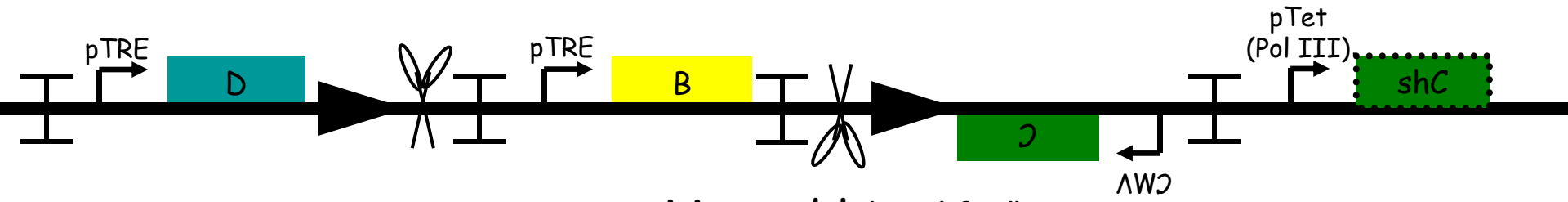


- Inversion model also expect to work,
  skip the details here due to limited time.

Project -- Simplified Form



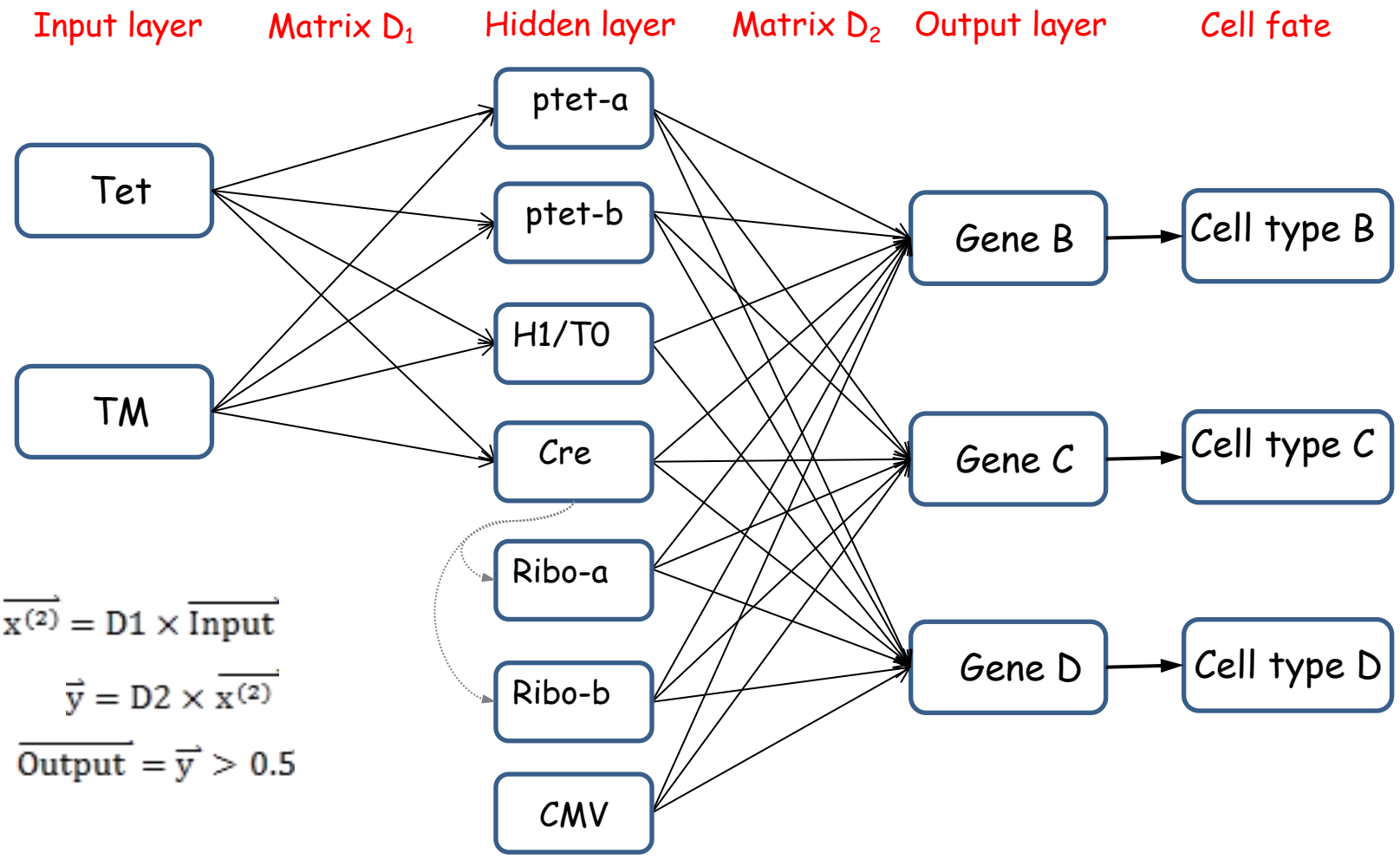
In the case of cell trans-differentiation,
"A" state is usually the initial cell,
so we can simplify the system into a 2-input-3-output one.



excision model (simplified)

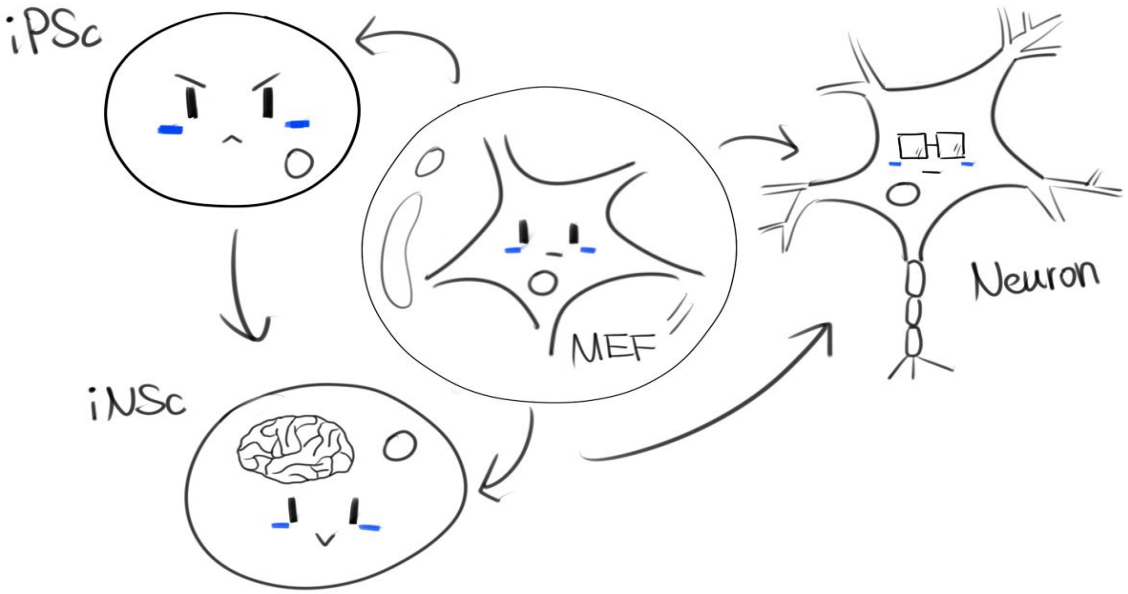
inversion model (simplified)

Project -- Modeling



Project -- Application

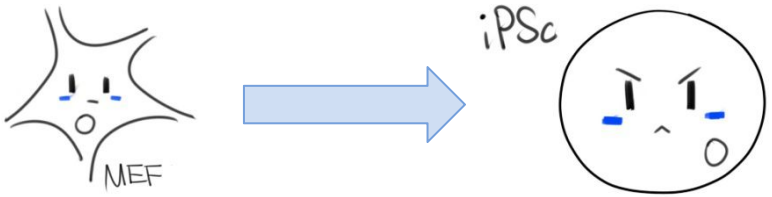
- Application: cell fate decision



Use this logic circuit ABCD gene replaced with certain genes to induce cell transdifferentiation and reprogramming.

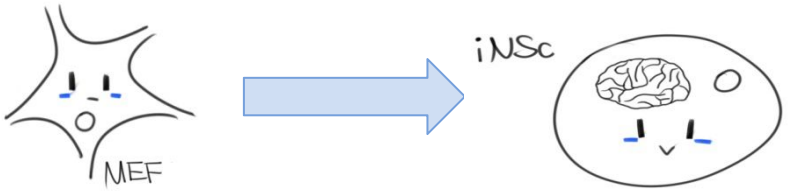
Brief introduction for application

- A→OSKM (four transcription factors linked with T2A ,used to somatic cell reprogramming to iPSc)



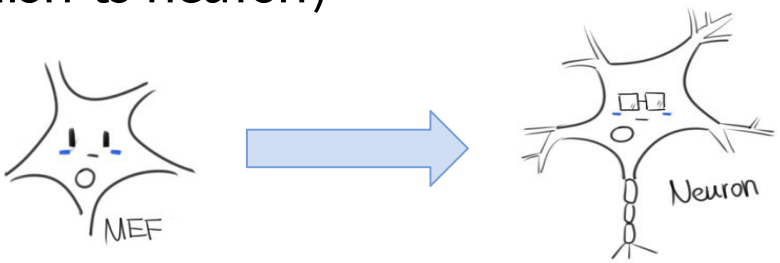
Takahashi K, Yamanaka S.. cell, 2006, 126(4): 663-676.

- B→ Sox2 (used to somatic cell reprogramming to iNSc)

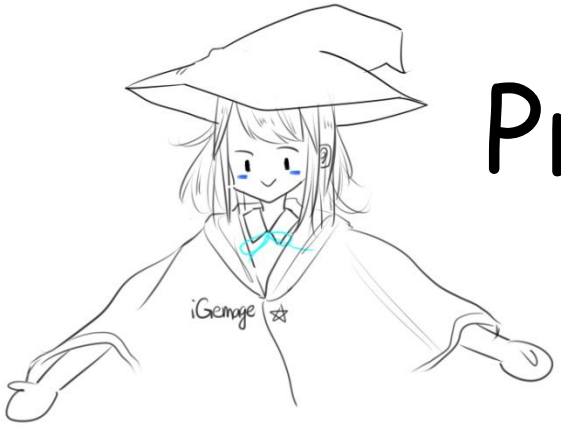


Ring K L, Tong L M, Cell stem cell, 2012, 11(1): 100-109.

- C→ shRNA for PTB (PTB gene knock down for somatic cell transdifferentiation to neuron)



Xue Y, Ouyang K, Huang J, et al Cell, 2013, 152(1): 82-96.



Project "iGemage"

Fibroblast

tamoxifen

tet

1. +tamoxifen
2. +tet

Change medium

iPS

iNSc

Neuron

iNS

iPSc

Neuron

astrocytes

Machine:"iGEMage"

others

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Results & Summary

- 1.Testing our parts

Useful information is on

http://Parts.igem.org

Fudan 2014 iGEM Team Parts

Favorite Fudan 2014 iGEM Team Parts *Edit*

		Name	Type	Description	Designer	Length

Fudan 2014 iGEM Team Parts Sandbox *Edit*

		Name	Type	Description	Designer	Length
	W	BBa_K1440000	Regulatory	Part2_pTRE promoter with cFP and ribozyme	Xuanye Cao	1291
	W	BBa_K1440066	Regulatory	reverse CMV->reverse RFP->pTRE->shRNA	Xuanye Cao	1192
	W	BBa_K1440088	Regulatory	long parts consist of part2 and part3	Xuanye Cao	2491

Use These three parts to test our three working systems:

Cre-ERTII system,

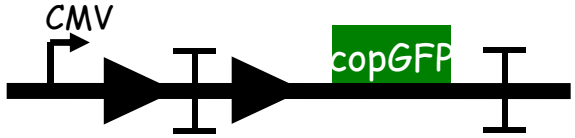
Tet-on system

Ribozyme system

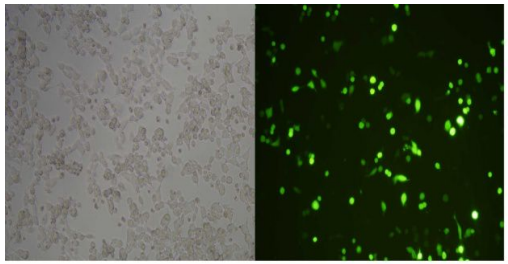
All the bio-bricks we used to test our systems are all included in the parts we submitted and registered.

Validation of Cre-TM system

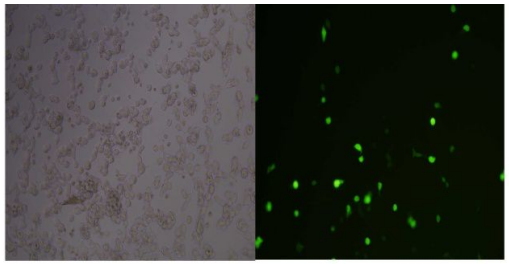
- Result of testing Cre-TM system



Control GFP



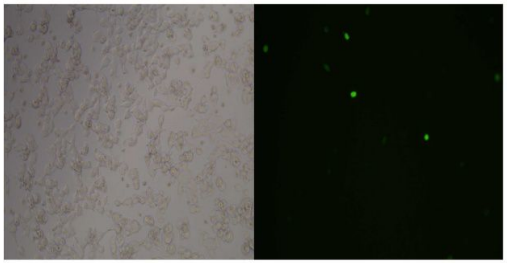
transfection with coGFP_control_36hr



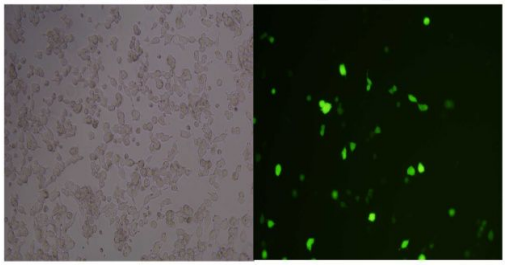
cotransfection with Cre-ERTII and part2 EGFP plasmid with loxp sites/ +TM_2umol_36hr

[TM] 2umol/ml

[TM] 0umol/ml



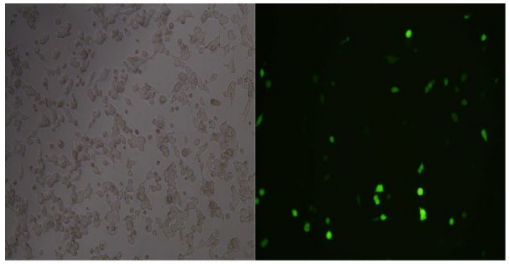
cotransfection with Cre-ERTII and part2 EGFP plasmid with loxp sites/ no_TM_36hr



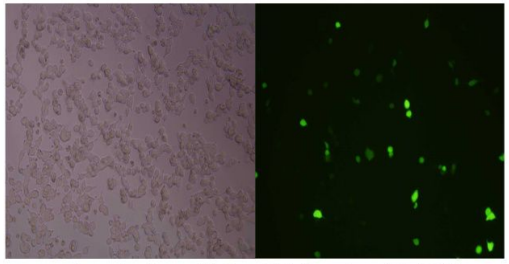
cotransfection with Cre-ERTII and part2 EGFP plasmid with loxp sites/ +TM_5umol_36hr

[TM] 5umol/ml

[TM] 1umol/ml



cotransfection with Cre-ERTII and part2 EGFP plasmid with loxp sites/ +TM_1umol_36hr



cotransfection with Cre-ERTII and part2 EGFP plasmid with loxp sites/ +TM_10umol_36hr

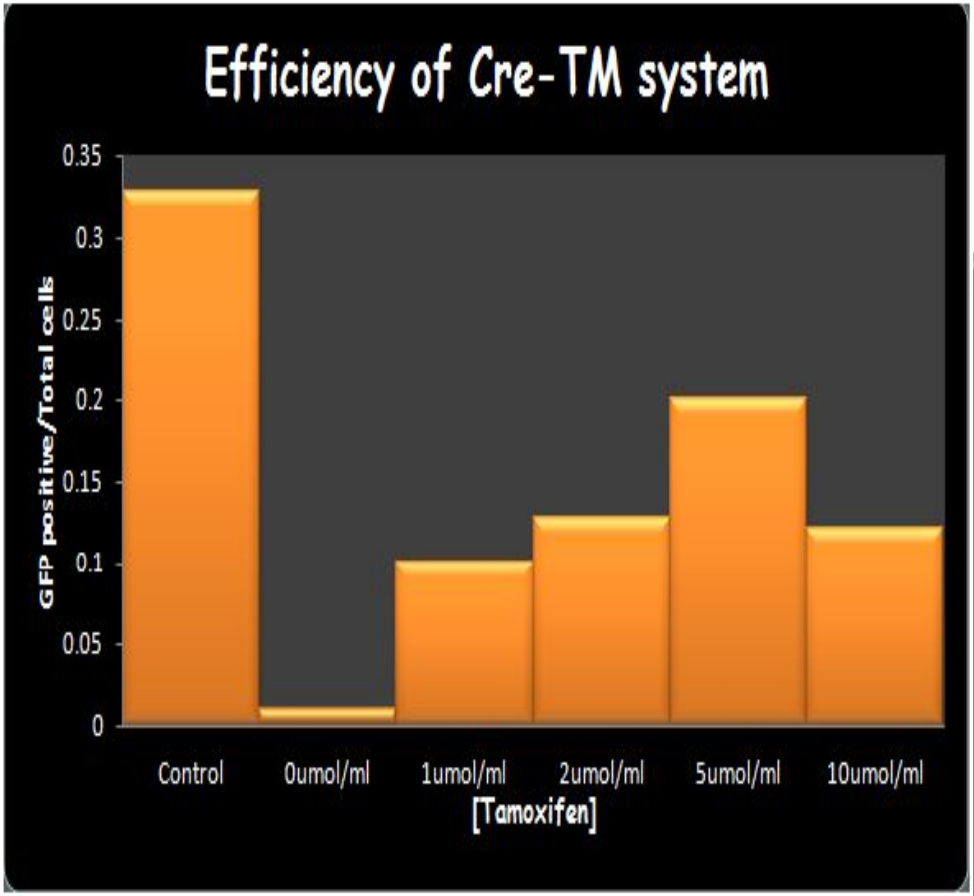
[TM] 10umol/ml

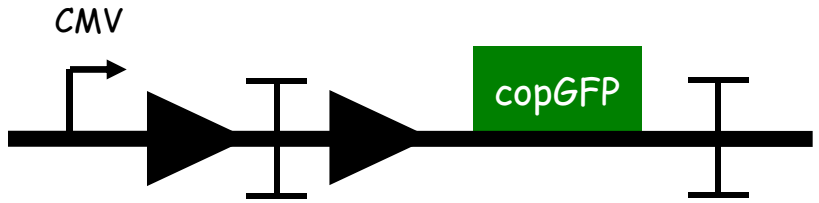
blood cell counting chamber

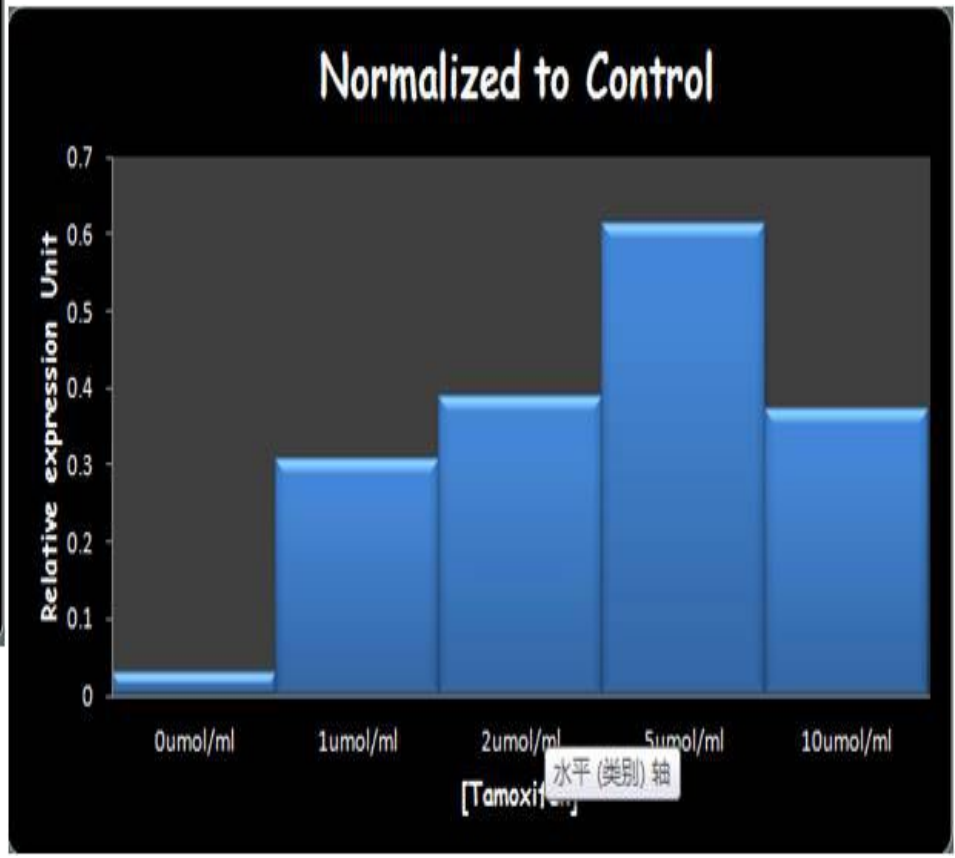
Efficiency Calculation:

GFP-positive cell numbers/ Total cell numbers

Validation of Cre-TM system







Conclusion:
Our Cre-TM system Works!

Validation of tet-on shRNA expression system

- Result of testing tet-on shRNA system

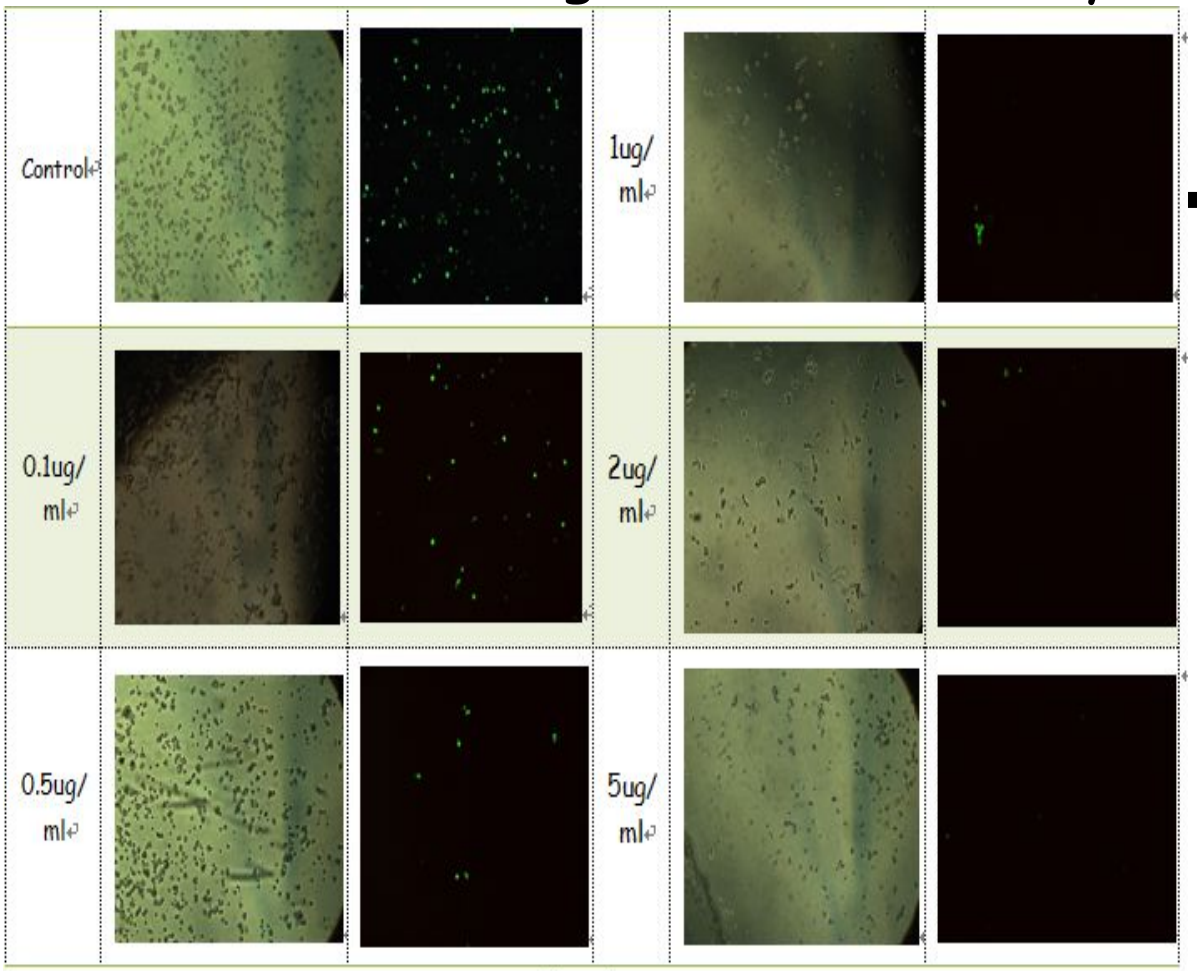
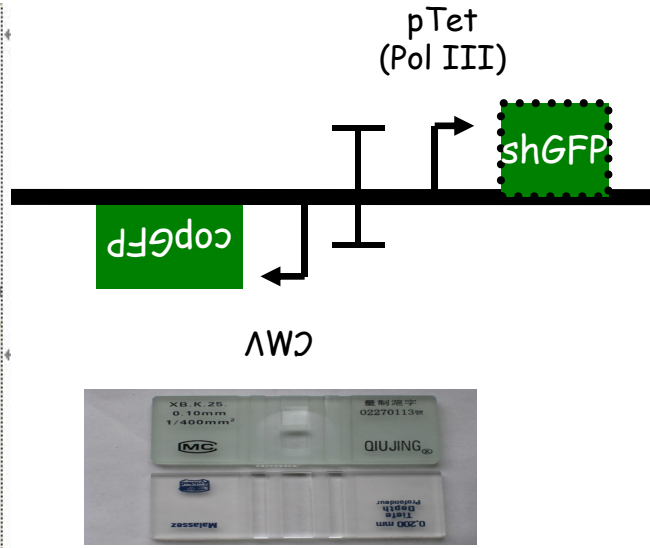


figure 1



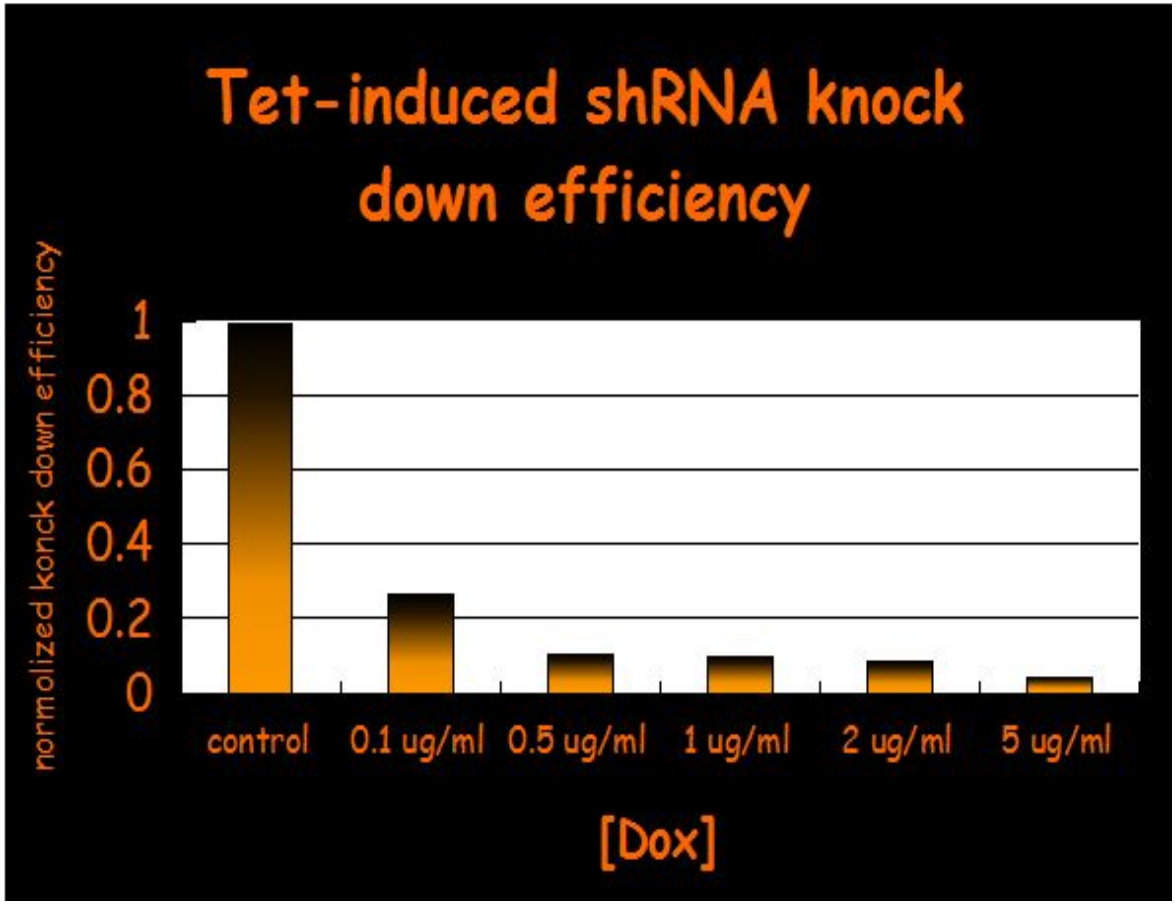
blood cell counting chamber

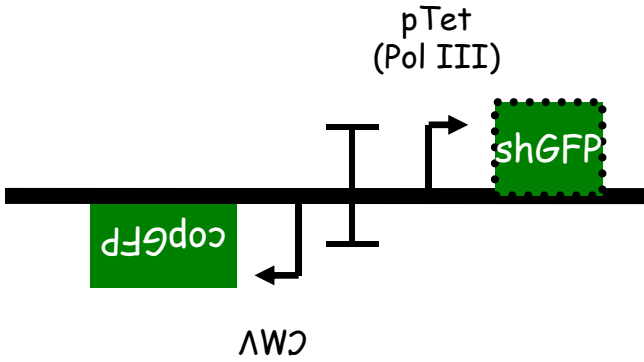
Efficiency Calculation:

GFP-positive cell numbers/ Total cell numbers

Validation of tet-on shRNA expression system

- Result of testing tet-on shRNA system

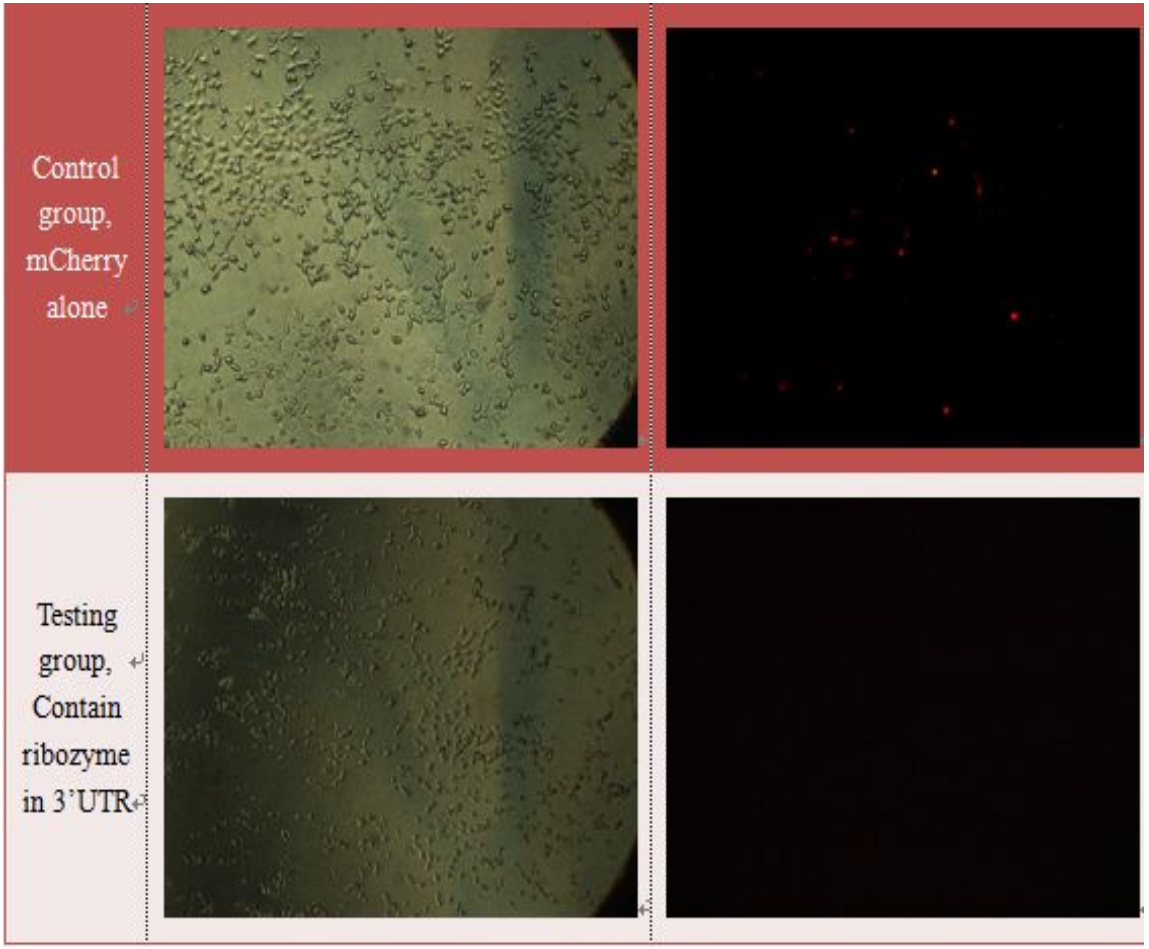




Conclusion:
Our tet-on shRNA system Works!

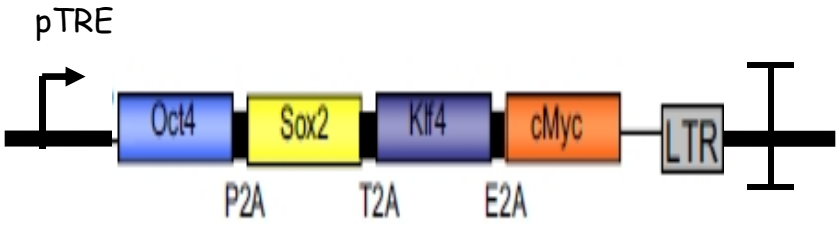
Validation of Ribozyme system

- Result of testing ribozyme system



Conclusion:
Our Ribozyme Works!

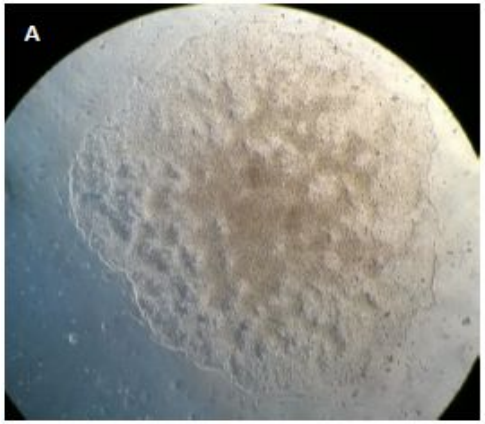
Use tet-on system to get iPSc!



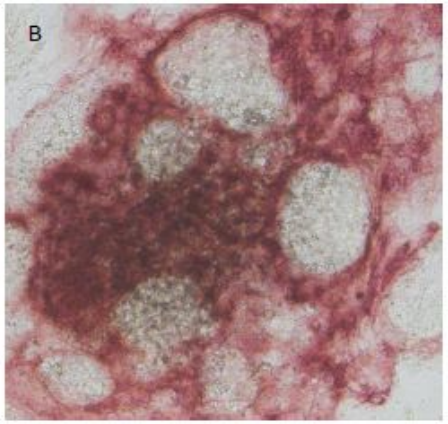
- Conclusion:

  We successfully get iPSc clone by our tet-on system.

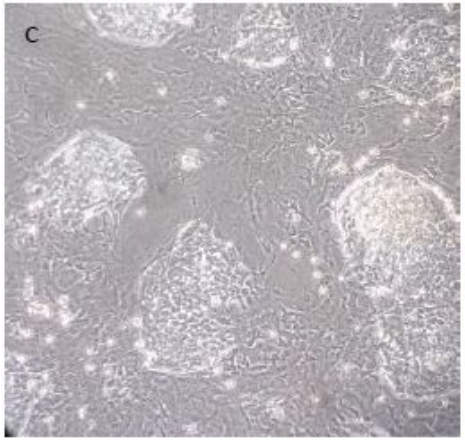
C (Dox) = 1ug/ml

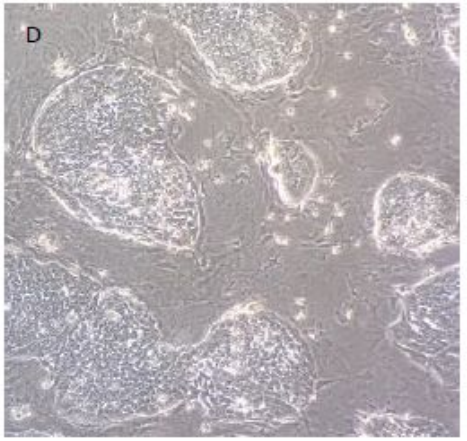


A. The single clone of iPS cells



B. Using the Alkaline Phosphatase kits(Sigma) to detect the iPS cells



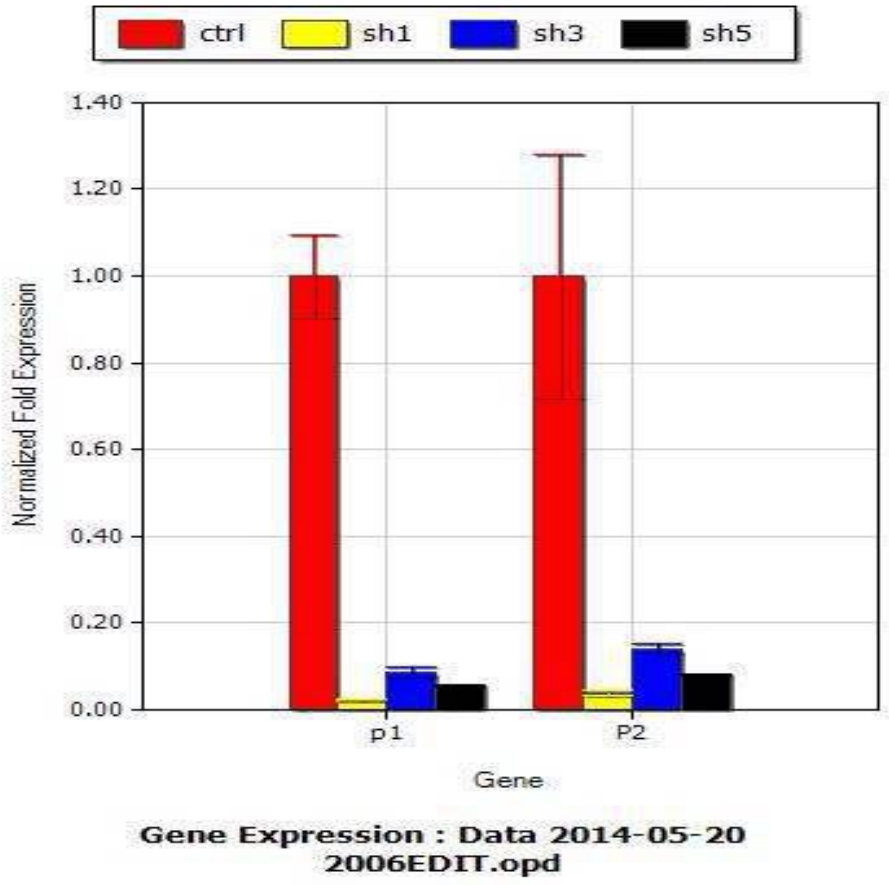


C&D. The tet-induced iPS cells with the OSKM plasmid.

Use tet-on shRNA system to get Neuron

- (b) using tet-on shRNA expression system to test neuron differentiation
- c(dox)=1ug/ml

- Conclusion:
- We successfully knock down PTB protein, with 3 designed shRNA.



qPCR for PTB knock down result

Results & Summary

- Summary:

(a) We successfully construct three parts for testing three systems which are most important to our design. And we get the quantitative data of three systems. However, because the limitation of time and many mistakes we made before, we have not assembled three parts together yet.

(b) We use three parts to test our application: cell fates' decision. We successfully get iPSc cell clone. For neuron and iNSc cell culture, although we have the help from Tongji University, we still cannot get the right result with our working-successfully parts because of limitation of time.

Outline

- Introduction
- Project & Modeling
- Results & Summary
- Human practice & safety
- Acknowledgements

Human practice & Outreach

- We help several teams, and also get their help;
- We join in the meets up in Taiwan
- We hold events like Bacteria Painting Competition to spread the spirit of iGEM and synthetic biology.
- We construct our own student association for iGEM competition.

- http://

With Tongji University

- **We offer help to Tongji University**

Http://

With Tongji University

They help us with the protocol of cell transdifferentiation and cell reprogramming.

With Zhejiang University

- We introduce our project to them, and give some advice to their project

With NYU Shanghai

- We help them build their "next-year" team and wet lab for iGEM

With Ann Yang

Meets up in NCTU-Formosa

We give a presentation about our project.

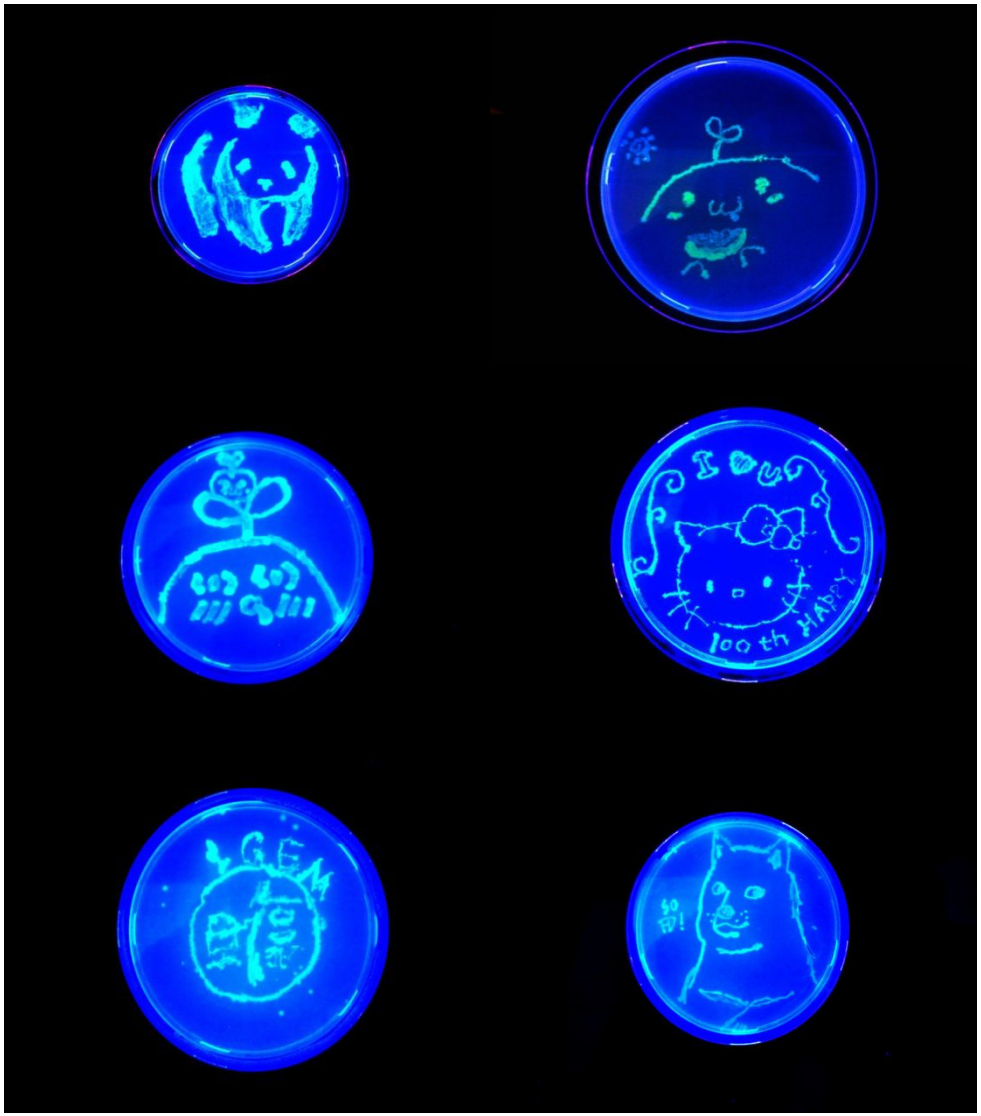
Certification!

We talk about our poster to the other 20 schools

Construct our own student's association for iGEM

- **Bertalanffy club**

Bacteria Painting Competition



Safety

	Advantages	Disadvantages
iPSc	strong self-renew ability	oncogenicity
	strong differentiation potential	low efficiency
somatic cell conversion	no oncogenicity	no self-renew abilities
	high efficiency	poor differentiation potential

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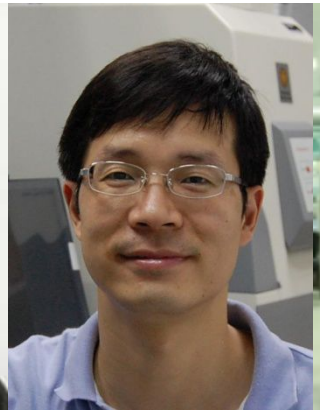
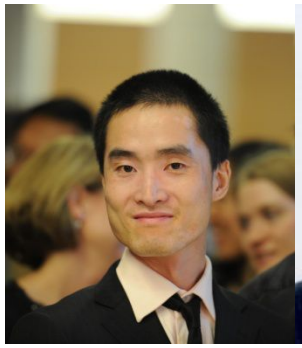
Acknowledgements

- Instructors:


Prof .Dr Lu Prof. T Ni Prof. F Lan

Thanks very much to them
for their help ~




Prof. W Yu Prof. JB Ma

Thanks to them!
for their offering the labs

☆Advisors:
BS Miao;
M Han;
XD Zhou;
DP Yun

Thanks to them for
their teaching 0 0~