

基于原核生物抑制子基因组 挖掘的正交逻辑门构建

改编自Brynne C Stanton, Alec A K Nielsen, Alvin Tamsir, Kevin Clancy, Todd Peterson & Christopher Avoigt在2014年发表于Nature Chemical Biology的论文Genomic mining of prokaryotic repressors for orthogonal logic gates



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目录

CONTENTS

01 研究背景

02 数据

03 讨论

04 结论



PART 01 ▶

研究背景

Background Information

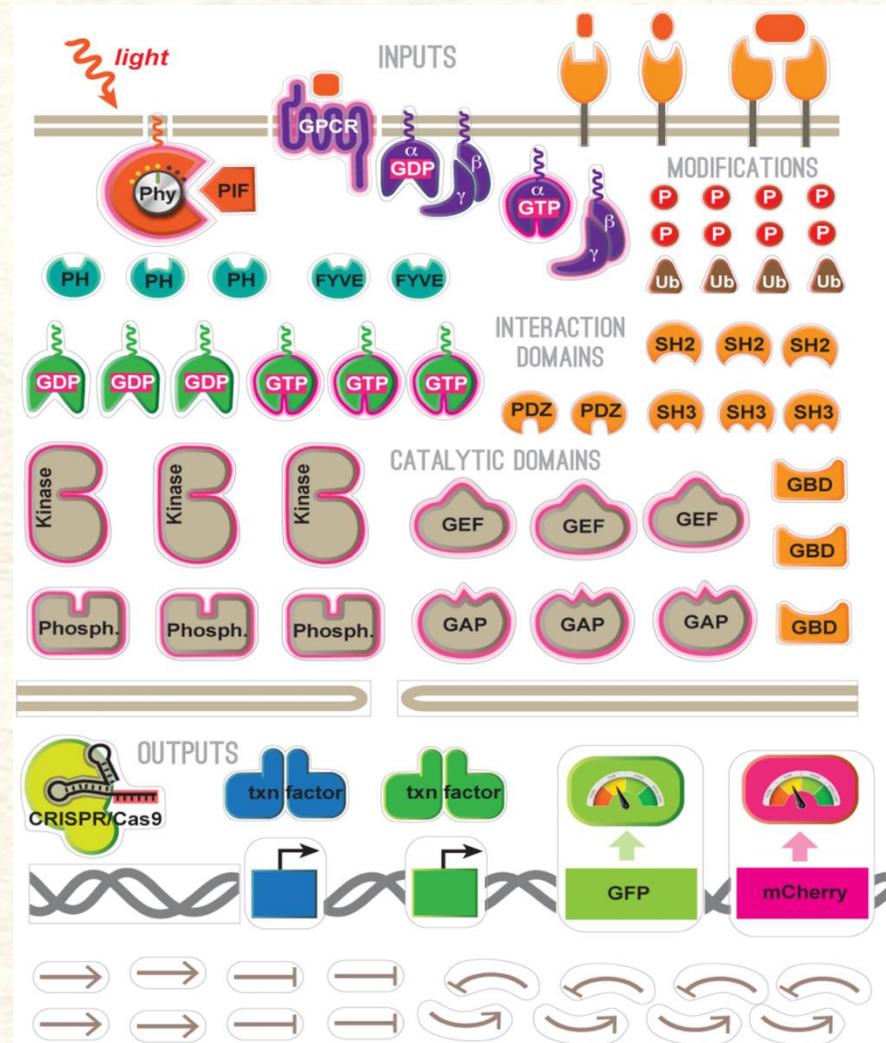
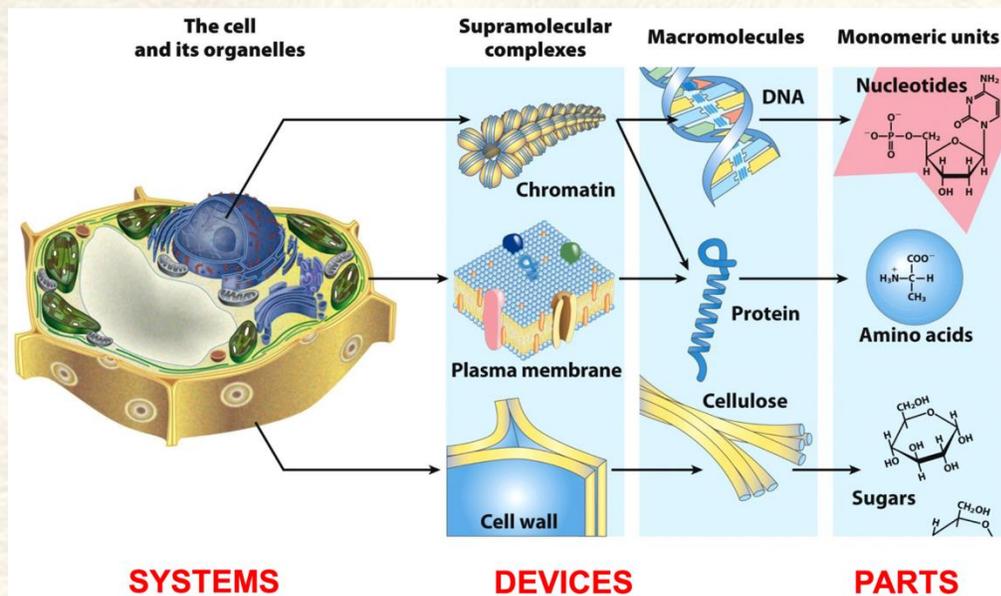
合成生物学概述

目标:

- ✓ 编程细胞执行复杂任务 (如生物计算、动态调控)
- ✓ 模块化设计遗传电路 (类比电子电路)
- ✓

瓶颈:

- 组件数量少
- 正交性不足
-



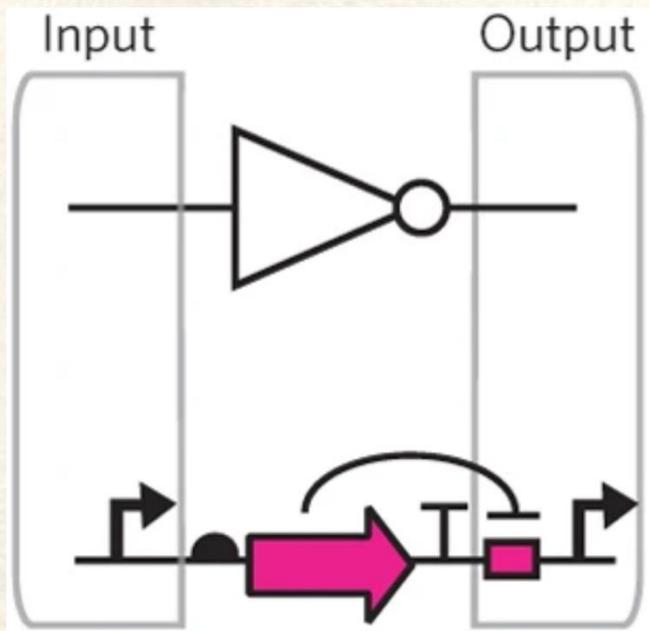
研究背景

实验数据

讨论

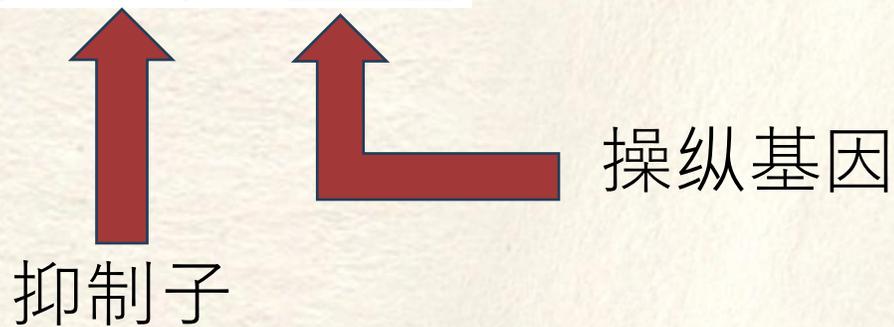
结论

NOT:



NOR: 串联另一启动子

例子：暗传感器  光传感器
雄传感器  雌传感器



研究背景

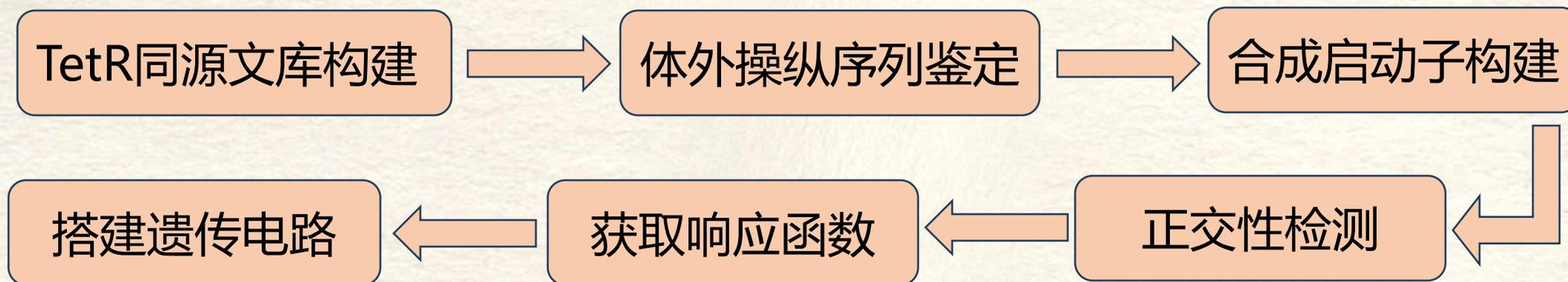
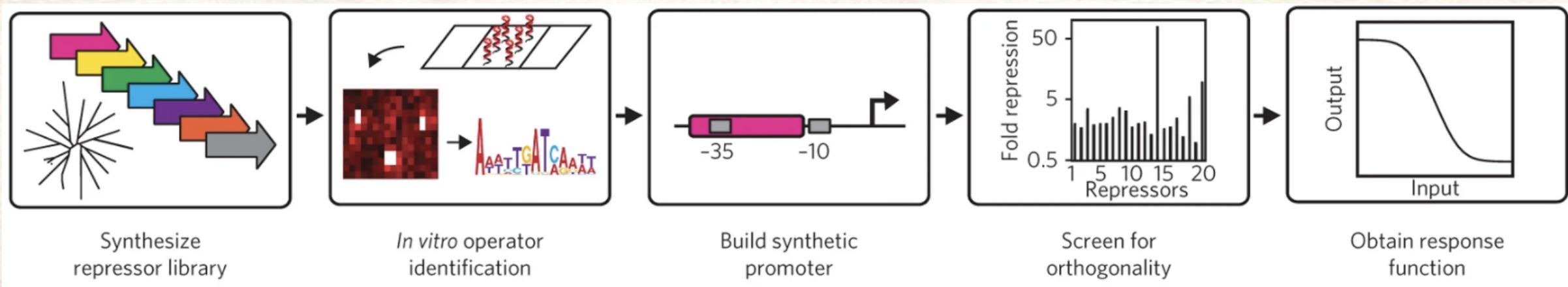
实验数据

讨论

结论

TetR

- 应用广泛
 - 细菌、真菌、昆虫、植物、哺乳动物细胞、活体动物
- 抑制模式简单
 - 二聚体结合启动子并物理阻断RNA聚合酶
- 用相对短的操纵序列实现特异性
- 从许多宿主生物中可以获得数万个同源物
 - 正交性潜力大

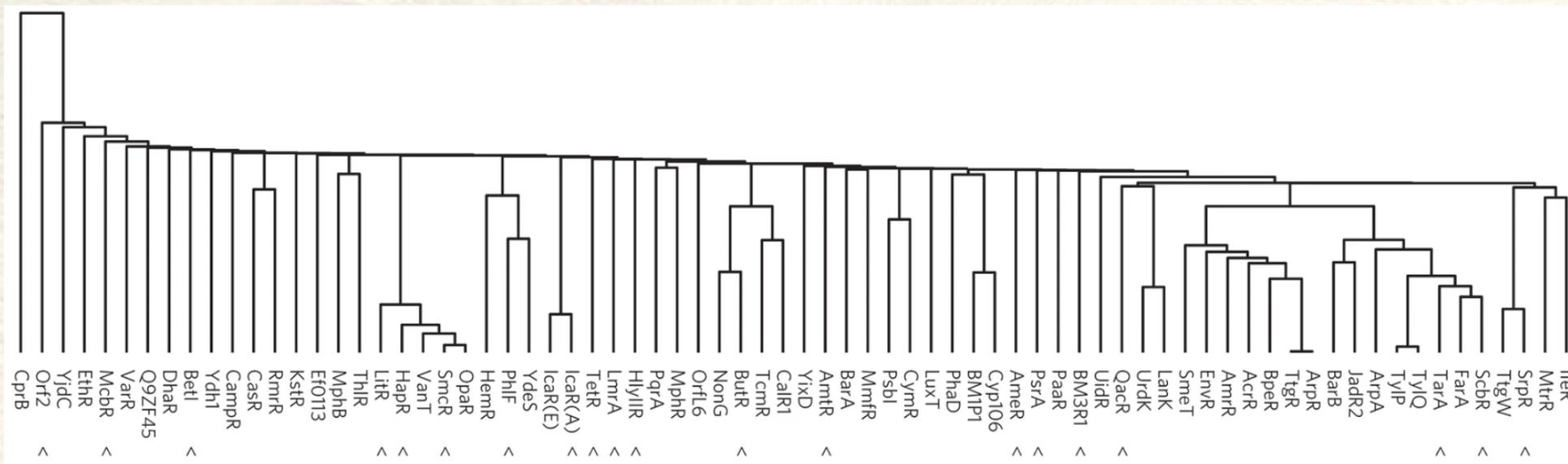


实验数据

Results

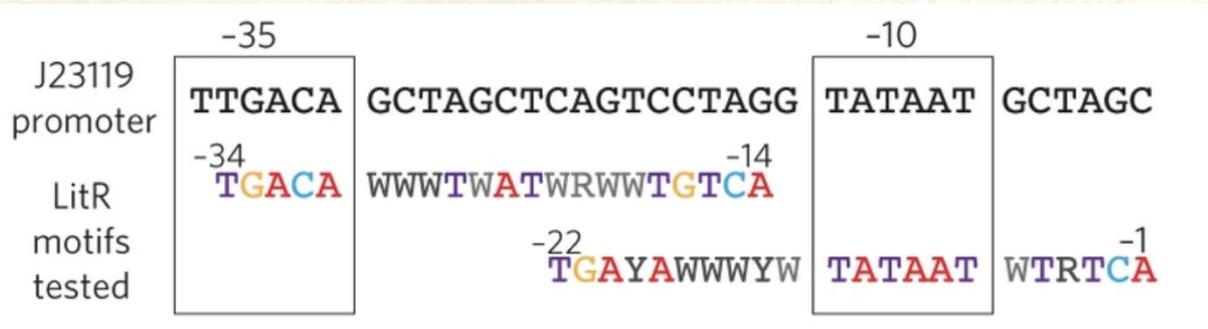
◀ PART 02

EMBL-EBI数据库
82017个TetR同源蛋白



73个已知调控功能的抑制子

合成启动子设计

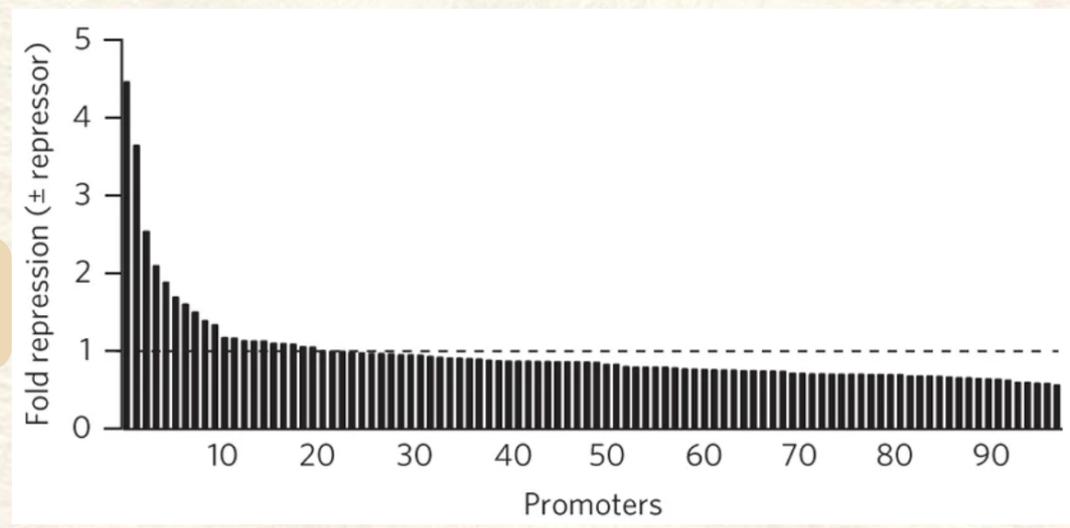


操纵基因

插入不同位置
构建启动子文库



流式细胞术



10个: CSI阵列
10个: 已知文献

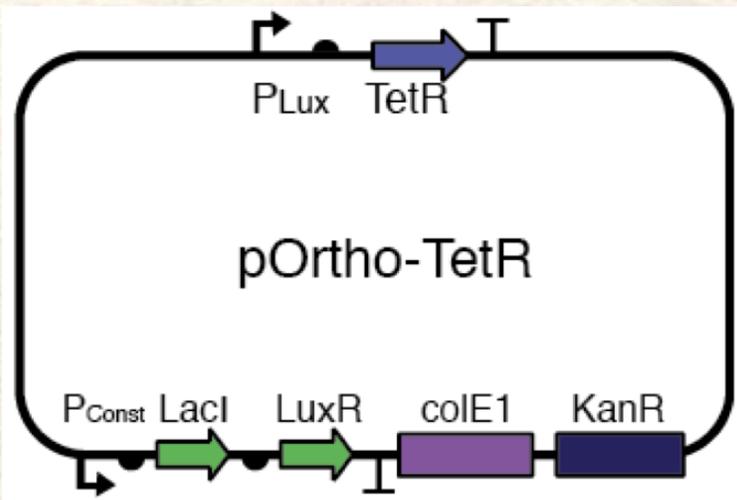
研究背景

实验数据

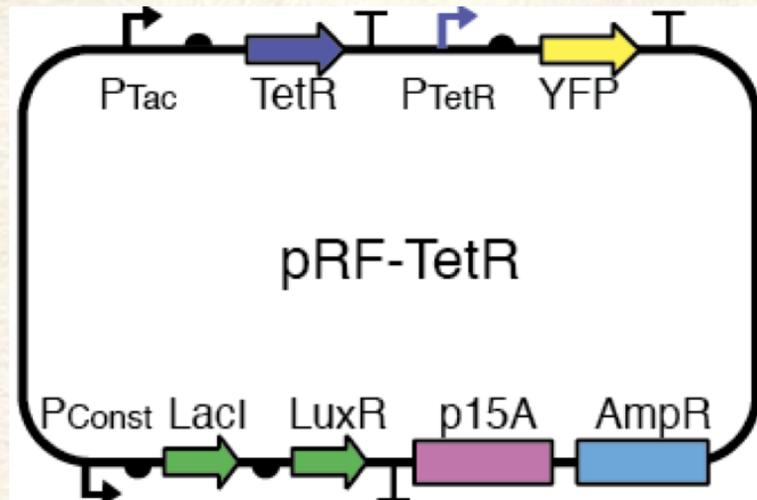
讨论

结论

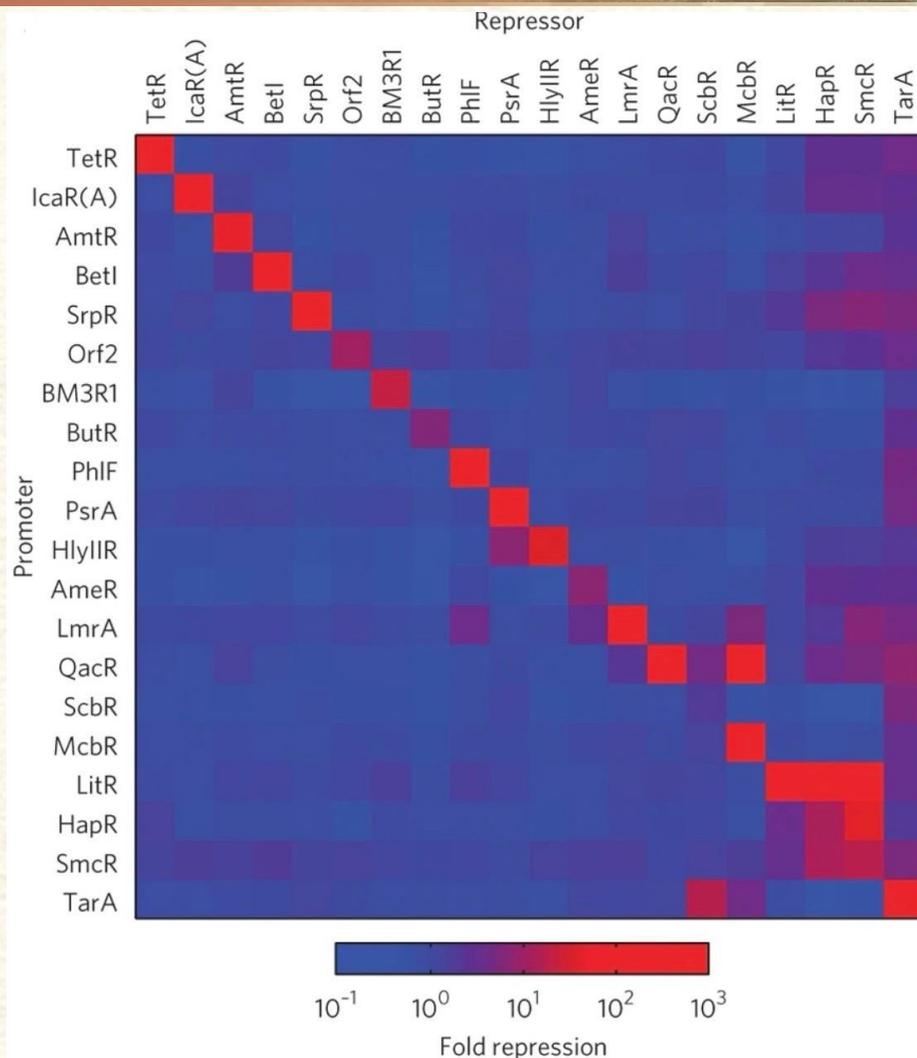
抑制子质粒

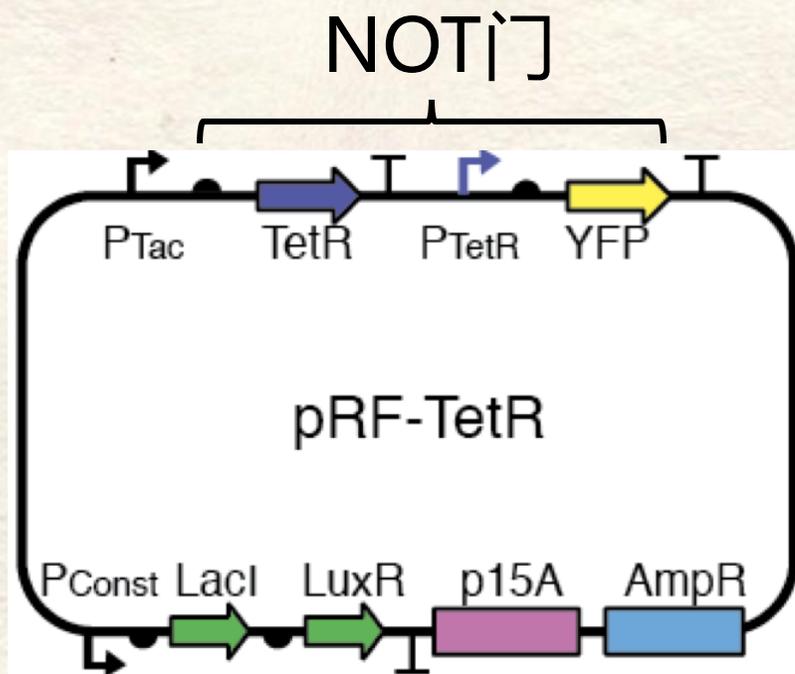


启动子质粒



正交性好!





RBS计算器

Repressor	RBS Library Sequence
AmeR	CTATGGACTATGTTTTTCACATACGAGGGGGATTAG ATG
AmtR	CTATGGACTATGTTTGAAGAGAGAATACTAG ATG
BetI	GCTACGACTTGCTCATTGACAGAGGATACTACTAG GTG
BM3R1	CTATGGACTATGTTTAACTACTAG ATG
ButR	CTATGGACTATGTTTTTCACACAGGAAATACTACG ATG
HapR	CTATGGACTATGTTTAAAGAGGACACATACTAG ATG
HyllR	CTATGGACTATGTTTGAAGAGGGACAAACACTAA ATG
IcaR (A)	CTATGGACTATGTTTTTCACACAGGGCCCG ATG
LitR	CTATGGACTATGTTTTTCACACAGGGTTTTTCACACAGGAGAAACCTCG ATG
LmrA	CTATGGACTATGTTTTTCACACAGGAAAGGCCTCG ATG
McbR	CTATGGACTATGTAGGAGAAATACTAG ATG
Orf2	CTATGGACTATGTTTTGAAGAGGAGAAACACTAG ATG
PhIF	CTATGGACTATGTTTGAAGGGGAGAAATACTAG ATG
PsrA	CTATGGACTATGTTTGAAGAGGATACGAATACTAG ATG
QacR	GCCATGCCATTGGCTTTTCACACAGGACACCGGTTAG ATG
ScbR	CTATGGACTATGTTTAAAGAGGAAAAGTACTAG ATG
SmcR	CTATGGACTATGTTTGAAGAGGAGAAATACTAG ATG
SrpR	CTATGGACTATGTTTTTCACACAGGAAATACCAGG ATG
TarA	CTATGGACTATGTTTTCAAAGAGGAGAAATACTAG ATG
TetR	CTATGGACTATGTTTTTCACACAGGAAAGCCTCG ATG

RBS文库：一系列预测表达水平从高到低的序列

NOT门对应的RBS序列

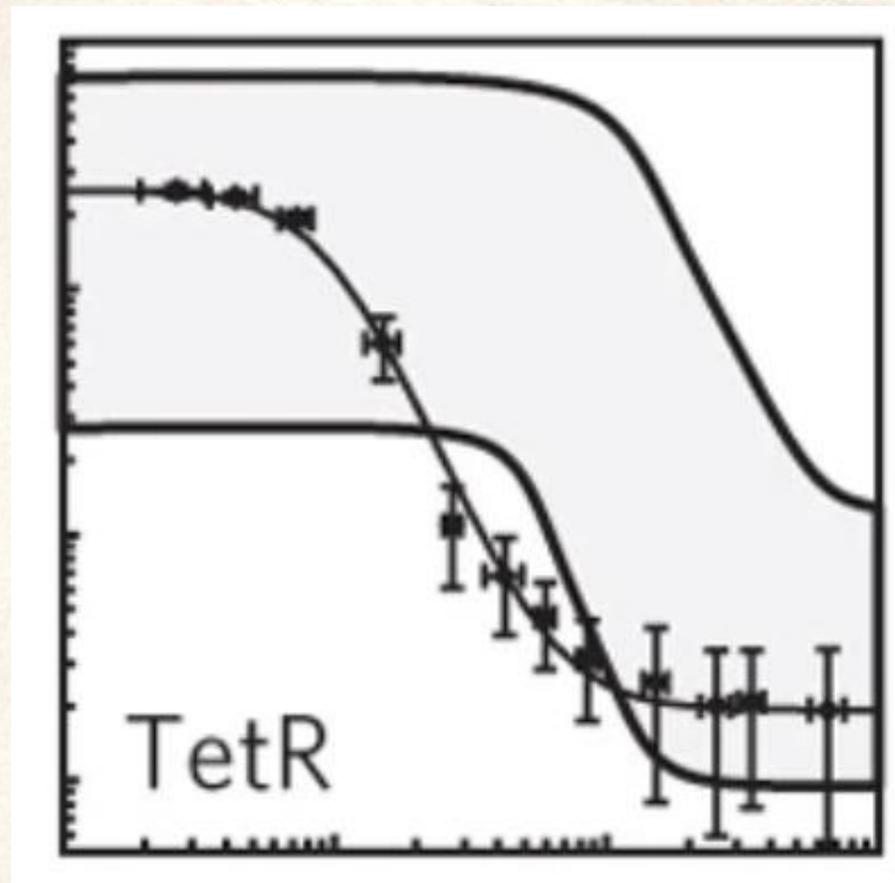
Hill 方程拟合输出启动子活性

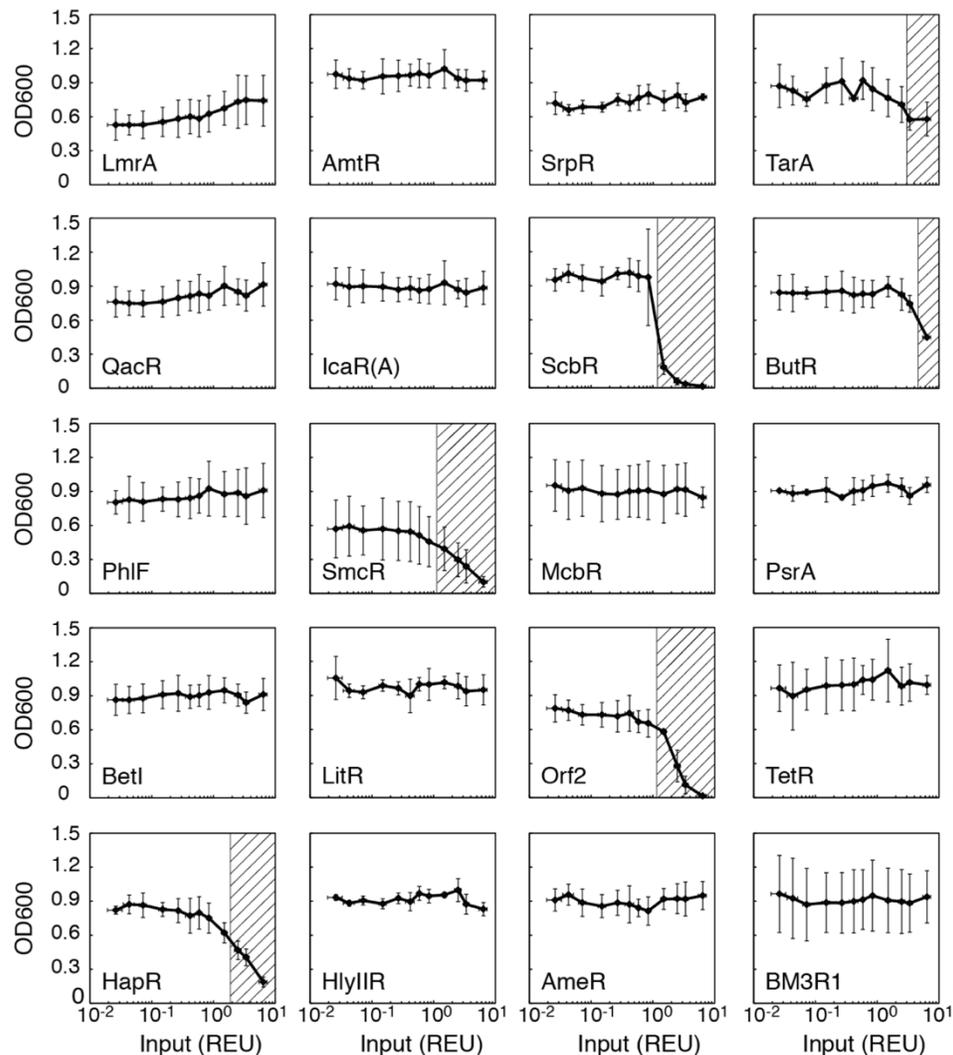
$$y = f(x) = y_{min} + (y_{max} - y_{min}) \frac{K^n}{K^n + x^n}$$

其中n是Hill系数，K是输出为最大值一半时的输入阈值水平

$$ON \in [3, 70]$$

$$OFF \in [1, 2.1]$$





培养6小时，测量OD600(细胞生长状况)

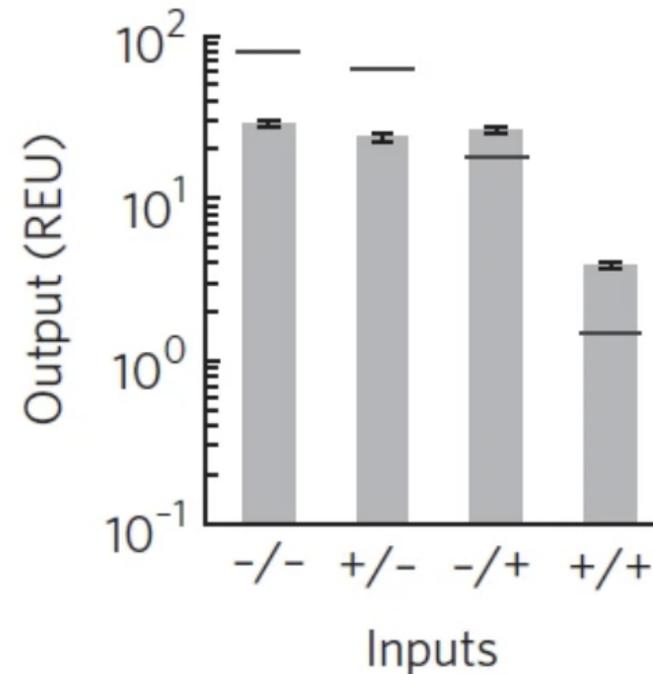
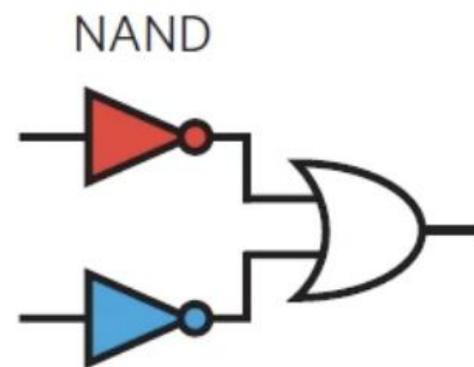
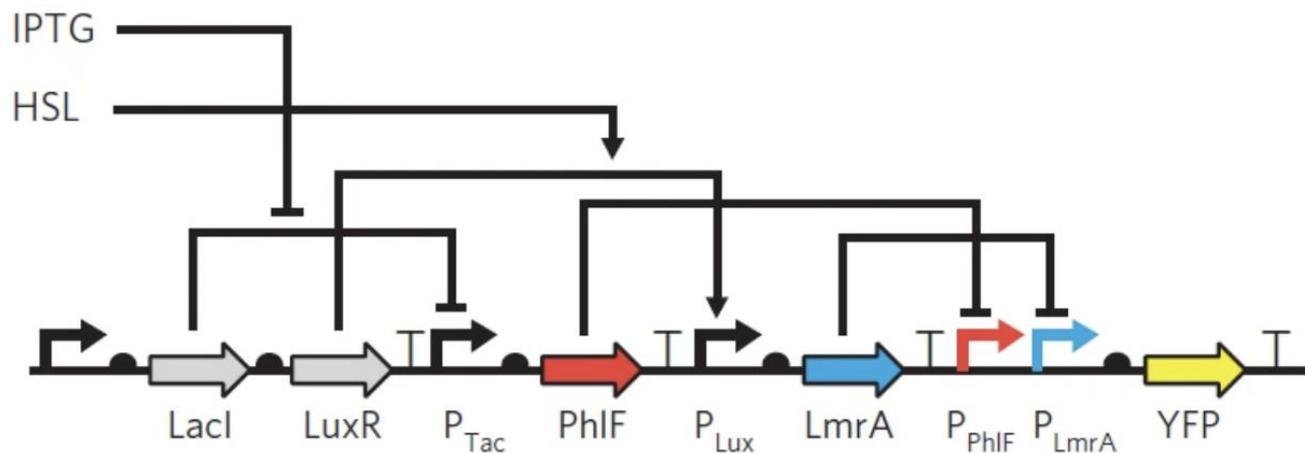
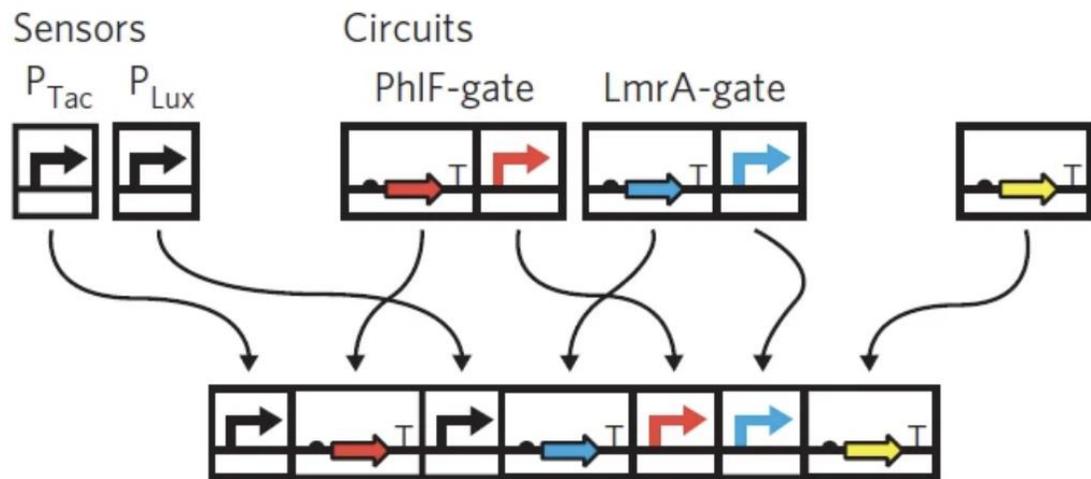
✓ 大多数抑制子无毒

✓ 6种抑制子 高输入 显示毒性

(毒性：细胞减少超过25%)

电路设计者：
通过量化计算，避免使用毒性区域

NAND门电路



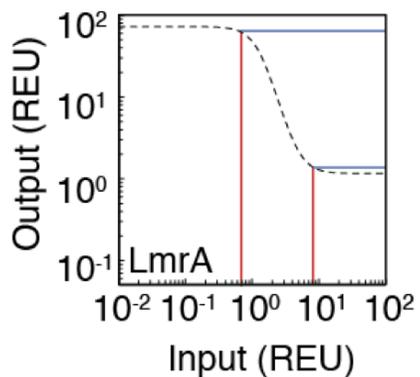
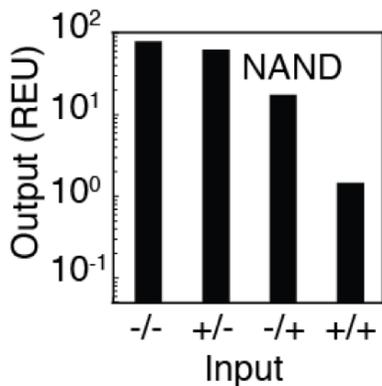
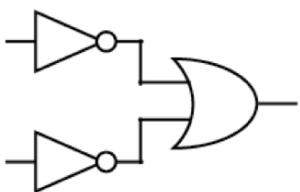
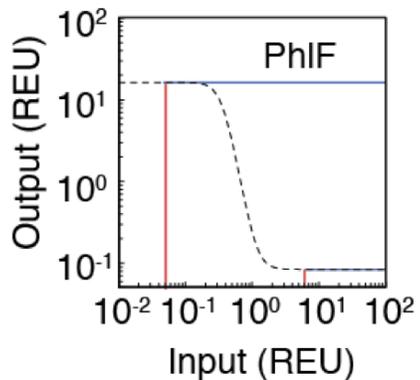
OFF态 (+/+) 与
最低ON态间相差
6倍

研究背景

实验数据

讨论

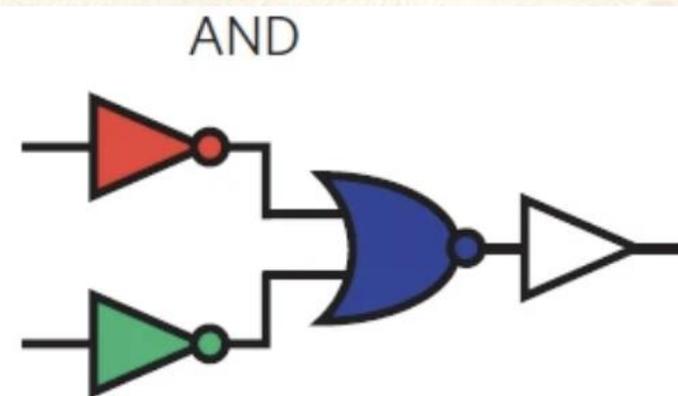
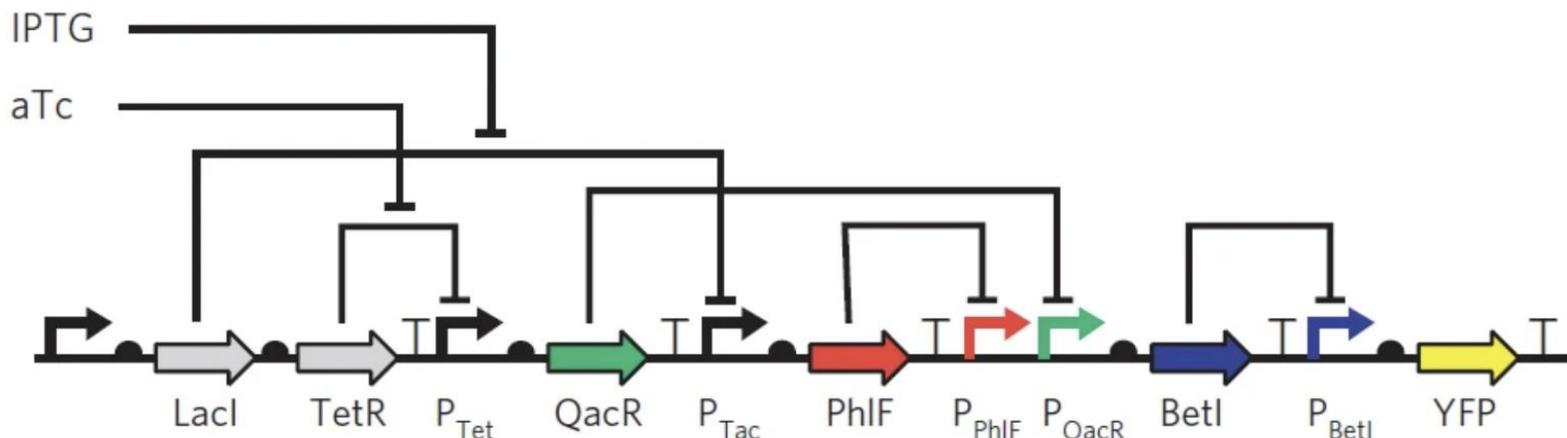
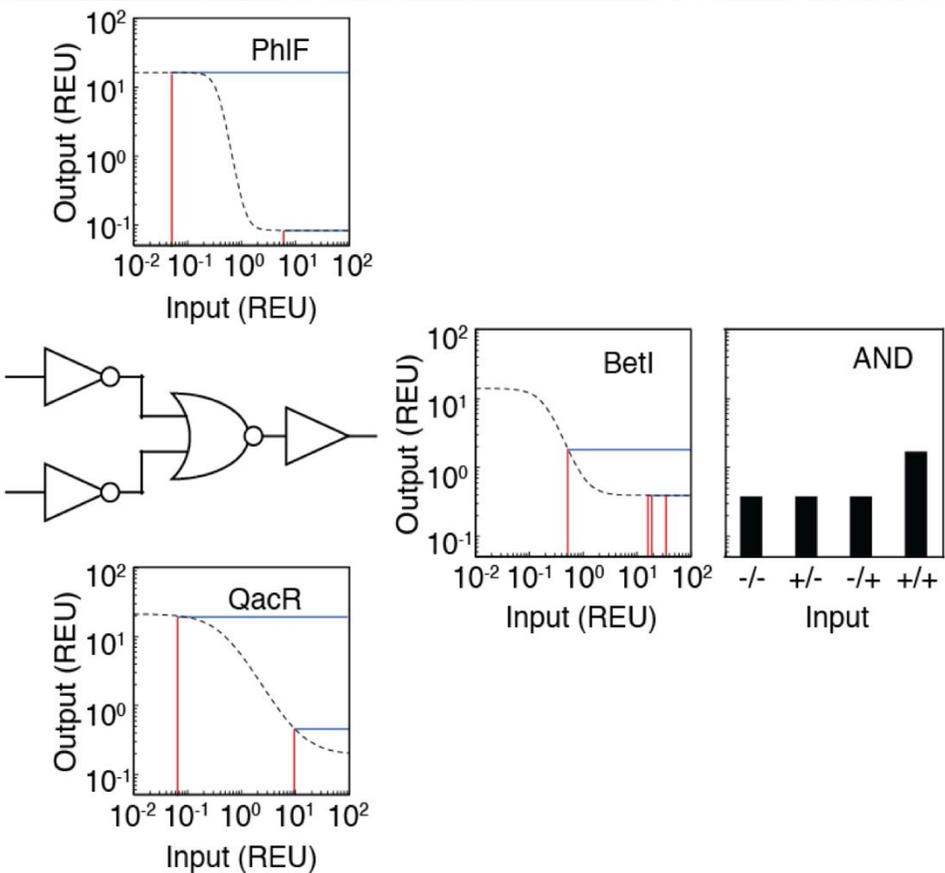
结论



$$\left\{ \begin{array}{l} Output_1 \in f_{PhIF}(P_{Tac}) = [0.1, 16] \\ Output_2 \in f_{LmrA}(P_{Tac}) = [1.4, 61] \end{array} \right.$$

$$Output = Output_1 + Output_2$$

AND门电路



OFF态 (+/+) 与
最低ON态间相差
4.4倍



PART 03 ▶

讨论

Discussion

一个粗略的组合估计:

$$N = \sum_{k=1}^n \frac{n! (3k + 1)^{2k}}{k! (n - k)!}$$

n: 总的抑制子数量
k: 电路中抑制子数量

当n=16时, $N > 10^{54}$

1

对遗传背景敏感，提高鲁棒性

2

降低OFF状态的表达

3

使用其它抑制子和生化机制

4

构建更大型的电路

结 论

Conclusion

◀ PART 04



1

从73个TetR同源抑制子开始，构建了一组16个逻辑门

2

测量交叉反应，确定逻辑门正交性

3

引入一种用NOT门和NOR门构建基因线路的通用方法

4

为合成生物学提供了可复用的标准元件



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感谢各位老师同学批评与指导



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