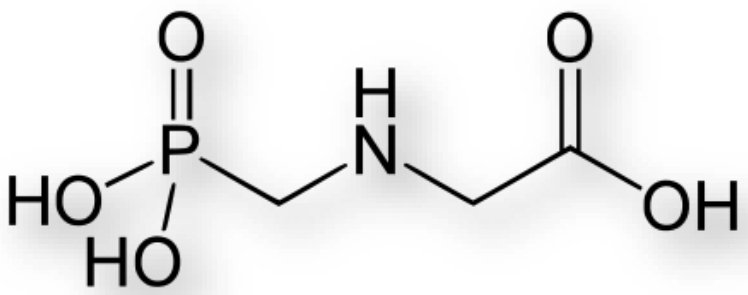
Antea-Glyphosate
Efficient Gly Detection & Degradation System



2020 XMU_China

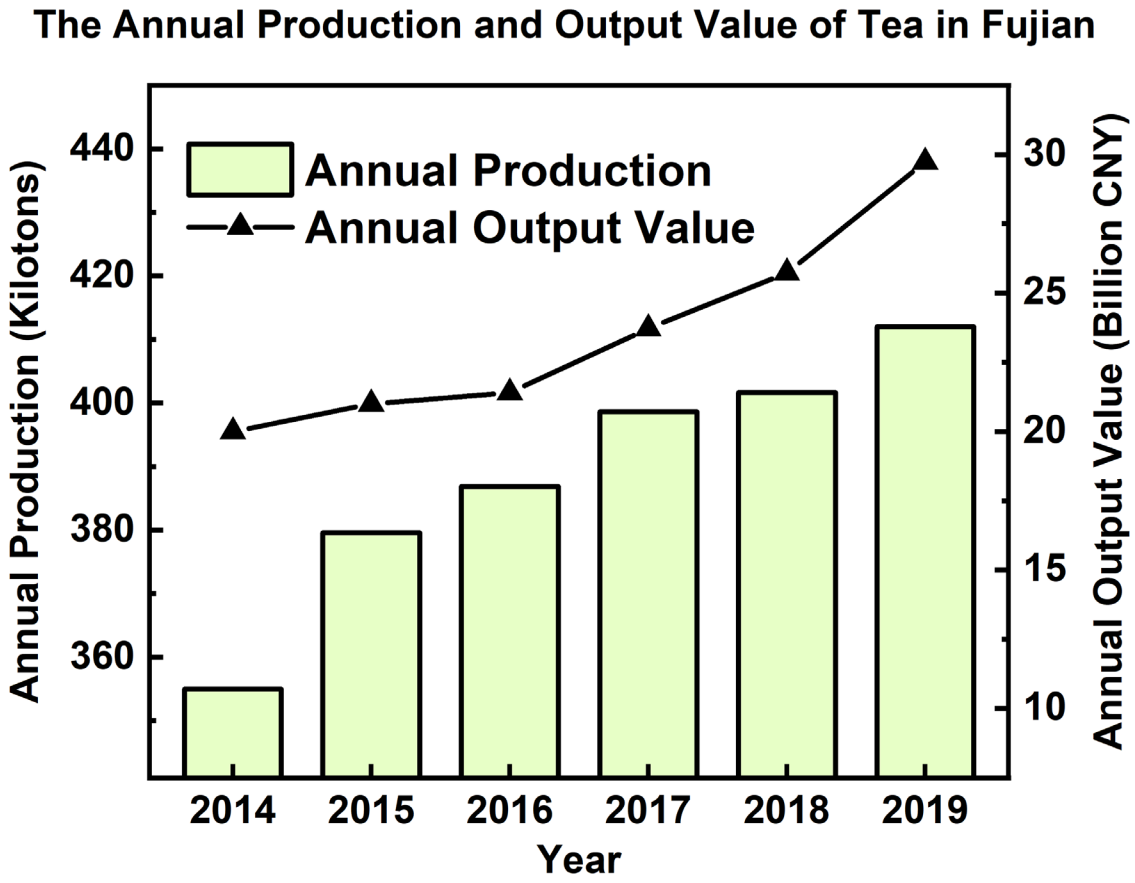
Track: Food & Nutrition

贺靖 2/22/2023

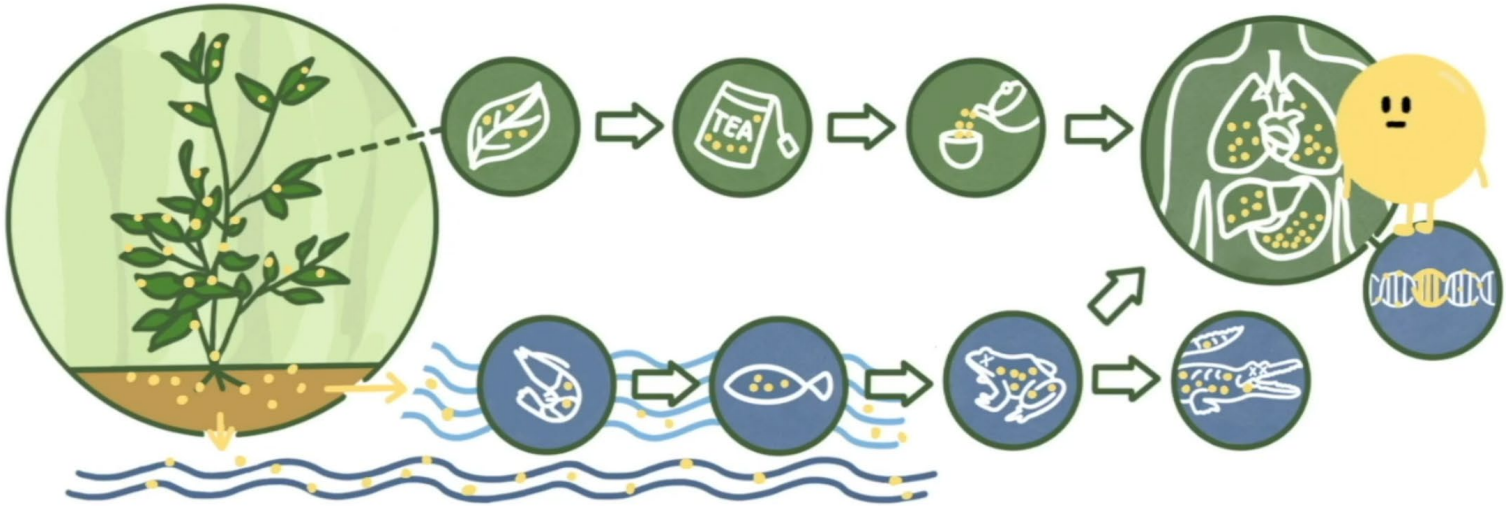
TOC

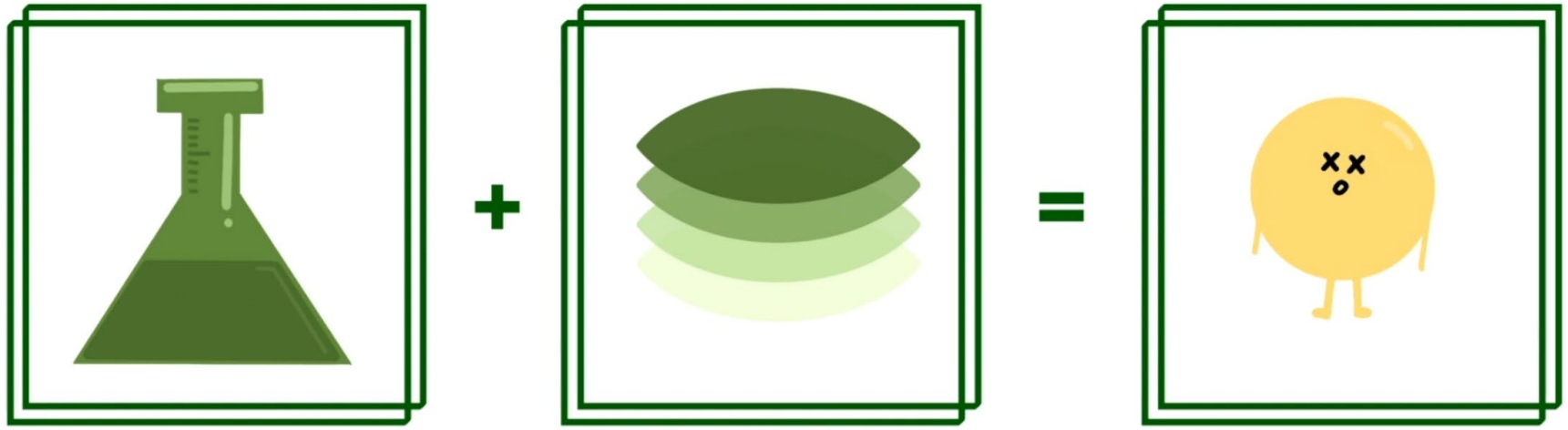
Background

- 随着现代茶叶种植业的发展，除草剂广泛运用于茶叶生产
- 农药&除草剂等残留存在较大的食品安全隐患
- 草甘膦 (Glyphosate) 等除草剂暂缺国家标准的农业残留检测方法



Description





Detection

- 传统检测方法：色谱法
- XMU_China 2020: GOX, GRHPR & iNAP System
- 通过测定草甘膦分解过程中产生的NADPH 间接测定草甘膦的含量
- Three Parts Detection
  I. Glyphosate 分解
  II. NADPH 测定
  III. GOX, GRHPR & iNAP System 固定

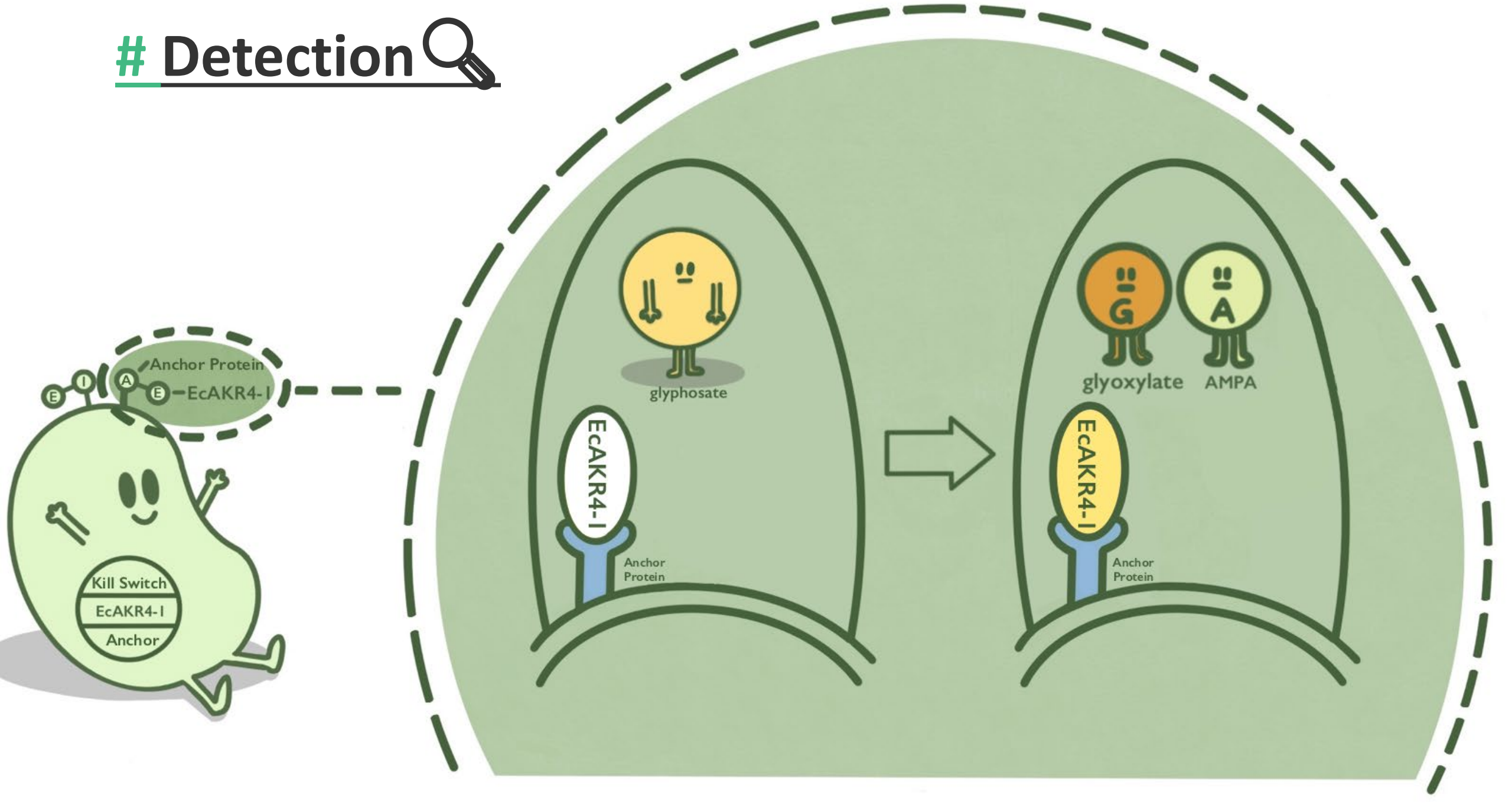
Detection

I. Glyphosate 分解

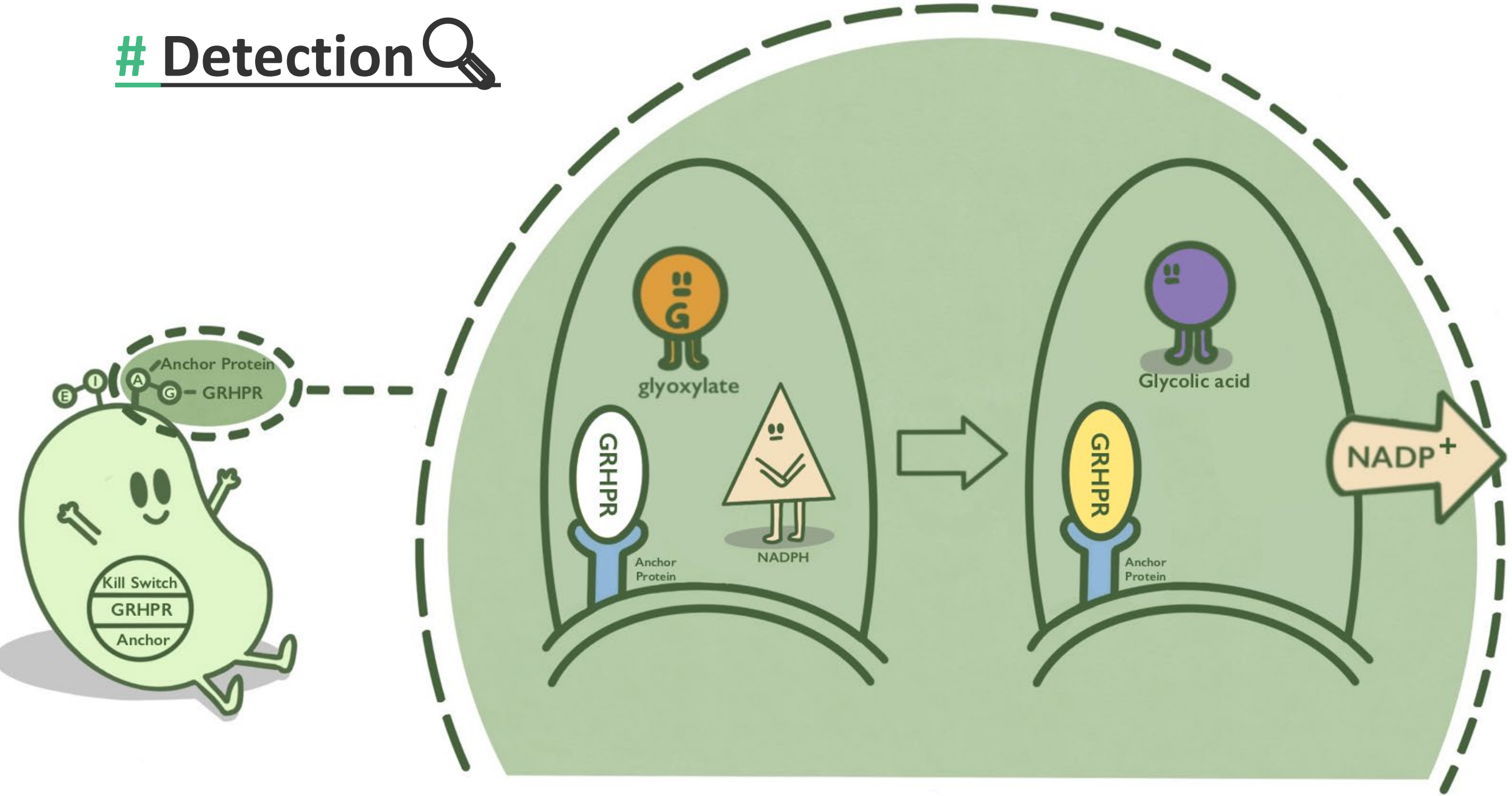
- GOX: 一种从名为 EcAKR4-1 的光头稗（草本）中提取的氧化酶
- 将草甘膦转化成 AMPA（氨甲基磷酸）和乙醛酸

- GRHPR: 人乙醛酸还原酶
- 将乙醛酸转化成乙醇酸，同时会有一分子的NADPH转化为NADP+

Detection


Anchor Protein
EcAKR4-1
Kill Switch
EcAKR4-1
Anchor
glyphosate
EcAKR4-1
Anchor Protein
G
A
glyoxylate
AMPA
EcAKR4-1
Anchor Protein

Detection

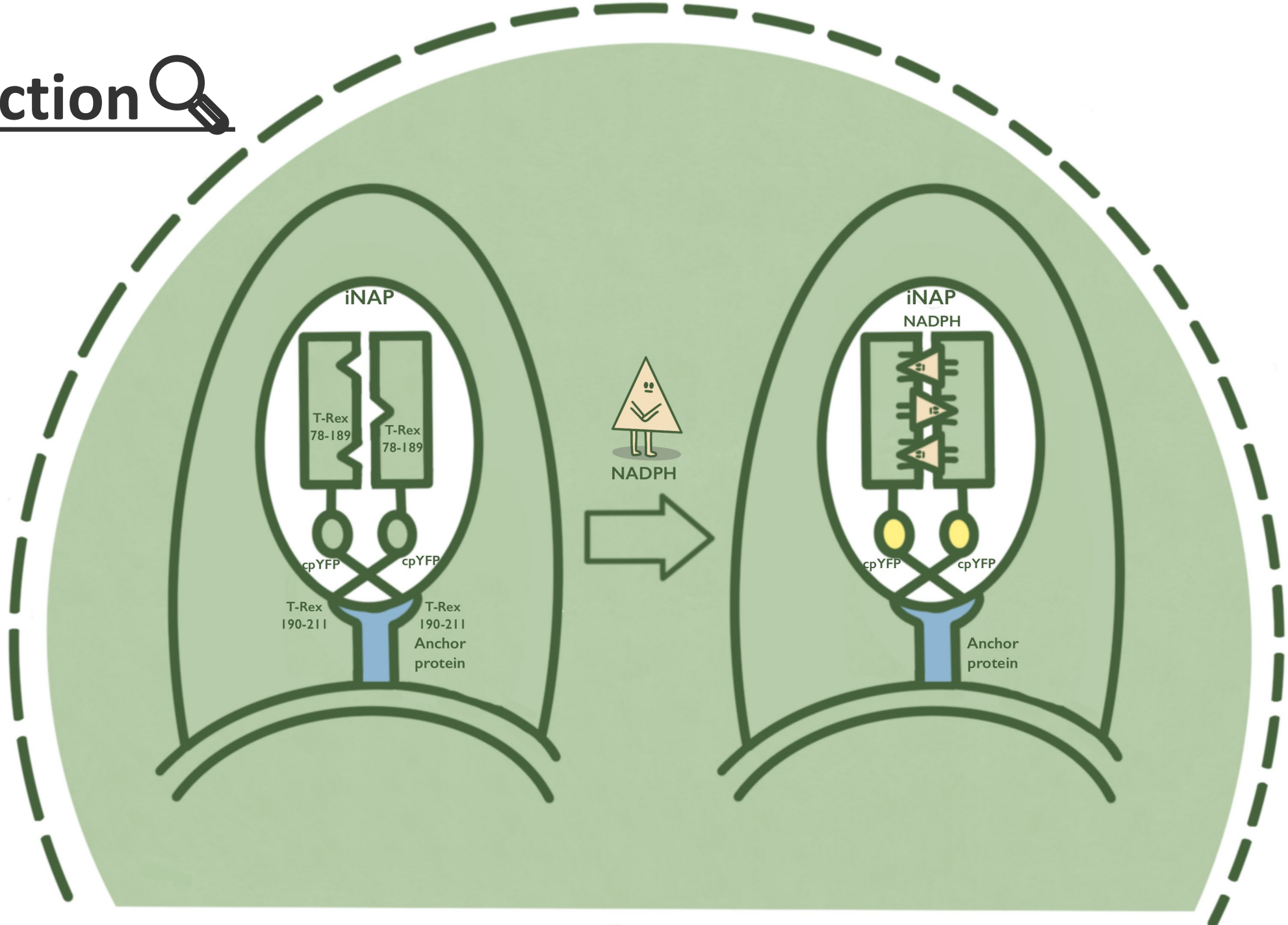

Anchor Protein
GRHPR
Kill Switch
GRHPR
Anchor
glyoxylate
GRHPR
Anchor Protein
NADPH
Glycolic acid
GRHPR
Anchor Protein
NADP+

Detection

II. NADPH 测定

- iNAP：NADPH 传感器（from T-Rex & cpYFP fusion protein)
- 存在NADPH时，NADPH 与 iNAP 结合使得 iNAP 发生构象变化，产生荧光，进而得到 [NAPDH]

Detection


iNAP
T-Rex
78-189
T-Rex
78-189
cpYFP
cpYFP
T-Rex
190-211
T-Rex
190-211
Anchor
protein
NADPH
iNAP
NADPH
cpYFP
cpYFP
Anchor
protein

Detection

III. GOX, GRHPR & iNAP 固定

- NADPH 作为代谢物普遍存在于细胞中，干扰草甘膦检测
- 将GOX、GRHPR、iNAP 与 Anchor Protein 融合，使其固定在细胞表面
- 三种可能的 Anchor Protein
  1. INPNC
  2. BrkA
  3. AIDA

Detection

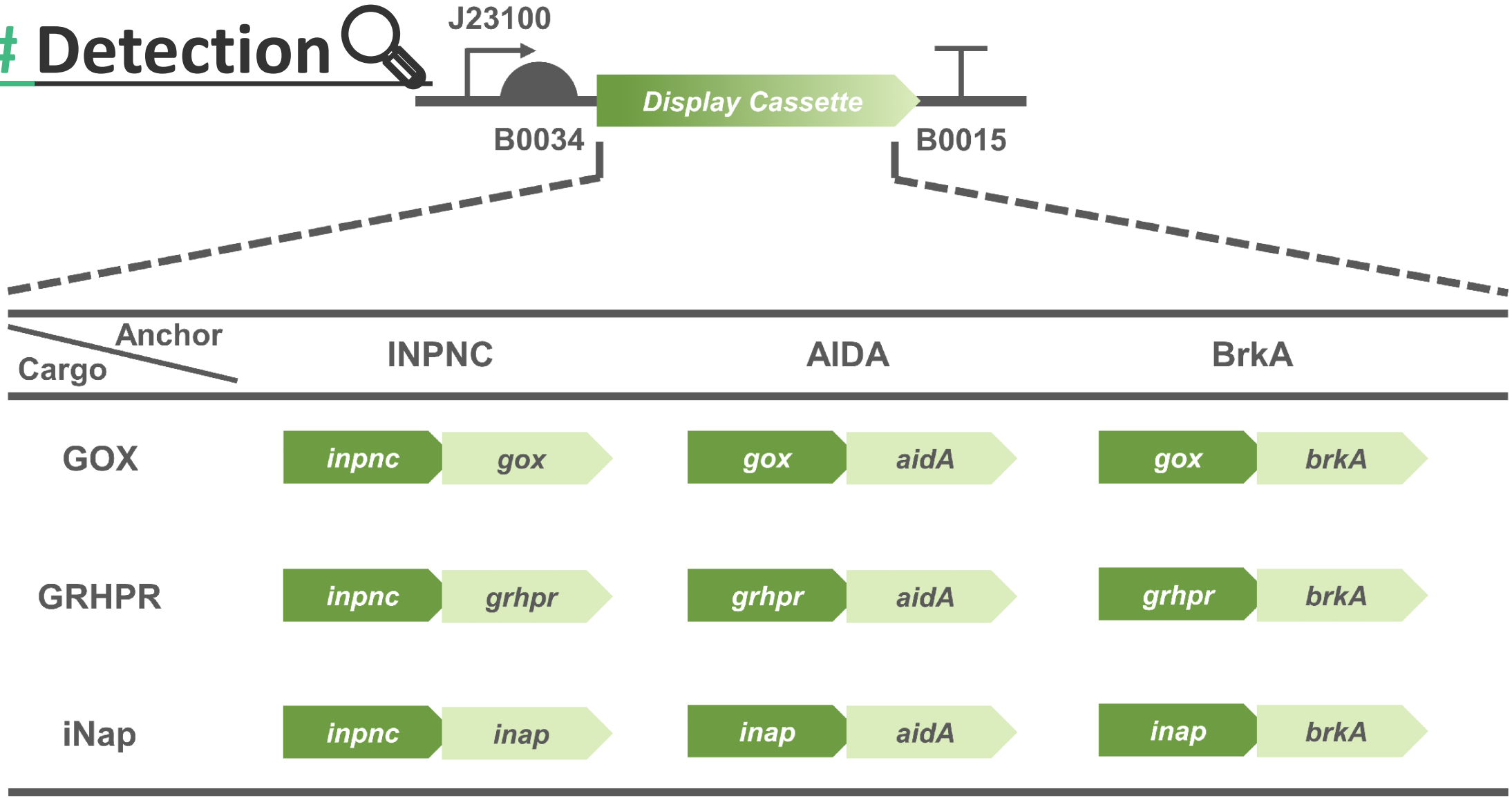
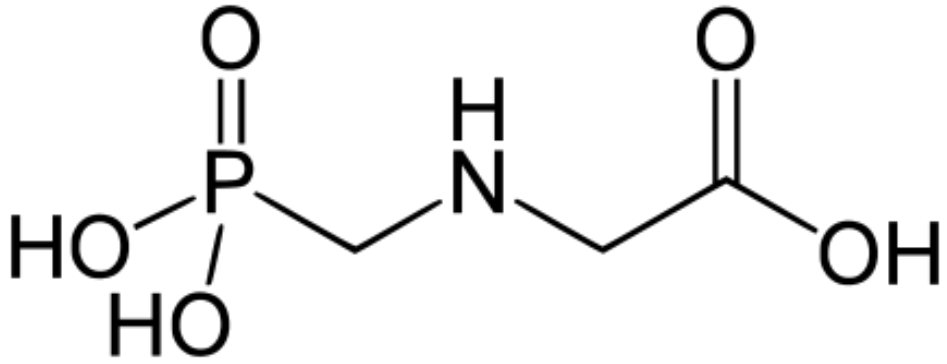


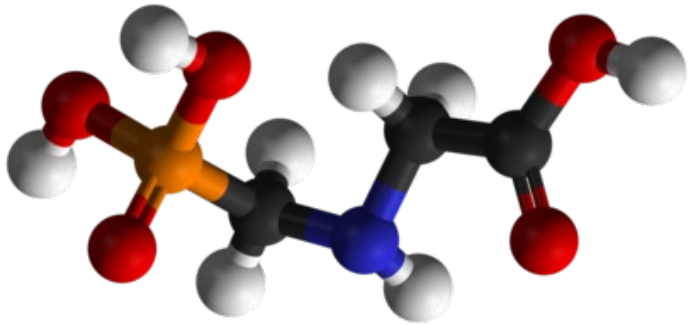
Fig | Nine tested fusion protein & Gene circuits of detection system

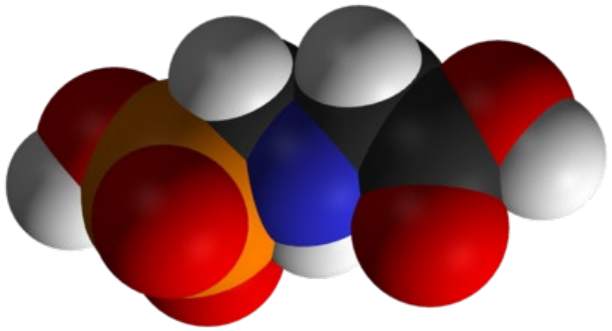
Degradation

I. Degradation Pathway of Glyphosate

- C-N 裂解：生成 AMPA 和 乙醛酸
- C-P 裂解：生成肌氨酸和磷酸

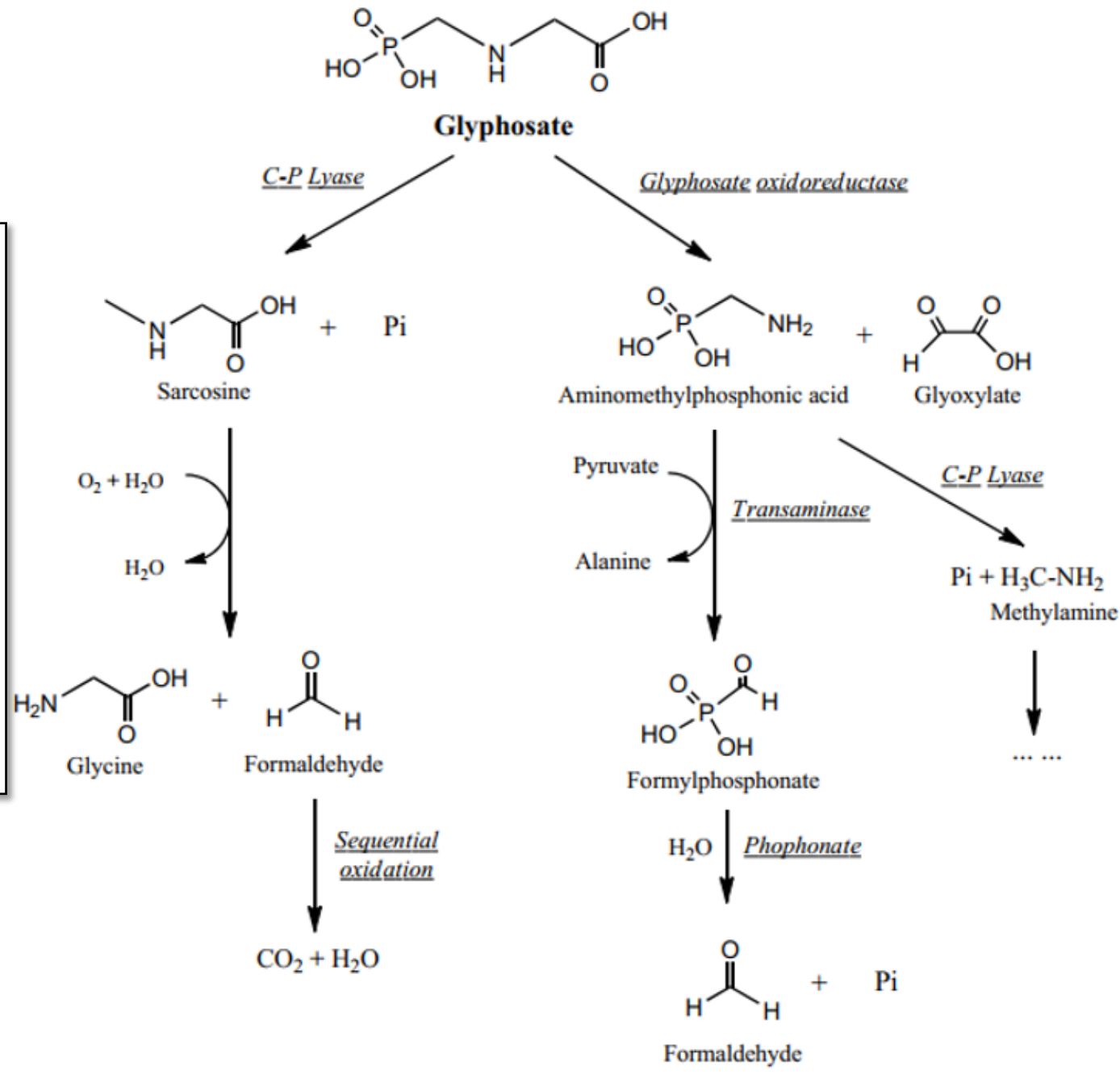






Degradation

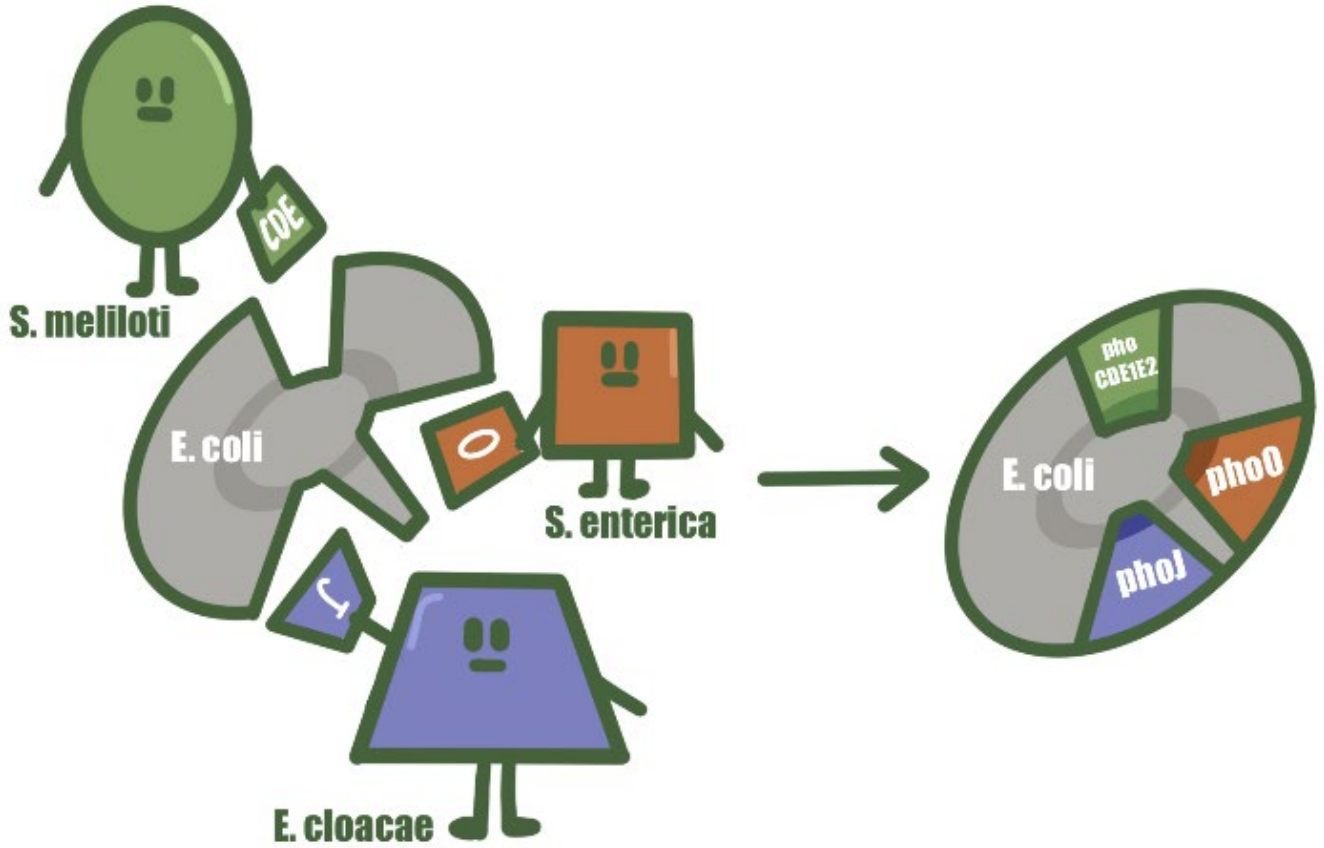
- Natural pathway: C-N Cleavage
- 产生的 AMPA 易残留且有害
- C-P 途径产生的 Sarcosine 易氧化形成可循环进出细胞的甲醛
- *E. coli* BL21(DE3) 存在功能失调的 C-P Cleavage System



Degradation

II. BL21(DE3) 三大改造

1. 磷酸的跨膜运输
2. 草甘膦的 C-P 裂解
3. AMPA 吸收与利用



Degradation

Proteins	Function of Proteins	Correlated Genes	Bacterial Strains*
Phosphonate acid transporter	Transport organophosphorus across membrane	***phnCDE***	*Sinorhizobium meliloti* 1021
Carbon-Phosphorus lyase	Degrade organophosphorus to small molecule	***phnGHIJK (core gene: phnJ)***	*Enterobacter cloacae* K7
Aminoalkylphosphonate N-acetyl-transferase	Derivatize AMPA into glyphosate analogue	***phnO***	*Salmonella enterica*

Fig | *phn* cluster

Degradation

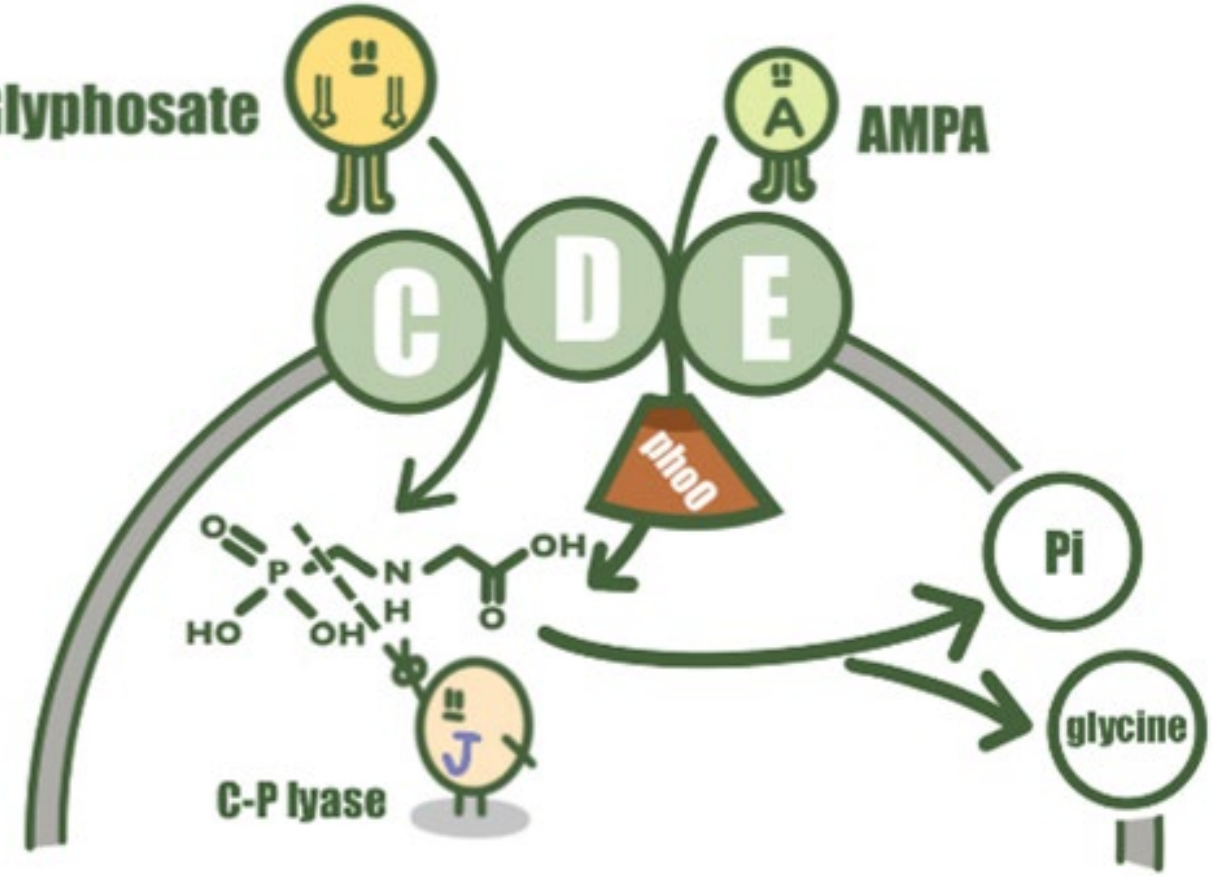


Fig | phn gene cluster mechanism

Degradation

III. The Application of RNAi

1. phnF(E. coli endogenous) 持续表达抑制 phn cluster
2. phnF targeted siRNA 阻碍 endo-phnF 的表达，激活草甘膦降解功能
3. Endo-phnJ targeted siRNA 确保 exo-phnJ 正常表达

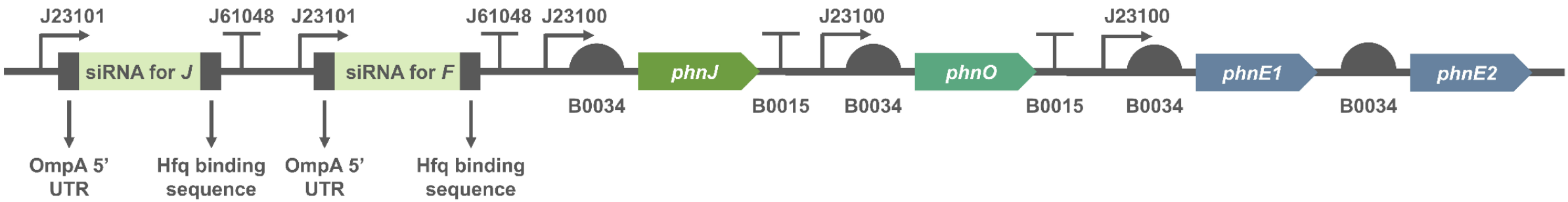


Fig | Gene circuit of degradation system

Kill Switches

I. kill switch in detection system

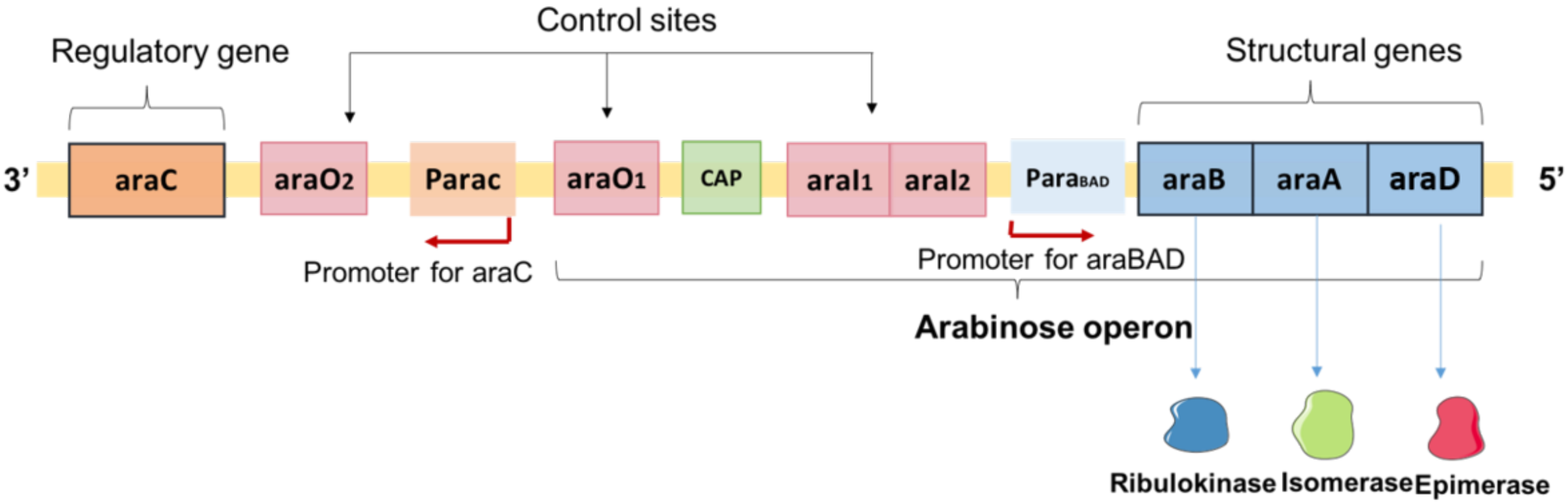


Fig | Structure of L-arabinose operon of E. coli.

Kill Switches

I. kill switch in detection system

- MazF: toxin protein in MazF-MazE (toxin-antitoxin module)
- CFU: colony forming units

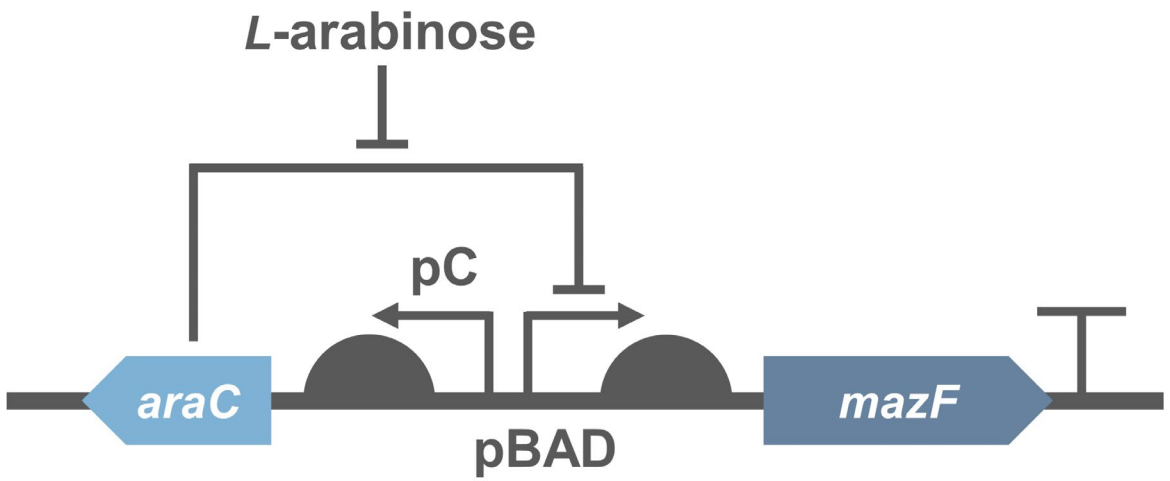
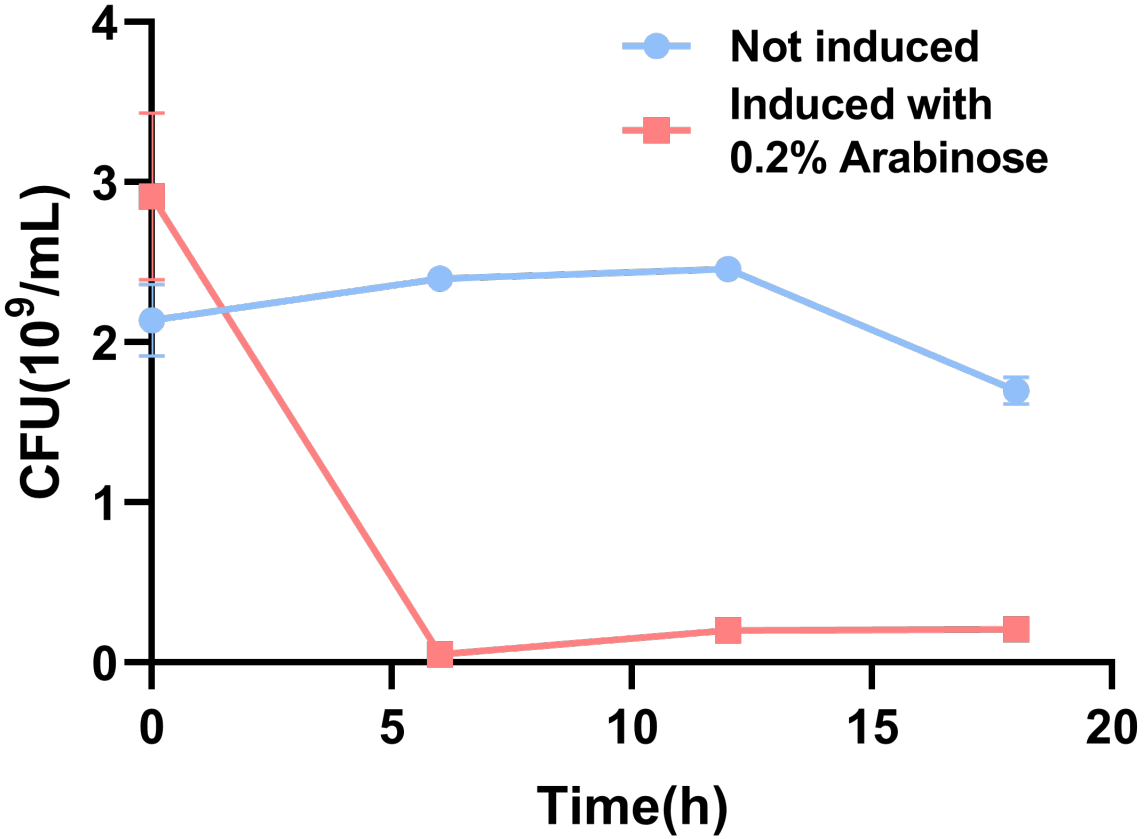


Fig | PBad/araC-RBS-MazF-terminator



Kill Switches

I. kill switch in detection system

- Inverter: cI repressor (from E. coli phage λ)
- + pR promoter which is inhibited by cI repressor

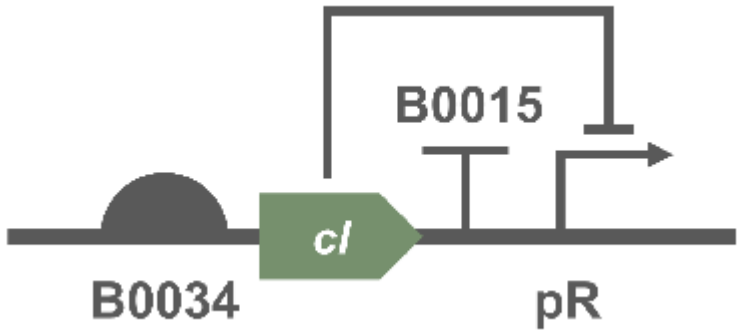
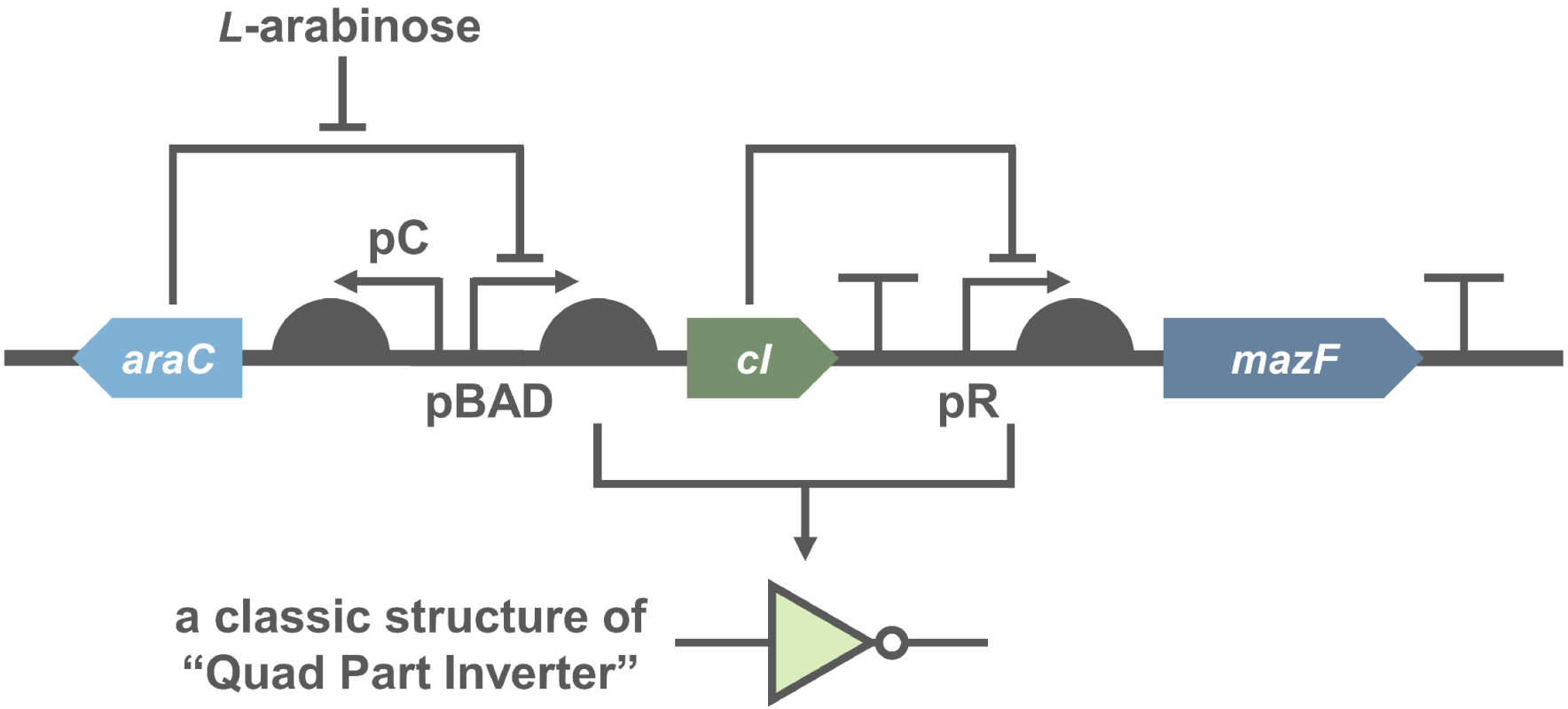


Fig | Inverter



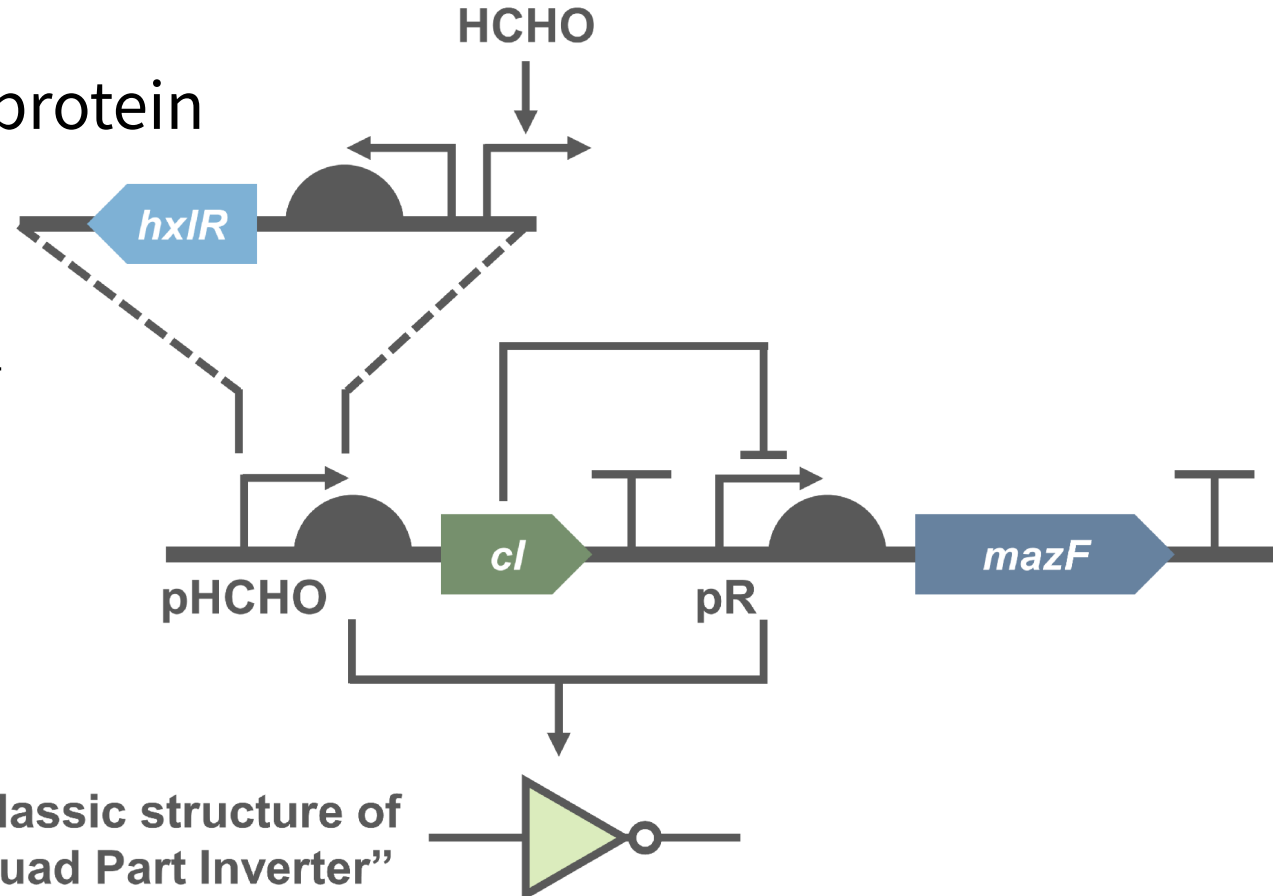
Kill Switches

II. kill switch in degradation system

- Quad Part Inverter: composed of 4 sub-parts

✓ promoter (e.g., pλ)
✓ regulated by the encoded repressor protein

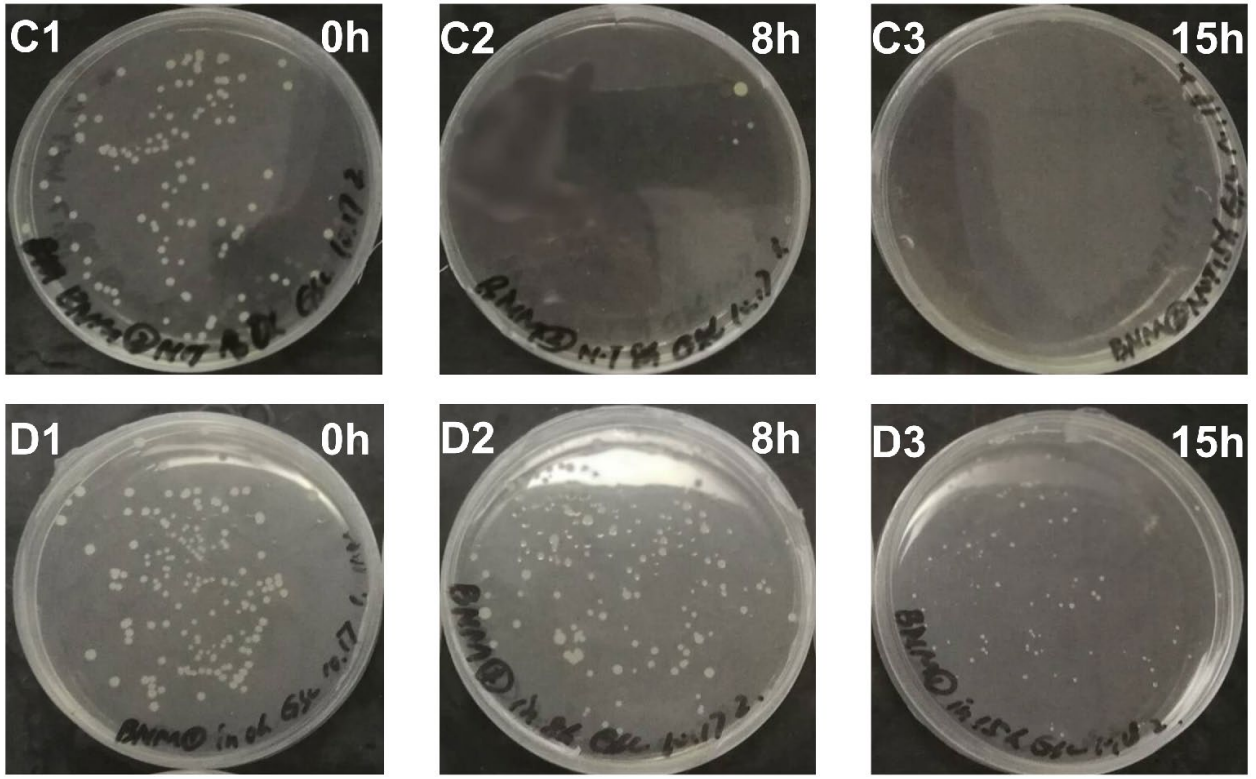
✓ RBS
✓ coding region for a repressor protein
✓ terminator



Kill Switches

II. kill switch in degradation

a



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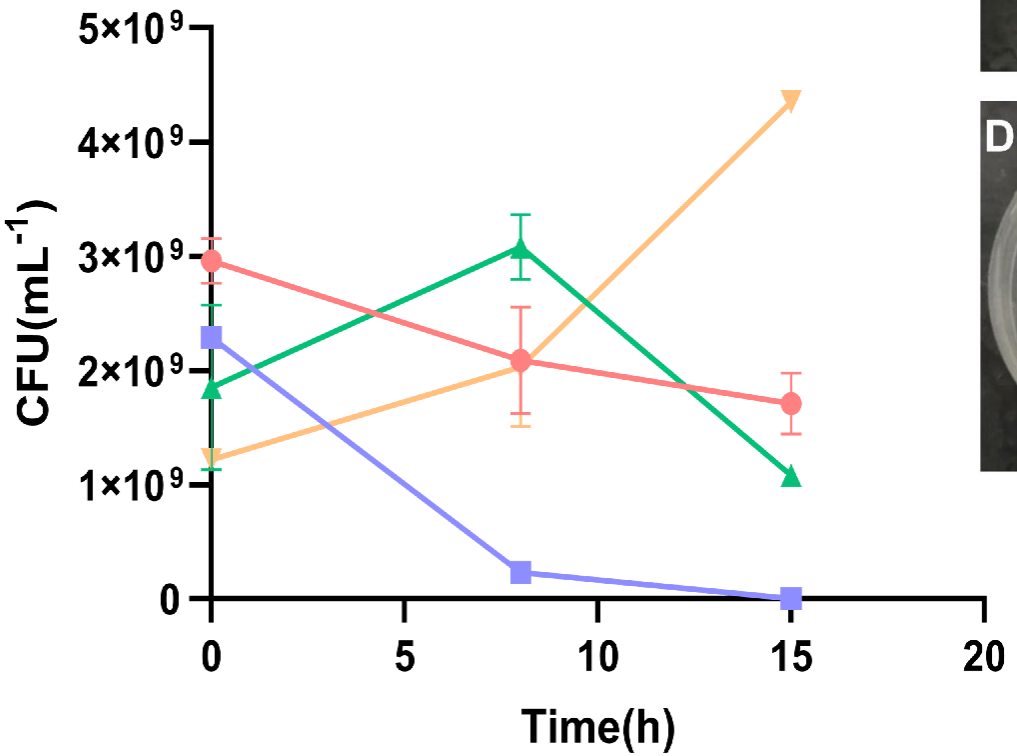

pBAD-Inverter-mazF with induction

pBAD-Inverter-mazF without induction

pBAD-mazF with induction

pBAD-mazF without induction

"C": non-induction group
"D": induction group

Resources

- https://2020.igem.org/Team:XMU-China
- https://en.wikipedia.org/wiki/Glyphosate
- https://en.wikipedia.org/wiki/RNA_interference
- https://en.wikipedia.org/wiki/L-arabinose_operon
- https://parts.igem.org/Part:BBa_K3332042
- https://parts.igem.org/Part:BBa_K1334002


END