Pathogen Detection Using an Engineered Contact-Dependent Inhibition System

John Errico
11/1/2014
iGEM
Human Practices

“I ♥ Nano Days”

SB County Science Fair
What is the Allosphere?

A simulated 3D immersive environment at UCSB

CDI stands for contact-dependent inhibition

• A bacterial defense and communication strategy

• Injected toxins act as non-specific DNases, RNases, etc.

• Mechanisms for self-strain protection against toxin (Cdil)

Ruhe, Low, & Hayes. Trends in Microbiology (2013)
CDI is composed of 3 genes

- CdiB is a membrane-bound ‘base’ for CdiA

- CdiA is the ‘stick’ with toxin attached to end, cleaved at conserved VENN sequence – binds BamA

- CdiI is an immunity protein specific for upstream toxin that prevents self-killing
CT may need a permissive factor to become active

When CT binds CysK (permissive factor), enzymatic activity is enabled

Both CysK and Immunity can bind CT to form a ternary complex
Bacterial two-hybrid systems detect protein-protein interactions

One half of Adenylate Cyclase (T25) fused to protein X, other half (T18) fused to Protein Y

If Proteins X/Y interact, Adenylate Cyclase forms cAMP

cAMP production identifies protein-protein interactions

Adapted from Karimova G et al. PNAS 1998;95:5752-5756
cAMP production turns on gene synthesis

Pap operon is a chromosomal promoter dependent on cAMP – only source of cAMP is from our system

Gene of interest must be transduced using a bacteriophage
CysK-T25 was made using OE-PCR

CysK forward primer w/ EcoRI site

PCR, longer extension period

Fused CysK-T25 product

T25 reverse primer w/ PstI site

Fused PCR product ligated into Tetracycline resistant backbone, transformed, checked with restriction analysis:

1.8Kbp = CysK-T25
I-T18 was extracted from pre-existing plasmid with PCR.

Ligation into Ampicillin resistant backbone, transformation, check with restriction analysis:

Faint 1Kbp = I-T18
Bacteriophage steal DNA randomly from a host

1. Introduce GFP-grown phage to cell line
2. Select for Kan^R colonies

Bacterial DNA  Viral DNA

Introduce GFP-grown phage to cell line, select for Kan^R colonies
Bacteria did not glow upon contact – Why?

Linker length may be not be long enough - Ternary vs. Binary

Endogenous CysK may be interfering – competitive antagonist

Couldn’t control by transfecting commercial binary two-hybrid construct – Conflicting antibiotic resistances

Adapted from Karimova G et al. PNAS 1998;95:5752-5756
The Future – a very customizable system

- Contact-dependent bacterial strain – Can include as many immunities as necessary
- Secondary messenger produced – cGMP?
- Gene synthesized – multi-gene cascade systems?
- Antibiotic production? – extremely specific killing
  ➫ Think T-cells
Acknowledgements

Advisors: David Low, Omar Saleh

Mentors: Christina Beck, Dan Nguyen

Lab space: Chris Hayes

The Team:
Zachary Haynes, Travis Smith, Hiro Sparks, Katie Lee, Sarah Lensch, Andrew Ballin, Daniel Reinhart, Colton Bracken, & Tsuyoshi Kohlgruber

And of course... iGEM!
Thank you! Questions?