Lambda phage killing: infection and “parts”

N Kuldell for 20.020
Spring 2009
Heidelberg iGEM 2008: ecolicense to kill

Part 2: DNA transfer, genetically programmed self-assembly and “parts”

Background information
DNA transfer
natural context: 3 mechanisms

Transformation
- Transfer of free DNA
- Resistant gene
- Dead bacterium

Conjugation
- Plasmid Donor
- Plasmid
- Resistant gene
- Gene goes to plasmid or to chromosome

Transduction
- Virus
- Bacterium receiving resistance genes
- Transfer by viral delivery

Courtesy of Fan Sozzi-Guo. Used with permission.
“Transduction” by bacteriophage

Bacteriophage: viruses that infect bacteria

Protein coat
encapsulates
nucleic acids

“Temperate” bacteriophage lambda

INFECT

REPLICATE

RELEASE

ASSEMBLE

lytic

Image courtesy of Gary Kaiser. Used with permission.

See http://escience.ws/b572/L17/L17.htm
“Temperate” bacteriophage lambda

Image courtesy of Gary Kaiser. Used with permission.
“Temperate” bacteriophage lambda

Some great ??s
What guides lytic/lysogenic decision?
What keeps lysogen stable?
What triggers lysogen to lytic cycle?

Image courtesy of Gary Kaiser. Used with permission.
“Temperate” bacteriophage lambda

Some great ??s the Heidelberg team asked:
Can we infect prey from lysogen (=predator)?
Can we keep lysogen from lysing itself?
Can we monitor lysis and lysogeny?

INFECT

REPLICATE

RELEASE

ASSEMBLE

Image courtesy of Gary Kaiser. Used with permission.
“Temperate” bacteriophage lambda

Some great ??s the Heidelberg team asked:
Can we infect prey from lysogen (=predator)?
Can we keep lysogen from lysing itself?
Can we monitor lysis and lysogeny?

Image courtesy of Gary Kaiser. Used with permission.
Genetically programmed infection

iGEM context: phage infection via conjugation

Courtesy of Fan Sozzi-Guo. Used with permission.
Genetically programmed infection
iGEM context: phage infection via conjugation

http://escience.ws/b572/L18/L18.htm

Courtesy of AJC1 on Flickr.

Courtesy of Stan Metzenberg. Used with permission.

Courtesy of DKFZ/Univ. Heidelberg/iGEM Team Heidelberg. Used with permission.
Genetically programmed infection
iGEM context: phage infection via conjugation

What “parts” are needed

Pilus… “F”
Origin for transfer… “oriT”
Selectable marker… “CamR”

“Part” is a genetically-encoded, human defined function

Model predicts: 10 killer cells kill
10^9 prey cells “in silico”
Genetically programmed infection
iGEM context: phage infection via conjugation

What “parts” are needed

Pilus…. “F’”

Origin for transfer… “oriT”

Selectable marker… “CamR”

“Part” is a genetically-encoded, human defined function

Courtesy of DKFZ/Univ. Heidelberg/iGEM Team Heidelberg. Used with permission.
“Temperate” bacteriophage lambda

Some great ??s the Heidelberg team asked:
Can we infect prey from lysogen (=predator)?
Can we keep lysogen from lysing itself?
Can we monitor lysis and lysogeny?

Image courtesy of Gary Kaiser. Used with permission.
Genetically programmed bi-stable switch
natural context: epigenetic regulation

Diagram removed due to copyright restrictions.
"Design of $\lambda$ lac." Fig. 1A and B in Atsumi, S., and J. W. Little.
"Regulatory Circuit Design and Evolution Using Phage $\lambda$."  
http://dx.doi.org/10.1101/gad.1226004
Genetically programmed bi-stable switch
iGEM context: flip and hold in one state

Diagram removed due to copyright restrictions.

"Design of Lambda lacI." Fig. 1B in Atsumi, S., and J. W. Little.
"Regulatory Circuit Design and Evolution using Phage Lambda."
http://dx.doi.org/10.1101/gad.1226004
“Temperate” bacteriophage lambda

Some great ??s the Heidelberg team asked:
Can we infect prey from lysogen (=predator)?
Can we keep lysogen from lysing itself?
Can we monitor lysis and lysogeny?
“Temperate” bacteriophage lambda

Some great ??s the Heidelberg team asked:
Can we infect prey from lysogen (=predator)?
Can we keep lysogen from lysing itself?
Can we monitor lysis and lysogeny?

Image courtesy of Gary Kaiser. Used with permission.
Fluorescence vs bioluminescence


Green fluorescent protein (GFP), courtesy of RCSB Protein Data Bank.

Courtesy of Bonnie Bassler. Used with permission.
Fluorescence vs bioluminescence


http://www.lifesci.ucsb.edu/~biolum/chem/

Image removed due to copyright restrictions. See Figure 4 in Schauder, S., and B. L. Bassler. "The Languages of Bacteria." Genes & Dev 15: (2001) 1468-1480

Green fluorescent protein (GFP), courtesy of RCSB Protein Data Bank.
Fluorescence vs bioluminescence


http://www.lifesci.ucsb.edu/~biolum/chem/

Image removed due to copyright restrictions. See Figure 4 in Schauder, S., and B. L. Bassler. "The Languages of Bacteria." Genes & Dev 15: (2001) 1468-1480

Green fluorescent protein (GFP), courtesy of RCSB Protein Data Bank.
One last thing about fluorescence

Green fluorescent protein (GFP), courtesy of RCSB Protein Data Bank.

Image removed due to copyright restrictions.


High resolution PDF poster available at http://jcs.biologists.org/cgi/data/114/5/837/DC1/1
Genetically programmed infection
iGEM context: phage infection via conjugation

What “parts” are needed

- Pilus…. “F’”
- Origin for transfer… “oriT”
- Selectable marker… “CamR”
- Stable lysogen… “cl”
- Monitor… “GFP”

Courtesy of DKFZ/Univ. Heidelberg/iGEM Team Heidelberg. Used with permission.
Predator cell
stable lysogen that infects by conjugation

+ CamR
+ GFP to follow process
20.020 Introduction to Biological Engineering Design
Spring 2009

For information about citing these materials or our Terms of Use, visit: [http://ocw.mit.edu/terms](http://ocw.mit.edu/terms).