

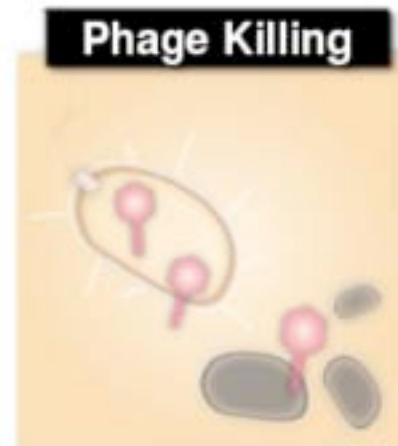
# Lambda phage killing: infection and “parts”

N Kuldell for 20.020

Spring 2009

# Heidelberg iGEM 2008: ecolicense to kill

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

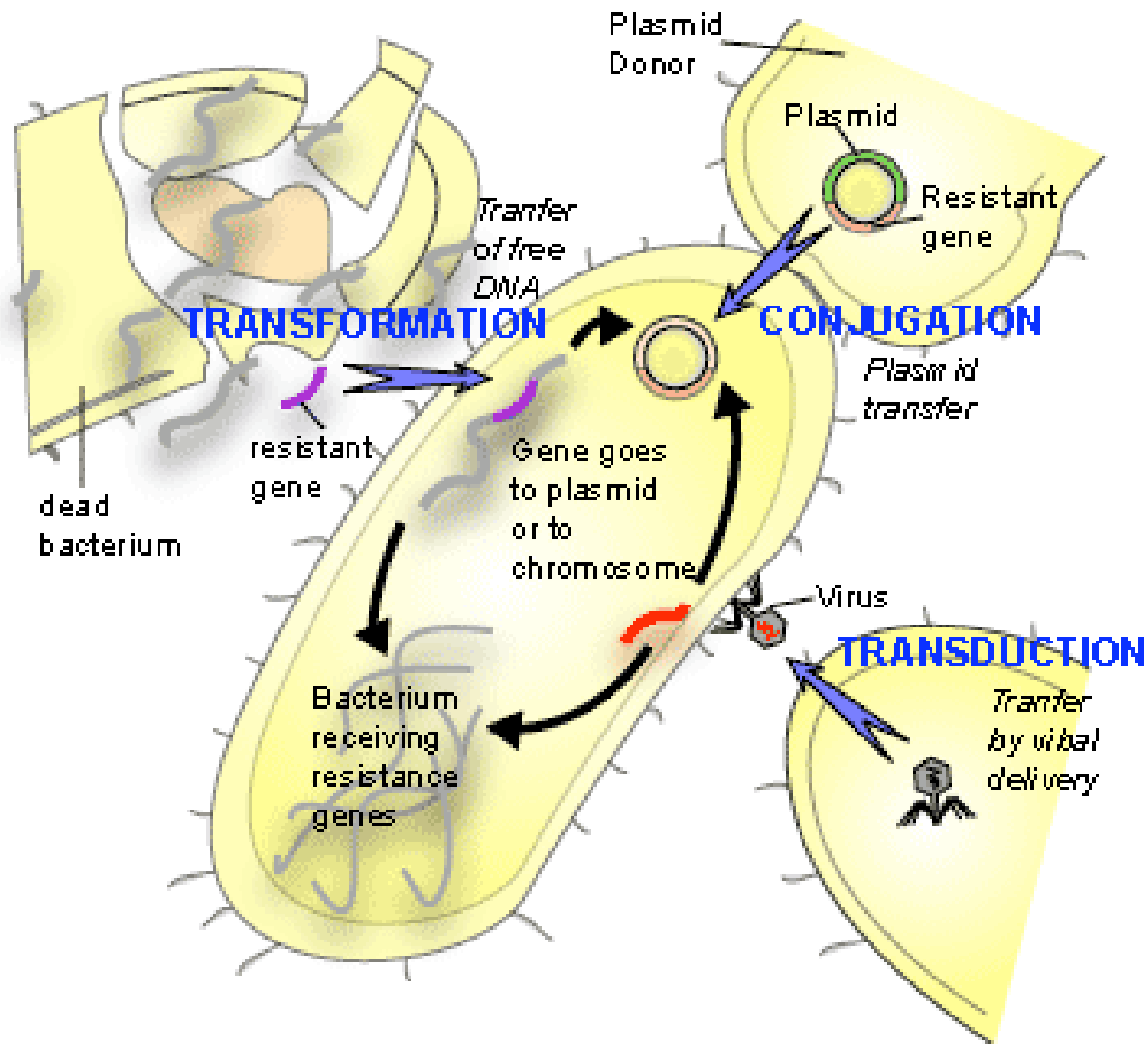


Courtesy of DKFZ/Univ. Heidelberg/iGEM Team Heidelberg. Used with permission.

## Background information

# DNA transfer

natural context: 3 mechanisms



Courtesy of Fan Sozzi-Guo. Used with permission.

# “Transduction” by bacteriophage

Bacteriophage: viruses  
that infect bacteria

Protein coat  
encapsulates  
nucleic acids

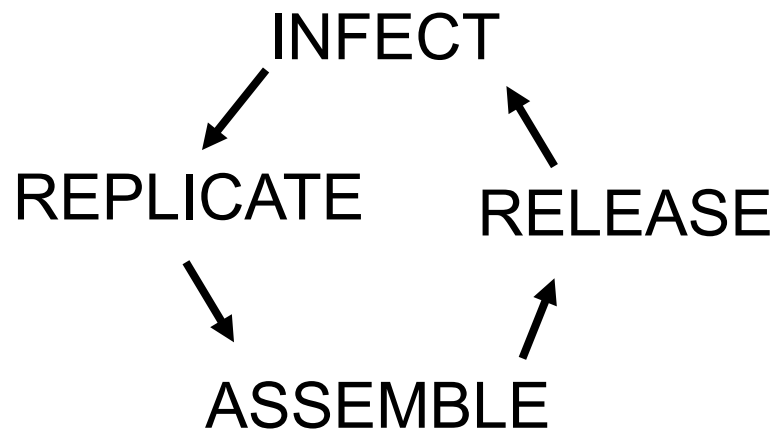
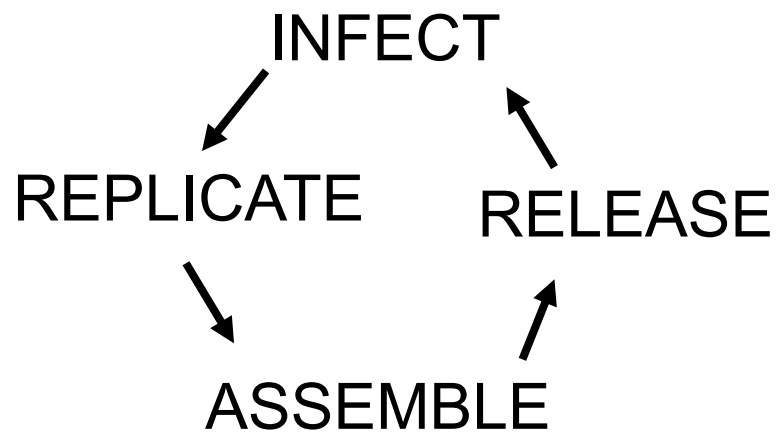


Image of bacteriophage removed due to copyright restrictions.  
See Phage Biotechnology website, <http://www.phagebiotech.com>.

# “Temperate” bacteriophage lambda



lytic

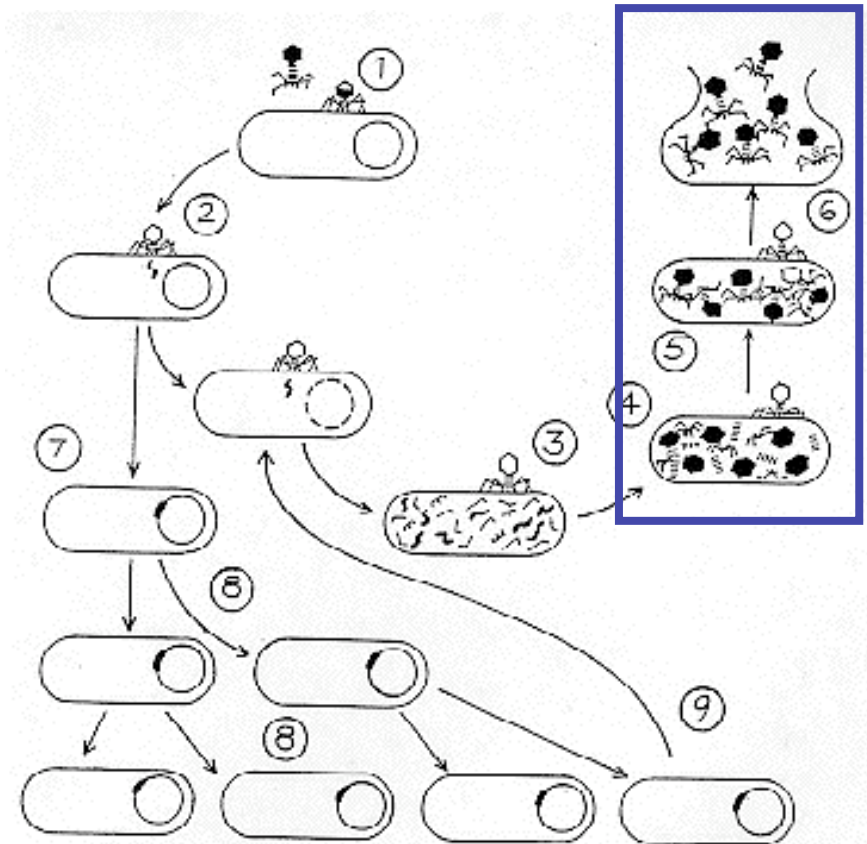
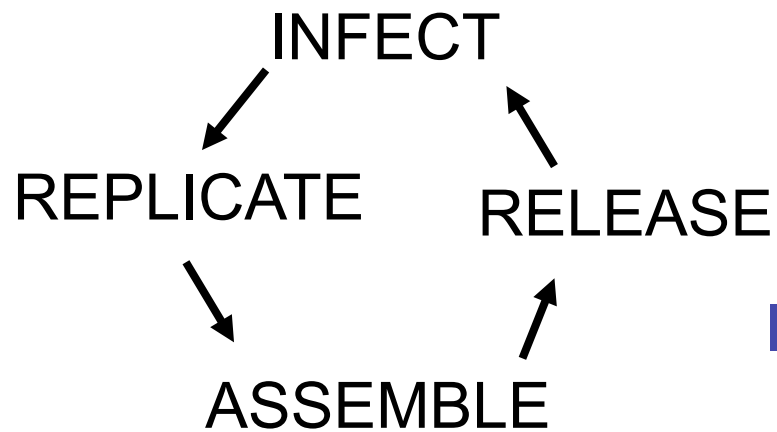


Image courtesy of Gary Kaiser. Used with permission.

See <http://escience.ws/b572/L17/L17.htm>

# “Temperate” bacteriophage lambda



lytic

lysogenic

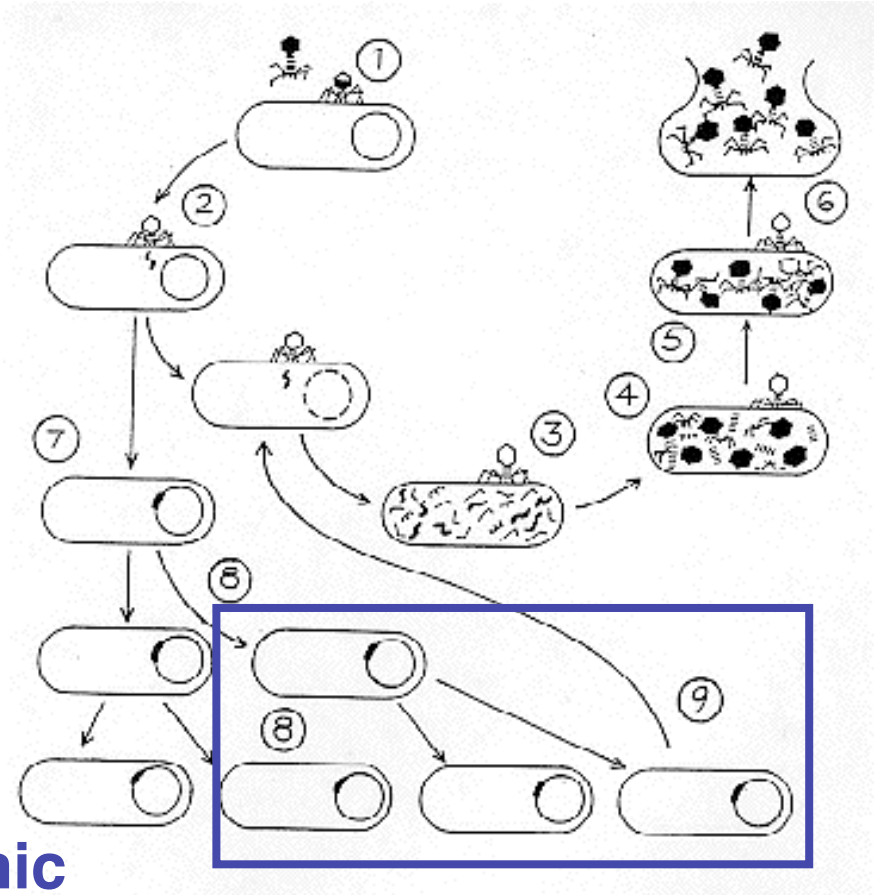


Image courtesy of Gary Kaiser. Used with permission.

# “Temperate” bacteriophage lambda

Some great ??s

What guides lytic/lysogenic decision?

What keeps lysogen stable?

What triggers lysogen to lytic cycle?

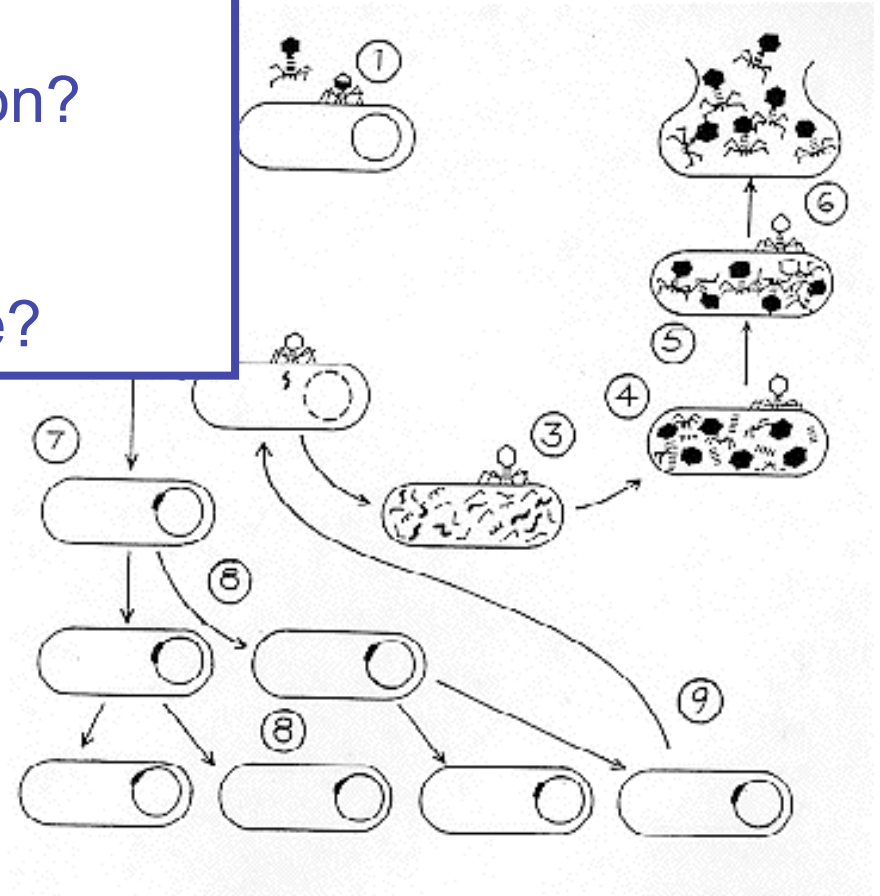
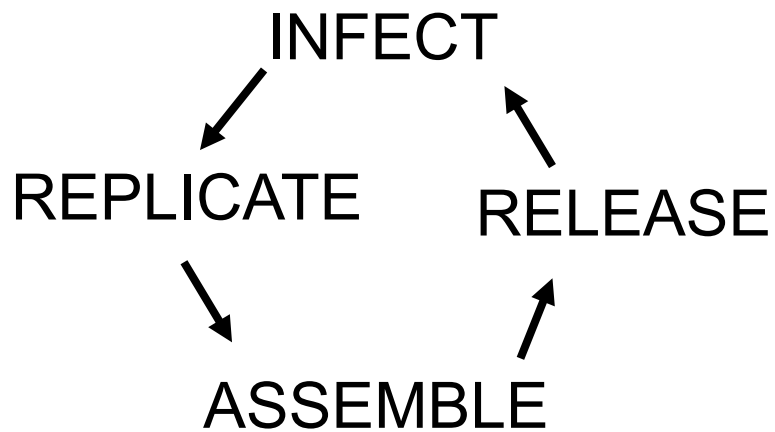


Image courtesy of Gary Kaiser. Used with permission.

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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?

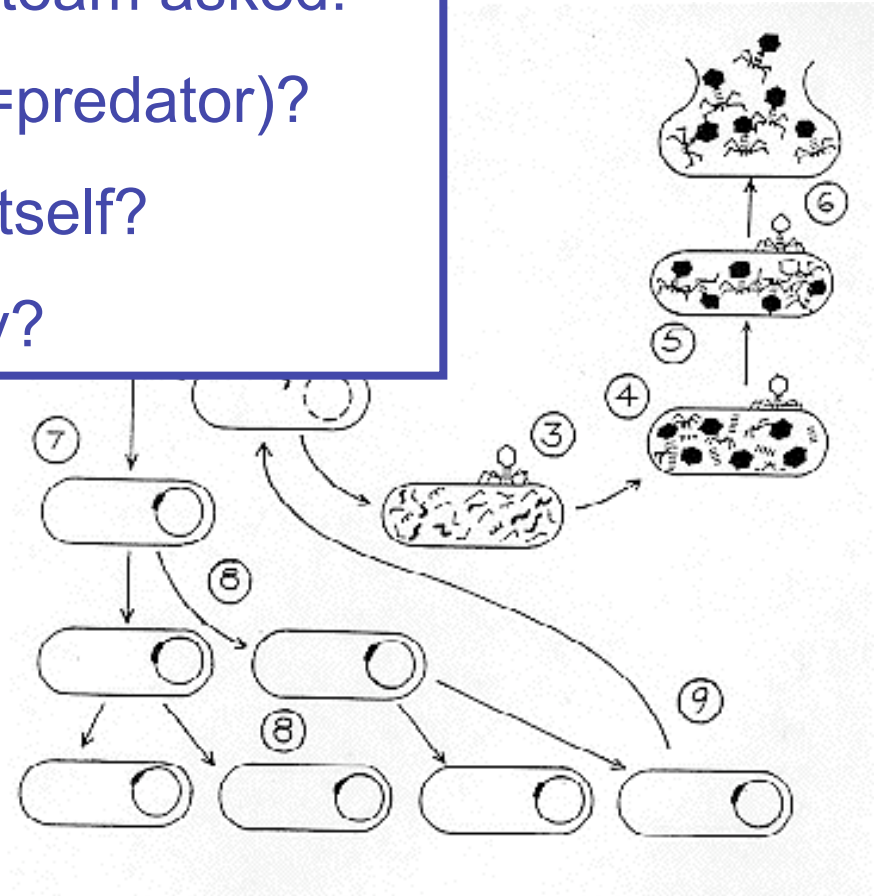
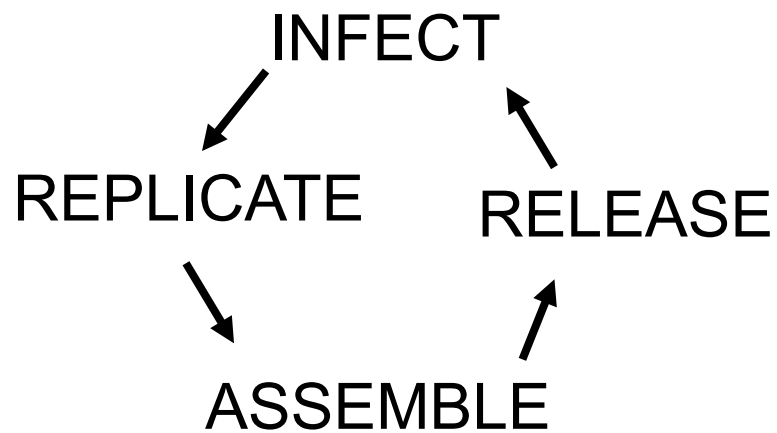


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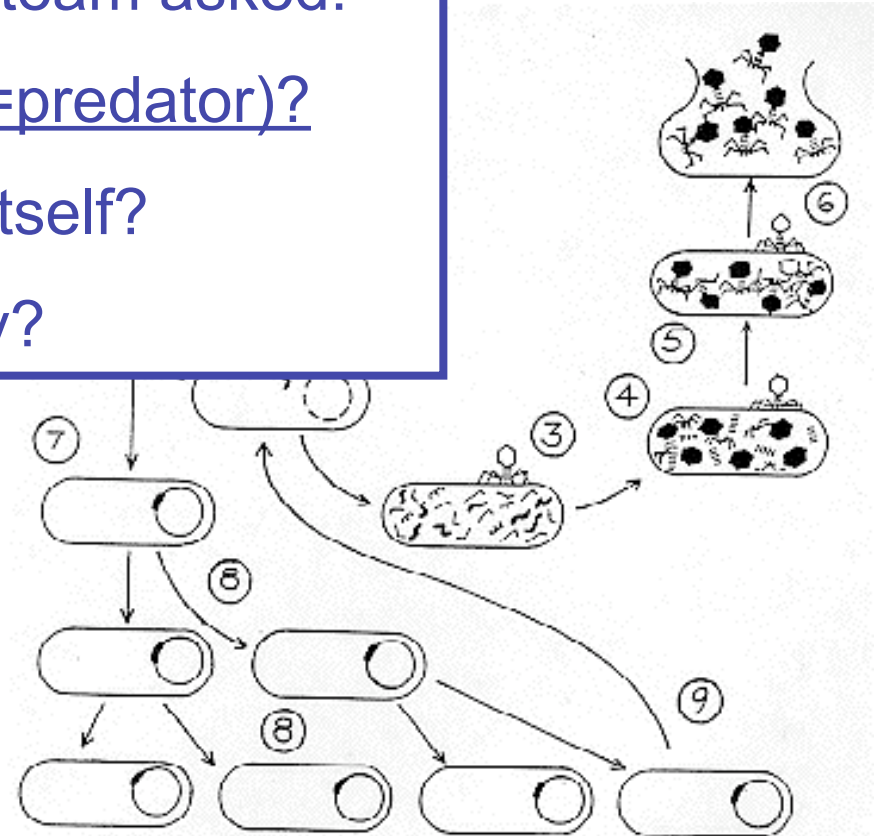
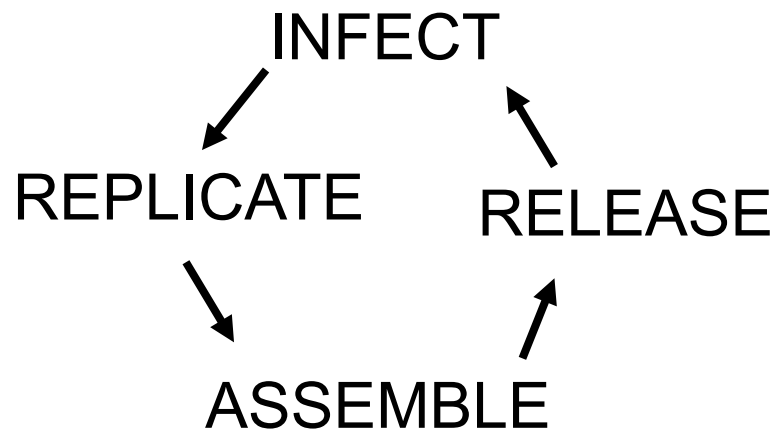
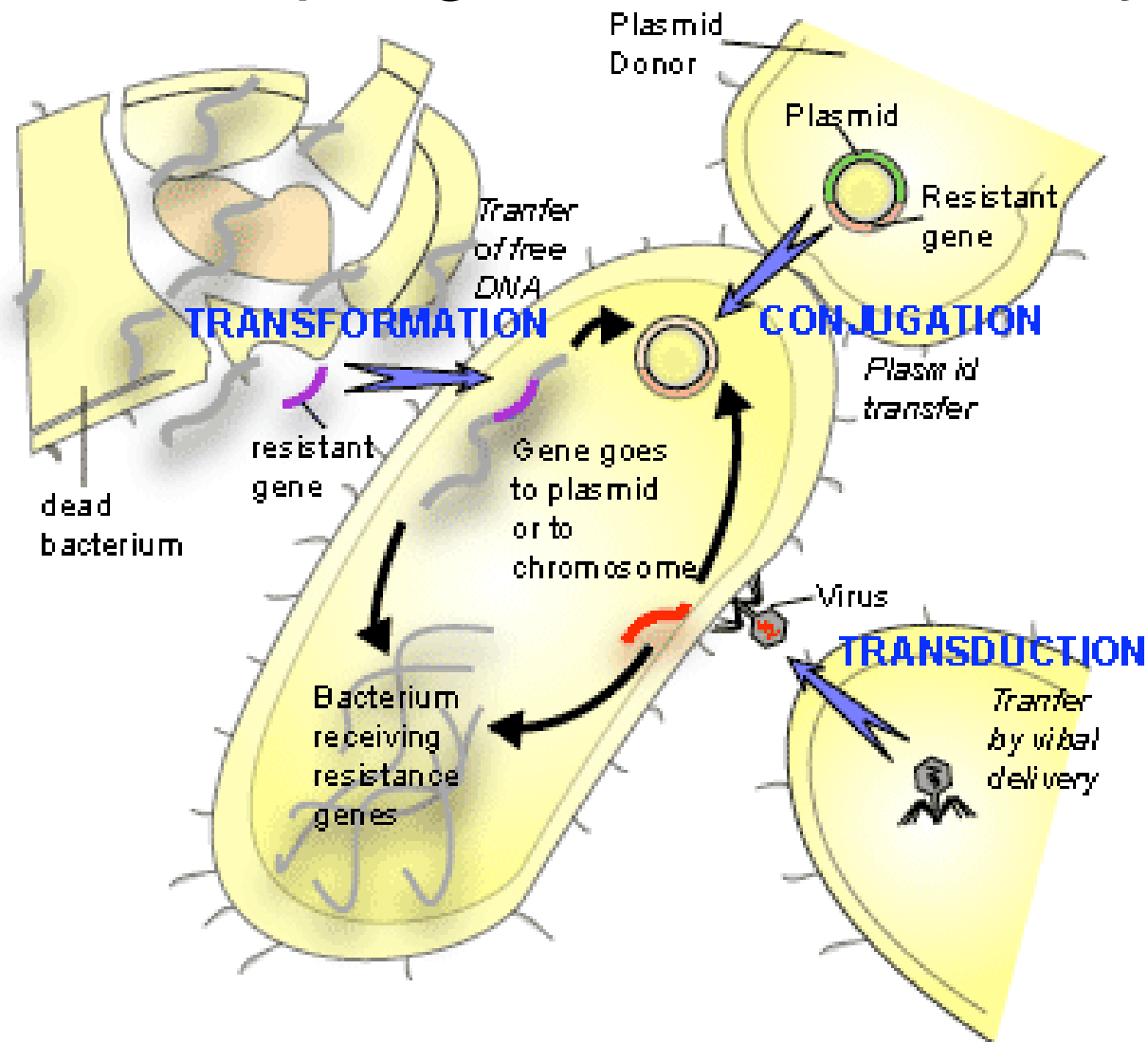


Image courtesy of Gary Kaiser. Used with permission.

# Genetically programmed infection

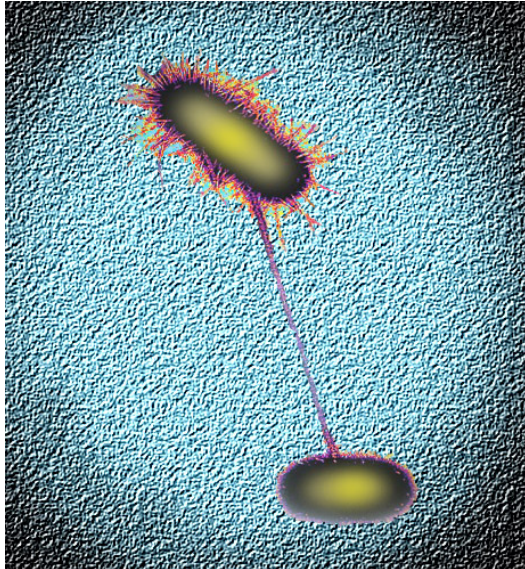
## iGEM context: phage infection via conjugation



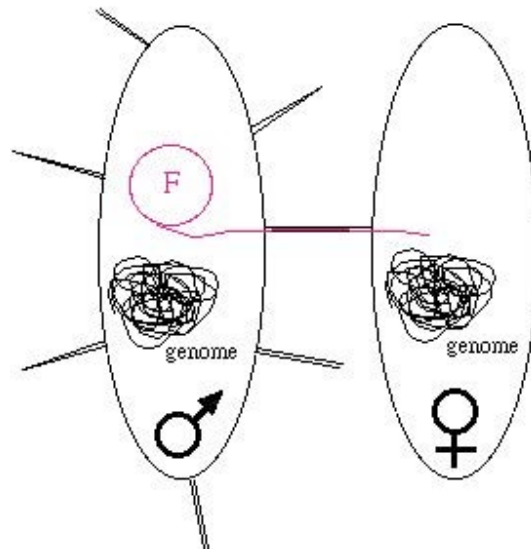
Courtesy of Fan Sozzi-Guo. Used with permission.

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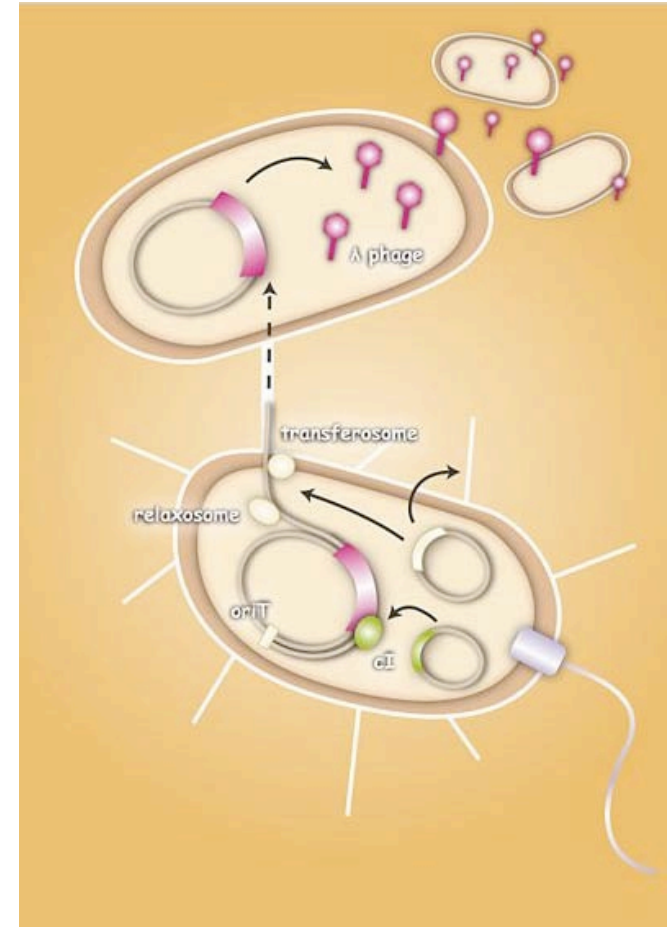


Courtesy of [AJC1](#) on Flickr.



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

Courtesy of Stan Metzenberg.  
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# Genetically programmed infection

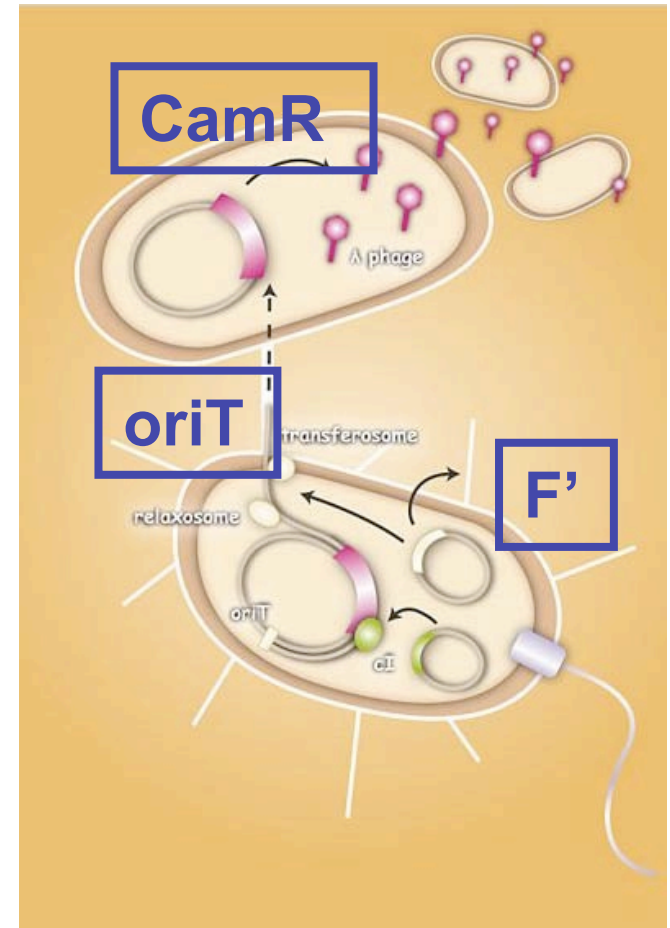
## iGEM context: phage infection via conjugation

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...

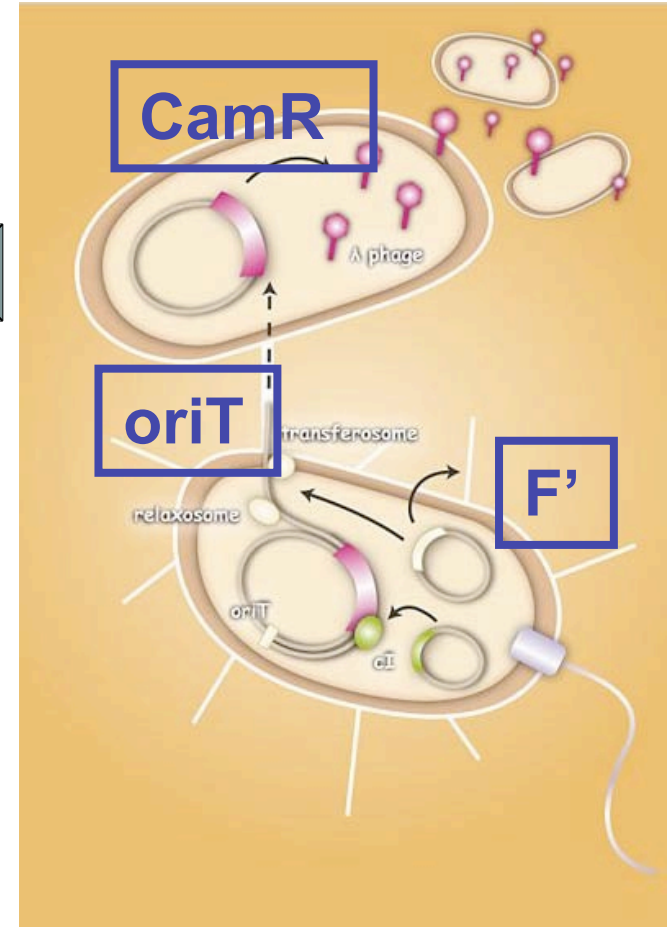
“oriT”

I714030

Selectable marker...

“CamR”

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Courtesy of DKFZ/Univ. Heidelberg/iGEM Team Heidelberg. Used with permission.

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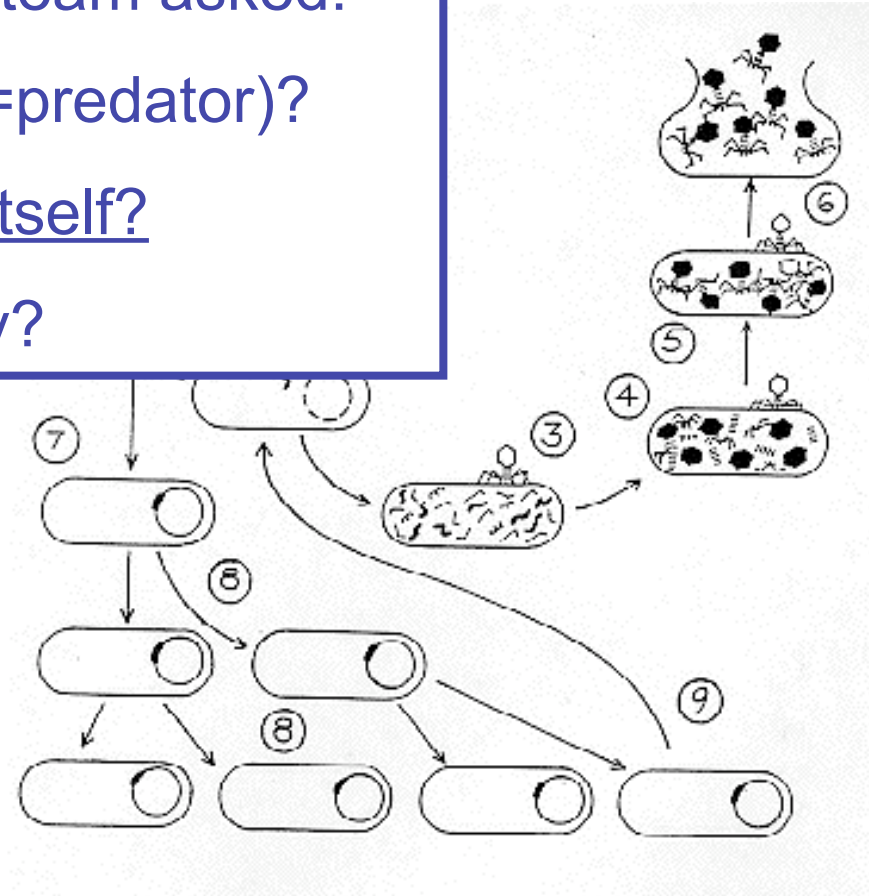
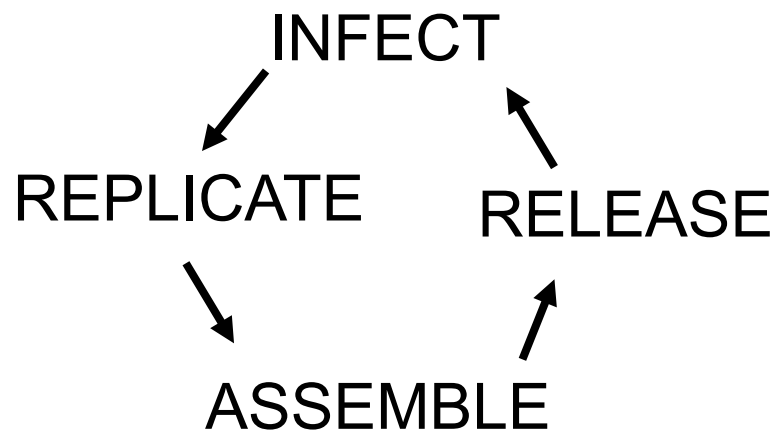


Image courtesy of Gary Kaiser. Used with permission.



# Genetically programmed bi-stable switch natural context: epigenetic regulation

Diagram removed due to copyright restrictions.

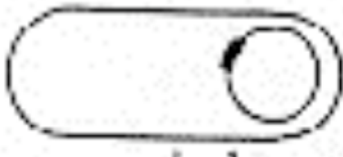
"Design of  $\lambda$  laci." Fig. 1A and B in Atsumi, S., and J. W. Little.

"Regulatory Circuit Design and Evolution Using Phage  $\lambda$ ."

*Genes & Dev* 18 (2004): 2086-2094.

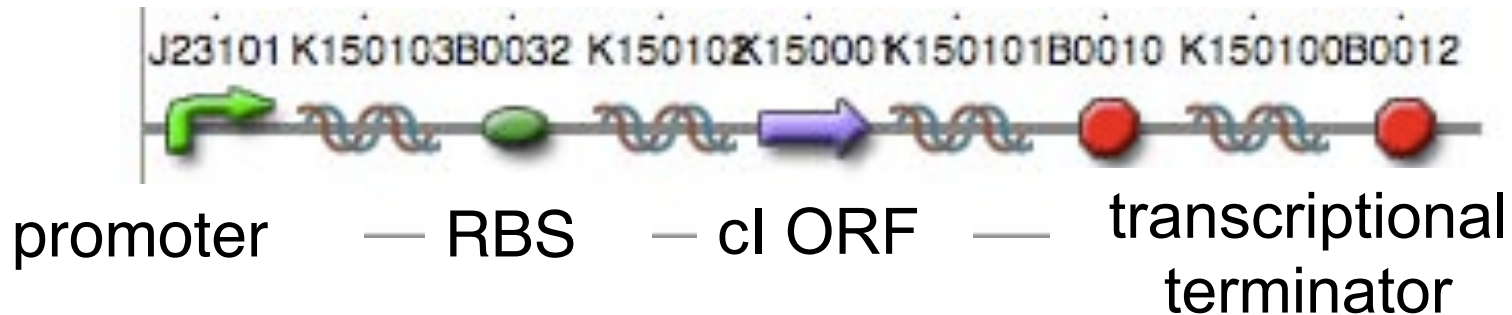
<http://dx.doi.org/10.1101/gad.1226004>

**lysogeny**



**lysis**

# Genetically programmed bi-stable switch iGEM context: flip and hold in one state



**lysogeny**

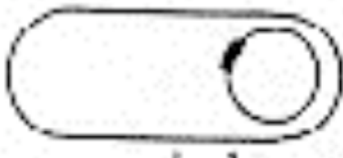


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"Design of Lambda *lacl*." Fig. 1B in Atsumi, S., and J. W. Little.

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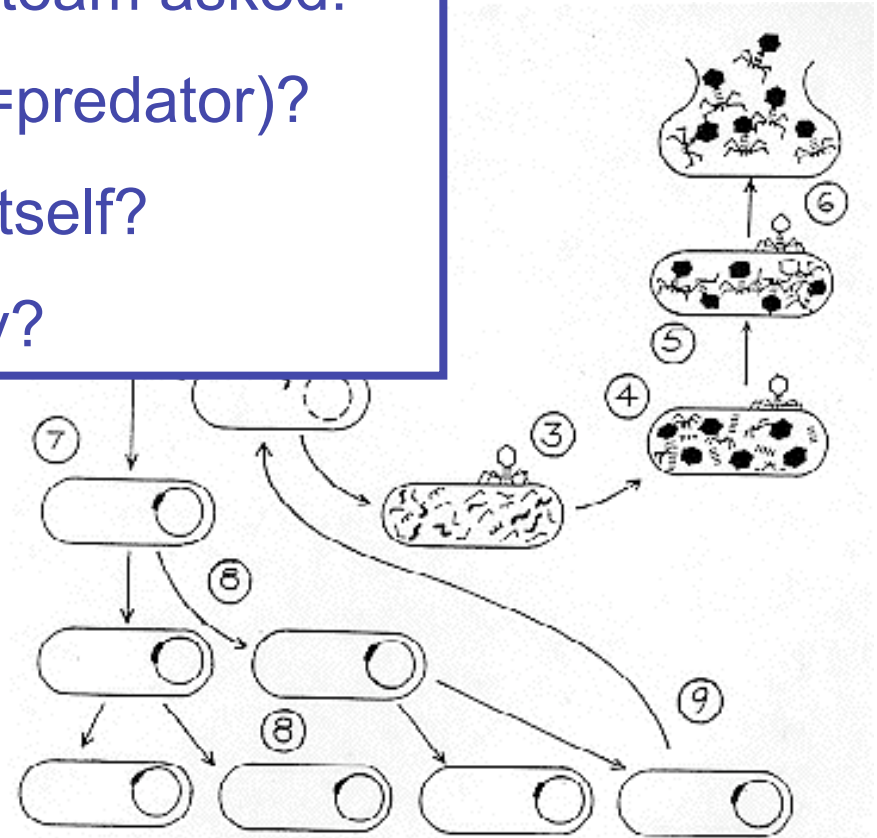
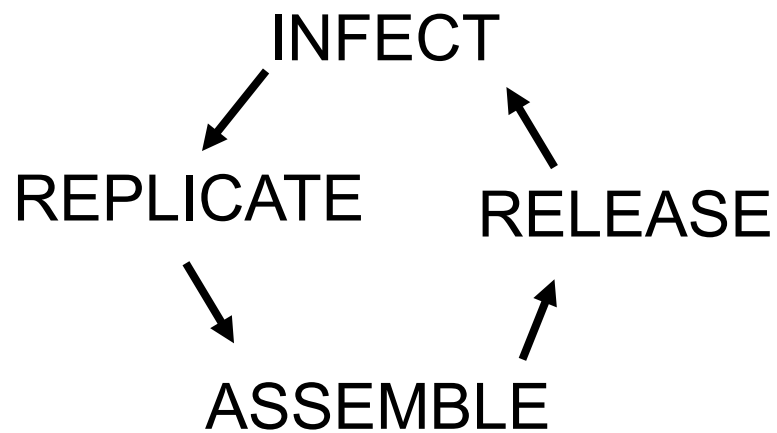


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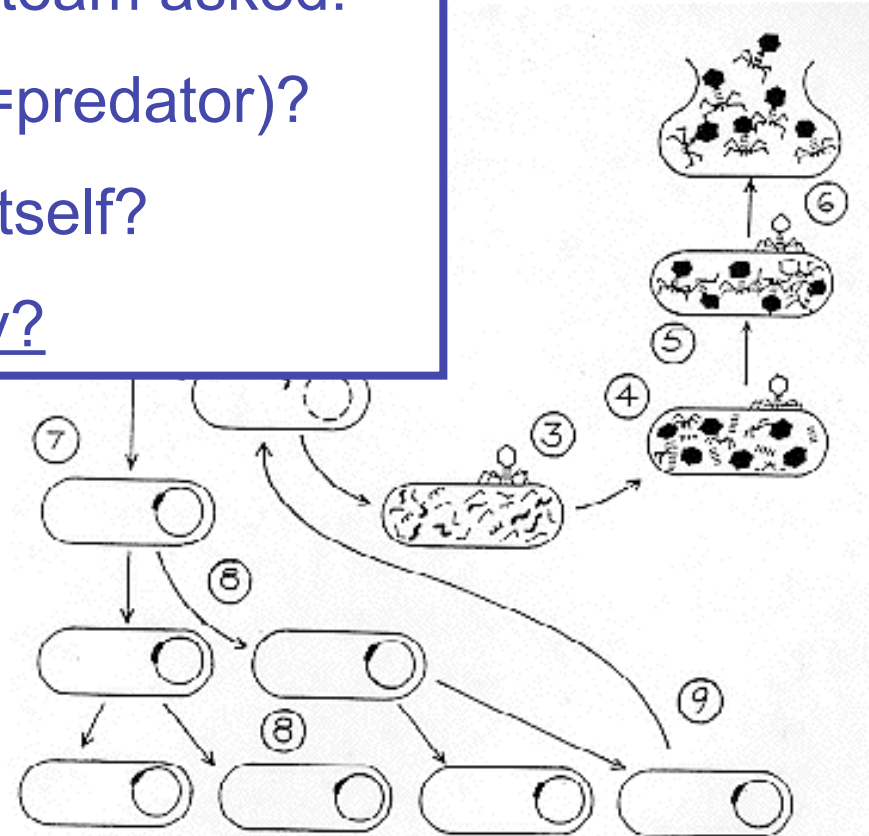
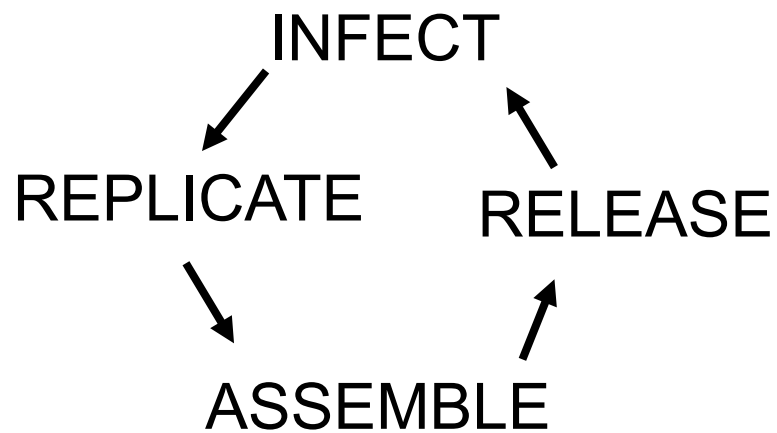
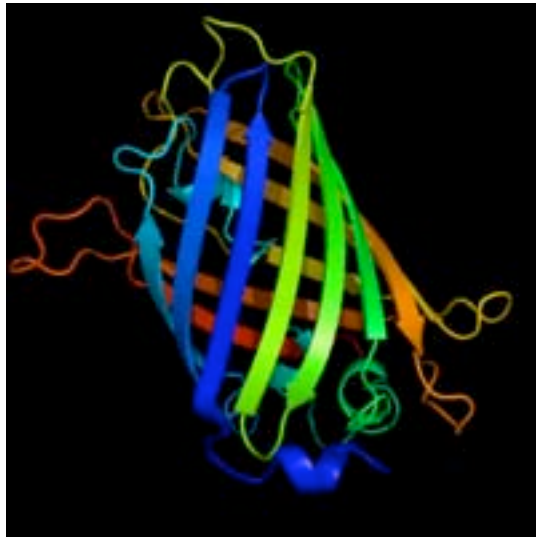


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# Fluorescence vs bioluminescence



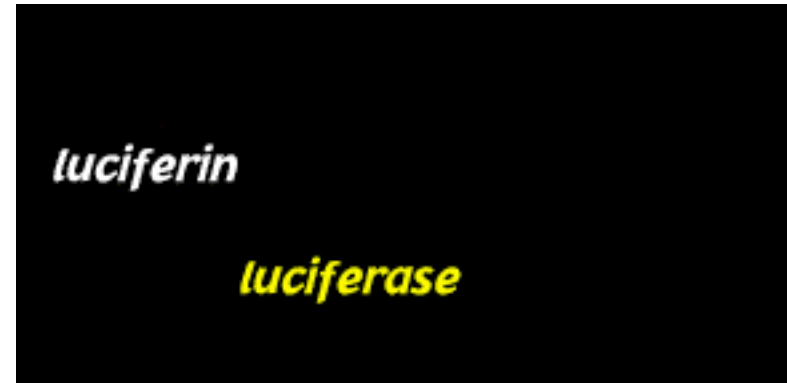
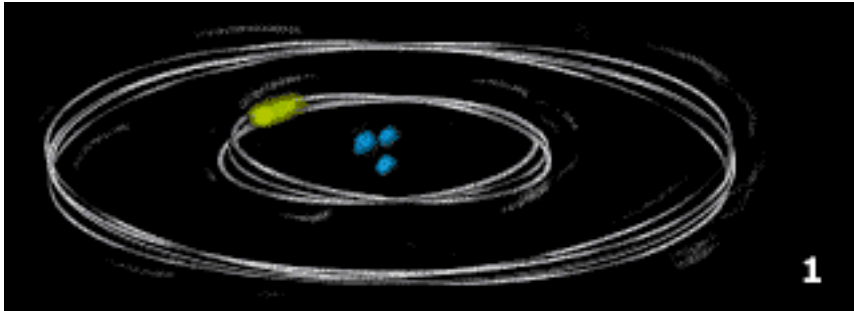
Courtesy of Bonnie Bassler. Used with permission.



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

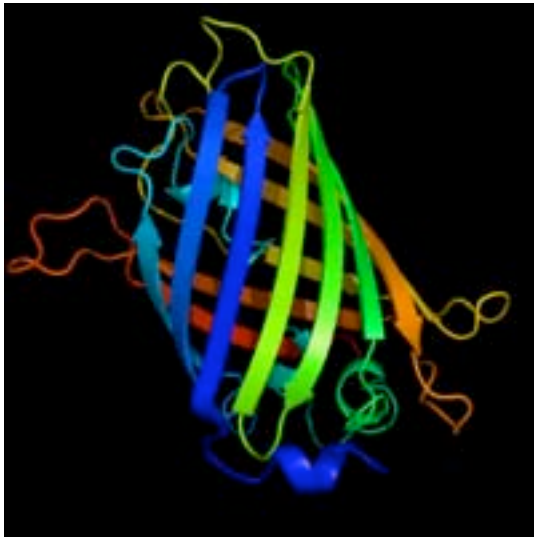
Image removed due to copyright restrictions. See Figure 4 in  
Schauder, S., and B. L. Bassler. "[The Languages of Bacteria.](#)"  
*Genes & Dev* 15 (2001): 1468-1480.

# Fluorescence vs bioluminescence



Courtesy of Steven Haddock. Used with permission. Source: Haddock, S.H.D.; McDougall, C.M.; Case, J.F. "The Bioluminescence Web Page", (created 1997; updated 2007; accessed Fall 2007).

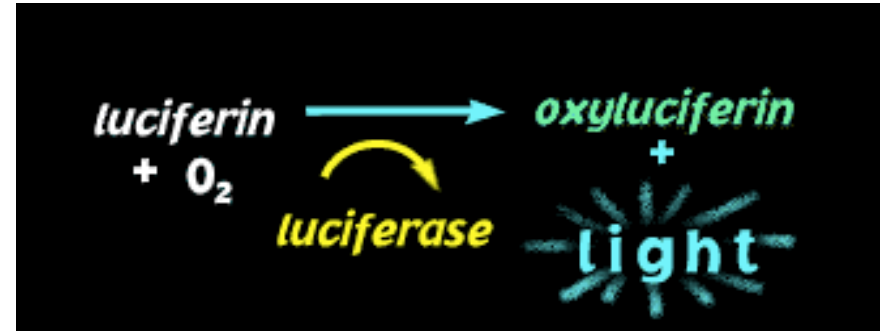
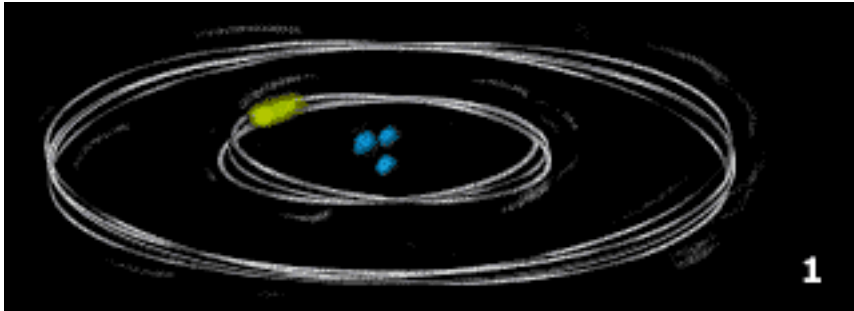
<http://www.lifesci.ucsb.edu/~biolum/chem/>



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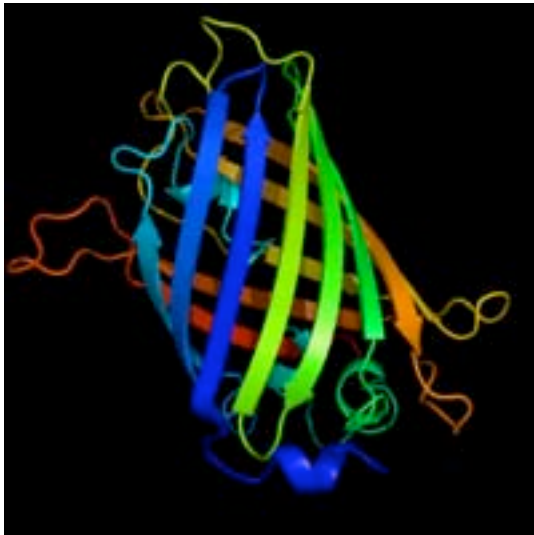
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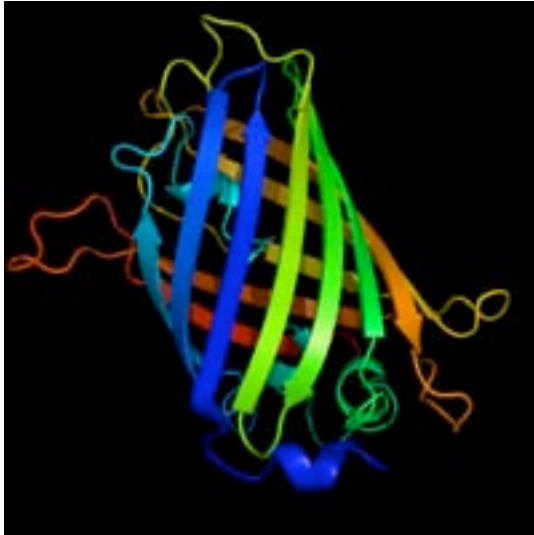
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Image removed due to copyright restrictions. See Figure 4 in Schauder, S., and B. L. Bassler. "The Languages of Bacteria." *Genes & Dev* 15: (2001) 1468-1480

# One last thing about fluorescence



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

Image removed due to copyright restrictions.

Agar plate of fluorescent bacteria colonies, forming  
a beach scene, from lab of Roger Tsien, UCSD.

[http://www.tsienlab.ucsd.edu/HTML/Images/IMAGE  
%20-%20PLATE%20-%20Beach.jpg](http://www.tsienlab.ucsd.edu/HTML/Images/IMAGE%20-%20PLATE%20-%20Beach.jpg)

Image removed due to copyright restrictions.

Figure 1 in Patterson, G., R. N. Day, and D. Piston.  
"Fluorescent Protein Spectra." *Journal of Cell Science*  
114 (2001): 837-838.

High resolution PDF poster available at

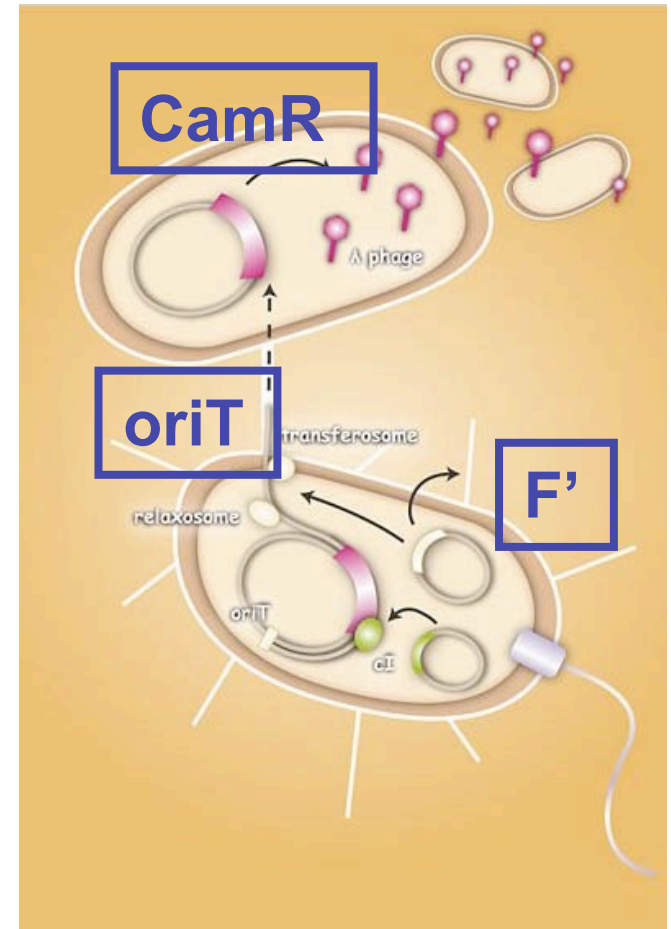
<http://jcs.biologists.org/cgi/data/114/5/837/DC1/1>

# Genetically programmed infection

## iGEM context: phage infection via conjugation

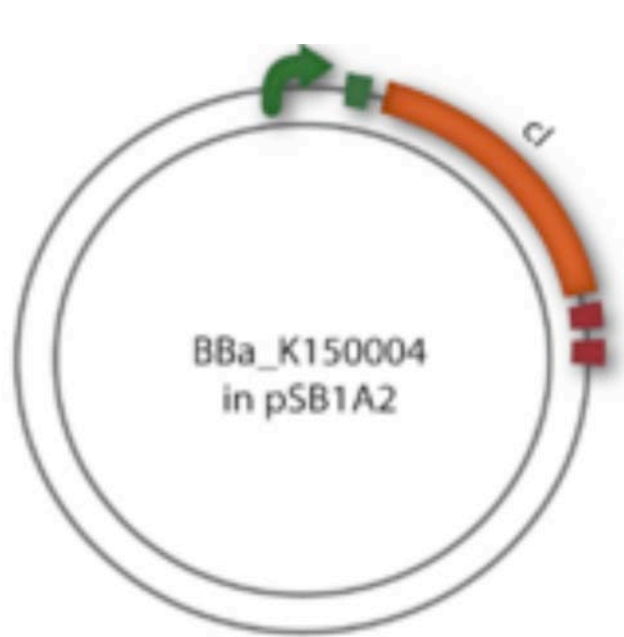
What “parts” are needed

Pilus....	“F”
Origin for transfer...	“oriT”
Selectable marker...	“CamR”
Stable lysogen...	“cI”
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





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Spring 2009

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