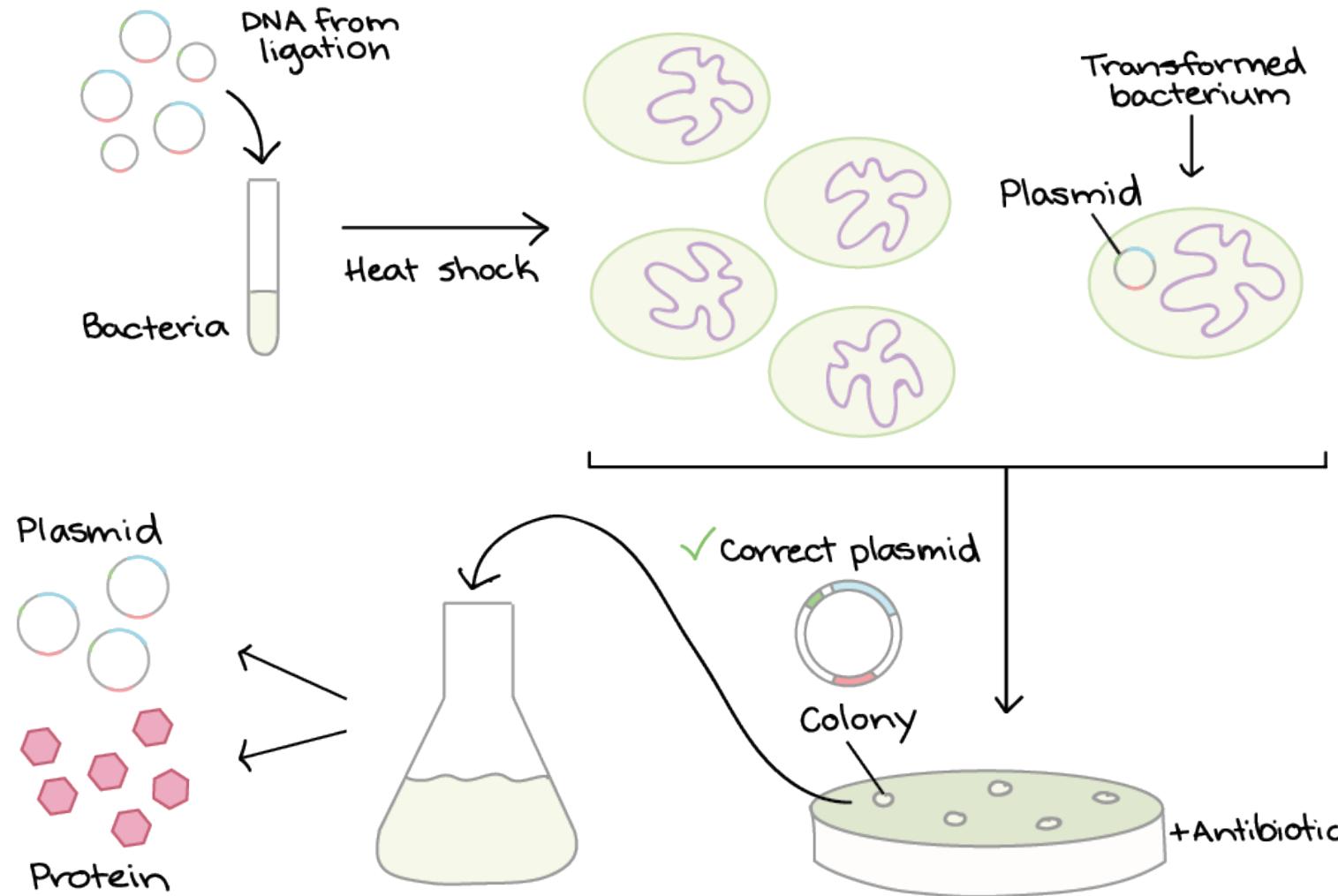


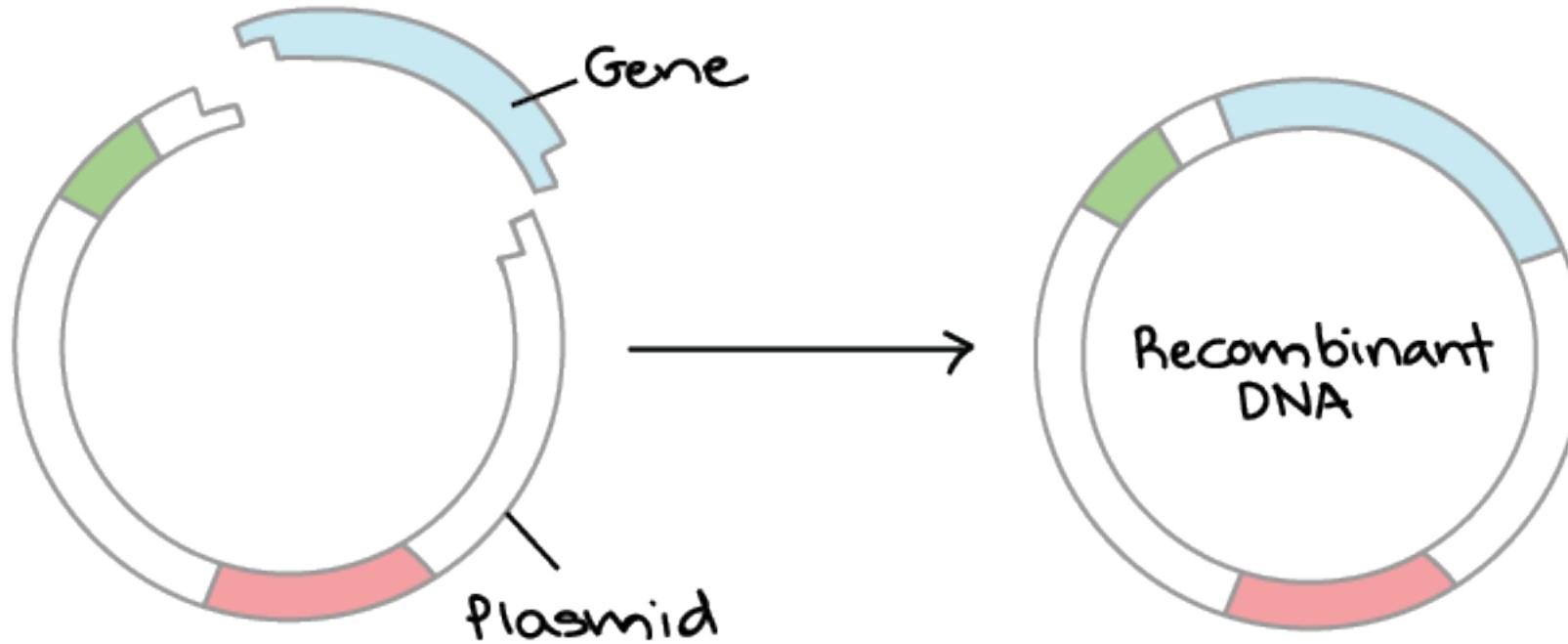
# Bacterial Transformation II

An experiment in genetic engineering

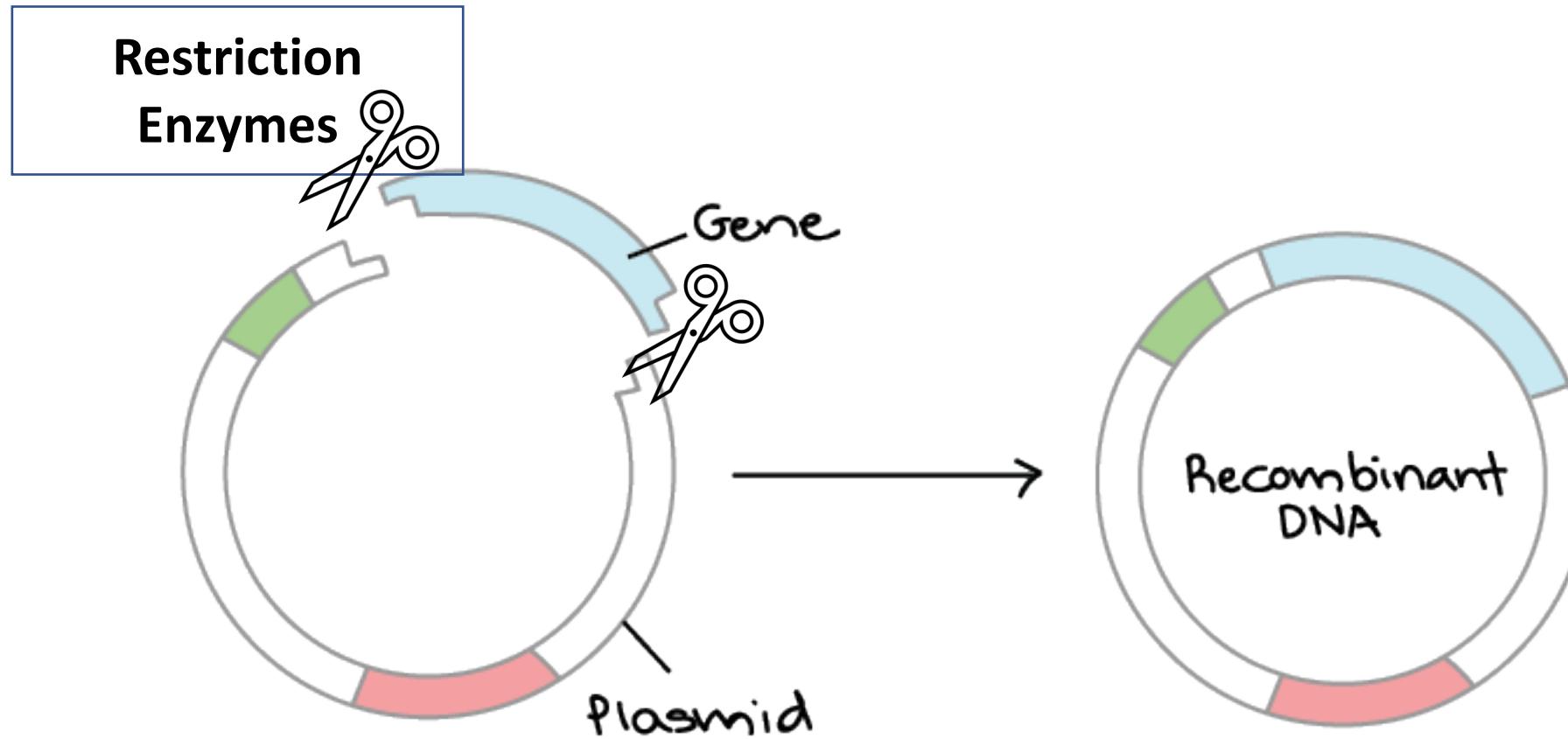
# Bacterial Transformation



# Review – How plasmids are made



# Review – How plasmids are made



# Review – How plasmids are made

Genus, species, strain, number

Eco R I

GAATTC  
CTTAAG

(Origins: *E. coli* RY13)

Hind III

AAGCTT  
TTCGAA

(Origins: *H. influenzae* Rd)

# Review – How plasmids are made

*Eco*RI cuts at **GAATTC**

TCGAT**GAATTCTAAGCTTAGTGAATTCTGTGCA**  
AGCTACTTAAGATTCGAATCACTTAAGACACGT

**AATTCTGTGCA**

**AATTCTAAGCTTAGTG**

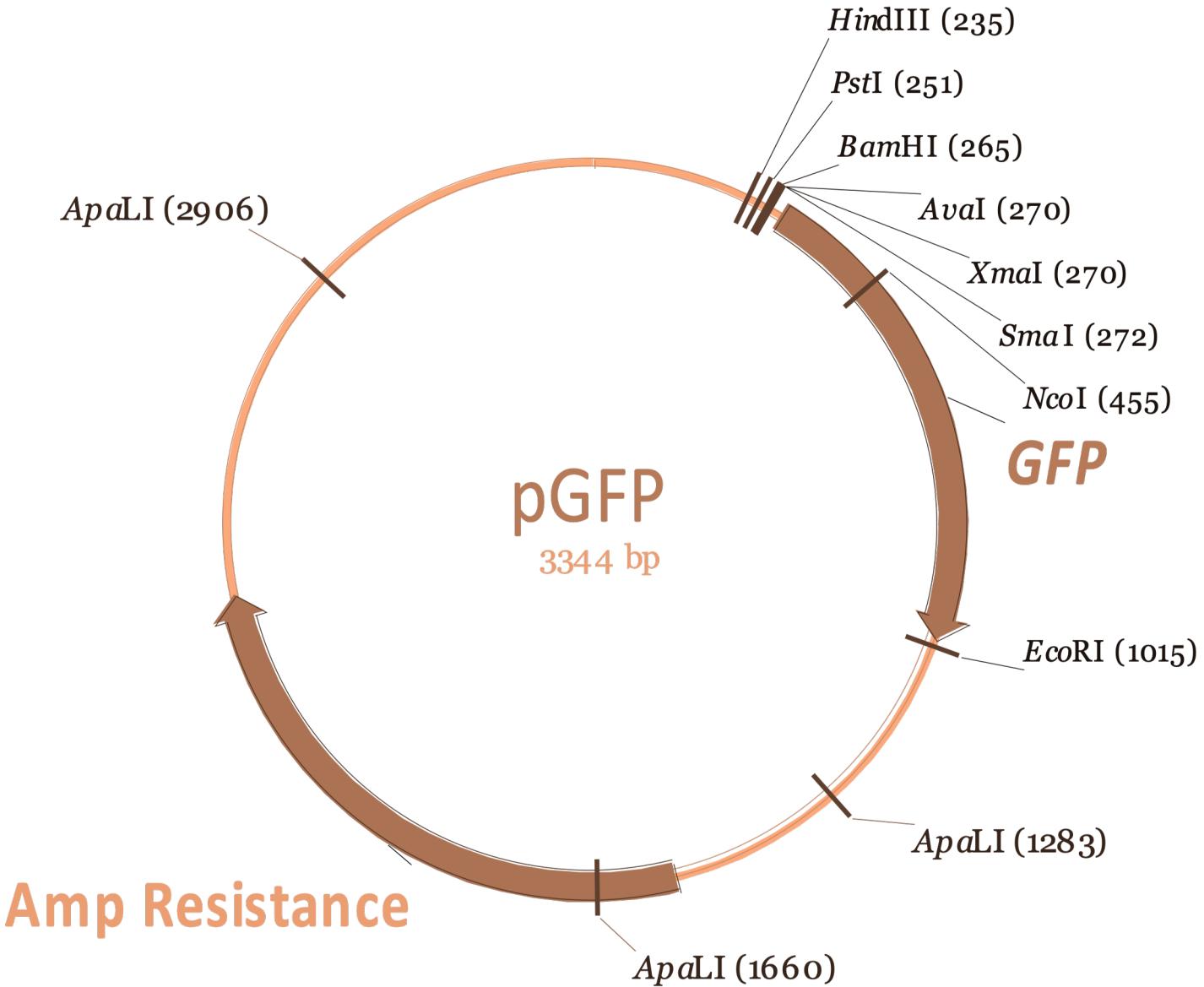
**GACACGT**

TCGAT**G**

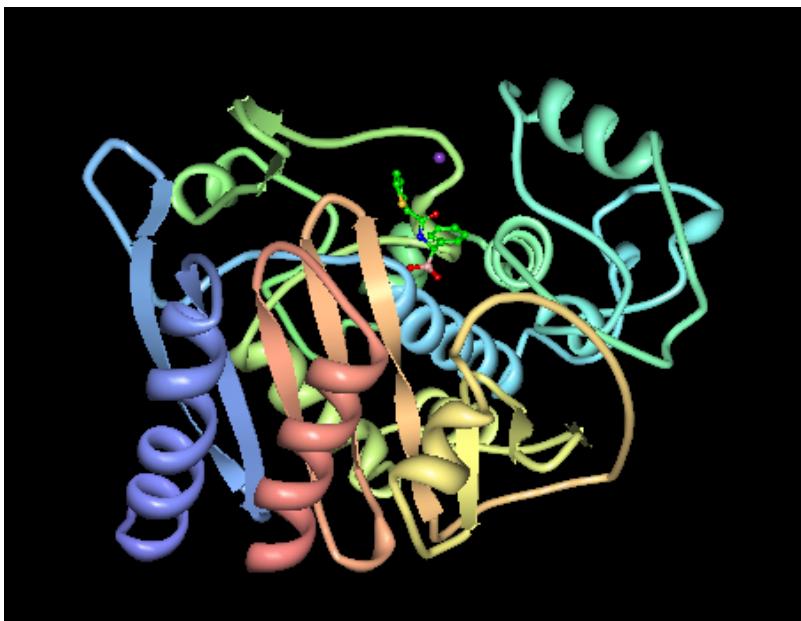
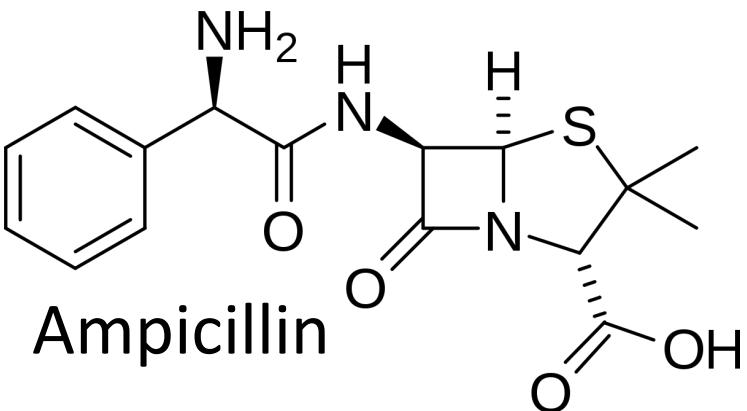
**GATTCGAATCACTTAA**

AGCTACTTAA

# pGFP plasmid



# pGFP plasmid

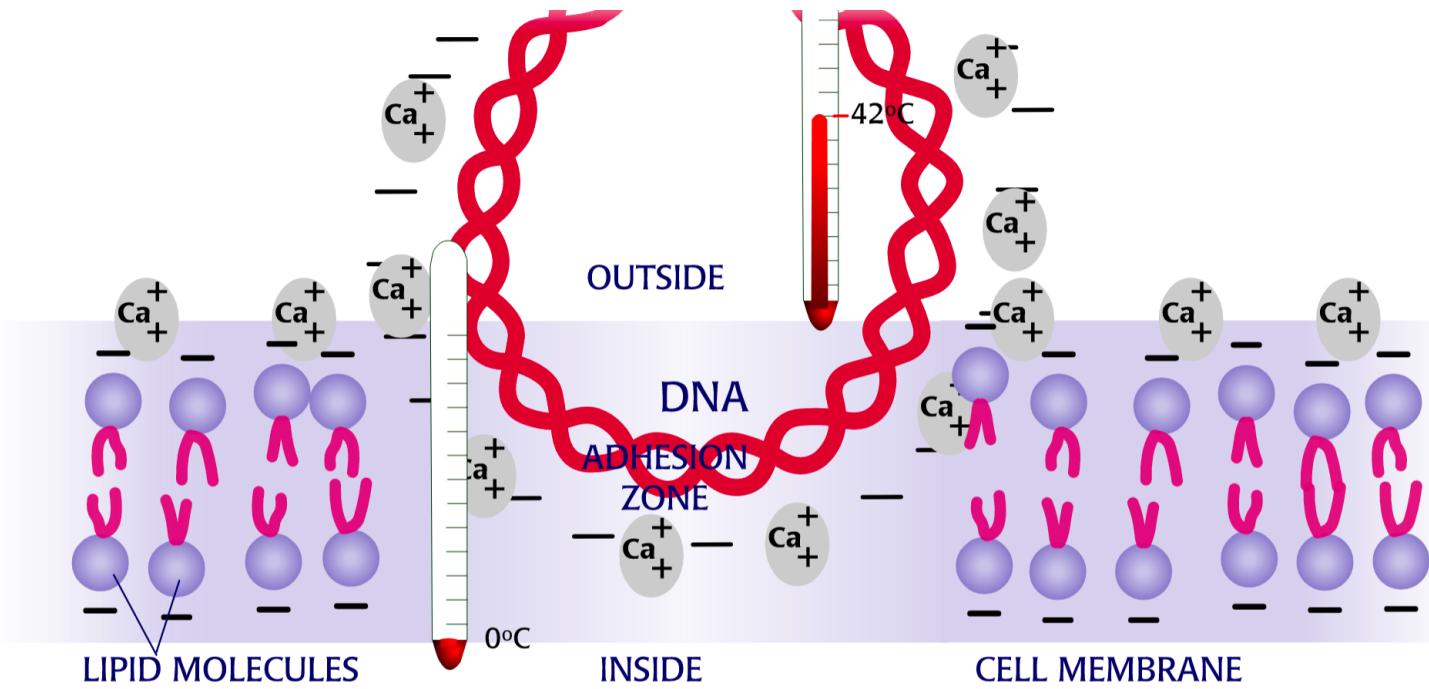


β-lactamase *bla* (TEM-1)



Transpeptidase

# During heat shock – (some) bacteria take up plasmid



# Results

# Controls

## Experiment:

At what temperature does  
ice ( $\text{H}_2\text{O}$ ) + Chemical “X”  
melt?

# Controls

**Positive control:** What does the effect look like if present?

# Controls

**Positive control:** What does the effect look like if present?

**Negative control:** What does the effect look like if absent?

# Controls

**Positive control:** What does the effect look like if present?

**Negative control:** What does the effect look like if absent?

**Sensitivity control:** Across what range of values can I measure the effect?

# Controls



**Positive control**



**Negative control**



**Sensitivity control**

**Photo credits**

[https://commons.wikimedia.org/wiki/File:Water\\_in\\_a\\_beaker.JPG](https://commons.wikimedia.org/wiki/File:Water_in_a_beaker.JPG)  
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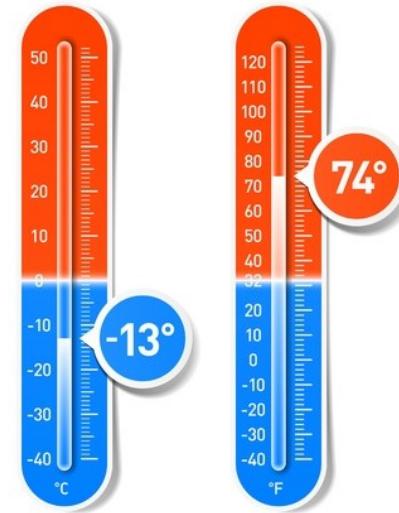
# Controls



**Positive control**



**Negative control**



**Sensitivity control**

**Photo credits**

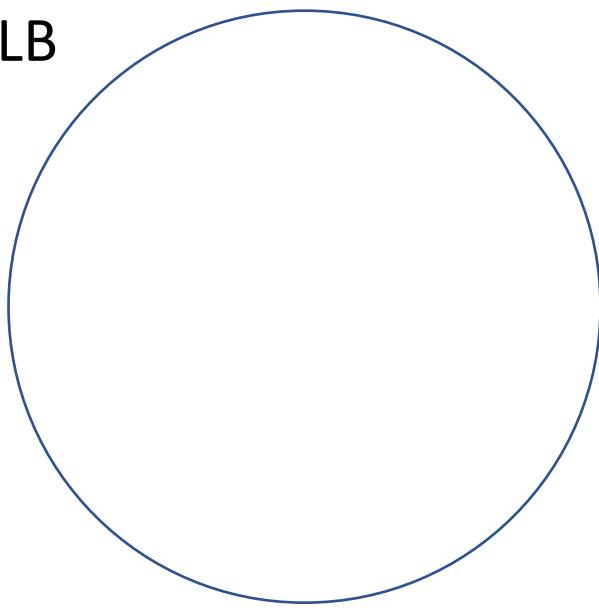
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<http://www.chem.uiuc.edu/webfunchem/temperature/Temp10.htm>  
<https://en.clipdealer.com/vector/media/A:17494508?>

# Results

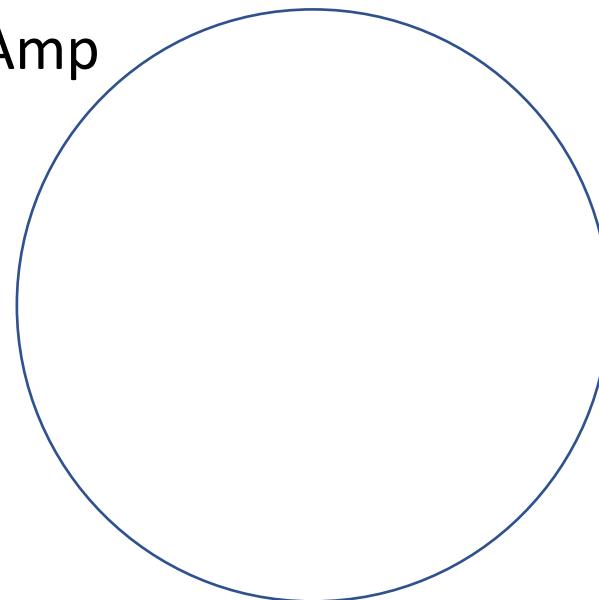
# Plating Diagram

(+) Bacteria

1 LB

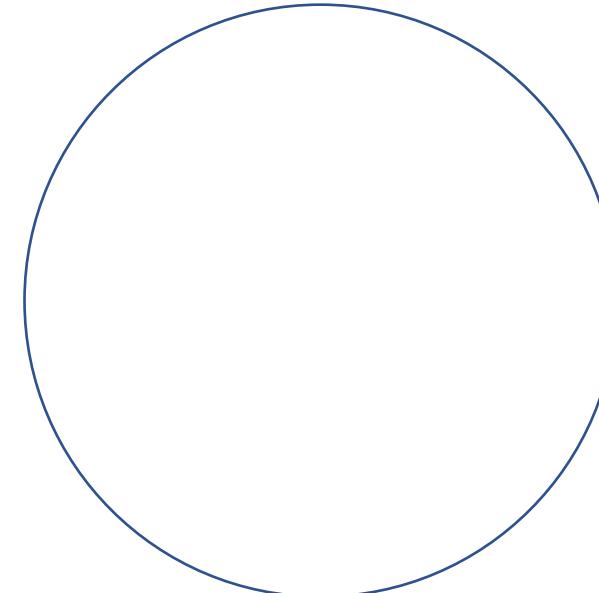


4 Amp

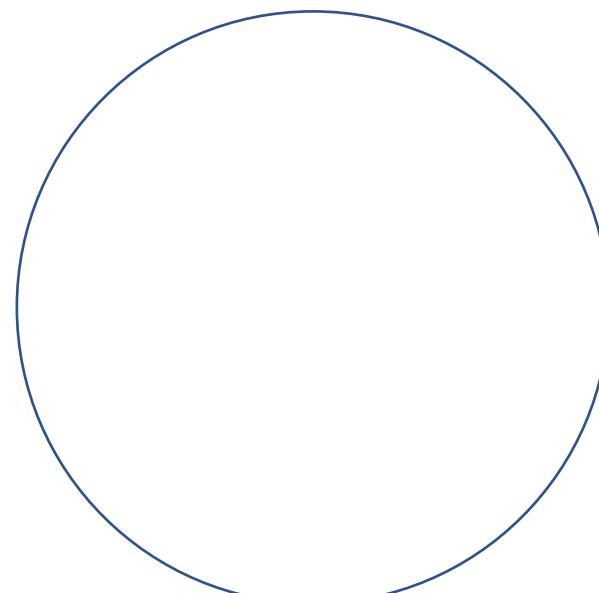


( - ) Bacteria

2



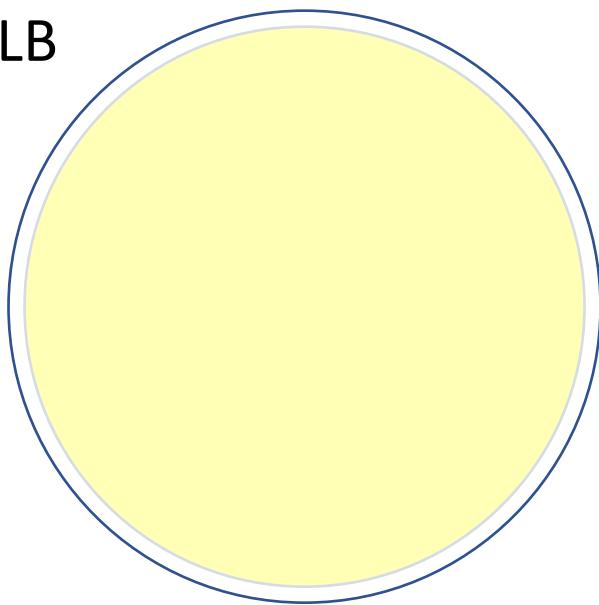
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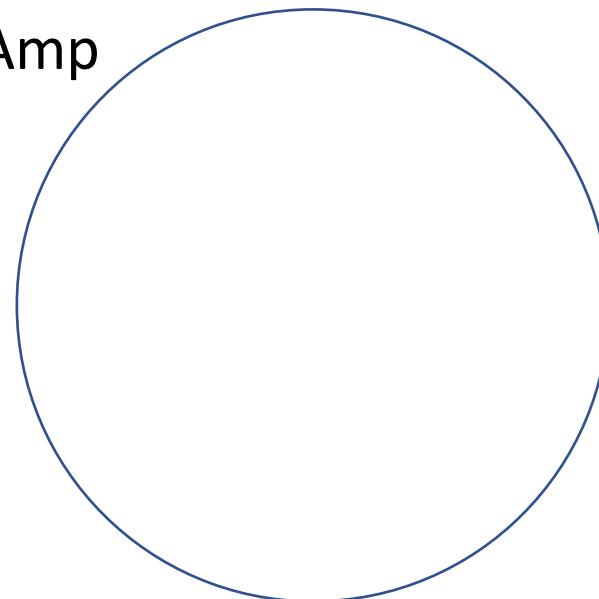
# Plating Diagram

(+) Bacteria

1 LB

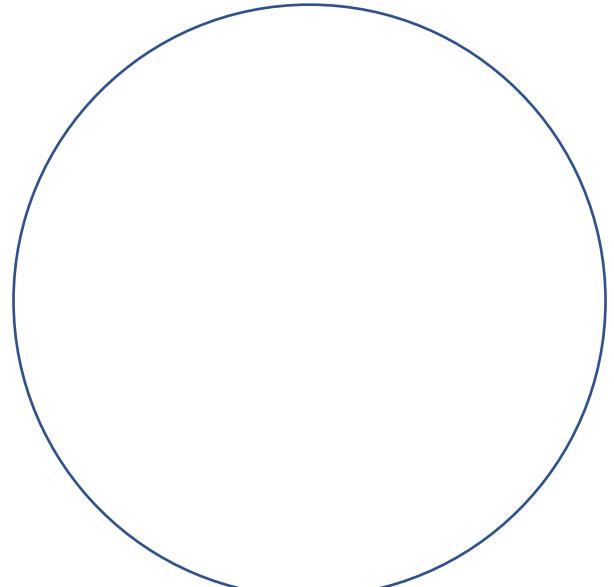


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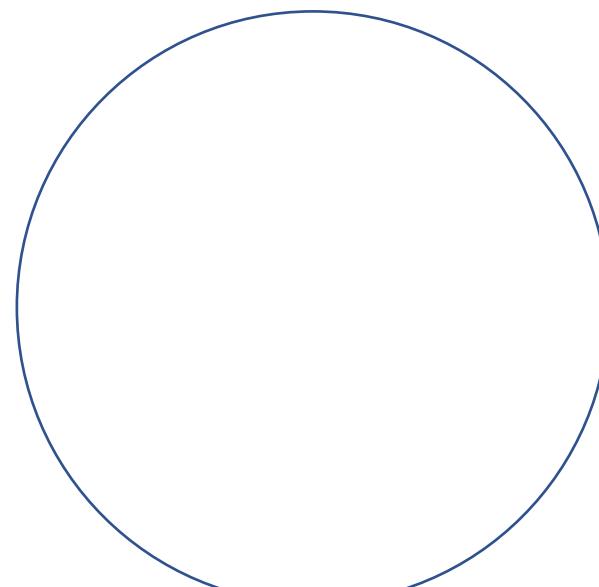


( - ) Bacteria

2



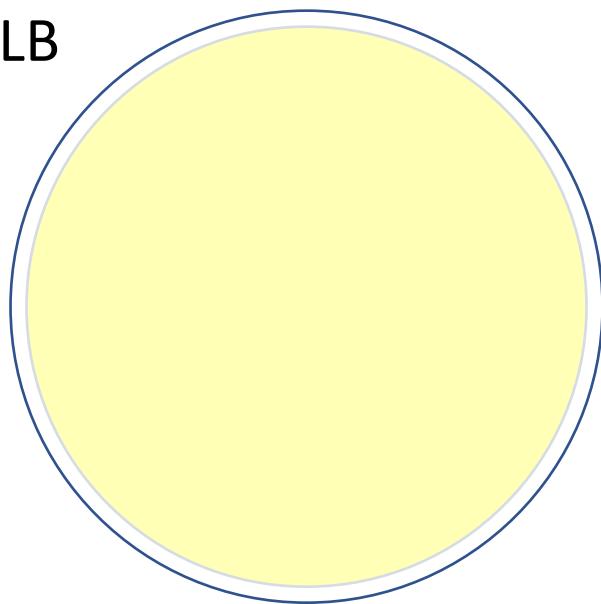
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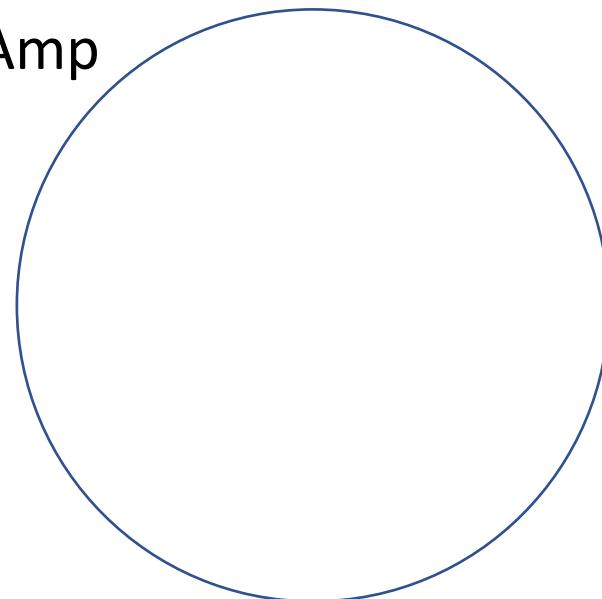
# Plating Diagram

(+) Bacteria

1 LB

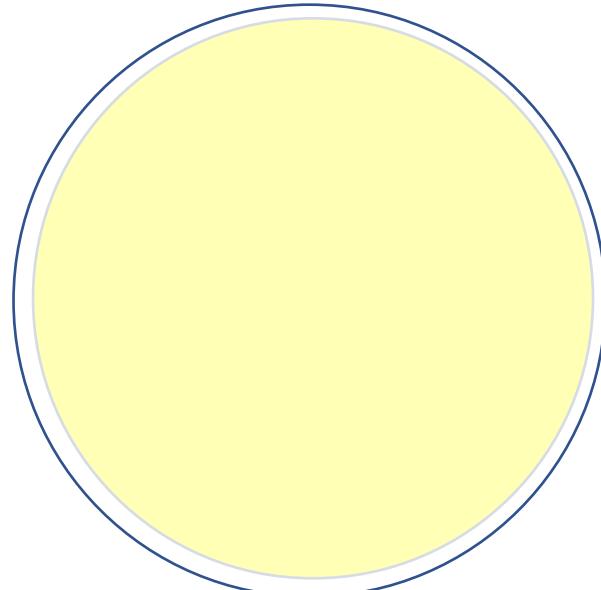


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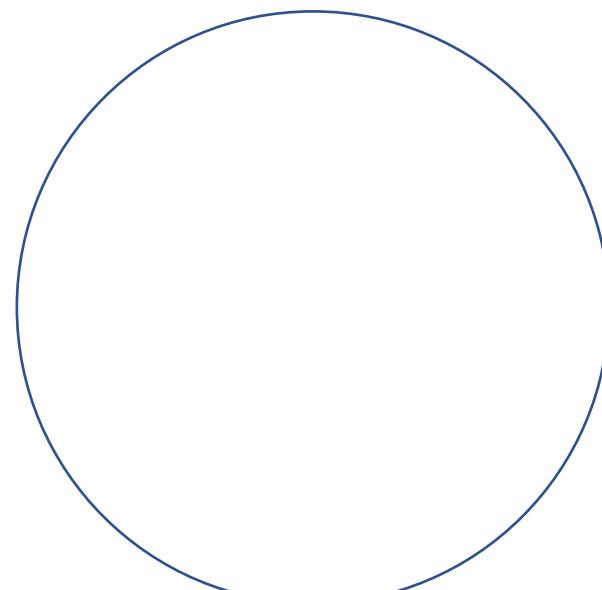


( - ) Bacteria

2



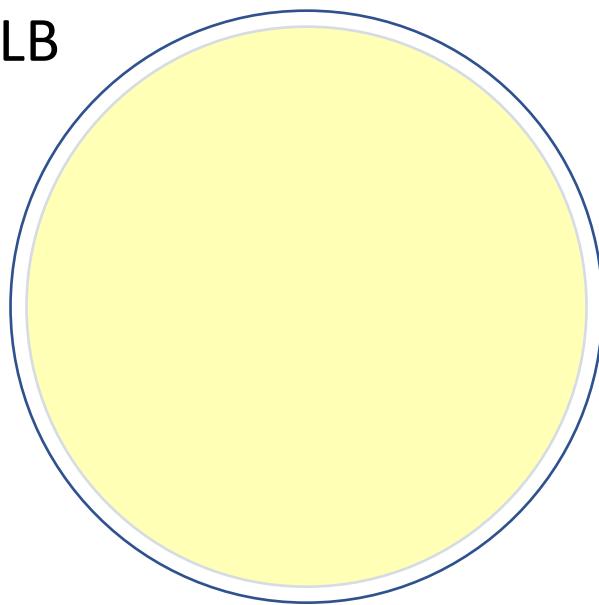
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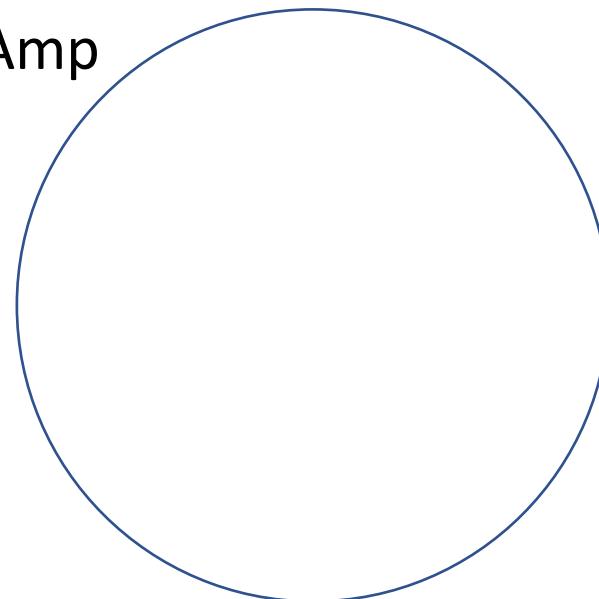
# Plating Diagram

(+) Bacteria

1 LB

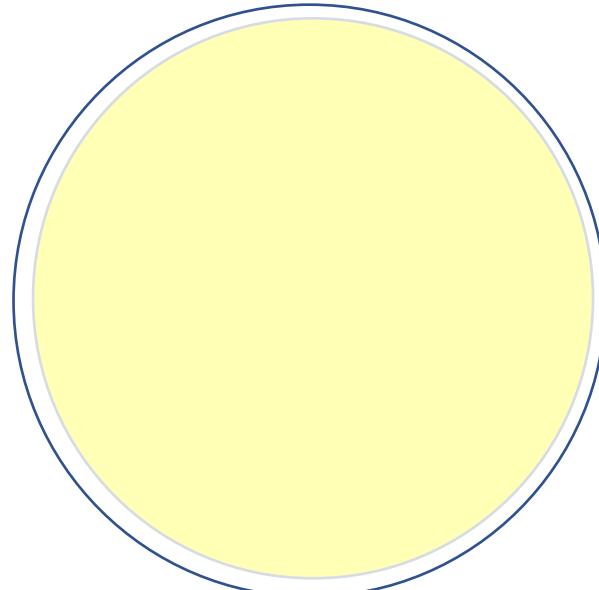


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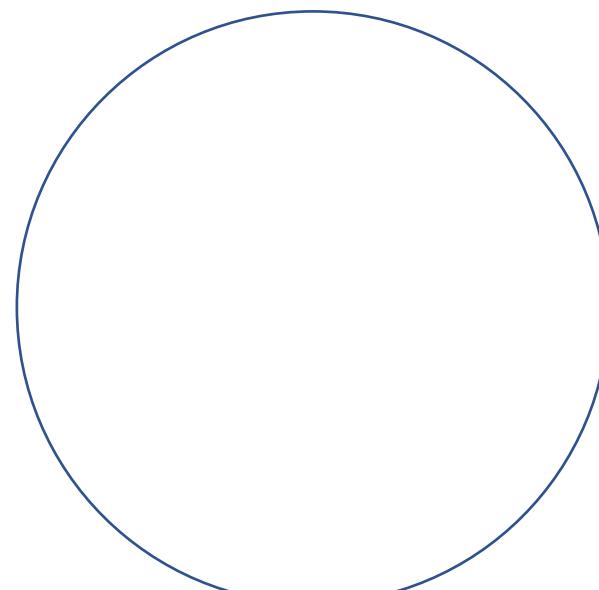


( - ) Bacteria

2



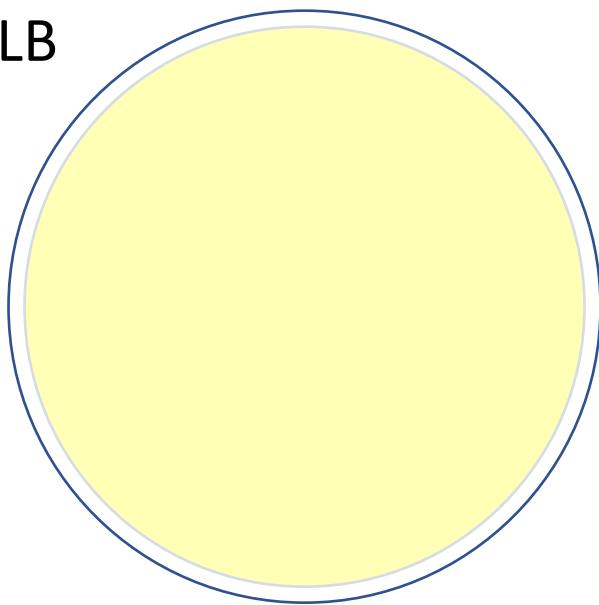
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# Plating Diagram

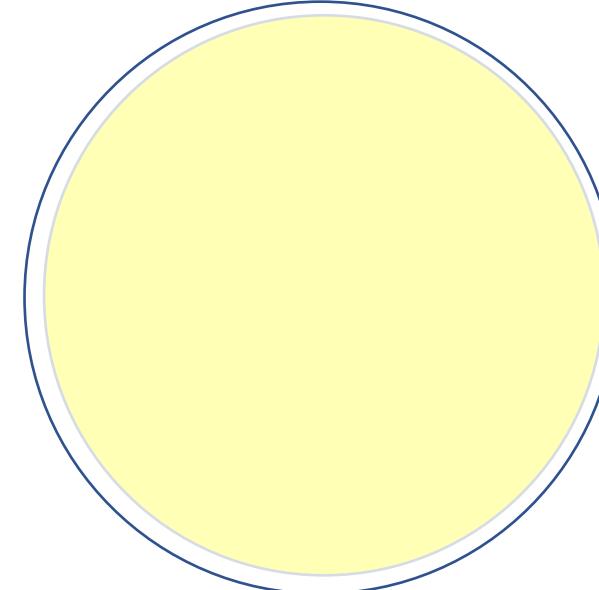
(+) Bacteria

1 LB

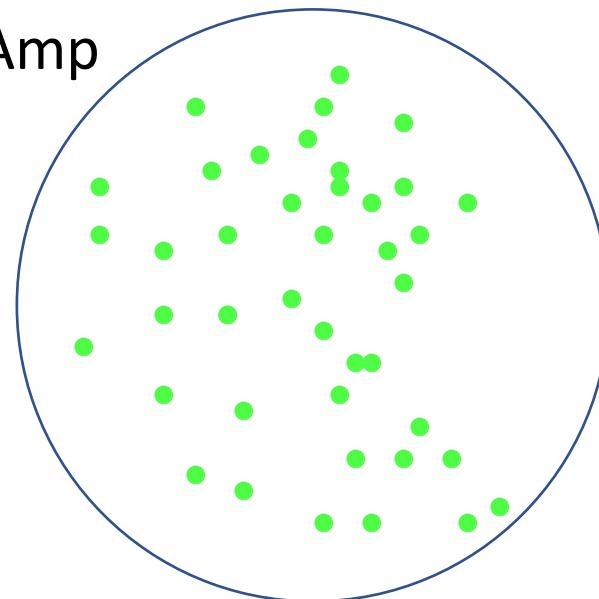


( - ) Bacteria

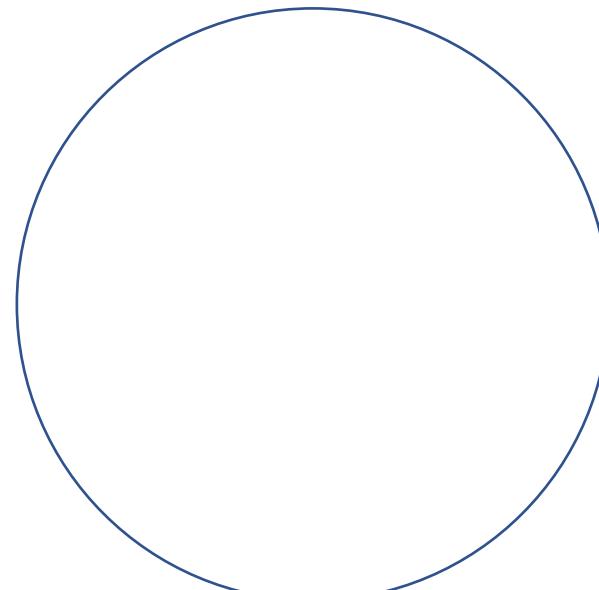
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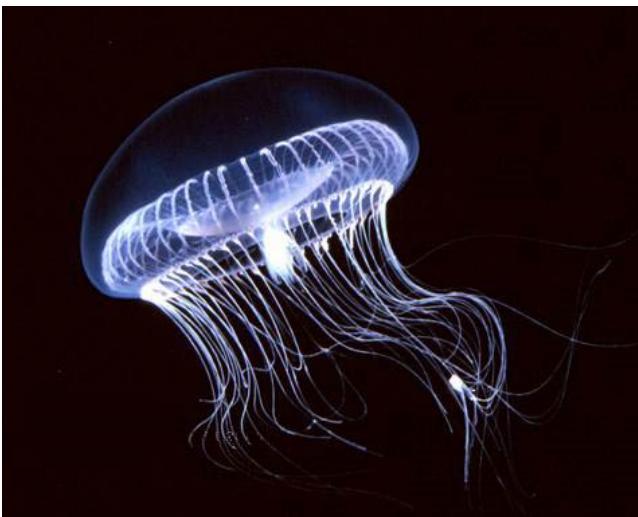
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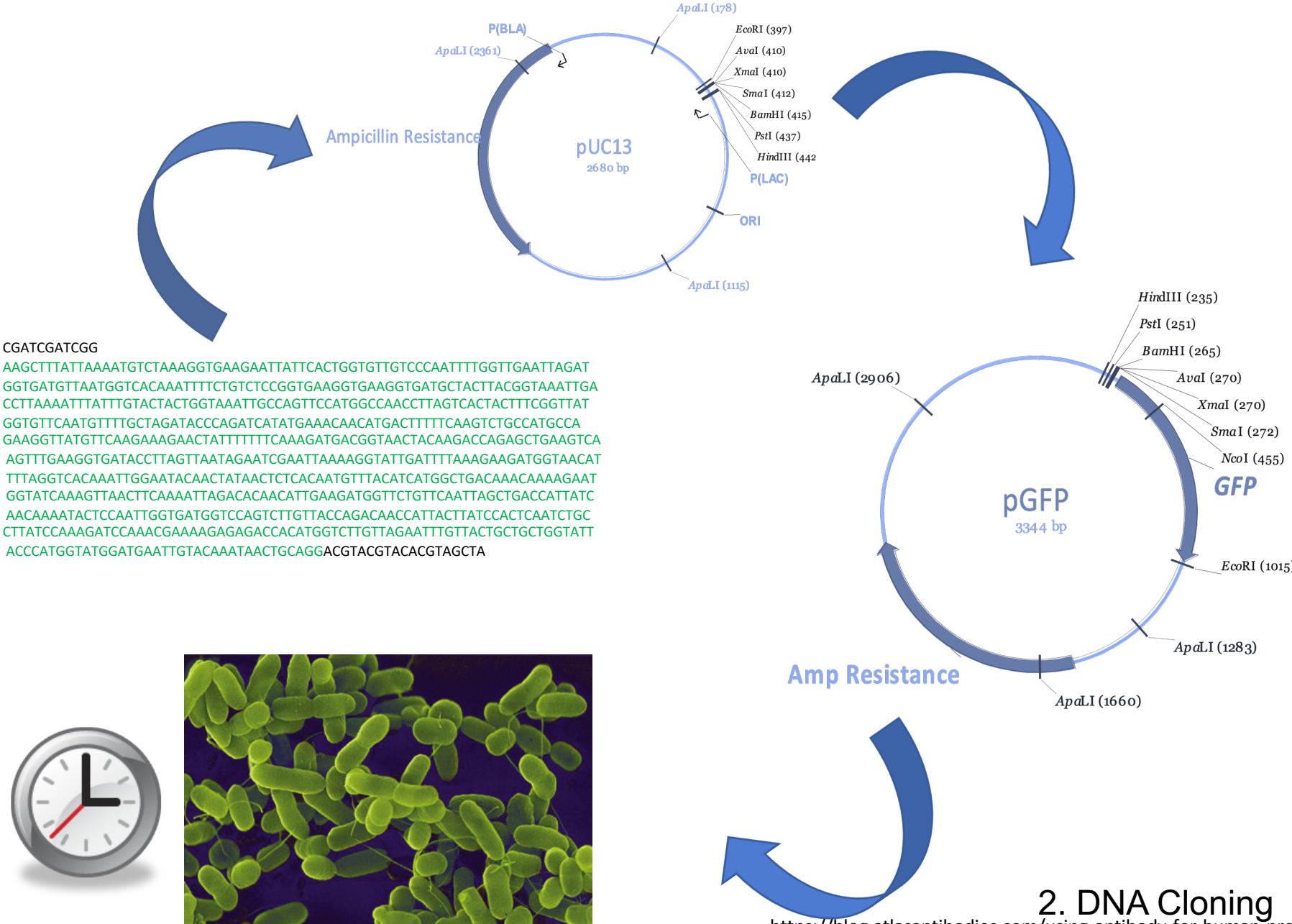
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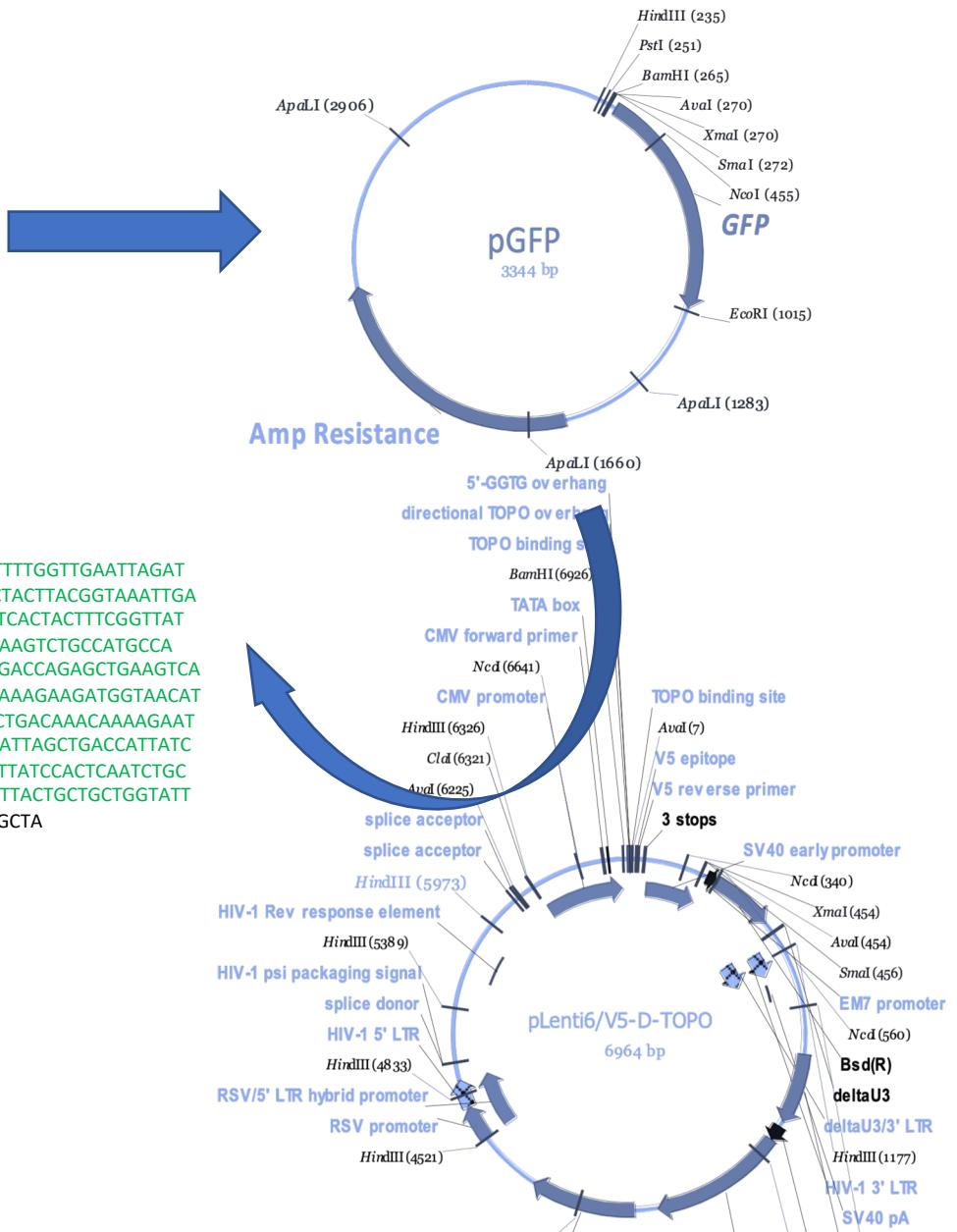
# Uses of GFP



# Uses of GFP



# Uses of GFP



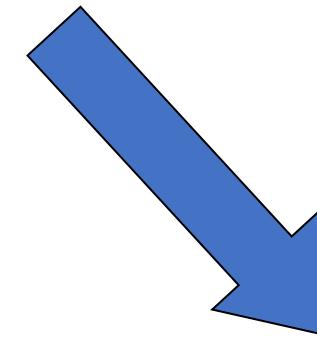
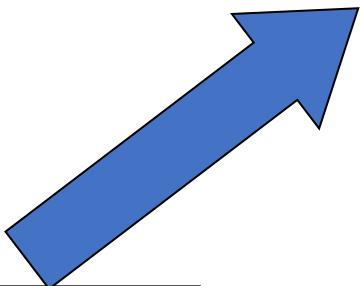
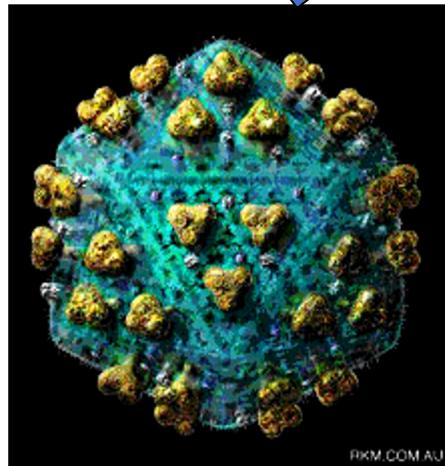
CGATCGATCGG

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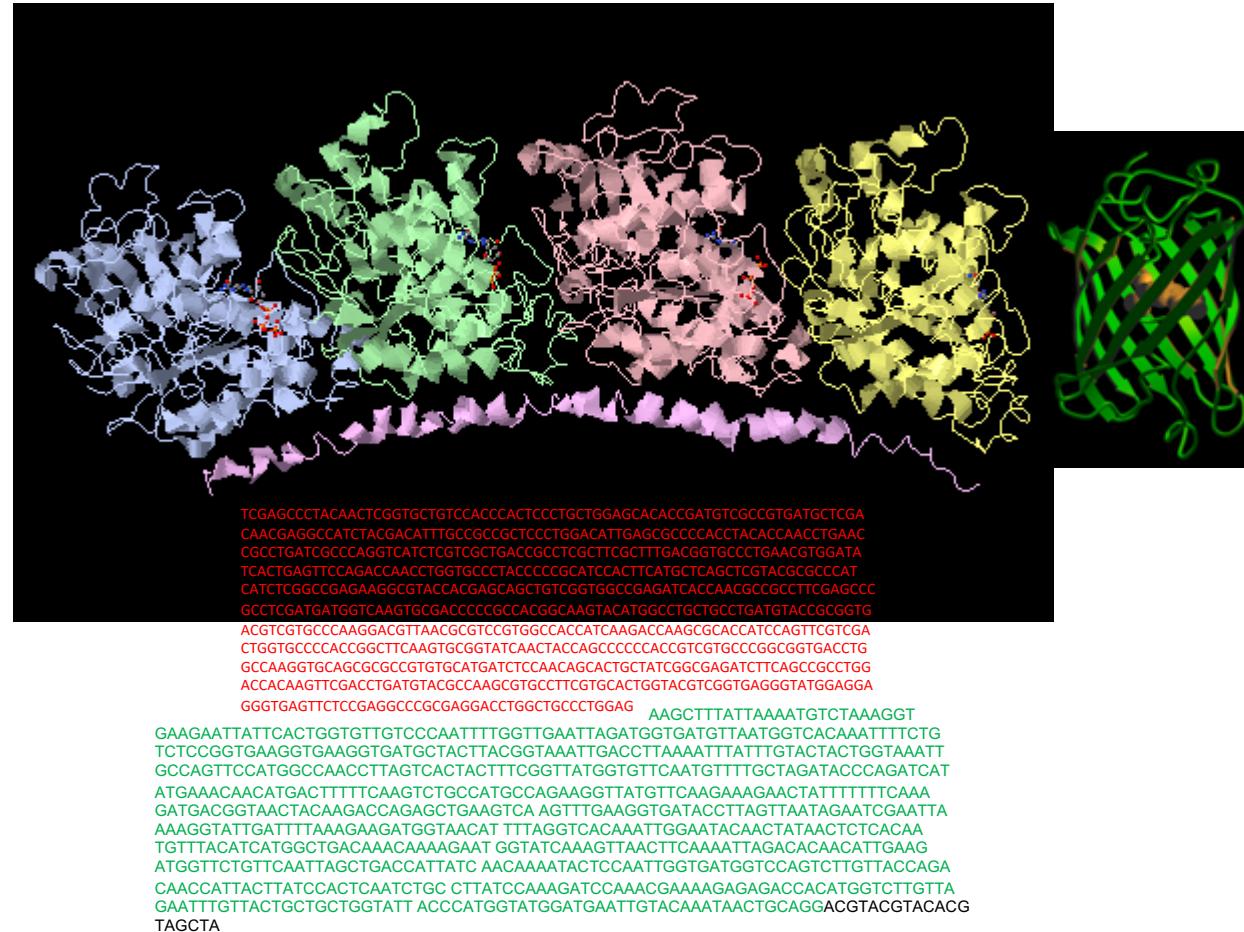
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# Uses of GFP



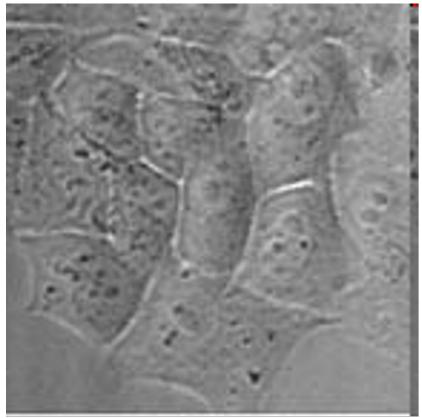
# Fusion Protein



$\alpha$ -Tubulin

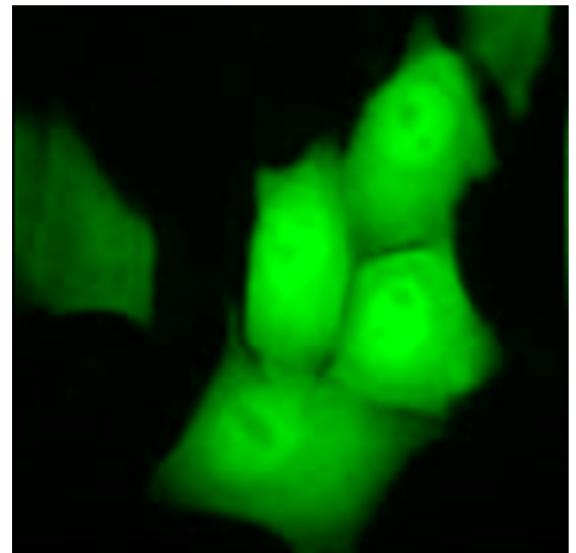
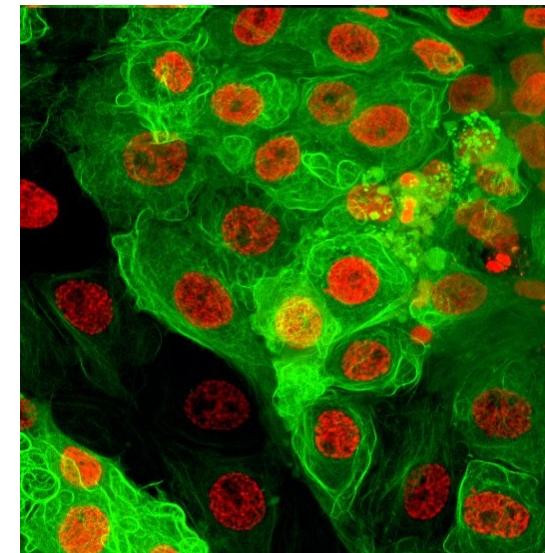
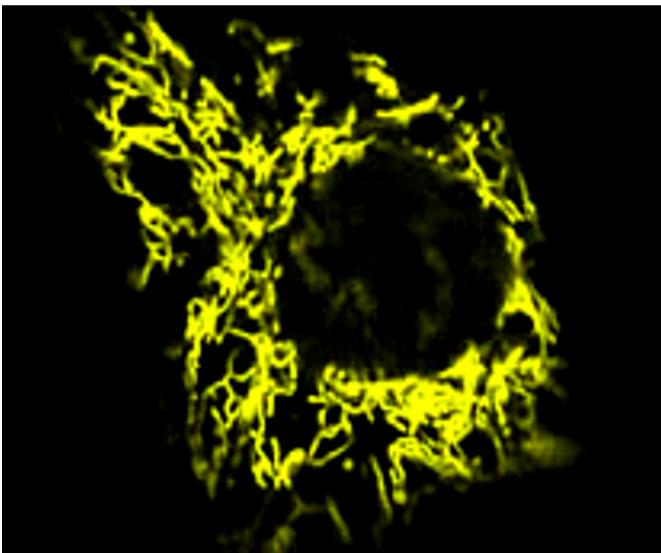
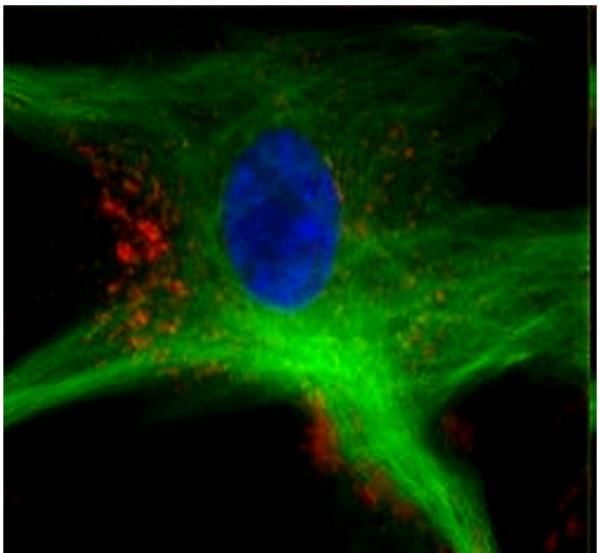


Normal view of cells – light microscopy

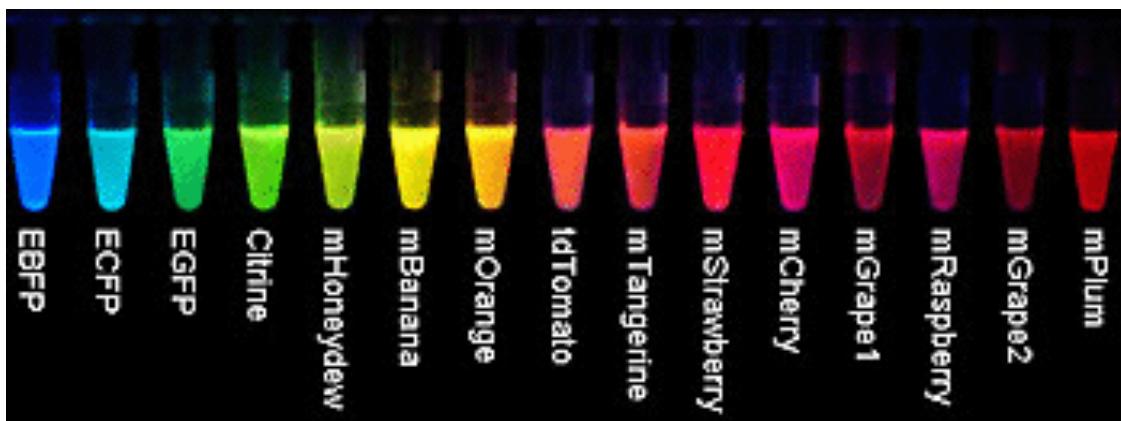
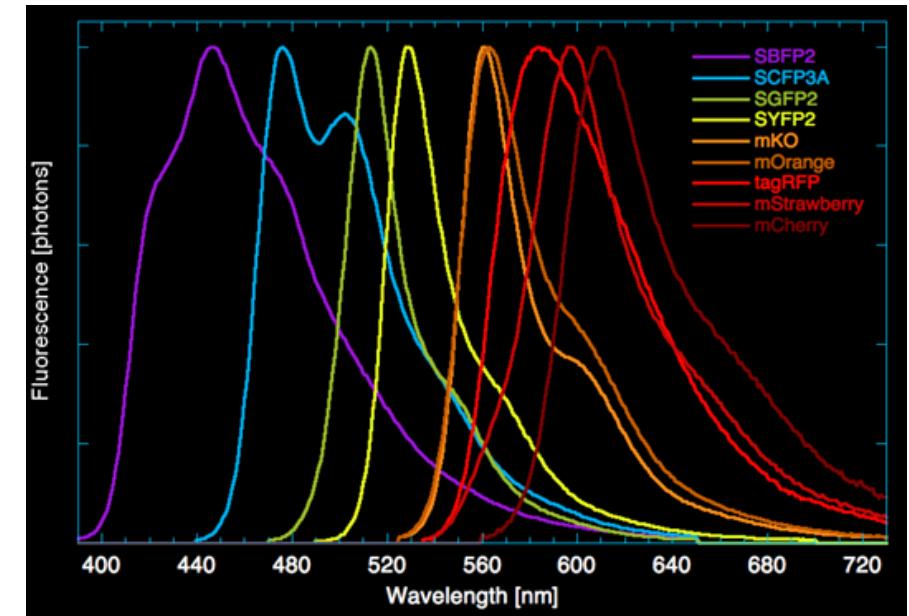
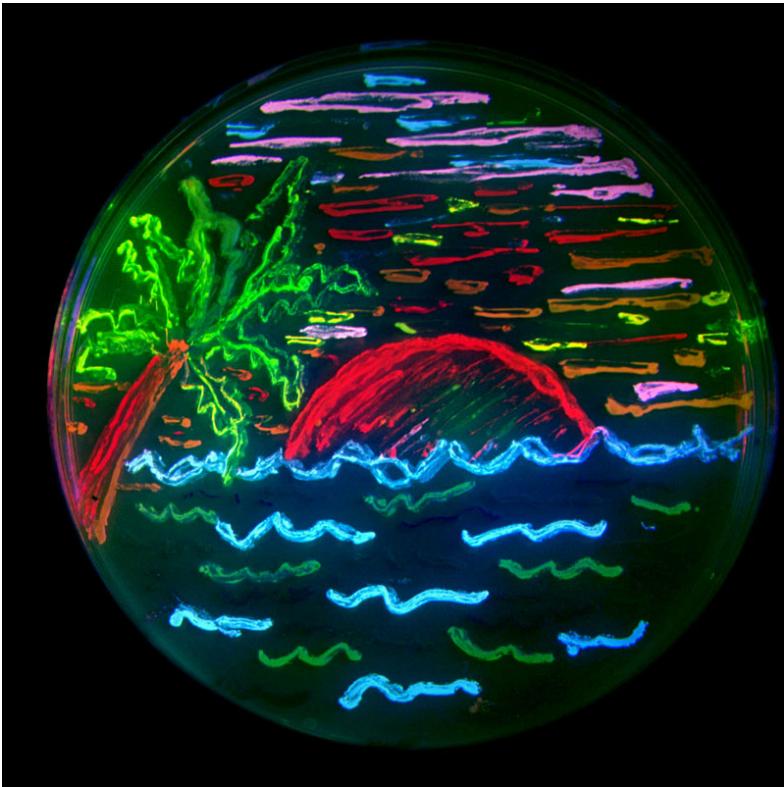


# Revealing cell dynamics

Cells tagged with fluorescent proteins – fluorescence microscopy



# Revealing cell dynamics

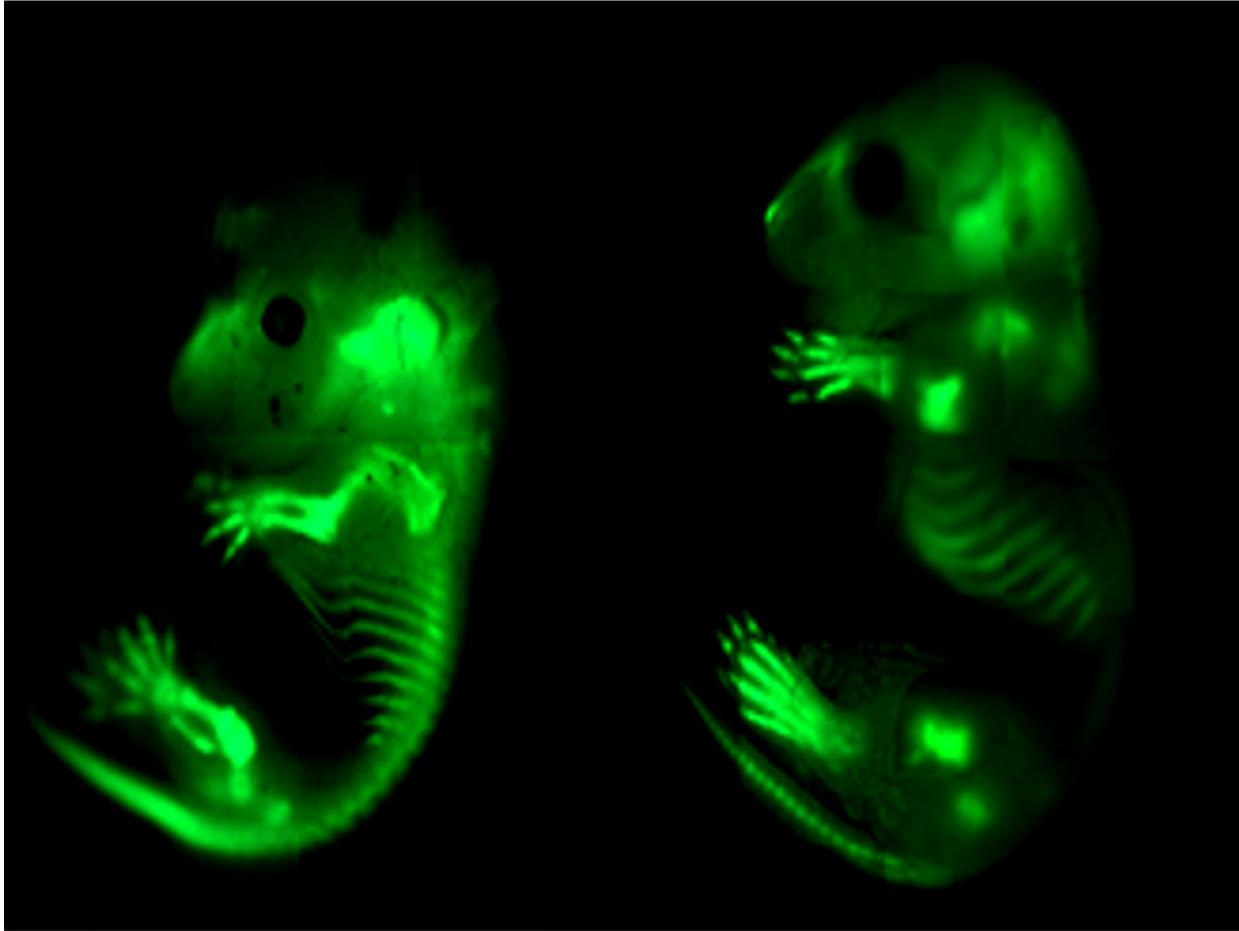


Shimomura      Chalfie      Tsien



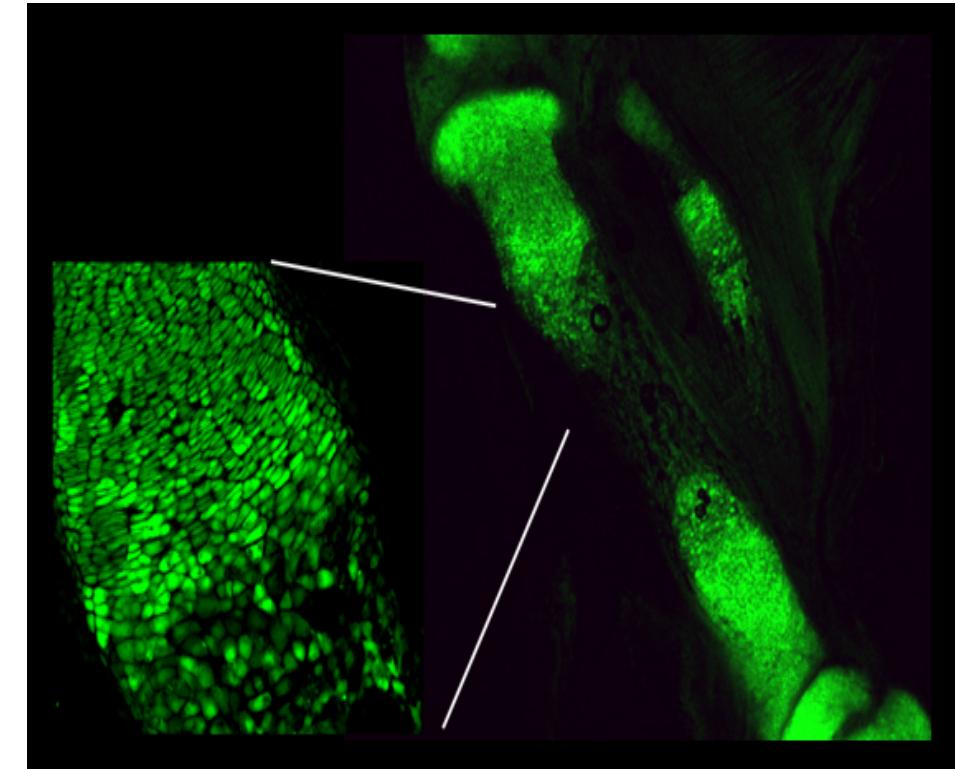
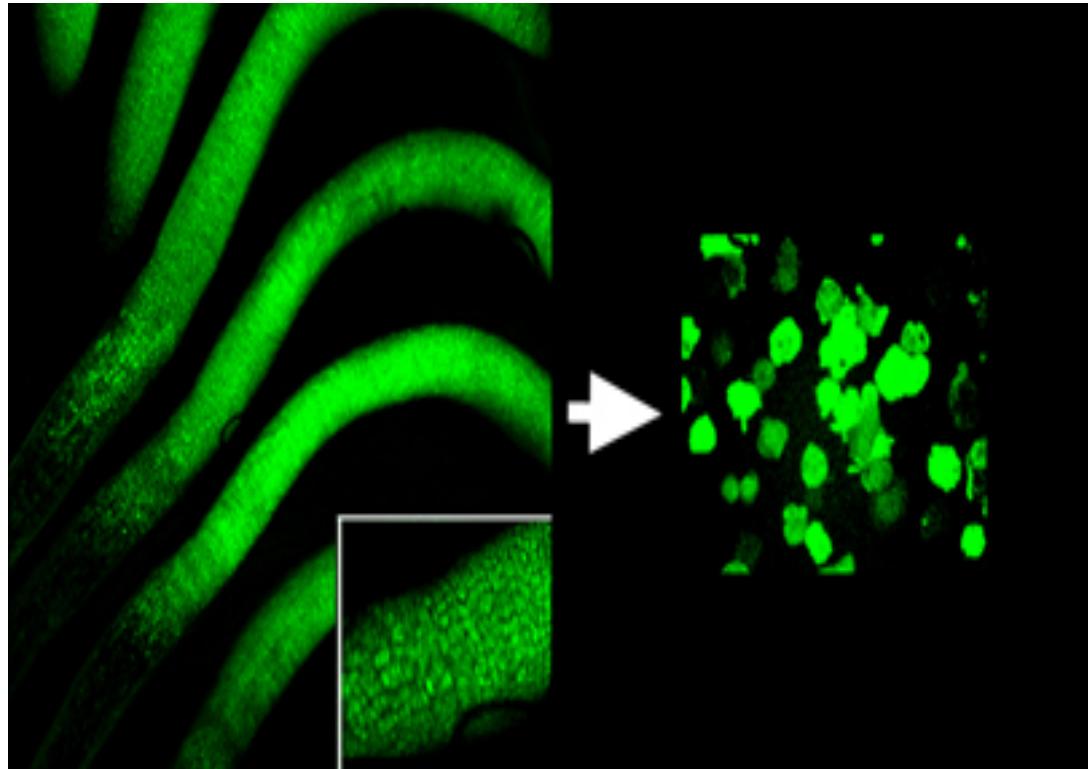
The Nobel Prize in Chemistry 2008

# Revealing cell dynamics



*Col2-GFP*

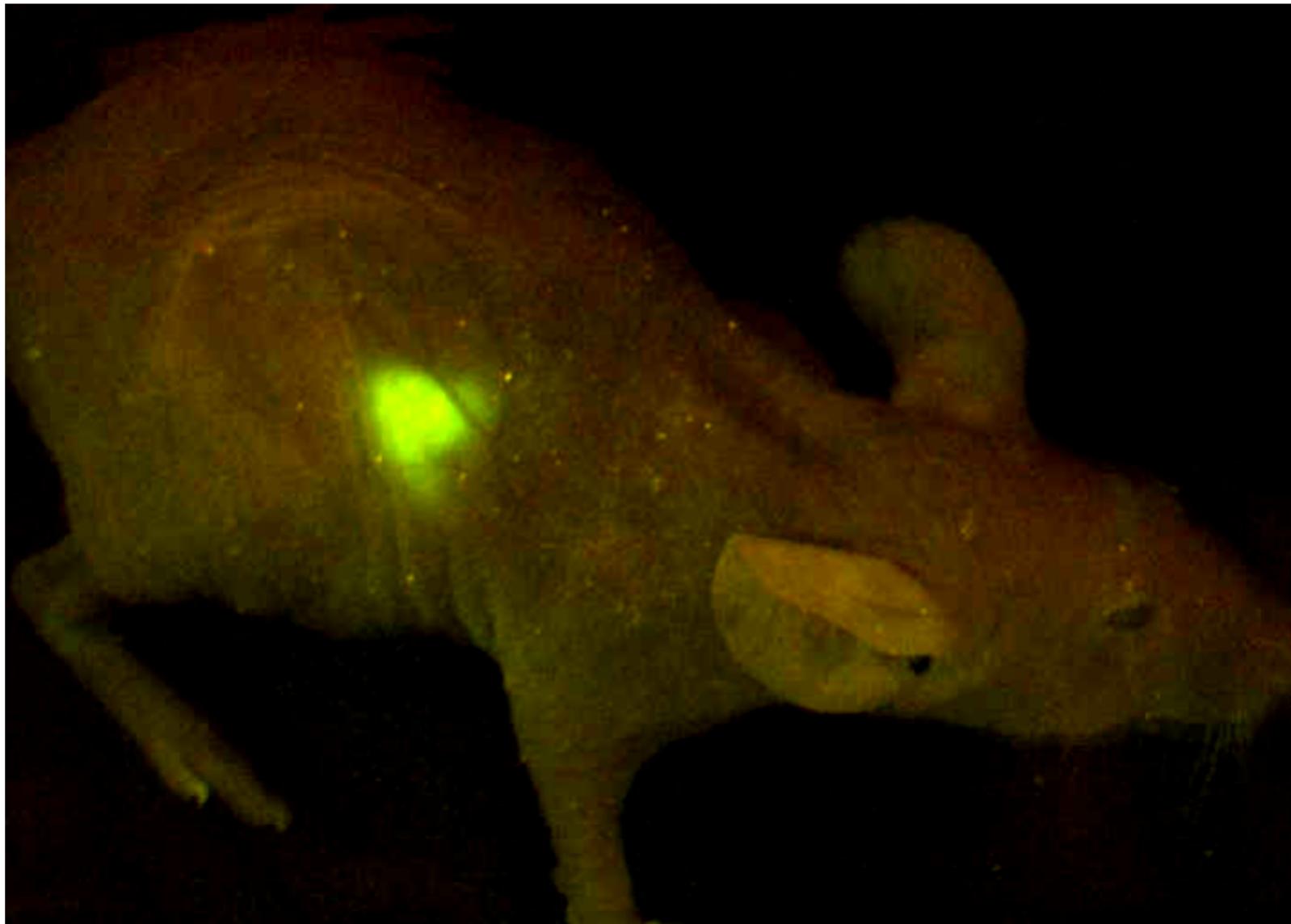
# Revealing cell dynamics



As cartilage is converted  
to bone, cells go dim

*Col2-GFP*

# Revealing cell dynamics



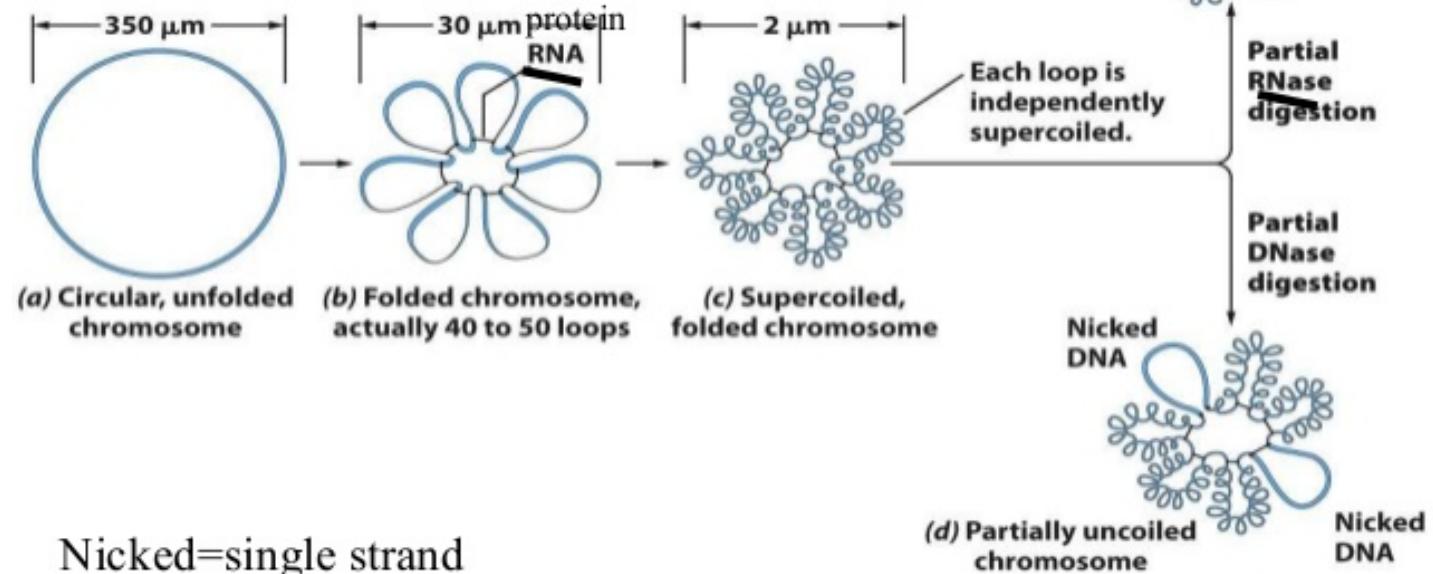
Through manipulation and study of model organisms  
we work to understand life

# Manipulation at the genetic level



## Model of *E. coli* Chromosome

folded=coiled



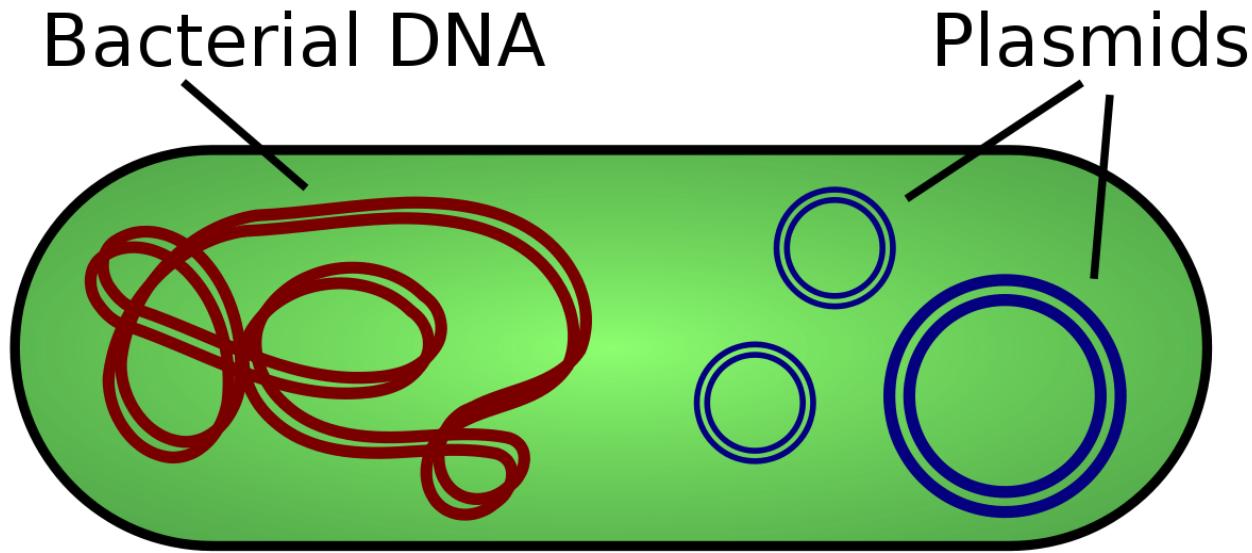
Nicked=single strand

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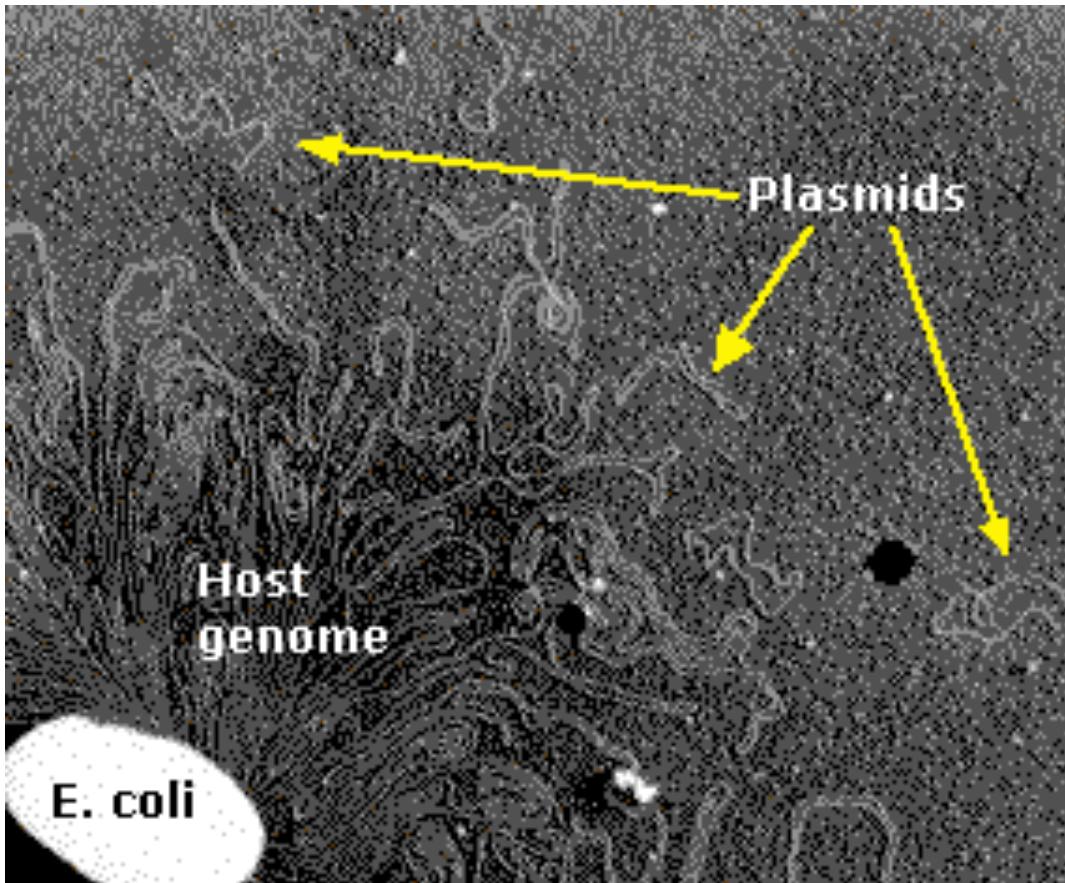
Image Credit

DR GOPAL MURTI / SCIENCE PHOTO LIBRARY

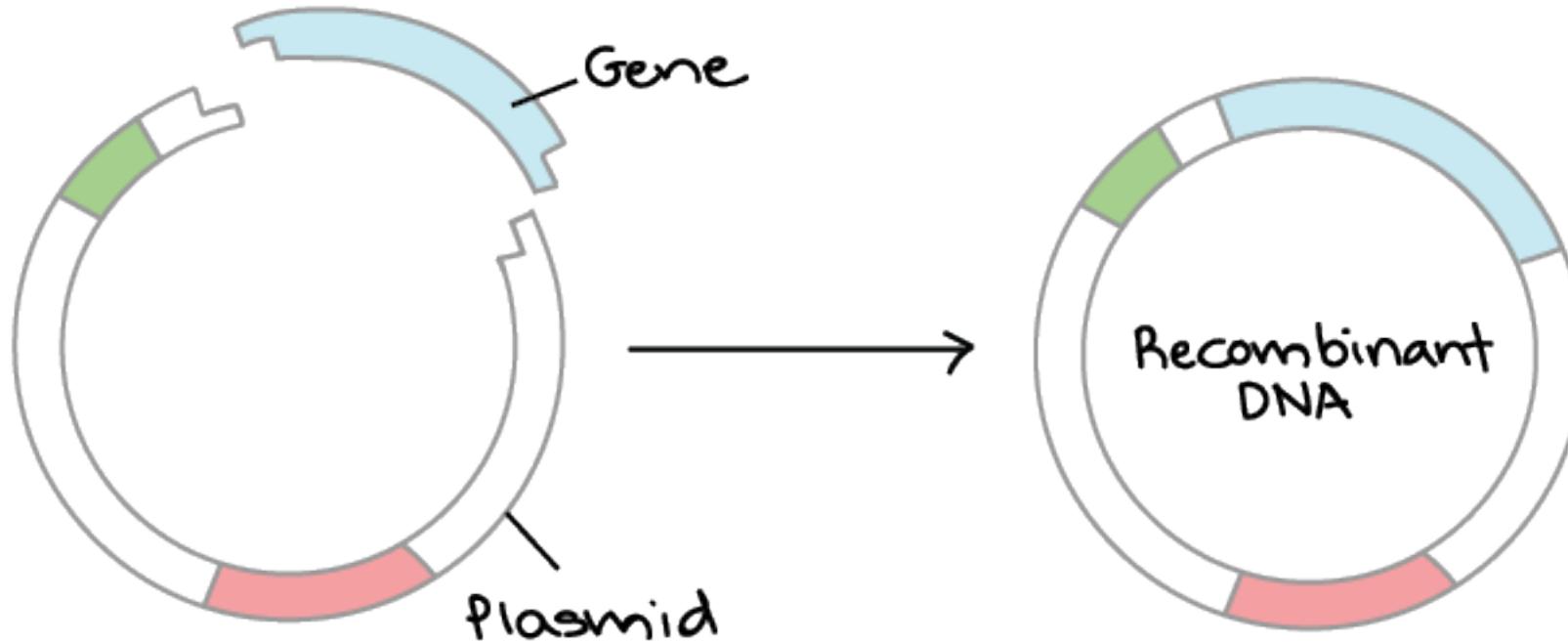
# Plasmids – short circular DNAs



# Plasmids – short circular DNAs



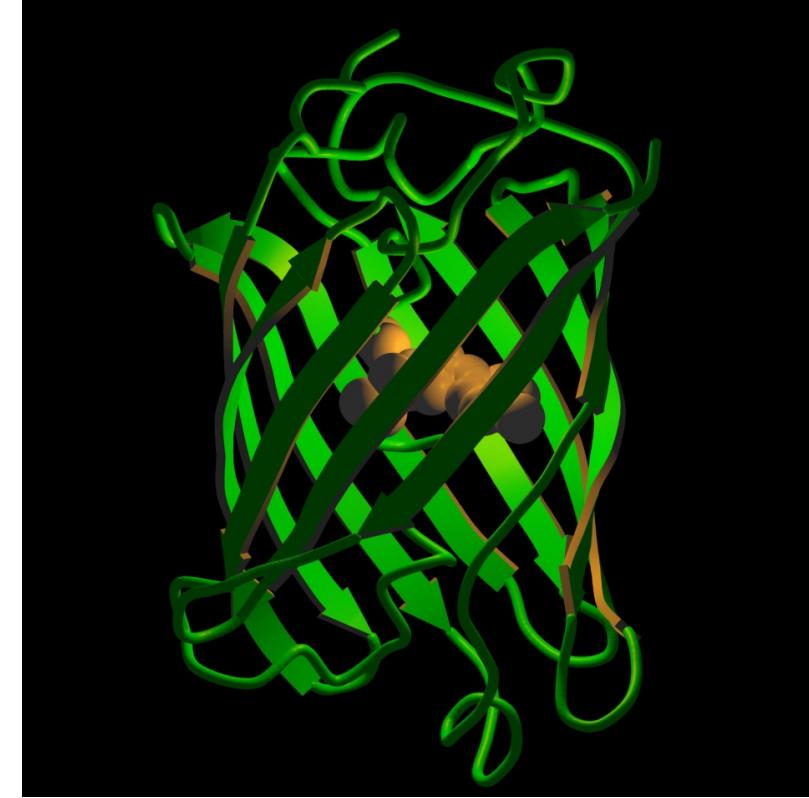
# Plasmid Manipulation



# Green Fluorescent Protein



*A. Victoria*



GFP Protein Structure