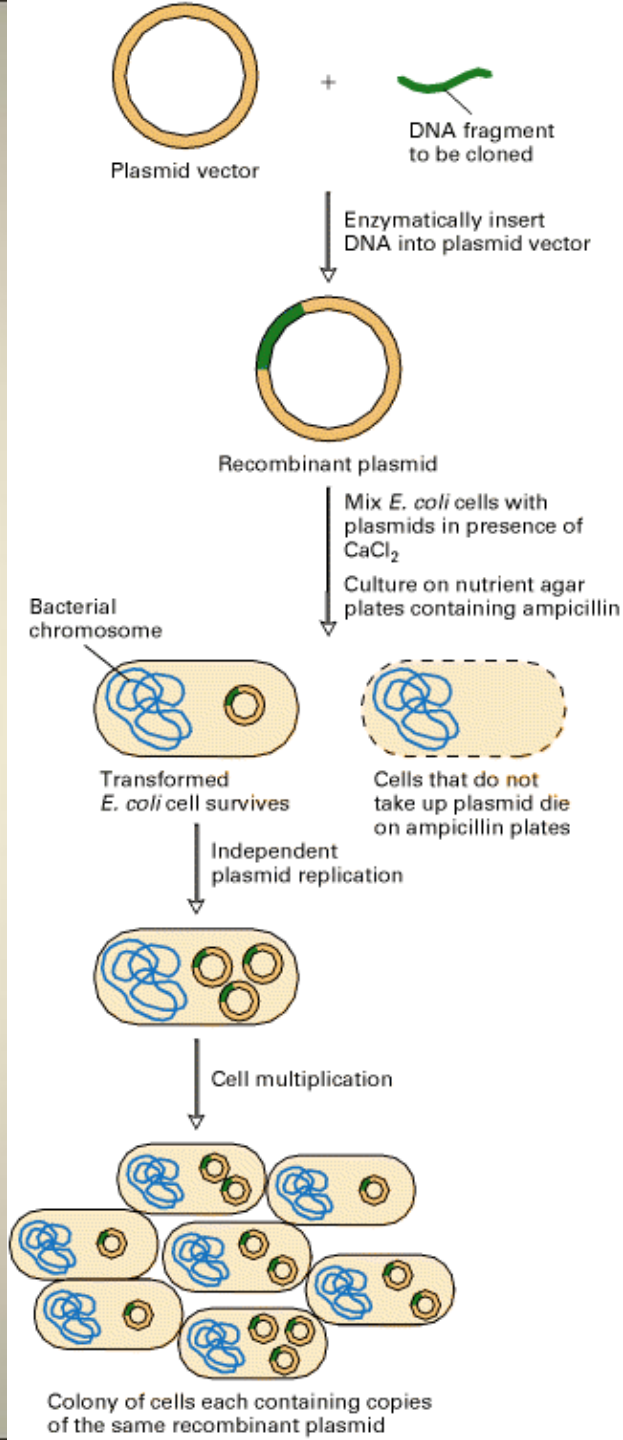
Gene Cloning

BIOTECHNOLOGY

Gene Cloning

- Making multiple copies of a single gene by using *in vivo* amplification

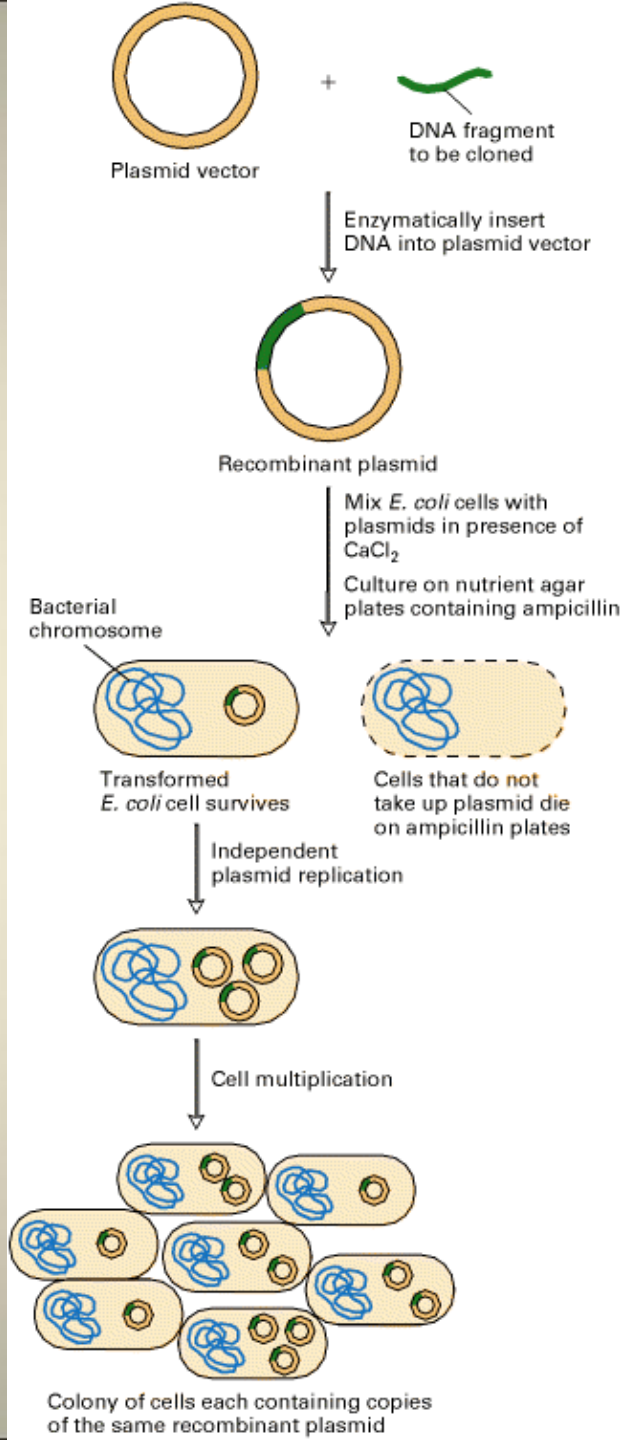
- Step 1: Forming recombinant DNA
- Step 2: Transformation (followed by many cell divisions)
- Step 3: Selection



http://tainano.com/Molecular%20Biology%20Glossary.files/image053.gif

Animation

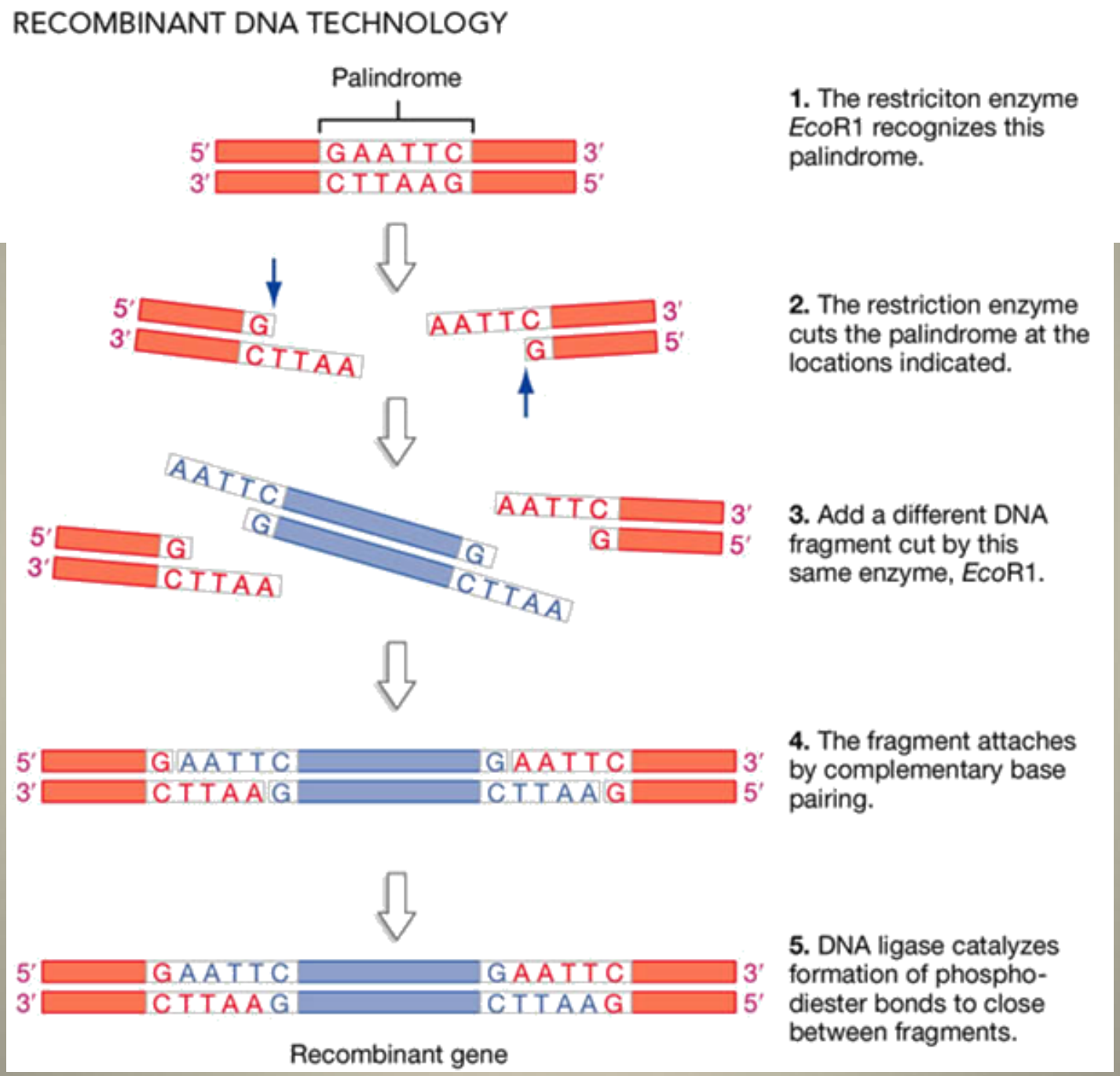
- Introduces gene cloning including information on forming a recombinant and transformation
- http://highered.mcgraw-hill.com/olc/dl/120078/micro10.swf



http://tainano.com/Molecular%20Biology%20Glossary.files/image053.gif

Step 1. Forming Recombinant DNA

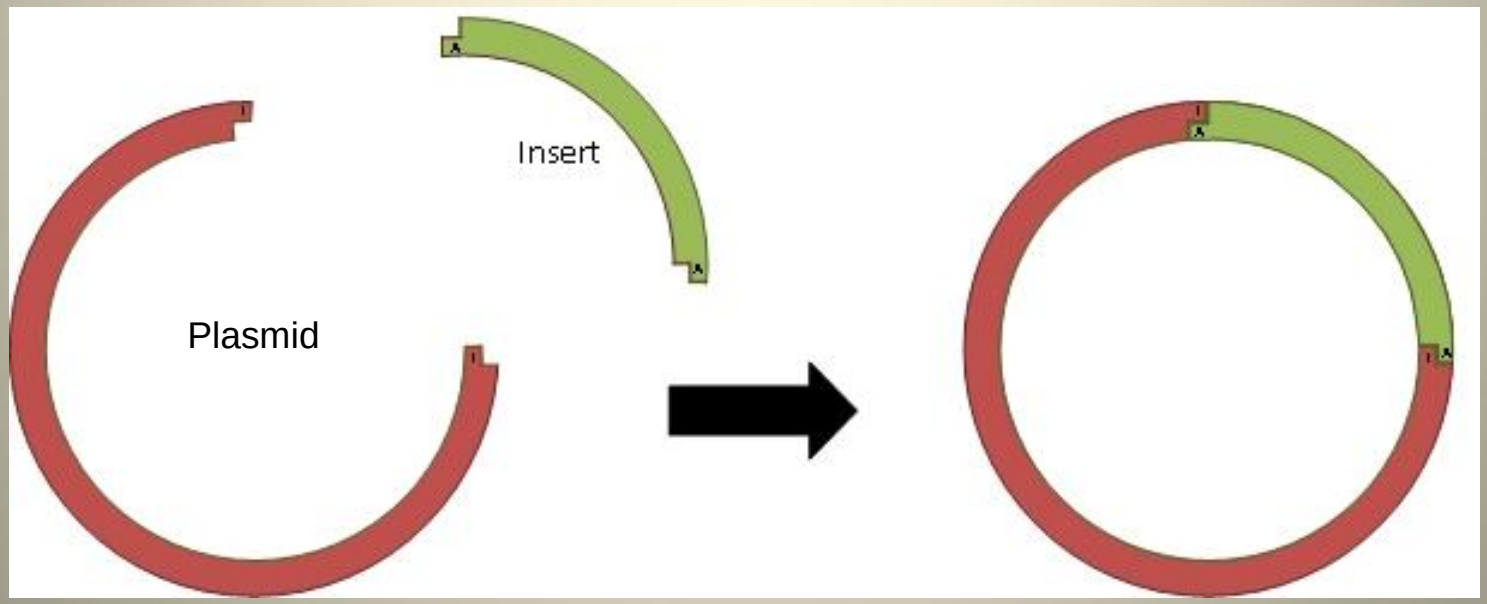
- **Recombinant DNA**: genes from two different sources (often different species) combined into one molecule
- Any DNA cut with the same restriction can be ligated together because they have the same sticky ends that are complementary



http://campus.queens.edu/faculty/jannr/Genetics/images/dnatech/bx15_01.jpg

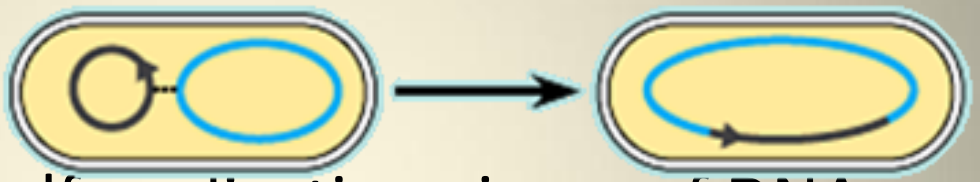
Step 1. Forming Recombinant DNA

- Usually the gene of interest is inserted into a bacterial **plasmid**



Bacterial Genome

- Genome contains one chromosomal DNA and many plasmids
- **Plasmids**:



  - small, circular, self-replicating pieces of DNA (separate from the bacterial chromosome)
  - Contain a small number of genes
  - Can incorporate themselves into the bacterial chromosome
- **Episome**: genetic elements that can exist either as a plasmid or as part of the bacterial chromosome

Advantage of Plasmids

- Plasmids are not required for bacterial cells to survive under normal conditions
- Under stress, genes on plasmids can confer advantages
  - Example: R (resistance) plasmid has genes that make bacteria resistant to antibiotics
- Plasmids increase genetic variation and thus the likelihood of survival in bacteria

Step 1. Forming Recombinant DNA

General steps:

- Restriction enzyme digestion of plasmid and gene of interest
- Hybridization of matching sticky ends on gene of interest and plasmid
- DNA ligase seals gene of interest with plasmid

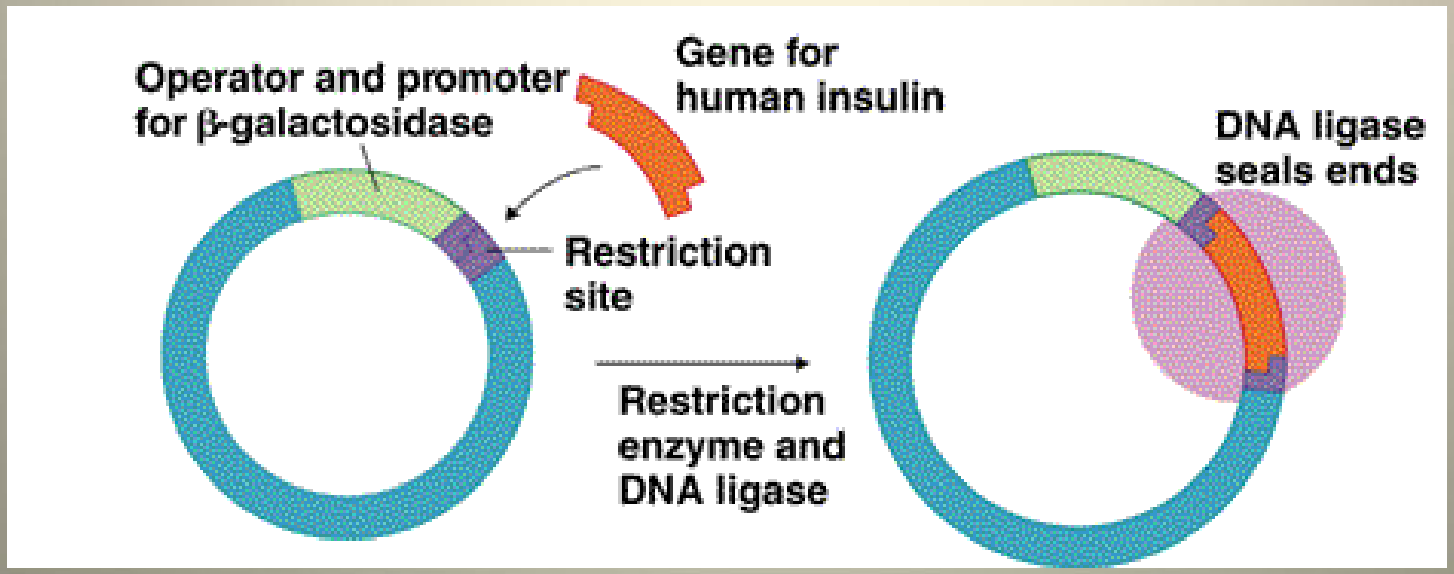


Fig. 20.3

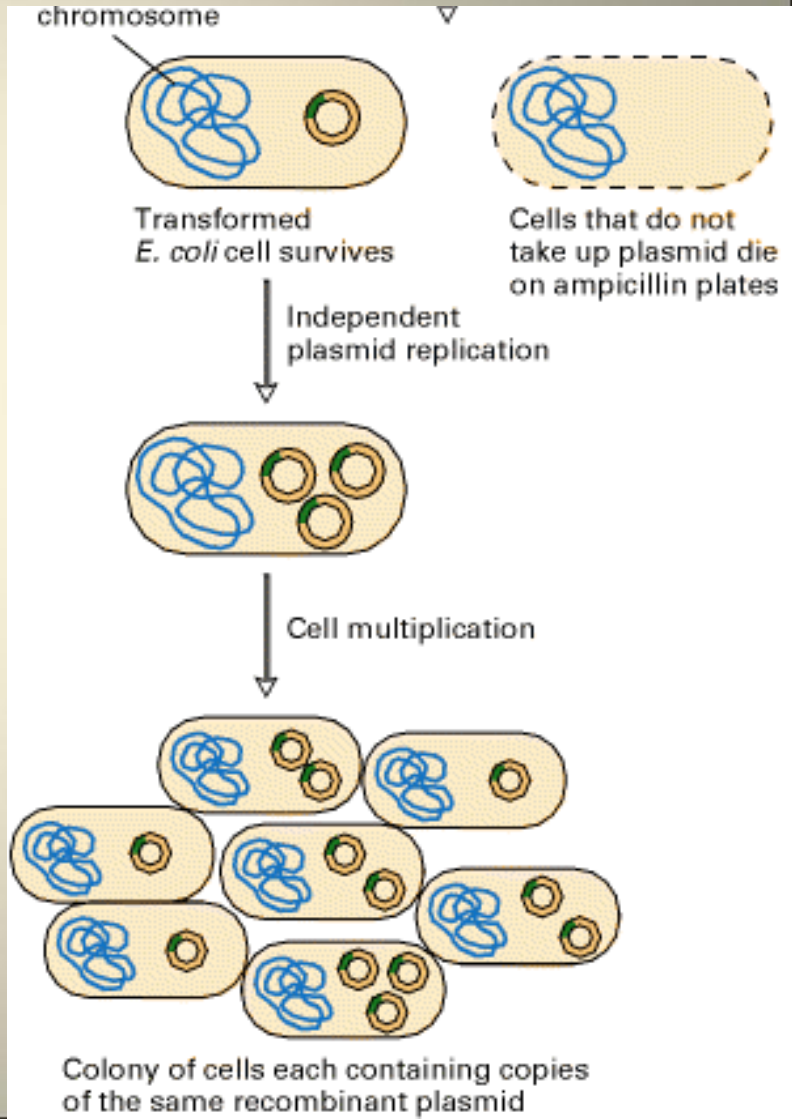
Activity: Recombinant DNA

- You are given either a gene of interest (linear) or a plasmid (circular)
- Cut out your DNA
- Digest it with the given restriction enzyme
- Find the person with a matching sticky end to form the recombinant

- Additional: Research the data pair. Prepare a short (3-4 sentence) write-up that relates to the two terms.

Step 2: Transformation

- **Transform** recombinant DNA into bacterial cell
- As bacterial cells multiply, the gene of interest will be replicated with each cell



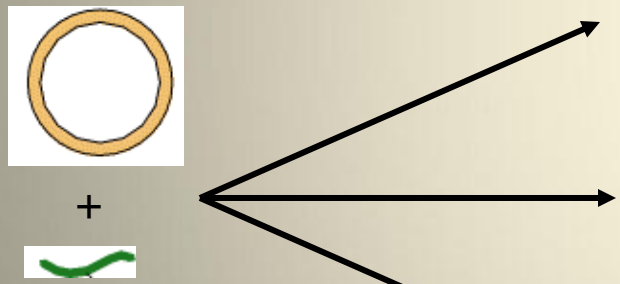
Step 2: Transformation

- Bacteria grown in flasks of liquid medium
- Incubate at optimal growing temperature

http://photos.news.wisc.edu/photos/8402/original/Shen_bacteria_flask08_8579.jpg?1286762168

Step 3: Selection

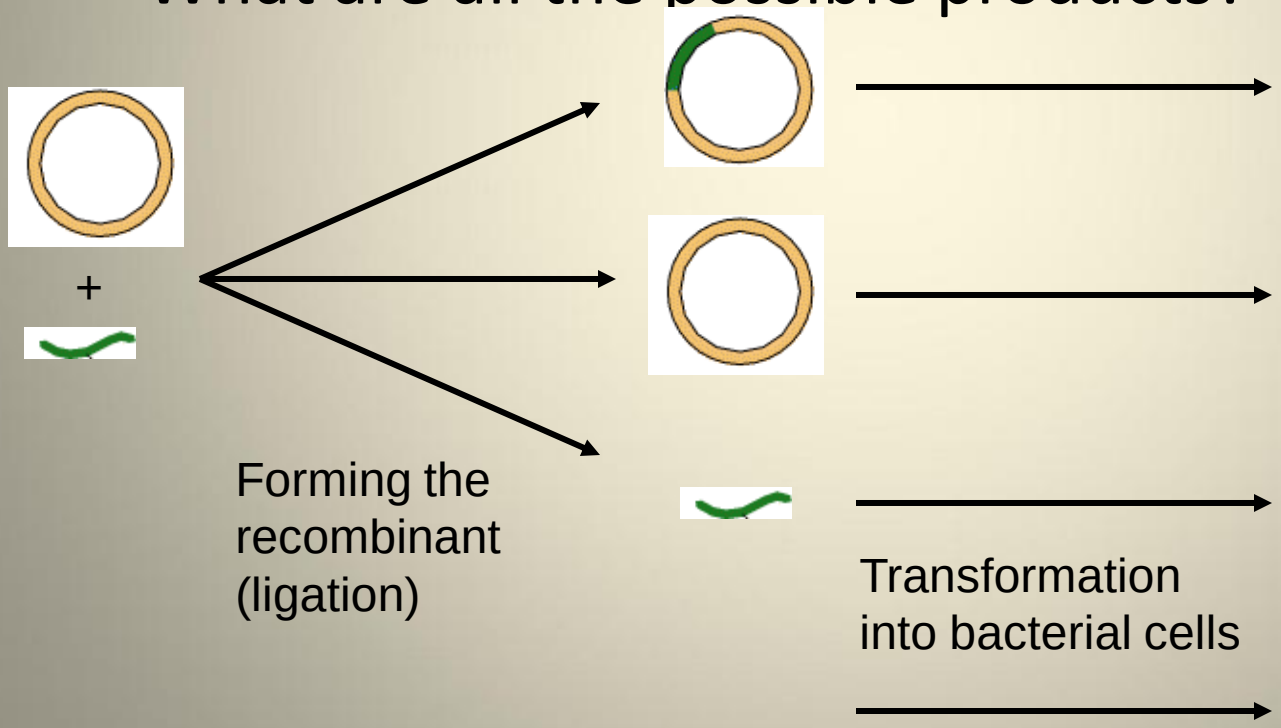
- **Selection**: Identify colonies of bacteria containing the recombinant DNA
- What are all the possible products?



Forming the recombinant (ligation)

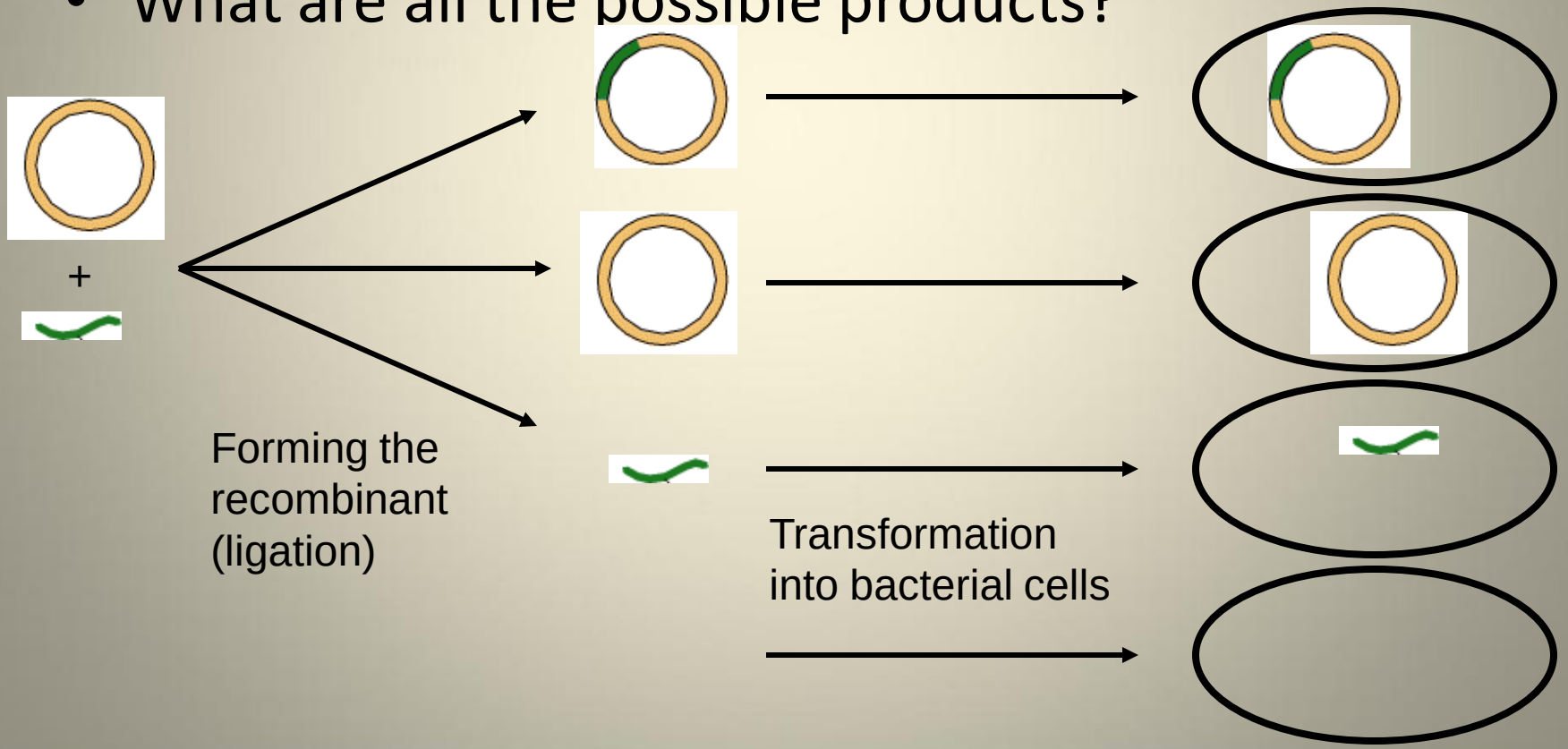
Step 3: Selection

- **Selection**: Identify colonies of bacteria containing the recombinant DNA
- What are all the possible products?

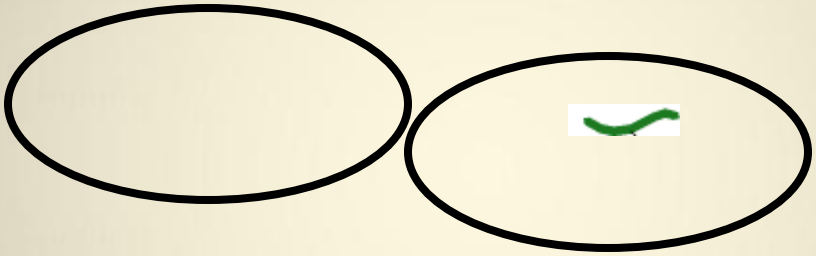
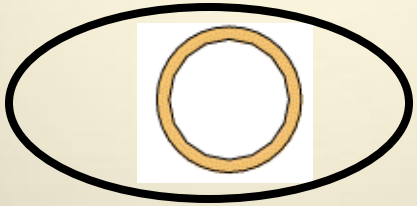
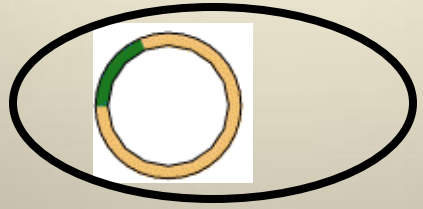


Step 3: Selection

- **Selection**: Identify colonies of bacteria containing the recombinant DNA
- What are all the possible products?

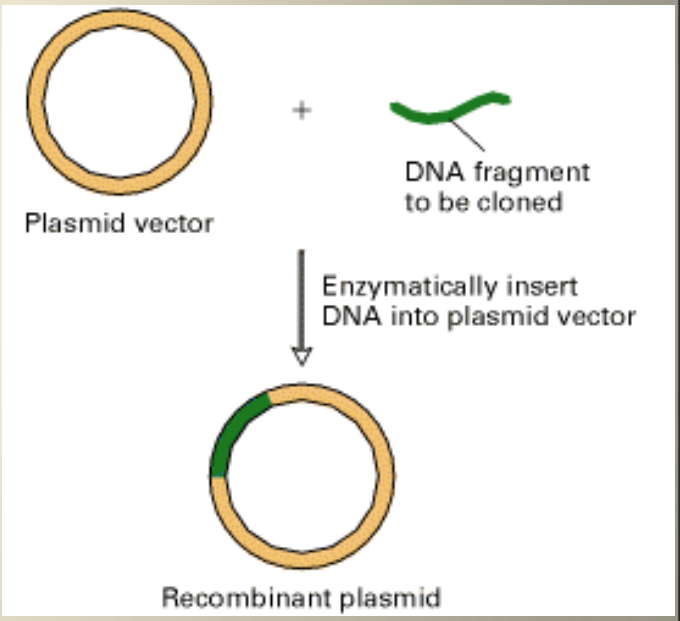


Step 3: Selection

Bacterial Products	Image	Cause
No vector		No transformation or transform without vector
Empty vector		Transform with unsuccessful ligation
Recombinant		Transformation and successful ligation

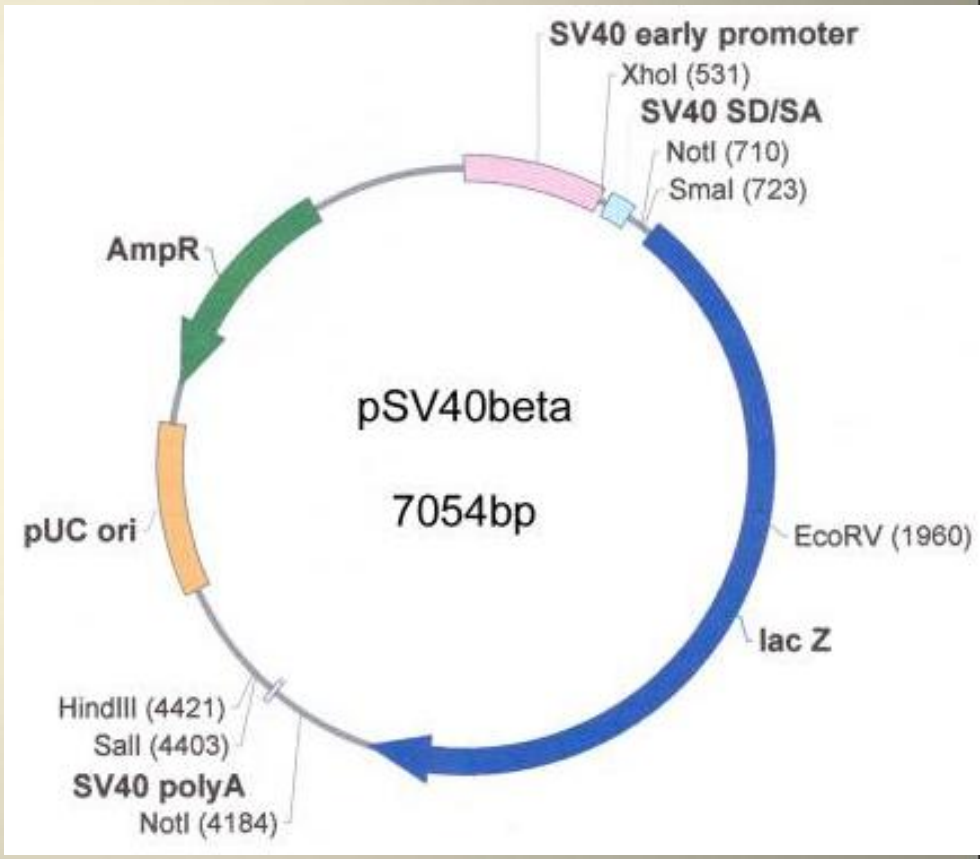
Cloning Vector

- A plasmid into which the gene of interest is introduced
- The vector has a number of specific sites useful in selection:
  - Ori
  - promoter
  - restriction sites / cloning site
  - ampR gene
  - lacZ gene



Cloning vector components

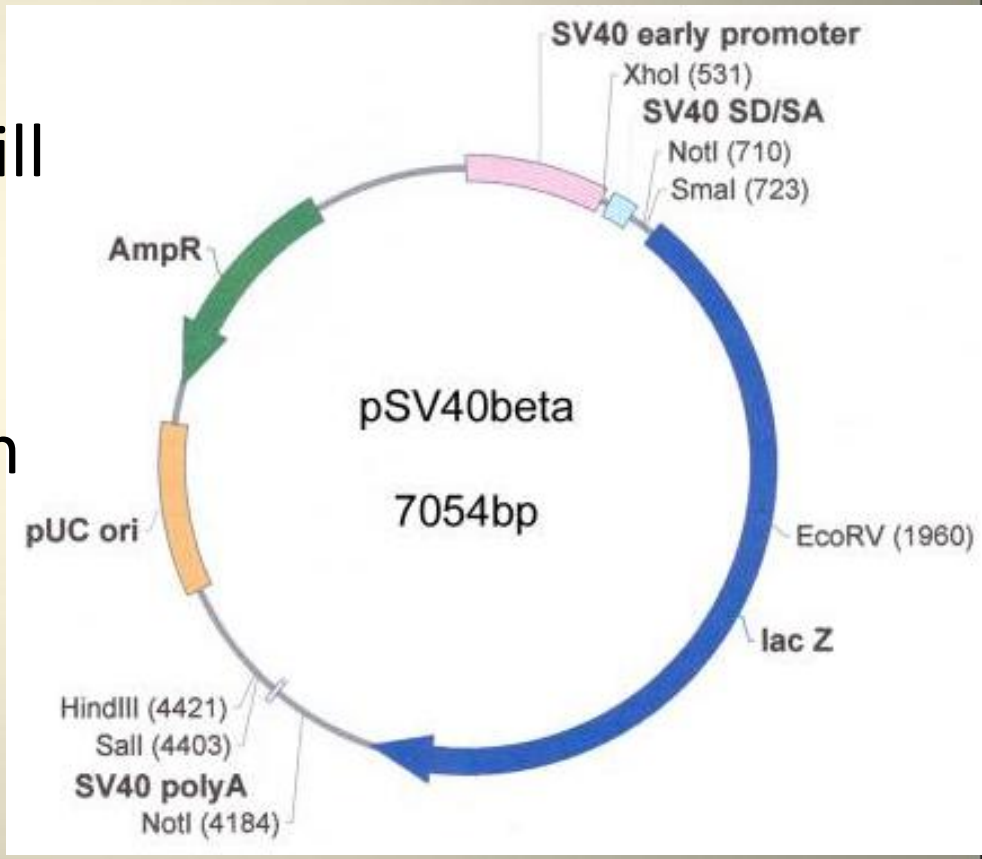
- **Replication origin (ori)**: allows plasmid to replicate in the host cell



Cloning vector components

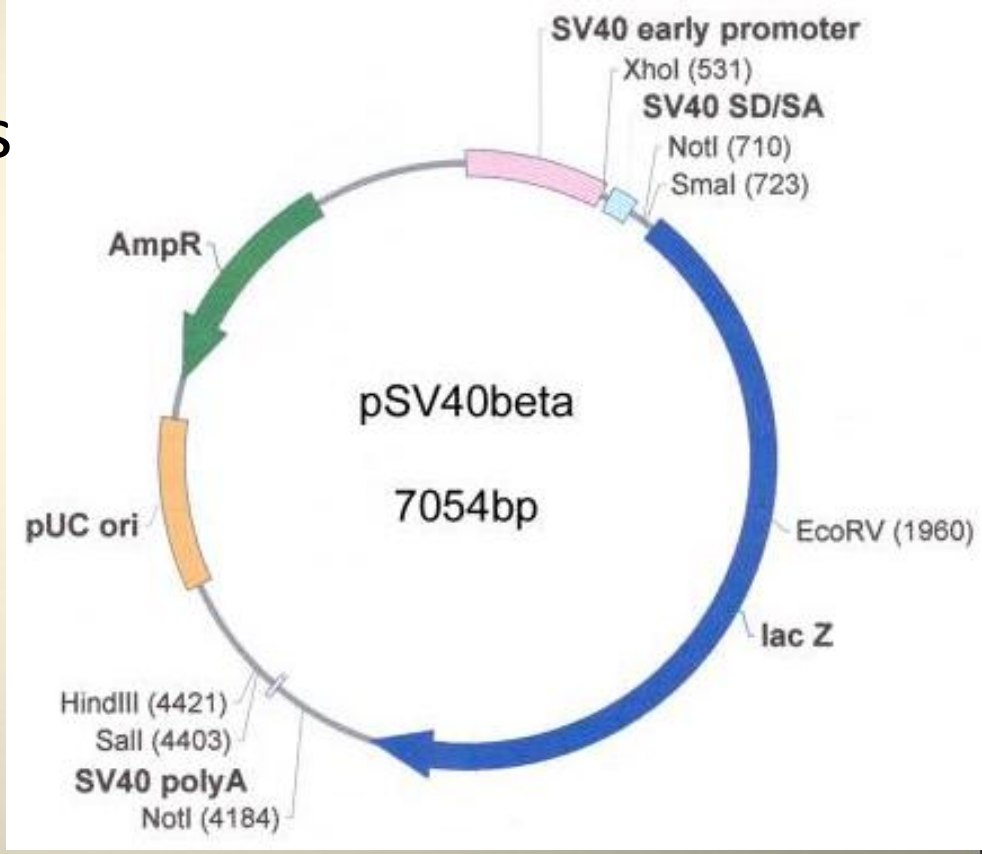
Cloning site:

- Where gene of interest will be inserted (ligated)
- Where transcription can occur because contains an **upstream promoter**



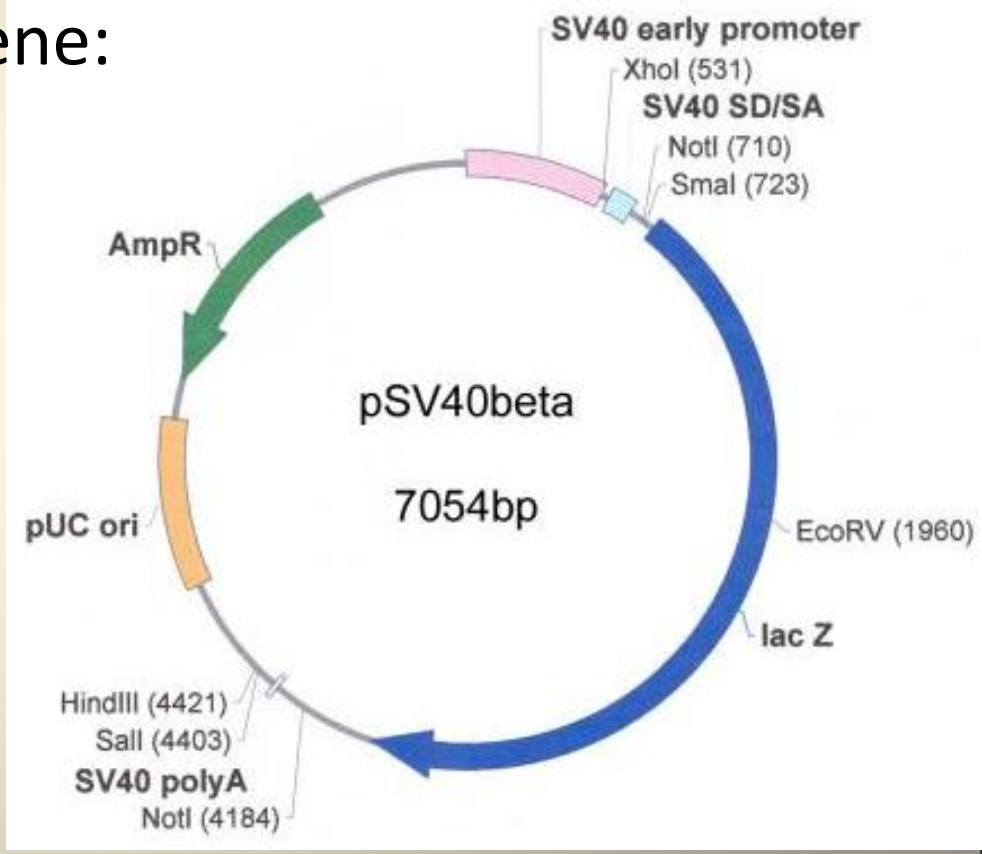
Cloning vector components

- **Antibiotic resistance (ampR)** gene: allows cells to be resistance to ampicillin (an antibiotic)
- Selection for host cells that have resistance
- Thus, selecting for transformation



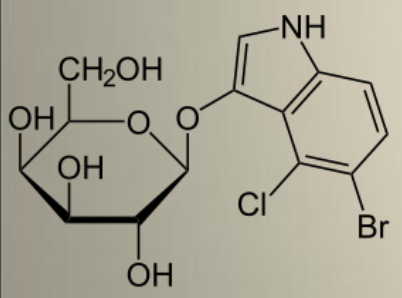
Cloning vector components

- **β-galactosidase (LacZ)** gene: enzyme produced will change a clear substrate called X-gal into a blue product



β-galactosidase Reaction

- β-gal acts on X-Gal (a clear soluble substrate) to produce a **blue** precipitate

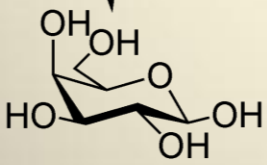


X-gal
(colourless)

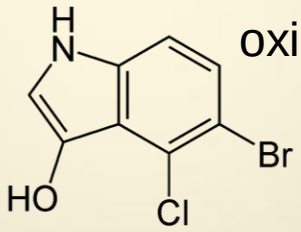
hydrolysis

β-Galactosidase

+ H_2O

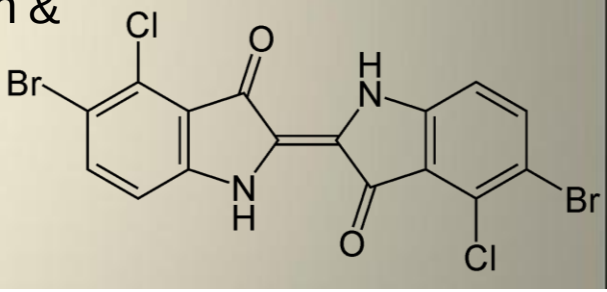


galactose



5-bromo-4-chloro-3-hydroxyindole

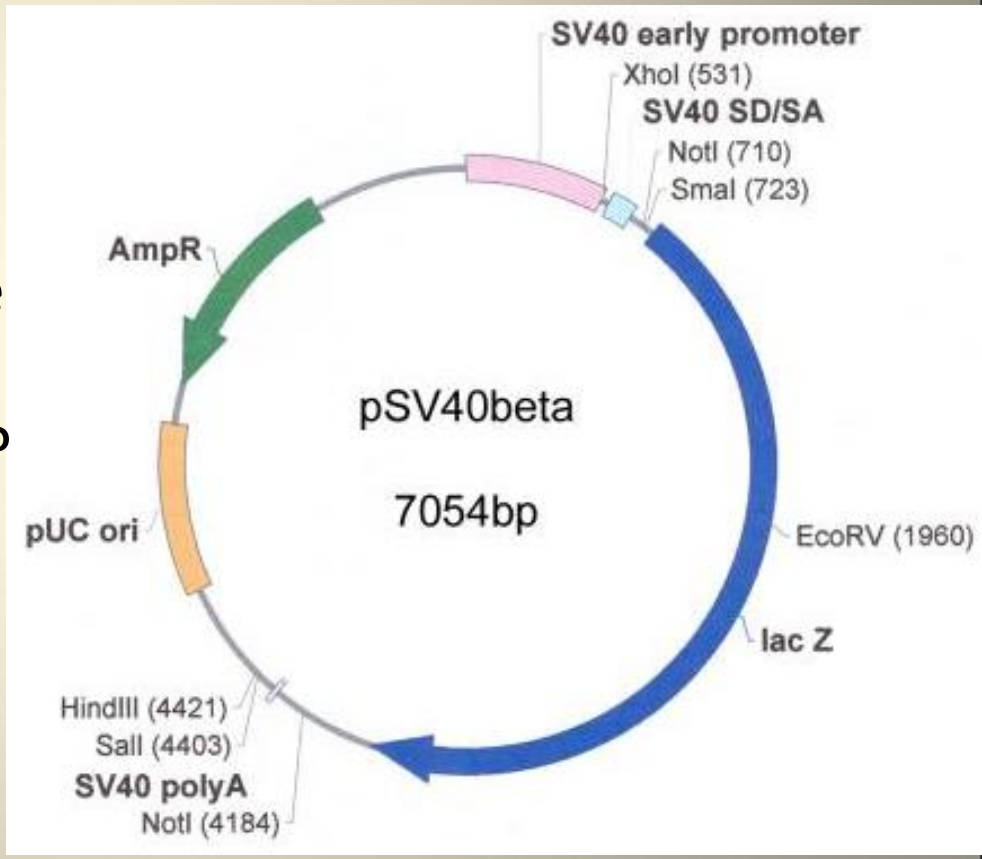
Spontaneous dimerization & oxidation



5,5'-dibromo-4,4'-dichloro-indigo, an intensely **blue** product which is insoluble

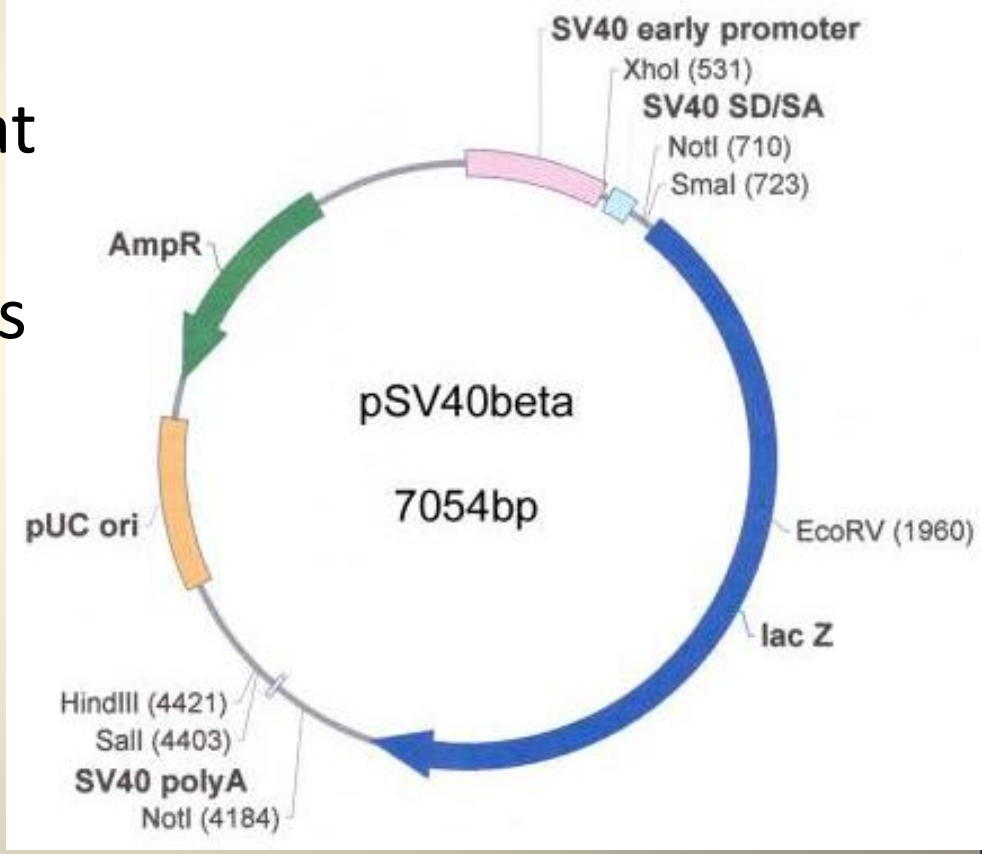
LacZ question

- The cloning vector on the right has a functioning lacZ gene.
- What will be the colour of the bacterial cell if it has this plasmid and is grown in X-gal?
- What would be the colour of the bacterial cell if it does not have this plasmid and is grown in X-gal?



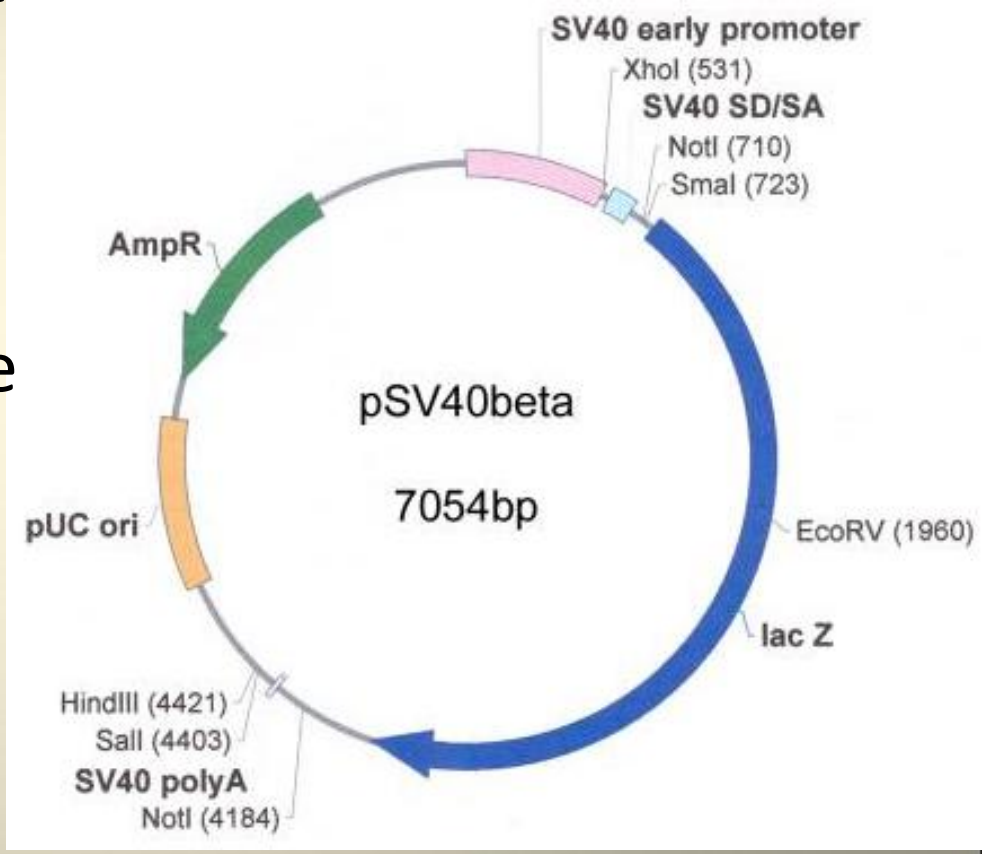
Step 1: Forming Recombinant DNA

- Where would you insert the DNA of interest so that you can “see” it in the bacterial cell (assume cells are grown in X-gal)?



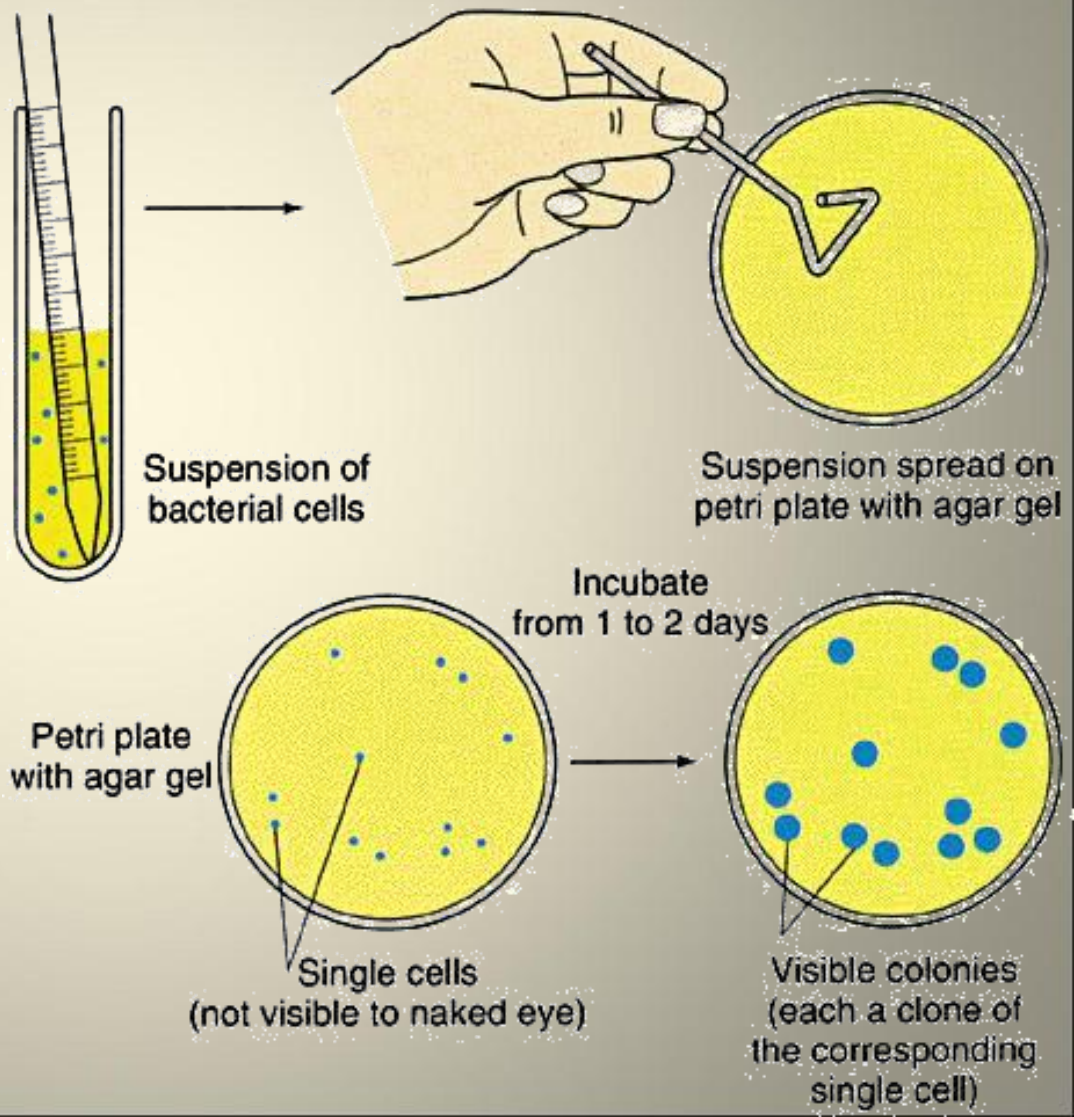
Step 1: Forming Recombinant DNA

- **Ligation**: joining different nucleic acids usually involving ligase
- Ligate the gene of interest into the vector such that it interrupts the lacZ gene
- Thus β–galactosidase is not made
- Question: What colour would the bacterial cells be if grown in X-gal?



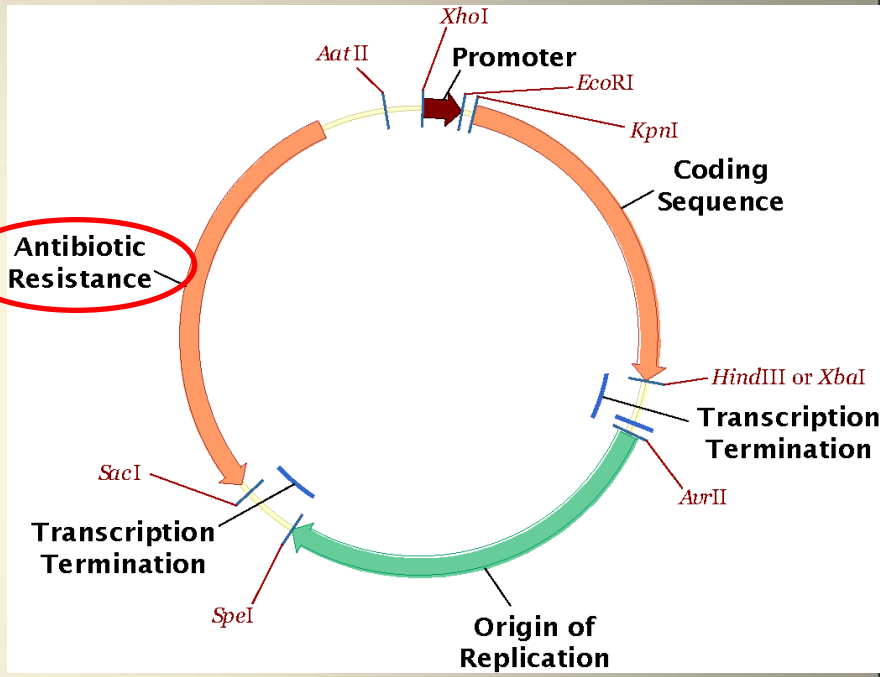
Step 3: Selection

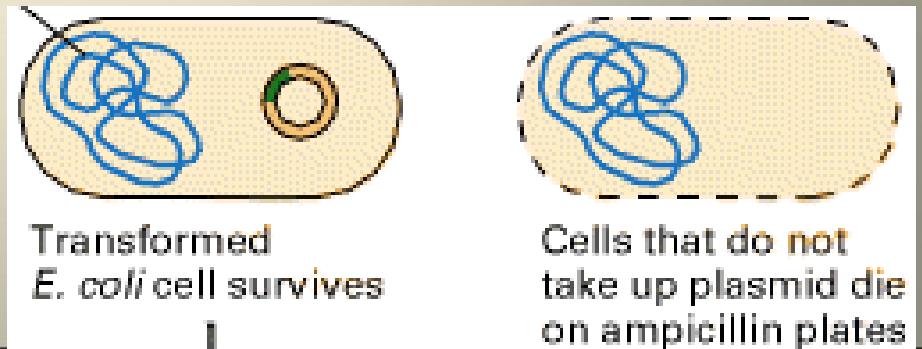
- **Plating**: taking a sample of the bacteria and growing them on plates
- Plates have a agar medium containing:
  - Antibiotics
  - X-gal



Selection Mechanism: Antibiotic Resistance

- Select for bacterial clones that **contain a vector** (select for **proper transformation**)
- Bacteria are grown on Petri plate containing a specific **antibiotic** (e.g. ampicillin)

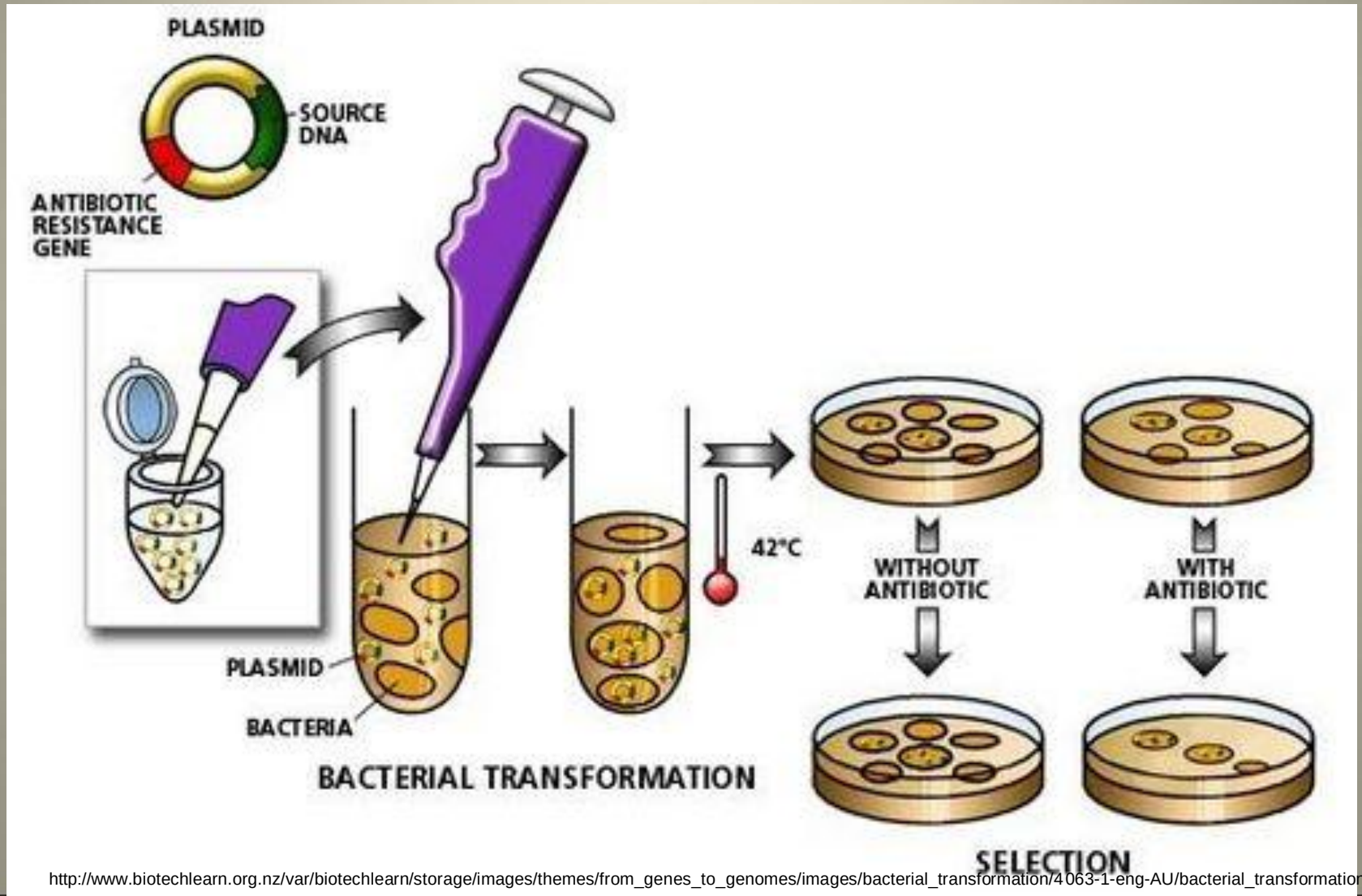




Antibiotic Resistance

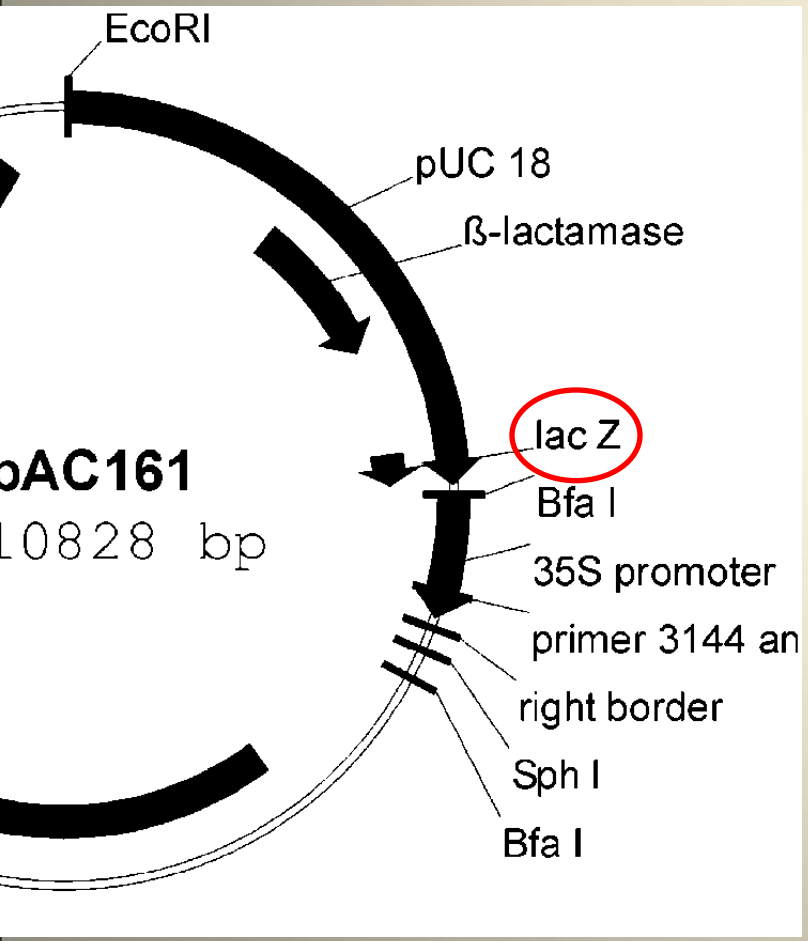
- Vector confers antibiotic resistant to bacteria because the vector contains an **antibiotic resistant gene (ampR)**
- Only bacterial cells that properly transformed the vector will live and grow on the plate

Selection for successful transformation



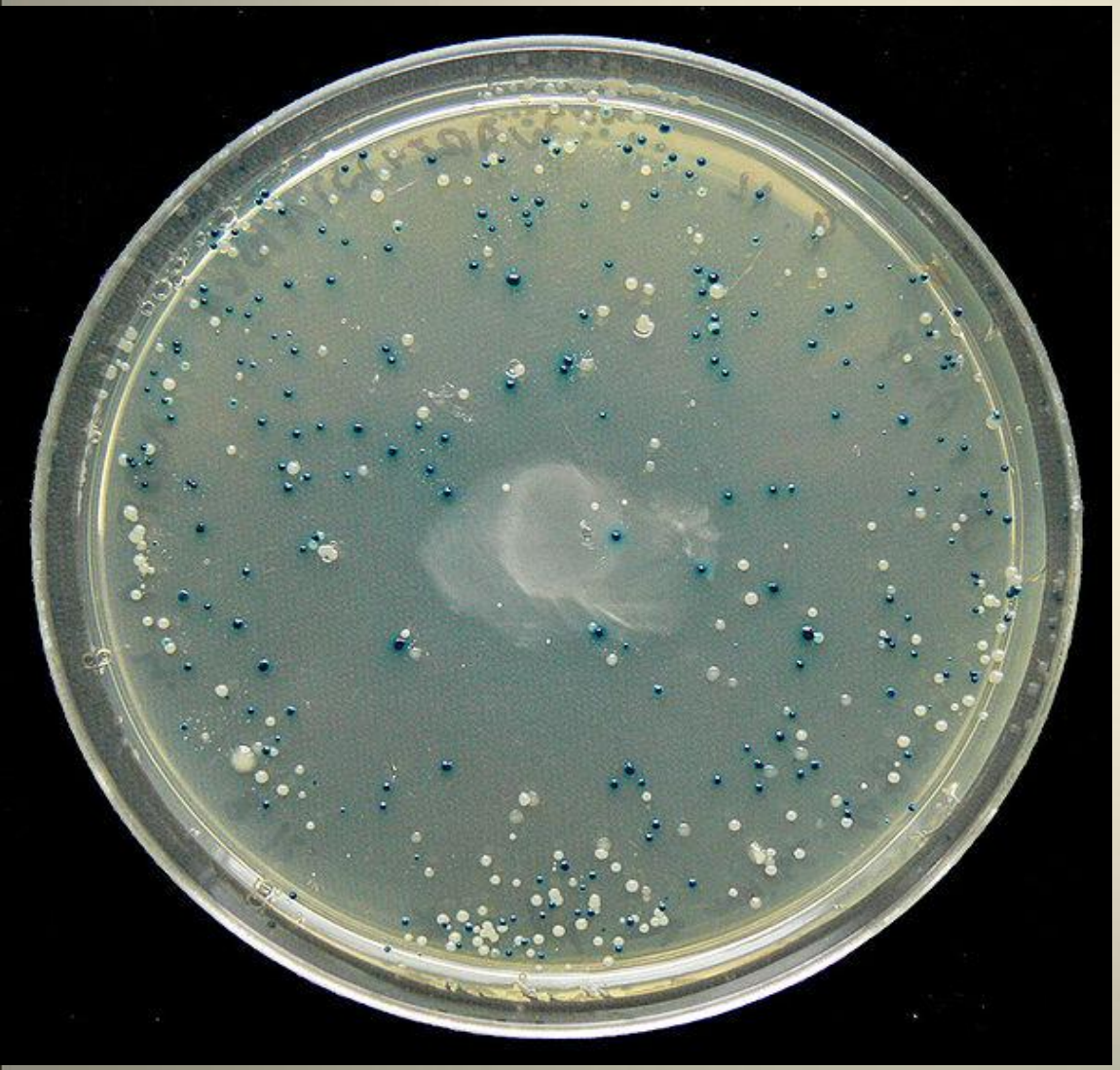
http://www.biotechlearn.org.nz/var/biotechlearn/storage/images/themes/from_genes_to_genomes/images/bacterial_transformation/4063-1-eng-AU/bacterial_transformation_large.jpg

Selection Mechanisms: β-galactosidase Screening



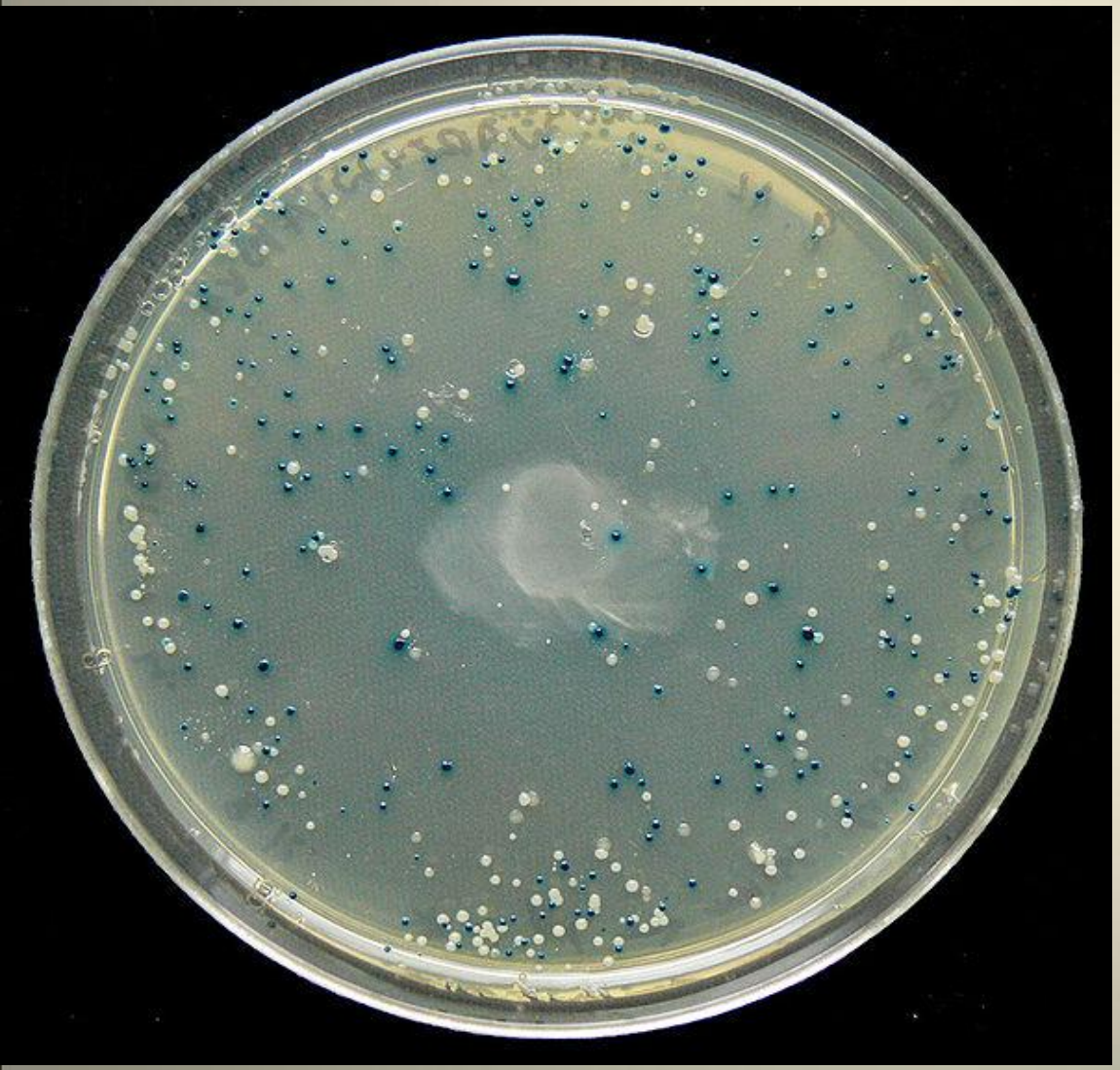
- Select for bacterial clones that **contain a vector with gene of interest** (select for **proper ligation**)
- Bacteria are grown on Petri plates containing **X-Gal**

Selection for successful ligation



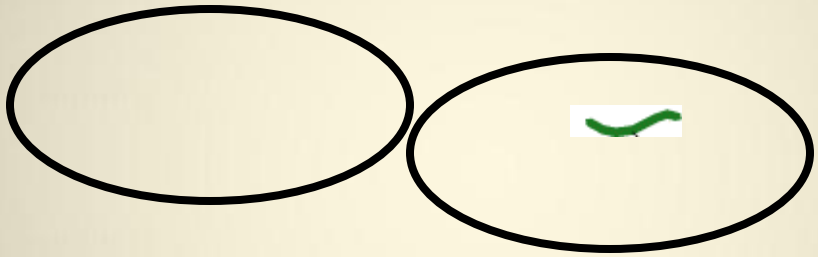
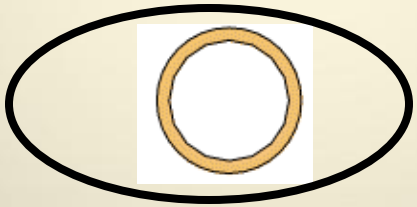
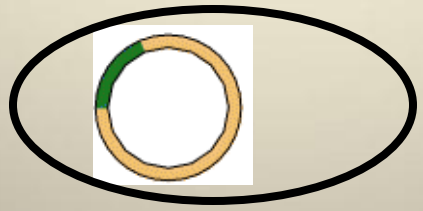
- Vectors contain lac Z gene that codes for the β-galactosidase (β-gal)
- Vectors that have the DNA insert won't have a functional β-gal enzyme
- These bacteria, when grown in X-gal, cannot process it and stays white

Selection for successful ligation

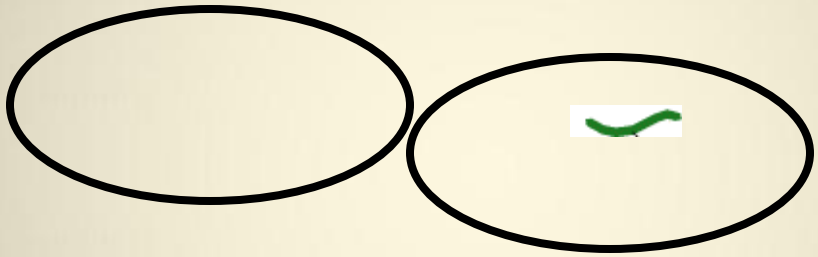
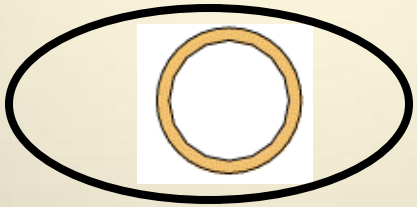
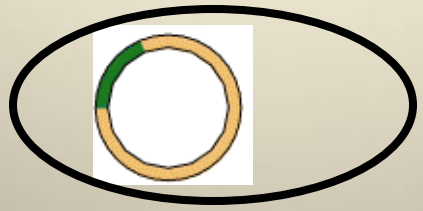


- Bacteria which accepted a vector WITHOUT the DNA of interest will have a working lacZ gene
- Gene codes for working β-gal enzyme which will process X-gal into a blue product

Step 3: Selection

Bacterial Products	Image	Selection Process
No vector		
Empty vector		
Recombinant		

Step 3: Selection

Bacterial Products	Image	Selection Process
No vector		Unable to grow in antibiotics
Empty vector		Grows in antibiotics Turns blue with X-gal
Recombinant		Grows in antibiotics Is white with X-gal

Possible Transformation Results

LB Medium additions	No vector	Empty vector	Recombinant DNA
Amp	No growth	White	White
X-gal	White	Blue	White
Amp + X-gal	No growth	Blue	White

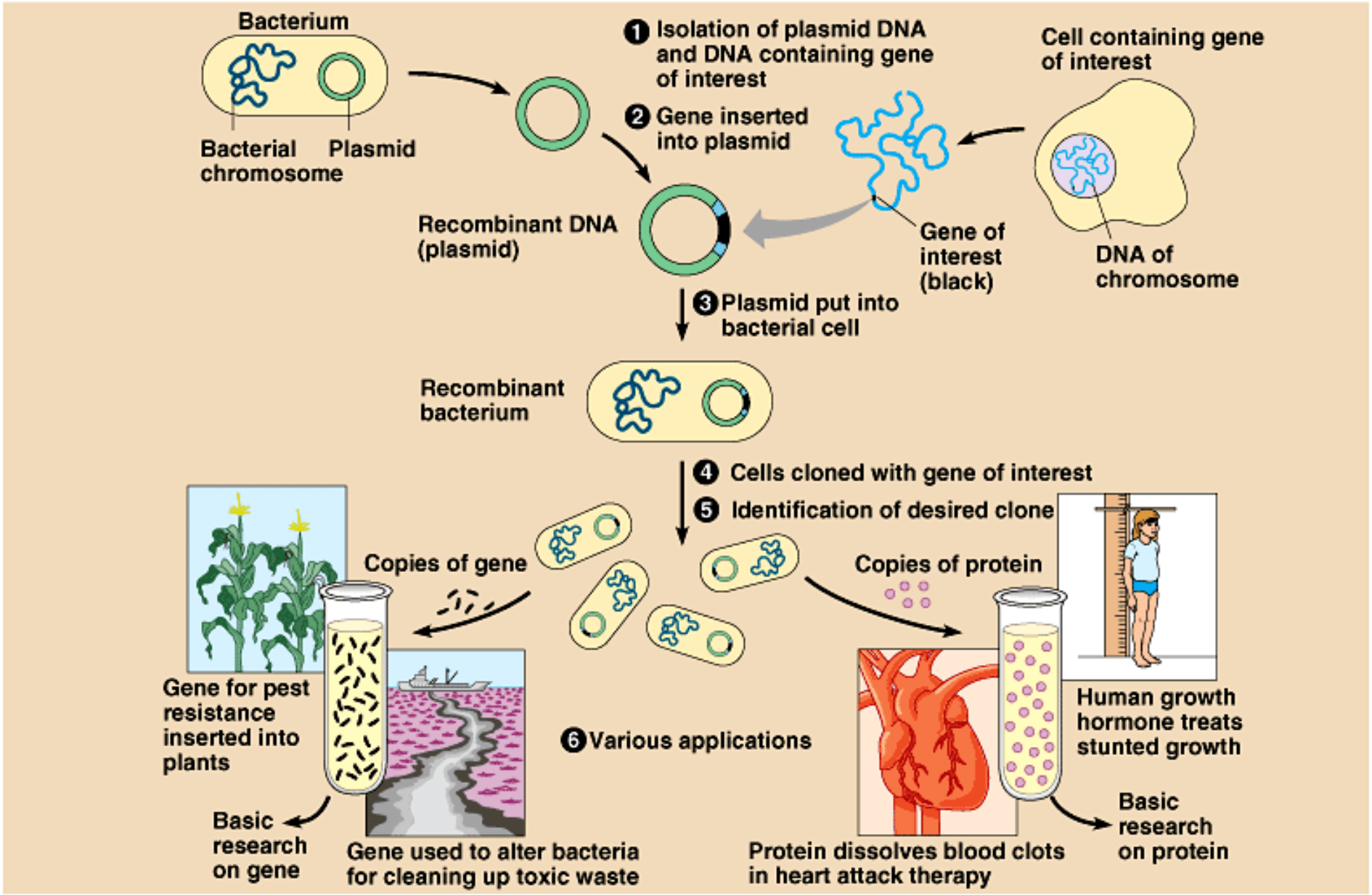


Fig. 20.1

Animation: Gene cloning

- http://www.sumanasinc.com/webcontent/animations/content/plasmidcloning.html (includes antibiotic resistance info)