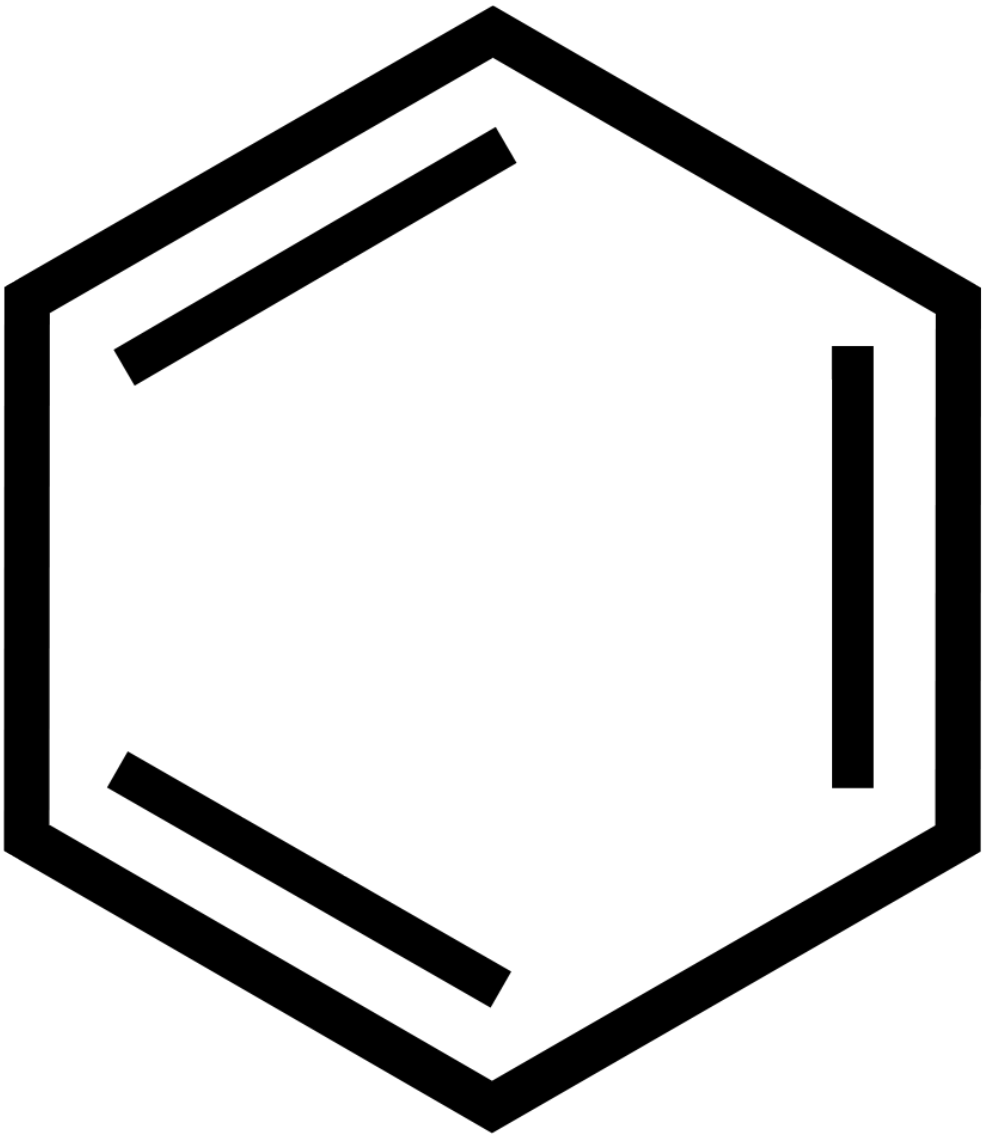

CLUTCH

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CONCEPT: WORKING WITH MICROORGANISMS

● Bacteria are easy to _____ with in a laboratory setting

□ They are fast dividing, take up little space, and are easily grown in a lab

- **Plating** is when bacteria in a liquid culture is put onto a petri dish containing *agar*

- Plated cells divide, but don't move so they become a clump of cells

- A **colony** is a clump of cells that can be seen with the eye (10^7)

- Generally, these are **clones**, because they are all derived from a single genetic ancestor

□ Bacteria are classified based on what they _____ to grow

- **Prototrophic** bacteria mean they grow on *minimal medium* (salts, carbon, and water)

- Generally WT, written like *lac*$^+$

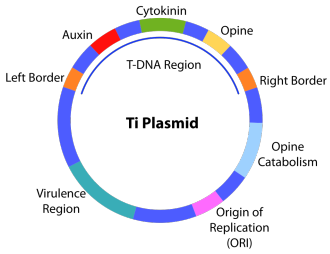
- **Auxotropic** bacteria only grow in one or more specific nutrients are present in the media (*complete)*

- Generally mutation, written like *lac*$^-$

EXAMPLE:

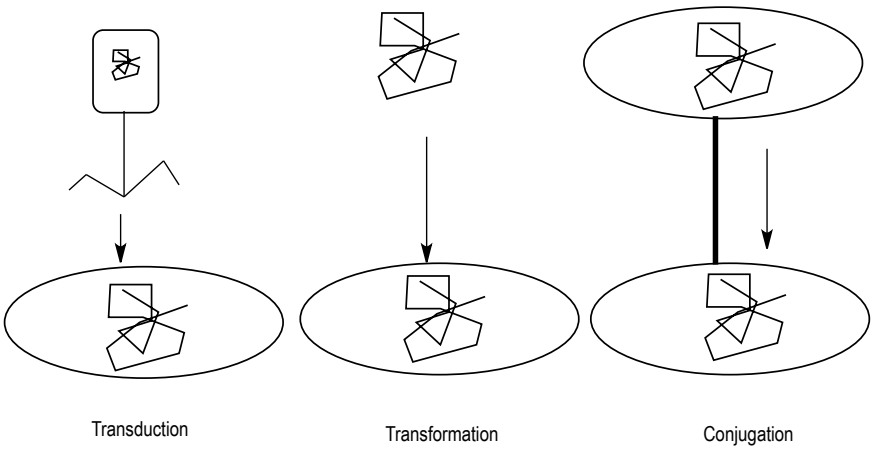
- The DNA found in bacteria comes in two sources
  - □ The bacterial chromosome is the _____ DNA molecule in the bacterium
  - □ The **plasmid** is a small, circular DNA found in bacteria in addition and outside of the main chromosome
    - Contains genes that are not essential to bacteria function
    - Plasmids are abundant (*E.coli* has 270 naturally occurring plasmids)
  - □ Mutations in bacterial DNA can be easily seen phenotypically
    - Effect colony morphology, causes *antibiotic resistance*, create auxotrophs, breakdown chemicals

EXAMPLE:



- Bacterial DNA can be _____ in three main ways
  - □ **Conjugation** is DNA transfer between contact and fusion of two different bacterial cells
  - □ **Transformation** is when a bacterium takes up DNA found in the external environment
  - □ **Transduction** is when phages transfer DNA into the bacterium
- These three are examples of **horizontal transmission** which transfers DNA between individual bacterium
  - Differs from **vertical transmission** which transfers DNA through bacterium division

EXAMPLE:



PRACTICE

1. What is a plasmid?
    a. A linear RNA molecule found in certain bacteria and plants
    b. Protein that is transferred between bacteria
    c. A small circular DNA found in bacteria
    d. A nutrient that bacteria need to grow

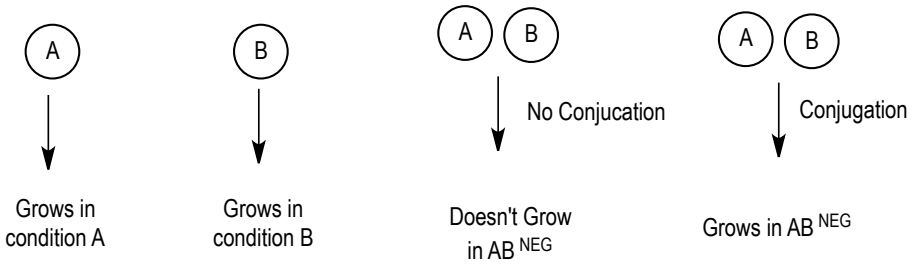
2. True or False: All bacterial cells within a bacteria colony grown on a plate in a laboratory are genetically identical.
    a. True
    b. False

3. Which of the following is NOT a way scientists can phenotypically identify a bacterial mutation?
    a. Changes in colony morphology
    b. Antibiotic resistance
    c. Creation of an auxotroph
    d. Sequencing the genome

CONCEPT: BACTERIAL CONJUGATION

- **Conjugation** is the physical union of bacterial cells to exchange genetic material
  - □ _____ in 1946 by Lederbergand and Tatum
    - They had two different *E. coli* strains: A and B
      - A only grows in medium with methionine and biotin
      - B only grows in medium with threonine, leucine, and thiamine
    - The two strains were mixed, and plated on a surface where neither would grow
      - But, some grew – meaning that DNA exchange had occurred between the two strains
  - □ The **Sex pili (F pili)** is the name of the structure that allows for conjucation
    - The **conjugation bridge** is the passageway for DNA transfer

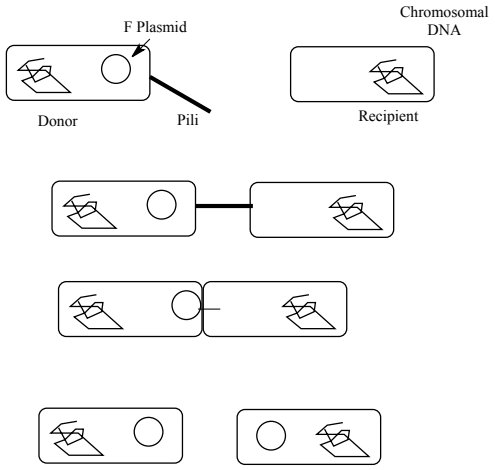
EXAMPLE:



F Factor

- The **F (fertility) factor** is a factor that confers the ability to swap DNA between bacteria
  - □ Bacteria with the F factor (**F^+**) can _____ genetic material, while bacteria without it (**F^-**) accept genetic material
    - The F factor, in this case, is a plasmid
    - The F^+ factor can be given to the F^- cell during conjugation
      - These recombinants form through conjugation, and not genetic recombination

EXAMPLE:



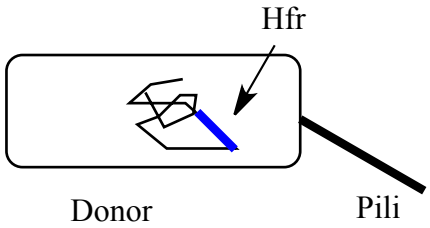
□ **Hfr (High frequency of recombination)** bacteria have the F factor integrated into the chromosome (not plasmid)

- The F factor, in this case, is a _____ gene in the chromosome

- The F^+ cannot be given to the F^- cell during conjugation

  - However, there are many more recombinants made by Hfr, but these occur via recombination

EXAMPLE:



□ **Hfr** bacteria can be used to _____ bacterial chromosomes

- You incubate Hfr cells with F^- bacterial cells (Hfr x F^-)

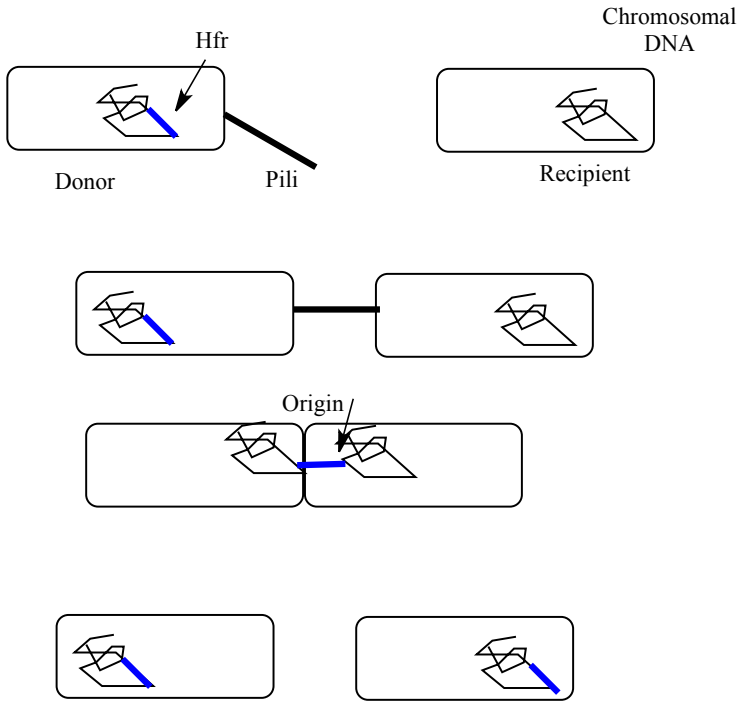
- The Hfr stimulates bacterial conjucation

  - The **Origin** is the area where the gene first transfers to the other cell

- You stop conjugation via **interrupted mating** where you use some kind of force to break the connection

- Genes close to Hfr, will have recombined before mating was interrupted. Genes far away, wont have.

EXAMPLE:



Other Plasmids

□ Bacteria contain other _____ in addition to the F factor

- **R plasmid** carries on it the genes that confer *antibiotic resistance*

- These plasmids can be transferred between bacterial species

- Often these contain a *transposon* (jumping gene) which assists in DNA transfer

PRACTICE

1. True or False: For conjugation to occur, bacterial cells must physically contact each other.
    a. True
    b. False

2. What is the name of the structure through which DNA is transferred?
    a. DNA bridge
    b. Sex Pili
    c. Genetic Material Passageway
    d. Fertility Factor

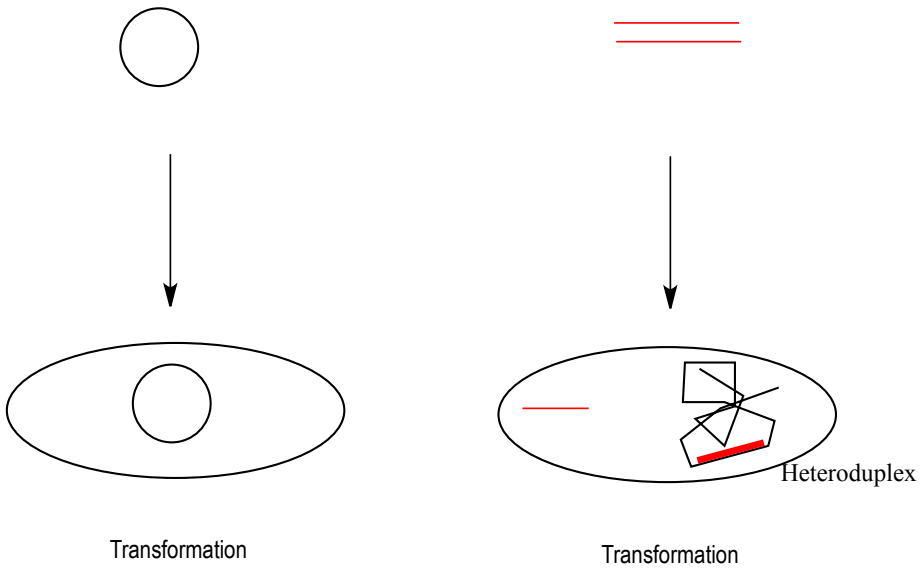
3. What property does the F factor give bacteria?
    a. Antibiotic resistance
    b. The ability to swap DNA via conjugation
    c. The inability to swap DNA via conjugation
    d. The ability to integrate the DNA into the chromosome

4. A F^+ bacterial cell can donate DNA to which type of bacterium?
    a. An F^+ bacteria
    b. An F^- Bacteria
    c. An Hfr Bacteria
    d. An R^+ bacteria

CONCEPT: BACTERIAL TRANSFORMATION

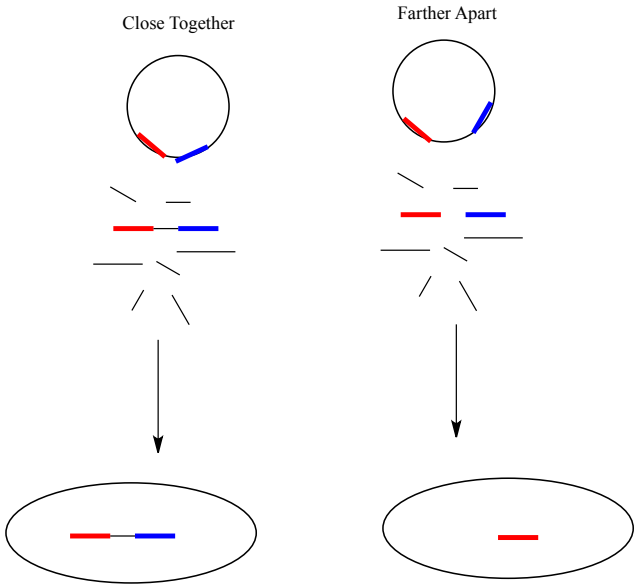
- **Transformation** is when a bacterium takes up DNA from the environment
  - □ DNA can be in the _____ due to:
    - Experimentally placed there
    - Death and bursting of nearby bacteria – which release DNA into the environment
  - □ Only **competent** cells are capable of transformation
    - Have a physiological state (natural, or experimentally induced) that allows the bacteria to take up DNA
  - □ Transformed DNA can stay in two _____
    1. It is a plasmid, that remains a plasmid once inside the bacterium
    2. The double helix is digest to a single strand, and that aligns with the bacterial chromosome
       - A **heteroduplex** is formed between the single strand and the complementary chromosome

EXAMPLE:



- Transformation can also be used to map genes
  - □ Introduce _____ DNA (through extraction, enzymes, etc…)
    - The closer the two genes are, the more likely they'll be taken up together
    - **Double transformation** is when two genes are taken up together

EXAMPLE:



PRACTICE

1. Transformation is the process of what occurring with DNA?
    a. Two DNA physically exchange DNA
    b. Bacteria take up DNA from the environment
    c. DNA is degraded
    d. DNA jumps from one bacteria cell to another

2. Scientists must use what type of cells to transform DNA in the laboratory?
    a. Viable cells
    b. Animal cells
    c. Competent cells
    d. Heat sensitive cells

3. The DNA from a bacterium with the genotype a^+ b^+ c^+ is used to transform a bacteria with the genotype a b c. Gene pairs were checked for cotransformation with the following results. Using these results determine which genes are linked.

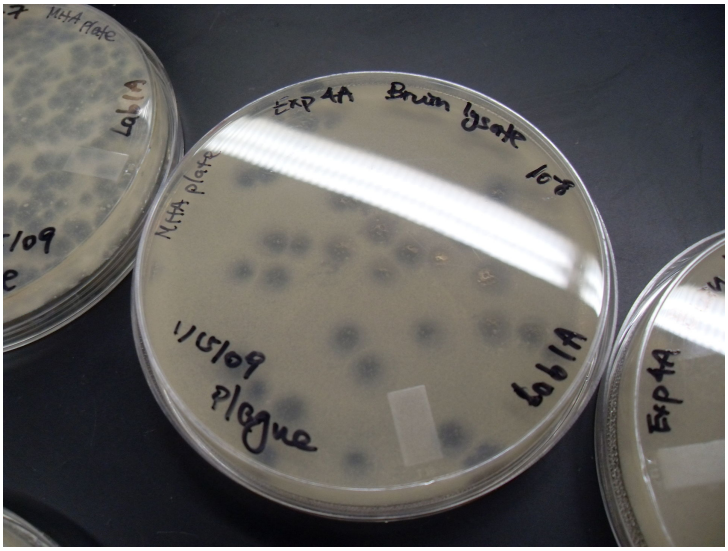
   a. A and B
   b. B and C
   c. A and C

Gene Pairs	Cotransformation
A and B	yes
B and C	no
A and C	no

CONCEPT: BACTERIOPHAGE GENETICS

- **Bacteriophages** are viruses that infect bacteria
  - □ A **Plaque assay** is used to study these viruses
    - Infect a bacterial culture with a _____
    - Plate the bacterial culture onto a petri dish that will grow the bacteria
    - Count the number of **plaques**, which form through **lysis** (breaking open) of infected bacteria
      - Lysis releases viral progeny into the environment

EXAMPLE:



□ There are three _____ of phages

- **Prophage** is a virus that has integrated its genetic material into the bacterial genome
- **Virulent phages** are those that immediately lyse and kill the host
- **Temperate phages** are those that remain inside the host for a period of time without lysing and killing it

Bacteriophages and Mapping

● Bacteriophage infections can be used to map bacteriophage _____

□ Recombination frequencies can be used to map genes

- Perform a **mixed infection** of a bacterial culture with two strains of bacteriophages

-Virus 1: H^+ and R^+ and Virus 2: H^- and R^-

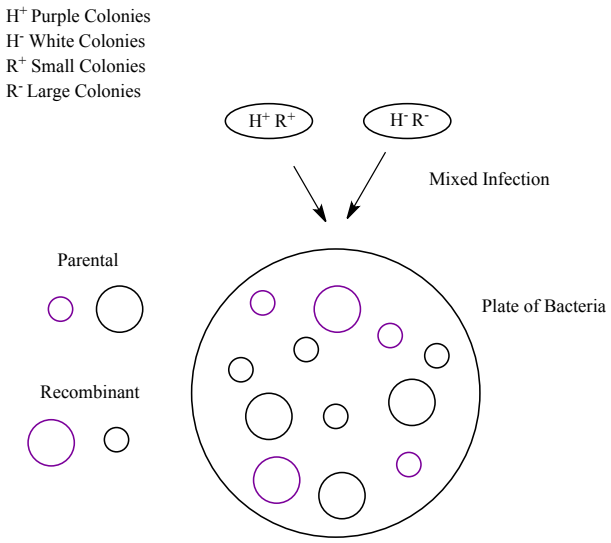
H^+ creates purple colonies, and R^+ creates small colonies

- Colonies will appear different than parental if they have recombined

- H^+/R^- or H^-/R^+

- RF = # number of recombined colonies / # number of colonies

EXAMPLE:



□ **Intragenic recombination** occurs when recombination occurs _____ a gene

- Usually this results in the ability to map the position of mutations inside a gene

- Benzer studied this in the r11 locus of T4 bacteriophage

- He collected over 20,000 independent r11 mutants, and crossed them

- By collecting the recombinants, he was able to map the individual mutations within the r11 gene

EXAMPLE:

r11 locus

PRACTICE

1. A plaque assay studies viruses through what measurement?
    a. The number of bacterial colonies that grow on a plate
    b. The number of bacterial colonies that are lysed upon infection of a bacteriophage
    c. Comparison between the number and size of bacterial colonies
    d. Rate of Infection

2. A mixed infection of two bacteriophage strains is often used for what purpose?
    a. To sequence bacteriophage genomes
    b. To measure bacteria resistance
    c. To study conjugation
    d. To map bacteriophage genes

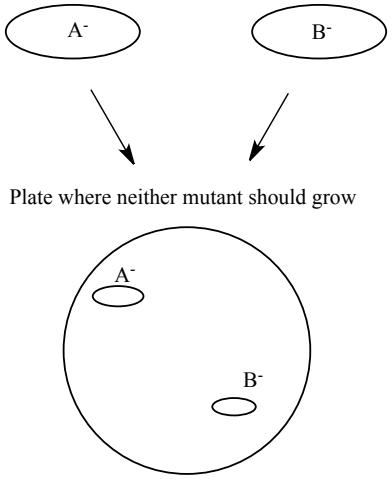
3. A mixed infection of two bacteriophage strains was performed. Infection of bacteriophage strain 1 causes the bacteria to be red and large, while infection of bacteriophage strain 2 causes the bacteria colony to be black and small. The following results were obtained. Using this data, determine the distance between the color and size genes.
    a. 45.3 m.u
    b. 15.7 m.u
    c. 7.9 m.u
    d. 2.2 m.u

Phenotype	# of Colonies
Red, Large	300
Black, Small	280
Red, Small	30
Black, Large	20

CONCEPT: TRANSDUCTION

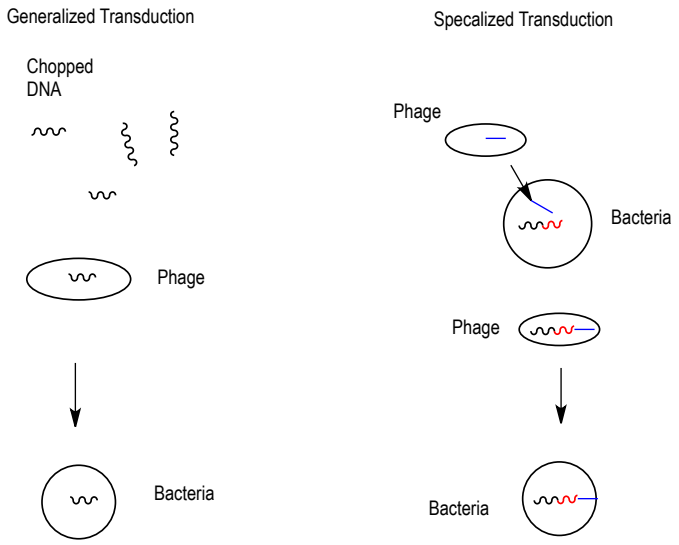
- **Transduction** is the process of a bacteriophage transferring foreign DNA into a bacterium
  - □ Discovered by Lederberg and Zinder in 1951
    - Two mutant *E.coli* strains that they _____ together
    - They plated the mixed *E.coli* on conditions where neither would grow (thinking they would die)
    - BUT, around 1 in 10^5 *E. coli* cells did grow – meaning that some kind of DNA transfer had to be occurring
      - Proved it wasn't conjugation by using a filter to prevent it – Found that it was a phage

EXAMPLE:



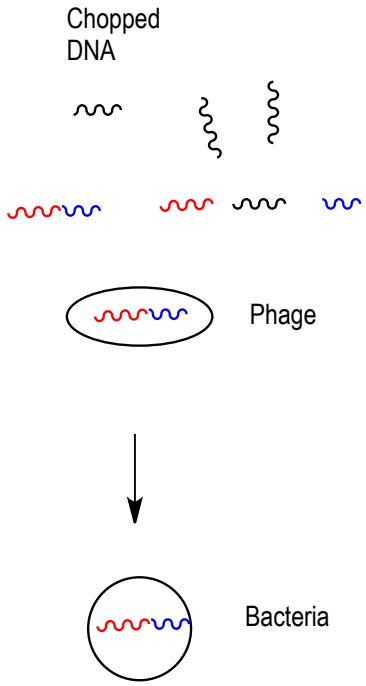
- There are two _____ of transduction
  - □ **Generalized transduction** is able to transfer any part of a bacterial chromosome
    - Lysed bacteria released cut up DNA into the environment – and a phage takes it up
    - Phage then transfers this into another cell
  - □ **Specialized Transduction** is able to transfer only specific parts of a bacterial chromosome
    - A specialized **transducer** inserts into only one place in the bacterial chromosome
      - When it is stimulated to leave, it picks up nearby genes

EXAMPLE:



- Generalized transduction can be used to _____ genes and study linkage
  - The closer two genes are, the more likely they'll be transduced together
    - **Cotransduction** occurs when a single bacteriophage carries more than one gene loci
  - The **cotransduction frequency** measures how often two gene loci are cotransduced
    - The closer two genes are, the more likely they'll be cotransduced together

EXAMPLE:



PRACTICE

1. True or False: Transduction uses viruses to transfer foreign DNA into bacteria
    a. True
    b. False

2. Specialized transduction differs from generalized transduction because specialized transduction is defined by what?
    a. The ability to only transfer specific DNA molecules
    b. The ability to transfer any DNA molecules
    c. The ability to transfer DNA and protein
    d. The ability to transfer specific RNA molecules

3. A cotransduction experiment was performed with two bacteria strains. The first train has the genotype l^+ g m^+ while the second strain has the genotype of l g^+ m. The researchers found that 46 colonies had cotransduced m^+ with l^+, while only 25 colonies had cotransduced g with l^+. Using this information determine which of the following gene pairs are closest together.
    a. l and m
    b. l and g
    c. m and g