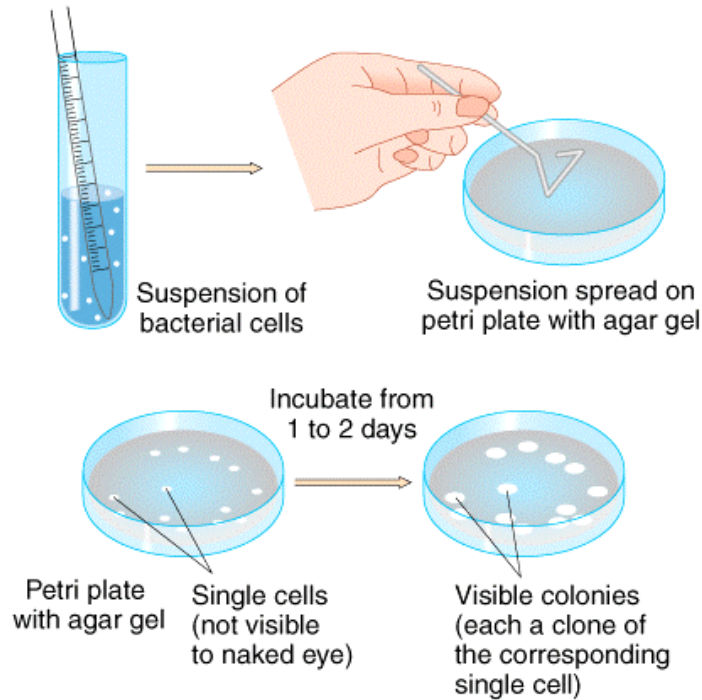


2/20/2002

Reminder: Feb. 28<sup>th</sup>, Dr. Pisano, Tulane Med., MO 157, 12:30

### Gene transfer and mapping in bacteria

Some terms to know: bacteriophages (phages), colony, clone, prototrophic (require minimal media), auxotrophic (require supplemented media).



**7-1** TABLE Some Genotypic Symbols Used in Bacterial Genetics

Symbol	Character or phenotype associated with symbol
<i>bio</i> <sup>-</sup>	Requires biotin added as a supplement to minimal medium
<i>arg</i> <sup>-</sup>	Requires arginine added as a supplement to minimal medium
<i>met</i> <sup>-</sup>	Requires methionine added as a supplement to minimal medium
<i>lac</i> <sup>-</sup>	Cannot utilize lactose as a carbon source
<i>gal</i> <sup>-</sup>	Cannot utilize galactose as a carbon source
<i>str</i> <sup>r</sup>	Resistant to the antibiotic streptomycin
<i>str</i> <sup>s</sup>	Sensitive to the antibiotic streptomycin

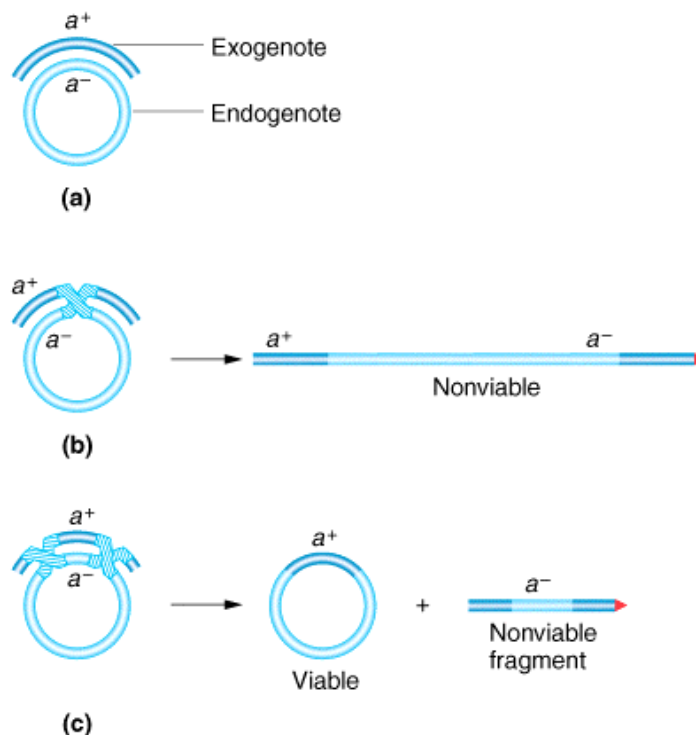
**Note:** Minimal medium is the basic synthetic medium for bacterial growth without nutrient supplements.

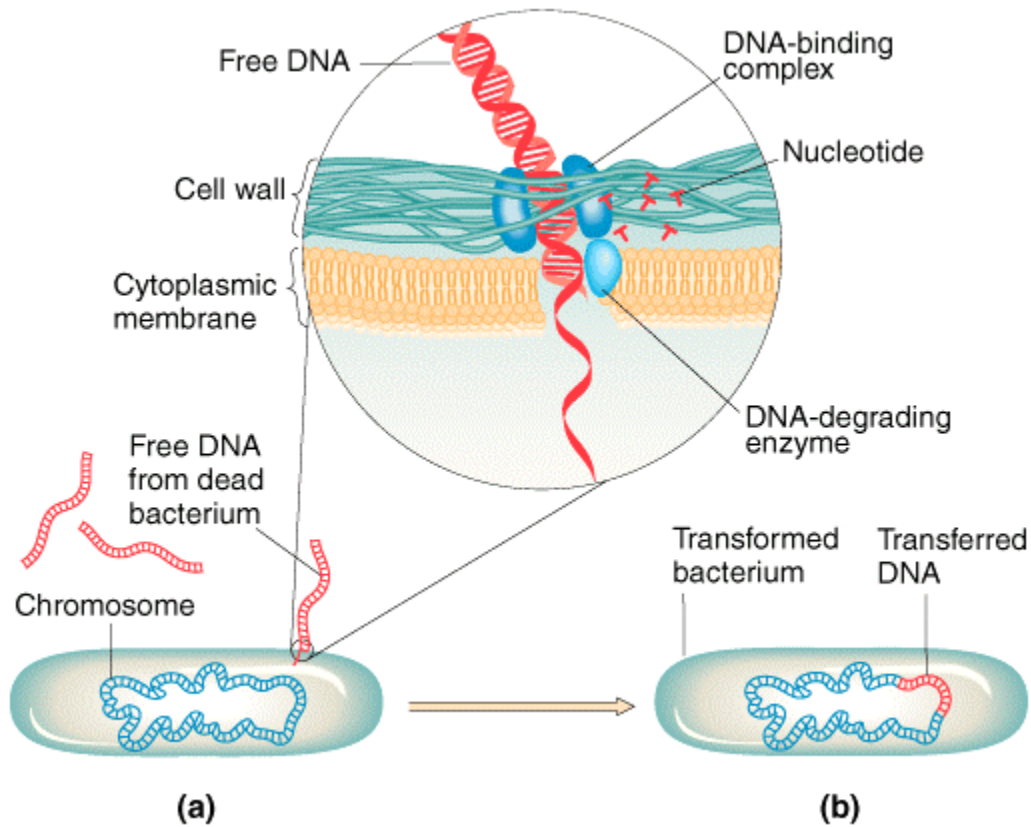
Methods of bacterial gene transfer to be studied. All may occur naturally in bacteria

- **Conjugation** - one bacterial cell transfer copies of DNA segments to another cell via **direct cell-to-cell contact**.
- **Transformation** - a bacterial cell can take up **extraneous DNA** and incorporate it into its chromosome
- **Transduction** - a **phage particle** can take up a piece of its host DNA and infect a second bacterial cell. The host DNA from the first cell can be incorporated into the chromosome of the infected cell

### Transformation

- Discovered by Griffith in *Streptococcus pneumoniae*.  
*Definition:* the uptake and stable integration of extraneous DNA from a donor bacterium by a recipient bacterium.
- Can be used to determine gene order by comparing genes two at a time and looking for double transformants.
- Show how homologous recombination involving crossing over must occur





- Do problem 18, p. 237 to demonstrate how relative gene linkage can be determined

Drugs added	# of colonies	Drugs added	# of colonies
None	10,000	BC	51
A	1156	BD	49
B	1148	CD	786
C	1161	ABC	30
D	1139	ABD	42
AB	46	ACD	630
AC	640	BCD	36
AD	942	ABCD	30

Problem 17, p. 236

Donor genotype:  $str^r mtl^+$ ; Recipient genotype:  $str^s mtl^-$

Streptomycin resistance ( $str^r$ ); able to use mannitol as an energy source ( $mtl^+$ )

Transforming DNA	% of cells transformed into		
	$str^r mtl^-$	$str^s mtl^+$	$str^r mtl^+$
$str^r mtl^+$	4.3	0.40	0.17
$str^r mtl^- + str^s mtl^+$	2.8	0.85	0.0066

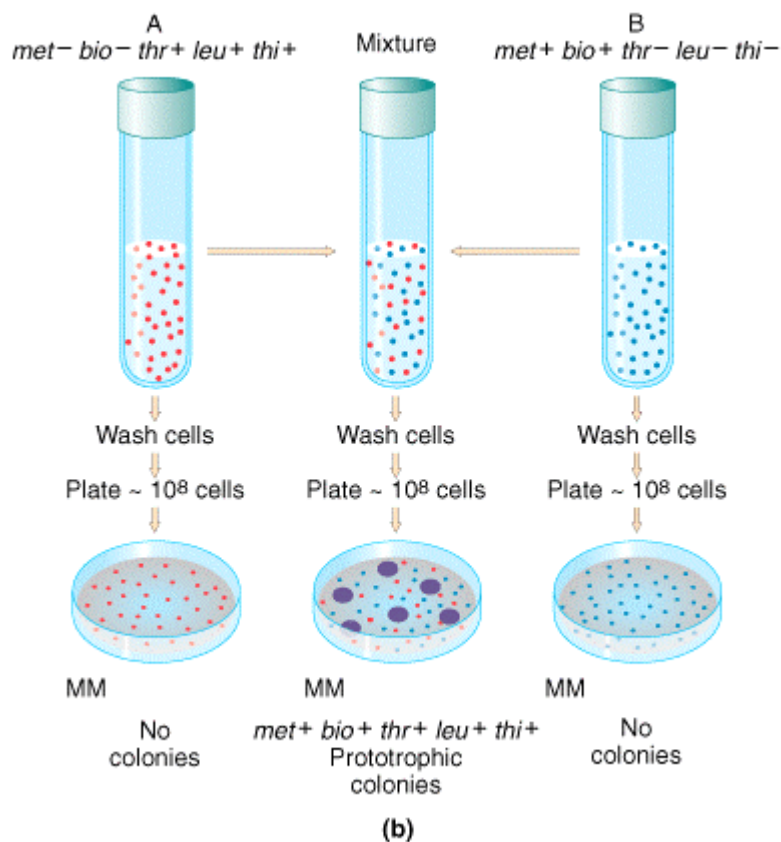
Describe media in each of the three columns of the table.

Conclusions from line 1 of the table?

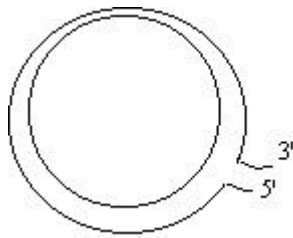
Conclusions from line 2 of the table?

## Conjugation

- Discovered by Lederberg and Tatum in *E. coli*. *Definition:* A mode of unidirectional transfer of genetic information involving direct cellular contact between a donor and recipient cell.



- Relies on the presence of a specific conjugative plasmid in the donor cell. Called the F factor in *E. coli* or simply, F.
- The presence of F causes the cell to be a donor cell and results in the formation of F pili on the outside of the cell wall. The lack of F causes the cell to be a recipient cell and no F pili are produced.
- The pilus is a hollow proteinaceous tube extending from the cell wall. When the pilus comes into contact with a recipient cell, the replication of the F plasmid takes place. Called transfer replication because it normally results in the transfer of a copy of the plasmid into the recipient cell.
- Transfer replication also called rolling-circle replication



- F may be autonomous (F<sup>+</sup> cell) or integrated into the bacterial chromosome (Hfr cells). Both cell types considered donor cells. The plasmid has a single ORI site and near this site is the integration sequence.
- Show how Hfr transfer genes first then F last. Explain crossing over in bacteria