Working with bacterial cultures

Avoid contamination!

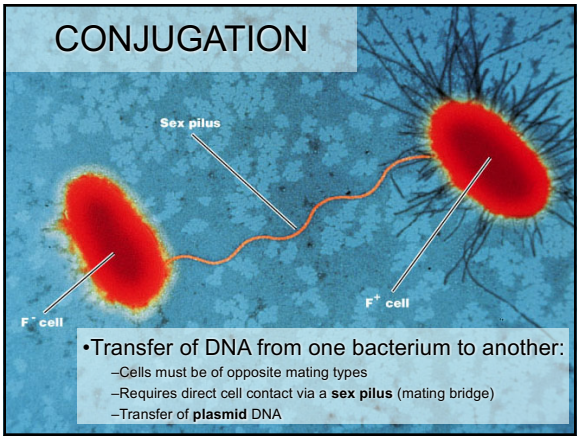
- Disinfect work area before working and again after working
- Keep work area clean and clear of clutter
- Pipette and transfer bacteria carefully
  - Avoid making aerosols
  - Keep instruments sterile — if in doubt, re-sterilize or replace
- **Don't let the world contaminate your experiment**
  — Don't let your experiment contaminate the world!

Working with bacterial cultures

Bacterial culture techniques and media

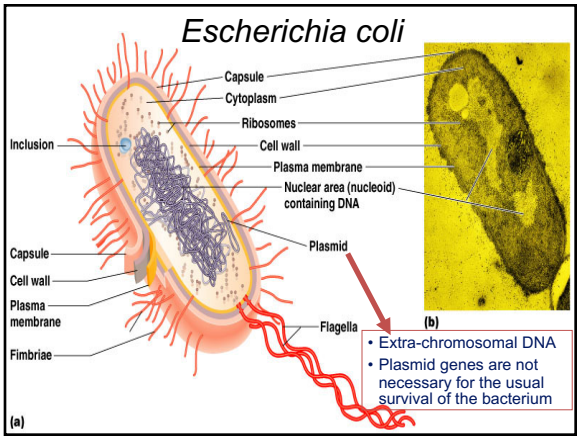
- A culture **medium** (plural: media) is the mix of ingredients used to grow bacteria in the lab. We will be using a recipe called **LB** (Luria-Bertoni), optimized nutrients for rapid growth of ***Escherichia coli*** bacteria.
- **Selective medium**: a specific variation of the medium that allows certain cells to survive or grow, while preventing other cells from growing. For example, when we add an antibiotic to the medium, we are selecting for the growth of antibiotic resistant strains, while preventing antibiotic-sensitive strains.
- When you add cells to a culture, you are **inoculating** the culture.
- **Liquid culture**: the cells are suspended in liquid medium. This is optimal for rapid growth of large number of cells. The cultures are incubated in a shaking incubator so the cells remain suspended with better exposure to nutrients and oxygen.
  - **Warning**: The caps on liquid culture tubes are deliberately loose for gas exchange.
    - Do not pick up tubes by their caps. Be careful not to switch caps on tubes.
    - Do not lay tubes on their sides. Carry tubes in a tube rack!
    - Label tubes (<u>not</u> caps) with tape. When done, remove tape and place upright in disposal rack pending autoclave sterilization.
- **Plate culture**: the media is mixed with warm agar, poured into a plate and cooled so the medium hardens into a gel layer. The cells are usually inoculated onto the surface of the gel. These are good for counting and analyzing bacteria and the resulting colonies and isolating specific clones.

CONJUGATION



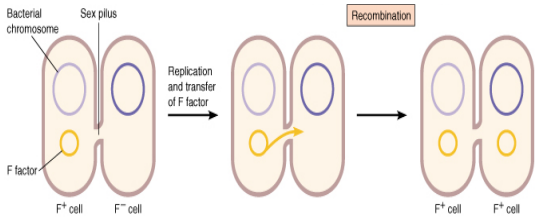
- Transfer of DNA from one bacterium to another:
  - –Cells must be of opposite mating types
  - –Requires direct cell contact via a **sex pilus** (mating bridge)
  - –Transfer of **plasmid** DNA

Escherichia coli



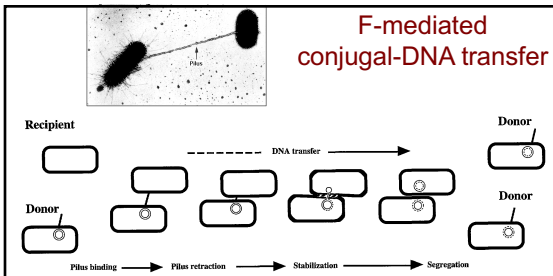
- Extra-chromosomal DNA
- Plasmid genes are not necessary for the usual survival of the bacterium

F plasmid Conjugation

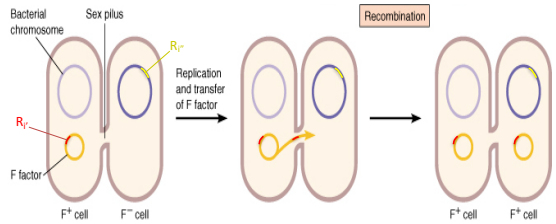


- "F factor" genes for sex pilus and "rolling replication" on F plasmid
- Donors with F plasmids (F^+ cells) transfer copy of plasmid to recipient cells (F^-)
- F^- cell becomes F^+

F-mediated conjugal-DNA transfer



Conjugal transfer of antibiotic-resistance genes (R_x)



- R_F: resistance to antibiotic A, gene on plasmid
  - Original F^+ cell resistant to antibiotic A
- $R_{F'}$: resistance to antibiotic B, gene on on chromosome
  - F^- cell resistant to antibiotic B
- Conjugate F^+ cell resistant to both antibiotics A & B!

Conjugation Experiments

- ***E. coli***, culture I (**cI**): strain **HB101**
  - resistant to streptomycin; sensitive to ampicillin
- ***E. coli***, culture II (**cII**): strain **S17**
  - resistant to ampicillin; sensitive to streptomycin

- **Experiment 1: Qualitative**. Did conjugation occur? Was antibiotic-resistance transferred?
- **Experiment 2: Quantitative**. How efficient was the conjugation process?
  What proportion of the population conjugated?
- **Experiment 3: Descriptive**. Which strain was the plasmid donor, and which was the recipient?