Why we use bacteria for genetic transformation?

• To genetically transform an entire organism, you must insert the new gene(s) into every cell in the organism. Therefore, **a single-celled organism** would be the best recipient for a genetic transformation, because it contains only one cell which needs to take up the new gene.

• Scientists often want to know if the genetically transformed organism can pass its new traits on to its offspring and future generations. To get this information, **an organism which reproduces quickly** would be a better candidate for your investigation. Fast production of offspring or new progeny will allow you to quickly assess if the new trait has been passed on.
Bacterial transformation

• The uptake of DNA by a bacterial cell.
  – Most species of bacteria are able to take up DNA molecules from the medium in which they grow.
  – Often DNA molecule taken up in this way will be degraded.
  – However, if the DNA molecule is a plasmid with an origin of replication recognized by the host, the DNA molecules can survive and replicate in the host cell.
  – Therefore, we can introduce DNA into bacteria after in vitro genetic manipulation.
Different types of transformation

• Natural competence
  – With the pneumococcus, cells spontaneously become competent to take up DNA. (*Bacillus subtilis* and *Hamophilus influenzae*)

• Protoplast transformation
  – Enzymatic removal of the cell wall can expose the cytoplasmic membrane and therefore the cells can take up the DNA in the presence of polyethylene glycol. (*Streptomyces* and *Streptococcus* species)

• Electroporation
  – The bacterial cells are mixed with plasmid DNA and are subjected to a brief pulse of high-voltage electricity.
Induced competence

- Most species of bacteria, including *E.coli*, take up only limited amounts of DNA under normal circumstances.

- In order to transform these species efficiently, the bacteria have to undergo some form of chemical treatment that will enhance their ability to take up DNA.

- Cells that have undergone this treatment are said to be competent.
Preparation of competent *E. coli* cells

Mid-log suspension culture

Competent cell yield
- 10% of mid-log cells

Timing of culture
- lag phase: 30 min after inoculation, for about 60 min
- log phase: the number of cells doubles every 20-25 min
- stationary phase: when the conc. reaches $10^9$ cells/ml
- death phase: waste products accumulate
Preparation of competent *E. coli* cells

- Soak the *E. coli* cells in an ice-cold salt solution (50 mM calcium chloride).
  - Neutralizes negative charges of the phosphate backbone of the DNA and the phospholipids of the cell membrane, allowing DNA to enter the cells
    - Cause the DNA to precipitate on to the outside of the cells
    - Change the cell wall property for better DNA binding.

- Heat shock the cells (42°C, 2 min)
  - Move the DNA into competent cells.
Recovery process

- If the transformed cells are plated on to the selective medium immediately after the heat-shock treatment, then only a limited number of the cells will survive.

- Place in a small volume of liquid medium, in the absence of antibiotic, and incubate for a short time to allow plasmid replication and expression to get started, and then plate on to the selective medium.
Selection for transformed cells

- Make use of a selectable marker carried by the plasmid.

- Plate cells on to an agar medium that contains the relevant antibiotic.

- The growth of *E.coli* colonies on the selective medium will confirm the antibiotic-resistance phenotype and the molecular genotypes of recombinant plasmids.

Figure 5.4 Selecting cells that contain pBR322 plasmids by plating on to agar medium containing ampicillin and/or tetracycline.