Transformation

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- Transformation refers to the gene transfer technique – in which, a host cell is transformed, by the introduction of a foreign DNA into it for expression and maintenance.
- As a general rule, cells do not accept extracellular DNA into them, because DNA is highly hydrophobic.
- During certain conditions, the cells get physiologically ready, to take up extracellular DNA. Such cells are termed. **Competent cells** and the process is termed **competency**.
- Competency in cells can be artificially induced by certain methods. This is termed **artificial transformation**.

- Some of the methods are as follows:

1. Heat-shock method of inducing artificial competence.
2. Electroporation (Electropermeabilization).
3. Microinjection.
4. Transfection.

Heat shock method of inducing artificial competence:

- This protocol is based on the principle that a sudden increase in temperature creates small transient pores on the cell wall of bacteria – allowing plasmid DNA, to enter the cell.
- The heat-shock method of bacterial transformation is carried out in a calcium-rich medium, using calcium chloride.
- This is the most efficient method for competent cell preparation.
- Addition of $CaCl_2$ to the cells, allow the binding of both the negatively charged DNA and the LPS of the cell.
- Thus, $Cacl_2$ counteracts the electrostatic repulsion between the DNA and the LPS.

- The steps involved include:
  A. Preparation of Competent cells
  B. Transformation by heat-shock
- To a mid-log phase culture of *E. coli* cells, $Cacl_2$ is added and incubated in ice for 30mnts. The DNA is added and the tubes are then transferred to a 42°C waterbath, for 2 minutes and then returned swiftly to ice.
- The contents are transferred to a 2ml LB broth and incubated at 37°C, in a shaker for 60-90 minutes.
- Then the cells are plated in LB agar plates containing selectable antibiotics (Kanamycin or ampicillin).

2. Electroporation (Electropermeabilization):

- Electropermeabilization is a technique for introducing genes into microbial, plant or animal cells.
- In this technique, a mild potential difference (pd) – is applied across the cell membrane of the cells.
- This results in the formation of temporary pores on the membrane, making the cells competent (ready to take up extracellular DNA).
- The new gene enters the host cell and get expressed, making the host cell – stably transformed.

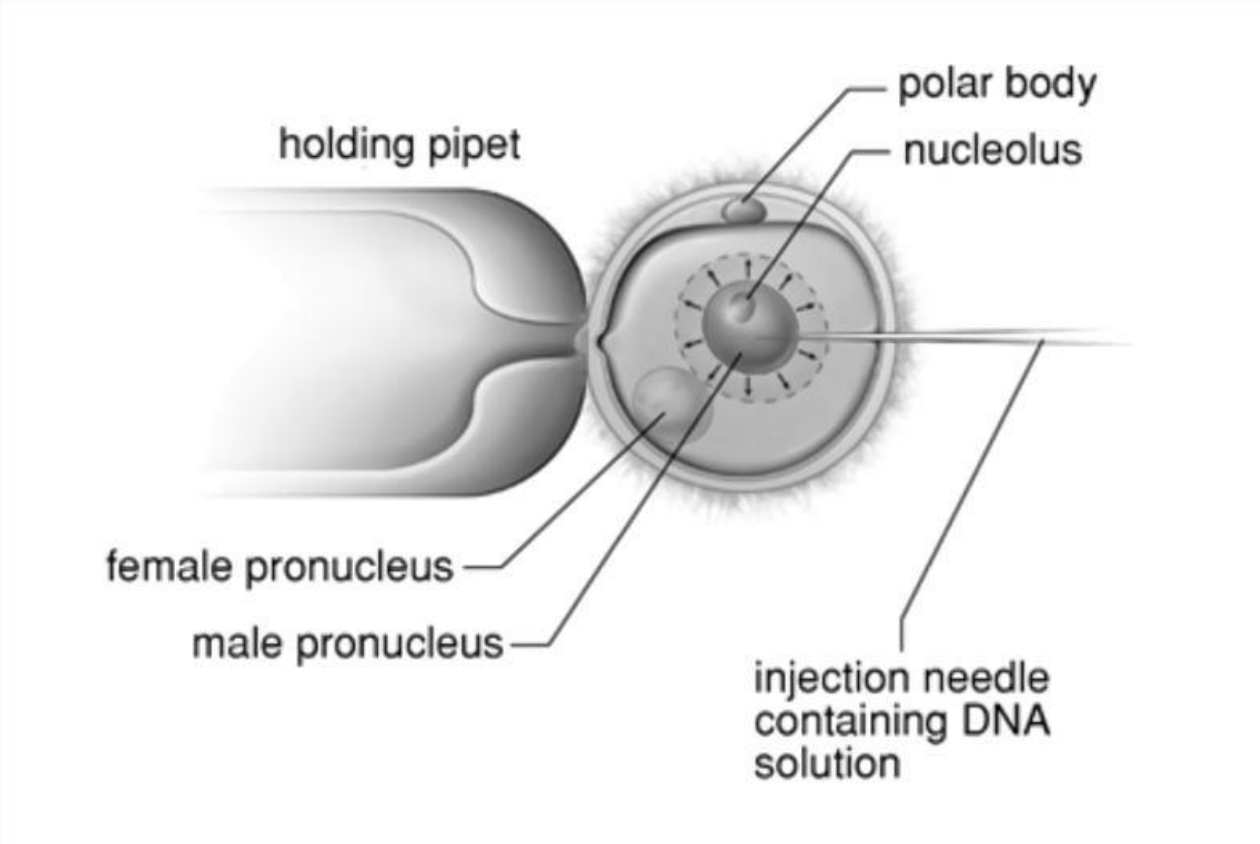
- Many factors affect the efficiency of transformation like temperature, volts, growth conditions, etc..
- Electroporation is a highly efficient method to introduce foreign DNA into tissue cultures.
- The process of introducing foreign gene into eukaryotic cells is called **Transfection**.
- Electroporation is done using electroporators – which can create an electrostatic field in a solution.
- The bacterial culture to be transformed, is taken in a glass or plastic cuvette – which is connected to two aluminium electrodes.

- The cell culture is mixed with the gene or plasmid and the mixture is pipetted into the cuvette.
- The voltage is set and applied for the specified time.
- Then the cells are plated immediately on an agar medium.

3. Transformation by Microinjection:

- DNA microinjection – also known as pronuclear microinjection, is a gene transfer technique in animals, for the creation of transgenic animals.
- It is a microsurgical procedure, conducted on a single cell.
- After fertilization of an animal (mice) egg, the male and female pronuclei (Pronucleus – the haploid nucleus of a male or female gamete, at the time of fusion, with the other gamete in fertilization), remain separated, for a few hours, before they fuse to produce the zygotic nucleus.

DNA microinjection



- This stage facilitates microinjection of the desired genes into the larger male pronucleus.
- Eggs that survive the injection (without destruction), are then transferred into a foster mother.
- The process involves the use of a glass needle, a micromanipulator (a positioning device) and a microinjector (micropipette).
- The micropipette is loaded with the genetic material and it uses hydrostatic pressure, to deliver the gene into the target cell, using a microscope.
- The steps are as follows:

1. The no. of available fertilized eggs that are to be injected, are increased, by stimulating the females to super-ovulate.
2. The super-ovulated females are mated and then killed.
3. The fertilized eggs are flushed from their oviducts.
4. Microinjection is carried out immediately after collection. The larger male nucleus is identified, using a dissection microscope.
5. A foster mother is induced pseudopregnancy by mating with a vasectomized male.
6. The eggs are then planted microsurgically, into the foster mother.

4. Transfection:

- Transfection refers to the process of transfer of extracellular genes (DNA) into eukaryotic cells.
- It can be done by physical, chemical or lipid-based methods.
- Cells – that have a foreign gene – that has been introduced by transfection, are termed transfectants.
- There are 2 types of transfectants – Stable transfectants and Trasnsient transfectants.
- Stable transfectants have the foreign DNA – integrated into their DNA
- Transient transfectants do not have the foreign DNA integrated into their DNA and so the foreign genes are expressed, only for a short period.
- Transfection can also be done by **bactofection** (infecting an animal cell with a bacterium), virus-mediated transfer, lipofection (lipid-mediated gene transfer), etc..