Gene transfer

II Physical method :-

1) Electroporation-

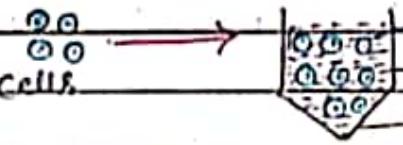
→ Electroporation is a process that is used to introduce foreign genes into a host cell.

→ It is based on the principle that high voltage electric pulses can induce all plasma membrane to fuse or also induce the cellular uptake of exogenous DNA (pores are created by this method)

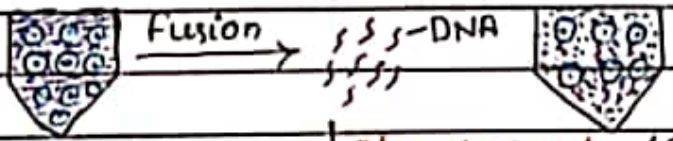
→ It is a simple or rapid technique for introducing genes into cells from various organism (microorganis, plant & animal).

⁂ procedure :-

cells are taken in solution in special cuvette.

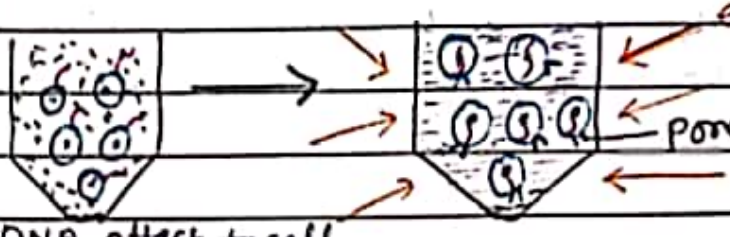


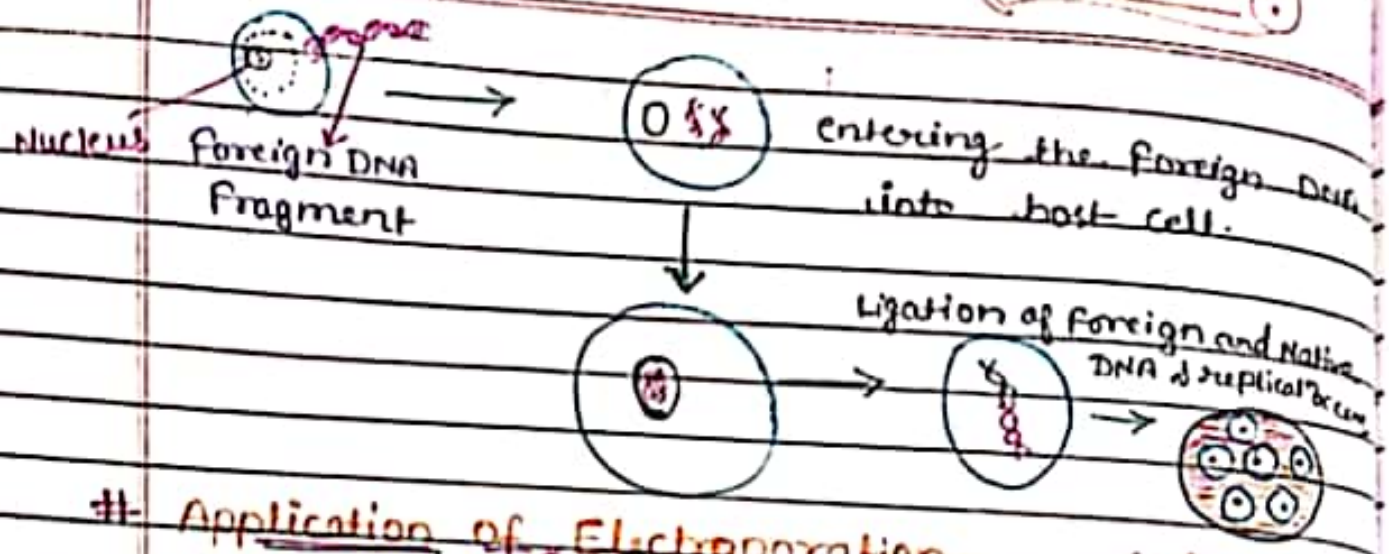
Fusion of foreign fragment of DNA which is desired and solution that contain cell.



Electric field (5sec electric shock is applied for)

Electric field is applied on the cell to increase permeability & create pore into the cell.





Application of Electroporation-

⇒ Electroporation is widely used in many areas of microbiology research and in medical field.

⇒ Formation of pores by high voltage pulse in lipid bilayers overcome the barriers of cell membrane.

⇒ It is an efficient process to transfer DNA into cells.

⇒ Electroporation has been reported to enhance the level of gene expression.

⇒ This technique significantly improve immune responses elicited to DNA vaccines in both small and large animals.

Advantage of electroporation-

1. Method is fast.
2. Less costly.
3. Applied for a number of cell types.
4. Simultaneously large no. of cells can be treated.
5. High percentage of stable transformants can be produced.

2) Microinjection :-

Microinjection is a technique of delivery of DNA into living cells (egg, foreign cacyle, embryo) through a glass micropipette.

⇒ The DNA solution is directly injected inside the nucleus of cell.

⇒ It uses glass capillary micropipette with the micromanipulators of microinjection assembly.

* principle -

Direct and precise delivery of DNA into the plant cells or its nucleus using microsyringe.

* Procedure.

1) 1st one end of glass micropipette is heated until the glass becomes liquid stressed.

2) It is quickly which forms a very fine tipat the heated end.

3) The tip of the pipette attened about 0.5 m diameter just like injection needle.

4) The process of delivery of foreign DNA done under powerful microscope.

5) cells to be microinjected are placed in container.

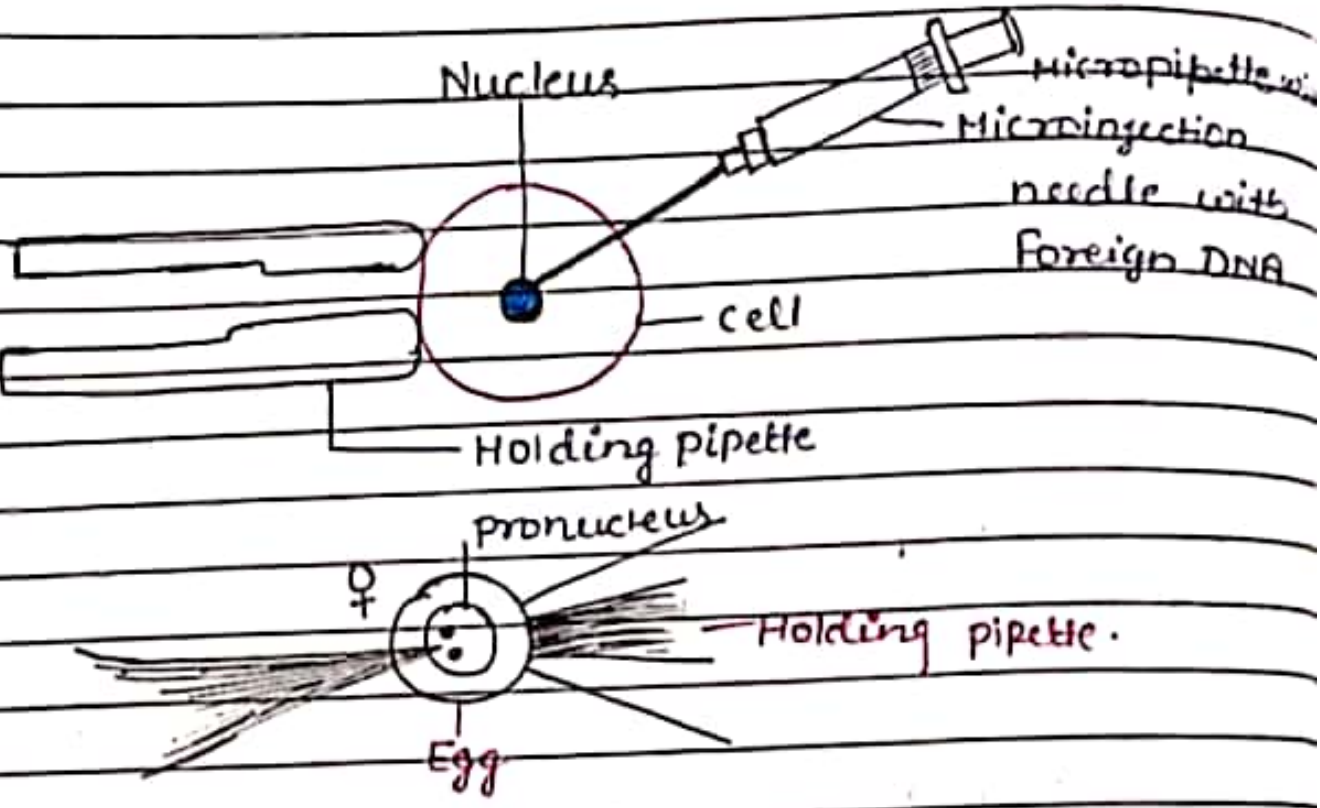
6) A holding pipette is placed in the filled of view of the microscope.

7) The holding pipette hold a target cell at the tip gently.

8) The tip of micropipette is injected the membrane of the cell.

9) Contents of needle are delivered into cytoplasm and the empty needle is taken out.

Fig - P.T.O



⇒ Transfer of DNA in early blastocyst stage by glass microneedle.

pronuclear Microinjection - It means before fusion of male nuclei with female nuclei.

Application of Microinjection:-

⇒ It is generally used for culture cells.

⇒ This process is applicable for plant cell as well as animal cell but more common in animals.

⇒ Technique is ideally used for producing transgenic animals quickly.

⇒ procedure is important for gene transfer to embryonic cells.

⇒ This technique is direct introduction of rDNA into host cell.