✡ GENE TRANSFER TECHNIQUE

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→ In 1900 century transformation technique was discovered.

→ After discovery of this technique various gene transfer methods have been developed.

Because of some problems desired DNA can not be enter inside the cell directly.

→ That's why scientists has developed so many techniques for DNA transfer known as transfection technique <in animal cell>.

→ There are 2 types of transfection techniques :

(i) Chemical
(ii) Physical

→ A special type of gene transfer technique known as biological gene transfer technique done via virus mediated also called transduction technique in animal cells.

(i) Chemical Methods

→ This technique use various chemicals for transfering desired DNA into

host cell.

** Types of Chemical Methods

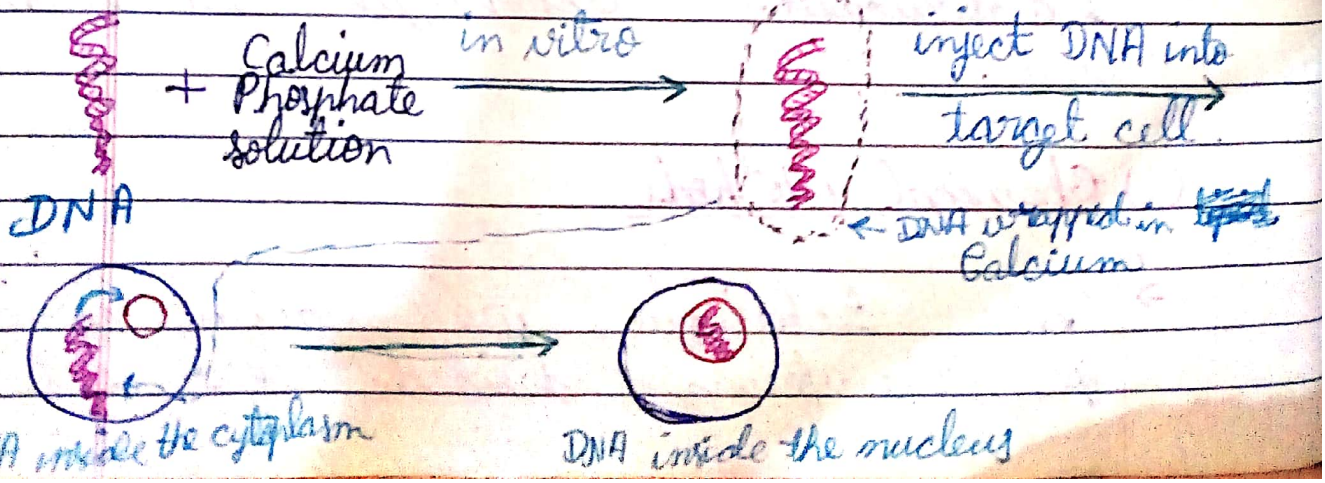
1) $CaCl_2$ mediated gene transfer technique

→ Take the desired DNA.

→ Leave this DNA in the prepared mixture of Ca^{++} and P^-.

→ This results in the coating of DNA by Ca^{++}.

→ Then this DNA (coated by Ca^{++}) can be easily transferred through the cell membrane because the lipid bilayer has no net charge so it can accept positively charged DNA easily. DNA is positively charged here because of the covering of Ca^{++} around it making it positively charged.



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27 Liposome ~~& Lipofection~~ mediated gene transfer technique

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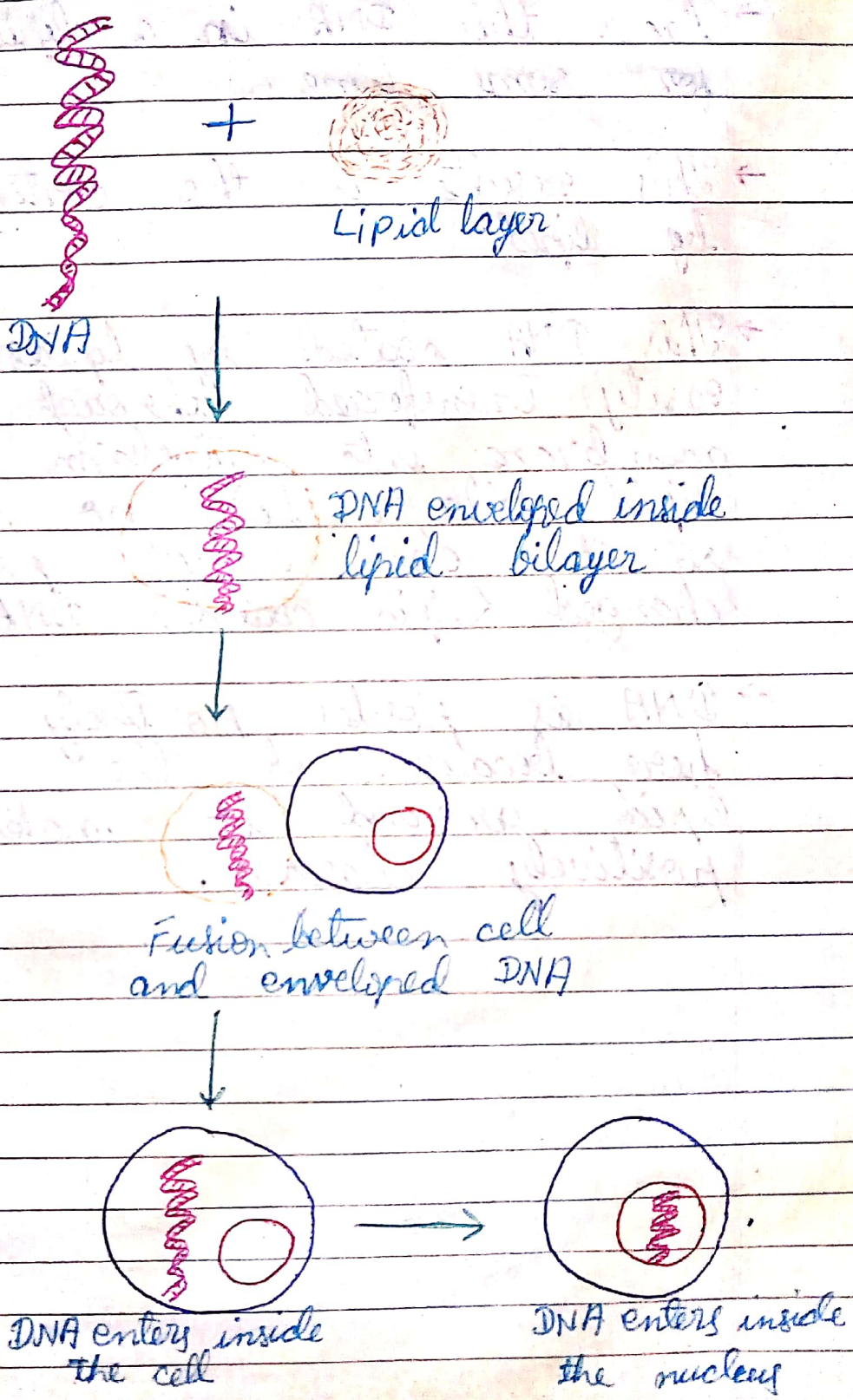

Sig.:- ~~LIPOFECTION OR~~ LIPOSOME MEDIATED GENE TRANSFER TECHNIQUE

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→ Take the desired DNA.

→ Leave this DNA in a lipid solution for some time.

→ This results in the coating of DNA by lipid.

→ This DNA coated by lipids can be easily transferred through the cell membrane into cytoplasm because the lipid bilayer has no net charge so it can accept positively charged (lipid coated) DNA easily.

→ DNA is posses positively charged here because of the coating of lipid around it making it positively charged.

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3) Lipofection or Phospholipid mediated gene transfer technique.

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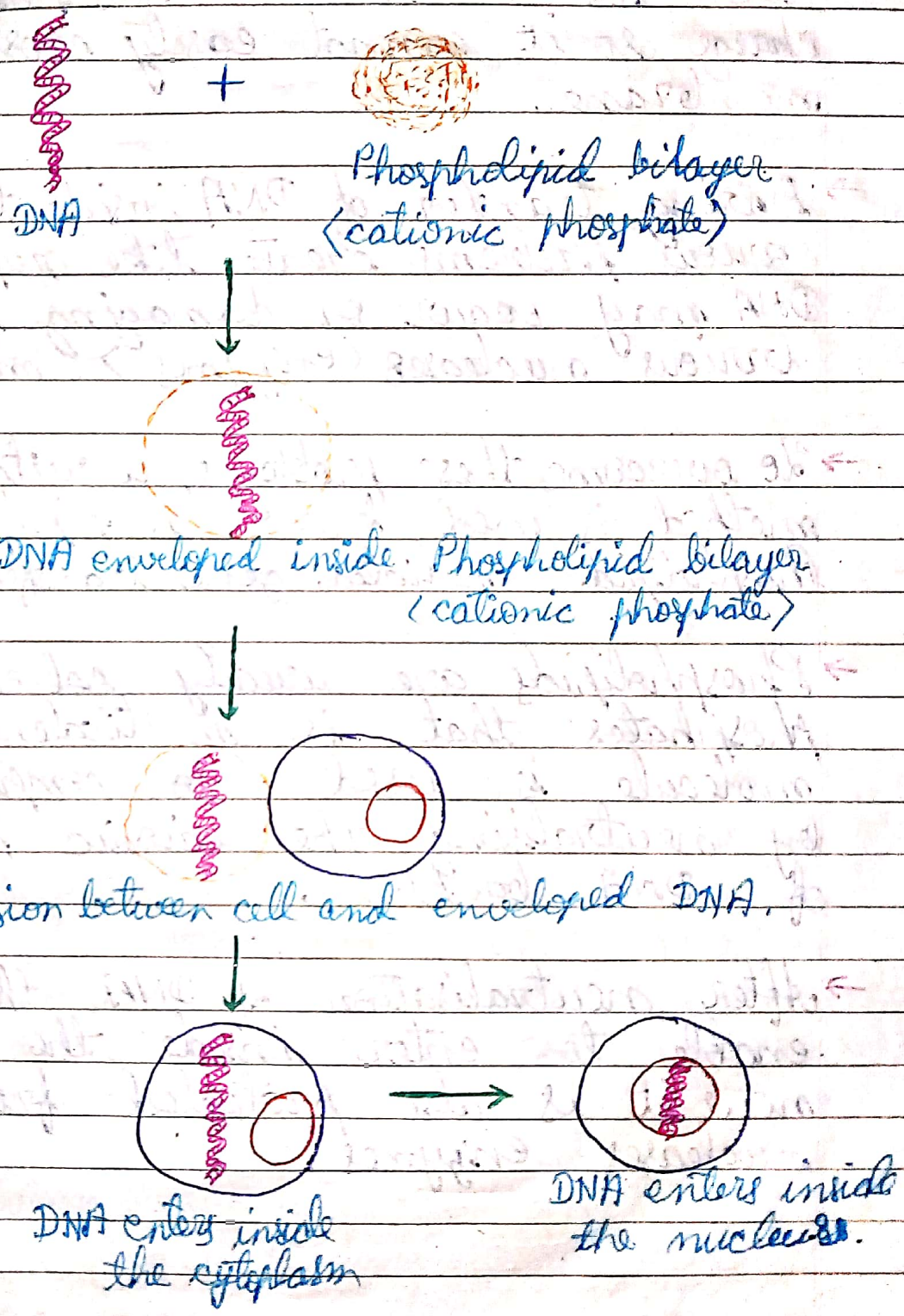

Fig.:- LIPOFECTION OR PHOSPHOLIPID MEDIATED GENE TRANSFER TECHNIQUE

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→ Take the desired DNA.

→ We know that DNA has ~~positive~~ negative charge so it cannot easily cross the cell membrane.

→ During transfer of DNA inside the cell, various problems creates like rupturing of DNA may occur or damaging of DNA by various nucleases (enzymes) may also occur.

→ To overcome these problems, a suitable chemical method is used known as Lipofection or Phospholipid mediated gene transfer method.

→ Phospholipids are usually ~~colour~~ cationic phosphates that is multivalent cation molecule is used to compact DNA by neutralising the anionic phosphates of DNA backbone.

→ After neutralisation of DNA, this DNA is enable to enter inside the cell and now it is also protected from different nucleases enzymes.

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4) DEAE dextran mediated gene transfer technique

→ Diethyl ammonium ethyl dextron is a cationic carbohydrate molecule.

→ We know that DNA has ~~positive~~ negative charge so it cannot easily cross the cell membrane.

→ During transfer of DNA inside the cell various problems creates like rupturing of DNA may occur or damaging of DNA by various nucleases (enzymes).

→ To overcome these problems a suitable chemical method is used known as DEAE dextran method.

→ In this method DEAE dextran containing solution is mixed with recombinant DNA or desired DNA fragment solution.

→ Because DEAE dextran is a type of cationic carbohydrate so it easily neutralise the negative charge of phosphate bond present on DNA.

→ When DNA incorporated with DEAE dextran then it becomes suitable particle to enter inside the selected cell or target cell.

→ After neutralising charges of DNA by DEAE dextran target cells are allowed to fuse with these neutralized DNA fragments.

→ After sometimes we saw that DNA enters inside the selected cell and finally, it transfer inside the nucleus.

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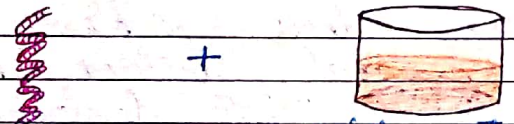
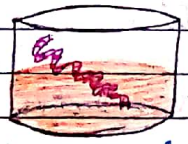
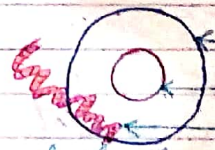




DNA inside the solution containing DEAE dextran

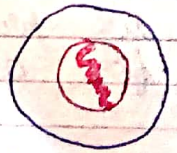
Neutralisation of the negative charge of phosphate bond present in DNA by DEAE dextran.

Neutralized DNA ready to incorporate into target cell.



Fusion of neutralised DNA and target cell

Neutralised DNA inside the cell.



Neutralised DNA inside the nucleus.

Fig:- DEAE DEXTRAN MEDIATED GENE TRANSFER TECHNIQUE

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(ii) Physical Methods

→ This technique use various physical methods for transferring desired DNA into target cell.

✱ Types of Physical Methods

1) Electroporation

→ Electroporation is a gene transfer technique in which a high electrical pulse is generated for generating pores through which the DNA enters inside the cell.

→ In this technique we select a (target) suitable cell and a desired DNA.

→ In vitro condition we allow for fusion of both cells and DNA.

→ For this purpose, we provide high electrical pulses for a few seconds (5-6 seconds)

→ Due to high electrical pulses, pores are created inside the cell membrane. ~~membrane~~ membrane.

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→ From these pores, our desired DNA fragments ~~or~~ enters inside the cell.

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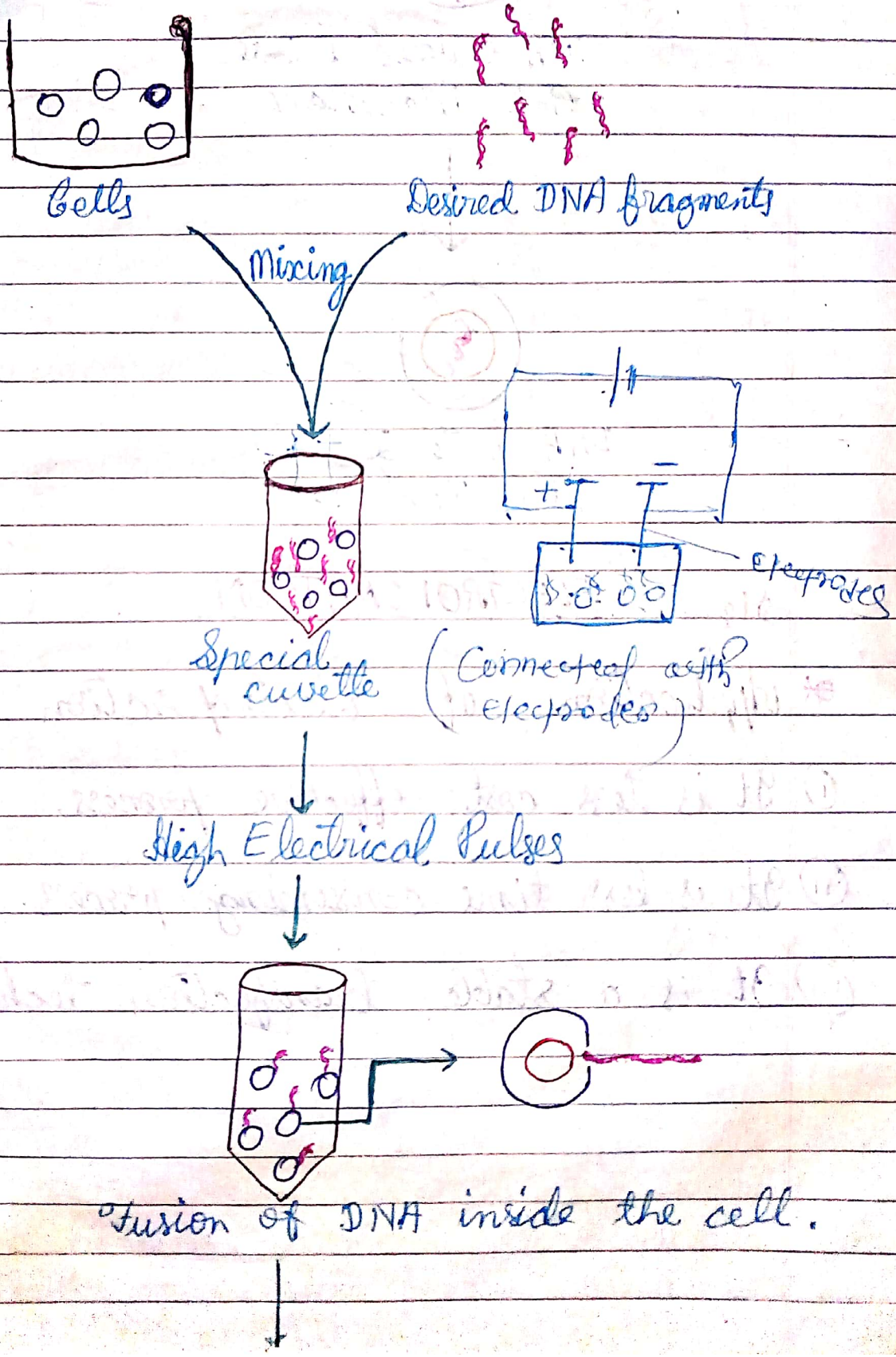

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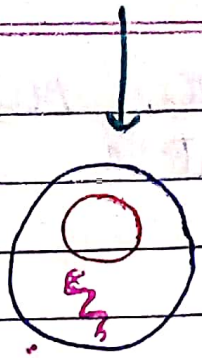
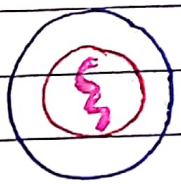


DNA entered inside Cell Membrane



DNA entered inside the Nucleus.

Fig :- ELECTROPORATION

Applications of Electroporation

(i) It is less cost effective process.

(ii) It is less time consuming process.

(iii) It is a stable transfection technique.

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2) Microinjection

→ Microinjection is a technique of delivering foreign DNA into a living cell (a cell - egg, oocyte, embryo of animals) through a glass micropipette.

* Procedure of Microinjection

(i) First of all we take a glass micropipette which is heated at one end upto it comes on semi-liquid state.

(ii) Streatching of glass micropipette at heated end to make a pointed tip just like an injection syringe.

(iii) A holding pipette holds the target cell that is used.
Target cell is placed in a container.

(iv) Glass pipette is called micropipette or mor microinjection that contains our recombinant DNA or desired DNA.

(v) The process of delivering foreign DNA is done under a powerful microscope.

(vi) A holding pipette is placed in the field of view of the microscope.

(vii) The holding pipette holds a the target cell at the tip when gently sucked.

(viii) The tip of the micropipette (0.5 mm diameter) is injected to the membrane of the cell.

(ix) Contents (DNA) of the needle are delivered in the cytoplasm and the empty needle is baked taken out.

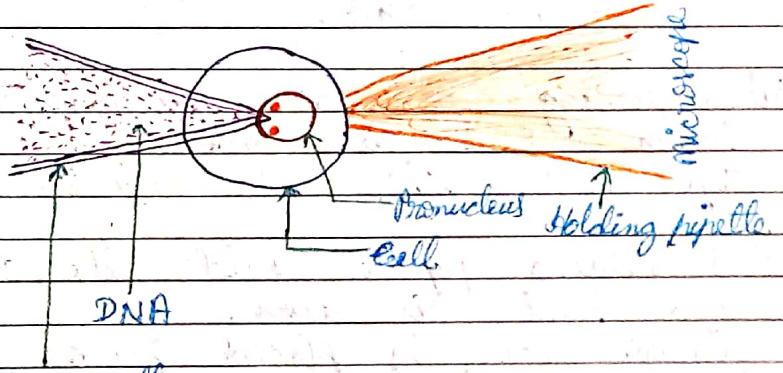

fig:- MICROINJECTION

* Applications of Microinjection

(i) It is a suitable gene the delivery method (Physical method).

(ii) This method is mostly used during animal gene transfer because here is no any cell barrier, but this technique is not successful in plant cells.

(iii) This is the very less time consuming technique that's why it is used at large scale in recombinant DNA technology field for the production of various desired products (vaccines clones).

Important :

(iv) It is the only technique where our recombinant DNA can be directly transferred inside the nucleus of the target cell.

(v) It is cost effective process than electroporation process.

(vi) By using this technique, we can apply quickly produce a desired product during any need or at adverse situation

like any uncommon diseases when come in future.

(vii) It is the most stable process than other physical processes.

3) Particle Bombardment method of gene transfer technique

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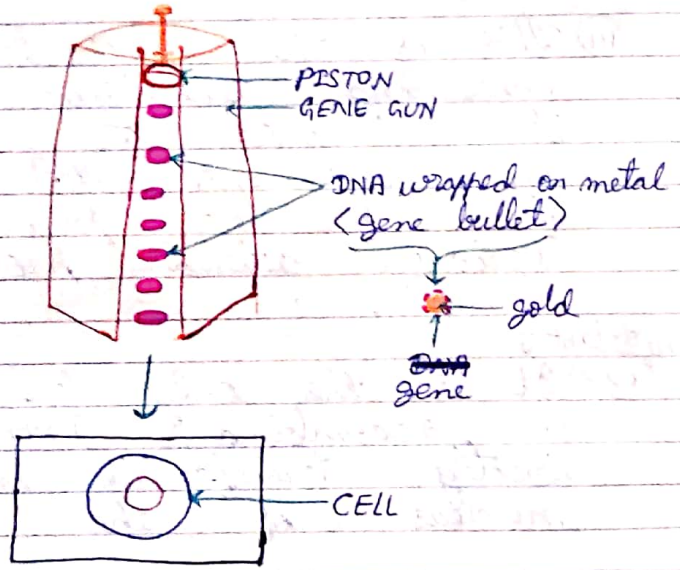

fig:- PARTICAL BOMBARDMENT METHOD
Gene-gun Method in Plants

→ Particle bombardment method has evolved into a useful tool for that allows direct gene transfer to a broad range of cells and tissues.

→ Particle bombardment method is one of the technologies for introducing foreign gene into cells.

→ This technique involves was developed by John Sanford and co-workers at Cornell University in the United States.

→ ~~But~~ This technique involves accelerating DNA-coated particles (microprojectiles) directly into intact tissues or cells.

→ Gene gun is made up of a piston and the whole setup containing the DNA wrapped on metal (called gene bullet).

→ The metal which is used ~~to~~ mostly for wrapping the DNA is gold.

→ DNA wrapped in gold acts like gene bullet. ~~is~~ This bullet is then bombard by the piston of the gene gun and made

it to strike on the cell at a high speed. These high speed bullets first enters in the cell and then enters inside the nucleus of the cell where it has to be integrated to its native DNA.

(iii) Biological Methods

→ Biological method is a method of direct gene transfer using bacteria into the target tissue, cell, organ or organism.

→ In Biological methods of gene transfer technique there comes:
i) Plasmid vector for DNA mediated gene transfer.
ii) Retrovirus mediated gene transfer
iii) SV-40 virus mediated gene transfer

→ In all biological methods of gene transfer technique ~~we use~~ vectors are used for transferring the genetic material

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from one organism to another.

→ Biological Method is the most suitable method for gene transfer; this means that it's success rate is high.

* Plasmid vector for DNA-mediated gene transfer technique

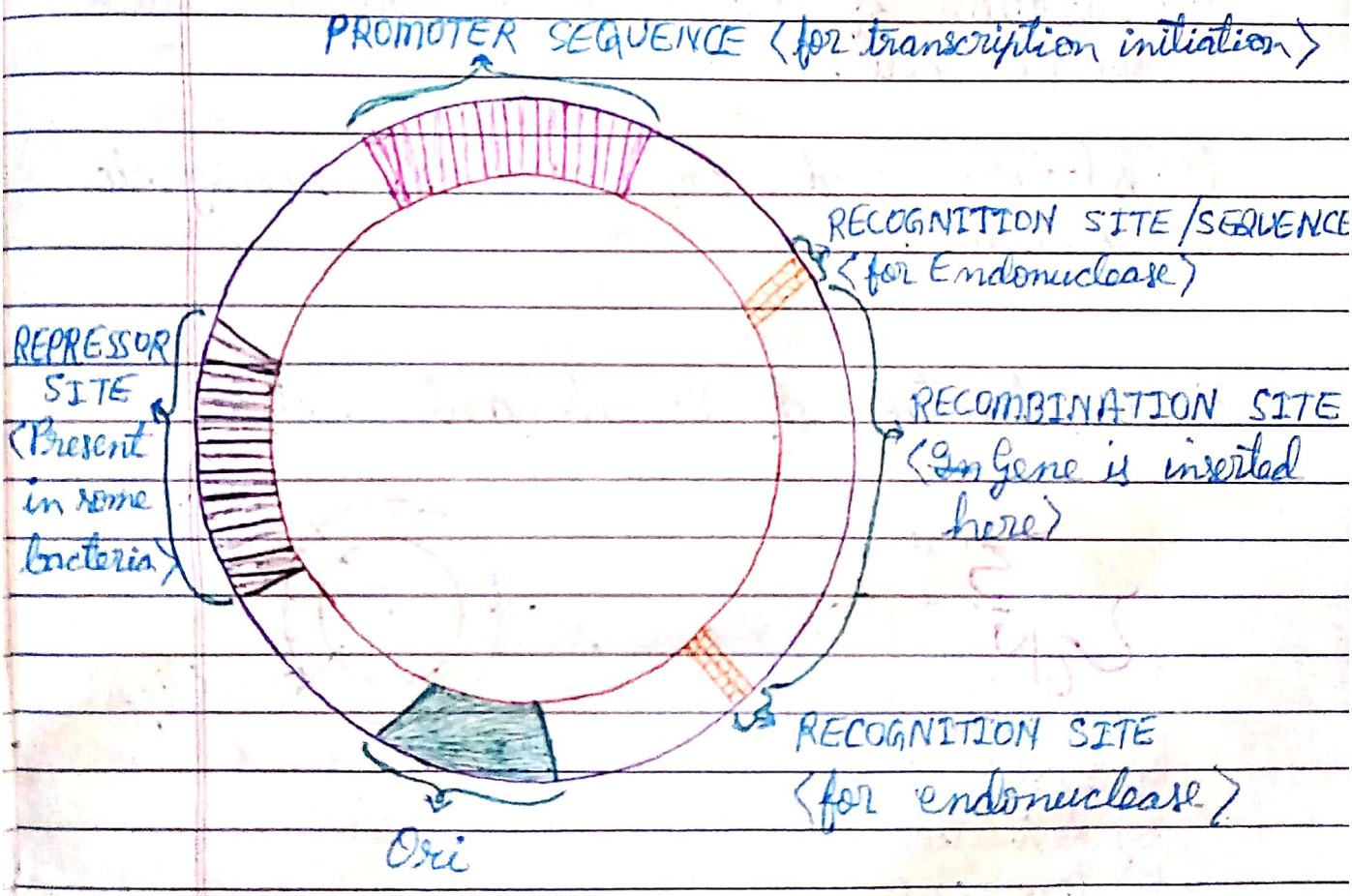

fig:- PLASMID VECTOR