INTRODUCTION OF PHAGE DNA INTO BACTERIAL CELLS

- There are two different methods by which a recombinant DNA molecule constructed with a phage vector can be introduced into a bacterial cell.

1. Transfection
2. invitro packaging

TRANSFECTION

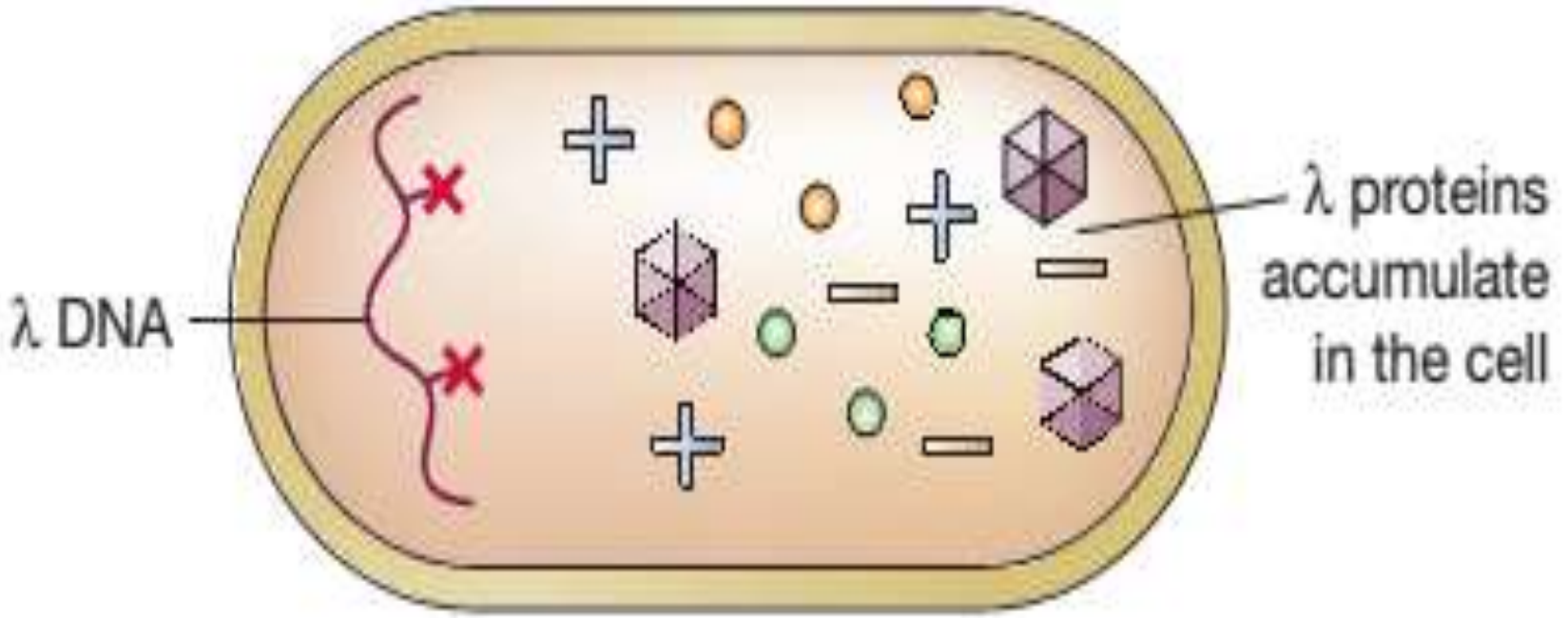
- Transfection is equivalent to transformation, the only difference being that phage DNA rather than a plasmid is involved.
- The purified phage DNA or recombinant phage molecule is mixed with competent E. coli cells and DNA uptake is induced by heat shock.
- Transfection is the standard method for introducing the double stranded RF form of an M13 cloning vector into E. Coli.

IN VITRO PACKAGING OF λ CLONING VECTORS

- Transfection with λ DNA molecules is not a very efficient process when compared with the infection of a culture of cells with mature λ phage particles.
- It would therefore be useful if recombinant λ molecules could be packaged into their λ head-and-tail structures.
- Packaging requires a number of different proteins coded by the λ genome.
- These can be prepared at a high concentration from cells infected with defective λ phage strains.
- Currently two different systems are in use.

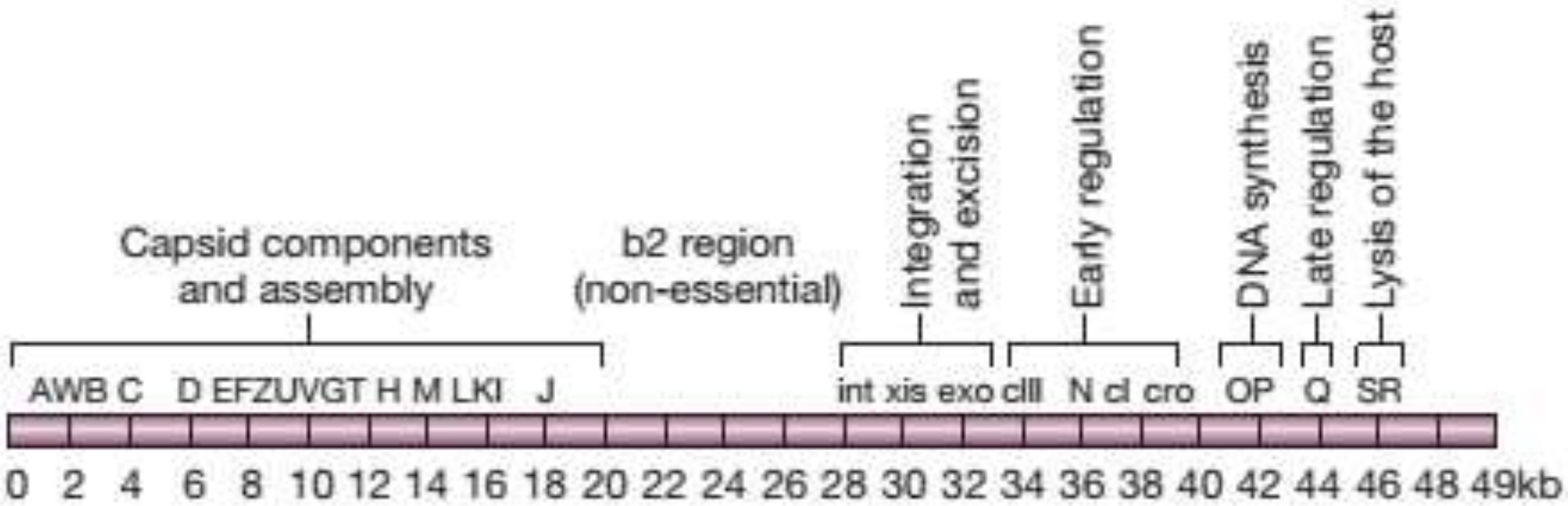
- With the single strain system, the defective λ phage carries a mutation in the cos sites, so that these are not recognized by the endonuclease that normally cleaves the λ catenanes during phage replication.
- This means that the defective phage cannot replicate, though it does direct the synthesis of all the proteins needed for packaging.
- These proteins accumulate in the bacterium and can be purified from cultures of E. coli infected with the mutated λ.
- The protein preparation is then used for invitro packaging of recombinant λ molecules.

(a) A single-strain packaging system



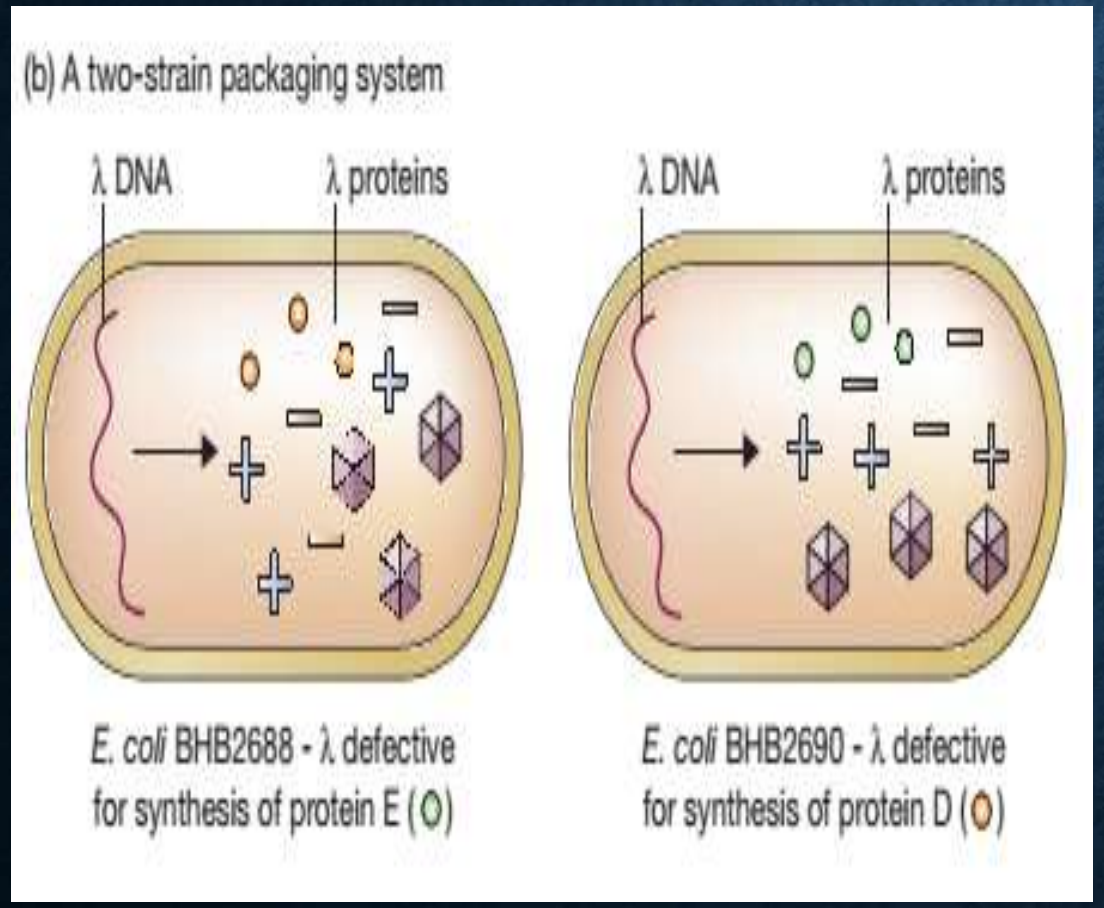
E. coli SMR10 – λ DNA has defective *cos* sites

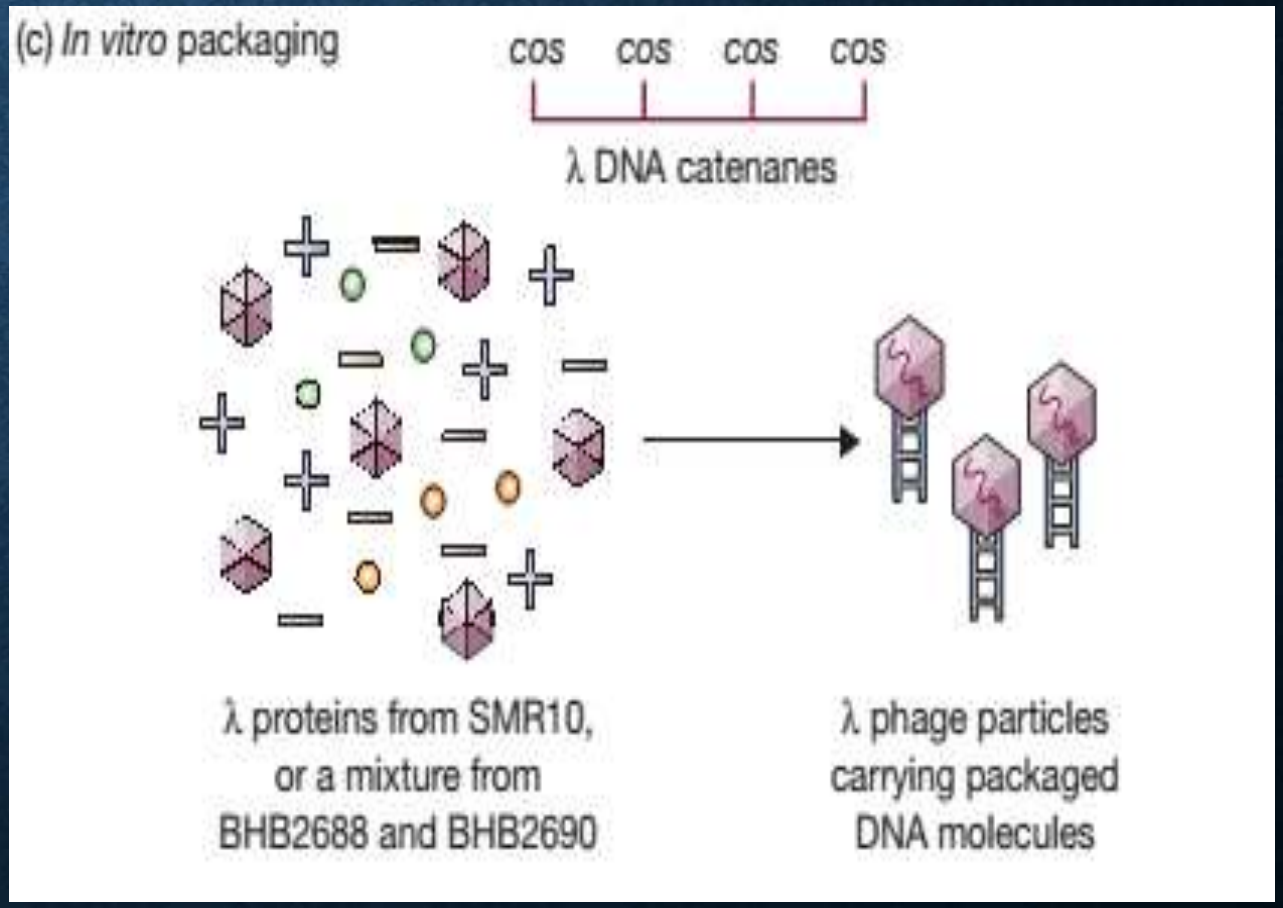
- With the second system, two defective λ strains are needed, both of which carry a mutation in a gene for one of the components of the phage protein coat.
- With one strain the mutation is in gene D, and with the second strain it is in gene E



- Neither strain is able to complete an infection cycle in E. Coli because, in the absence of the product of the mutated gene the complete capsid structure cannot be made.
- Instead the products of all the other coat protein genes accumulate.
- An in vitro packaging mix can therefore be prepared by combining lysates of two cultures of cells,
- One infected with the λ D−strain the other infected with the E- strain.
- The mixture now contains all the necessary components for in vitro packaging
- Formation of phage particles is achieved simply by mixing the packaging proteins with λ DNA, because assembly of the particles occurs automatically in the test tube

- The packaged λ DNA is then introduced into E. Coli cells simply by adding the assembled phages to the bacterial culture and allowing the normal λ infective process to take place.

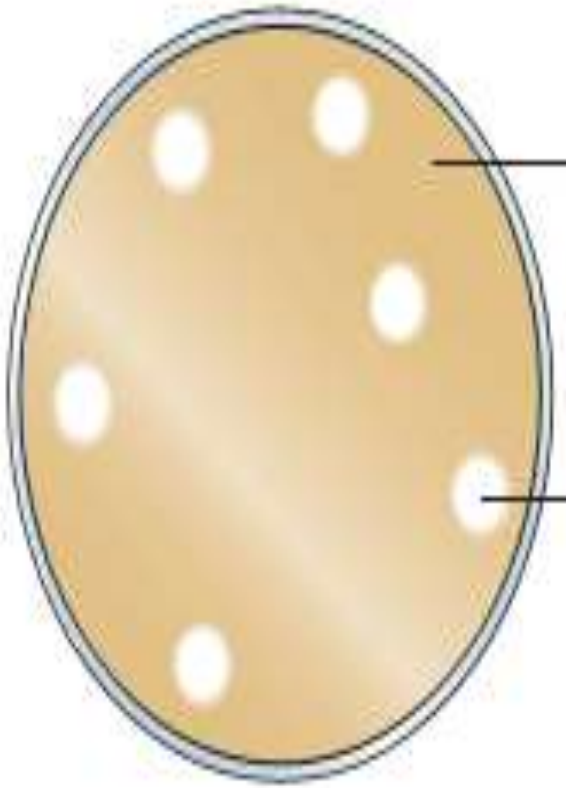




PHAGE INFECTION IS VISUALIZED AS PLAQUES ON AN AGAR MEDIUM

- The final stage of the phage infection cycle is cell lysis.
- If infected cells are spread onto a solid agar medium immediately after addition of the phage particles, or immediately after transfection with phage DNA, cell lysis can be visualized as plaques on a lawn of bacteria.
- Each plaque is a zone of clearing produced as the phages lyse the cells and move on to infect and eventually lyse the neighbouring bacteria.

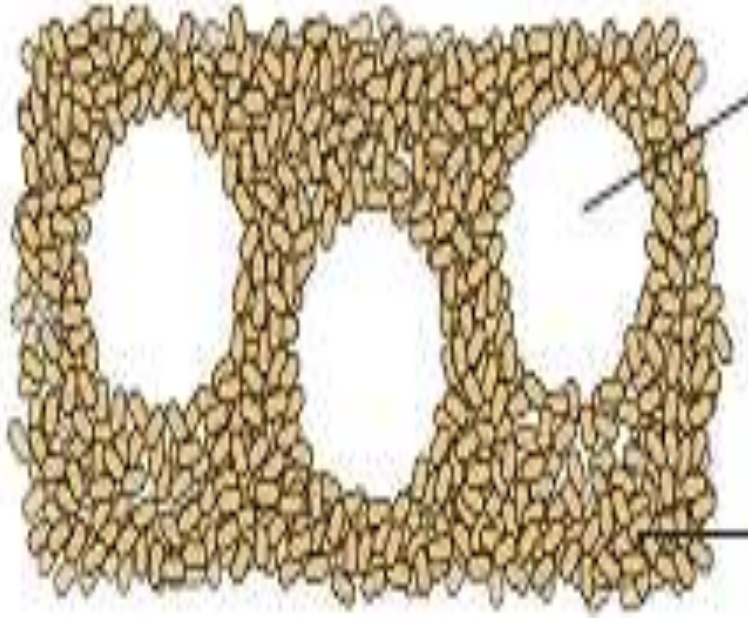
(a) Plaques on a lawn of bacteria



Lawn of confluent bacterial growth

A plaque - a zone of clearing

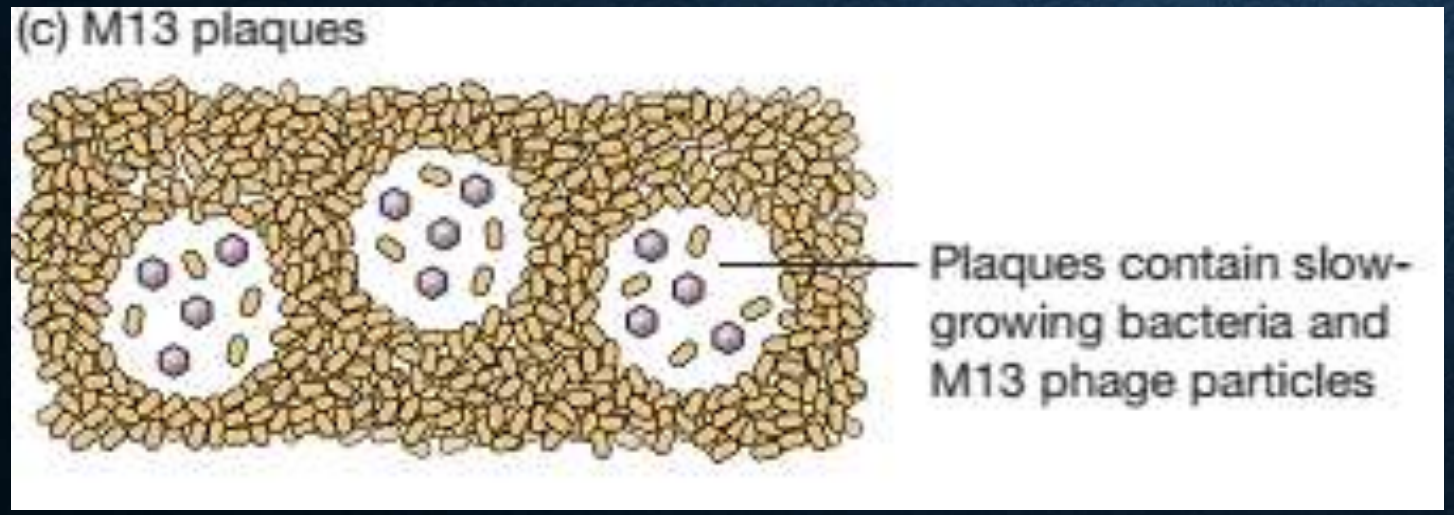
(b) Lytic plaques



Plaque - all the bacteria are lysed, leading to a high titre of bacteriophages

Lawn of bacteria

- Both λ and M13 form plaques. In the case of λ these are true plaques produced by cell lysis, but M13 plaques are slightly different as M13 does not lyse the host cells .
- Instead, M13 causes a decrease in the growth rate of infected cells, sufficient to produce a zone of relative clearing on a bacterial lawn. Although not true plaques, these zones of clearing are visually identical to normal phage plaques.
- These plaques may contain self ligated vector molecules, or they may be recombinants



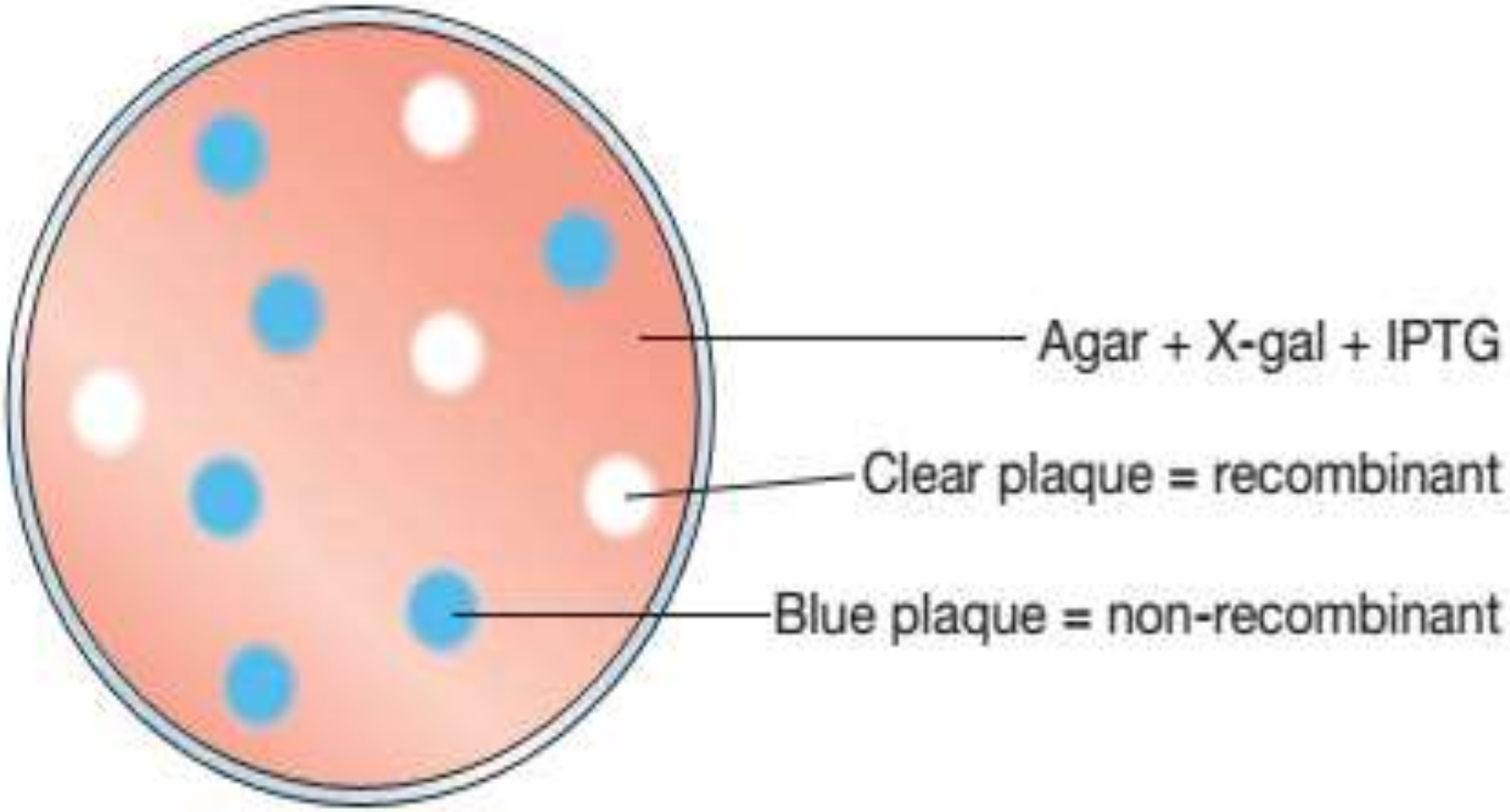
IDENTIFICATION OF RECOMBINANT PHAGES

- A variety of methods for distinguishing recombinant plaques have been devised, the following being the most important

INSERTIONAL INACTIVATION OF A LACZ′ GENE CARRIED BY THE PHAGE VECTOR

- All M13 cloning vectors as well as several λ vectors carry a copy of the lacZ gene.
- The insertion of new DNA into this gene inactivates β-galactosidase synthesis just as with the plasmid vector pUC8.
- Recombinants are distinguished by plating cells onto X-gal agar. Plaques comprising normal phages are blue; recombinant plaques are clear

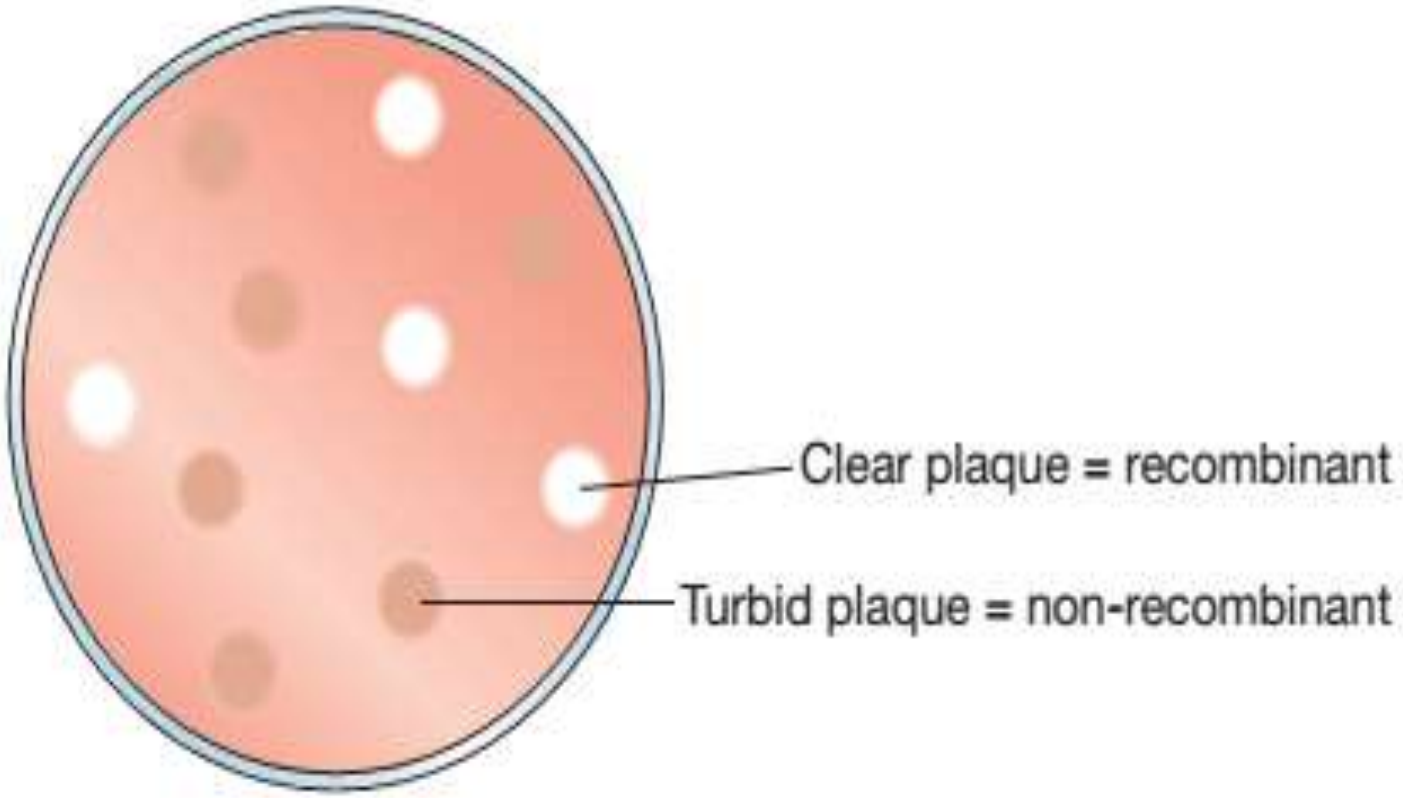
(a) Insertional activation of the *lacZ'* gene



INSERTIONAL INACTIVATION OF THE λ CI GENE

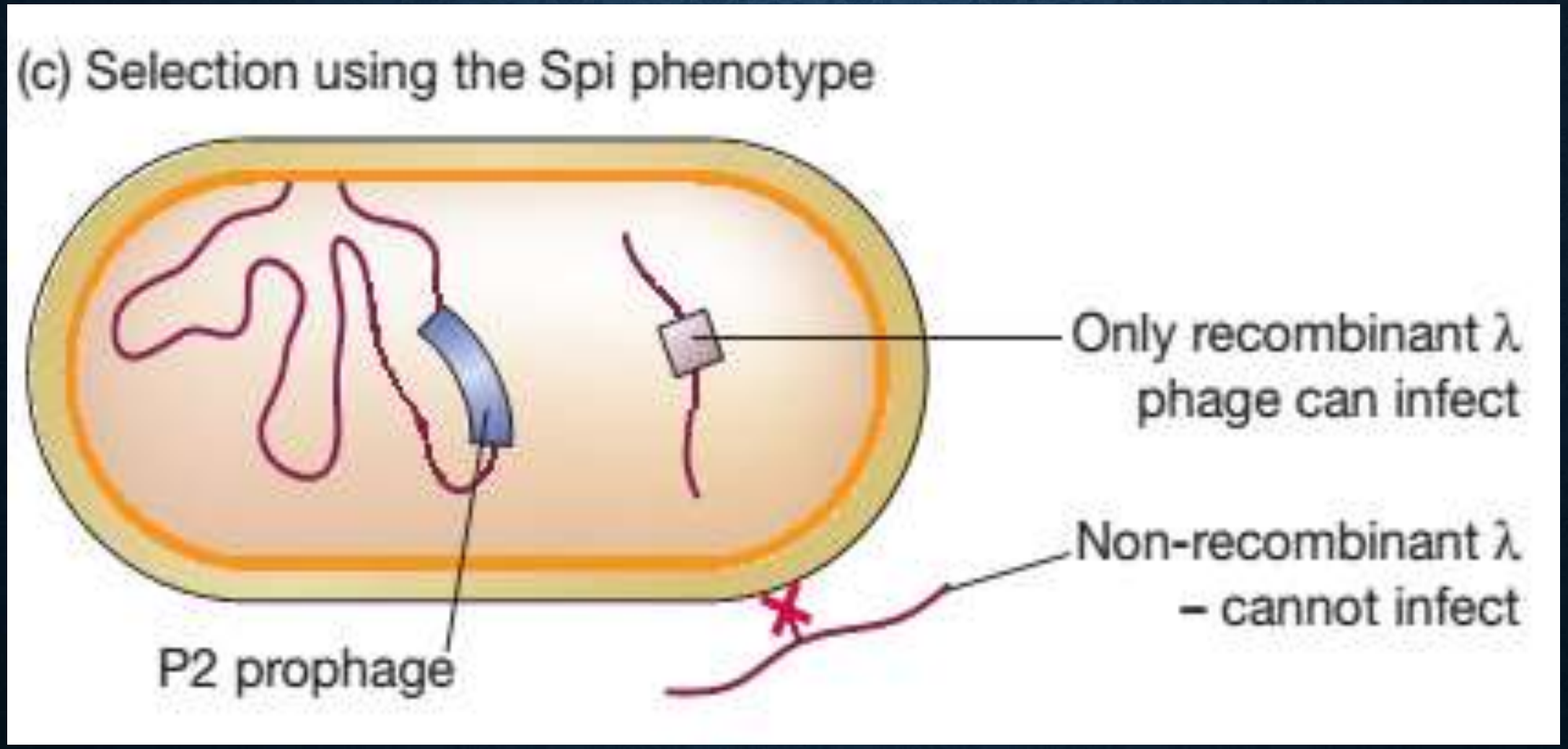
- Several types of λ cloning vector have unique restriction sites in the cI gene.
- The insertional inactivation of which causes a change in plaque morphology.
- Normal plaques appear ‘turbid’ whereas recombinants with a disrupted cI gene are clear .
- The difference is readily apparent to the experienced eye

(b) Insertional activation of the λ *cI* gene



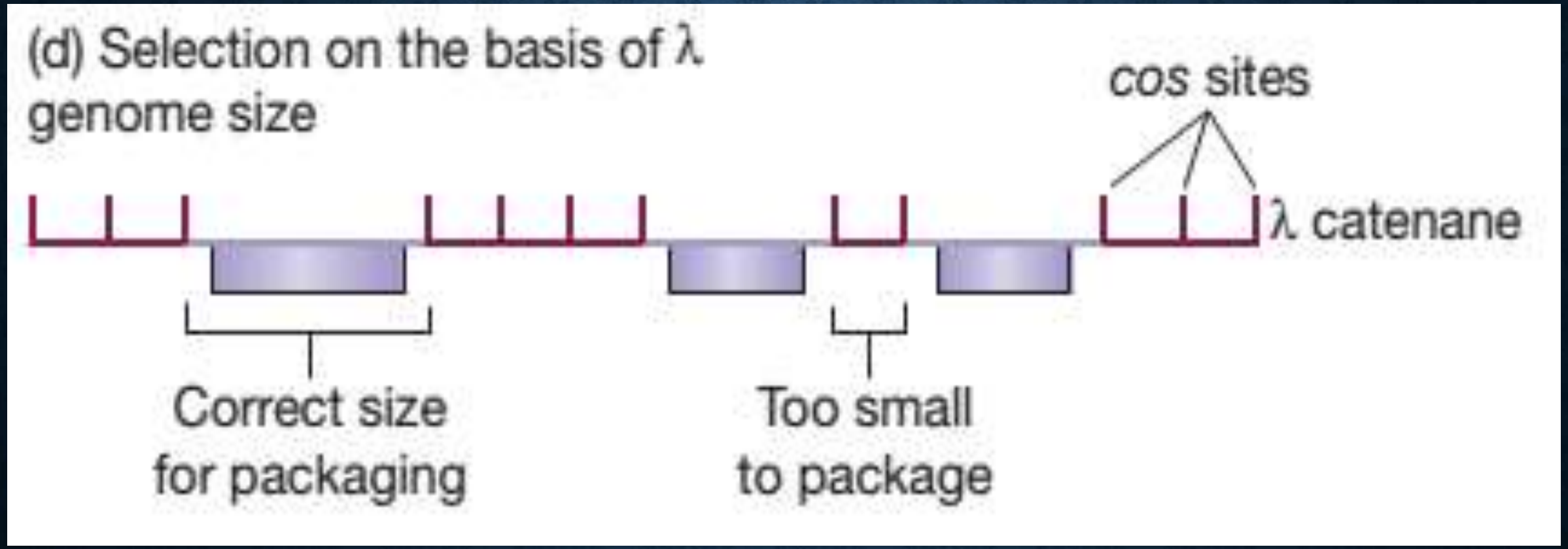
SELECTION USING THE SPI PHENOTYPE

- Λ phages cannot normally infect E.coli cells that already possess an integrated form of a related phage called P2;λ is therefore said to be Spi+(sensitive to P2 prophage inhibition).
- Some λ cloning vectors are designed so that the insertion of new DNA causes a change from Spi+ to Spi− enabling the recombinants to infect cells that carry P2 prophages.
- As only the recombinants are Spi−
- Only recombinants will form plaques


(c) Selection using the Spi phenotype
Only recombinant λ phage can infect
Non-recombinant λ – cannot infect
P2 prophage

SELECTION ON THE BASIS OF λ GENOME SIZE

- The λ packaging system which assembles the mature phage particles can only insert DNA molecules of between 37 and 52 kb into the head structure anything less than 37 kb is not packaged.
- Many λ vectors have been constructed by deleting large segments of the λ DNA molecule and so are less than 37 kb in length.
- These can only be packaged into mature phage particles after extra DNA has been inserted bringing the total genome size up to 37 kb or more.
- Therefore only recombinant phages are able to replicate.

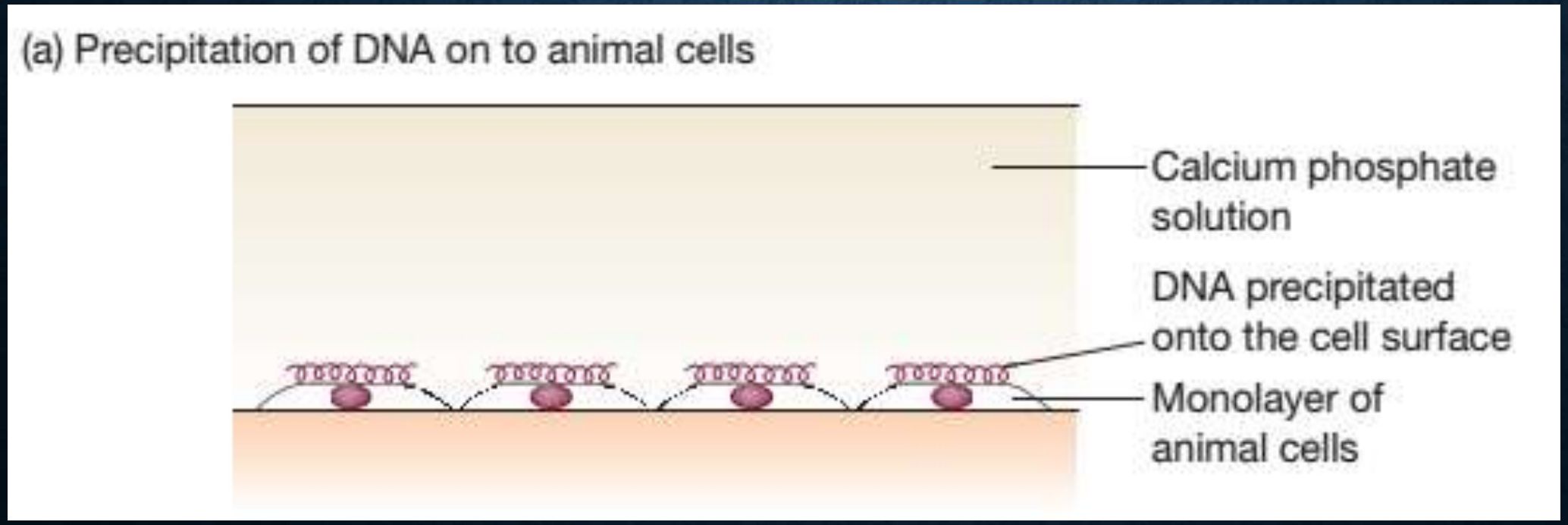

(d) Selection on the basis of λ genome size
cos sites
λ catenane
Correct size for packaging
Too small to package

INTRODUCTION OF DNA INTO NON-BACTERIAL CELLS

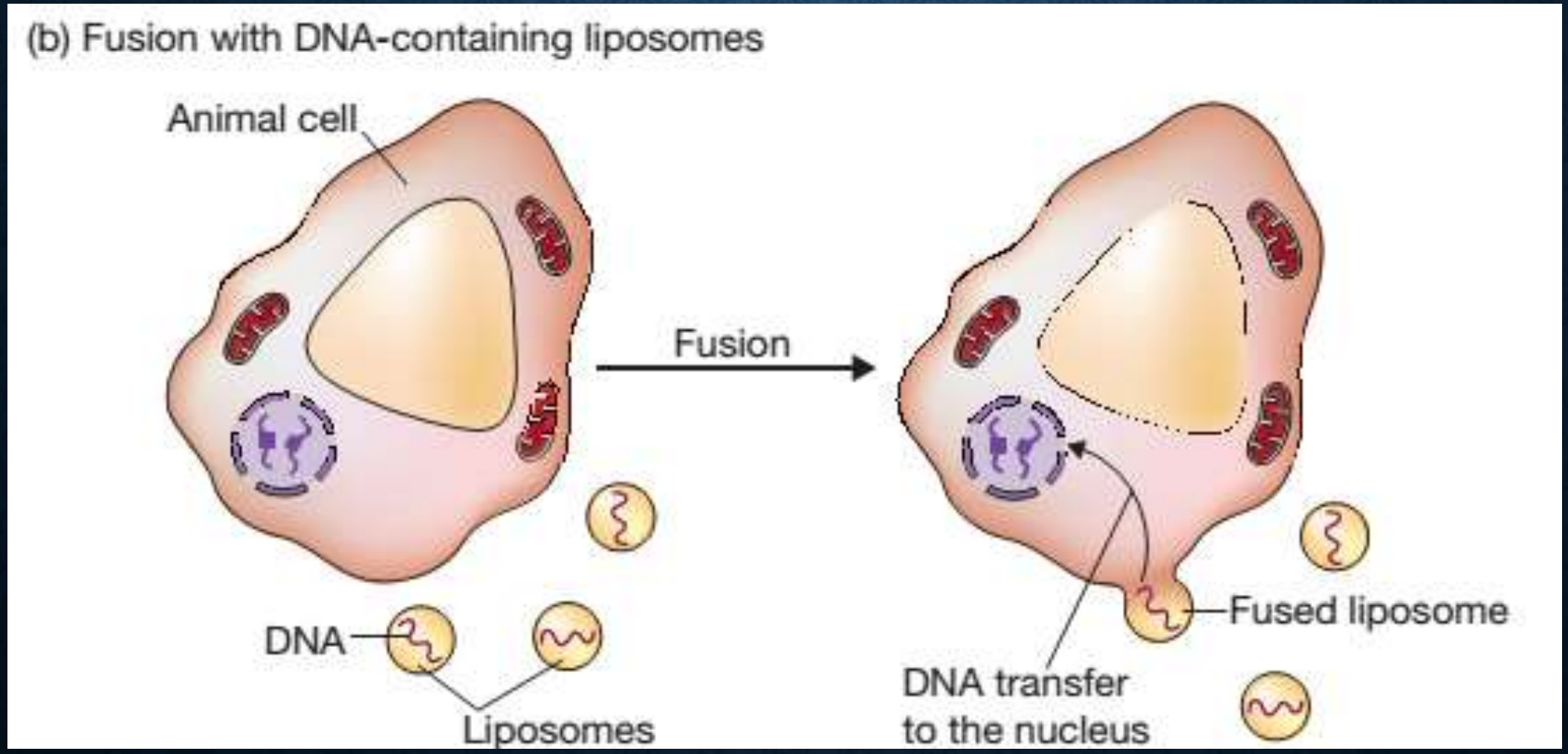
- Various ways of introducing DNA into yeast, fungi, animals and plants are also needed if these organisms are to be used as the hosts for gene cloning.
- Strictly speaking, these processes are not ‘transformation’, as that term has a specific meaning that applies only to the uptake of DNA by bacteria, but molecular biologists have forgotten this over the years and ‘transformation’ is now used to describe uptake of DNA by any organism.
- In general terms, soaking cells in salt is effective only with a few species of bacteria
- Although treatment with lithium chloride or lithium acetate does enhance DNA uptake by yeast cells, and is frequently used in the transformation of *Saccharomyce scerevisiae*.
- However, for most higher organisms, more sophisticated methods are needed.

TRANSFORMATION OF INDIVIDUAL CELLS

- With most organisms the main barrier to DNA uptake is the cell wall.
- Cultured animal cells which usually lack cell walls are easily transformed.
- DNA is precipitated onto the cell surface with calcium phosphate.



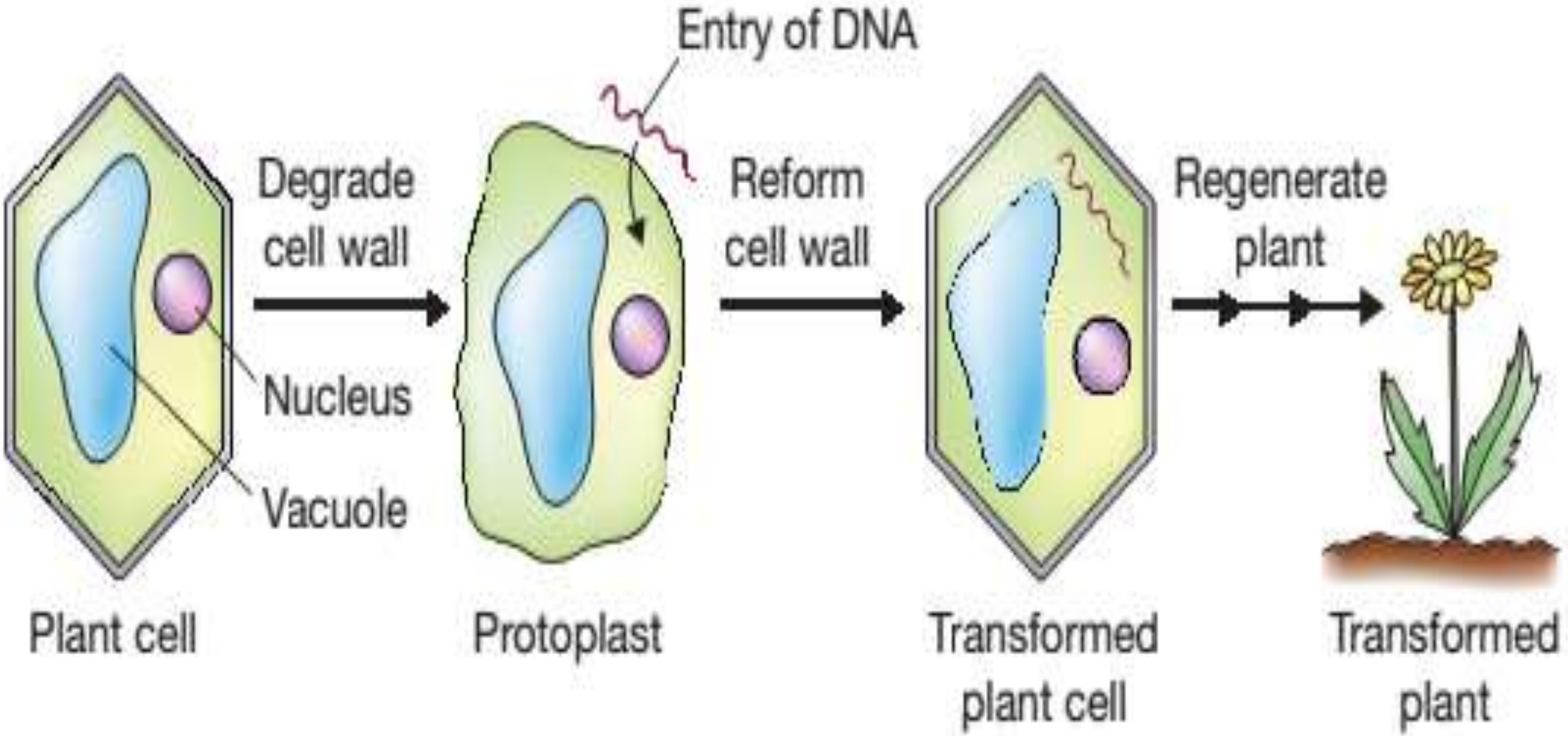
- Or enclosed in liposomes that fuse with the cell membrane.



- For other types of cell the cell wall must be removed . And enzymes that degrade yeast, fungal and plant cell walls are currently available.
- Under the correct conditions, intact protoplasts can be obtained that generally take up DNA quite readily

- Transformation can be stimulated by applying specialized techniques such as electroporation whereby the cells are subjected to a short electrical pulse
- That is thought to induce the transient formation of pores in the cell membrane through which DNA molecules are able to enter the cell.
- After transformation the protoplasts are washed to remove the degradative enzymes, after which their cell walls are re-formed spontaneously

(c) Transformation of plant protoplasts



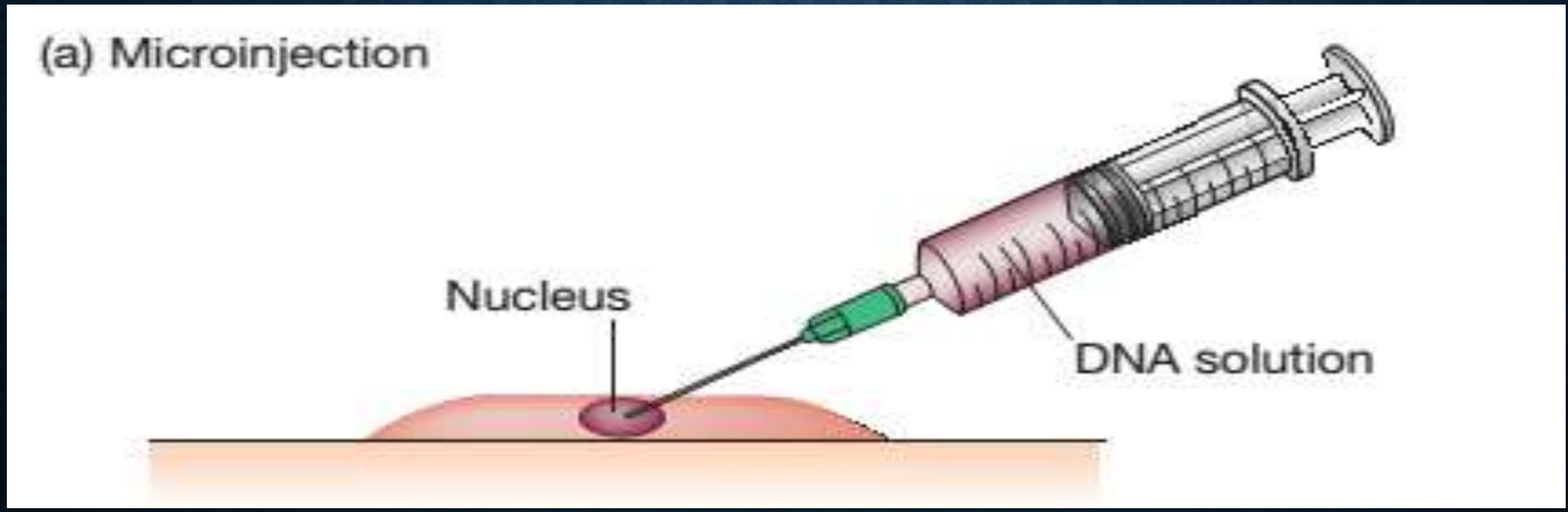
- In contrast to the transformation systems described above, two physical methods are available for introducing DNA into cells

1. Micro injection

2. Biolistics Method

MICRO INJECTION

- In this method a very fine pipette is used to inject DNA molecules directly into the nucleus of the cells to be transformed .
- This technique was initially applied to animal cells but has subsequently been used successfully with plant cells

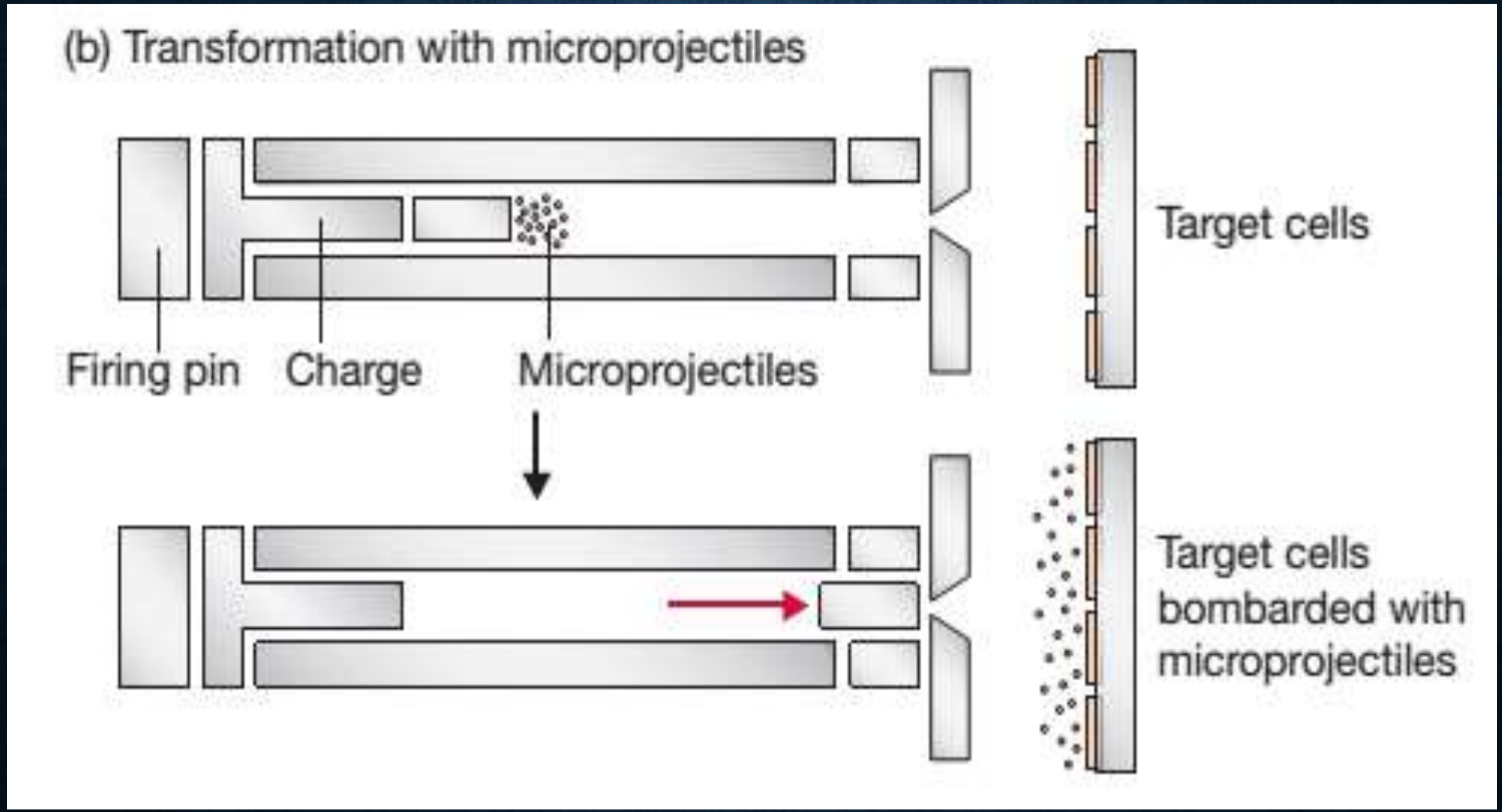


BIOLISTICS METHOD

- This method involves bombarding the cells with high-velocity microprojectiles usually particles of gold or tungsten that have been coated with DNA.

- The microprojectiles are fired at the cells using a particle gun .

- This unusual technique has been used with several different types of cell.


(b) Transformation with microprojectiles
Firing pin
Charge
Microprojectiles
Target cells
Target cells bombarded with microprojectiles