Ministry of Higher Education & Scientific Research

Salahaddin University

College of Science

Biology Department

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Preparation of Competent Cells

and

Heat-Shock Transformation

Lab.1 Practical Molecular Biology 14.02.2022

1. Human insulin is extracted from pancreas cells and an insulin-producing gene is isolated.

2. A plasmid DNA is extracted from a bacterium and cut with restriction enzyme, forming plasmid vector.

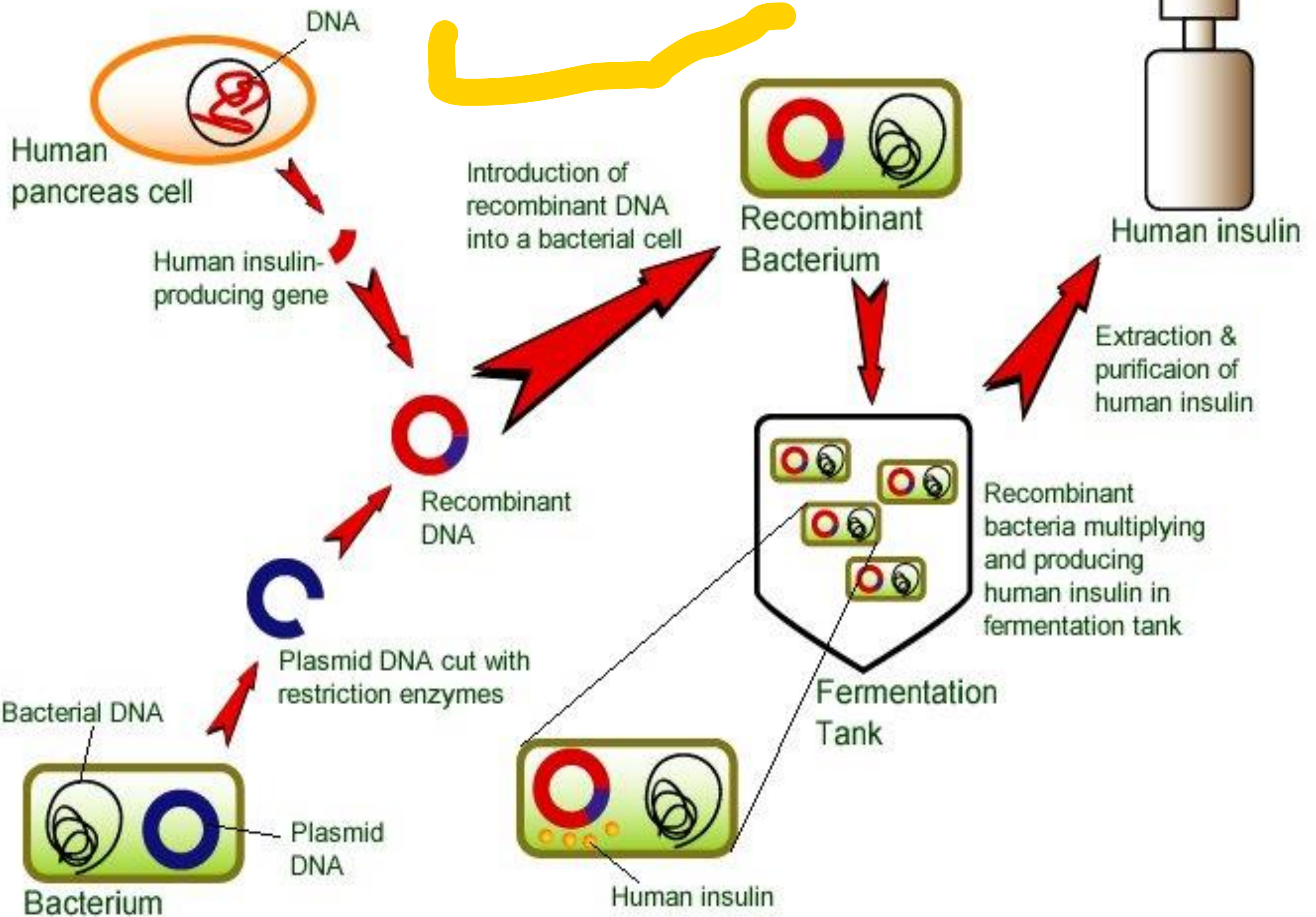
3. Insert human insulin-producing gene into the bacterial plasmid vector to form the recombinant DNA.

4. Introduce this recombinant DNA into a bacterial cell to form the recombinant bacterium.

5. The recombinant bacteria multiply and produce human insulin.

6. Insulin is extracted, purified and bottled. It is then ready to be injected into diabetic patients.

Human Insulin Production



Human Cell

Gene for human growth hormone

*Eco*RI

Recombinant DNA

Gene for human growth hormone

Sticky ends

DNA recombination

DNA insertion

Bacterial Cell

Bacterial chromosome

*Eco*RI

Plasmid

Bacterial cell for containing gene for human growth hormone

E. coli bacterium

Plasmid

Bacterial chromosome

1 Isolate plasmid

Cell with DNA containing gene of interest

2 Isolate DNA

DNA

3 Cut plasmid with enzyme

Gene of interest

4 Cut cell's DNA with same enzyme

Gene of interest

5 Combine targeted fragment and plasmid DNA

6 Add DNA ligase, which closes the circle with covalent bonds

Recombinant DNA plasmid

Gene of interest

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7 Put plasmid into bacterium by transformation

Recombinant bacterium

8 Allow bacterium to reproduce

Clone of cells

DNA CLONING

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The Learning App

Cloning vector (Plasmid)

Eukaryotic Chromosome

DNA fragment of interest is obtained by cleaving chromosome with restrictions endonuclease

Cleaved with restriction endonuclease

DNA ligase

DNA fragments are ligated to the prepared cloning vector

Recombinant vector DNA is introduced into the host cell

Propagation (cloning) produces many copies of recombinant DNA

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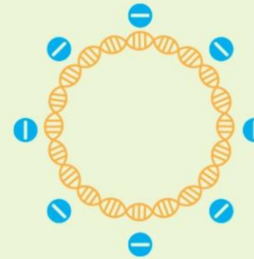
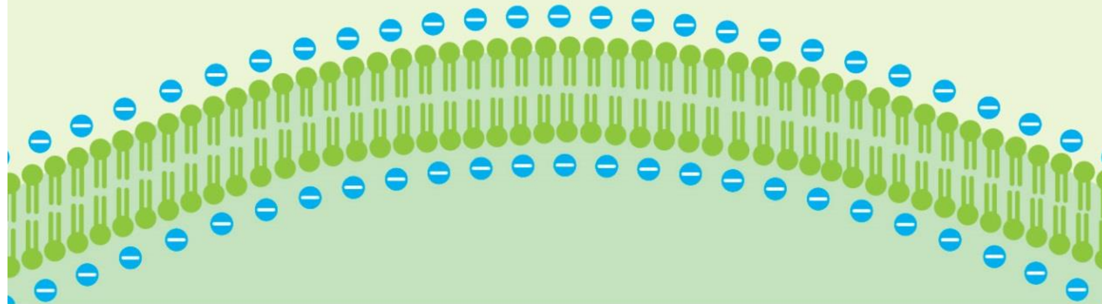
Prior to $CaCl_2$ Treatment

Negative charges cause natural repulsion between the cell membrane and the plasmid DNA.

GOLDBIO





After $CaCl_2$ Treatment

Calcium from $CaCl_2$ interact with the negative charges, which leads to an electrostatically neutral environment.

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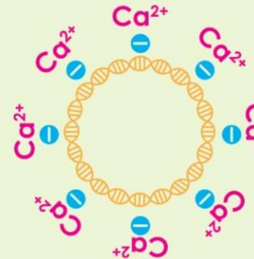
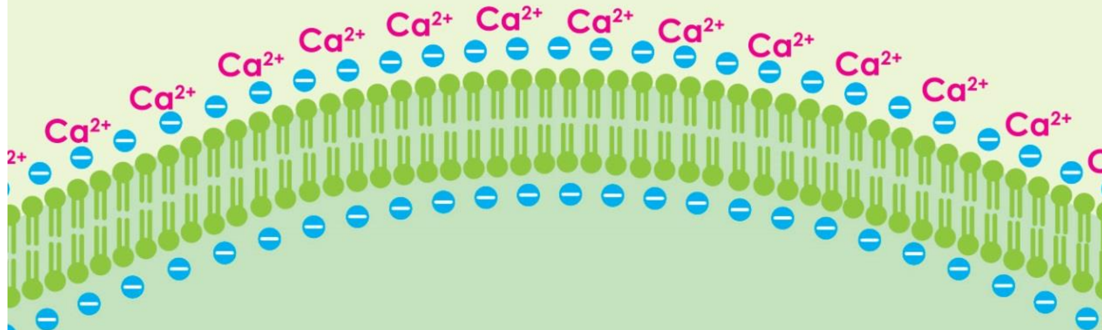
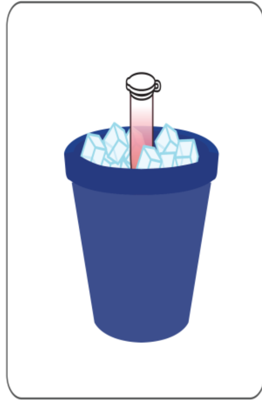
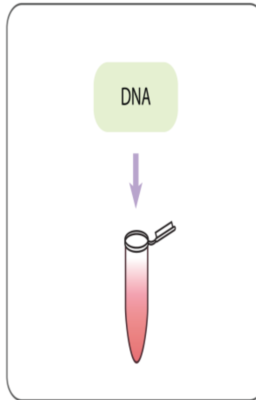




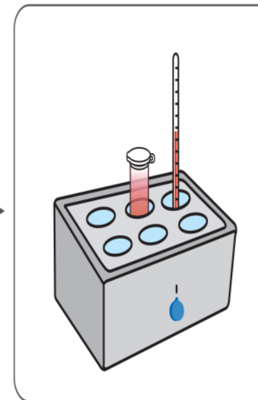
Competent Cells Workflow



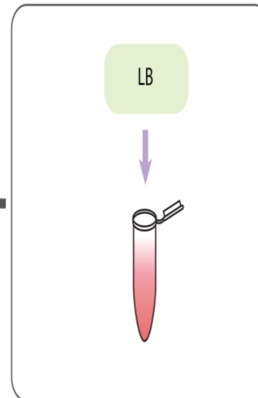
Thaw aliquoted ProClone™ Competent Cells (Cat. No. E003) on ice for 10 to 15 min



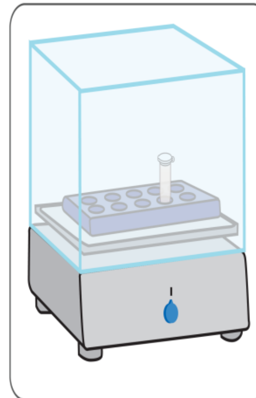
Add DNA to the cells and mix gently then incubate on ice for 30 min



Heat Shock at 42˚C for 45 sec



Cool on ice for 1 to 2 min then add 150 µl of LB to ProClone™ Competent cells (Cat. No. E003)



Incubate at 37˚C for 1 hr on shaker

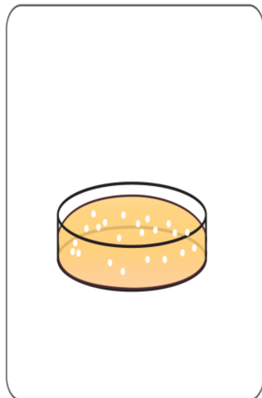


Plate on LB agar with appropriate antibiotic and incubate overnight at 37˚C

Noncompetent *E. coli*

E. coli that has not been made competent will not take up plasmids introduced into their enviroment.

Competent *E. coli*

E. coli made competent either through $CaCl_2$ and heat-shock or through electroporation will have better membrane permeability (pores), enabling plasmid uptake.

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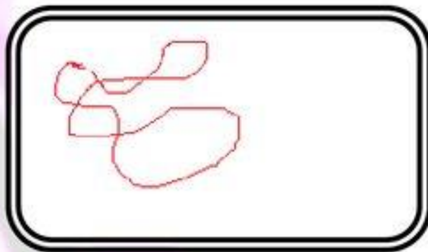
Principle of the experiment:

[Chemical transformation]

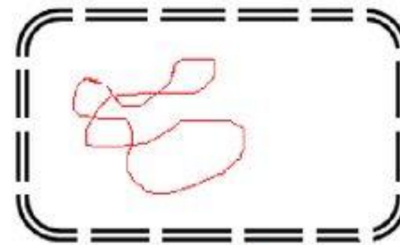
Recombinant Plasmid

Insertion



1.CaCl2 treatment
To permeabilize
the bacterial cell
membrane

2.Brief heat shock
to facilitate
The DNA up take.



Competent
Bacterial cell

Transformed bacteria

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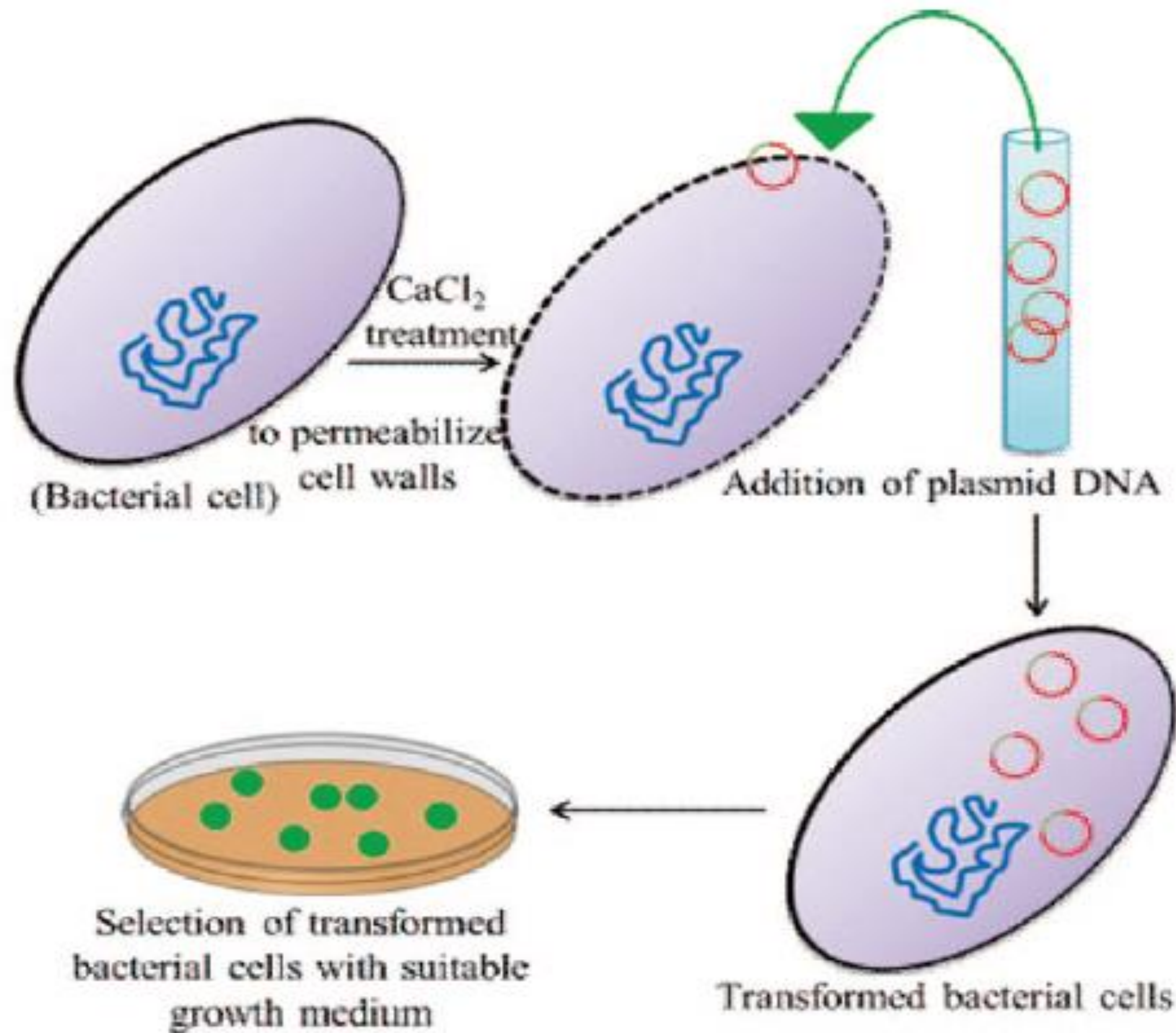

Fig. 1: Preparation of competent cells by $CaCl_2$ treatment and transformation

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