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Transformation V.4

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Protocol status: Working
We use this protocol and it's working

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Abstract

Transformation is a direct modification of the genotype of a cell from a different one by extracellular applications using recombinant DNA techniques. Transformation refers generally to the integration of exogenous DNA into the cell and its integration into the genome. Before starting the transformation process, the gene of interest is ligated with the plasmid vector via the ligase enzyme. This process is called ligation. Generally, nucleic acids or plasmids cannot enter into bacterial cells by themselves. The stimulatory effect is required for that purpose. This means that the cell membranes to be transformed must be pre-arranged. Bacteria that can contain free DNA are called competent bacteria. Some bacteria are highly competitive in normal growth conditions; but some must be treated with chemical or physical methods to gain competitive properties. Competent cells which are cells are generally stimulated with calcium chloride about chemically and the cell membranes are arranged such that the plasmid vector containing the gene of interest can harbor the vector.
Some bacterial cells are lasting competent, however; most of them need to be influenced for being competent. There are two ways about this transformation process that;
• Chemical Transformation
• Electrical Transformation (Electroporation)
In general, chemical transformation is used. In chemical way, some special chemicals are used to open the pores which are found in cell membrane. This is because, opening the pores represents the availability of being permeable. Most of the time, divalent ions like Ca++ are used with assistance of heat-shock.

Materials

STEP MATERIALS

LB Broth **Amresco Catalog #**J106-2KG

Protocol materials

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LB Broth **Amresco Catalog #**J106-2KG

Add DNA to competent cells

1

Let stand on ice for 30 min

2 00:30:00 On ice

Heat shock

3 00:01:00

42 °C

Equipment

new equipment NAME

Grant Bio PHMT Thermoshaker BRAND

125-0649 SKU

Leave on ice

4 00:01:00

Add LB

5 900 µL for 100 µl cells

800 µL for 200 µl cells

LB Broth **Amresco Catalog #J106-2KG**

Equipment

new equipment

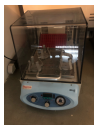
NAME

Shaker

BRAND

-

SKU



Put in a shaker

6

37 °C

00:50:00

Centrifuge at 7000 rpm

7

00:01:00

Equipment

new equipment

NAME

Centrifuge

BRAND

-

SKU

Discard LB

8

Resuspend cells and plate them all

9