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HIMEDIA® Product Information

HiPer® Yeast Transformation Teaching Kit

Product Code: HTBM025

Number of experiments that can be performed: 10

Duration of Experiment: 4 days

Day 1- Preparation of media and revival of Yeast host
Day2- Inoculation of host strain from a single colony
Day 3- Protocol
Day 4- Observation and calculation of transformation efficiency

Storage Instructions:

- The kit is stable for 6 months from the date of manufacture
- Store Yeast host strain at 2-8°C
- Store Carrier DNA and plasmid DNA at -20°C
- Other kit contents can be stored at room temperature (15-25°C)

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Aim:

To prepare *Saccharomyces cerevisiae* competent cells and transform a yeast plasmid DNA.

Introduction:

Yeasts are eukaryotic heterotropic organisms classified in the kingdom fungi. Most of the fungi grow as multicellular filaments called hyphae forming a mycelium. Some species also grow as single cells e.g. Yeast, which possess a chitinous cell wall. Yeasts are chemoorganotrophs as they use organic compounds as a source of energy and do not require sunlight to grow. Yeasts grow best in a neutral or slightly acidic pH environment. Yeasts can grow over an optimal temperature range of 30°C to 37°C, depending on the type of species. Genetic engineering, i.e. transformation of yeast cells with recombinant DNA, became feasible for the first time in 1978. Since then, recombinant DNA technology in yeast has established itself, and a multitude of different vector constructs are available. Generally, these plasmid vectors also called shuttle vectors contain genetic material derived from the *E.coli* vector pBR322 (or its derivatives) and a genetic element (origin of replication) which enable them to be propagated in *E.coli* cells prior to transformation into yeast cells and a selectable marker (mainly the ßlactamase gene, *amp*) for the bacterial host. Additionally, the shuttle vectors provide a selectable marker to be used in the yeast system.

Principle:

Transformation of yeast cells may be achieved by three principal approaches:

- Permeabilization of cells by treatment with Lithium acetate
- Electroporation
- Bombardement of cells by DNA-coated tungsten micro projectiles

Lithium acetate-mediated yeast transformation is the most widely used method for introducing DNA into yeast cells. This method was first performed by Ito in 1983. Polyethelene glycol (PEG) is absolutely required for the binding of DNA to the surface of intact yeast cells or yeast protoplasts, and has no effect on the surface topography of intact yeast cells or yeast protoplasts. In the presence of PEG, Lithium ions could greatly enhance the binding of plasmid DNA to the surface of intact yeast cells, increase their transformation frequency, and affect their surface topography. Lithium ions increase the permeability of yeast cell wall, and then increase the exposed sites of DNA binding on intact yeast cells. The main role of PEG is to induce DNA binding to cell surface. During yeast transformation, single stranded DNAs are absolutely essential and these are called carrier DNAs. During yeast transformation the selection of the plasmids are based upon the use of auxotrophic mutant strains which are unable to grow without a specific amino acid. When the yeast strain which contains an auxotrophic mutation (in a specific gene) is transformed with a plasmid containing the wild type copy of that gene, the transformed colonies can be selected by growing the cells on the specific dropout media. A leucine auxotrophic yeast mutant cannot grow on a growth medium which is devoid of leucine but a wild-type or a leucine prototrophic yeast strain can grow. The leucine auxotroph has a mutation in a gene (e.g. *LEU2*) of the leucine synthesis pathway and this mutant strain will grow in this medium if leucine is supplied from outside e.g. from a plasmid which contains *LEU2* gene.

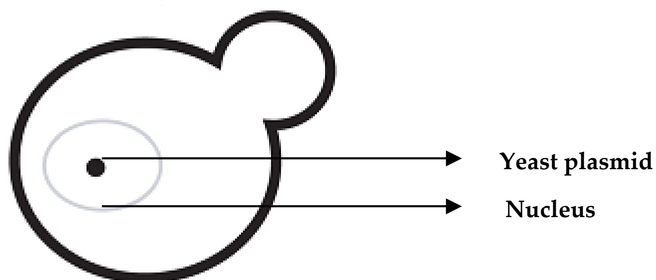


Fig 1: After yeast transformation the plasmid is maintained within the nucleus

Kit Contents:

This kit can be used to prepare *S. cerevisiae* competent cells by Lithium acetate method and perform transformation using a *LEU2* containing yeast plasmid.

Table 1: Enlists the materials provided in this kit with their quantity and recommended storage

Sr. No.	Product Code	Materials provided	Quantity 10 expts	Storage
1	TKC214	70% PEG Solution	1.1 ml	R T
2	TKC215	YT Buffer (Sterile)	230 ml	R T
3	ML024	Molecular Biology grade Water	2.5 ml	R T
4	M1363	Yeast Extract Peptone Dextrose Broth	45 g	R T
5	G065	SD Growth Medium w/o LEU	12.5 g	R T
6	MB053	Agar Powder, Bacteriological	10 g	R T
7	TKC216	Yeast host	1 No.	2-8 °C
8	TKC217	Carrier DNA	0.11 ml	-20 °C
9	TKC218	Plasmid DNA	0.055 ml	-20 °C
10	PW1139	Collection Tubes, Polypropylene (2.0 ml)	20 Nos.	R T

Materials Required But Not Provided:

Glasswares: Conical flask, Measuring cylinder, Beaker
Other requirements: Heater, Micropipettes, Tips, 50 ml Centrifuge Tubes, Water bath (42°C), 30°C Incubator, 30°C Shaker, Centrifuge, Crushed ice, Sterile loop and Spreader

Storage:

HiPer® Yeast Transformation Teaching Kit is stable for 6 months from the date of manufacture without showing any reduction in performance. On receipt, store Yeast host at 2-8°C. Plasmid DNA and carrier DNA should be stored at –20°C. All other kit contents can be stored at room temperature (15-25°C).

Important Instructions:

1. Read the entire procedure carefully before starting the experiment. The entire procedure should be carried out under sterile conditions.
2. Transformation should be carried out as soon as possible after the competent cells are prepared. Storage of competent cells leads to poor or no transformants.
3. Use cut tips while pipetting 70% PEG Solution, as it is too viscous.
4. PEG is having the tendency to precipitate down. Prior to use, warm the solution at 50^{0}C, till it becomes clear.
5. Concentration of plasmid is 50 ng/µl.
6. **Preparation of YPD broth (100 ml):** Dissolve 5 g of YPD media in 100 ml of distilled water. Sterilize by autoclaving.
7. **Preparation of YPD agar plates (20 ml):** Dissolve 1 g of YPD media and 0.3 g of agar in 20 ml of sterile distilled water. Sterilize by autoclaving and pour on sterile petri plate.
8. **Preparation of SD Growth Medium w/o LEU agar plates (100 ml):** Dissolve 2.74 g of SD Growth Medium w/o LEU and 1.5 g of agar in 100 ml of distilled water. Sterilize by autoclaving and pour on sterile petri plates.

Procedure:

Day 1:

1. Streak a loopful of culture from the stab (Yeast host) on to YPD Agar plate.
2. Incubate the plate overnight at 30°C.

Day 2:

1. Inoculate a single colony from the revived plate in 1 ml of YPD broth.
2. Incubate in a 30°C shaker for overnight.

Day 3:

1. Take 50 ml of YPD broth in a sterile flask. Transfer 1 ml of overnight grown culture into this flask.
2. Incubate at 30°C shaker at 250 rpm for 2-3 hours till the O.D reaches ~ 1.0.

A) Preparation of Competent Cells:

1. Transfer the above culture into a 50 ml polypropylene tube (not provided).
2. Centrifuge at 5000 rpm for 10 minutes at RT.
3. Decant the medium completely. No traces of medium should be left.
4. Resuspend the cell pellet in 20 ml of YT Buffer.
5. Centrifuge at 5000 rpm for 10 minutes at RT.

6. Decant the medium completely. No traces of medium should be left.
7. Resuspend the pellet in 1 ml of YT Buffer.
8. This cell suspension contains competent cells and can be used for transformation.

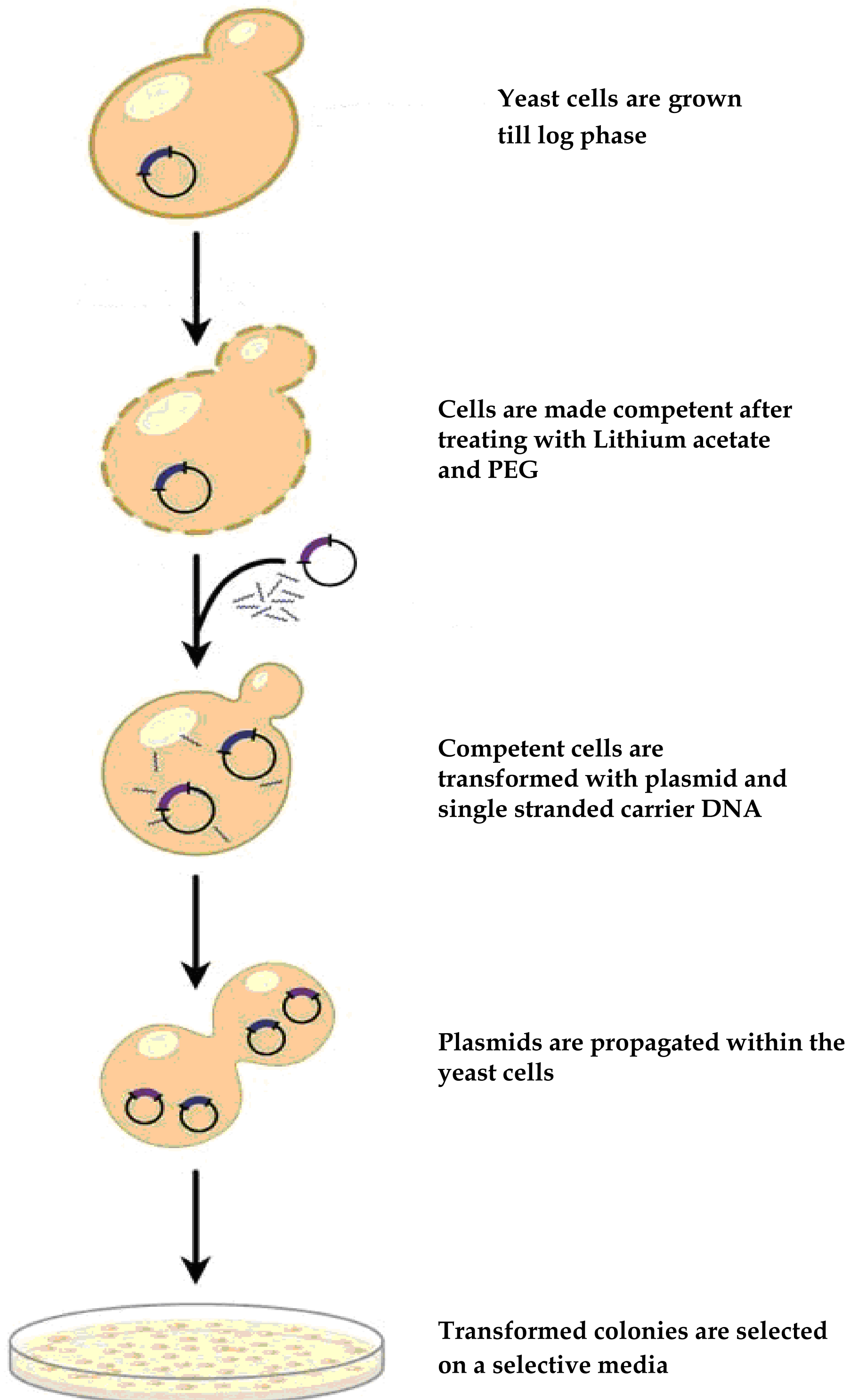
B) Transformation of cells:

1. Take 200 μl of the above cell suspension in two 2.0 ml collection tubes and label them as control and transformed. Add 10 μl of carrier DNA to each tube and mix well.
2. Add 5 μl of plasmid DNA to the tube labeled as transformed and mix well.
3. Add 100 μl of 70% PEG solution to each tube.

   Note: Cut the tip while pipetting PEG, as it is very viscous.

4. Mix all the contents of the tube by vigorous vortexing.
5. Incubate the tubes at 30 °C for 30 minutes.
6. Transfer the tubes to a preheated water bath set at a temperature of 42ºC for 15 minutes (heat shock).
7. Centrifuge the tubes at 2000 rpm for 5 minutes. Aspirate out the supernatant and add 100 μl of sterile water to both the tubes and resuspend the pellets.
8. Plate the cells from each tube on SD Growth Medium w/o LEU using a sterile spreader.
9. Store at room temperature till the plates are dry and Incubate the plates for 2-3 days at 30ºC

Flowchart:



Observation and Result:

After incubation observe the plates for the yeast growth and count the number of visible colonies.
Calculate the efficiency of transformation.
Record your observations as follows:

Sr. No.	Plate	Growth	Number of colonies	Transformation Efficiency
1	Control plate			
2	Transformed plate			

Denote +ve when you observe yeast growth, -ve when there is no growth

Calculation of transformation efficiency:

Transformation efficiency is defined as the number of yeast cells transformed per microgram of plasmid DNA in a transformation reaction.
Transformation Efficiency = Number of colonies x 1000 ng/ Amount of DNA plated (ng) = /µg

Interpretation:

On transformation of *leu2* auxotroph cells with the *LEU2* containing yeast plasmid, the host is able to synthesize leucine as this plasmid carries the wild type copy of *LEU2* gene. As a result, those cells that grow in absence of leucine are transformed cells.

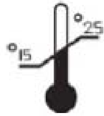
Troubleshooting Guide:

Sr. No	Problem	Possible Cause	Solution
1	Few or no transformants	PEG was not added in proper amount	Make sure that only required amount of PEG is added
		Heat shock treatment not done accurately	Follow the procedure, with exact time duration for heat shock treatment
		Plates were not incubated at proper temperature for sufficient time	Incubate the plates at 30ºC for minimum 48 hours
2	Contamination observed on plates	Sterility was not maintained during the experiment	Make sure that the entire procedure is performed aseptically

Please refer disclaimer Overleaf.

Technical Assistance:

At HiMedia we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance, mail at mb@himedialabs.com



Storage temperature

Do not use if package is damaged

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