Prokaryotic Cells Transformation Using Simple, Rapid And High Efficiency Method

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ABSTRACT
Transformation is the basic experiment in molecular biology laboratory thus the present study was carried out to find new and rapid methods for performed it with low cost and time, Luria broth was used instead of other compromised media, the results show that Luria media have high efficiency to transform bacteria than other media. The results show that using Luria medium suitable and easier for transformation experiment.

KEY WORDS: Transformation, Luria medium.

INTRODUCTION
Transformation has been used in all laboratories of molecular biology and genetic engineering lab, its used for amplification vector and plasmids, also for methylated DNA and repair plasmid nicked after DNA assembly methods, furthermore it used as a screening system for recombinant DNA. In all previous studies the stander methods of transformation used SOB, SOC and SOB agar media for bacteria growth, these media needed long time for preparing its, thus present study aims to use simple and high efficiency media for transformation.

LB is a widely used in bacterial molecular biology it discovers by Giuseppe Bertani (1952) when trying to optimize plaque formation on a Shigella indicator strain (Bertani, 1952). LB has been variously misconstrued to stand for “Luria Broth”, “Luria-Bertani” medium, and “Lennox Broth”; however, the acronym originally stood for “Lysogeny Broth” (Bertani, 2004). The agar form of the medium should be designated LA but it is often referred to as LB. Although originally developed for bacteriophage studies and Shigella growth, LB subsequently became the medium of choice for growth of Escherichia coli and other related enteric species.

MATERIALS AND METHODS
Solution preparation:
1- Vector dilution: Turbu- GFP vector diluted by adding 1 µl of stock solution to 99 µl of dH2O.
1- Buffers prepare as follows:
1- CaCl2 0.1 M, 1.1098 g was added to 90 ml of dH2O then complete to 100 ml.
2- KC1 250 mM, 1.86 g was added to 90 ml of dH2O after dissolved it complete to 100 dH2O.
3- MgCl2 80 mM, 1.62 g was added to 90 ml of dH2O after dissolving it complete to 100 ml.
4- CaCl2 20 mM, 0.221 g was added to 90 ml of dH2O after dissolving it complete to 100 ml.
5- MgCl2-CaCl2 buffer, prepared by equal volume of 3 and 4.
6- MgSO4 20 mM, 0.492 g was added to SOB medium, then it sterilize by Millipore 0.20 Nm.
7- SOB was prepared by add 5 g trypton, 1.25 yeast extract, 0.125 g NaCl to 200 ml dH2O, the mixture was shaken to dissolve contain 2.5 ml of 250 mM KCl was added, then the pH of media was adjusted to 7.06, mixture was completed to 250 ml of dH2O and sterilize by autoclave.
8- SOB agar, 15 g of agar was added to 1000 ml of SOB broth.
9- Glucose 1M prepared by added 1.98 g to 10 ml dH2O, after it dissolving sterilized by Millipore 0.20 nm.
10- SOC it prepared by 250 ml of SOB which added 5 ml of 1M glucose.
11- Bacterial growth, stander bacterial cell E coli HB101 was cultured on Luria agar for 18 hours.
12- Kanamycin, it prepared by added 30 mg to 1ml of dH2O as an aliquot solution (30µg/µl) then it sterilized by Millipore and store at -10°C.
13- Luria broth it prepared by adding 2,5 g to 100 ml dH2O.
14- Luria agar with kanamycin , 2.5 g was added to 100 ml dH2O, 3.7 g of agar was added then it sterilize by autoclave after this kanamycin was added.

Competent cell preparation: It performed according to (Cohen, et al 1972) with modification
1- Culture one colony of bacteria on 25 ml of SOB in 250ml flask, at 37°C with shaking for 3 hours, then OD of culture was measured at 600nm. OD was 3.2nm.
2- Polyethylene tubes were chilled on ice for 5 minutes or until just thawed.
3- Tube was centrifuged at 4100 rpm for 10 min. in 4C°.
4- Media was discarded, and the tube was inverted for 2 min.
5- Pellet was re-suspended gently with 4.2ml of ice cooled of MgCl2-CaCl2.
6- Tubes were centrifuged as in step 3.
7- Buffer was discarded and tubes were inverted for 4 min.

Plasmid : green fluorescence protein (GFP –Evrogen ), E coli HB101 (Promega ), Luria broth (high media).

Experimental design: GFP was transformed using three methods as follows: Transformation according to Cohen et al (1972) with using SOB, SOC and SOB agar with kanamycin this is the first method; the second was the same method but used Luria broth and Luria agar with kanamycin; the third method was according to Promega (cat#L2011) Every equipment used in transformation must be at -4C°, and polyprobelen tubes must be used because polyethylene decreased transformation efficiency.

A- The first methods as following:
1- A 200 µl of competent cell was transferred to a polyprobabene tube that chilled in ice for 10 min.
2- A 3 µl of dilution vectors was added to the tube, with gently mixed. Put on ice for 30 min.
3- Tube was transferred to water bath for heat shock at 42°C° for 90 sec. without shaking.
4- Tube was transferred to ice for 2 min.
5- Then 800 µl of SOC was added to the tube, Tubes were incubated at 37°C° for 45 min in a water bath with shaking.
6- A 200µl of culture was transferred to SOB agar contain 20 mM of MgSO4 and kanamycin for positive selection, also it cultured at Brian heart fusion broth as negative control.
7- Petri dish left at Rome temperature for 3 min to absorbent liquid, and then it incubated at 37°C° for 18 hours.
8- Pellet was re-suspended in 1.2 ml of ice cooled CaCl2 0.1M.

B- The second method as the previous method but Luria broth and Luria agar was used

C- Third methods according to Promega (Cat#L2011) as follows:
1- A 17 × 100mm polypropylene culture tubes were Chilled sterile on ice.
2- frozen Competent Cells was Removed from -20°C, and placed on ice for 5 minutes or until just thawed. Once the cells have thawed, use chilled (4°C) pipette tips
3- Gently mix the thawed Competent Cells by flicking the tube, and transfer 100µl to each chilled culture tube.
4- A1–50ng of DNA was added per 100µl of Competent Cells.
5- Immediately return the tubes to ice for 10 minutes.
6- Heat-shock the cells for 50 seconds in a water bath at exactly 42°C.
7- Immediately place the tubes on ice for 2 minutes.
8- A 900µl of cold (4°C) SOC medium was added to each transformation reaction, and incubate for 60 minutes at 37°C with shaking.
9- For each transformation reaction, 100µl of undiluted cells dilutions on antibiotic plates then it Incubate at 37°C for 12–14 hours.

Transformation used Luria broth it performed as (Sambrook and Russell, 2001) method but used Luria broth in bacterial growth and Luria agar with kanamycin for transformation selection.

Equation for Transformation Efficiency (cfu/µg)  
\[
\frac{\text{cfu on control plate} \times 1 \times 10^7}{\text{ng of Competent Cells Control DNA plated} \mu g} 
\]

RESULTS AND DISCUSSION

The results show that the transformation efficiency of Luria broth was 2.5x10^8ng/µg with kanamycin, while transformation efficiency used SOB, SOC, SOB agar was 1x10^7ng/µg while the transformation using Promega protocol was a failure.

The uptake of exogenous DNA by cells depending on the permeability of cell walls of bacteria thus bacterial cells Permeability depending on microenvironments ,thus bacterial cell can be up take Molecules when
it exposure to stress transformation use different methods to create stress such as heat shock, electricity pulsed and chemical compound.

All transformation methods need long time for preparing media and high cost thus Luria broth which used in this study easy in preparation and don’t need to adjust pH, so it used for molecular biology experiment such as DNA and RNA extraction from different type of bacterial species.

LB medium is a rich medium used in the culture of the Enterobacteriaceae bacteria and for coli phage plaque tests. LB and media (SOC, Terrific Broth, 2xYT which related with it are used in genetic engineering experiments and in other molecular biology procedures. Sometimes an antibiotic can be added to medium for selection cells that contain a specific genetic element like a plasmid, a transposon. X-Gal (5-bromo-4-chloro-3-indolyl-beta-D-galactoside) can be added to medium if the blue-white screen was used for plasmids bearing the alpha fragment of the beta-galactosidase gene. IPTG (isopropyl-beta-D-thiogalactopyranoside) also added for inducing the expression of genes that controlled by the lac promoter. LB has also been used as a general-purpose bacterial culture medium for many types of facultative organisms.

Many types of the LB recipe found in the literature and lab procedures, such as use of LB ("Lennox Broth"), it used 5g of NaCl grams per liter of (Lennox, 1955; Gerhardt, et al. 1994). In some studies a diluted version of LB (0.1% LB) used in environmental microbe isolates (Lee, 2000). In order to isolate marine microbes like Vibrio spp., the investigators used 30 g of NaCl per liter (Nandi, et al., 2000). To maximize growth, especially when a carbon source such as glucose is at a final concentration of 1 to 2g per liter of medium, phosphate buffer or Tris-HCl buffer may be added to maintain the pH.

Present study suggested using Luria broth without any additive for transformation also the results of plasmid profile after transformation by three methods show that no effect on its features thus Luria broth is suitable for rapid transformation by competent cells.

REFERENCES


