

Lab:8

Bacterial transformation

Transformation is the process by which foreign DNA is introduced into a cell. Transformation of bacteria with plasmids is important not only for studies in bacteria but also because bacteria are used as the means for both storing and replicating plasmids. Because of this, nearly all plasmids (even those designed for mammalian cell expression) carry both a bacterial origin of replication and an antibiotic resistance gene for use as a selectable marker in bacteria.

Scientists have made many genetic modifications to create bacterial strains that can be more easily transformed and that will help to maintain the plasmid without rearrangement of the plasmid DNA. Additionally, specific treatments have been discovered that increase the transformation efficiency of the bacteria and make them more susceptible to either chemical or electrical based transformation, generating what are commonly referred to as 'competent cells.

Standard heat-shock transformation of chemically competent bacteria

1. Take competent cells out of -80°C and thaw on ice (approximately 20-30 mins).
2. Remove agar plates (containing the appropriate antibiotic) from storage at 4°C and let warm up to room temperature and then (optional) incubate in 37°C incubator.
3. Mix 1-5 μL of DNA (usually 10 pg - 100 ng) into 50-100 μL of competent cells in a microcentrifuge or falcon tube. GENTLY mix by flicking the bottom of the tube with your finger a few times.

Note: Transformation efficiencies will be approximately 10-fold lower for ligation of inserts to vectors than for an intact control plasmid.

4. Incubate the competent cell/DNA mixture on ice for 20-30 mins.
5. Heat shock each transformation tube by placing the bottom 1/2 to 2/3 of the tube into a 42°C water bath for 90 secs.
6. Put the tubes back on ice for 1-2 min.

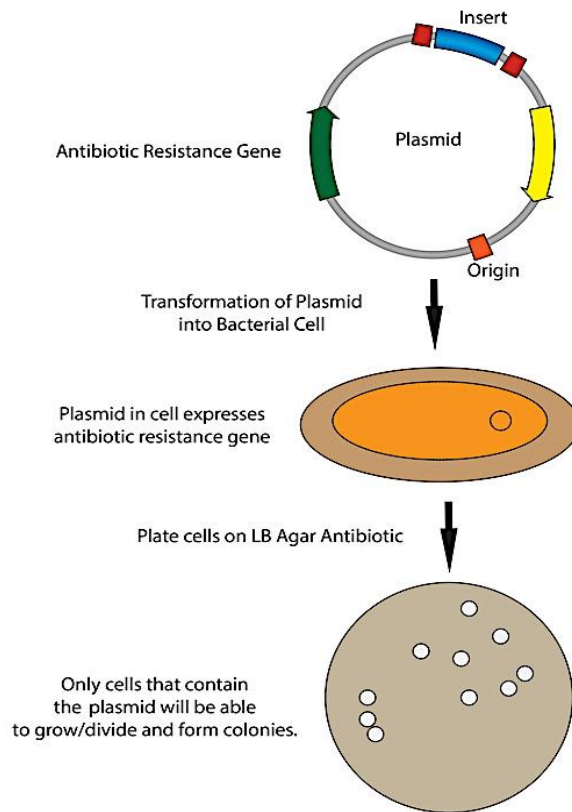
7. Add 800 μL LB or SOC media (without antibiotic) to the bacteria and grow in 37°C shaking incubator for 45 min- 1 hour.
8. Plate some or all of the transformation onto a LB agar plate containing the appropriate antibiotic. Following day, calculate number of colonies on the plates
9. To determine the efficiency of transformation, a positive control transformation should be using 1 ng of control plasmid (e.g:- puc19). The efficiency of transformation is calculated as below:

$$\frac{\text{Number of colonies on Plate}}{\text{Amount of DNA plated (ng)}} \times 1000 \text{ ng}/\mu\text{g}$$

Note: it is recommend to plate 50 μL on one plate and the rest on a second plate. This gives the best chance of getting single colonies, while allowing you to recover all transformed cells.

Note: If the culture volume is too big, gently collect the cells by centrifugation and re-suspend in a smaller volume of LB so that there isn't too much liquid media on the agar plates. If the agar plate doesn't dry adequately before the cells begin dividing, the bacteria diffuse through the liquid and won't grow in colonies.

10. Incubate plates at 37°C overnight.
11. It is recommended to use competent cells only (with out DNA) as a control for the transformation.



Electrocompetent Cells Transformation Protocol

- 1-Prechill the cuvettes on ice.
- 2-Thaw the electrocompetent *E. coli* cells on ice and transfer 40 μL in the cuvette.
- 3-Add the DNA solution (ex. 1 μL of plasmid DNA) and mix gently by shaking the cuvette (do not pipet the cells up and down).
- 4-Dry the cuvette with a tissue to avoid a bypass.
- 5-Insert the cuvette into the electroporation chamber of the pulse control module, and electroporate at $v=2.5\text{kV}$.
- 6-Add immediately 1 mL SOB (no antibiotics!) and mix by gentle pipetting.
- 7-Incubate the cuvette 30-45 minutes at 37°C .
- 8-Plate 10 or 100 μL on LB agar plates containing an antibiotic.
- 9-Incubate o/n at 37°C .
- 10 -Inoculate 3 mL LB (with antibiotic) with a single colony.

Factors Affecting Transformation Efficiency

1- DNA used for transformation reaction

- **The concentration of DNA** must be carefully quantified and the same DNA must be used for all transformations. The pUC19 DNA (used as control) is accurately quantitated and is suitable to maintain the amount of plasmid DNA used in transformation reactions. The transformation reaction is efficient when <10ng of DNA is used. pUC19 DNA (0.1ng) is suitable as control.
- **The form of DNA:** Supercoiled DNA is most efficient for transformation compared to linear or (single strand) ssDNA that has the transformation efficiency of <1%.

2- **Heat shock:** Optimal heat shock set up is as follows:

42°C for 45 seconds for PCR tubes or thin-walled tubes

37°C for 60 seconds for microfuge tubes or thick-walled tubes

It is better to use the 60 seconds at 37°C.

3- Time between transformation and plating: The transformation efficiency is significantly decreased as the time between the transformation reaction and the plating is increased. This, however, also depends on the strain and the plasmid used.

4- Microbial medium used: Super Optimal Broth (SOB rich medium) is most suitable for transformation and gives almost two-fold higher transformation efficiency when compared to LB medium

5- Selective plates: some of commercially prepared agar plates are most are better than others. Plating large number of cells may give rise to satellite colonies that are not true transformants. Streaking the colonies on selective agar plates is recommended to identify true transformants.

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6- Freeze/thawing of cells: Activity of cells that are refrozen and thawed is significantly reduced resulting in at least two-fold decrease in transformation efficiency.