Enhancing Transformation Efficiency

Transformation efficiency is defined as the number of colony forming units (cfu) that would be produced by transforming 1 µg of plasmid into a given volume of competent cells. However, in practice, 1 µg of plasmid is rarely transformed. Instead, efficiency is routinely calculated by transforming 100 pg–1 ng of highly purified supercoiled plasmid under ideal conditions. Transformation Efficiency (TE) is calculated as: TE = Colonies/µg/Dilution. Efficiency calculations can be used to compare cells or ligations. Our recommended protocols and tips are presented here to help you achieve maximum results.

Recommended Protocols

High Efficiency Transformation Protocols

1. Thaw cells on ice for 10 minutes.
2. Add 1 pg–100 ng of plasmid DNA (1–5 µl) to cells and mix without vortexing.
3. Place on ice for 30 minutes.
4. Heat shock at 42°C for 10–30 seconds or according to recommendations.
5. Place on ice for 5 minutes.
6. Add 950 µl of room temperature SOC.
7. Place at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
8. Mix cells without vortexing and perform several 10-fold serial dilutions in SOC.
9. Spread 50–100 µl of each dilution onto pre-warmed selection plates and incubate overnight at 37°C (30°C for SHuffle™ strains) or according to product recommendations.

5 Minute Transformation Protocol

(10% efficiency compared to above protocol)

1. Thaw cells in your hand.
2. Add 1 pg–100 ng of plasmid DNA (1–5 µl) to cells and mix without vortexing.
3. Place on ice for 2 minutes.
4. Heat shock at 42°C for 30 seconds or according to recommendations.
5. Place on ice for 2 minutes.
6. Add 950 µl of room temperature SOC. Immediately spread 50–100 µl onto a selection plate and incubate overnight at 37–42°C. NOTE: Selection using antibiotics other than ampicillin may require some outgrowth prior to plating.

DNA Contaminants to Avoid

CONTAMINANT	REMOVAL METHOD
Detergents	Ethanol precipitation
Phenol	Extract with chloroform and ethanol precipitate
Ethanol or Isopropanol	Dry pellet before resuspension
PEG	Column purify or phenol/chloroform extract and ethanol precipitate
DNA binding proteins (e.g., Ligase)	Column purify or phenol/chloroform extract and ethanol precipitate

(see other side)

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Transformation Tips

Thawing

- Cells are best thawed on ice.
- DNA should be added as soon as the last trace of ice in the tube disappears.
- Cells can be thawed by hand, but warming above 0°C decreases efficiency.

Incubation of DNA with Cells on Ice

- Incubate on ice for 30 minutes. Expect a 2-fold loss in TE for every 10 minutes this step is shortened.

Heat Shock

- Both temperature and time are specific to the transformation volume and vessel. Typically, 30 seconds at 42°C is recommended.

Outgrowth

- Outgrowth at 37°C for 1 hour is best for cell recovery and for expression of antibiotic resistance. Expect a 2-fold loss in TE for every 15 minutes this step is shortened.
- SOC gives 2-fold higher TE than LB medium.
- Incubation while shaking or rotating the tube results in a 2-fold higher TE.

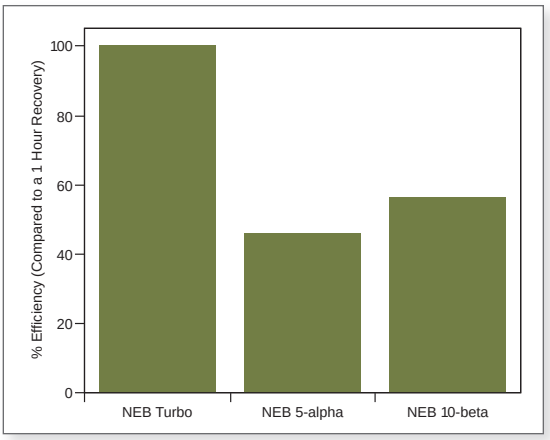
Plating

- Selection plates can be used warm or cold, wet or dry with no significant effects on TE.
- Warm, dry plates are easier to spread and allow for the most rapid colony formation.

DNA

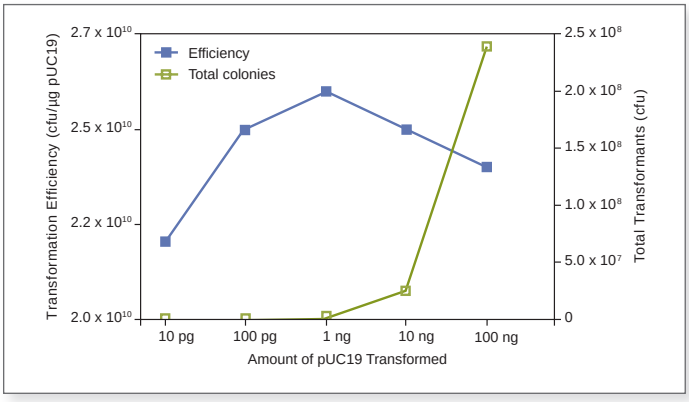
- DNA for transformation should be purified and resuspended in water or "TE" Buffer.
- Up to 10 µl of DNA from a ligation mix can be used with only a 2-fold loss of efficiency.
- Purification by either a spin column or phenol/chloroform extraction and ethanol precipitation should be performed.
- The optimal amount of DNA is lower than commonly recognized. Using clean, supercoiled pUC19, the efficiency of transformation is highest in the 100 pg–1 ng range. However, the total number of colonies that can be obtained from a single transformation reaction increases up to approximately 100 ng.

Effect of Outgrowth Period on Electroporation Efficiency of Electrocompetent Cells



Strains were electroporated with 10 pg of pUC19 DNA suspended in deionized water. Immediately after electroporation, SOC (pre-warmed to 37°C) was added to the cuvette to a final volume of 1 ml. The cells were diluted 1:1000 in SOC and plated on LB-ampicillin plates pre-warmed to 37°C. The electroporation efficiency of NEB Turbo is not dependent on the outgrowth period. Elimination of the 1 hour outgrowth period results in an 56% and 46% reduction in electroporation efficiency for NEB 5-alpha and NEB 10-beta, respectively.

DNA Effects on Transformation Efficiency and Colony Output



The optimal amount of DNA to use in a transformation reaction is lower than commonly recognized. Using clean, supercoiled pUC19 and NEB 5-alpha electrocompetent cells, the efficiency of transformation is highest in the 100 pg–1 ng range. However, the total colonies which can be obtained from a single transformation reaction increase up to about 100 ng.

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NEW ENGLAND BioLabs® Inc. enabling technologies in the life sciences
New England Biolabs, Inc., 240 County Road, Ipswich, MA 01938-2723
Telephone: (978) 927-5054 Toll Free: (USA Orders) 1-800-632-5227 Toll Free: (USA Tech) 1-800-632-7799 Fax: (978) 921-1350 e-mail: info@neb.com