# **Enhancing Transformation Efficiency**

Transformation efficiency is defined as the number of colony forming units (cfu) that would be produced by transforming 1  $\mu g$  of plasmid into a given volume of competent cells. However, in practice, 1  $\mu g$  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/ $\mu g$ /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.
- 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.

CLONING & MAPPING

DNA AMPLIFICATION & PCR

RNA ANALYSIS

PROTEIN EXPRESSION & ANALYSIS

GENE EXPRESSION & CELLULAR ANALYSIS

#### 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)



## 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.

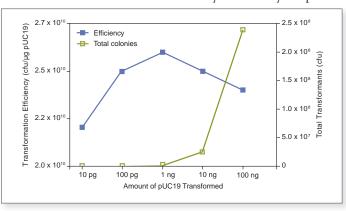
#### Plating

- · 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.

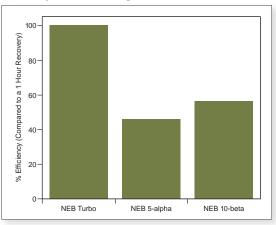
## DNA Effects on Transformation Efficiency and Colony Output



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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.

# 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.