Enhancing Competent Cell Transformation Efficiency

INTRODUCTION

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 Protocol

1. Thaw competent 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 outgrowth medium.
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 outgrowth medium.
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 competent 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 outgrowth medium. 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.

TABLE 1: **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 (e.g. Monarch® Spin PCR & DNA Cleanup Kit (5 µg), NEB #T1130) or phenol/chloroform extract and ethanol precipitate
DNA binding proteins (e.g., ligase)	Column purify (e.g. Monarch Spin PCR & DNA Cleanup Kit (5 µg), NEB #T1130) or phenol/chloroform extract and ethanol precipitate

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 (or NEB 10-beta/Stable outgrowth medium) 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.
- For best results, the volume of transformed DNA should not exceed 10% of the total volume of cells.
- Column purify (e.g. Monarch Spin PCR & DNA Cleanup Kit (5 µg), NEB #T1130) or phenol/chloroform extract and ethanol precipitate.
- 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.