Transformation Protocol

1. Thaw a tube of NEB 5-alpha Competent E. coli cells on ice until the last ice crystals disappear. Mix gently and carefully pipette 50 µl of cells into a transformation tube on ice.
2. Add 1–5 µl containing 100 pg–1 µg of plasmid DNA to the cell mixture. Carefully flick the tube 4–5 times to mix cells and DNA. Do not vortex.
3. Place the mixture on ice for 30 minutes. Do not mix.
5. Place on ice for 5 minutes. Do not mix.
6. Pipette 950 µl of room temperature SOC into the mixture.
7. Place at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
8. Warm selection plates to 37°C.
9. Mix the cells thoroughly by flicking the tube and inverting, then perform several 10-fold serial dilutions in SOC.
10. Spread 50–100 µl of each dilution onto a selection plate and incubate overnight at 37°C. Alternatively, incubate at 30°C for 24–36 hours or 25°C for 48 hours.

5 Minute Transformation Protocol

A shortened transformation protocol resulting in only 10% efficiency compared to the standard protocol may be suitable for applications where a reduced total number of transformants is acceptable. Follow the transformation protocol above with the following changes:

1. Steps 3 and 5 are reduced to 2 minutes.
2. Omit outgrowth (Step 7) completely for ampicillin-resistant plasmids or reduce the outgrowth time for other selective media as appropriate.

Transformation Protocol Variables

Thawing: Cells are best thawed on ice and DNA added as soon as the last bit of ice in the tube disappears. Cells can also be thawed by hand, but warming above 0°C will decrease the transformation efficiency.

Incubation of DNA with Cells on Ice: For maximum transformation efficiency, cells and DNA should be incubated together on ice for 30 minutes. Expect a 2-fold loss in transformation efficiency for every 10 minutes this step is shortened.

Heat Shock: Both the temperature and the timing of the heat shock step are important and specific to the transformation volume and vessel. Using the transformation tube provided, 30 seconds at 42°C is optimal.

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 transformation efficiency for every 15 minutes this step is shortened. SOC gives 2-fold higher transformation efficiency than LB medium; and incubation with shaking or rotating the tube gives 2-fold higher transformation efficiency than incubation without shaking.

Plating: Selection plates can be used warm or cold, wet or dry without significantly affecting the transformation efficiency. However, warm, dry plates are easier to spread and allow for the most rapid colony formation.

Effect of DNA Purity on Transformation Efficiency and Colony Output: The total colonies which can be obtained from a single transformation reaction with NEB 5-alpha Competent E. coli (Subcloning Efficiency) are not significantly reduced when using miniprep DNA. Using 10 ng–1000 ng of clean, supercoiled pUC19 or pUC19 isolated with a QiAprep Spin Miniprep Kit, total colonies increase with increasing DNA concentration.
DNA Contaminants to Avoid

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Removal Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detergents</td>
<td>Ethanol precipitate</td>
</tr>
<tr>
<td>Phenol</td>
<td>Extract with chloroform and ethanol precipitate</td>
</tr>
<tr>
<td>Ethanol or Isopropanol</td>
<td>Dry pellet before resuspending</td>
</tr>
<tr>
<td>PEG*</td>
<td>Column purify or phenol/chloroform extract and ethanol precipitate</td>
</tr>
<tr>
<td>DNA binding proteins* (e.g. Ligase)</td>
<td>Column purify or phenol/chloroform extract and ethanol precipitate</td>
</tr>
</tbody>
</table>

*Ideally, DNA for transformation should be purified and resuspended in water or TE. However, up to 10 µl of DNA directly from a ligation mix can be used with only a two-fold loss of transformation efficiency. Where it is necessary to maximize the number of transformants (e.g. a library), a purification step, either a spin column or phenol/chloroform extraction and ethanol precipitation should be added.

Calculation of Transformation Efficiency

Transformation efficiency is defined as the number of colony forming units (cfu) which would be produced by transforming 1 µg of plasmid into a given volume of competent cells. The term is somewhat misleading in that 1 µg of plasmid is rarely actually transformed. Instead efficiency is routinely calculated by transforming 100 pg–1 ng of highly purified supercoiled plasmid under ideal conditions. If you plan to calculate efficiency to compare cells or ligations, keep in mind the many variables which affect this metric.

Transformation efficiency (TE) equation:

$$ TE = \frac{\text{Colonies}}{\mu g \text{ DNA}} \times \frac{1}{\text{Dilution}} $$

Colonies = the number of colonies counted on the plate
µg = the amount of DNA transformed expressed in µg
Dilution = the total dilution of the DNA before plating

TE calculation example:

Transform 1 µl (1 µg) of pUC19 DNA into 50 µl of cells, outgrow by adding 950 µl of SOC and dilute 10 µl up to 1 ml in SOC before plating 100 µl. If you count 250 colonies on the plate, the TE is:

Colonies = 250
µg DNA = 1.0
Dilution = 10/1000 x 100/1000 = 0.001
TE = 250/1/0.001 = 2.5 x 10^5 cfu/µg

Solutions/Recipes

SOB:

- 2% Vegetable peptone (or Tryptone)
- 0.5% Yeast Extract
- 10 mM NaCl
- 2.5 mM KCl
- 10 mM MgCl₂
- 10 mM MgSO₄

SOC:

- SOB + 20 mM Glucose

LB agar:

- 1% Tryptone
- 0.5% Yeast Extract
- 0.17 M NaCl
- 1.5% Agar

Blue/White Screening:

- X-gal 80 µg/ml
- IPTG* 0.3 mM

*Omit IPTG for potentially toxic genes

Antibiotics for Plasmid Selection

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Working Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>100 µg/ml</td>
</tr>
<tr>
<td>Carbenicillin</td>
<td>100 µg/ml</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>33 µg/ml</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>30 µg/ml</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>25 µg/ml</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>15 µg/ml</td>
</tr>
</tbody>
</table>

Genotype: **thuA2 Δ(araF-lacZ)U169 phoA glnV44 80Δ (lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17**

Strain Properties

The properties of this strain that contribute to its usefulness as a cloning strain are described below. The genotypes underlying these properties appear in parentheses.

Blue/White Screening (**80Δ (lacZ)M15**): makes the ω-fragment of β-galactosidase (β-gal); (**argF-lacZ**) deletes the β-gal gene on the chromosome. pUC19 and similar plasmids show for the ω-peptide of β-gal (**lacZ**). The α-peptide can combine with the ω-fragment of β-gal that is carried on **80** (α-complementation). When β-gal is reconstituted in this manner it can cleave 5-bromo-4-chloro-3-indolyl-β-D-galactosidase (X-gal) and results in blue colonies on an X-gal plate. Inserts cloned into the plasmid polylinker disrupt the α-peptide gene and the colonies are white.

Recombination Deficient (**recA1**): E. coli has a repair system that will recombine homologous sequences. Genomic clones often have duplicated regions, and RecA mediated rearrangements can be problematic, particularly when regions of homology are longer than 50 bp. Strains which have the RecA function deleted tend to grow more slowly than recA strains.

Endonuclease I Deficient (**endA**): The periplasmic space of wild type E. coli cells contains a nonspecific endonuclease. Extreme care must be taken to avoid degradation of plasmids prepared from these cells. The endA mutation deletes this endonuclease and can significantly improve the quality of plasmid preparations.

Restriction Deficient (**hsdR17**): Wild type E. coli K12 strains carry a restriction endonuclease which cleaves DNA with sites (AAC(N6)GTGC and GCAC(N6)GTT. While E. coli DNA is protected from degradation by a cognate methyl-transferase, foreign DNA will be cut at these sites. The hsdR mutation eliminates this endonuclease activity. However, this strain has functional methyl restriction systems and may not be suitable for direct cloning of eukaryotic DNA.

T1 Phage Resistant (**thuA2**: T1, an extremely virulent phage requires the E. coli ferric hydroxamate uptake receptor for infectivity. Deletion of this gene confers resistance to this type of phage, but does not significantly affect the transformation or growth characteristics of the cell.

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