High Efficiency Transformation Protocol

Perform steps 1–7 in the tube provided.
1. Thaw a tube of NEB 5-alpha F’Iq Competent E. coli cells on ice for 10 minutes.
2. Add 1–5 µl containing 1 pg–100 ng 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 5 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.

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, 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.
DNA Contaminants to Avoid

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Removal Method</th>
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<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 resuspension</td>
</tr>
<tr>
<td>PEG*</td>
<td>Column purity or phenol/chloroform extract and ethanol precipitate</td>
</tr>
<tr>
<td>DNA binding proteins*</td>
<td>Column purity or phenol/chloroform extract and ethanol precipitate</td>
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</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:

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

Colonies = the number of colonies counted on the plate

µg = the amount of DNA transformed expressed in µg

Dilution = total dilution of the DNA before plating

TE calculation example:

Transform 2 µl (100 pg) of control pUC19 DNA into 50 µl of cells, out-grow by adding 250 µl of SOC and dilute 10 µl up to 1 ml in SOC before plating 30 µl. If you count 150 colonies on the plate, the TE is:

$$\text{Colonies} = 150$$

$$\mu g \text{DNA} = 0.0001$$

$$\text{Dilution} = 10/300 \times 1000 = 0.001$$

$$\text{TE} = \frac{150/0.0001/0.001}{1.5 \times 10^9} \text{cfu/µg}$$

Antibiotics for Plasmid Selection

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Working Concentration</th>
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<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>
</tbody>
</table>

Genotype: F’ proA B’ lacIq Δ(lacZ)M15 zxf::Tn10 (TetR) / fhuA2ΔargF-lacZ

U169 phoA glnV44 o805(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 (F’ Δ(lacZ)M15): makes ω-fragment of β-gal; Δ(lac-proAB) deletes the β-gal gene on the chromosome. pUC19 and similar plasmids code for the α-peptide of β-galactosidase (lacZ). The α-peptide can combine with the ω-fragment of β-galactosidase which is carried on the F’ (α-complementation). When β-galactosidase is reconstituted in this manner it can cleave 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 that have the RecA function deleted tend to grow more slowly than recA strains.

Endonuclease I Deficient (endA1): 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 endA1 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 hsdR1 mutation eliminates this endonuclease activity. However, this strain has functional methyl restriction systems and may not be suitable for direct cloning of eukaryotic DNA.

M13 phage sensitive (F’): Infection by M13 and other similar phage requires E. coli surface features conferred by the F plasmid carried by some E. coli strains. Infection by these phage allows production of single-stranded DNA and the generation of phage display libraries. The F plasmid is frequently modified to carry other useful DNA in the cell [e.g. Δ(lacZ)M15 in this cell line] and when modified is called F’.

T1 Phage Resistant (fhuA2): 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.

Lac Promoter Control (lacIq): The lac repressor blocks expression from lac, tac and trc promoters frequently carried by expression plasmids. If the level of lac repressor in E. coli cells is not sufficient to inhibit expression via these promoters during transformation or cell growth, even low levels of expression can reduce transformation efficiency and select against desired transformants. The extra molecules of lac repressor in lacIq strains help to minimize promoter activity until IPTG is added.

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Companion Products Sold Separately:

SOC Outgrowth Medium

#B9020S 4 x 25 ml medium