Phage T1 resistant (\(\text{dam}^-/\text{dcm}^-\))

Allows for growth of plasmids free of Dam and Dcm methylation

- Activity of nonspecific endonuclease I
- Free of animal products

Transformation Efficiency: 1–3 \(\times 10^8\) cfu/\(\mu\)g pUC19 DNA

1. Remove cells from \(-80^\circ\text{C}\) freezer and thaw in your hand.
2. Add 1–5 \(\mu\)l 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 2 minutes. Do not mix.
5. Place on ice for 2 minutes. Do not mix.
6. Pipette 950 \(\mu\)l of room temperature SOC into the mixture. Immediately spread 50–100 \(\mu\)l onto a selection plate and incubate overnight at 37°C. **NOTE:** Selection using antibiotics other than ampicillin may require some outgrowth before plating on selective media. Colonies develop faster at temperatures above 37°C, however some constructs may be unstable at elevated temperatures.

**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.

**DNA Effects on Transformation Efficiency and Colony Output:**

<table>
<thead>
<tr>
<th>Amount of DNA Transformed ((\mu)g)</th>
<th>Transformation Efficiency (T/(\mu)g (\times) 10^9)</th>
<th>Total Transformants (cfu (\times) 10^7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 pg</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>100 pg</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>1 ng</td>
<td>0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>100 ng</td>
<td>0.6</td>
<td>0.5</td>
</tr>
</tbody>
</table>

**NOTE:** Selection using antibiotics other than ampicillin may require some outgrowth before plating on selective media. Colonies develop faster at temperatures above 37°C, however some constructs may be unstable at elevated temperatures.

**Quality Control Assays**

**Transformation Efficiency:** 1 ng of pUC19 plasmid DNA was used to transform \(\text{dam}^-/\text{dcm}^-\) Competent E. coli following the high efficiency protocol provided. Greater than 1–3 \(\times 10^6\) colonies formed/\(\mu\)g after an overnight incubation on LB-ampicillin plates at 37°C.

Untransformed cells were also tested for resistance to phage \(\phi\)80, a standard test for resistance to phage T1, and sensitivity to ampicillin, kanamycin, spectinomycin and tetracycline. Cells are resistant to chloramphenicol and streptomycin.

**Transformation Protocol**

1. Thaw a tube of \(\text{dam}^-/\text{dcm}^-\) Competent E. coli on ice for 10 minutes.
2. Add 1–5 \(\mu\)l 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 30 minutes. Do not mix.
5. Place on ice for 5 minutes. Do not mix.
6. Pipette 950 \(\mu\)l 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.

**Storage and Handling:** Competent cells should be stored at \(-80^\circ\text{C}\). Storage at \(-20^\circ\text{C}\) will result in a significant decrease in transformation efficiency. Cells lose efficiency whenever they are warmed above \(-80^\circ\text{C}\), even if they do not thaw.
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* (e.g. Ligase)</td>
<td>Column purify or phenol/chloroform extract and ethanol precipitate</td>
</tr>
<tr>
<td>DNA binding proteins</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:

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

TE calculation example:
Transform 2 µl (100 pg) of control pUC19 DNA into 50 µl of cells, outgrow by adding 950 µl of SOC before plating 100 µl. If you count 20 colonies on the plate, the TE is:

Colonies = 20
µg DNA = 0.0001
Dilution = 100/1000 = 0.1
TE = 20/0.0001/0.1 = 2 x 10^4 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

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>Kanamycin</td>
<td>30 µg/ml</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>15 µg/ml</td>
</tr>
</tbody>
</table>

Genotype: ara-14 leuB6 fhuA31 lacY1 tsx78 glnV44 galK2 galT22 mcrA dcm-6 hisG4 rhd11 R (1024B::Tn10) Terd endA1 rpsL136 (Str⁶)
dam13::Tn9 (Cam⁴) xylA-5 mtl-1 thi-1 mcrB1 hsdR2

Strain Properties

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

dam and dcm Methylation Deficient (dam13::Tn9 (Cam⁴), dcm-6): Most laboratory strains of E. coli contain both Dam methylase and Dcm methylase. Dam methylase transfers a methyl group to the adenine in the sequence GATC. Dcm methylase methylates the internal cytosine residues in the sequences CCAG and CCTGG. Several restriction endonucleases will not cleave sites with these modified bases. The damdcm strain allows growth and purification of DNA free of Dam and Dcm methylation

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 endA mutation deletes this endonuclease and can significantly improve the quality of plasmid preparations.

Restriction Deficient (hsdR2): Wild type E. coli K12 strains carry the EcoK Type I restriction endonuclease which cleaves DNA with sites (AAC(N6)GTGC and GCAC(N6)GT). While E. coli DNA is protected from degradation by a cognate methyl-transferase, foreign DNA will be cut at these sites. The hsdR2 mutation described above eliminates the endonuclease.

Partially Methyl Restriction Deficient (mcrA, mcrB1): E. coli has a system of enzymes, mcrA, mcrB and mrr which will cleave DNA with methylation patterns found in higher eukaryotes, as well as some plant and bacterial strains. DNA derived from PCR fragments, cDNA or DNA previously propagated in E. coli will not be methylated at these sites and will not be cleaved. This strain has a functional Mrr endonuclease and may not be suitable for direct cloning of eukaryotic DNA.

T1 Phage Resistant (fhuA31): 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.

Companion Products Sold Separately:

SOC Outgrowth Medium
#B9020S 4 x 25 ml medium