8. Warm selection plates to 37°C.
7. Place at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
6. Pipette 950 µl of room temperature SOC into the mixture.
5. Place on ice for 5 minutes. Do not mix.
3. Place the mixture on ice for 30 minutes. Do not mix.
2. Add 1–5 µl containing 1 pg–100 ng of plasmid DNA to the cell mixture.

Transformation Efficiency:
1 ng of pUC19 plasmid DNA was used to transform dam–/dcm– strains. The 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: 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.
**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:**

\[
TE = \frac{\text{Colonies}}{\mu g / \text{Dilution}}
\]

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 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:

\[
\text{Colonies} = 20
\]

\[
\mu g \text{ DNA} = 0.0001
\]

\[
\text{Dilution} = \frac{100}{1000} = 0.1
\]

\[
TE = \frac{20/0.0001/0.1}{2} = 2 \times 10^6 \text{ cfu/µg}
\]

**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

**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 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>
</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.

**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 thiA1 lacY1 tsx78 glnV44 galK2 gagT22 mcrA dcm-6 hisG4 rhlD1 R(zgb210::Tn10) Tn10 endA1 rpsL136 (Str*) dam13::Tn9 (Cam*) xylA-5 mtl-1 thi-1 mcrB1 hisG4

**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 (Cam*), 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 CCAGG 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)GTT). 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