The Inoue Method for Preparation of Competent E.Coli
“Ultra-competent” Cells

Buffers & Solutions

1. High quality DMSO
2. .5M PIPES (piperazine-1,2-bis[2-ethanesulfonic acid]) at pH 6.7
   i. Dissolve 15.1g of PIPES in 80mL Milli-Q H₂O
   ii. Adjust pH of solution to 6.7 (with KOH or HCl)
   iii. Add Milli-Q H₂O to final volume of 100mL
   iv. (optional: sterilize solution by vacuum filtration in pre-rinsed Nalgene filter of .45µm pore size)
3. Inoue Transformation Buffer (chill to 0°C before use)
   i. Dissolve solutes below in 800mL of Milli-Q H₂O
      | Reagent      | Qty/L   | Final Conc. |
      |--------------|---------|-------------|
      | MnCl₂·4H₂O   | 10.88g  | 55mM        |
      | CaCl₂·2H₂O   | 2.20g   | 15mM        |
      | KCl          | 18.65g  | 250mM       |
   ii. Add 20mL PIPES (.5M, pH 6.7)
   iii. Adjust total volume to 1L with Milli-Q H₂O
   iv. Sterilize Inoue transformation buffer by filtration through pre-rinsed .45µm Nalgene filter

Media

1. LB or SOB for initial culture growth

Centrifuge & Rotors

1. Sorvall GSA rotor or equivalent

Special Equipment

1. Liquid nitrogen
2. Polypropylene tubes
3. Shaking incubator (18°C)
4. Water bath at 42°C
5. Chilled microfuge tubes
Day 1: Growing Bacterial Cultures

1. Pick a single bacterial colony (2-3 mm in diameter) from a plate that has been incubated for 16-20 hours at 37˚C.
2. Transfer colony into 25mL of LB broth or SOB medium in at 250mL flask.
3. Incubate culture for 6-8 hours at 37˚C with vigorous shaking (250-300rpm).
4. Inoculate three 1L flasks of 250mL SOB using the below volumes of this starter culture.

<table>
<thead>
<tr>
<th>Flask #</th>
<th>Vol. of Starter Culture</th>
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<tbody>
<tr>
<td>1</td>
<td>1mL</td>
</tr>
<tr>
<td>2</td>
<td>50µL</td>
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<tr>
<td>3</td>
<td>25µL</td>
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5. Incubate all three flasks overnight at room temperature (18-22˚C) with moderate shaking.

Day 2: Harvesting Cells and Freezing Competent Cells

1. Read the OD₆₀₀ of all three cultures. Continue to monitor every 45min until reading is at 0.55.
2. Transfer the culture vessel to ice water bath for 10min.
3. Harvest cells by centrifugation at 2500g (3900 rpm in Sorvall GSA rotor) for 10 minutes at RT in 50mL falcon tube.
4. Pour off medium and dry the tube (inverted) on paper towels for 2min (use vacuum aspirator to remove any drops of remaining medium adhering to walls of the centrifuge bottle or trapped in its neck).
5. Resuspend the cells gently (by swirling) in 80mL of ice-cold (0˚C) Inoue transformation buffer.
6. Harvest cells by centrifugation at 2500g for 10 minutes at room temperature.
7. Pour off the medium and dry the tube on paper towels for 2min (use vacuum aspirator to remove any drops of remaining medium adhering to walls of the centrifuge bottle or trapped in its neck).
8. Resuspend cells GENTLY in 20mL of Inoue transformation buffer (0˚C).
9. Add 1.5mL of DMSO (swirl to mix bacterial suspension).
10. Store on ice for 10min.
11. Quickly dispense 50µL aliquots of suspensions into chilled, sterile microfuge tubes. (20mL of suspension -> 400 tubes of 50µL).
13. When needed, remove tube of competent cells from freezer, thaw in hand, use immediately.