Transformation Protocol

**Purpose:** To transform particular DNA into a desired cell line. For example, transforming a kanamycin resistance plasmid into E. coli cell line IR9-50-1.

---

### Solutions

**Keep all solutions on ice for the duration of this experiment.**

<table>
<thead>
<tr>
<th>Name</th>
<th>Recipe</th>
<th>Date or Lot #</th>
<th>Volume Needed*</th>
</tr>
</thead>
<tbody>
<tr>
<td>100mM CaCl₂</td>
<td>1.11 g CaCl₂ 100ml diH₂O</td>
<td></td>
<td>833ul/tube</td>
</tr>
<tr>
<td>Plasmid or DNA</td>
<td>N/A</td>
<td></td>
<td>1.5ul/tube</td>
</tr>
<tr>
<td>SOC</td>
<td>Aliquot from stock in fridge</td>
<td></td>
<td>950ul/tube</td>
</tr>
</tbody>
</table>

*You will most likely have 3 tubes.

---

### Supplies and Equipment

<table>
<thead>
<tr>
<th>✓</th>
<th>Equipment</th>
<th>Miscellaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>~20ml Test tubes (3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pipette gun</td>
<td>10ml glass pipettes</td>
</tr>
<tr>
<td></td>
<td>p1000 and p10 pipettes</td>
<td>white and yellow tips</td>
</tr>
<tr>
<td></td>
<td>2ml Eppendorf tubes (3)</td>
<td>rack and float</td>
</tr>
<tr>
<td></td>
<td>1.5ml Eppendorf tubes (3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mixing Bath</td>
<td>set to 37°C</td>
</tr>
<tr>
<td></td>
<td>Water baths (2)</td>
<td>set to 37°C and 42°C</td>
</tr>
<tr>
<td></td>
<td>Spectrophotometer</td>
<td>cuvettes</td>
</tr>
<tr>
<td></td>
<td>Centrifuge</td>
<td>in 4°C cold room</td>
</tr>
<tr>
<td></td>
<td>Vacuum apparatus (for aspirating)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ice bucket</td>
<td>ice</td>
</tr>
<tr>
<td></td>
<td>Bunsen burner</td>
<td>striker</td>
</tr>
<tr>
<td></td>
<td>Agar plates</td>
<td>with appropriate antibiotic</td>
</tr>
</tbody>
</table>

---

**Notes:**

Check with someone if you are unsure of which plasmid/DNA sample or plates to use. For this example, a kanamycin resistance plasmid is being transformed. We will plate on kanamycin plates which selects for bacteria that contain the transformed resistance plasmid.

Always wipe cuvettes with a kimwipe before taking a spec reading.

Supernatant is the top layer of liquid in an Eppendorf tube.

Aspiration is removing the supernatant with the vacuum apparatus (to waste beaker).

To transfer supernatant use the 1000ml pipette.

---

**Next Step:**
1. Prepare an overnight culture (day before) (see separate protocol)

2. Prepare and incubate test tubes:

   Pipette the following amounts into each of three test tubes:
   - 5.0ml LB broth (this is a blank/control) (Label Tube #1/Ctrl)
   - 4.9ml LB broth and 100ul overnight culture (Label Tube #2)
   - 4.9ml LB broth and 100ul overnight culture (Label Tube #3)

   Incubate all 3 tubes in the 37°C mixing bath until optical density of 0.3-0.5 at 600nm. (see step 3)

3. Read optical density (start around 60 minutes of incubation in mixing bath) (be sure spec is set to 600nm):

   - Pipette 800ul from tube #1 into a cuvette to use as a blank. Record the absorbance: __________
   - Pipette 800ul from tube #2 into a cuvette to use as the sample and record the absorbance: __________
   - Take readings at 10 minute intervals until the difference between sample and blank is between 0.3 and 0.5

   Final sample reading: _______ Blank reading: _______ Difference: _______ Total time: _______ min

4. Centrifuge and Resuspend:

   - Label three 2ml Eppendorf tubes A, B, and C.
   - Pipette 1.6ml from tube #3 into each of the three tubes.
   - Spin the tubes at max speed for 5 minutes using the centrifuge in the 4°C cold room
   - Aspirate supernatant

   **KEEP EVERYTHING ON ICE FROM THIS POINT FORWARD**

   - Resuspend pellets from step 4 by adding 333ul of ice cold CaCl₂ to each tube
   - Incubate on ice for at least 20 minutes
   - Spin the tubes at max speed for 5 minutes using the centrifuge in the 4°C cold room
   - Aspirate supernatant
   - Resuspend in 500ul ice cold CaCl₂

5. Transform:

   - Label three 1.5ml Eppendorf tubes A', B', C'; Pipette the following amounts into each tube:
     - A': 70ul from tube A, 1.5ul plasmid
     - B': 70ul from tube B, 1.5ul plasmid
     - C': 70ul from tube C, 1.5ul diH₂O (this is your control)

   - Incubate A', B', and C' on ice for at least 30 minutes
   - Incubate at 42°C for exactly 90 seconds
   - Incubate on ice for at least 2 minutes
   - Pipette 850ul SOC into each tube
   - Incubate 45 minutes at 37°C
   - Spin all three tubes at max speed for 5 minutes using the centrifuge in the 4°C cold room
   - Aspirate supernatant
   - Resuspend in 100ul SOC each for full plates (only use 50ul SOC if using half plates in step 6)

6. Plate:

   - Label three plates A, B, and C/Ctrl along with date, initials, agar type, incubation temperature, etc.
   - Pipette 100ul of transformed cells from A' onto plate A
   - Pipette 100ul of transformed cells from B' onto plate B
   - Pipette 100ul of control cells form C’ onto plate C/Ctrl

7. Incubate plates at 37°C overnight or as needed.