1. **Purpose**
   Allowing DNA to be introduced into the cell. Insertion of DNA onto the bacterial chromosome.

2. **Area of application**
   All bacteria.
3. Apparatus and equipment

<table>
<thead>
<tr>
<th>Apparatus/equipment</th>
<th>Location (Room number)</th>
<th>Check points</th>
<th>Criteria for approval/rejection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ice</td>
<td>Across V18-403b-2</td>
<td>●</td>
<td></td>
</tr>
<tr>
<td>Heating block</td>
<td>Laboratory 1. Floor</td>
<td>●</td>
<td></td>
</tr>
<tr>
<td>Pipettes (1000)</td>
<td></td>
<td>●</td>
<td></td>
</tr>
<tr>
<td>Pipettes (100)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pipettes (10)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4. Materials and reagents – their shelf life and risk labelling

<table>
<thead>
<tr>
<th>Name</th>
<th>Components (Concentrations)</th>
<th>Manufacturer / Cat. #</th>
<th>Room</th>
<th>Safety considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Purple pipette tips</td>
<td>Contact lab-manager</td>
<td>Micro storage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green pipette tips</td>
<td>Contact lab-manager</td>
<td>Micro storage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue pipette tips</td>
<td>Contact lab-manager</td>
<td>Micro storage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 % arabinose</td>
<td></td>
<td></td>
<td>Chemical room</td>
<td></td>
</tr>
</tbody>
</table>

5. QC – Quality Control
   Colony PCR

6. List of other SOPs relevant to this SOP
   iGEM2017_SOP12_v02_EG_Making_LB_and_LA_media

7. Environmental conditions required

8. Procedure
   Preparation of electro competent cells

   Overnight culture of strain BW25113/pKD46.
   Dilute ONC 100-fold in 50 mL LB in a sterile glass flask and add appropriate antibiotica. Grow at 30 °C until OD₄₅₀ has reached 0.4-0.6.

   Be attentive to place the culture at 30 °C.

   Place a bottle of sterile H₂O on ice.

   1.1 When the cells have reached the desired OD, induce the culture with 500 µL 20 % arabinose. (500 µL pr 50 mL culture).
1.2 Shake for 30 minutes. Be precise and use a timer!

1.3 While the cells are inducing get an ice bucket with ice and water and cool down a centrifuge to 4 °C.

1.4 Immediately after 30 minutes of arabinose-induction, rapidly cool culture-flask in ice water.

1.5 Leave on ice for 5-10 minutes, while gently shaking once in a while.

**Washing to make competent cells.**

2.1 Transfer cells to sterile 50 mL plastic centrifuge tubes.

2.2. Spin at 6500 rpm for 10 minutes.

2.3 Decant supernatant. Pellet can be soft, so be careful.

2.4 Add 1 mL cold water to tube and gently resuspend cells by gently shaking the tubes by hand. If the pellet does not dissolve, resuspend the cells gently with a pipette.

2.5 Add another 40 mL cold water to tube and invert several times to mix.

2.6 Spin as before. Pellet will be soft.

2.7 As soon as centrifuge comes down remove tubes and decant supernatant.

2.8 Add 1 mL cold water and resuspend. Transfer to an eppendorf tube.

2.9 Spin for 1 minute.

2.10 Aspirate off supernatant with a pipette.

2.11 Carefully resuspend pellet in 300 µL cold water.

**Electroporation.**

3.1 Mark and place 3 cuvettes on ice. Special cuvettes: BioRad 0.1 cm gap.

3.2 Mark 3 eppendorf tubes and place on ice.

3.3 Add 10 ng DNA to tube no. 2 and 100 ng DNA to tube no. 3.

3.4 Add 100 µL cells to each tube and transfer content to the cold cuvettes.

3.5 Wipe ice off cuvettes and zap using a Gene Pulser Electroporation System. On the Gene Pulser the settings should be on 1,8 kW and 25 VFD and on the controller 200 Ω.

3.6 Add 1 mL pre-warmed LB to the cuvettes and transfer to clean eppendorf tubes.

3.7 Place the tubes for phenotypic expression at 37 °C for 1-2 hours.

3.8 Spread approximately 75 µL cells on selection plates and incubate overnight at 37 °C. Optional: place at 42 °C to ensure that the bacteria loose the pKD46 plasmid.
9. **Waste handling**

<table>
<thead>
<tr>
<th>Chemical name</th>
<th>Concentration</th>
<th>Type of waste (C, Z...)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pipette tips</td>
<td>N/A</td>
<td>GMO waste (yellow bags)</td>
<td></td>
</tr>
</tbody>
</table>

10. **Time consumption**

11. **Scheme of development**

<table>
<thead>
<tr>
<th>Date / Initials</th>
<th>Version No.</th>
<th>Description of changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.09.10 / SJ</td>
<td>01</td>
<td>The SOP has been written</td>
</tr>
<tr>
<td>17.09.10 / EG</td>
<td>01</td>
<td>The SOP has been approved</td>
</tr>
</tbody>
</table>

12. **Appendices**

BW25113/pKD46 expresses the $\lambda$red gene form an arabinose inducible promoter. The plasmid pKD46 has a temperature sensitive replicon.