Transfection of Sf9 cells with baculovirus DNA (P1 virus)

1. Culture Sf9 cells in advance, make sure the cell density is in the range of 1.5–3 × 10⁶ cells/ml (SF900II + 5%FBS + antibiotics), prepare (2 × n) ml plating media, unsupplemented (w/o FBS or antibiotics). n=constructs number

2. Seed 10 × 10⁵ Sf9 cells (e.g. 0.5ml if the cell density is 2 × 10⁶ cells/ml) per well into a 6-well plate to achieve ~70% confluence, and add media up to 1ml to cover the surface. Allow cells to attach for 10-30 minutes at 28°C. Remove the media. Wash with plating media once. Add 1 ml plating media per well.

3. Prepare Transfection Mix A:

   8ul CellfectinII + 100ul plating media, unsupplemented.

4. Mix well CellfectinII and plating media by pipetting and Incubate Transfection Mix A at room temperature in a hood for 30 minutes.

5. Prepare Transfection Mix B:

   1.5ul midi-prep DNA (~1µg/ul conc.) + 1.5ul linearized baculogold DNA.

   DO NOT pipette, just swirl gently to mix.

   Incubate Transfection Mix B at room temperature in a hood for 5 minutes and add 100ul plating media, unsupplemented. Pipette only once, so as not to damage the linear DNA.

6. Combine Transfection Mix A and B (Add A to B). Mix gently by swirling and pipetting only once, and incubate for 30 minutes at room temperature.

7. Add all transfection mixture (~210µl) dropwise onto the cells from Step 2. Incubate cells at 28°C for 4 hours.

8. Remove the transfection mixture, wash with 1ml complete growth media twice, and replace with 2 ml of complete growth media (SF900II+5% FBS+antibiotics).

9. Incubate cells at 28°C for 5 days.

The cell densities that are typically obtained from confluent cultures:
<table>
<thead>
<tr>
<th>Flask Size (cm²)</th>
<th>Sf9</th>
<th>Sf21</th>
<th>High Five™ Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>4.0 x 10⁶</td>
<td>3.8 x 10⁶</td>
<td>3.0 x 10⁶</td>
</tr>
<tr>
<td>75</td>
<td>1.2 x 10⁶</td>
<td>1.1 x 10⁶</td>
<td>9.0 x 10⁶</td>
</tr>
<tr>
<td>150</td>
<td>2.4 x 10⁵</td>
<td>2.3 x 10⁵</td>
<td>1.8 x 10⁵</td>
</tr>
</tbody>
</table>

**P2 baculovirus amplification**

1. If Sf9 cell density is in the range of 1.5–2.5 x 10⁶ cells/ml, use 5ml (for 2 x 10⁶ cells/ml density) in a T75 flask to achieve ~70% confluence.
2. Add media to total 10ml and allow cells to attach for 1 hour.
3. Remove the media from the plate using the pump and add 1ml new media.
4. Add all P1 virus (~1.5ml) dropwise and mix gently by inverting 4-6 times.
5. Incubate at 28°C for 1 hour, invert several times every 20 minutes.
6. Add 10ml new media.
7. Harvest the P2 baculovirus after 2-3 days. Collect all P2 virus into a 15ml falcon tube. Use 1ml P2 baculovirus for P3 amplification. Store the rest at 4°C.

**P3 baculovirus amplification**

1. If cell density is in the range of 1.5–2.5 x 10⁶ cells/ml, use 10ml (for 2 x 10⁶ cells/ml density) in T175 flask to achieve ~70% confluence.
2. Add the media to total 20ml and allow cells to attach for 1 hour.
3. Remove the media from the plate using the pump and add 2ml new media.
4. Add 1ml P2 virus dropwise and mix gently by inverting 4-6 times.
5. Incubate at 28°C for 1 hour, invert several times every 20 minutes.
6. Add 20ml new media.
7. Harvest the P3 virus after 2-3 days.

Use 1.5ml for expression test (30ml cells). Store the rest at 4°C for large prep.

**P4 baculovirus amplification** (for large prep)

1. Prepare 400ml Sf9 cells in a 2.8L flask.
2. If cell density is in the range of 1.5–2.5 x 10⁶ cells/ml, add 10ml P3 virus.
3. Incubate the Sf9 cells at 90rpm, 28°C.
4. Harvest the P4 virus after 3 days at 4,000rpm for 20mins in an autoclaved bottle.

5. Store the virus at 4°C before use. Or if less than 300ml virus is needed, do P4 amplification as P3 with more T175 flasks.