

## Selection of Stable Transfected Cell Lines

### Background

Once cells have been successfully transfected, the next step is to seed and select the transfected cell line in 96-well plates to select pure colonies by limited dilution as outlined below:

### Protocol:

1. Seed the transfected cells in 96-well plates in 10% conditioned medium:
  - a) Dilution conditions:
    - 2x96 well plates with 0.1 cell per well
    - 2x96 well plates with 0.5 cell per well
    - 2x96 well plates with 1 cell per well
2. Incubate the cells for 24h.
3. Remove medium and add conditioned selection medium containing selection antibiotic at the predetermined concentration required for your cell line. Incubate 96-well plates at 37°C with CO<sub>2</sub>.
4. Check the plates every day for colonies. Colony formation depends on proliferation rate of the cell line and can take anywhere from 3 days to 1 week.
5. Refresh selective medium every 3-4 days until colonies appear.
6. Select the wells with only one single colony. Make sure colonies are not growing in clumps as they will be less sensitive to the antibiotic.
7. When a well contains a single colony, transfer the colony to a 24-well plate in selection medium and repeat until you have enough cells for freezing and storage in liquid nitrogen. Use the appropriate antibiotic concentration as determined from the kill curve.

### Quality Control

Seed 24-well plates with insert and determine the transfection efficiency by immunostaining.

1. Grow cells on insert in a 24-well plate until well is confluent.
2. Remove medium and wash cells with 1X PBS.
3. Fix cells with methanol or paraformaldehyde and wash with 1X PBS.
4. Add primary antibody in 24-well plate against protein of interest and incubate at 37°C for 1 hour (depending on antibody).
5. Wash cells with 1X PBS.
6. Add secondary antibody in 24-well plate and incubate at 37°C for 1 hour (depending on antibody).
7. Wash with 1X PBS.
8. Remove insert from 24-well plate and affix to a microscopy slide with nail polish or other suitable adhesive.
9. Determine the percentage of transfected cells with a fluorescence microscope.