TOKU-E

TOKU-E Plasmid DNA Transfection Protocol

Background

Once the appropriate antibiotic concentration to use for selection of the stable transfected cells has been determined by performing a kill curve, the next step is to generate a stable cell line by transfection of the parental cell line with a plasmid containing the gene of interest and an antibiotic resistance gene.

Plasmid DNA Transfection Protocol

1. Seed the parental cell line in a 24-well plate and incubate for 24h at 37°C.

2. Transfect the parental cell line the next day at 80% confluency with the construct (e.g. using calcium phosphate etc…) and include a sample of un-transfected cells as a negative control. Incubate at 37°C in $C0_2$.

3. After transfection (6h to 24h depending on the transfection method used), wash the cells once with 1X PBS and add fresh medium containing the selection antibiotic to the cells. Use the appropriate antibiotic concentration as determined from the kill curve.

4. Check, refresh, and expand the cells in selection medium every 2-3 days until you have enough cells for limited dilution (confluency in T25 flask or 10 cm dish).

Quality Control

Seed 24-wells 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 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 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 microscopy slide with nail polish or other suitable adhesive.

9. Determine the percentage of transfected cells with fluorescence microscope.

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