Transient Transfection of HEK293s GnTi- Cells and Screening of Constructs by FSEC

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Cell seeding (day 1)

1. Add $1x10^6$ attached HEK293S GnTI- cells in 2 ml of DMEM:F12, supplemented with 10% FBS/1% Anti-Anti to each well of each six-well culture plate. Incubate at 37 °C with 5% CO_2/humidity for 16-24 h.

Small scale transient transfection to screen constructs (day 2)

2. For each well, prepare an autoclaved 1.5 ml centrifuge tube. Using a pipette, add 4 uL of Lipofectamine 2000 into 50 uL of Opti-MEM I.
3. Add 1 ug of purified DNA into 50 uL of Opti-MEM I in a separate 1.5 ml centrifuge tube.
4. Add DNA/Opti-MEM I mixture to the Opti-MEM/Lipofectamine mixture, gently mix and incubate for 20 min at RT.
5. Pipette the Opti-MEM I-DNA mixture drop wise onto well containing 70-80% confluent HEK293S GnTIcells. Ensure even dispersal.
6. After 8-24 hours, replace the medium with supplemented DMEM:F12 plus 10 mM sodium butyrate.
7. Incubate the cells at 37 °C with 5% CO_2 and humidity for 2 days.

Screen constructs by FSEC for monodispersity and expression level (day 4)

8. Aspirate off the medium and wash the transfected adherent cells carefully with 2 ml DPBS.
9. Add 1 ml DPBS to each well, collect the cells and transfer them to a 1.5 ml centrifuge tube, or pool all well into 15-mL Falcon tube.
10. Centrifuge the cells at 1,500xg for 5 min, 4°C.
11. Remove the supernatant and store at -80°C. To screen, resuspend the cell pellet in 200 uL solubilization buffer.
12. Nutate samples for 2 h at °C.
13. Centrifuge the solubilized sample at 50,000 rpm in a TLA 55 rotor for 40 min, 4°C.
14. Collect supernatant and analyze 100 uL by FSEC. Allow 1hr for each sample to be analyzed by FSEC. Samples should be stored at 4°C until analysis.
15. Identify the best expressed and monodisperse candidate via FSEC