Hek 293S Transfection

The day before transfection

Culture 293 cells to a density of $2x10^6$ cells/ml in DMEM-F12 + 10% FBS + 1% anti-anti

I.Transfection day {Morning procedure to avoid late night} (Invitrogen)

1.)Count the cells (1.5-2 x 10^6 cells/ml to make sure they're in log phase)

2.)Seed each well of a 6-well plate with 2ml of 0.6 x 10^6 cells/ml to achieve ~70% confluence. Allow the cells to attach for ~15 minutes at 27-28 C {Note: Prepare transfection mixture in waiting time.}

3.)Remove the old medium, wash with Opti-MEM medium once

4.)Add 2ml of Opti-MEM medium

5.)Add combined transfection mixture (~200ul) dropwise onto each well, and incubate at 37C overnight at 5% CO_2.

Preparation of Transfection mixture for each well

1.)Prepare (a) Lipofectamine 2000: 8ul (Lipofectamine) + 100ul Opti-MEM medium in a microfuge.

2.)Prepare (b) DNA: 1ul (1-2.5ug pQCXIP-GOI) + 100ul Opti-MEM in a microfuge.

3.)Add preparation (a) and (b) and incubate 5 min at room temperature in the fume hood.

Tube (a)=(100 x 6 =600) + (8 x 6 =48) = 648ul

Tube (b)= (100 x 6 =600) = (1x6=6)= 606ul

Tube (a) +(b) = ~1200ul

Harvesting

1.)After 12-16 hours incubation time, aspirate the transfection mixture. Add DMEM-F12 + 10% FBS + 1% anti-anti to the cells (2ml in each well). No wash step necessary.

2.)Add 10mM sodium butyrate and drop the temperature to 30 C

3.) Harvest 48 hours later