

ES cell transfection with RNA oligos
Make gelatin plates, sit in RT.
Change media for ES cells 2 hours before transfection

Day 1

Making media without antibiotics

Making mix A (For each 24 well transfection)
20ul RNA oligo (1uM concentration) added to 30ul OPTiMEM

Making mix B (For each 24 well transfection)
1ul Lipofectamine2000 in 50ul OptiMEM (for 24 well plate 12ul lipo into 600 optiMEM)

- 1 Make mix A
- 2 start trypsin (2ul trypsin/10cm dish)
3. Make mix B (5' + Vortex)
4. mix A+B (20')
5. Aspire plates.
6. count cells
7. resuspend in media with no antibiotics. To 100,000cells/ml.
8. Add mix A+B to wells
9. Add 0.5ml of cell suspension.

Day 2:

For every duplicates, change media into ES cell media with Penn&Strep into one well
In the second well, change media into ES cell media with Penn&strep with 1uM RA

Day 3

Same as day 2

Day 4

Trypsinize cells and FACS for GFP.