

Collecting and Transfecting Cells

- 1) Depending on cell type do what ever is necessary to collect all the cells to be used into a 50ml tube.
- 2) Count the cells. **MAKE SURE THE TUBE IS STIRRED BEFORE TAKING SAMPLE.**
- 3) Aliquot the cells into 15ml tubes so that there are 10 million cells per tube.
- 4) Spin down at 1800rpm for 8 mins.
- 5) While spinning down fill one 100mm culture dish with 10 mls of media for each transfection.
- 6) Aspirate off the supernatant.
- 7) Resuspend the cells in 400ul of medium and then transfer to the electroporation cuvetts with the same pipet.
- 8) Electroporate 4 samples at a time at 210V and 975 uF.
- 9) Transfer the samples to the culture dish as quickly as possible after they have been electroporated.

Harvesting Transfected 293 Cells and Running a Luciferase Assay

- 1) Collect the cells from the culture dishes but pipeting the medium over the plate while holding it at an angle to see that the cells are washed off.
- 2) Collect the cells and media in a 15 ml tube.
- 3) Spin down at 22C and 1800 rpm for 8 mins.
- 4) Aspirate off the supernatant.
- 5) Wash the cells in 5 to 7 ml PBS.
- 6) Spin down at 22C and 1800 rpm for 8 mins.
- 7) Aspirate off the supernatant.
- 8) Mix the luciferase lysis buffer to 1x. (Make about 500ul per sample)
- 9) Resuspend the cells in 400ul of lysis buffer and then put in 1.7 ml tubes.
- 10) Flash freeze the cells in a dry ice/isopropanol bath for 5 mins, using a float to hold up the tubes.
- 11) Then immediately after place the tubes in the 37C bath for 3 minutes.
- 12) Remove from bath and spin down for 5 mins at 15000rpm.
- 13) Samples are now ready for testing in the luminometer.