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 cuvets 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.