Transient Transfection of Suspension Cells by Electroporation

1. Count cells. The cells should be in the exponential phase of growth.

2. Collect 4 x 10^6 cells per transfection. Wash cells twice with Opti-MEM media without FBS (Life Technologies). Resuspend cell pellet in 0.1 ml Opti-MEM (**without FBS**) per 1 x 10^6 cells.

NOTE: Omit Penicillin-Streptomycin from the Opti-MEM transfection media, as electroporated cells are exquisitely sensitive to the antibiotics, reducing transfection efficiency.

3. DNA preparation: Use only high quality DNA preparations (e.g. Qiagen Midi-Prep kit). Precipitate DNA with ethanol. Wash DNA pellet with 70% ethanol and let the DNA dry under the laminar flow hood. Resuspend DNA pellet with Opti-MEM media. The amount of DNA used will depend on specific applications.

4. Combine 0.4 ml cell with 10 μl of DNA solution. Add to an electroporation cuvette with 0.4 cm electrode gap. Incubate on ice for 10 min

5. Pulse once (1 kv, 3 μF). Monitor the time constant, which will tell you whether the electroporation worked or not. Time constants will vary depending on cell type, cell density and the nature of the electroporation media. For this protocol, the time constant should be around 0.14 (K-562 cells) or 0.20 (Sp2/0-Ag14 cells).

6. Incubate 10 sec at room temperature.

7. Pulse a second time (1 kv, 3 μF). Monitor the time constant (it should be similar to the first pulse).

8. Place on ice for 10 min.

9. Resuspend the cells in 5-10 ml culture media containing 10 % FBS and the necessary antibiotics. Place in culture flask. Test for ectopic gene expression after 24, 48 and 72 hrs post-transfection.