Mammalian Cell Transfection

Date _____

Cell line for the transfection _____

1. Inoculate a 100mm culture dish with exponentially growing cells at 5 X 10^5 cells per dish no more than 24 hours before the transfection (for L293 cells, pass 1:6 at the end of the day for transfection the next day).
2. The transfection should be done at the end of the day so that they will sit in the $CaCl_2$ and BBS only overnight. Follow the steps below to set up the transfection.
   A. Dilute the desired amount of DNA with distilled, deionized, sterile water to 450 µL. (for L293 cells, 10 µg DNA works well). ***USE ONLY POLYSTYRENE TUBES*** !!!
   B. Add 50 µL of the sterile 2.5 M $CaCl_2$ solution.
   C. Set a Vortex mixer to #3 and while vortexing the tube, slowly add 500 µL sterile 2XBBS.
   D. Allow the mixture to incubate for 20 minutes at room temperature.
   E. Gently mix the precipitate to ensure adequate suspension of the precipitate. Then add the suspension dropwise to the media while swirling the plate to ensure even distribution.
   F. After 12-24 hours (overnight), gently remove the media and wash the cells twice with PBS (without Ca or Mg) and add fresh complete media.
3. For transient transfections, the cells may be collected 48 hours after the transfection.

2.5 M $CaCl_2$	**2 X BBS**	**for 500 mL**
18.37 g	**50 mM BES**	**5.33 g**
Bring up to 50 mL w/ddH_2O	**280 mM NaCl**	**8.18 g**
filter sterilize	**1.5 mM Na_2HPO_4**	**0.1065 g**
	pH to exactly 6.95 with HCl	
	filter sterilize	

Tube #	DNA Description	Amount of DNA for ___µg	Water	2.5 M $CaCl_2$	2 X BBS
1				50 µL	500 µL
2				50 µL	500 µL
3				50 µL	500 µL
4				50 µL	500 µL
5				50 µL	500 µL
6				50 µL	500 µL
7				50 µL	500 µL
8				50 µL	500 µL
9				50 µL	500 µL
10				50 µL	500 µL
11				50 µL	500 µL
12				50 µL	500 µL
13				50 µL	500 µL
14				50 µL	500 µL
15				50 µL	500 µL
16				50 µL	500 µL
17				50 µL	500 µL
18				50 µL	500 µL
19				50 µL	500 µL
20				50 µL	500 µL