Cell Transfection (Novachoice) Kenneth Eum (5/04/2012)

- 1. Prepare a master mix consisting of serum free media (SFM), Novachoice transfection reagent, and the Novachoice booster reagent
 - a. In a 1.5ml tube, add the appropriate amount of pre-warmed SFM
 - i. 100µl of SFM per transfection
 - b. Add the appropriate amount of the Novachoice transfection reagent
 - i. 1µl of Novachoice transfection reagent per transfection
 - c. Add the appropriate amount of the Novachoice booster reagent
 - i. 0.5µl per transfection
- 2. In a new 1.5ml tube, add the appropriate amount of DNA
 - a. lug of DNA per transfection
- 3. Add the master mix into the 1.5ml tube containing the DNA
- 4. Incubate for 30 minutes
- 5. In the 35mm dishes where the cells are growing, remove 1.1ml of media leaving only 0.9mL of media in the dish with the cells.
- 6. Add the appropriate master mix + DNA prepared from step 3
- 7. Repeat steps 5 and 6 until all cells of interest have been transfected
- 8. Incubate the cells at 37°C with 5% CO2 for at least 4 hours
- 9. After 4 hours remove the media from the cells and replace with 2ml of the appropriate fresh media
- 10. After 24 hours, begin to select with selection agents
 - a. If using blasticidin, use 10µg/ml (50µl blasticidin in 50ml of media)
 - b. If using zeocin, use 250 $\mu g/ml~(125 \mu l~of~zeocin~to~50 ml~of~media)$
 - c. If using geneticin, use $1000\mu g/ml$ ($1000\mu l$ of geneticin to 50ml of media)