Cell Transfection (Lipofectamine)

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1. Prepare a master mix consisting of serum free media (SFM), Plus reagent, and the Lipofectamine LTX reagent
    a. In a 1.5ml tube, add the appropriate amount of DNA (1μg)
    b. Add the appropriate amount of the Plus reagent
        i. 1μl of Plus reagent per transfection
    c. Add the appropriate amount of pre-warmed SFM
        i. 100μl of SFM per transfection
    d. Incubate for 5-10 minutes
    e. Add the appropriate amount of the Lipofectamine LTX reagent
        i. 3μl per transfection
    f. Very gently mix the cocktail

2. Incubate for 30 minutes

3. In the 35mm dishes where the cells are growing, remove 1.5ml of media leaving only 0.5mL of media in the dish with the cells.

4. Add the appropriate master mix + DNA prepared from step 1

5. Incubate the cells at 37°C with 5% CO2 for at least 4 hours

6. After 4 hours remove the media from the cells and replace with 2ml of the appropriate fresh media

7. 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 250μg/ml (125μl of zeocin to 50ml of media)
    c. If using geneticin, use 1000μg/ml (1000μl of geneticin to 50ml of media)