Laboratory maintenance, short procedures and housekeeping:

1. Sterilizing glass pipettes
2. Making complete growth media (DMED, 10% FBS, antibiotics)
3. 70% ethanol, 20% bleach and 1% detergent
4. Aliquoting
5. Freezing cells
6. Starting cells from new vial
7. Monitor CO2 tank and cell incubator
8. Making PBS (phosphate-buffered saline)
9. Operation of laminar flow hood and waste flask
10. Proper startup and shutdown of microscope
11. Reusing Bioptechs dishes
12. 37C water bath
13. Routine cell splitting/passage
14. Cell transfection

1. Place 9" glass Pasteur pipettes into metal container, stick autoclave tape to side of container and place into autoclave. Do not over tighten the autoclave door (the "door closed" light will appear when tightened sufficiently). Be sure water level inside the autoclave is at bottom of valve mechanism (opening is on top of incubator). Press dry program (scissors icon on autoclave) and then start button. After cycle is complete place metal container into oven for drying (be sure to use protective gloves or mitts when handling hot metal container).

2. Thaw FBS and antibiotics (PSA) in 37C water bath. Remove approximately 75 ml from the DMEM 1X bottle (volume should be 450 ml after removal). Add 50ml FBS and 5 ml PSA solution to the DMED 1X bottle and mix. Filter the complete media using the filter and vacuum system in the cell culture hood. Label, date and store media in refrigerator (media is good for 1 month after preparation)).

3. Use 70% (v/v) ethanol for cleaning inside the cell culture hood and other surfaces. Use 20% (v/v) bleach for decontaminating old tissue culture flasks, extra trypsinized cells and dishes. For proper disposal of old flasks, add 20 % bleach into flask, dump into drain and flush with tap water. Make 1% (1 gram/ 100 ml) detergent solution (for cleaning glassware and Bioptechs dishes, see 11) by dissolving powered lab detergent in tap ddH20.

4. As a general rule, monitor stock of aliquots (FBS, PSA, trypsin and other reagents). Restock aliquots as needed and monitor inventory for reordering.

5. Add 75 µl DMSO/ cryovial. As a reference, a single confluent 25cm2 flask is sufficient for 5-6 vials of cells. Wash flask with PBS and add standard amount of trypsin solution (~1ml, same as when splitting cells). After cells are completely trypsinized (removed from bottom of flask surface) add 5-6 ml of complete media to flask and mix. Add 1ml of cell suspension to the cryovials containing 75 µl DMSO and mix by inversion. Immediately place vials in Nalgene Freezing container (be sure container has proper volume of isopropanol) and place into -80C freezer for a minimum of 3 hours, but not longer than 2 days. Remove vials from -80C freezer and place into liquid nitrogen tank (caution: wear gloves and protective glasses when transferring cells into liquid nitrogen).

6. First prepare a 25cm2 flask with complete growth media (5ml). Remove vial from liquid nitrogen tank (caution: wear gloves and protective eye glasses); be sure to replace cap and close liquid nitrogen tank

properly. Immediately place the vial of cells into 37C water bath using a small flotation foam piece. Immediately after vial has thawed, pipette vial contents (to ensure all cells are recovered from vial and cell clumps are dispersed), place the cells in the flask with media. Gently mix the flask with cells and place in incubator.

7. The CO2 tank pressure should be approximately 750 psi when full. One tank is sufficient for about one month. As tank is near empty the pressure will decline below 750 psi over several days. Do not allow tank to completely empty.
On the incubator, %CO2 should read 5 and temperature should read 37C. Values will fall slightly below after incubator has been open during normal use. Important: check level of water in pan located inside bottom of incubator (also examine for microbial contamination/growth). Be sure to clean any spills inside the incubator using 70% ethanol.

8. Non-sterile PBS (not suitable for cell culture) can be made by diluting the 10X PBS with Millipore filtered water (located above sink in lab 1040).

9. The cell hood should be sterilized with UV light for approximately 30 minutes (longer is fine, but do not leave on overnight). Turn off UV light, turn on working light, open hood to just below line and turn on laminar flow air supply. After working in hood, wipe working surface with 70% ethanol. Close hood when finished. Check waste flask below incubator (must empty flask before reaches 600ml).

10. When is system completely shut down (no components are on except for the interfaced computer), **first** turn on the mercury arc lamp (only when needed for fluorescence). Wait for 10 seconds before turning on any other components. Next, turn on power supply for the microscope. Wait for the microscope to initialize. Last, open the Leica software on the computer. Shut down the system in the opposite order: close software > shut off power to microscope > turn off mercury lamp.

11. The Bioptechs dishes should cleaned immediately after use: 1) flush dish with tap ddH20, 2) soak in 1% detergent solution for at least 1 hour, but not longer than over night, 3) rinse with tap ddH20 very thoroughly to remove all residual detergent, 4) spray dish with 100% ethanol and wipe glass surface and dish with Kimwipe until dry. Store the clean dishes in plastic bag.

12. The water bath should be kept at 37C. Be sure to monitor water level. As water evaporates, replace with tap ddH20 as needed. Monitor water for contamination and cleanliness. If water appears cloudy or turbid, a short cleaning procedure should be performed: 1) turn off water bath and unplug power cord, 2) dump water into sink and rinse with tap ddH20 (some water can be removed first with a small beaker before carrying the water bath over to the sink), 3) clean the water bath with 70% ethanol, 4) place empty water bath onto bench, 5) using a beaker, fill the water bath with tap ddH20 (approximately ½-full). Note: be sure exterior of water bath and surrounding bench top is dry before plugging in power cord and turning on the water bath.

13. Remove media from flask using vacuum system. Immediately add sterile PBS (5ml for 25cm2 flasks). Remove PBS with vacuum system and immediately added 1-2 ml trypsin/EDTA solution. Place flask/dish in incubator for 0.5-1 min. While flask/dish is in incubator, may prepare new dish/flask with fresh media. Firmly tap flask/dish to dislodge cells from surface. Pipette trypsinized cells to break up cell clumps. Add desired number of drops of trypsinized cells to the new flask/dish with fresh media. Mix and place in incubator.

14. This procedure is specific for cells in one 35mm diameter dish with 2ml media. First add 100ul sterile DMEM (without antibiotics, without FBS) in a sterile 1.5 ml tube. Next, next add 1ug plasmid DNA and mix. Then add 3.5ul Fugene transfection reagent and mix. Incubate for 20 minutes at RT to allow reagent-DNA binding. Add the mixture to the dish and gently rock dish to disperse.