MANDATORY RULES FOR ALL USERS OF THE DAI TISSUE CULTURE LABORATORY

This set of rules is intended to be supplemented with the tissue culture manual. And these practices must be strictly followed by everyone who wishes to use the culture facility.

I. **General**.

A. Entering the tissue culture room and preparing it for use:

Turn off the UV lights in the lateral flow hoods. Attach vacuum flask for suctioned media to vacuum outlet. Swab the inside of the hood and all small equipment placed in the hood with 10% chlorox first and then 70% ethanol to remove any residual chlorox. * this is important as it will prevent possible cross-contamination from prior use of the hood. Turn on the gas burner only when you are sitting down at the hood and NEVER leave it unattended even if you momentarily leave the culture room. There are air currents in the hood at all times and a fire could ensue. Check the temperature of the bath. (Water should be 37°C).

If it is the weekend, always check the water level of the pan on the bottom shelf of the incubator to make sure there is plenty of water (should be 2/3 full). This is the water that supplies the incubator with constant high humidity. It is autoclaved distilled water. If you need to use the last flask of autoclaved water to fill the pan, please autoclave some new flasks. Add a small scoop of methyl 4-hydroxybenzoate to the pan. It will not readily dissolve but this is okay. If you suspect that something is wrong and you cannot solve the problem by yourself, please call someone who can help you out.

Sign up for the TC hood sign-up sheet whenever you use the hood no matter whether it's busy or not. We need to know who was the last person in the hood when something wrong happens. If your work involves any viruses, sign up by the day before the procedure and indicate the type of virus on the sign-up sheet. In general, please plan ahead and sign up early when it's possible.

B. Thawing and handling media and other culture reagents.

1. Media- put all bottles of media that you wish to use in the 37°C bath to thaw or to warm up. Cells do not like cold showers! Separate bottles of media are to be used for each cell type, even if two cell types require the same type of media. If you have any excess media, store it at 4°C and label all partially used bottles with your **name, date and the cell type**. If the media is bluish or if there is a precipitate at the bottom, the media must be regassed with CO2 (set-up in main lab), and REFILTER-STERILIZED before use. This condition indicates that the bottle was not sealed tightly before freezing and if air can get in so can bacteria. Do not thaw more bottles of media than you need. Essential media such as DMEM with 10% FBS will be made as common stocks. Experienced TC users take turns to prepare such common media. These media have to be tested for

bacterial contamination in the CO2 incubator at least for three days. List of shared media may be updated depending on the cell types frequently used in the lab. Special medium specific for certain cell types has to be made by each user. When you thaw fetal bovine serum (FBS) stock, please make 100-mL aliquots and freeze them back. Indicate on each FBS aliquot the type/brand, lot number if necessary, and whether or not it is already heat inactivated. Use up these FBS aliquots before thawing/purchasing a new commercial bottle! Take extreme care to avoid contamination when you prepare aliquots for TC reagents such as FBS. Please consult experienced TC users in the lab when you are not sure how to perform the procedure.

*****NOTE: Some cells require high quality FBS or specially treated FBS, whereas others do not. For example, primary skin keratinocytes need the expensive, characterized FBS from Hyclone, and the FBS needs to be chelated to remove Ca (to be used only in making E low Ca media). 293T cells grow well in cheaper, medium quality FBS such as from Germini. Pay attention to detailed labels on the FBS bottle so you do not use the wrong one for your cells.

2. Trypsin. Experienced TC users take turns to make aliquots. Aliquots should be stored in -20°C. Recipe is in the Dai lab protocol. Thawed aliquots should be kept in 4°C as a working solution. Use up or discard working aliquots after a period of 1 month. – it loses its enzymatic activity upon multiple freezing and thawings. 0.25% trypsin is for tissues and for certain epithelial cell lines which are hard to harvest with 0.05% trypsin; 0.05% trypsin is for cultured cells. Recipe is in the handbook.

3. Penicillin/streptomycin. Make 10-mL aliquots when you thaw the original stock. And store in -20°C freezer.

4. Other. Cholera toxin must never be frozen unless in media. Hydrocortisone is stored in 95% ethanol. Mitomycin C is toxic and light sensitive and care should be taken when handling it. Make certain that you familiarize yourself with the tissue culture handbook for more detailed instruction.

C. **Sterile Technique**.

Note: A detailed description of sterile technique is supplied in the manual. Take the time to read it.

The sterility of the culture room is absolutely essential. Once the culture room has been contaminated with mold or sporulating bacteria/yeast, etc, it is a major task of great expense and effort to decontaminate it. **It could literally mean months of effort and wasted work for others.** Sloppiness of any kind will result in loss of tissue culture privileges and this will be strictly enforced.

1. New members will be instructed in sterile technique before they are allowed to use the culture room and everyone is expected to follow the guidelines. Any major deviations will not be tolerated and any minor deviations are at your own risks.

2. Any media/cell spills in the hood, which carry over or splatter into the filters in the hood, must be cleaned immediately – what is the best way to do this?. They can be a source of serious contamination. Serious spills should be reported to TC supervisor (Kazu) and/or Xing.

3. Only materials needed to carry out each current operation should be in the hood. Pipets should be taken out as needed. When opening sterile boxes of pipets, jars, etc., always make certain that the item is placed well into the hood and that the airflow is not blocked. Always try to work towards the back of the hood and in a free airflow. Minimize motion across the blower to limit contamination. If you are ever in doubt as to the sterility of any item you have opened, discard it or put it in the wash bin. Do not put it back for someone else to use.

4. Do not block the UV light with extra stuff in the hood. Only minimum equipments (e.g., small racks, burner, suction tubes) are allowed to be left in the hood at the end of the day.

5. Place all garbage into the garbage can immediately after use. Do not overfill the garbage can.

6. Do not leave garbage in the hood even for a minute or two. Do not leave dirty pipets in the hood even while you are working.

7. Rinse bottles and tubes with 10% bleach for a few minutes then rinse with water and place into the TC bin.

8. Viral users (e.g., retro, lenti, adenoviruses) must pay extreme attention not to contaminate any equipment in the TC room. Virus-containing solutions can only be exposed inside the hood. Most of these viruses can infect human cells and are potentially harmful to us.

9. Frequent testing of cell lines is essential to prevent large-scale contamination (as well as wasted effort culturing contaminated cells). Cell lines should be tested for mycoplasma (once a year), and new cell lines coming into the lab should also be tested immediately. Test cells using PlasmoTest mycoplasma testing kit (Invivogen, cat #rep-pt 2). *the protocol for this is online and comes with the kit.
http://www.invivogen.com/PDF/PlasmoTestrep-pt2_TDS.pdf

D. Cleaning up.

When you are finished using the hood, remove all tubes, pipets, etc. and dispose of them properly. Rinse the VACUUM LINE WITH 10% CHLOROX AND CLEAN

THE WORK SURFACE OF THE HOOD WITH CHLOROX AND THEN 70% ETHANOL AS YOU DID WHEN YOU BEGAN. There should be no messes of any sort left by any user.

1. If you are the last person to use the culture facility: Make certain the gas line is shut off. Check to make certain that the door of the incubator is securely latched and that the temperature is 37 ^{0}C and the CO2 level is correct. Do not leave without making sure that these conditions are met. Make certain that the light of the scope is turned off. Turn on the UV light in the horizontal flow hood.

2. When the garbage can is full, seal the bag with tape and replace with a new can. EH&S pick-up the biological waste on Wednesday (is this still the case?Yes). So make sure not to carry over the filled garbage bag to the next week.

3. Replace the liquid waste bottle when it is filled up to 2/3 from the bottom (over-filling the bottle will increase the chance of cross-contamination). Put 50-mL undiluted bleach (don't we put bleach into an empty flask now?Yes) and cover the top with foil and write down the date/time and your name. Leave the waste with bleach at least for overnight and dump the contents down the drain Set up a new waste bottle with 50-mL undiluted bleach.