REU Bootcamp Checklist

Activities

Calculations

- Instructions
 - Dilutions
 - Percentage Concentrations
 - Molarity
 - Normality
- Set-up
 - Print calculation sheets for everyone (15 sheets)
 - Print periodic table sheets (4 per session)
 - Provide pencils and calculators (4 per session)
 - Dry-erase markers and erasers to demonstrate some calculations in front of the students

Pipetting

- Instructions
 - Use of micropipettes and pipet guns (20 μL, 200 μL, 1000 μL, 5 mL, 10 mL, 25 mL, 50 mL)
 - Explain how to read each of the instruments, associated lines on tips, and stripettes
 - Explain how to use tips in a sterile way (by not touching tips as you attach
 it to the micropipette or pipet gun)
 - Explain to pipet slowly, and to not get liquid inside the instrument by taking up too much liquid
 - Demonstrate how to change speed on pipette guns
 - Complete all of these steps with food-colored water
 - Demonstrate/discuss all of the above, and have each student practice by pipetting at least once with each micropipette and the pipet gun
- o Set-up
 - Find enough pipets at least one of each size 20 μL, 200 μL, 1000 μL, and pipet gun, and make sure pipet gun is charged
 - Create 3 50 mL conical tubes of food-colored water, and label as such
 - Find at least one bag of each type (5 mL, 10 mL, 25 mL, 50 mL), and two 200 μL and 1000 μL tip boxes
 - Find 50 15 mL conical tubes, 30 50 mL conical tubes, and 100 1.5 mL microfuge tubes for students to pipet samples into

Sterile Technique

- Instructions
 - Biosafety cabinet
 - Proper lab attire: gloves, goggles, lab coats
 - Disinfecting material entering the cabinet (EtOH) to minimize culture contamination, and clean cabinet following work
 - Materials should be placed as far back in the cabinet as possible, and work at least 4 inches inside of the hood

- Laminar air flow
 - Downward laminar airflow prevents ambient air from entering
 - Sterile technique no hands over open containers, etc., especially due to downward airflow
- Aspirating solutions
 - Use of the vacuum
 - How to aspirate from the TC flask without affecting cells
- Working with tissue culture flasks
 - How to open and close tissue culture flasks sterilely
 - Exchange of nutrients and oxygen in a tissue culture flask
 - Taking out and putting back tissue culture flasks in incubators (don't talk, breathe, etc. while incubator door is open)
- Students
 - Use of pipet in hood with media bottle
 - Use of vacuum
 - Wipe down hood before/after use, and EtOH hands
- Set-up
 - Gloves (S, M, L, XL), goggles (4 pairs), and lab coats (2 M, 2 L, 2 XL) for students, plus whatever is needed for the trainer
 - EtOH and kimwipes for disinfection
 - Bottles with media or PBS for examples of opening containers, and pipet gun and stripettes for pipetting
 - Tissue culture flasks with media or PBS
 - Vacuum set-up and 200 µL pipet tips for vacuum
 - Waste container for used tips

Cell Culture and Harvest

- Instructions
 - General passaging procedure enzyme detachment, spinning down cells, counting cells, reseeding
 - Use of a hemacytometer and how to calculate number of cells in flask
 - Trypan blue exlusion for cell counting
- Set-up
 - Gloves (S, M, L, XL), goggles (4 pairs), and lab coats (2 M, 2 L, 2 XL) for students, plus whatever is needed for the trainer
 - EtOH and kimwipes for disinfection
 - Cells to be passaged and passaging materials, including hemacytometer

Imaging

- Instructions
 - Bright field microscopy
 - Phase contrast microscopy
 - Epifluorescence microscopy
 - Cell labeling technique (IHC, Dapi, PI, etc.)
- Set-up
 - Brightfield and phase contrast samples to image (3T3 fibroblasts)
 - Stained cells, etc. to image (GFP fibroblast, spinal cord tissue on slides)

Dapi stain for mouth swipe of cells

Image Analysis

- Instructions
 - Using image analysis software (primarily ImageJ)
 - Demonstrate color merging
 - Generally best w/ 8bit etc. images
 - Grayscale intensities with pseudocolor/'look up tables' LUT
 - Also demonstrate this with phase images and phallodin stained
 - Show pseudocoloring
 - Demonstrate image processing on a split channel (ex. Live/dead)
 - Subtract background
 - Set threshold
 - Adjust brightness contrast
 - Binary operations (erode erode dilate dilate)
 - Analyze particles/ show outlines
 - Measuring cell morphology (turn on shape descriptors in set measurements)
- Set-up
 - Images to analyze for above
 - ImageJ set-up on computers
 - Reserve computer lab in BME