

EXERCISE

1

Introductory Laboratory: Basic Handling and Passage of Cells in Culture

PURPOSE

The purpose of this laboratory is to get individuals acquainted with the most basic aspects of cell culture. Originally, this particular laboratory exercise was combined with the next one. However, in our experience, many students found it too confusing and encountered a substantial amount of cell loss and/or contamination of cultures. We utilize both a continuous cell line that does not exhibit contact inhibition (the neuroblastoma line) and one that does (3T3 fibroblasts), in order to provide first-hand evidence of differing morphologies and behaviors as confluency is reached. Any combination of contact-inhibited and non-contact-inhibited lines will achieve this purpose. Should you wish to “go slower,” one might start out with, for example, just the fibroblast line, or whatever line you have chosen that exhibits contact inhibition.

Central tasks of this lab:

- Familiarize yourself with working in a hood.
- Familiarize yourself with the operation of “pipette aids.”
- Familiarize yourself with writing a protocol, which must appear in the form of “Step 1,” “Step 2,” etc.

Important: A protocol in your own words should be written in your lab notebook prior to the lab. We reserve the right to limit access to this and all subsequent laboratory exercises to those students who can provide a protocol prior to entry.

PROCEDURE

You will receive 2 “P₀” NB2a/d1 neuroblastoma cells (“NB”) and 1 P₀ 3T3 fibroblast per person (3 plates per group). While most labs are designed for working in pairs, it is important in this particular lab to work individually, so that each of you acquires the “baseline” experience of this laboratory exercise.

Examine your plates; record the different shape of these two cell lines; record percent confluency.

The next task is to generate 2 new plates of NBs per group and keep the fibroblast cells alive until next week. Therefore, you need to trypsinize (as per introductory chapter), count cells (using a hemacytometer, as per the introductory chapter), and split your cells into new plates.

This will invoke calculating your own dilutions for the desired confluency. Do not ask your laboratory instructor to do this for you; he or she will not. The calculation is an essential part of the exercise. Come up with your own calculations, and request that they be reviewed. It does not matter if you were incorrect; it matters if it is clear that you tried. Conversely, DO NOT begin work until your calculations have been approved.

In this and in all subsequent exercises, you are expected to use the minimum amount of laboratory consumables, such as pipettes, sterile dilution tubes, etc. Therefore, you are to figure out (prior to the lab) how many pipettes can be used in order to carry out your experiment without causing contamination.

NOTE: NBs can “get by” just fine on horse serum, while 3T3 fibroblast cells require fetal bovine serum (FBS). *Be sure to use the correct media for each cell type.* NBs, and any other cells that can get by on “lesser” serum types still will grow well if given FBS. While it may seem easier simply to use fetal bovine serum for both lines, this can be cost-prohibitive over the long-term, especially for a smaller, academic laboratory. Cultivate habits that do not waste resources or grant funds; these will often be the same funds that pay your salary.

To optimize your chances for success, you should make 2 different dilutions (for example, 1:20 and 1:50). Re-feeding will be necessary (every 3 days). Re-passage will likely be needed.

OUTCOME

No report is required for this preliminary exercise, BUT in order to participate in next week’s lab (which will require a report), you will need to generate plates of cells—read the next exercise to figure out the number of plates you will need.

IF your cells do not survive, it is your responsibility to contact your laboratory instructor in the hope that you have time to make replacement plates.

SIMPLIFIED PROCEDURE FOR PASSING CELLS

Remove the media from the “stock” plate. Tip the plate slightly away from you, wait a minute (keeping it tipped), then remove any of the residual media from the plate.

Rinse the plate with 1 mL of SERUM-FREE MEDIUM: to do this, add 1 mL of serum-free medium to the plate, swirl the plate gently, then remove this medium.

Add 1 mL of trypsin to the plate, gently swirl, and incubate the plate at 37°C for approximately 5 minutes, after which, examine the plate under the microscope. Look for cells that are floating. You may need to tap the edge of the plate gently. When you see a majority of the cells are detached and floating, add 1 mL of serum-containing medium.

**** NOTE:** NOT EVERY CELL WILL DETACH.... YOU ARE LOOKING FOR A MAJORITY OF THEM TO DETACH.

When making more than 1 plate: remove the serum-media/trypsin solution and place contents into a centrifuge tube. Using the tabletop centrifuge, pellet the cells by spinning for 3-4 minutes at 5-6 rpm. You may not see a pellet at the bottom, so carefully remove 1.5 mL of supernatant. Then resuspend the pellet in the appropriate amount of serum-containing media.

HINTS

- Use a fresh culture, i.e., a culture that was previously frozen and stored in either liquid nitrogen or -80°C .
- All media should also be made fresh—if the media contains L-Glutamine it only has a shelf life of 6 weeks before it becomes inactivated.
- All media prior to use must be warmed in a water bath heated to 37°C . Use the inside of the wrist to test for warmth.
- When working in the hood, the entire surface should first be sprayed down with 70% ethanol and left to evaporate for 1-2 minutes before placing any cultures inside the hood. To minimize contamination, keep the lids on the plates as much as possible.
- In making media using calf serum or horse serum, the serum-containing media must be filtered prior to use.

MATERIALS

10% serum-containing media

Trypsin

NB2a plates (2/group)

- To make 10% serum-containing media:
 - Thaw the following: 1 L bottle of DMEM-only media (this may or may not contain L-Glutamine), antibiotic/antimycotic solution, and serum (either horse serum or iron supplemented calf).
 - For 1 L: remove 100 mL of DMEM only.
 - Add 100 mL of serum.
 - Remove 10 mL of solution and use it to dissolve L-Glutamine.
 - a. Use 2 vials per 1 L bottle.
 - Add back the dissolved L-Glutamine vials into DMEM bottle.
 - Add 10 mL of antibiotic/antimycotic solution.
 - Store at 4°C .
- To make Trypsin:
 - Trypsin/EDTA comes as needed for cell detachment; therefore, it only needs to be dispensed into aliquots.

