Answers of the Lab2 Questions

I. Primary culture of hippocampal neurons

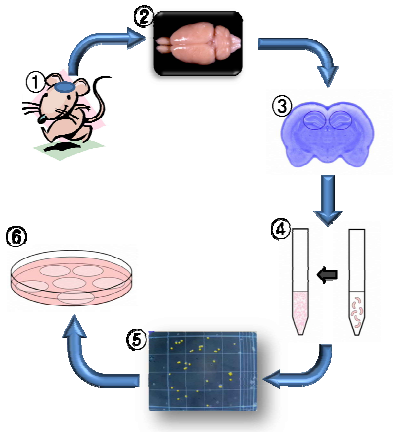
1. What are the purpose and advantages of culture study against animal study?

   - Avoid of ethical issues
   - physicochemical environment accurately controlled
   - homogeneity (cell type well defined)
   - many cellular functions can be investigated
   - less costly for screening assays

2. Compare the primary culture with the cell line culture.

- **Primary culture** : Limited growth potential & Limited life span
  - Advantages
    - May represent the best experimental *in vitro* models
    - May retain characteristics of normal cells from that organ
  - Disadvantages
    - Difficult to obtain
    - Susceptible to contamination
- **Cell line culture** : Immortal, Fast Growth, Grow upto higher cell density
  - Advantages
    - Easy to maintain in culture & Easy to obtain large quantities
    - Typically easy to manipulate
  - Disadvantages
    - Cell line may change overtime - genetically unstable
    - Unclear how well they represent function of original cell type

3. Describe the procedure of primary culture of hippocampal neurons.



① Rat embryo

② Brain 채취

③ Hippocampus 채취

④ Dissociation

⑤ Cell counting

⑥ Cell Seeding

II. Microcontact printing

1. What are the advantage and the disadvantage of microcontact printing?

- **Advantages**
  - Fast, simple, and inexpensive
  - High resolution
  - Not require clean room instrumentation
  - Not require absolutely flat surface
- **Disadvantages**
  - Dry surface
  - Require alignment with MEAs
  - Restriction in fine controls (ex. cell positioning,…)

2. Imagine problems that can occur when protein patterns become complex such as SNU logo.

- Crosstalk between cell patterns like jumping of neuritis
- Paring, Sagging, Shrinking by elastomeric property of PDMS

3. Imagine methods that can improve protein transfer from the microstamp onto the substrate.

- improvement of surface property into Hydrophilic by plasma cleaning
- using chemical reaction like covalent bonding

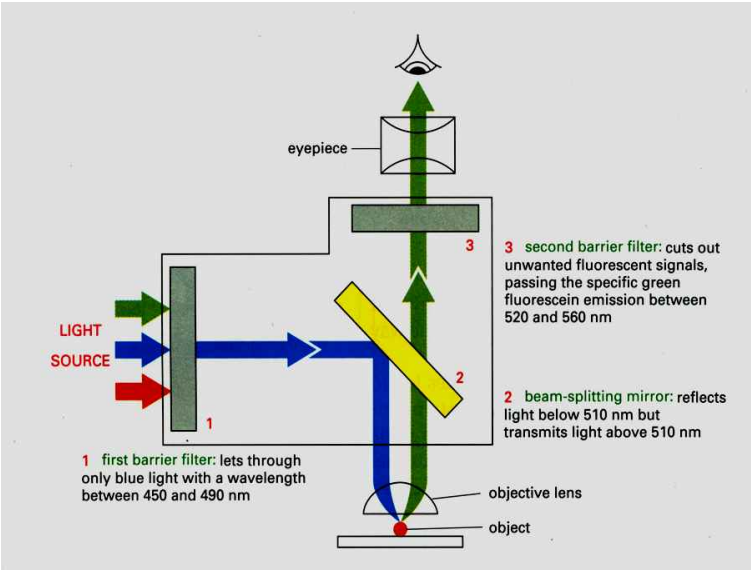
III. Immunostaining

1. Explain the principles and the methods of immunostaining.

- Compared to others, Immunostaining technique is fairly easy to perform and straightforward, with not TOO much variability of whether it works or not because of using antibody-antigen reaction. We can identify the positions of target proteins(antigens) with fluorescence microscope by reacting these with antibodies labeled with fluorescence dyes. And we can amplify the fluorescence signal through the double antibody technique. The procedure is as follows.

  - ▶ Prepare sample
  - ▶ Fixation to preserve the cells/tissue and to immobilize the antigen
  - ▶ Permeabilization to "punch holes" in the cell membrane so antibodies can diffuse in to bind the target protein
  - ▶ Blocking to eliminate non-specific binding of antibodies
  - ▶ Primary antibody incubation
  - ▶ Secondary antibody incubation
  - ▶ Prepare for viewing (mounting)

2. Draw the structure of Fluorescence microscope and explain the principle of it.



In most cases, a component of interest in the specimen is specifically labeled with a fluorescent molecule called a fluorophore (such as green fluorescent protein (GFP), fluorescein or DyLight 488).

The specimen is illuminated with light of a specific wavelength (or wavelengths) which is absorbed by the fluorophores, causing them to emit longer wavelengths of light (of a different color than the absorbed light). The illumination light is separated from the much weaker emitted fluorescence through the use of an emission filter.