MOLECULAR MEDICINE

BICD145

WINTER 2019

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GRADES

The final grade will be based on the following:

1. There will be a final exam to test the understanding of the course material.
2. Lab write-ups. ***NOTE: a first priority in good documentation of an experiment is that someone else should be able to reproduce the same experiment given the information that is provided in the notebook.***
3. Lab conduct [preparation, courtesy, cooperativity (being a team player), cleanliness].
4. Results (how successful the experiments were).

***DON'T FORGET TO BRING SAFETY GLASSES, LAB NOTEBOOK, AND LABCOAT ***

Organization of the course

1. The class will be divided into groups of 2 people working as lab partners.
2. This lab course is very different than most other lab courses. We will be doing a combination of immunology, virology, molecular biology and biochemistry. What is unique to this course that it applies modern techniques in different areas of biomedical research.
3. One of the aims of this course is to provide an atmosphere that is much more like a real research environment than what is experienced in a standard "cookbook" lab course. The ideal student is someone who is trying to prepare for a career in the biological and/or biomedical sciences.

NOTE: It is our expectation that the students prepare thoroughly for the lab (like they are really doing an original experiment). This preparation includes not only reading over the experimental procedures before class, but it also includes doing background reading and research that extends beyond the recommended reading.

4. Note that the students will be responsible for providing the funds to replace broken equipment. Before each experiment, Lollie/Diane will go over a checklist of equipment for which the students are responsible. This checklist will be signed by the students before each experimental section. Then, after each experimental section, the equipment on the checklist will be checked again, and the students will be responsible for the replacement cost of any broken or missing items.

5. There are 10 weeks to the quarter. The first week is devoted to classroom instruction and laboratory demonstrations. The next 9 weeks will include laboratory sections.

Practical considerations for immunology and molecular biology in the laboratory

Cells

Cells and lymphocytes in particular are fragile. Treat cells as labile reagents. Many precautions, as listed below, should be taken to keep cells alive -- keep them happy!

1. Handle cells on ice or at 4˚C. This means that work with celss must be performed in either an ice bucket (ice bath) or in the cold (4˚C) room. The lower temperature helps to keep cells alive in an unnatural environment. Of course, you should never heat up cells.

2. Do not subject cell suspensions to vigorous stirring, mixing, or vortexing. The shearing forces may kill the cell.

3. The buffer appropriate for cell suspensions should be around physiological pH (pH 7) and salt (around 100 mM monovalent salt). If the pH is too low or high, or if the salt is too low or high, cells will undergo apoptosis. Frequently, it is advisable to add fetal calf serum to the medium. As a salt solution phosphate buffered saline (PBS) is used.

4. Cells sometimes undergo cell death when they are dilute (≤ 100.000 cells/ml). Keep cell density high.

5. Storage of cells in the frozen state. Most cells can be stored at ≤ -70˚C in 10 % DMSO in the frozen state. Cells must be slowly-frozen and quick-thawed at 37˚C.

DNA and RNA

1. Wear gloves when handling nucleic acids! There are nucleases on your fingers. Also, do not use any buffers lacking EDTA or high salt that have been standing around at room temperature for more than one week. Microorganisms tend to grow in the buffers, and they secrete nucleases that will degrade your DNA. Buffers that are particularly a problem are TE, 0.3 M NaOAc, distilled water, and Hepes buffers.

2. Setting up a standard reaction with a 10 x buffer. Keep the volume of enzyme at 10% or less of the total reaction volume. Add the enzyme last, and do not vortex the tube containing the enzyme too vigorously.

3. Deproteinization of solutions with phenol-chloroform. Remember that the aqueous phase is on top and the organic phase is on the bottom.

4. Precipitation of DNA with NaOAc (1/10 volume of 3 M NaOAc) and ethanol (2.5 to 3 volumes).

DNA modifying enzymes

1. Restriction enzymes. Need Mg(II). Blunt end or 5' or 3' overhang. Note that the phosphate is almost always on the 5' end.
2. DNA polymerases. Need DNA template, primer, and dNTPs. 5' to 3' synthesis. Sometimes have exonuclease activities (3' to 5' or 5' to 3').
3. RNA polymerases. Need DNA template and rNTPs. 5' to 3' synthesis. Recognize specific promoter sequences.
4. Alkaline phosphatase. Will remove 5' phosphates.
5. T4 polynucleotide kinase. Will transfer gamma phosphate of ATP onto the 5' end of DNA.
6. T4 DNA ligase. Joins DNA fragments. Has a large preference for cohesive ends over blunt ends. Requires ATP. Also requires 5' phosphate and 3' OH.
7. Reverse transcriptase. Make complementary DNA from RNA template. Needs RNA template, primer, and dNTPs.
8. Bal 31. Double-stranded exonuclease.
9. ExoIII (E. coli). 3' to 5' exonuclease.
10. Lambda exonuclease. 5' to 3' exonuclease.
11. S1 and mung bean nucleases. Prefer single-stranded DNA over double-stranded DNA.

Proteins

Since there are 20 amino acids (which are highly variable in structure and properties) of which proteins are composed and only 4 nucleotides (of similar chemical structure) of which nucleic acids are composed, the structures and properties of proteins are much more varied than those of nucleic acids. In the lab, the physical properties of different DNAs are quite consistent, whereas the physical properties of proteins is highly variable. Thus each protein must be treated in a unique manner.

1. Treat proteins as labile species. Many precautions, as listed below, should be taken to maintain proteins in their native structure (secondary, tertiary, and quaternary). It sometimes doesn't hurt to anthropomorphize proteins.
2. Handle proteins at 4˚C. This means that work with proteins must be performed in either an ice bucket (ice bath) or in the cold (4˚C) room. The lower temperature helps to minimize unfolding or any sort of thermal denaturation of the protein from its native conformation. Never heat up any proteins.
3. Wear gloves when handling proteins. Your fingers contain proteases that degrade proteins.
4. Do not subject protein solutions to vigorous stirring, mixing, or vortexing. The shearing forces may denature the proteins.
5. In crude extracts, it is useful to include various protease inhibitors (PMSF, EDTA, benzamidine, sodium metabisulfite, pepstatin, leupeptin, etc.). Note that there are many different types of proteases in crude

cell extracts, and any given protease inhibitor may work on a few of the many proteases. In general, it is wise to use a mixture (or cocktail) of many protease inhibitors to prevent proteolysis by the many different proteases that may degrade your desired protein.

6. The buffer that a protein is should be around physiological pH (pH 7) and salt (around 100 mM monovalent salt). If the pH is too low or high, or if the salt is too low or high, the proteins will often precipitate in either the native or denatured state.

7. Proteins are charged species, and they have patches of positively and negatively charged regions. For any protein, there exists a particular pH at which the net charge of the protein is 0. This special pH, which is a unique property of each protein, is called the pI (isoelectric point). Proteins are attracted to each other by hydrophobic interactions and are repelled from each other by electrostatic repulsion. At pH = pI, the electrostatic repulsion is at a minimum, and thus, pure proteins will sometimes preciptitate (self-aggregate) when pH = pI.

8. Proteins sometimes become unstable when they are dilute (≤ 1 mg protein/ml). Remember that the intracellular protein concentration (which is what the proteins normally experience) is very high. If protein instability in dilute solution is a problem, it can be sometimes overcome by the addition of a neutral carrier protein, such as insulin or bovine serum albumin (MW = 66 kD).

9. Water is highly structured, and often proteins are denatured in water, possibly due to the water forcing the proteins to conform to the structure of the water. To destabilize the structure of water and to stabilize the native structure of proteins in water, it is a general practice to add about 10 to 15% (v/v) glycerol to buffers in which proteins are stored. One drawback to the addition of too much glycerol is that it is viscous and is an impediment to chromatography and dialysis. [Glycerol is normally used at 10%, but people occasionally use it at 15% or 20%.] Carrier proteins, such as insulin or bovine serum albumin, also probably destabilize the structure of water.

Molecular Medicine-Syllabus

Instructor: Kees Murre (534-8796)
Staff: Dennis Hickey (534-3891)
Laboratory telephone: (534-2097)

Week 1- Introduction
First Class
Overview of the course/bureaucratic issues
Determine lab groups
Check out of equipment to students
Safety lecture

Second Class
Lecture: Animal Care
Biohazard
Tissue Culture

Week 2- The bone marrow
First Class
Lecture: Lymphocyte biology
Remove bone marrow
Isolate lymphocyte populations from the bone marrow
Characterization of the bone marrow population

Second Class
Lecture: The innate and adaptive immune system
Characterization of the peripheral lymphoid compartments

Week 3- The T cell lineage
First Class
Lecture: T cell development
Prepare thymocyte populations for analysis by flow cytometry

Second Class
Lecture: B cell development
Flow cytometric analysis of lymphoid populations

Week 4- Lymphocytes in aged and immunodeficient mice
First Class
Lecture: Stem cells
Analysis of aged and immunodeficient thymocytes

Second Class
Lecture: Immunodeficient murine models
Flow cytometric analysis of aged and immuno-deficient lymphoid populations

Week 5- Genomic stability and thymocyte survival
First Class
Lecture: Molecular mechanisms that underpin lymphocyte survival
Thymocyte survival upon exposure to ultraviolet radiation

Second Class
Analysis of survival versus cell death

Week 6- Introduction to Molecular Biology
First Class
Lecture: Introduction to molecular biology techniques
Making competent bacteria

Second Class
Lecture: Bacterial transformation
Transformation of retroviral construct

Week 7- Preparation of DNA for transfection
First Class
Lecture: The biology of retro- and lentiviruses
Large-scale retroviral construct preparation

Second Class
Lecture: Chromosomal and plasmid DNA
Cesium chloride centrifugation
Isolation of plasmid DNA

Week 8- Characterization of retroviral DNA
First Class
Lecture: Gene transfer
Characterization of retroviral DNA by restriction enzyme digestion

Second Class
Lecture: Introduction to tissue culture
Prepare cells for transfection

Week 9- Transfection of DNA encoding tumor suppressors into embryonic kidney cells
First Class
Lecture: Proto-oncogenes and tumor suppressors
Transfection of tumor suppressors into embryonic kidney cells

Second Class
Lecture: Role of tumor suppressors and developmental progression
Harvest retroviral preparations.
Flow cytometric analysis of RAG-deficient lymphoid populations

Week 10- Analysis of lymphomas infected by retroviruses- Gene Therapy
First Class
Lecture: Tumor biology
Infect T cell lymphomas with retroviral supernatant.

Second Class
Lecture: Leukemias and lymphomas
Analysis of lymphomas transduced with retrovirus
Final quiz