California State University, Los Angeles
Department of Biological Sciences
FALL, 2015

Course title, number and units: Animal Cell Culture Theory and Technique, Biology 481 (4)

Instructor: Robert M. Nissen, PhD
Office Location: BioSci 143
Telephone: 323-343-5104
Email: rnissen@calstatela.edu
Website: www.calstatela.edu/faculty/rnissen/ Moodle for course

Class Location: ASCL230
Time: MW 1:30-2:20PM
M 2:30-5PM; WF 2:30-3:45PM
Office hours: F 11AM - 12PM
or by appointment

Description: Topics include extra-cellular matrix, signaling, stem cell biology, differentiation, primary cells and immortalized cells as applied to cells cultured in vitro. Basic techniques including counting, passage, storage and medium preparation. Lecture 2 hours, laboratory 6 hours.

Prerequisites: BIOL 380 or MICR 300A or CHEM 431A or CHEM 435.

Course Requirements:

Textbook: Freshney, R. Ian, Culture of Animal Cells. John Wiley & Sons, Hoboken, NJ (5th or 6th edition). Multiple copies of this book will be available for use in the laboratory. PURCHASE IS NOT REQUIRED. The Karp book used in Cell Biology will also be available as an in-class reference as needed.

Additional Reading Materials: Check the Moodle course for lecture slides, lab protocols, and additional reading materials.

Attendance: Students are responsible for all material presented in class, including announcements about changes in course procedures. A fair calculation for the time required for this class should take into account the need to spend at least 1 hour of independent study for each lecture hour. As a lab intensive course, make up labs are not possible and participation in lab exercises is essential.

Evaluation:

Tests: There will be a midterm, a final, and a lab practical. The tests will be a mix of short answer and short essay questions. If evidence of emergency can be provided for a missed final, an Incomplete will be given.

Lab Reports: As a lab intensive course, a substantial fraction of your grade is derived from the writing of lab reports. See later sections for more detail on this.

Grading:

5%	checks on notebook use during class
5%	lab report 1: hemocytometer use
10%	lab report 2: genome engineering
15%	midterm
5%	lab report 3: cryopreservation
5%	lab report 4: transient transfection
10%	lab report 5: directed differentiation
15%	lab report 6: deletion characterization
10%	lab practical
20%	final
100%	total

Grades in this course are not curved. Course grades will be assigned as follows:

A:	89% - 100%	C:	64% - 73%
A-:	86% - 88%	C-:	61% - 63%
B+:	84% - 85%	D+:	58% - 60%
B:	79% - 83%	D:	50% - 57%
B-:	76% - 78%	F:	below 50%
C+:	74% - 75%		

ADA Compliance: Reasonable accommodation will be provided to any student who is registered with the Office of Students with Disabilities and requests needed accommodation.

ACADEMIC HONESTY: Students are expected to read and abide by the University's Academic Honesty Policy, which can be found at http://www.calstatela.edu/academic/senate/handbook/ch5a.htm as well as in the current Schedule of Classes. Students who violate this policy will be subject to disciplinary action, and may receive a failing grade in the course for a single violation.

Late assignments will not be graded, resulting in a loss of points. Arrangements for "excused lateness" must be made in advance and approved by the instructor.

General Advice: To ensure that you will be seen promptly during office hours, arrange an appointment with me by email in advance. Drop-ins are fine, but if I am already meeting with another student you will have to wait. If you are unable to meet with me during regular office hours, we can arrange an appointment at another time. If you put in the effort required, you should learn a lot from this course. If you are having trouble, or are not learning what you hoped to learn, talk to me. I benefit from your feedback.

Lecture Course Schedule:

Week	Day	Date	Lecture Topic
1	M	28-Sep	Intro to Cell Culture
	W	30-Sep	Microscopy
2	M	5-Oct	Math and stats review, lab reports, hemocytometer use
	W	7-Oct	Transient transfection and reporter assays
3	M	12-Oct	Lentiviral structure and principles of transduction
	W	14-Oct	Cryopreservation
4	M	19-Oct	Analysis of Signaling Pathways
	W	21-Oct	Gene regulation: transcription and post-translation
5	M	26-Oct	MIDTERM EXAM
	W	28-Oct	BMP signaling
6	M	2-Nov	Lab
	W	4-Nov	Lab
7	M	9-Nov	Stem Cells and Directed differentiation
	W	11-Nov	Veteran's Day observed - campus closed
8	M	16-Nov	microRNAs, CRISPR/Cas9, and Indel analysis
	W	18-Nov	Flow Cytometry
9	M	23-Nov	Lab
	W	25-Nov	Lab
10	M	30-Nov	Bioethics and Cell Culture
	W	2-Dec	review
	F	**4-Dec**	**Lecture Final Exam**
11	M	**7-Dec**	**LAB PRACTICAL (FINAL PERIOD) 1:30-4PM**

Learning Outcomes

This course will provide you with experience and information so that you can become:

1. competent with the basic methods of mammalian cell culture,
2. knowledgeable regarding the cell biology that influences how cells are cultured,
3. more practiced at planning ahead for a laboratory procedure,
4. more skilled at reporting and analyzing procedural and experimental observations in a cell culture context,
5. more practiced at working in a team,
6. and aware of a variety of approaches in which cell culture can be used to address biological questions.

Required Supplies

1. Laboratory notebook: 8 1/2" X 11" page size, 4 or 5 squares/inch
2. memory stick for saving material from your own or another computer

Highly Recommended

Laptop computer (wireless internet access a plus)

Collegiality

Make a commitment to developing a sense of community with the entire class. Think of ways to enhance this sense of community, perhaps even outside of class time. Get to know and appreciate each member of the class.

Lab Course Schedule:

Week	Day	Date	Lab Exercises	
0	F	25-Sep	Class intro	
1	M	28-Sep	Aseptic technique; thaw C2C12 cells	
	W	30-Sep	Splitting Cells	
	F	2-Oct	Splitting Cells	
2	M	5-Oct	Expt 1: Using the hemocytometer (**Due Oct 14**)	
	W	7-Oct	continue 1	
	F	9-Oct	complete expt 1	
3	M	12-Oct	Expt 2: Genome engineering (**Due Nov 25**)	
	t	13-Oct	cont 2 - change medium (10min)	
	W	14-Oct	cont 2 - clear 293T supernatants and transduce C2C12	
	F	16-Oct	cont 2 - C2C12 puro selection	
4	M	19-Oct	cont 2 - refeed	Expt 3: Cryopreservation experiment (**Due Oct 28**)
	t	20-Oct		cont 3 - transfer to liq N2 (15 min)
	W	21-Oct	cont 2 - refeed	cont 3
	F	23-Oct	cont 2 - refeed	complete expt 3
5	M	26-Oct	cont 2 - subclone by serial dilution	Expt 4: Transient Transfection BMP reporter assay (**Due Nov 4**)
	t	27-Oct		cont 4 - change medium (10 min)
	W	28-Oct	cont 2 - refeed	cont 4 - harvest for extracts
	F	30-Oct	cont 2 - refeed	Expt 5: Directed differentiation - myogenesis (**Due Nov 18)**
6	M	2-Nov	cont 2 - refeed	expt 5 - myogenesis ICC1
	W	4-Nov	cont 2 - expand into 6-well plate	expt 5 - complete myogenesis ICC2
	F	6-Nov	cont 2 - expand into 10cm plates	confocal imaging to complete expt 5
7	M	9-Nov	cont 2 - freezebacks for KO lines	
	W	11-Nov	Veteran's Day observed - campus closed	
	F	13-Nov	cont 2 - refeed	
8	M	16-Nov	cont 2 - extracts for Western blot screening	
	W	18-Nov	Expt 6: characterization of KO lines (**Due Dec 7)**	
	F	20-Nov	cont 6	
9	M	23-Nov	cont 6	
	W	25-Nov	cont 6	
	F	27-Nov	Thanksgiving - Campus Closed; cell line maintenance	
10	M	30-Nov	cont 6	
	W	2-Dec	cont 6	
	F	**4-Dec**	**Lecture Final Exam**	
11	**M**	**7-Dec**	**LAB PRACTICAL (FINAL PERIOD) 1:30-4PM**	

Course Requirements

1. The laboratory notebook will be graded in class. You will use your laboratory notebook to prepare in advance for performing experiments, make any calculations for solutions you will be making, and keep notes during the course of your laboratory work. You will keep your notebook up to date. It must give good evidence that you know what you are doing and why you are doing it. Plan and record directly into your notebook; do not copy from scratch paper. You may take the notebook home with you! **Your notebook will be checked at the beginning of class on the day any new procedure is to be started to determine whether or not you have completed Sections A-F (see below). You will not be allowed to start a procedure unless those sections are complete. Failure to complete these sections in advance of class can cause a considerable delay, and may make it impossible for you to finish.** A notebook should be written clearly enough that someone else can refer to it and repeat your experiments a year or more from now. However, if it "looks perfect", you are probably not using it correctly. Notebooks will be collected and graded unannounced periodically during laboratory periods.

   Reserve the first two pages of your notebook for a table of contents. Keep your notebook **as you work**. In the Experimental Record section, make the following entries for each of the procedures you carry out in class. Your instructor will inform you what constitutes a procedure. Be sure to ask at the end of each period if you are unclear on what is required in your notebook for the next period.

   A. Date(s), including year – entered each different day you work on a procedure, at the point where you begin taking notes on that day.
   B. Title
   C. Objective – what are the scientific and technical purposes of the experiment or exercise. When appropriate, state the objective as a hypothesis.
   D. Controls for i) technical success and ii) accurate interpretation of results
   E. Your predicted outcome if the technique for the day works as expected
   F. Methods and Materials. Write out the protocol, in your own words. It can be much condensed relative to the handouts. For Laboratory Reports (see below), this section should be written in paragraph form and should reflect what you actually did in lab.
   G. Results/Data and Calculations. Example: a count of the number of stained and unstained cells is **Data**; determining the percentage of cells that are stained is **calculations**. In the Laboratory Report, the Results section should be modeled after a journal article, with figures and legends, tables, and a narrative that describes the results, pointing out what the reader should observe in the figures and tables.
   H. Discussion – Make notes about what your results (presented in Section G) mean. Compare your results with predicted outcomes, make notes about any discrepancies from your prediction, the significance of your results, what the results mean biologically, and/or what the results mean technically. This is an appropriate place to also discuss questions provided at the end of some of the lab protocols. In the Laboratory Report, this entire section should be made up of well-written paragraphs.

2. Laboratory Reports must be turned in on paper as well as emailed to the instructor on the day they are due in lecture. Each laboratory report will include Dates, Title, Introduction, Materials and Methods, Calculations, Results, Discussion and may not exceed 6 pages in length, including references, the complete narrative, and all tables and figures. Be sure to state the hypothesis tested and point out any relevant controls essential to the interpretation of the data. The Laboratory Report should be written much like a journal article and this will be discussed in class. The Methods section should be written in paragraph form and should reflect what you actually did in lab. The Results section should include any figures, tables, and legends, and a narrative that describes the results, pointing out what the reader should observe in the various figures and tables. Similarly, the Discussion should be composed of well-written paragraphs.
   You may find this online freeware for image editing helpful: http://pixlr.com/
3. Making media and other solutions: Make calculations for **all** recipes in your lab notebook. Calculations will be checked each time your notebook is checked. Label all solutions with the contents, date, and team #. Most labels should be written on laboratory tape, not directly on the container.
4. Wear a labcoat in the lab. No open-toed shoes. All food and drink must be left outside the lab.

SAFETY ISSUES

SAFETY WITH GAS JETS

The handles for the gas jets in this room are set in groups of 4. If the valves are not all turned on in the same direction or all turned off in the same direction, the handles bump into each other and the valves do not fully open or close. This is a potential hazard because a jet you think is off may not be off and gas may be coming into the room. Please be sure that all handles for valves that are in the off position are pointed in the same direction, and please be sure that all 4 handles for all 4 valves are turned off in the SAME direction when you leave the lab. The last person out of the lab each night must check this.

SAFETY WITH UV LIGHT

Biosafety hoods used for cell culture are often equipped with UV lights to use as a method of sterilizing the air and exposed work services in a laminar-flow hood between uses. The light does not effectively reach shadow areas such as crevices and so is of somewhat limited usefulness. Short wavelength UV light irreversibly damages the DNA in microorganisms that are within range of the energy making it impossible for the cells to survive. Spores, however, are resistant to UV. A typical UV light in the hood emits light at a wavelength of 254 nm, 50-100W, and is left on for 30 minutes for routine sterilization.

UV light is a powerful source of UV radiation, which can cause serious irreversible damage to unprotected eyes and skin. Always read the instructions before using a UV unit and associated equipment, and be sure all personnel in the area are properly protected. The UV lights in the hoods in this room do not operate unless the hood is closed. The sash of the hood is UV opaque, that is UV light can not penetrate the sash, so persons in the room are safe even when the light is on.

WHAT IS UV RADIATION?

Ultraviolet (UV) rays are invisible electromagnetic rays lying beyond the violet end of the visible spectrum of light. UV rays of different wavelengths have different effects on us, and are commonly categorized as UV-A, UV-B and UV-C.

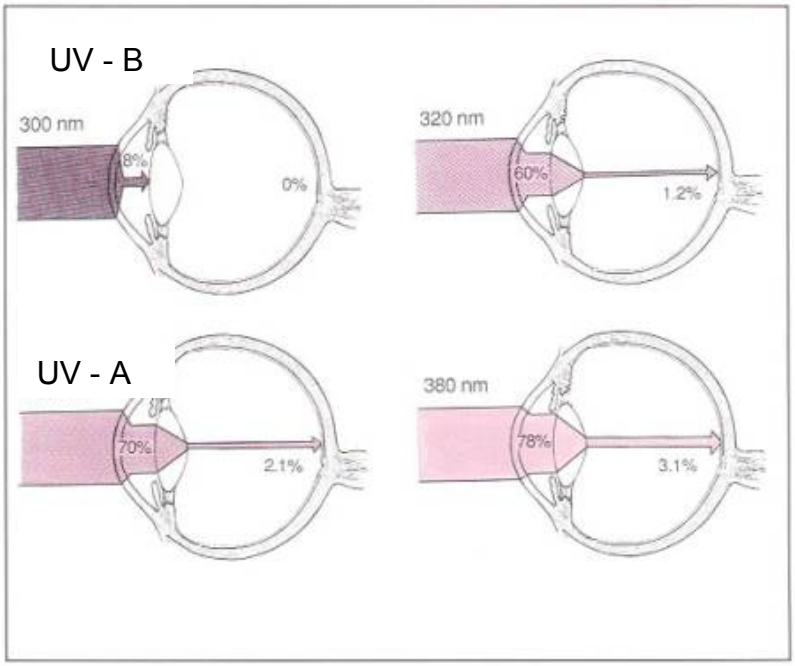
UV-C (200nm-290nm) is the shortest wavelength range and is present in greatest quantity above the earth's atmosphere because the earth's ozone layer absorbs the UV-C radiation in solar energy. However, UV-C is also given out from welding arcs, from the sunlight's reflection from snow at high altitudes, and is used in *instrumentation for sterilization.*

UV-B (290-320) and UV-A (320-390) are present in our living environment in direct sunlight or in reflection from snow, water, sands, glass windows, concrete walls, etc. *Transilluminators used to visualize ethidium bromide stained gels use UV in the UV-B range.*

WHAT ARE THE HARMFUL EFFECTS OF UV RAYS ON HUMAN EYES?

UV-C is absorbed by the ozone layer and normally does not present any threat. However, man made sources of UV-C, such as open lamps used for DNA/membrane cross-linking, are very harmful to the eyes, if you do not use the proper protection. UV-A and UV-B are not absorbed by the ozone layer and scientific evidence now shows that exposure to both UV-A and UV-B can have damaging long and short term effects on your eyes and vision.

The different layers of ocular tissue absorb different wavelengths of UV rays, as shown in the diagram below.



If you are exposed, unprotected, to excessive amounts of UV radiation over a short period of time, you are likely to experience a condition called photokeratitis. Like a "sunburn of the eye," it may be painful and you may have symptoms including red eyes, a foreign body sensation or gritty feeling in the eyes, extreme sensitivity to light and excessive tearing. Fortunately, this is usually temporary and rarely causes permanent damage to the eyes.

Long term exposure to UV radiation can be more serious. Scientific research has shown that exposure to even small amounts of UV radiation over a period of many years may increase your chance of developing a clouding of the lens of the eye called a cataract and can cause damage to the retina, the nerve-rich lining of your eye that is used for seeing. Damage to the lens or the retina is usually not reversible.

The effects of UV radiation are cumulative. The longer your eyes are exposed to UV radiation, the greater the risk of developing conditions such as cataracts in later life.

WHAT ARE BIOSAFETY LEVELS (BSL)?

The Centers for Disease Control (CDC) established four levels of biosafety based on the degree of hazard associated with an organism, to describe the combination of laboratory practices and techniques, safety equipment, and facilities needed to protect against exposure. These four biosafety levels (BSL) require successively more restrictive practices and facilities as work moves from the least restrictive BSL1 to work with the highest hazard level of BSL4. Exposure to biohazardous agents is intended to be prevented or limited by establishing and following the appropriate biosafety level practices and conditions. Our cell culture lab will involve techniques at BSL1 and BSL2.

BSL1 applies to the basic level of containment and essentially represents good microbiological laboratory practice with no special primary or secondary barriers required. This applies to work with defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans. This includes organisms such as host/vector strains of E coli, the human 293T cell line, and the mouse C2C12 cell line. The following considerations apply:

1. Immediately notify the laboratory supervisor in case of an accident, injury, illness, or overt exposure associated with laboratory activities.
2. Do not eat, drink, smoke, chew gum, handle contact lenses, or apply cosmetics in the laboratory. Persons wearing contact lenses in the laboratory should also wear goggles or a face shield.

3. Do not bring any food, medications, or cosmetics, into the laboratory for storage or later use.
4. Do not pipette by mouth; only mechanical pipetting devices are permitted.
5. Perform all procedures carefully to minimize the creation of splashes or aerosols.
6. Establish and follow policies for safe handling of sharps. Use a high degree of caution when handling any contaminated sharp item, such as needles and syringes, slides, pipettes, capillary tubes, and scalpels. Substitute plasticware for glass whenever possible. Handle broken glassware with brush and dustpan, tongs, or forceps - not directly with hands.
7. Do not bend, shear, break, recap, or remove used needles from disposable syringes or otherwise manipulate such units by hand before disposal. Dispose of needles and syringes in the puncture resistant container provided in the laboratory for this purpose. Place full containers in an autoclave bag and sterilize before disposal in medical waste boxes.
8. Work on open bench tops is permitted; use of special containment equipment such as a biological safety cabinet (BSC) is not generally required for agents assigned to BSL1.

This guideline is focused on the safety of the user. We will still conduct BSL1 work within the laminar flow hoods to protect our cell cultures from contamination.

BSL2 applies to work with a broad spectrum of moderate-risk agents that are generally present in the environment at large and are associated with human disease of varying severity. All the lentivirus work to be undertaken in this course falls within the BSL2 level of work. Other microorganisms assigned to this containment level include HIV, salmonella, toxoplasma, hepatitis B and HeLa (the cell line harbors human papillomavirus). In addition to BSL1 conditions, this level of work also requires that:

1. Laboratory personnel have specific training in handling any pathogenic agents used.
2. Access to the laboratory is limited to only authorized personnel when BSL2 work is being done.
3. Wear gloves, lab coats, gowns, smocks, or other provided protective garments while working with hazardous materials. When leaving the lab, remove and leave coats and other protective clothing in the lab.
4. Extreme precautions are taken with contaminated sharps.
5. Biosafety cabinets are used when there is potential for splash or aerosol creation.

Air sampling studies have shown that most common manipulations of bacterial and viral cultures in research laboratories release aerosols of viable organisms. Therefore, no manipulation of lentivirus particles or cell lines exposed to them will occur outside the four main biosafety cabinets located along the walls of ASCL230.

BSL3 and BSL4 apply to work with exotic agents of increasingly greater potential for causing serious human illness or death. No work at BSL3 or higher is currently being done and facilities that would meet the requirements of these biosafety levels are not currently available at CSULA.

REFERENCES

http://www.atcc.org
http://www.cdc.gov/biosafety
http://www.alanoptics.per.sg/uv.htm
http://www.uwo.ca/ophthalmol/GR/99-00/8mar00_files/image006.jpg

I have read the above information on turning off the gas jets, UV light exposure, and biosafety levels and will exercise care with the jets, UV light and biohazard materials.

Your printed name _____

Your signature _____