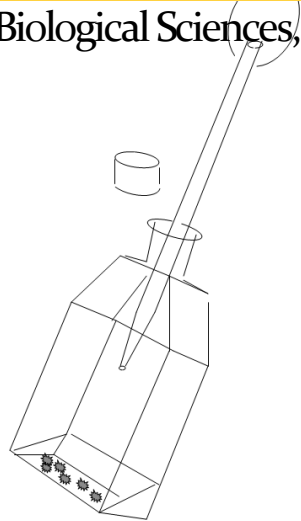




Department of Biological Sciences, KCC, CUNY



**Syllabus:**  
**Biology 57, Biotechnology: Cell Culture and Cloning**

**KINGSBOROUGH COMMUNITY COLLEGE**  
**THE CITY UNIVERSITY OF NEW YORK**

Professor Sarwar Jahangir, *Ph.D.*  
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Access-Ability Services (AAS) serves as a liaison and resource to the KCC community regarding disability issues, promotes equal access to all KCC programs and activities, and makes every reasonable effort to provide appropriate accommodations and assistance to students with disabilities. Please contact this office if you require such accommodations and assistance. Your instructor will be glad to make the accommodations you need, but you must have documentation from the Access-Ability office for any accommodations.

**Access-Ability Services is always looking to hire student aides/federal work study students to help provide certain services for our students with disabilities, if you are interested please stop by D205 to find out more.**

## Disability Related Services D205

### Office Hours:

Monday	9am-5:00pm
Tuesday	9am-8:00pm
Wednesday	9am-8:00pm
Thursday	9am-5:00pm
Friday	9am-5:00pm

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## Course Description

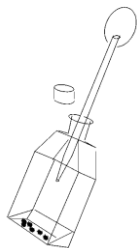
Biology 05700 - Biotechnology: Cell Culture and Cloning (4 crs. 6 hrs.)

Biological principles underlying animal and plant tissue culture and cloning techniques are taught using current cell culture research publications. The formation and maintenance of primary and continuous culture, monolayer and suspension cultures, cell separation techniques, and cell cloning studies are conducted. Cell morphology in vitro, callus formation, cytochemistry, immunocyto-chemistry, autoradiography, chromosome spread preparation and karyotyping, western blotting and enzyme-linked immunoabsorbance assays are performed and analyzed.

A one semester upper level course, teaching cutting edge science in biotechnology for those who wish to major biology leading to an A.S. degree in Biotechnology for a career in the biotechnology industry in NY City and elsewhere. It also can be taken as a required course to complete A.S. in Biology with Concentration in Biotechnology.

The course is divided into 2 hours lecture, 1 hour recitation and 3 hours laboratory each week for 12 weeks. Both the lectures and the laboratory exercises are geared to grow animal cells including mammalian/human cells. Students will grow primary and secondary cells, learn their nutritional requirements, conduct cell differentiation and make cell cloning along with ethical, legal, and social concerns. This is a prime course for completing A.S. in Biotechnology. Upon completion of the Biotechnology program, students will have the option to transfer to Brooklyn College for completion of a B.A. or B.S. degree in Biology taking additional 60 credits.

Prerequisites: Bio 14, Chem 11, and Math 20, and passing CUNY Reading, Writing and Math exams or departmental permission.



DEPARTMENT OF BIOLOGICAL SCIENCES  
KINGSBOROUGH COMMUNITY COLLEGE  
BROOKLYN, NEW YORK 11235  
AUGUST, 2009  
EDITION 1001

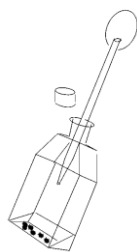
## Biology 57: Biotechnology: Cell Culture and Cloning

Cell culture and cloning is a major pillar of biotechnology. The importance of cell culture for studying cell biology for human benefit was realized long time ago. Although it started in 1880's with the development of Ringer's solution that was able to maintain a heart beating outside of the body, quickly followed by Roux in 1885 who succeeded holding chicken embryo medulla in a saline for several days, but it was not complete until Harrison. In 1907, it was Harrison who succeeded growing neurons in hanging drop culture which is considered as the beginning of cell culture.

Today, it has become routine to grow and maintain cells, including human cells, under controlled conditions. Using cell culture a great variety of vaccines (e.g., measles, mumps, rubella, polio, and Hepatitis A), range of therapeutic proteins (e.g., EPO, TPA and  $\gamma$ -interferon), monoclonal antibodies, anticancer drugs, and cells for therapeutic uses are routinely produced. The scope of cell culture now extends across the phylogenetic tree for therapy to agriculture, industry to environment, and biology to evolution.

Today, transformation of cells with desirable qualities is frequently being addressed. Scientists were able to transform pancreatic non-beta cells into insulin producing beta cells, umbilical mesenchymal cells into neuron cells, bone marrow cells into muscle cells and retinal cells with the help of cell culture techniques.

With the growth of human population, rise in longevity, environmental degradation and declining resources, application of cell culture is expanding everyday across the globe. This requires learning and developing techniques for cell isolation and *in vitro* cell culture; aseptically and in disease free conditions. In order to remain competitive we must learn these techniques as soon as possible so that we can join the workforce as producers, researchers and educators.



**FACULTY**

Professor Sarwar Jahangir

Office: S 206, Tel: 718-368-5743  
Bio Office 718- 368-5502

PROFESSOR

Name: \_\_\_\_\_  
Office Hours: \_\_\_\_\_

Office: \_\_\_\_\_  
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PROFESSOR

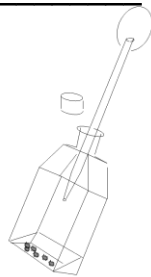
Name: \_\_\_\_\_  
Office Hours: \_\_\_\_\_

Office: \_\_\_\_\_  
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TUTOR

Name: \_\_\_\_\_  
Hours: \_\_\_\_\_

Location: \_\_\_\_\_  
Telephone: \_\_\_\_\_

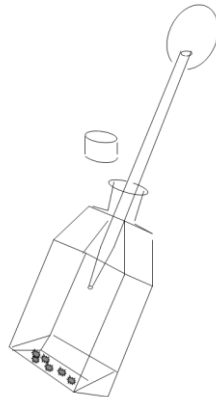


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**Course Goals For Student Outcomes**

1. Learn basic techniques and applications of cell culture for biotechnology.
2. Understand the biology of cultured cells and its application in biotechnology.
3. Learn the application of equipment, lab design and lay out, aseptic technique, and safety.
4. Preparation and sterilization of lab equipment, culture vessels, and media.
5. Culture of cell lines and primary cell culture and quantitation of cells.
6. Cell separation, selection, and cloning.
7. Characterization, differentiation and transformation of cells.
8. Contamination, cytotoxicity, and cryopreservation.
9. Specialized cells, organ culture and molecular techniques.
10. Problem solving.

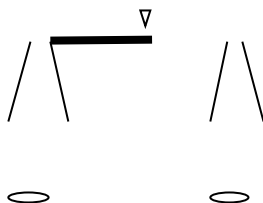


**TEXTBOOK AND GRADING POLICIES****Textbook:**

Culture of Animal Cells: A Manual of Basic Technique, 5th Edition. R. Ian Freshney. July 2005. Pp 672. ISBN: 978-0-471-45329-1

**Laboratory Manual:**

Same as above and supplemented with Cell Culture Manual 3<sup>rd</sup> Edition of the Sigma-Aldrich Cell Culture Manual available free on line: <http://www.sigmaaldrich.com/life-science/cell-culture/learning-center/cell-culture-manual.html>

**GRADING POLICIES**

Lecture:

Two Lecture Exams @ 10% X 2	= 20%
Written review: Frontiers in biotechnology	= 10%
<u>One Final Exam @ 20% X 1</u>	= 20%
Total	= 50%

Laboratory:

Preparatory Homework assignment	= 10%
Laboratory reports	= 15%
<u>Laboratory Quizzes @5% x 4</u>	= 25%
Total	= 50%

Lecture (50%) + Laboratory (50%)

= 100%

**It is the College's policy that every student must wear a laboratory coat during every laboratory session. The coat must be of cotton, knee high, full sleeve and buttoned in front. In addition, each student must have a pair of laboratory safety goggles and disposable non-latex hand gloves to wear in the laboratory as needed.**

Each student must prepare a neat and clean lab report in a lab notebook and submit at the end of the semester for grading.

**EXTRA CREDIT:** Students may earn extra credit worth 5 points by submitting a term paper at the end of the 9<sup>th</sup> regular semester week. The term paper must be:

1. At least 5 typed or printed - double spaced pages.
2. Topic to be selected with the approval of the instructor.
3. Use bibliographic format and at least three (5) sources.
4. It must be your original work. Presenting someone else's work, ideas or words as your own is plagiarism. You must give credit to those authors and researchers, whose ideas, words and research you used, in your term paper.



## **STUDENT RESPONSIBILITIES**

### **Absence Policy**

A student who has been absent 15% of the total number of instructional hours in BIOLOGY 57 is deemed to be excessively absent. The 15% excessive absence policy applies to either the laboratory or lecture component. Each lateness will be counted as ½ hour of absence. Excessive absences may result in the instructor assigning either a lower grade or a “WU” grade for that course.

The absent student must take the initiative for remaining up to date in the course and is responsible for all covered material and assigned work. The student must discuss absences from laboratory activities with his or her professor. This should be done prior to an anticipated absence or immediately following a missed activity session.

### **Cell Phones and Beepers**

The use or ringing of cell phones and beepers in the classroom during class sessions is a disruption of class and a violation of the Henderson Rules. Violation of this policy may result in a disciplinary referral.

### **Written Assignments**

Based on your experiment, you will prepare a laboratory reports each week. The problems and questions will be based on the observations and experiments that you complete. Although you will be completing experiments in groups, **your reports must demonstrate your independent observations, interpretations, and conclusions.**

### **Plagiarism**

You can find the CUNY and KCC’s Academic Integrity Policies are in:

- Kingsborough Community College Catalogue
- Kingsborough Community College Student Handbook
- [www.kingsborough.edu/Academic\\_Integrity\\_Policy.pdf](http://www.kingsborough.edu/Academic_Integrity_Policy.pdf)

“Plagiarism as a **violation of academic integrity** is the intentional use of another’s intellectual creation(s) without attribution. Determination and penalty—ranging from grade reduction to course failure—is at the sole discretion of the faculty member.”

*In addition, your instructor will inform you of his or her policy regarding academic integrity at the beginning of the semester.*

### **Assessment**

You will be given a 20 minutes assessment test during the last week of the semester in order to test the effectiveness of teaching and learning in this course. Your instructor will decide on the content of this test and may assign a credit value, if appropriate.

# Week 1

## Introduction:

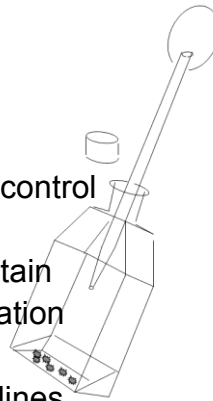
### Background and advantages of cell and tissue culture, and biology of cultured Cells.

#### READING ASSIGNMENT

Textbook: Chapters 1 and 3. Pages 1-9 and 31-42.

#### LEARNING OBJECTIVES

1. Learn the history of tissue culture, applications, achievements and limitations.
2. Understand the need for and advantages of tissue culture:  
How to: Control environment,  
Characterize samples  
Maintain sample homogeneity, and  
Attain economy of operation.
3. Learn the major differences between *in vitro* and *in vivo* growth.
4. Explain the need for and analyze the cell culture environment.
5. Describe and identify cell adhesion, and also learn the roles of:  
Adhesive molecules,  
Intercellular junctions,  
Extracellular matrix,  
Cytoskeleton, and  
Cell motility.
6. Explain cell proliferation, cell cycle, and how to control cell proliferation
7. Understand cell differentiation and how to maintain differentiation and what leads to dedifferentiation
9. Describe cell signaling and energy metabolism.
10. Explain initiation of cell culture, evolution of cell lines and senescence
13. Distinguish between the origins of culture cells and cell lines.



## Week 2

### Design and layout for a dedicated cell culture lab.

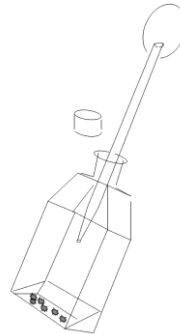
#### READING ASSIGNMENTS

1. Textbook Chapters 4 and 5: Pages 43 -53 and 55-72.

#### LEARNING OBJECTIVES

1. Understand the requirements for a cell culture laboratory:
  - a. Make a planning,
  - b. Explain the services and supports needed,
  - c. Explain an aseptic room and suite, and
  - d. Define a preparation area.
2. Prepare a list of essential equipment needed for cell culture and describe:

a laminar flow hood, an incubator,  
a CO<sub>2</sub> Incubator, an autoclave,  
refrigerators and freezers,  
an aspiration pump, a centrifuge,  
a cryostorage container,  
a water purification unit,  
an inverted microscope,  
a dissecting microscope,  
a hemocytometer, cell counter, colony counter,  
a pH meter, conductivity meter, osmometer,  
temperature recorders and others.



3. Learn how to handle fluids including pipetting and micropipetting.

## Week 3

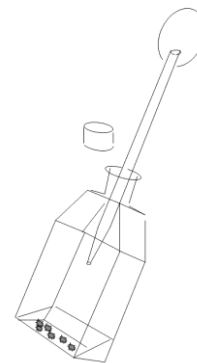
### Aseptic technique, culture vessels and laboratory safety.

#### READING ASSIGNMENT

Textbook Chapters 6 and 7: Pages: 73-85; 87-103.

#### LEARNING OBJECTIVES

1. Understand the objectives of maintaining sterility.
2. Explain the elements of an aseptic environment:
  - a. What is a quiet area?
  - b. What is a work surface?
  - c. What is personal hygiene?
  - d. How to maintain media and cultures?
3. Describe sterile handling that will include:
  - a. Swabbing
  - b. Capping
  - c. Flaming
  - d. Pippeting, and
  - e. Pouring.
4. Understand the functioning of a laminar flow apparatus and other equipment and procedures applied in cell culture.
5. Prepare a list of culture vessels and their qualities:
  - a. What is a substrate?
  - b. What vessels to choose and why?
  - c. What are the specialized systems?
  - d. What is a treated surface?
6. Define and understand laboratory safety:
  - a. What are biohazards?
  - b. What are the safety regulations?
  - c. What is bioethics?



## Week 4

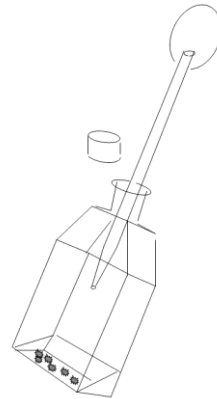
### Cell culture media and requirements.

#### READING ASSIGNMENT

1. Textbook Chapters 8 and 9: Pages 105-113, and 115-128

#### LEARNING OBJECTIVES

1. Describe the development of media.
2. Learn the physico-chemical characteristics of media that will include the following:
  - a. pH,
  - b. CO<sub>2</sub> and bicarbonate,
  - c. Buffering,
  - d. Oxygen requirements,
  - e. What is osmolality,
  - f. What are the roles of temperature
  - g. What is the function of viscosity, and
  - h. What are the roles of surface tension.
3. Explain balanced salt solutions.
4. Describe a complete media and the roles of:
  - a. Amino acids,
  - b. Vitamins,
  - c. Salts,
  - d. Glucose,
  - e. Organic supplements,
  - f. Hormones, growth factors, and
  - g. Antibiotics.
5. Understand the roles played by serum and its constituents including proteins, growth factors, hormones, nutrients, lipids, minerals and inhibitors.
6. Analyze the procedure for medium and serum selection.



## Week 5

Lecture Exam 1.

**Followed by a lecture on:**

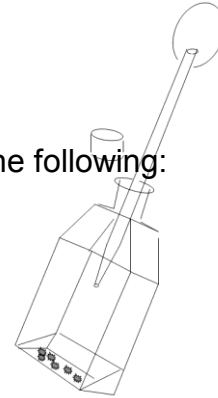
**Serum-free media.**

### READING ASSIGNMENT

2. Textbook Chapter 10: Pages 129- 143.

### LEARNING OBJECTIVES

1. Define serum free media.
2. Describe the disadvantages of serum that will include the following:
  - a. Physiological variability,
  - b. Shelf life and consistency,
  - c. Quality control,
  - d. Specificity,
  - e. Availability
  - f. Downstream processing,
  - g. Contamination,
  - h. Growth inhibitors, and
  - i. Standardization.
3. Explain the advantages of serum free media, including:
  - a. Selective media, and
  - b. Proliferation and differentiation regulation,
4. Describe the disadvantages of serum free media including, multiplicity, selectivity, reagent purity, cell proliferation and availability.
5. Explain the essential factors in replacement serum and their roles in:
  - i. Subculture as adhesion factors, protease inhibitors,
  - ii. Hormones,
  - iii. Growth factors,
  - iv. Nutrients,
  - v. Proteins and polyamines, and as
  - vi. Matrix
6. Describe and analyze the selection and development of serum free medium.



## **Week 6**

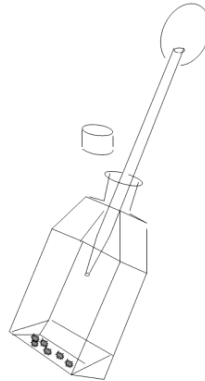
### **Primary Cell culture**

#### READING ASSIGNMENT

Textbook chapter 12: Pages 175-197.

#### LEARNING OBJECTIVES

1. Define primary cell culture and their types.
2. Describe isolation of tissue for cell culture.
3. Explain an embryo cell culture protocol
4. Describe a chick embryo cell culture protocol and compare.
5. Explain human biopsies, compare and contrast with others.
6. Describe the following in relation to primary culture:
  - a. Primary explants,
  - b. Enzymatic disaggregation,
  - c. Mechanical disaggregation, and
  - d. Determination and separation of viable cells.



## Week 7

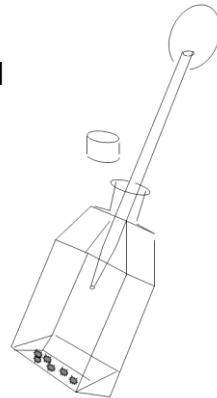
### Subculture and Cell lines.

#### READING ASSIGNMENT

1. Textbook Chapter 11: Pages 199- 215.

#### LEARNING OBJECTIVES

1. Define and describe the cell line nomenclature.
2. Explain subculture and propagation that will include:
  - a. Culture age,
  - b. Line designations, and
  - c. Choosing cell lines.
3. Describe routine maintenance based on:
  - a. Cell morphology,
  - b. Replacement of medium in relation to:
    - i. Drop in pH
    - ii. Cell count
    - iii. Cell types, and
    - iv. Morphological changes, and
  - c. Volume depth and surface area.
4. Explain criteria for subculture including:
  - a. Growth cycles and split ratio,
  - b. Suspension propagation, and
  - c. Procedure for data recording.





## Week 8

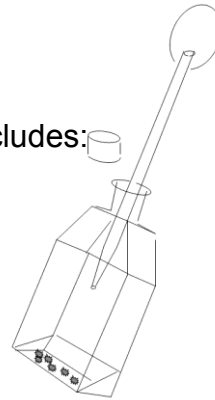
### Cell separation and Characterization.

#### READING ASSIGNMENT

Textbook Chapters 15 and 16: Pages 237-246; 229-257.

#### LEARNING OBJECTIVES

1. Describe techniques of cell separation including:
  - a. Cell density measurement, isopyknic sedimentation,
  - b. Cell size and sedimentation velocity,
  - c. Antibody based techniques,
  - d. Fluorescence activated sorting, and
  - e. Others
2. Explain the need for cell characterization that includes:
  - a. Record keeping,
  - b. Authentication, and
  - c. Identification and lineage determination,
3. Explain morphological characterization applying:
  - a. Microscopy, and
  - b. Staining techniques.
4. Describe chromosomal characterization including:
  - a. Contents,
  - b. Banding, and
  - c. Analysis.
5. Define DNA Characterization including:
  - a. Hybridization,
  - b. Fingerprinting, and
  - c. Profiling.
6. Describe RNA and Protein based characterization.



## Week 9

Lecture Exam 2

To be followed by a lecture

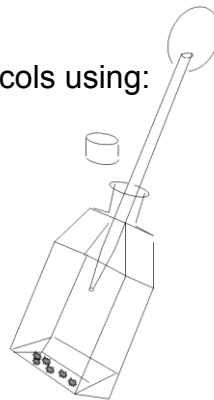
### Cloning and Selection.

#### READING ASSIGNMENT

Textbook Chapter 14. Pages, 217-235.

#### LEARNING OBJECTIVES

1. Define cell cloning.
2. Describe protocols for cell cloning.
3. Explain stimulation of plating efficiency including:
  - a. Improving clonal growth,
  - b. Conditioned medium, and
  - c. Feeder layers.
4. Define suspension cloning and describe its protocol using:
  - a. Cloning in agar, and
  - b. Cloning in methocel
5. Describe isolation of clones with protocols using:
  - a. Cloning rings,
  - b. Irradiation cloning, and
  - c. Suspension clones.
6. Define replica plating.
7. Explain isolation of genetic variants.



## Week 10

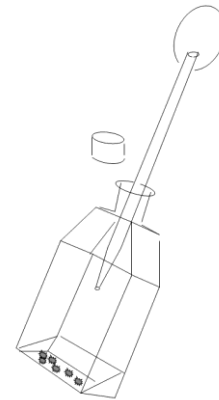
### Cell Differentiation.

#### READING ASSIGNMENT

Textbook Chapter 17. Pages 281-267

#### LEARNING OBJECTIVES

1. Describe expression of *in vivo* phenotype.
2. Define stages of commitment and differentiation.
3. Explain stem cell plasticity
4. Describe markers of differentiation.
5. Describe induction of differentiation with roles of:
  - a. Soluble inducers,
  - b. Cell interaction,
  - c. Paracrine growth factors,
  - d. Negative paracrine factors,
  - e. Cell-Matrix interactions, and
  - f. Systemic or exogenous factors, as:
    - i. Physiological, and
    - ii. Non-physiological.
6. Distinguish differentiation and malignancy.



## Week 11

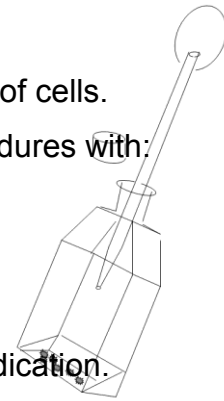
### Transformation, immortalization, contamination and cryopreservation

#### READING ASSIGNMENTS

Textbook chapters 18, 19 and 20. Pages 291-306; 307-319; 321-334.

#### LEARNING OBJECTIVES

1. Define cell transformation addressing:
  - i. Genetic instability,
  - ii. Chromosomal Aberration, and
  - iii. DNA content.
2. Describe immortalization of cells and
  - i. Control of senescence,
  - ii. Use of viral genome,
  - iii. Telomerase induced immortals, and
  - iv. Human fibroblast immortals.
3. Explain aberrant growth and tumorigenicity of cells.
4. Define contamination and monitoring procedures with:
  - i. Sources,
  - ii. Types, and
  - iii. Monitoring techniques.
5. Describe procedures for contamination eradication.
6. Explain cryopreservation with:
  - i. Rationale,
  - ii. Medium,
  - iii. Stages of cryopreservation and
  - iv. Cell banks.



## **Week 12**

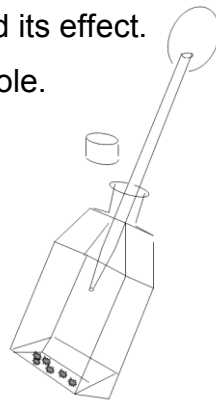
### **Cell Quantitation.**

#### READING ASSIGNMENT

Textbook Chapter 21: Pages: 335-358.

#### LEARNING OBJECTIVES

1. Explain cell counting and the use of:
  - a. Hemocytometer,
  - b. Electronic counting,
  - c. Monolayer staining.
2. Describe cell weight determination.
3. Explain DNA contents and rates of synthesis.
4. Define protein contents and rates of synthesis.
5. Describe procedures to measure cell proliferation and growth with:
  - a. Experimental design, and
  - b. Growth cycle.
6. Describe plating efficiency and its effect.
7. Define labeling index and its role.



**TENTATIVE WEEKLY LABORATORY SCHEDULE**

**Laboratory Manual:** Culture of Animal Cells: A Manual of Basic Technique, 5th Edition. R. Ian Freshney. July 2005. Pp 672. ISBN: 978-0-471-45329-1. Suggested supplement: Cell Culture Manual 3<sup>rd</sup> Edition Cell Culture Manual on line: <http://www.sigmaldrich.com/life-science/cell-culture/learning-center/cell-culture-manual.html>.

Week	Date	Exercise	Handouts will be provided
1	TBA	Practice aseptic technique and autoclave labware.	Do
2	TBA	Grow CHO-S Cells in suspension culture	Do
3	TBA	Continue CHO-S cell culture. Observe and record growth, morphology and other microscopic characteristics.	Do
4	TBA	<b>Quiz 1 on Exercises 1-3.</b> Start growing CHO-S Cells in adhesive culture.	Do
5	TBA	Continue CHO-S cell culture. Observe and record growth, morphology and other microscopic characteristics.	Do
6	TBA	Begin Sf9 Cell culture Record observations	Do
7	TBA	<b>Quiz 2 on Exercises 4-6</b> Continue SF9 cell culture. Observe and record growth, morphology and other microscopic characteristics.	Do
8	TBA	Begin Human Adipose Derived Stem cell Culture Record all observation.	Do
9	TBA	Continue Human Adipose Stem cell culture. Observe and record growth, morphology and other microscopic characteristics.	Do
10	TBA	<b>Quiz 3 on Exercises 7-9</b> Observe and record growth, morphology and other microscopic characteristics. Begin differentiation of stem cells One sample for chondrogenesis One sample for adipogenesis	Do
11	TBA	Continue differentiation of stem cells Observe and record growth, morphology and other microscopic characteristics.	Do
12	TBA	<b>Quiz 4 on Exercises 10-12</b> Complete stem cell differentiation. Observe and record growth, morphology and other microscopic characteristics.	Do
	TBA	<b>Submit Final Laboratory Notebook</b>	

**Cell Culture Resource Links**

<http://www.jove.com/index/details.stp?ID=1267>

<http://www.bdbiosciences.com/support/resources/cellculture/index.jsp>

<https://www.atcc.org/Science/tabid/203/Default.aspx>

<http://books.google.com/>

[http://cshprotocols.cshlp.org/cgi/collection/plant\\_cell\\_culture](http://cshprotocols.cshlp.org/cgi/collection/plant_cell_culture)

<http://vision.berkeley.edu/>

**Cell Culture Opportunities Link**

<http://www.careerbuilder.com/>

<http://www.job-search-engine.com/keyword/cell-culture>

<http://www.indeed.com/q-Cell-Culture-jobs.html>

<http://us.jobrapido.com/Cell-Culture-jobs>

<http://www.jobs.net/>



**Notes**

