

**Molecular Diagnostics, Spring 2012
Syllabus**

Dr. Sandra Sharp LKH 316 (323) 343-2072 email: ssharp@calstatela.edu
Office hours: Drop-in for BIOL 413 only: M, 3:00-4:00; T, 1:30-2:30

Required text: Handouts; Online Protocols and Lectures

<http://www.calstatela.edu/faculty/ssharp/slides/>

Password: _____

Required supplies: two (2) small bluebooks (for quizzes) and bluebooks for midterm and final

Week 1	April 2	Introduction Begin Unit I: HLA-DQ alpha assay
	April 4	HLA-DQA1 assay (cont'd)
Week 2	April 9	HLA-DQA1 assay (cont'd)
	April 11	HLA-DQA1 assay (cont'd) Introduce Abstracts and HIV ELISA protocol
Week 3	April 16	Unit IV: Mock HIV ELISA
	April 18	Draft of HLA-DQA1 paper due Analysis of HLA-DQA1 assay results – bring your laptops HIV – ELISA continued Introduce SOPs
Week 4	April 23	ELISA - HIV abstract due Present HLA-DQA1 results Begin Unit III: Cancer and the Cell Cycle; Western analysis
	April 25	Draft SOP due p53; Western analysis (cont'd)
Week 5	April 30	HLA-DQA1 final report due p53; Western analysis (cont'd)
	May 2	Midterm ; Analyze p53 Western analysis results
Week 6	May 7	Begin Unit IV: Leukemias and lymphomas; DNA-based assays for them
	May 9	p53 – Western analysis final SOP due DNA-based assays for leukemias and lymphomas (cont'd.)
Week 7	May 14	DNA-based assays for leukemias and lymphomas (cont'd.)
	May 16	DNA-based assays for leukemias and lymphomas (cont'd.) Introduce presentation assignment: MoDi for infectious disease
Week 8	May 21	Analyze Results for DNA-based leukemias and lymphomas Begin Unit V: Bacterial identification; Real time PCR
	May 23	Bacterial identification; Real time PCR (cont'd.)
Week 9	May 28	Memorial Day Holiday
	May 30	Leukemia/lymphoma Abstract due Bacterial identification; Real time PCR (cont'd.)
Week 10	June 4	Work on bacterial ID abstracts and/or infectious disease presentation
	June 6	Oral reports from independent assignment
June 8		Bacterial identification abstract due at noon
Monday, June 11 or Wednesday, June 13		Final Examination, 8 – 10:30 AM

**Molecular Diagnostics - Spring, 2012
Course Requirements**

I. Grading

One midterm - 15%
Lab report - 15% (Draft = 5%; Final = 10%)
Abstracts - 15% (3 X 5%)
Standard Operating Procedure (SOP) – 5%
Oral Infectious Disease Presentation – 10%
Quizzes - 10%
Cumulative final exam - 30%

Lab report, abstracts, and SOP are due as indicated on the syllabus. Each assignment must be turned in as a word document to Dropbox before 8 AM on the day it is due. Late assignments will not be accepted. It is your responsibility to learn how to insert figures into a word document. Appropriately labeled figures must be part of the word document. All documents must be titled as follows: **YourLastName_Title**. If everyone uses this convention, it is much easier for me to sort saved documents. For the paper draft, you should also include a completed self-critique: **YourLastName_Self Critique**.

A quiz will be given every day at the beginning of the period. It will test preparedness for that period's lab work as well as knowledge of the previous period's lecture and lab work. Quiz questions will be answered in bluebooks. The lowest two quiz grades will be dropped.

Midterm and final exam questions are to be answered in bluebooks. There will be no make-ups for the midterm. In the event of an excused, and only an excused, absence from the midterm, the final exam will be counted for **45%** of the grade. Excused absences include: 1) notice in advance of a graduate or professional school interview or 2) illness, documented by a physician. After one unexcused absence from class, each additional unexcused absence from class will result in a deduction of 2 percentage points from the overall grade.

Grades will be based on an overall percentage, 90-100 = A; 80-89 = B; 65-79 = C; 50-64 = D. Plus/Minus grades will be given within the specified ranges.

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>. Students who violate this policy will be subject to disciplinary action, and may receive a failing grade in the course for a single violation.

II. Lab Write-ups

For the HLA-DQ alpha - Sequencing assay, a lab report is required in the form of a paper.

For the Mock HIV-ELISA, 14/18 Translocation – PCR-Southern, and Bacterial identification – real time PCR assays, abstracts are required.

For the p53-Western, a Standard Operating Procedure (SOP), with as a final SOP with added results and discussion.

(Total: 1 report, 3 abstracts, 1 SOP)

Further details and scoring for each write-up will be announced in class and on online handouts.

III. The oral presentation on a molecular assay for infectious disease will serve to introduce you to molecular tests that are variations on the techniques we do in class. More information will be provided during the quarter.

Molecular Diagnostics, Spring, 2011, Course Requirements (cont'd.)

IV. Laboratory rules.

1. A lab coat must be worn at all times.
2. No eating or drinking or gum chewing in the lab.
3. No food or drink in the lab except in a backpack.
4. Gloves will be provided and required for some procedures.
5. You must bring your laboratory protocols, either printed out or on your laptop, to each class.

V. Upon completion of this course, you should have:

1. Knowledge of the molecular characteristics upon which molecular diagnostic techniques are based.
2. Knowledge of a spectrum of DNA- and immuno-based assays and the molecular techniques used in such assays.
3. Knowledge of current applications of molecular diagnostic assays.
4. Awareness of the importance of controls and careful interpretation of results.
5. Knowledge of the biology underlying the conditions for which our laboratory assays test.
6. Improved scientific writing skills.
7. Awareness of career opportunities as a clinical genetic and molecular biology scientist.

Unit I – HLA-DQA1 Assay

Reading: Handouts; Online Protocols and Lectures

- April 2 Class basics
Lecture: Background for HLA-DQA1
Lecture: Cell extract
Lab check-in, assembly of teams, assignment of team letters
Preparation of extract containing genomic DNA from buccal cells
Lecture: Basic concepts of molecular assays
- April 4 Lecture: PCR, Basics of Hybridization
Set up and run PCR
Lecture: Immunology I

A fraction of your PCR reaction will be run on a gel and documented by the Instructional Support Technician, Daisy Chau, for us to analyze. You will get to run your own agarose gel for Unit IV.

- April 9 Lecture: agarose gel electrophoresis
Analysis of gel results
Lecture: column cleanup of PCR products
Lecture: Spectrophotometric analysis to determine DNA concentration
Column cleanup of PCR products and spectrophotometric determination of concentration
Determine details for samples to be sequenced
Lecture: Immunology II
Pointers on writing papers
- April 11 Lecture: Sequencing
Set up sequencing reaction
Visit our sequencing instrument
Lecture: Immunology II, III
Introduce Abstracts and HIV ELISA protocol
- April 16 Lecture: Immunology III
Introduce HIV – ELISA
Perform ELISA
- April 18 **Draft of HLA-DQA1 paper due**
Analyze HLA-DQA1 sequencing results – **bring your laptops**
Lecture on HIV
Introduce SOPs

Protocols:

Preparation of Extract Containing Genomic DNA from Cheek Cells

HLA-DQA Assay

- PCR amplification
- Check gel: Agarose Gel Electrophoresis: Casting and Running the Gel
- Column clean-up of PCR products
- Spectrophotometric determination of PCR product concentration
- Sequencing
- Preparation of samples for sending out
- Analysis of Sequencing Results
 - Choosing sequence for analysis
 - Chromatogram – location of sites with two base calls
 - Sequencing alignment – using the HLA database; tabulation of results

Unit I: HLA-DQA1 Assay

Preparation of Extract Containing Genomic DNA from Buccal (Cheek) Cells

First and foremost, it is necessary to have a sample of your nuclear DNA, which contains within it the sequence of DNA you wish to amplify. Your nuclear DNA is called the template for the PCR reaction because it provides the pattern of base sequence to be duplicated. The source of DNA is a sample of several thousand cells from your cheek, obtained by saline mouthwash. The cells are collected by centrifugation and resuspended in a solution containing the resin "Chelex," which binds metal ions that inhibit the PCR reaction. The cells are lysed by boiling, the metal ions, are chelated, and the lysate/bead mixture is centrifuged to remove cell debris and the Chelex beads. A sample of the supernatant containing DNA from the nucleus will be mixed with the other reagents for the PCR reaction.

Materials to be provided

Microcentrifuge for 1.5 ml tubes and with adaptors for PCR-sized tubes

Thermocycler set at 99°C

Thermocycler position grid sheet

Vortexers

Labeled storage box for storage of collected, labeled, individual DNA samples.

For each student:

200 microliters 5% Chelex in a PCR tube (the suspension must be visually inspected after the beads have settled to be sure each student has the same total volume and volume of beads)

0.9% saline – 10 ml in a sterile paper cup

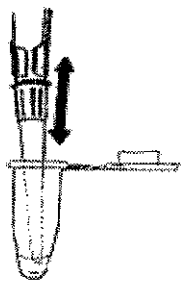
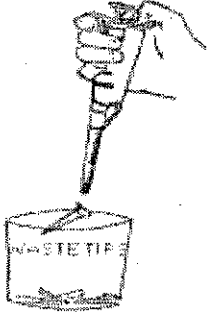
1.5 ml microfuge tubes (1 to spin cells and 1 for final sample)

For each group of 4 students:



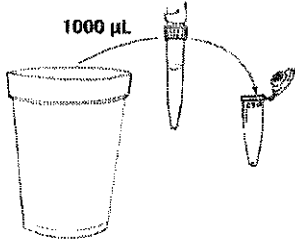
150 ul 0.9% saline in a microfuge tube

Permanent marker

Procedures: Take notes as we go over the procedures.

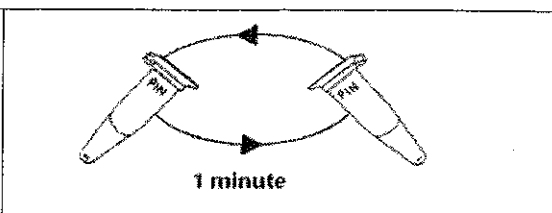
IMPORTANT LABORATORY PRACTICES	
<p>Add reagents to the bottom of the reaction tube, not to its side. You should add each additional reagent directly into previously-added reagent and pipet the combined liquid up and down several times to ensure proper mixing. Pipet slowly to prevent contaminating the pipette barrel.</p> 	<p>Change pipette tips between each delivery. You should change the tip even if it is the same reagent being delivered between tubes.</p> 

Place a check mark in the box of each step as it is completed.

DNA Preparation Using a Saline Mouthwash	
<p>1. Swirl 10 mL of 0.9% saline in your mouth for 30 seconds.</p> <p><i>Note to teachers: Make sure you use sterile containers for the saline.</i></p>	
<p>2. Expel saline into a cup and swirl to mix the cells.</p>	
<p>3. Transfer 1000 μL of the liquid into a 1.5 mL microfuge tube, labeled with your PIN.</p> <p><i>Note to teachers: If you don't have p1000 micropipets, students can use 1 mL transfer pipets or the p200 set at 200 μL (five times).</i></p>	

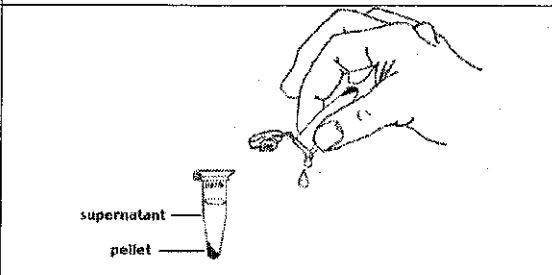
4. In a balanced centrifuge, spin sample for 1 minute.

Note to teachers: With the small, black microcentrifuges, you may need to spin for 3-5 minutes.



5. Observe your cell pellet at the bottom of the tube. Pour off the supernatant, being careful not to lose your cell pellet.

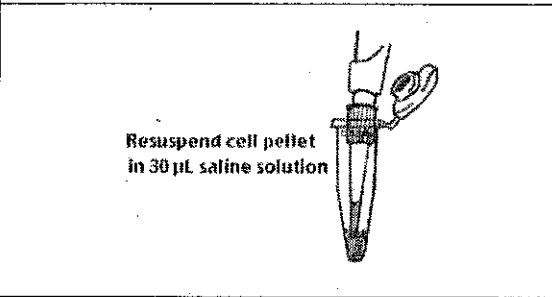
Note: It is okay if some supernatant is left in the tube.



6. Resuspend your cell pellet in 30 μL of saline. Make sure the entire cell pellet is thoroughly mixed by vortexing, pipeting up and down several times, or "racking" your tube.

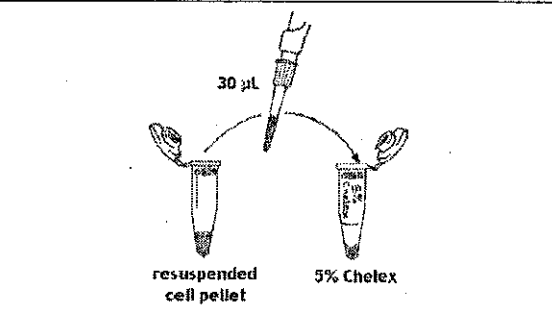
Note: To "rack" your sample, be sure the top of the tube is closed, hold tube firmly at the top, and pull it across a microfuge rack 2-3 times.

Note to teachers: If there is still $\sim 100\mu\text{L}$ of saline in the cell pellet tube, do not add more saline. Simply resuspend the pellet in the existing volume.



7. Withdraw 30 μL of the cell suspension and add it to a 0.2 mL tube containing 200 μL of 5% Chelex.

Note: Do not pipet up and down at this step or else you will clog the tip with Chelex beads.

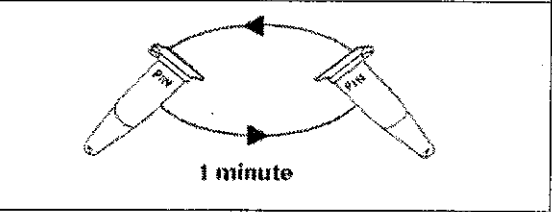


8. Place your 0.2 mL tube with 200 μL of Chelex and 30 μL of cell suspension in the 99°C thermal cycler for 10 minutes.

Note: Remember to record the location of your tube in the thermal cycler.

		2	3	4	5	6	7	8
A	1012					0820		
B					1027			
C		0724						

9. Shake your tube well or briefly vortex it and then place it in a balanced centrifuge. Spin for 1 minute.



10. Withdraw 60 μ L of supernatant (no Chelex beads) to a clean tube, labeled with your PIN.

Note: This stored sample is your "DNA" tube.

***Note to teachers:** This step allows you to ensure that no Chelex beads have been transferred into the DNA tube. If you see beads, have students put the DNA back into their chelex tube, re-spin, and transfer 60 μ L into a NEW tube.*

60 μ L supernatant

