5-2018

LABORATORY MANUAL ANT 101: Introduction to Biological Anthropology

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LABORATORY MANUAL

ANT 101:
Introduction to
Biological Anthropology

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Version: May 2018

Developed with support from a National Science Foundation TUES Award (DUE-1245013)

Table of Contents

(List of Laboratory and Class Exercise Worksheets)

Name: _____ Lab Date: _____
Note: Each student must complete his/her own lab worksheet and include it in a lab notebook.

ANT 101
Evolution and Scientific Thinking

In this lab, you will practice thinking and working like a scientist and become more familiar with the scientific method. This builds on what you read in Ch. 1 and 2 of the textbook and what we discussed in class about ideas like 'hypothesis' and 'fact'. The challenge presented in today's lab is also relevant to understanding how science differs from other ways of comprehending the world, as discussed in that chapter.

Work with your lab team to discuss and answer the following questions and complete the challenge.

Challenge: You are a biological anthropology professor at a state university in Missouri. You just received a shipment of two new human skeletons (casts) for use in teaching evolution and comparative anatomy – one male, one female. Unfortunately, the identifying labels did not get shipped with the skeletons, and there was no information on the boxes to indicate which of the two was male and which one female. How are you going to figure this out?

(Note: You may not utilize personal screen devices/computers during class today to assist in answering these questions. Turn them off.)

Steps in lab:

1) *Hypotheses & Methods*: Generate two or three hypotheses about skeletal differences between males and females. With your lab team (group of three or four at one end of the lab table), discuss and write below **a)** what you think would be different in a male and female skeleton, and **b)** what you would measure or examine to test your hypotheses. Be clear and specific about both the differences you hypothesize and how you think you might test that hypothesis.

a) What would be different?
(Be clear about which sex you are referring to.)

b) How would you detect this or measure it?
(Be as specific as you can about technique.)

2) *Observations/Results:* When instructed, go with other teams, as assigned, to the designated skeleton and conduct your planned observations and measurements, recording your findings here (state whether observing skeleton #1 or #2):

Skeleton # _____

3) *Observations/Results:* When instructed, go with your large group to the second skeleton and conduct your observations and measurements, recording your findings here (state whether observing skeleton #1 or #2):

Skeleton # _____

When you finish examining the second skeleton, return to your lab table.

Based on what you've seen so far, if you could now go back to the first skeleton and re-measure anything, what would it be? (Don't actually do this).

4) *Discussion:* Based on your results, what conclusions do you draw about the sex of each skeleton? Explain/justify using your evidence (do this briefly).

Full Class Discussion (make your notes here and on the back of this page if you need more space)

Keep this lab worksheet in your lab notebook, and make sure it is fully completed.

Name: _____ Lab Date: _____

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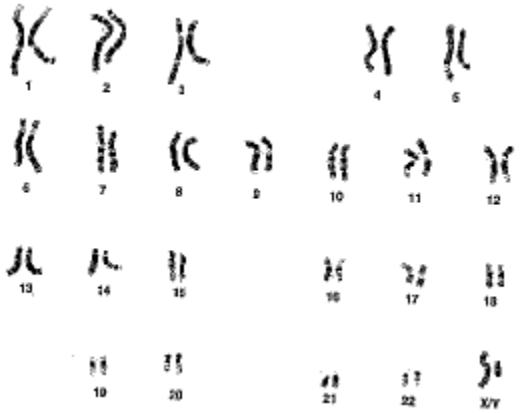
ANT 101
Genes and Variation

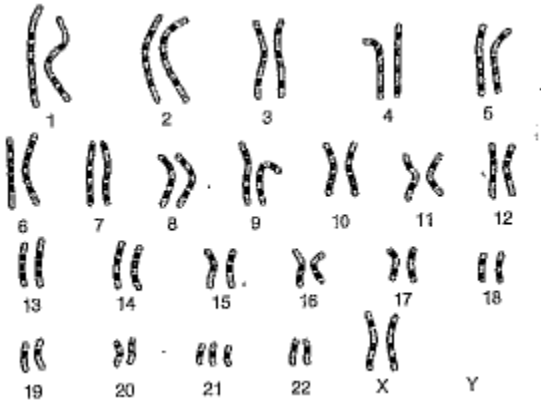
Work with your lab team to discuss and answer the following questions and challenges. This lab is intended to help you become more familiar with the basic methods used to study questions in Mendelian and population genetics that you learned about in class and in your textbook. Note: the questions and challenges in today's lab relate to chapters 3, 4, 5.4, and Appendix B in your textbook.

Part 1, Exercise 1 – Karyotypes: The first figure below is of a normal human karyotype (chromosome summary). It shows the 23 pairs of chromosomes found in somatic cells.



Courtesy: National Human Genome Research Institute

Now look at this second image, and identify which are the autosomes (non-sex chromosomes) and which are the sex chromosomes. Answer the following questions:



Courtesy: National Human Genome Research Institute

a. Is this a male or female? How do you know?

b. What is abnormal about this sequence (describe what you see that appears abnormal)?

c. Can you diagnose the syndrome? What is it?

Exercise 2 – Mendelian Genetics: The ABO blood group is a simple Mendelian trait. (**Pre-lab**): Read the material provided at the start of the lab explaining the ABO blood system, then answer the following questions.

Pre-lab Questions (answer briefly)

1. What is the human ABO blood system?

2. What kinds of surface markers are present on red blood cells?

3. How many blood phenotypes are there in the ABO blood group system, and what are they?

Scenario:

Kevin has been in the hospital a couple of times recently for stomach problems. While there, he was told that his ABO blood type is different from both of his parents' blood type, and that neither of his parents would be able to donate blood if he ever needed a transfusion. He is trying to understand how this could possibly be, so he seeks help from a friend, who tells him that his biological anthropology professor can probably help them both understand the situation. Kevin meets the professor and tells her the blood types the hospital said he and his parents have. The professor sets up an experiment using simulated blood to help them understand the underlying physiology and genetics.

What is your research question? (In this case, it might be difficult to state a single hypothesis initially, so you can ask a specific question instead.)

Methods

Materials:

Simulated blood and anti-sera for blood typing; blood typing slides; toothpicks; marker; gloves

Procedure:

1. Label each blood typing slide:
   #1 for Kevin's mother's sample
   #2 for Kevin's father's sample
   #3 for Kevin

2. Place 3 to 4 drops of Kevin's mother's sample in each of the A and B wells of Slide #1.
3. Place 3 to 4 drops of Kevin's father's sample in each of the A and B wells of Slide #2.
4. Place 3 to 4 drops of Kevin's sample in each of the A and B wells of Slide #3.

5. Place 3 to 4 drops of the simulated anti-A serum in each A well on the three slides.
6. Place 3 to 4 drops of the simulated anti-B serum in each B well on the three slides.

7. Obtain 2 toothpicks per blood typing slide. Stir each well with a separate clean toothpick for 30 seconds. To avoid splattering the simulated blood, do not press too hard on the typing tray.

8. Observe each slide and record your observations (results) in the table below. To confirm agglutination, try reading text through the mixed sample. If you cannot read the text, assume you have a positive agglutination reaction.

	Anti-A Serum	Anti-B Serum	ABO Phenotype
Slide #1: Kevin's mother			
Slide #2: Kevin's father			
Slide #3: Kevin			

Discussion:

1. What is Kevin's genotype? How do you know?

2. What are Kevin's parents' genotypes? Predict all of the genotypes probabilities of their offspring. (*Hint*: use a method that you have learned about in this genetics unit to answer this question, using Kevin's genotype and the other results from your experiment.)

3. Explain in a few words how it is possible that Kevin could have a different ABO blood type than either parent.

4. If Kevin had a sibling with one of the other genotypes shown in your Punnett square, could he or she receive blood from one or both parents? Be specific for each other genotype shown and state each corresponding phenotype (blood type).

Challenge 1:
Come up with another ABO scenario where a child could have a different blood type from either parent that would make it impossible for her to receive a transfusion from either parent. Show your work here and on first half of the next page, as needed.

Stop here for today. Bring this worksheet back to class on Tuesday for the second part of the lab (continued below).

Part 2

Exercise 3 – Population Genetics. Earlobe attachment is considered to be a simple Mendelian trait. The contrast is between direct attachment of the lower part of the earlobe to the head (the 'attached' phenotype, fig. 1 below) and a free-hanging lobe ('non-attached', fig. 2). The attached earlobe is inherited as a recessive. Use 'E, e' as your lettering system for this trait.

1

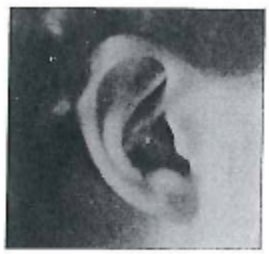


FIGURE Attached earlobe (recessive). *Photo, D. L. France*

2

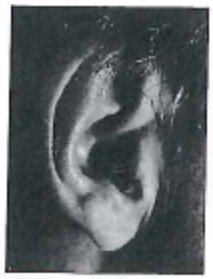


FIGURE Free-hanging earlobes (dominant). *Photo, D. L. France*

What is your own phenotype? _____ Your possible genotype(s)? _____

Challenge 2: Figure out what is the predicted genotype distribution of the earlobe trait in this class, and what is the frequency of the two alleles. (Note: For purposes of this challenge, assume that your class is a biological population, which, of course, it is not.)

What is your research question? (In this case it might be difficult to state a single hypothesis initially, so you can ask a specific question instead.)

What method/technique that you have learned about would you use to answer this question? Why would you choose that method?

Now, apply this method, show your work here, and report your results on the next page where prompted. **Hint:** Have one person collect the phenotype counts for your table and write them on the white board next to your team number; when all of the teams have reported, use the phenotype totals for the whole class to answer this challenge.

(Report your results on the next page.)

Report your results here:

a. What are the phenotype frequencies in this population (be sure to label each frequency)?

b. What are the genotype frequencies you obtained (be sure to label each frequency)?

c. What are the gene (allele) frequencies you obtained (be sure to label each frequency)?

Discussion (must be completed):
Have you answered your research question? Have you met the challenge? Explain.

(Space for class discussion notes or your additional work on back of page.)

Space for additional work and class discussion notes:

Name: _____ Lab Date: _____

Note: Each student must complete his/her own lab worksheet and include it in a lab notebook.

ANT 101

Human Variability – Anthropometric Measurement

Body size and shape vary across and within human populations. As you have learned, adult body height (stature) is determined by both genes and environment, particularly the environment during child growth and development; once adult height is reached, height is no longer subject to environmental effects. On the other hand, adult body weight (mass) is affected by body height and by both short and long term environmental effects (e.g., diet, activity level, disease state, etc.). Anthropometry includes a set of tools and techniques for accurately measuring various body traits; anthropologists use these methods to better understand human population variation. **For today's lab you'll be working with your small lab team. After being instructed, make the measurements on each other and record them (A and B below). Refer to the handout on your table as needed for additional assistance. Then try calculating one or two indices (C) and do the cranial capacity estimate (D). After you have completed this part, proceed to the challenges.** This lab is related to chapters 1.2 and 6 in your textbook.

A. Procedures: Follow the procedures as demonstrated in the videos and instructed in class. On the next page, record measurements in the appropriate units as outlined below. **NB:** when striving for precision in a research setting, measurements are not made through clothing (other than a light gown), and we typically measure 2 or 3 times and use the average. For today's class, measure carefully; repeat a measurement if you or your teammates think your technique wasn't accurate. Team members should 'spot' and assist each other during measurements.

1. **Stature (Standing Height)**. Using the stadiometer, measure and record the distance between the top of the head and the standing platform (subject should have shoes removed). **Note:** You need to make sure that your eyes are level with the pointer when making the reading. Measure in centimeters. Record number to the nearest 0.1 cm.

2. **Weight (Optional - e.g., if you want to be able to calculate your BMI)**. Using the scale, measure the weight (subject should have shoes removed). Convert pounds to kilograms (lb/2.2) and record.

3. **Head Length.** Using the calipers, measure and record the distance between the most prominent point between the eyebrows and the most backward projection of the head. Measure in millimeters (to the nearest 0.5 mm).

4. **Head Breadth**. Using the calipers, measure and record the greatest transverse (side to side) diameter of the head, which is usually just over the parietal bones, above and possibly slightly behind the ears. Measure in millimeters and record to the nearest 0.5 mm.

5. **Ear-Head Height**. Using the calipers or other device provided, measure the distance between the top of the auditory meatus (ear canal opening) and the top of the head. Measure in millimeters; record to the nearest 0.5mm.

6. **Head Circumference**. Using the tape measure, measure and record the maximum circumference of the head, not including the brow ridges. Measure in centimeters. Record number to the nearest 0.1 cm. (Note: this measurement is usually only done in children, to follow their cranial growth or in millinery – hat – stores).

B. Record the measurements (measure each member of your team, recording the person's initials):

	Person's Initials: ____	____	____	____
1. Stature (cm)	_____	_____	_____	_____
2. (optional) Weight (kg)	_____	_____	_____	_____
3. Head length (mm)	_____	_____	_____	_____
4. Head breadth (mm)	_____	_____	_____	_____
5. Head Height (mm)	_____	_____	_____	_____
6. Head circumference (cm)	_____	_____	_____	_____

C. Anthropometric indices

An anthropometric index is a ratio between two measurements, sometimes expressed on a scale of 100. It is possible to calculate a couple of indices from the measurements you took today. You can try calculating these indices for your own measurements.

1. **(Optional)** Body Mass Index (BMI): a measure of body weight controlling for height = $\frac{\text{weight (kg)}}{\text{height (m}^2\text{)}}$

Note: you have to convert centimeters to meters before calculating.

Your BMI: _____

In anthropological contexts, BMI is used as a measure of inter- and intra-population variation in body size in adults.

In medicine and public health, BMI is used to assess nutritional status and risk for chronic disease. In this context, BMI value range interpretations (for adults) currently are:

<18	underweight
18-24.9	normal weight
25-29.9	overweight
≥30	obese

2. Cephalic Index (a measure of **head shape, not size**) = $\frac{\text{Head Breadth}}{\text{Head Length}} \times 100$

Your CI: _____

Types: ("dolicho" refers to long, "meso" refers to medium, and "brachy" refers to broad or wide)

Dolicocephalic: up to 75.9
Mesocephalic: 76.0 to 80.9
Brachycephalic: 81 and over

Your head shape type: _____

Head shape has both a significant genetic component and is influenced by long-term adaptation to climate (as is body shape/size and nose shape). Compare the head shape types of people in your team. Do you see any differences by region of population ancestry? Describe.

D. Cranial Capacity. It is also possible to use the three cephalic measurements you took to estimate **cranial capacity** (volume) using a formula (Lee-Pearson). Use mm in this formula and be very careful about decimal points. Results will be in cc (cubic centimeters).

Males: 0.000337 (head length – 11) X (head breadth – 11) X (ear-head height – 11) + 406.01

Females: 0.0004 (head length – 11) X (head breadth – 11) X (ear-head height – 11) + 206.60

Record your Cranial Capacity: _____

Challenge #1:
You are hanging out with your friends, and the conversation wanders onto the topic of brain size and what it's related to. One guy asserts that people with bigger brains are smarter than people with smaller brains. Another friend said that is silly, that brain size is mainly affected by body size, and since big people aren't always smarter than small people, the first guy's idea doesn't make sense. Since you just learned how to do a little bit of anthropometry in your bioanthro class, you decide to take a look at your friend's claim that brain size is related to body size using a convenience sample (your classmates).

What is your prediction (hypothesis) about the relationship of brain size to body size?

How could you test your hypothesis using the skills you just learned? Which measures or indices would you use, and why? Be specific about how each measure you choose relates to brain size or body size.

Go ahead and collect/assemble your data. What are your results? Report them in an organized format here, and add them to the class results in the spreadsheet on the instructor's computer.

Challenge #2:
One of your other friends who was in on that discussion said that she thought brain size was probably mostly influenced by sex. Since you're on a roll, you decide to test this relationship, too.

What is your prediction about the relationship of sex and brain size? Why do you think this?

Report your results by sex here, and add them to the class results.

NOTE: YOU MUST PRINT OUT THE CLASS DATA TABLE AND GRAPHS TO INCLUDE WITH THIS LAB IN YOUR NOTEBOOK!! The table/graph file will be in the lab folder for Week 4 as soon as I have time to load it.

Discussion (respond to these questions in your lab teams, before the larger class discussion):

Which is the better predictor of cranial (and brain) size: body size, or sex? Are these two variables (body size and sex) related to each other? How might they each be related to cranial size?

How does cranial size differ from brain size?

Think about the measure of body size that you used. What influences does it reflect? What does it actually measure?

Room for extra notes (on techniques and/or notes from team or class discussion):

Name: _____ Lab Date: _____

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ANT 101

Human Adaptability – Temperature Stress Response

In today's lab you will learn how to assess surface temperature of the skin and examine the relationship of this measure to temperature stress. As you read in the pre-lab, many of the chemical reactions and cellular processes necessary to sustain human life occur most readily at a body temperature of approximately 37.0°C (98.6°F). Homeostatic mechanisms work to maintain this temperature, regardless of changes in the external environment. Changes in temperature are sensed by the skin, which is well-designed to counteract these changes. Beneath the protective epidermal layer of the skin lies the dermis, which contains sweat and oil glands and a rich blood supply.

You'll be working in your small teams today. After you learn the basic technique of taking surface temperature using the Vernier physiology interface in your small teams, and we have discussed your findings, you will work with your team to develop a hypothesis about temperature response and collect data to test that hypothesis. You will then create two PowerPoint slides (hypothesis and results) and briefly present your group's experiment to the rest of the class (at the beginning of our next lab class, in one week). This lab relates to the following chapter 6 sections in Revel: 6.5.

I: Pre-Lab Reading about the background: done before class. Refer to this information as needed during the lab.

II: Lab experiment: Skin Temperature Response to Cold Stress (30 minutes)
In this experiment, you will

- Learn to measure skin temperature
- Obtain graphical representation of skin temperature.
- Compare temperature before and after exposure to a cold stimulus.
- Use these skills to test a hypothesis generated by your lab team.
- Present these results to the class with two slides.

METHODS

Materials

computer	alcohol wipes
Vernier computer interface	cellophane tape
Logger *Pro* software	ice cubes/ice water
Vernier Surface Temperature Sensor	towel (paper or cloth)

Procedures

Select one person from your lab group to be the initial study subject. Follow the steps below.

1. Connect the Surface Temperature Sensor to the Vernier computer interface using the 'Go-Link' interface currently inserted in one of the USB ports on your laptop.
2. Open the file "02 Skin Temperature" from the *Human Physiology with Vernier* folder.

3. Remove excess oil from the skin over the **inside of the forearm** with soap and water or alcohol. Tape the Surface Temperature Sensor to the arm, over the area you cleaned. Be sure to tape the thermistor end (the tip) of the sensor directly to the arm (see Figure 1, but use the location described in this experiment, the forearm).

4 Click ▶ Collect to begin data collection. Collect data for **50 s** to obtain a baseline recording of the temperature. Click ■ Stop to end data collection. Record the initial baseline temperature in Table 1.

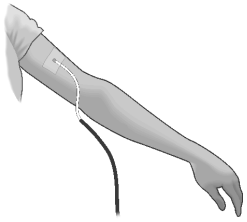


Figure 1

5. Choose 'Store Latest Run' from the Experiment menu to store the data.

6. Remove the Surface Temperature Sensor from the arm. Obtain a piece of ice and hold the ice over the area of the lower arm to which the Surface Temperature Sensor was affixed. Hold the ice cube in place for **30 s**.

7. Remove the ice and quickly blot the area dry with a towel. DO NOT RUB as friction can cause an increase in skin temperature.

8. Tape the Surface Temperature Sensor to the forearm again, in the same area where the ice was held.

9. Click ▶ Collect to begin data collection. Data will be collected for 120 s. Click ■ Stop to end data collection. Record the initial temperature after cold exposure in Table 1, and record the final temperature at 120 s.

10. Store this run by choosing 'Store Latest Run' from the Experiment menu.

11. Determine the rate of recovery.

    a. Select the data collected from 0–50 s by clicking and dragging to highlight this region.
    b. Click on the Linear Fit button, , and check the box next to Run 2.
    c. Click OK and a best fit linear regression line will be shown for the selected run. The linear regression statistics are displayed in a floating box.
    d. In Table 1, record the value of the slope, *m,* for the run.

12. Store the data by choosing 'Store Latest Run' from the 'Experiment' menu.

13. **Tip:** if you want to save data for later use in a presentation or additional experiment, choose 'Save As' in the File menu and give it a name; you can save it to the desktop or to a storage device.

RESULTS

Table 1		
Run	**Temperature (°C)**	**Slope(m), or rate (°C/s) at 50 sec**
Baseline		n/a
Right after cold exposure		n/a
At 120 seconds after exposure		

DISCUSSION

1. Was the baseline temperature recovered within the 2 minutes during which post-exposure data were collected? Estimate how long it would take for full recovery to be achieved. Relate this to everyday experiences where you have been exposed to cold.

2. What is going on structurally or physiologically that can explain these results?

PAUSE – FULL CLASS DISCUSSION (15 minutes) – use p. 5-6 for any notes you want to take

III. Group Mini Project -- Part A: hypothesis and data collection (45 minutes):

Identify (as a team) a hypothesis related to human adaptability and temperature that you can examine now, in class, using the methods you learned in this lab; develop your specific methods approach; and collect data among your team members to test that hypothesis using the Vernier software. Report your results in a data table in this worksheet (see next couple of pages). You may also wish to graph your results.

1) State your **hypothesis** (one sentence).

2) How will you test this? Briefly describe your **methods**.

3) Present your **results** in table format here.

4) **Discussion:** Was your hypothesis supported or refuted? Explain.

IV. Group Mini Project – Part B: literature search and slide creation (final 30 minutes of class)
1) Do some research on the web in the scholarly literature regarding population or individual differences in temperature response. Identify your sources and what you learned here.

2) **Make two PowerPoint slides to use during your presentation to the class**: one stating the hypothesis, and one presenting the results in table and/or graphic form. You will use these in your team's class presentation at the beginning of next week's lab class. In your presentation, you should be prepared to state whether your results supported or refuted your hypothesis.

Class Discussion: Notes from instruction on techniques and/or notes from large class discussion (continue on the back if you need more room)**.**

Name: _____ Lab Date: _____

Note: Each student must complete his/her own lab worksheet and include it in a lab notebook.

ANT 101
Classification & Tree Building

Today's lab is part 1 of a 2-part lab. The second part will happen during the next course topic area, on primates (next week). Lab teams should have the same members for both labs, so the classmates you work with today will be on your team next time as well. Read through the paragraph below, and after a brief introduction by your instructor, work with your team to discuss and answer the following questions and challenges.

For many students, the principles of classification and how they relate to evolutionary relationships may seem difficult to understand in the abstract. One purpose of this lab is to help you to understand them better through guided practice. Another purpose is to engage you in solving problems or challenges using the scientific process. Note: the questions and challenges in today's lab relate to the following secttions in your textbook: 5.2, 5.3, the phylogenetics section of 6.3.

***(Lab Introduction – slides*)**

1. Look at the 3 skulls of different primate species, and develop, **with your lab team**, a hypothesis about how they are related (which two are more closely related, which more distant, etc.). Briefly state your hypothesis, then provide your reasoning (what you observed that helped to form your hypothesis – be specific).

(Instruction in tree-building – slides)

2. Now look at the faces on the piece of paper (Problem 1) provided to your table. Think about how they are 'related', and construct an evolutionary 'tree' to show what you have come up with. We will discuss this after you finish it, so do not go on to the next item yet.

3. Now do the same thing for Problem 2.

4. Now do the same thing for Problem 3.

Challenge, Part 1: Based on the ability you and your team just demonstrated in the techniques of classification, your colleagues at the university have decided your team is ready to take a crack at constructing an evolutionary tree depicting your hypothesis about the relationships among the 3 skulls you observed earlier (question 1). Show that tree here.

Preparation for Part 2 of the Challenge

The next phase of this lab (next week's lab) will involve using mitochondrial DNA (mtDNA) sequences to build phylogenies (trees) for primate species in order to test your hypothesis based on the skulls. As a class, we will be working with 10 primate species: humans and 9 others. As you noticed in the exercise you did with the faces, the more species you are comparing, the more possibilities there are for how to build a tree, so we are going to break this down into smaller samples of species. Each of you will receive a set of mtDNA sequences for 3 primate species to take home with you and work on as homework between now and the next lab (each student on your team will get a different set). I am going to show you in lab today what you need to do for that assignment, which you will complete at home and bring back with you to the next lab class.

(Class demo – slides)

Example of table (from class demo for cytochrome oxidase 1, subunit 1 sequence):

	Human	Orangutan	Howler
Human	----	5	12
Orangutan		----	11
Howler			----

Homework (must do at home and bring the completed assignment to next week's lab class):

1) On the sheet of DNA sequences you receive, circle or highlight using different colors the base pairs where there is a difference between pairs of species (do this for all combinations of 2 species in your assigned group of 3 primates, for the entire two-page, 956-nucleotide NADH sequence);

2) Count the number of nucleotide (base) differences between each pair of species and write those numbers on the DNA sequence sheet;

3) Draw a 4-column, 4-row table on the next page of this lab worksheet to report those counts from your 3 DNA sequences (use the example above as your model). **Be sure to bring this lab worksheet and your DNA sequences to next week's lab.**

HOMEWORK for Week 7 Lab:
Draw your 4-column, 4-row table here and report your pairwise difference counts from the DNA sequences assignment you did. Bring this worksheet and your DNA sequences to next week's lab class.

Class Discussion/notes (be sure to label what question above they belong to; continue on the back if you need more space):

Name: _____ Lab Date: _____

Note: Each student must complete his/her own lab worksheet and include it in a lab notebook.

ANT 101
Primate Classification

Today's lab is part 2 of a 2-part lab. Lab teams should have the same members as for the lab last week on Classification and Tree Building. Read through the paragraphs below, then work with your team to discuss and answer the following questions and challenges.

Today's lab will help you continue to build your understanding and skills related to classification while beginning to engage you with how evolution is manifested at the molecular (genetic) level as well as in the phenotype. It will also help you to better understand the relationships among the living primates, including humans. Note: Today's lab relates to the following material in your textbook: Ch. 7, especially sections 7.1-7.3 (in addition to the material referenced in last week's lab).

Recap: In the last lab, your team generated a hypothesis, expressed both in words and, later, in a proposed phylogenetic tree, about the relationships among three species represented by three skulls (monkey, ape, and human). After some instruction and practice in the method of classifying and tree building using observed traits, your team was given some genetic data to use to test your hypothesis, and you were instructed in the method to use with these data. You applied that method in a homework assignment in which you were to identify and count pair-wise differences in a 956-base segment of mtDNA for three primates. **You should have your week 6 lab worksheet with you today, including your completed table of counts (partial results) on page 5, as well as the sheet of DNA sequences you were assigned.**

Challenge, Part 2: Your colleagues who asked you to classify the three primate skulls are wondering if you were correct. They gave you some DNA sequences to work with in the hope that you could use them to help address this question. **Work with your team to discuss and answer the following steps of the challenge. Note:** Steps 1-3 below are relevant to pooling your team's results. The later steps are relevant to interpretation/discussion.

Results:

1. Look at your own counts (the table you made on p. 5 of the week 6 lab worksheet) and think about what the numbers say about which species are more closely related. Then draw a 3-species **phylogenetic tree** based on your own table/data, and state briefly the basis for grouping the species the way you did. You can discuss this with your teammates if you're not sure.

2. Now, with your team members, pool your individual counts in a larger **table** (list all the species examined by your team members across the top and left side of the table, and enter the counts just as you did in your own table). Make sure you know whether each non-human primate species is an ape or a monkey; it would be easiest if you group them by primate type in your table.

3. Look at the pooled data and think about what they mean, discussing them with your teammates. Propose hypotheses as a team about the relationships among the three primate groups – monkeys, apes, and humans – based on the genetic counts. Draw a simple, 3-group (monkeys, apes, humans) tree to show those proposed relationships. This may be the same as the one you drew in #1 based just on your own three assigned species, or it may be different because you now have more data.

Discussion:

4. Now, compare your genetics-based hypothesis to the hypothesis your team developed when you looked at the skull anatomy last week (bottom of p. 3 on the week 6 lab worksheet). Compare the two trees, genetics v. anatomically based. Are the hypotheses/trees based on genetics and anatomy the same? Different? Explain.

5. Can you be sure which one of these hypotheses is “correct”? Why or why not?

6. How could you further test your anatomically-based hypothesis?

7. How could you further test your genetically-based hypothesis?

8. **Entire class:** We will bring the various teams' ideas together and look at a table and tree for the full sample of mtDNA from 10 primate species. **Use this space for any notes/diagrams you wish to record from that discussion (more space on the back of this page**). **Some of these ideas may be relevant to your lab report assignment; see below.**

Homework Assignment: Write a group lab report, due in class next **Thursday, 10/19,** on this two-part lab. The structure should be like a scientific paper, i.e.: Introduction, Methods, Results, Discussion. Each student will draft one section of the report, but **all team members are responsible for the final product**. The report should be typed, double-spaced, and no less than 2-3 pages (not including the header). **This is a graded assignment; guidelines about what each section should include and a rubric for how it will be graded are posted on the D2L site.**

Additional space for notes from team or class discussion:

Name: _____ Lab Date: _____

Note: Each student must complete his/her own lab worksheet and include it in a lab notebook.

ANT 101

Primate Skeletal Anatomy & Locomotion

As a group, primates have a variety of ways of moving around in their habitats, i.e., locomotion. Each primate species utilizes a particular locomotor style, and there is a relationship between mode of locomotion and habitual posture including relative lengths of fore- and hindlimbs. These locomotor behaviors and habitual postures represent, in part, adaptations to environmental conditions in which the species has evolved. Today's lab will help you better understand these patterns and how to utilize limb measurement to identify an unknown primate's locomotor pattern and general group affiliation.

For today's lab you'll be sharing the specimens with the other team at your large table. After you have been instructed in the techniques being used today, discuss and answer the following challenge and its associated questions with your smaller team. Note: Today's lab relates to the following chapter sections in your textbook: 7.2, 7.3 , Appendix A.

Challenge:

You are a field primatologist who discovered some bones of a deceased primate in a forest on your latest expedition in the Old World. You are anxious to figure out what kind of primate this was and how it moved around in its environment. Fortunately, there were forelimb and hindlimb bones among those you found (Sample A). Your colleague back in the lab gave you another set of bones (Sample B) to compare with the ones you found, thinking that this might help you with your analysis.

1. Examine Sample A. What is your hypothesis about the locomotor behavior of primate 'A'? (use the locomotor pattern descriptions we discussed in class). Explain very briefly your choice of hypothesis.

2. How will you test this hypothesis (what is your method)? Describe the steps you will follow, including how you will use sample B – and be thorough. What kind of evidence will you be looking for?

3a. Collect your data and report your results here in table form, making sure to label which bone set, and bone, you're describing. You can use your textbook and the other resources provided to help you identify the bones. (Note: you will need to share the two boxes of bones with the other team at your large table.)

3b. Using the computer assigned to your team, enter the data you obtained in Excel and figure out how to present the results in graphic form. Explain here briefly what you did. **(Note: when you have it looking the way you want, either print out a copy for each team member or save a copy to the desktop and email it to all of the team members. Each person should print a copy and insert it with this lab worksheet in his/her lab notebook.)**

3c. Have you finished testing your hypothesis yet? Is the question about creature A's locomotor behavior answered? What more could you do?

4. Figure out how you would use the measurements you made to predict the kind of locomotor behavior these primates engaged in. **(Show your calculations and how you are using the measurements here.)** What is your prediction for each primate? Explain.

5. Can you be sure that you now have the answer to the original question (what was the locomotor behavior of primate A)? What additional information would you like to have to be able to further test your predictions about A and B?

6. (After I give you some additional information and a handout): Plot your data and label your two points 'A' and 'B'. Where do your two primates fall in relation to the locomotor patterns shown on the graph? **(Note: keep this graph and put it with this lab worksheet in your lab notebook. You should have two graphs for this lab in your notebook – the one from 3b and this one.)**

Discussion:

7a. Were your predictions in #4 about locomotor behavior supported or refuted? Explain.

7b. Was your original hypothesis (in Item 1) supported or refuted? Explain.

7c. Looking at your graph and the version shown on the screen in the front of the classroom, what can you deduce about the general relationship between positional behaviors of primates and

limb length?

body size?

limb length and body size?

7d. Based on all of this, what larger group(s) of primates do you think your two primate samples belong to?

Class Discussion: Room for any notes from instruction on techniques and/or large class discussion after the exercise. Continue on the back of this page as needed.

Name: _____ Lab Date: _____

Note: Each student must complete his/her own lab worksheet and include it in a lab notebook.

ANT 101
Human Osteology & Forensics

One area of expertise in biological anthropology is human osteology, the scientific study of human bones (morphology, pathology, etc.). Osteologists require a deep understanding of variation in bone characteristics, including those shaped by sex and age. Forensic anthropologists must be very knowledgeable about human osteology, in addition to having other knowledge and skills. Today you will use what you read about osteological sex differences in the pre-lab readings to try to solve a mystery. **You will be working with your lab team using the specimens assigned to your large table. After you have been instructed in the relevant techniques, discuss and answer the following challenge and questions with your team.** Note: today's lab relates to the following sections in your textbook: 15.5, 15.6, 15.7, 15.8, 15.9, Appendix A.

Challenge:
A shallow grave containing a few bones has been discovered in a wooded area near Jonesburg. A few years ago, a 28 year old woman from another town in the vicinity was reported missing by her friends, and there has been no contact or information about her whereabouts since then. She was in good health at the time. As a forensic anthropologist, you have been asked to do a preliminary examination of the bones to determine whether they could possibly be those of the missing woman. One of the first steps would be to determine the sex of the remains.

Indicate which sample your team is studying (by letter): _____

1. What is your initial hypothesis?

2. How will you test this hypothesis (what is your method)? Describe the steps you will follow. What kind of evidence will you be looking for?

3. Collect your data, making sure to be deliberate and methodical in your observations. Report your results here in an organized format (e.g., a table), and label each characteristic or trait you are describing.

4. Discussion:

a. What is your interpretation of these results? On what basis do you think that? How sure are you of this interpretation?

b. (Depending on your answer in #4 above): What additional information would you like to have to be able to further test your hypothesis?

c. In a few brief sentences, relate your results to the pre-lab reading you did and to the exercise we did in the early lab where you were trying to determine which skeleton was female and which was male.

Notes from instruction on techniques and/or large class discussion after the exercise (attach an additional sheet of paper if needed):

Name: _____ Lab Date: _____

Note: Each student must complete his/her own lab worksheet and include it in a lab notebook.

ANT 101

DNA Fingerprinting Lab

In today's lab you will learn how to use DNA fingerprinting, or typing, to solve a problem. This technique is used in forensics, anthropology, and conservation biology not only to determine the identity of individuals but also to establish relatedness. It is also being used to find genetic linkages to inherited diseases. In addition, scientists are learning a great deal about our evolutionary history from DNA analysis. **You'll be working in your usual small teams today. I will give you guidance on the various steps as you work through the lab, and it will be important that you follow directions, verbal and written, very carefully. NOTE: You will write an individual lab report on this lab, due in class on Tuesday, 11/14**. Today's lab relates to the following chapter sections in your textbook: 15.8, 15.9.

Part 1: Pre-lab reading about the background and methods: done before class. Refer to this information as needed during the lab, and use it to help you with your report, especially with providing background as part of the **Introduction**.

Part 2: Lab challenge and procedures.
Challenge: Who was this woman? Or, at least, to whom is she related?

A shallow grave containing a few bones was discovered in a wooded area near Jonesburg. A few years ago, a 28 year old woman from another town in the vicinity was reported missing by her friends, and there has been no contact or information about her whereabouts since then. She was in good health at the time. As a forensic anthropologist, you have been asked to determine whether these remains could be those of the missing woman. Your first step (Week 9 Lab) was to examine the bones to determine the sex; based on that study, we decided that the deceased was female. Now you hope to make a more definitive determination of her identity by comparing DNA you have been able to extract from the remains to DNA from hair and other samples provided by the families of women who have gone missing within fifty miles of Jonesburg in the last 15 years. The technique you decide to use is often called DNA fingerprinting. In particular, you decide to 'snip' DNA segments using restriction enzymes to create restriction fragment length polymorphisms, or RFLPs, and then compare the prepared DNA samples using gel electrophoresis.

(Note: You will be working with DNA that is from plasmids, i.e., it is not human DNA.)

Here are your samples:

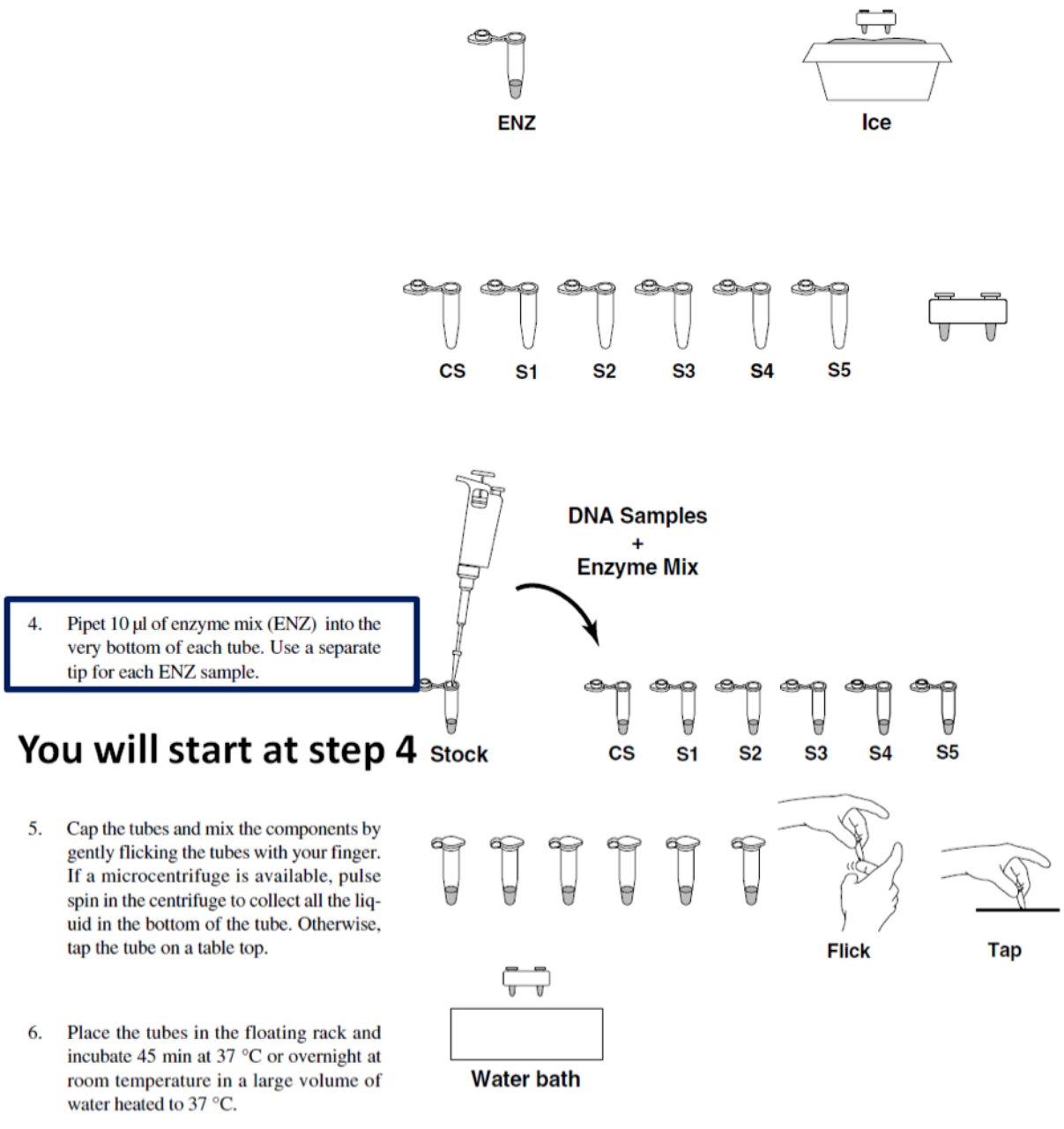
CS = the unknown crime victim
S1 = known subject 1 (a 30 year old business woman from Smithtown)
S2 = known subject 2 (a 20 year old student from Harris College)
S3 = known subject 3 (a 28 year old woman from Adamsville)
S4 = known subject 4 (a 31 year old homemaker from the other side of the county)
S5 = known subject 5 (a 35 year old musician from Jonesburg)

Other tubes:
ENZ = enzyme (to cut the DNA into RFLPs)

LD = loading dye (to stain the DNA fragments)
M = marker (to help you measure)
H_2O = water (to hydrate the electrophoresis wells)

Methods

Quick Guide for DNA Fingerprinting Kit



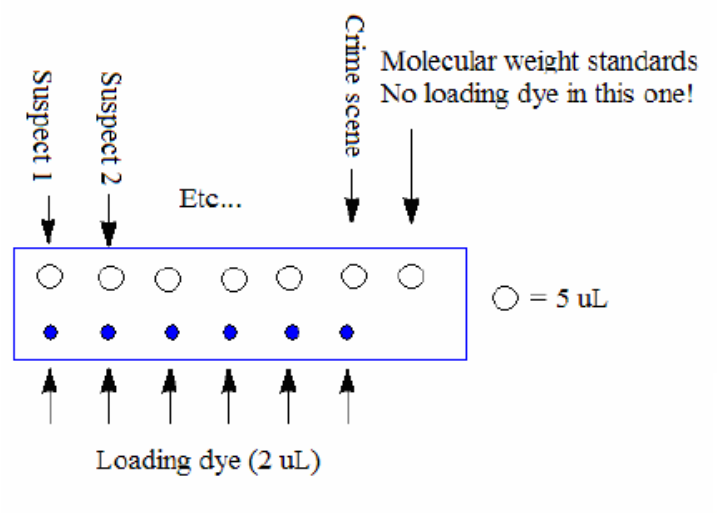
Be sure to put one of your group's initials, or the group's number, on one of the <u>tubes</u> (not on the floating rack) so the group samples don't get mixed up.

THE FOLLOWING WILL BE DONE BY ME

- Insert cassette into dock
- Plug in electrophoresis power supply

THE FOLLOWING WILL BE DONE BY YOU

- Obtain a piece of parafilm and "spot" **2 µl** of the loading dye (LD), using an automatic micropipet and a fresh tip, in 6 separate areas as shown on the figure below, then discard that tip.

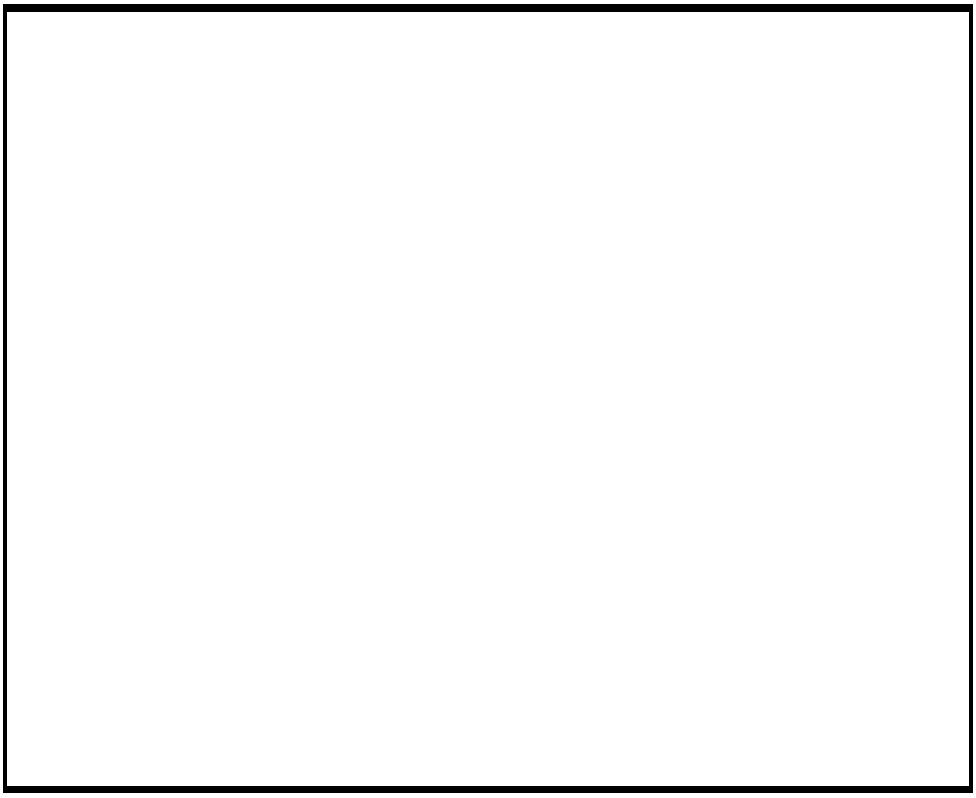


- Using a new tip, load **6 µL** of distilled water in <u>each</u> well of the flash gel (FG) system. Discard the tip, cap the water, and put it aside.
- Using a new tip, load **5 µl** of marker (M) into well #1 of the FG system. Discard the tip.
- Using a new tip, load **3 µl** of the enzyme/sample from the crime victim (CS), deposit it on the first spot of loading dye, collect the combined dye/sample into the micropipet, and transfer it into well #2. Discard the tip. Continue this procedure for each of the Known Subject samples in this order: 1-5.
- The final order will be: M, CS, S1, S2, S3, S4, S5.
  <u>USE A FRESH TIP FOR EACH SAMPLE!!!</u>

- **ASK ME FOR HELP WITH THIS STEP IF YOU HAVE ANY QUESTIONS:** Plug the gel dock cables into the power supply (red in red, black in black), then turn on the power supply (switch on the side), set the voltage at 275 V, turn on the gel dock light, and push 'run'.
- Watch until desired separation is achieved (5-7 min). Record your observations (your results) in the next section below (if you have a cell phone in your team, take a photo of the gel to report with your results).
- When finished, first turn off the light on the gel dock, then turn off the power supply. <u>Only then</u> disconnect the dock cables from the power supply (to avoid an electric shock!).

Results

Sketch the pattern resulting from the gel electrophoresis in the box below, or include a print-out of the photo your team took with this lab worksheet in your lab notebook (each student needs one!). **Also include either the sketch or the photo of the gel in the Results section of your lab report (you can paste it in)**. **Be sure to label all of the results/runs on your sketch or photo.**



Discussion: Work with your teammates to answer these questions.

Compare the fragment sizes of the known subjects and the unknown (crime) victim.
Is there a match? If so, which subject matches?

How sure are you that this subject matches? Why or why not?

What would you conclude about the identity of the unknown victim?

What next steps would you take, if any, to further investigate this question?

Class Discussion: Notes from instruction on techniques and/or notes from large class discussion (use back of page if you need more room).

Name: _____ Lab Date: _____

Note: Each student must complete his/her own lab worksheet and include it in the lab notebook.

ANT 101

Primate/Hominin Identification - Bipedalism

The fundamental trait that defines hominins is habitual bipedalism. Habitual bipedal locomotion is made possible by multiple skeletal adaptations, literally from head to toe. When a new fossil hominoid discovery is made, a key question that will be addressed is whether or not there is morphological evidence of bipedalism – i.e., whether this was a hominin (versus another kind of ape). This lab will help you apply what you have learned about bipedal skeletal characteristics so that you can become more familiar with how these kinds of determinations are made by paleoanthropologists. **For today's lab your team will be visiting each lab station as part of a larger group. After you have examined the specimens at each station, return to your seats and discuss and answer the following questions/challenges with your usual team members. I'll tell you when to move to the next station. In each case, you are being asked to focus on specimen A and to use the other specimens (B and C) to help you answer the challenge about A.** Note: Today's lab relates to the following chapter sections in your textbook: Ch. 10, Introduction through 10.4.

Challenge #1 (Station 1):

You are an up and coming paleoanthropologist. Because of your reputation, one of your colleagues from another university contacts you about a skull he found (A). He thinks it might be a hominin, but he wants to get your opinion. You have some other skulls in your collection that you can use to help you figure this out (B and C).

1a. Examine 'A'. What is your <u>hypothesis</u> about the locomotor behavior of creature 'A'?

1b. How will you test this hypothesis (outline your <u>method</u>)? What kind of evidence will you be looking for?

1c. What were your results?

1d. Discussion – What did you conclude about your hypothesis from your testing? What else would you like to know?

Challenge #2 (Station 2):
Another paleoanthropologist gets in touch and wants you to give her an opinion about some fossils she has been puzzling over. She brings you the collection you see at station 2 (sample A), and you pull out some similar materials from your own collection (B and C).

2a. Examine 'A'. What is your hypothesis about how creature A moved around in its environment?

2b. How will you test this hypothesis (outline your method)? What kind of evidence will you be looking for?

2c. What were your results?

2d. Discussion – Was your hypothesis supported or refuted? Explain

2e. Thinking about what you've learned in previous labs, is there another way you could test your hypothesis? What would you need to know in order to do that?

Challenge #3 (Station 3):
Another colleague recently unearthed an amazing find (A), which he has made a cast of so that he can show it to you and some other scientists. He wants to know if these prints were made by a hominin. You have a couple of other specimens that might help you figure this out (B and C).

3a. Look closely at 'A'. What is your hypothesis?

3b. How will you test this hypothesis (outline your method)? What kind of evidence will you be looking for?

3c. What were your results? (more room next page)

3d. Discussion – Was your hypothesis supported or refuted? Explain.

Reflection: (to be completed in class after you visit all 3 stations)

Did it matter in what order you visited the stations? Did that influence your data-gathering or conclusions? Did you want to revisit a station after visiting other stations?

Class Discussion: Notes from instruction on techniques and/or notes from large class discussion.

Name: _____ Lab Date: _____

Note: Each student must complete his/her own lab worksheet and include it in a lab notebook.

ANT 101

Hominin Evolution – Skulls, Teeth, and Diet Lab

As you have learned, multiple hominin species were extant around 3 million years ago. It has been suggested that this represented an adaptive radiation to different dietary environments/niches. The different types of diets helped to shape a diversity of skull and dental morphologies. In this lab, you will practice comparing cranial and dental characteristics of several hominins and hominids and generate and test hypotheses about their diets. **For today's lab you'll be visiting each lab station with other teams. I'll tell you when to move to the next station. After you have examined the specimens from each station, discuss and answer the questions about the specimens and start to work on the challenge with your usual team members.** This lab relates to textbook chapter-sections 10.4, 10.5, 11 Intro through 11.4, and 11.7.

STATION 1 (samples A and B) – 3-4 small teams, as assigned
STATION 2 (samples C and D) – 3-4 small teams, as assigned

Observations:

1. Work at your assigned station until l direct you to switch with the other group. Compare the four skulls and the associated teeth on the following traits (very brief, 1-3 word descriptions).

	A	B	C	D
Sagittal crest				
Facial prognathism/ robustness				
Brow ridges				
Postorbital constriction				
Shape of skull				
Cranial capacity				

	A	B	C	D
Size and shape of canines				
Presence of a diastema				
Size and shape of incisors				
Size and shape of premolars				
Size and shape of molars				
Species or type?				

2. When you have examined all 4 specimens, indicate what species or type of hominid or hominin you think each one is (discuss with your team members), entering that label in the last row of the table. Describe here briefly what evidence led you to the identifications you made.

A

B

C

D

Challenge 1: What did they eat?

1. Based on your observations of the specimens above, and the information you read in your textbook and the pre-lab reading (also provided as a handout on your table) about the relationship of primate dental characteristics and diet types, generate a hypothesis about what kind of diet each of them ate habitually (the type or properties of the foods).

A

B

C

D

2. Which of the four species do you think ate the most fibrous or hard foods? Why do you think that?

3. Let's test your hypothesis.
As it turns out, the size of a primate's cheek (premolar and molar) teeth relative to its body mass can indicate dietary specialization. Diets that involve habitual chewing of tough foods are associated with molar teeth that are absolutely large and large relative to body mass. Orangutans are an example. They eat a diet that includes a lot of hard-shelled nuts and fruits compared to the diets of other hominoids. On the graph provided to your team (and shown on the projected slides), notice the relative position of the point for the orangutan. If you were to draw a straight (best fit) line connecting the points for the Asian and African hominoids and human, the point for the orang would fall above the line, indicating that its cheek tooth area is larger than would be predicted by its weight.

After instruction on the relevant technique, go to STATION 3 or STATION 4, as assigned. On the maxilla or mandible provided, measure the maximum bucco-lingual (cheek side to tongue side) breadth and the maximum mesio-distal (front to back) length of P4, M1, and M2, **in mm**. Record your measurements. Multiply the two measurements for each tooth to get the surface area. Add the three surface areas together.

	bucco-lingual (mm)	mesio-distal (mm)	surface area (mm^2)
P4			
M1			
M2			
Total Cheek Tooth Area (CTA) (mm^2)			

Log transform the CTA (log 10) (you can do this easily on a calculator): _____

The estimated body weight of the hominin whose jaw this was = 48 kg. Do the log 10 transform for this weight:

_____

Now, work with your usual team to plot the data for this hominin on the 'Body Weight vs. Cheek Tooth Area' graph.

Does the body mass (wt) of this hominin fall within the range of body mass of the other primates?
If so, we can have some confidence about our effort to interpret the dental adaptation of our target hominin.

Draw the best fit line for the hominoids (the original points on the graph) as described above. What would the <u>predicted</u> cheek tooth area be for a hominoid/hominin of 48 kg?

_____

How does this compare with the <u>actual</u> CTA (how much larger or smaller)?

_____

Does this support your hypothesis in question 2 of this challenge about which hominin ate the toughest diet? Explain.

Challenge 2: What did they eat, and how did they obtain it?

1. Restate your skull and tooth-based hypothesis about the diet of species D.

2. A fossilized faunal bone was found near that skull. Go back to your lab table and look at the sample under the microscope. What do you see (results), and what do you think this is or might be (interpretation)?

3. Does this support your hypothesis about the diet of species D? Why or why not?

4. What other kinds of information would you like to have to help you figure out the kinds of foods each of these species ate?

Additional Notes: from instruction on techniques and/or from large class discussion (add a blank page if you need more room).

ANT 101

In-Class Exploration: Population History and Ancestry
Week 14

Today's activity builds on skills you learned in the earlier labs on evolutionary relationships among primates. You will learn a bit about bioinformatics internet resources (where DNA databases can be found and how they can be used to analyze sequences for patterns). Note: Today's exercise relates to the following section in your textbook: 13.7, as well as to what you have learned previously about molecular genetics and phylogenetics.

I. Introduction

- The technical revolution in molecular biology in the last couple of decades has produced enormous amounts of information about sequences of amino acids in proteins and sequences of bases in nucleic acids (DNA). As you know, some genes code for proteins (amino acids), while others do not.

- A variety of computer techniques have been developed to help us understand the meaning of these genetic sequences. These techniques are collectively known as bioinformatics, and they include databases of sequences and computer programs that allow us to look for patterns.

- Through bioinformatics, sequences are being used by scientists to understand relationships among different life forms, in a sense to deconstruct and find the rules and meanings embedded in the language of DNA.

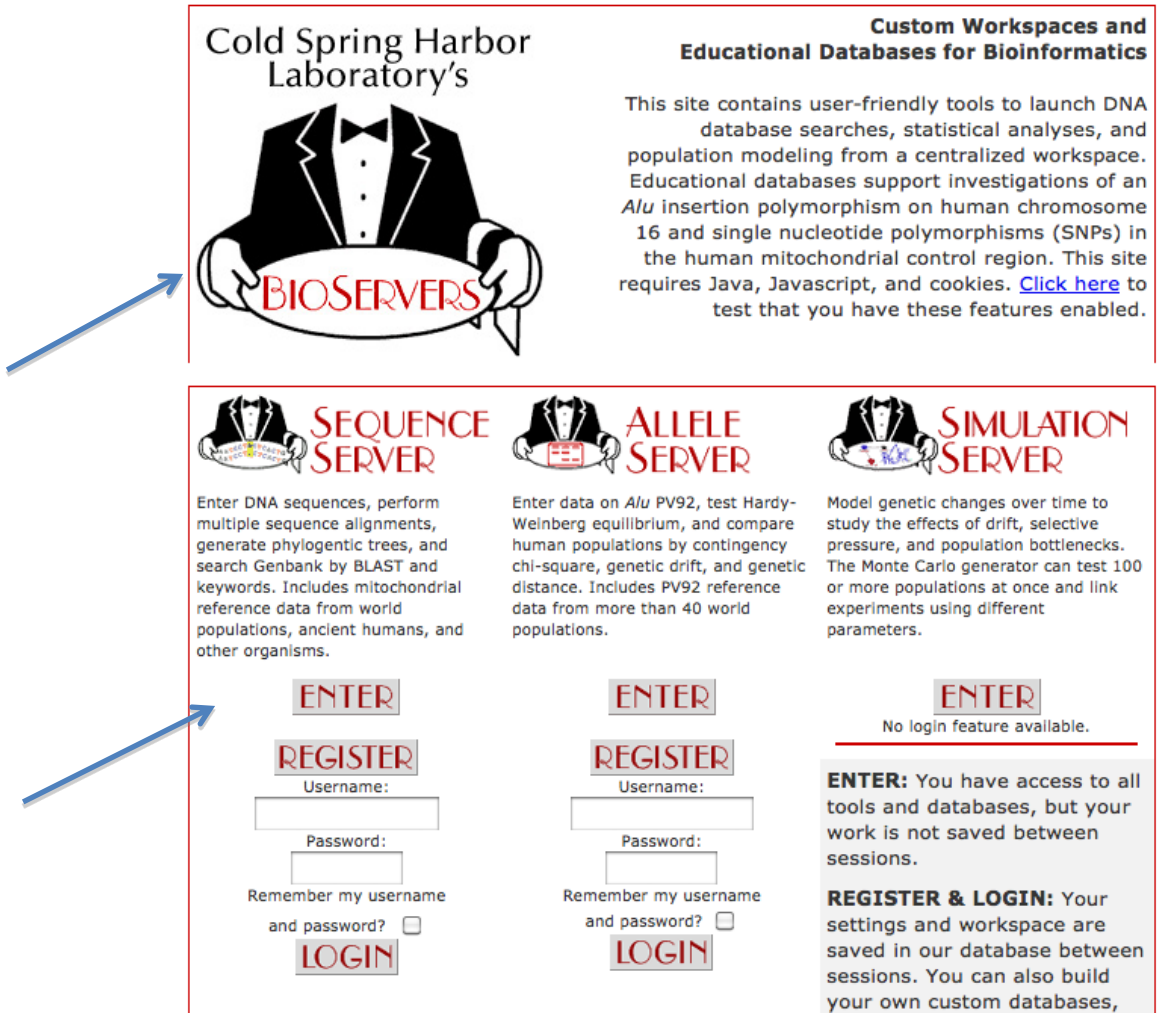
- In earlier labs, you used one 956 base pair (bp) mitochondrial DNA sequence that had been generated through bioinformatics in order to examine the evolutionary relationships among different primate groups. Imagine taking the next step that we discussed during the second lab on classification: testing our hypothesis (our phylogenetic tree), by using more and different sequences of DNA. Imagine trying to compare hundreds of nucleotide sequences – yikes! In today's activity, you will visit a DNA database that will allow you to compare amino acid or nucleotide sequences quickly, and you will learn to construct a basic query (in this case about human population ancestry).

II. Instructions – Learning the Method

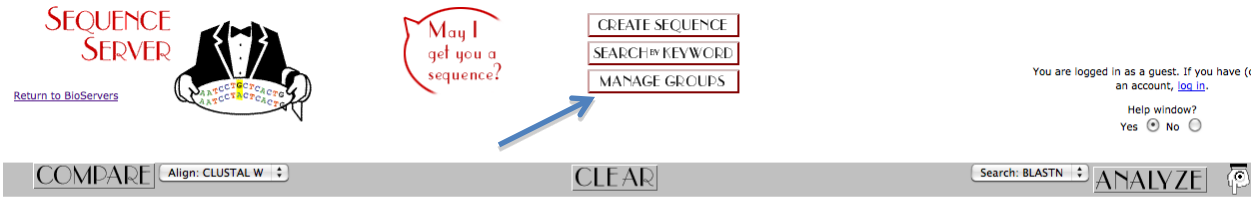
Get a laptop computer (one per 2 students, i.e., 2 computers to a 4-person team). Using internet resources, you will compare mitochondrial DNA sequences. One reason scientists like to use mtDNA is that it mutates more rapidly than nuclear DNA (and, remember: we can use the accumulated mutations to infer recency of shared ancestry). Scientists often use mtDNA to study the evolution of humans, including different populations of modern humans.

Go to one of the Dolan DNA Learning Center's bioinformatics databases: http://www.bioservers.org/bioserver/. (The Dolan DNA Learning Center is part of the Cold Spring Harbor Laboratory, www.dnalc.org.)

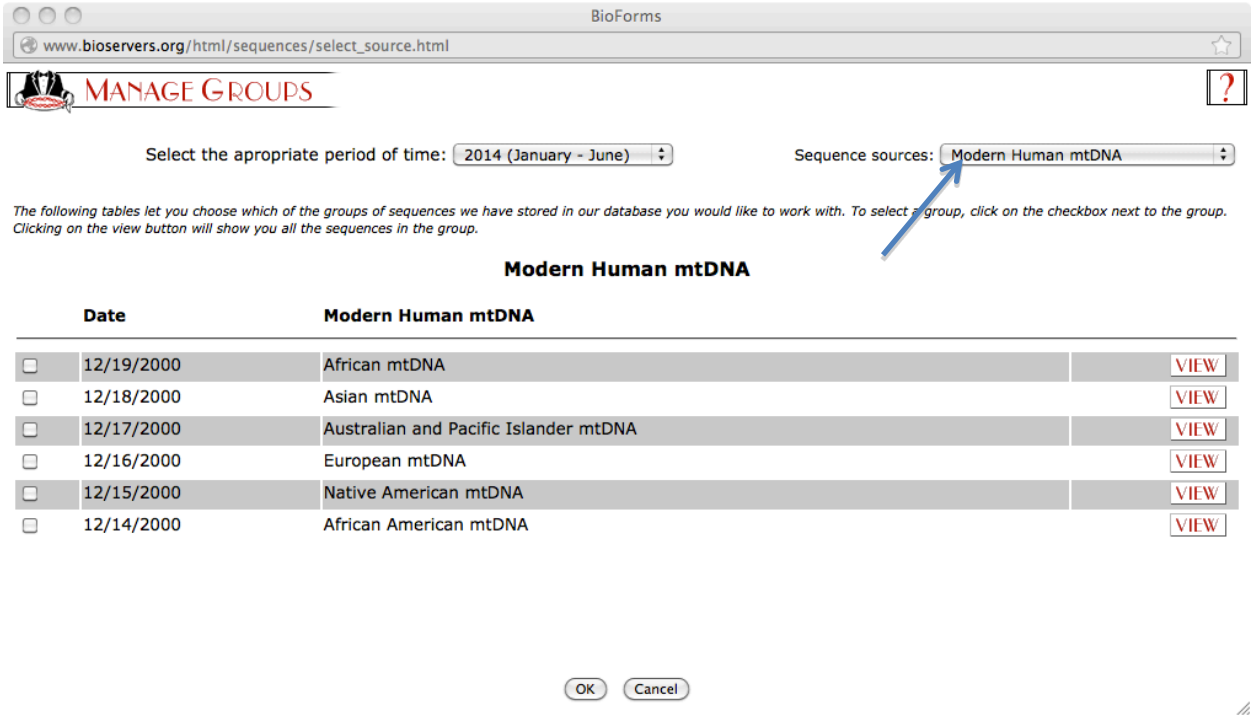
After you enter the BioServers site (click on 'BioServers'), enter the Sequence Server (click on 'Enter'; you can register if you want the website to remember and store your search information).



Click on Manage Groups:



Click on the pull-down menu on the top right called “Sequence Sources” and briefly look at the wide range of sequence choices. Choose “Modern Human mtDNA”. Click in all of the boxes to the left of the six choices so that you can see all of the different human samples, or click on the ones you know you want to compare. Click on the ‘?’ in the upper right corner for help.



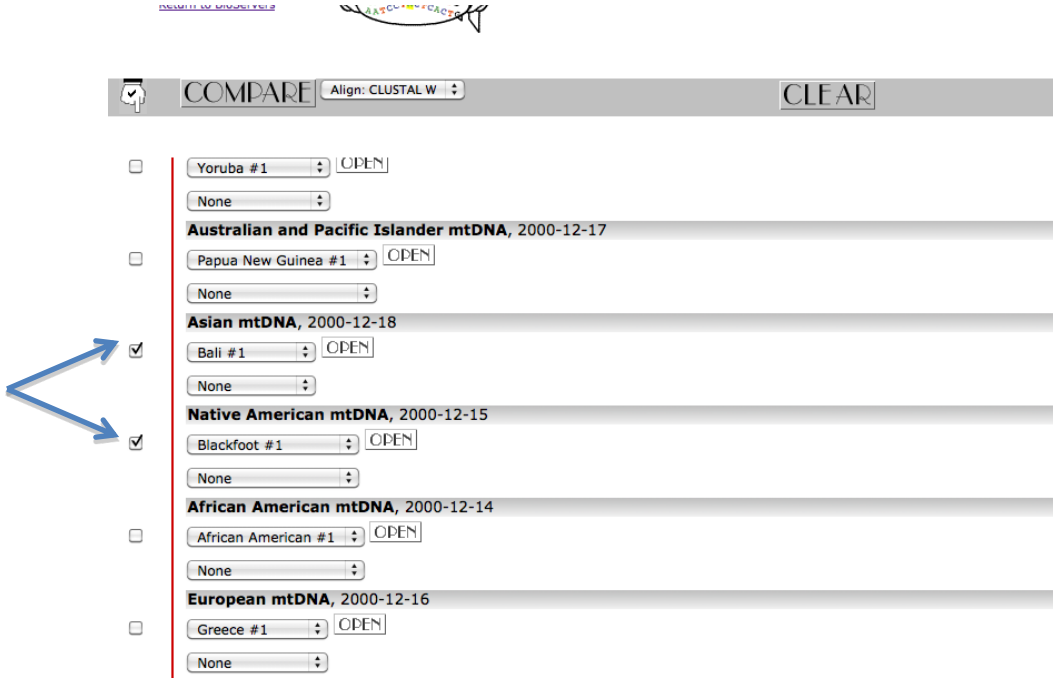
Here is an example (do this in the program as you read each step):

Sample question: What is the origin of Native Americans – with what other human population do they share the closest ancestry?

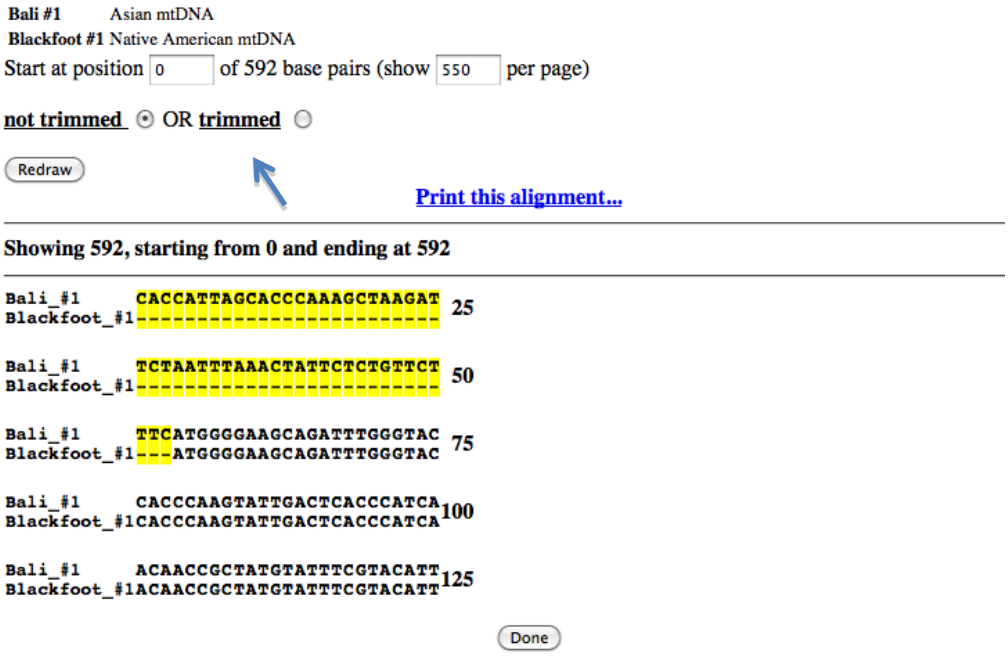
My initial hypothesis*: The mitochondrial DNA (mtDNA) of Native Americans is more closely related to the mtDNA of Asians than it is to African American mtDNA.*

Here is how I will test my hypothesis:

1) I will compare the sequence of an individual Native American mtDNA to an individual Asian mtDNA and count the number of differences in the nucleotide sequence (base pairs) of the mtDNA.
2) I will compare the sequence of a Native American mtDNA to an African American mtDNA and count the number of differences in the nucleotide sequence (base pairs) of the mtDNA.
3) I will compare the results, making sure that I am accounting for the total length of the sequences that are being compared (e.g., 10 bp differences out of 400 bp does not carry the same weight as 10 bp differences out of 300 bp). I must 'control' for the length of the total sequence being compared. You can use relative proportions or percentages to do this.



The initial portions of the sequence are shown below. As you scroll down through the sequence, you will see that there are yellow highlighted areas that show which nucleotide sequences are different. In this example, I choose to ignore the beginning part of the sequence where there is no overlap (the first 53 bases) and the end of the sequence (after nucleotide 444). NB: The computer program will do this for you if you choose to 'trim' the sequence. You can then count the number of differences that are left. Also note that you want to set the number in the second box (where it says 550 below) to a slightly higher number than the total base pairs in that alignment in order to see all of the sequence. In the example below this was not done.



I then repeat the process to compare the Native American sample to the African American sample (I used #1; steps not shown here, but you should do them for practice).

Here are my results:
Number of nucleotide differences between Asian Bali #1 and Native American Blackfoot #1 = 7 (out of 390 bp).
Number of nucleotide differences between African American #1 and Native American Blackfoot #1 = 9 (out of 381 bp).

Based on these results, can I conclude that my hypothesis is supported? Or do I need to collect more data? **Answer:**

Note about printing/saving: When you have the comparison you want, click the 'Print this alignment' link that appears right above the sequence alignment. You can send your sequences to the lab printer.

III. Your inquiry:
Now it's your turn. Using this same database, develop a hypothesis with your team about modern human population relationships, test it, and report your results.

Your team's hypothesis:

How you will test your hypothesis (your methods)? Which samples will you choose?

Your results:

Your analysis/discussion: Was your hypothesis supported or refuted? Explain. Do you need to collect more data?

If time allows, explore other DNA "groups" available on the Dolan Bioservers site. Feel free to go back to the site on your own time and explore the many possible DNA and amino acid sequence databases it has to offer, ask queries, and test hypotheses.

Name: _____ Lab Date: _____

Note: Each student must complete his/her own lab worksheet and include it in a lab notebook.

ANT 101

Human Adaptability – Cardiovascular System

In today's lab you will learn how to assess pulse, respiration, and blood pressure and examine the relationship of these measures to cold stress. Human blood pressure is highly responsive to environmental conditions and varies normally throughout the day. Along with pulse and respiratory rate, blood pressure can be used to evaluate human adaptability to a range of stressors. The human stress response involves a cascade of neuro-hormonal changes that affect the cardiovascular system, among other physiological functions.

You'll be working in your usual small teams today. After you learn the basic technique of taking blood pressure using the Vernier physiology interface in your small teams, as well as checking pulse and respiratory rate, you will work with your team to develop a group experiment that will serve as the basis for your group presentation next Thursday, 12/7. You will find steps related to the group project in the later part of this lab worksheet. This lab relates to the following chapter sections in your textbook: 6.5 (adaptability); 15 through 15.4 (biomedical anthropology).

I: Pre-Lab Reading about the background and methods: done before class. Refer to this information as needed during the lab.

II: Lab experiment: Cardiovascular Response to Cold Stress.
In this experiment, you will

- Learn to take pulse and respiration rates as well as blood pressure
- Obtain graphical representation of blood pressure.
- Compare pulse and respiratory rates and blood pressure before and after exposure to cold stimulus.
- Observe an example of sympathetic nervous system activation ("fight or flight" response).

METHODS

Materials

computer
Vernier computer interface
Logger *Pro* software
watch or timer
Vernier Blood Pressure Sensor
ice water bath
towel (paper or cloth)

Procedures

Select one or more persons from your lab group to be the study subject(s).

Part 1: Baseline Pulse, Respiration, and Blood Pressure

1. Connect the Blood Pressure Sensor to the Vernier computer interface. There are two rubber tubes connected to the pressure cuff. One tube has a white connector at the end and the other tube has a bulb pump attached. Insert the white connector into the 'Go-Link' interface currently inserted in one of the USB ports on your laptop.

2. Open the file "07 Blood Press Vital Sign" from the *Human Physiology with Vernier* folder in the Logger Pro documentation.

3. Attach the Blood Pressure cuff firmly around the upper arm, approximately 2 cm above the elbow. The two rubber hoses from the cuff should be positioned over the biceps muscle (brachial artery) and not under the arm (see Figure 1). Feel for the brachial pulse, on the inside of the subject's arm just above and medial to the crease, to assist you with positioning.

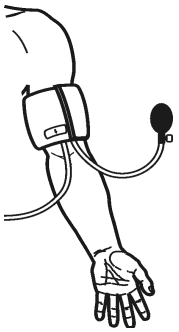


Figure 1

4. Have the subject sit quietly on a stool with his or her forearm resting on a table surface. *The person having his or her blood pressure measured must remain still during data collection; there should be no movement of the arm or hand during measurements.*

5. Pulse: Determine the person's pulse rate by palpating (feeling for) the radial artery on the wrist just below the thumb. Use your index and middle finger to feel the pulse, and count the pulse for one minute; memorize that number. The normal pulse rate for a resting adult is between 60 and 80 beats per minute.

6. Respiration: With your hand still in place on the radial artery, count the number of times the person breathes for one minute. By leaving your hand in place, the person will not know you are counting their respirations; people can become anxious and breathe faster when they know that someone is counting their breaths. The normal respiratory rate for resting adults is between 12 and 16 breaths per minute. Record the pulse and respiration rate in the first two cells of Table 1.

7. Click ▶ Collect to begin data collection. Immediately pump the bulb until the cuff pressure reaches at least 160 mm Hg. Stop pumping. The cuff will slowly deflate and the pressure will fall. During this time, the systolic, diastolic, mean arterial pressures, and pulse will be calculated by the software. These values will be displayed on the computer screen. When the cuff pressure drops below 50 mm Hg, the program will stop calculating blood pressure. At this point, you can terminate data collection by clicking ■ Stop. Release the pressure from the cuff by pressing the release button, but do not remove the cuff.

8. Enter the pulse and the systolic, diastolic, and mean arterial pressures from the screen readout in Table 1.

9. Store the data by choosing' Store Latest Run' from the 'Experiment' menu.

Part 2: Pulse, Respiration, and Blood Pressure Response to Cold

10. Collect data to examine the body's response to cold.
    a. With the cuff still attached, have the subject put the hand of his or her non-cuffed arm in the ice water bath, up to the wrist if room allows.
    b. As soon as the subject's hand enters the ice water bath, click ▶ Collect.
    c. Pump the bulb until the cuff pressure reaches at least 160 mm Hg, then stop pumping.
    d. When data have been collected for 15 seconds, have the subject remove his or her hand from the ice water bath. One team member should immediately take the radial pulse in that arm for 20 seconds; multiply by three to get the rate while another counts the respirations for one minute.
    e. The systolic, diastolic, and mean arterial pressures will be calculated by the software. These values will be displayed on the computer screen. When the blood pressure readings have stabilized (after the pressure drops to 50 mm Hg), the program will stop calculating blood pressure. At this point, you can terminate data collection by clicking ■ Stop. Release the pressure from the cuff, and remove the cuff from the subject's arm.

11. Enter the manually obtained pulse and respiratory rates, and the program-generated systolic, diastolic, and mean arterial pressures, and the pulse in Table 2.

12. If you want to keep this trial, choose 'Store Latest Run' from the Experiment menu, as before. When you want to keep data, choose 'Save' or 'Save As' under the 'File' menu.

13. Note: there are two pages to the display (see item in menu bar that denotes the page number). Page 1 is the graphic representation of your results, and Page 2 provides the data table. You may want to make use of these features in the presentation of your group project results.

RESULTS

Table 1–Baseline Pulse, Respiration, and Blood Pressure					
Pulse rate from palpation	Respiratory rate from observation	Systolic pressure (mm Hg)	Diastolic pressure (mm Hg)	Mean arterial pressure (mm Hg)	Pulse from blood pressure readout (beats/minute)

Table 2–Pulse, Respiration, and Blood Pressure Response to Cold					
Pulse rate from palpation	Respiratory rate from observation	Systolic pressure (mm Hg)	Diastolic pressure (mm Hg)	Mean arterial pressure (mm Hg)	Pulse from blood pressure readout (beats/minute)

DISCUSSION

1. Describe the trends that occurred in the respiratory rate, systolic pressure, diastolic pressure, mean arterial pressure, and pulse rate with cold stimulus. How might these be useful in a "fight or flight" response?

2. What is going on physiologically that can explain these results?

III. Group Project Challenge:
Identify (as a team) a research question or hypothesis related to human adaptability and the cardiovascular system that you can examine using the methods you learned about in this lab. Research the background literature (scholarly articles) related to this question, develop your specific methods protocol, test your hypothesis/answer your question using the Vernier software, report your results, and discuss them. You will do a group oral presentation on next Thursday, the last class day, in scientific format. Guidelines are provided on the D2L site in the lab project/presentation folder.

Tasks for this project: Work on steps 1-3 below and as many of the other steps as time allows today. Note that you must receive my approval before moving on to step 4. **Fill in the relevant areas on the next page** (1-3 to show me for approval, 4-5 as you complete your data gathering and analyze your results).

1) Identify a cardiovascular adaptability **question** and related **hypothesis** you want to investigate. **Today**

2) Find at least two scientific (peer-reviewed) articles about this particular topic or question to provide **background**. **Report at least one of these for item 1 on p. 5**. Continue literature search outside of class as needed. **Today+**

3) Write a one sentence statement of your **question**/**hypothesis** and a brief description of your planned **methods** in this lab worksheet (complete p. 5, items 2 and 3). **Today**

4) Once your proposal has received approval: Use the Vernier blood pressure lab interface (using the general steps outlined above). You should plan to run multiple comparisons to thoroughly test your hypothesis if time allows. If your hypothesis is not supported, those are your results, so report them as such in the **Results** section of your presentation and discuss them and the implications in the **Discussion** section. **Today+**

5) Make at least one slide to show me so I can see that you're on the right track with that part of the project. Preferably do so in today's class if you are able to get that far. If not, I will want to see it next Tuesday in class. **Today+**

<u>Your project inquiry documentation:</u> Complete the sections below as you work through the tasks above. You will state your team's question and/or hypothesis about cardiovascular adaptability, list a related reading, and describe your proposed methods (briefly). **The parts you need to show me in order to receive approval before collecting your data are in bold, below.** <u>All</u> steps below (1-5) must be filled in on this worksheet when you hand in your lab notebook at the end of the semester.

1) List one scientific article (author, title, and source) you have found that relates to your question about cardiovascular adaptability and that will provide background for your presentation (you will need at least 2 for your presentation):

2) Your question and/or hypothesis:

3) How you will test your hypothesis or answer your question (what are your methods)? What data will you collect and how? How many replications will you do?

4) Your results: Do you need to collect more data?

5) Your discussion: What do you conclude? Are your results definitive? Relate them back to the research question or hypothesis you developed. In your presentation, also relate your findings to the literature you found. What would be some next steps?

Class Discussion: Notes from instruction on techniques and/or notes from large class discussion (attach more paper if you need more room)**.**

Name: _____ Lab Date: _____

Note: Each student must complete his/her own lab worksheet and include it in a lab notebook.

ANT 101

Genetic Adaptation: Determination of CCR5-Δ32 Genotype in a Population Using Enzyme Linked Immunosorbent Assay (ELISA)

Humans have a suite of adaptive capabilities, both behavioral and biological. One of these, at a population level and over generations, is genetic adaptation. Some genetic variation among human populations is a result of natural selection acting on phenotypic variation that is relevant to mortality and/or fertility. Infectious disease has been a potent selective agent in human and primate evolution. By now you are familiar with the example of the sickle cell balanced polymorphism that is found in some malarial environments. Today we will focus on a different locus, the CCR5 gene. **You will do today's lab steps with your usual lab team. The entire class will combine results in the later part of the lab.** Today's lab relates to the following pages in your textbook: 62-74; 362-377 (p. 368 specifically relates to CCR5).

Part 1: Pre-Lab Reading about the background and methods: done before class. Refer to this information as needed during the lab.

Part 2: Lab challenge and procedures.

Challenge:

Your team of bioanthropologists has been given the task of determining the likely frequency of the CCR5-Δ(delta)32 gene variant in a population of European ancestry exposed to HIV. The technique you decide to use is called **Enzyme Linked Immunosorbent Assay (ELISA)**. It allows you to test samples for the presence of antigens (direct ELISA) or antibodies (indirect ELISA). In this case, you decide to use ELISA to test for the presence of HIV, and from that to deduce the frequency of the CCR5-Δ32 allele (abbreviated as 'Δ32' in this lab), which in some populations appears to provide resistance to HIV.

Methods:

General Procedure:

Each student will be given a simulated sample (**NOTE: <u>there are no HIV antigens in the sample)</u>** from subjects who were previously infected with HIV. You each will test your sample for the presence of HIV. You may get no reaction, a weak reaction, or a strong reaction depending on the quantity of antigen in your sample. In this scenario, an individual with no reaction is resistant to the infection, one with a weak response will develop the disease but it will be less severe, and a person possessing a high concentration of antigen will develop the most severe form of the disease. You will qualitatively determine the amount of antigen by comparing your sample's color intensity to the positive and negative controls.

Specific Procedures:

1) Get a subject sample and write down the number of your sample _____

2) Label your 12-well strip. On each strip label the first 2 wells with a "+" for the positive controls and the next 2 wells with a "-" for the negative controls. Label the remaining wells with your sample number and the sample number for the other members of your team (two samples for each member). For example, if your team members have samples 1-4, the wells would look like this:

+ + - - 1 1 2 2 3 3 4 4

3) Use a fresh pipet tip on the pipetter to transfer 50 µl of the positive control (+) into wells 1 and 2.

4) Use a fresh pipet tip to transfer 50 µl of the negative control (-) into wells 3 and 4.

5) Transfer 50 µl of each team member's sample into the appropriately numbered wells using a fresh pipet tip for each sample (2 wells for each sample).

6) WAIT 5 minutes for the samples to bind to the wells.

7) WASH - Tip the microplate strip upside down onto a stack of paper towels and gently tap the strip a few times. Be careful not to splash the samples back into the wells. Discard the top paper towel.

Use a transfer pipette to fill each well with wash buffer but be careful not to overfill or spill liquid from one well into another. You can use the same transfer pipet for the wash buffer for all wells. Tip the strip upside down on the paper towels and tap. Discard the top paper towel.

Repeat the wash step (for a total of 2 washes).

8) Use a fresh pipet to transfer 50 µl of primary antibody (PA) into all of the wells you have used on the microplate strip. The primary antibody will bind to the HIV antigens that are bound to the well.

9) WAIT 5 minutes for the antibodies to bind.

10) WASH all wells 2 times by repeating the steps outlined in step 7.

11) Use a fresh pipet to transfer 50 µl of secondary antibody (SA) into all of the wells you have used of the microplate strip. The secondary antibody binds to the primary antibody and also has an enzyme attached to it.

12) WAIT 5 minutes for the secondary antibody to bind.

13) WASH all wells **3 times** by repeating the steps outlined in step 7.

14) Use a fresh pipet tip to add 50 µl of enzyme substrate (E) into all of the wells you have used of the microplate strip.

15) Wait 5 minutes and record your results in Table 1 (next page) on your lab sheet, and add them to the results table for the whole class (front of the room). It is important that you not wait more than 5 minutes to read your results.

Results:

Table 1: Subject results from ELISA assay

Sample number	ELISA results (-, +, ++)	HIV? (no, less severe, more severe)	Genotype Δ32/Δ32; +/ Δ32; +/+
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			

Count the number of subjects with negative, weak positive, or strong positive reactions and record the numbers in Table 2.

Table 2: What are the CCR5 Δ32 genotype frequencies?

HIV antigen	Genotype Δ32/Δ32; +/ Δ32; +/+	No. of individuals	% of total
none			
Low levels			
High levels			

Based on your data, if you had 100 individuals, how many would be completely resistant to HIV infection? _____

Determine the allele frequency of the Δ32 allele. To do this (refresher from Ch. 3 in the textbook):

A) Take the total number of subjects and multiply by 2 (this is the total number of alleles). _____

B) Each subject who is resistant to the disease has 2 copies of the Δ32 allele.
Number of resistant subjects X 2 = _____

C) Each subject who has a low level of antigen has 1 copy of the Δ32 allele.
Number of subjects with low levels X 1 = _____

D) Add up the number of alleles from (B) and (C) and divide by the total number of alleles from (A). Multiply by 100 if you wish to convert frequency to percentage (%).
What is the allele frequency of the Δ32 gene in our subject population? _____

Discussion:

1) What do you conclude about the likely prevalence of the Δ32 allele in the sampled population?

2) What next steps would you like to take, if any, to further investigate the question of Δ32 allele prevalence in this population?

(For questions 3 and 4): Below is a table taken from the article you read by Hummel et al. (2005) entitled "Detection of the CCR5-Δ32 HIV resistance gene in Bronze Age skeletons."

Table 1 Distribution of CCR5-Δ32 in modern, historic and prehistoric European populations[a]

Population (location, date)	*No. studied*	*CCR5-Δ32*			*Allele frequency (%)*
		wt/wt	*wt/mut*	*mut/mut*	
Göttingen, central Germany, 2000	346	287	54	5	9.2
Goslar, central Germany, 1750–1810	19	12	7	—	18.4
Alia, Sicily, 1837	19	18	1	—	2.6
Lübeck, northern Germany, Black Death mass grave, 1350	14	10	4	—	14.2
Lübeck, northern Germany, famine mass grave, 1316	20	15	5	—	12.5
Lichtenstein cave, central Germany, 900 BC	17	13	4	—	11.8

3) Based on the data shown in this table, how do your results compare with the results of individuals tested for the Δ32 allele in the year 2000?

4) Based on the data from the Hummel et al. paper, is there any difference in allele frequency in samples from 900 BC versus 1350? What do these results suggest about the appearance of the Δ32 allele? Specifically, what selection events in the past may have led to the appearance of the Δ32 allele?

Class Discussion: Notes from instruction on techniques and/or notes from large class discussion