

Science Research Mentoring Program

LAB SKILLS: MOLECULAR BIOLOGY

This course introduces students to basic techniques in molecular biology, through extracting their own DNA and genotyping themselves at a mtDNA locus by restriction digestion.

- 2 **Session 1: Laboratory Math**

- 11 **Session 2: Pipetting and Making Solutions**

- 12 **Session 3: DNA Extraction**

- 15 **Session 4: PCR Amplification of mtDNA**

- 18 **Session 5: Restriction Digestion and Making an Agarose Gel**

- 19 **Session 6: Analysis of Results by Gel Electrophoresis**

- 20 **Session 7: Working with raw sequence data**

Session One: Laboratory Math

LEARNING OBJECTIVES

Students should understand the common SI units and prefixes used in the lab, be able to convert between SI units and solve basic dilution problems, and do basic molarity calculations.

KEY TOPICS

- SI units and prefixes
- Solutions in molecular biology
- Dilutions in molecular biology
- Molarity

CLASS OUTLINE

TIME	TOPIC	DESCRIPTION
15 minutes	Lab Safety	Review lab safety rules.
10 minutes	Lecture: SI	Explore why scientists use SI units, commonly used prefixes in molecular biology, and conversions.
10 minutes	SI: Practice	Students complete conversion worksheet; share answers.
15 minutes	Lecture: Dilutions and Stock Solutions	Units of concentration (w/v, v/v, M, X), calculating dilution volumes and concentrations using $C_1V_1=C_2V_2$.
20 minutes	Dilutions and Stock Solutions: Practice	Students complete dilution worksheets; share answers.
15 minutes	Lecture: Molarity	Definition of molarity; difference between moles and molarity; calculating molarity.
20 minutes	Molarity: Practice	Students complete molarity worksheet; share answers.

MATERIALS

- Worksheets
- Calculators

PREPARATION

None required

HOMEWORK

None

Session One: Laboratory Math: HANDOUT**Lab Safety Rules**

1. Conduct yourself responsibly at all times.
2. Never work alone. No student may work in the science classroom without a teacher present.
3. Do not eat, drink, or chew gum. Do not use laboratory glassware to hold food or beverages.
4. Hang up all backpacks and coats properly; never hang them on the back of your chair. Work areas and floor space should be kept clear and tidy.
5. Wear safety goggles whenever using chemicals, heat, or glassware.
6. Don't wear contact lenses.
7. Dress properly. Long hair, and dangling jewelry and clothing are hazards in a laboratory setting. Long hair must be tied back.
8. Shoes must cover the foot. No sandals.
9. Examine glassware before each use. Never use chipped, cracked, or dirty glassware. Observe a liquid by placing the glassware on a table; never hold it overhead.
10. Remember that heated glassware remains very hot for a long time. Set it in a designated place to cool – not directly on the laboratory desk – and always on an insulated pad. Allow plenty of time for hot apparatus to cool, and handle with tongs or gloves if necessary.
11. Never look into a container that is being heated. Do not immerse hot glassware in cold water, as it may shatter.

Session One: Laboratory Math: HANDOUT**Metric System Prefixes**

Most of the world uses the metric system as the standard set of units, as does the scientific community worldwide. In the metric system, each type of measurement has a base unit (e.g. meter for distance, liter for volume, gram for mass).

Prefixes are then added to the base unit to specify how much of that unit is present. The value of the base unit is multiplied by the value signified by the prefix to obtain the value of the full measurement. The most common prefixes are:

TERA: multiplies a metric unit by 10^{12} , or 1000000000000

GIGA: multiplies a metric unit by 10^9 , or 1000000000 (one billion)

MEGA: multiplies a metric unit by 10^6 or 1000000 (one million)

KILO: multiplies a metric unit by 10^3 or 1000 (one thousand)

HECTO: multiplies a metric unit by 10^2 or 100 (one hundred)

DEKA: multiplies a metric unit by 10^1 or 10 (ten)

DECI: multiplies a metric unit by 10^{-1} or 1/10 (one tenth)

CENTI: multiplies a metric unit by 10^{-2} or 1/100 (one hundredth)

MILLI: multiplies a metric unit by 10^{-3} or 1/1000 (one thousandth)

MICRO: multiplies a metric unit by 10^{-6} or 1/1000000 (one millionth)

NANO: multiplies a metric unit by 10^{-9} or 1/1000000000 (one billionth)

PICO: multiplies a metric unit by 10^{-12} or 1/1000000000000

FEMTO: multiplies a metric unit by 10^{-15} or 1/1000000000000000

Session One: Laboratory Math: HANDOUT**Molarity and Solutions**

INTRODUCTION

Molarity is a measure of the concentration of solute in a solution, where the solute is measured in moles. Moles are the molecular weight of a substance expressed in grams. Molarity equals moles (mol) per liter (L). 1 molar = 1 mol/L = 1 M. Prefixes can be added to molarity (M) as with the standard metric units.

Be careful to distinguish between moles and molarity. "Moles" measures the amount or quantity of material; "molarity" measures the concentration of that material in a solution.

One mole of a substance is defined as Avogadro's number of molecules of that substance. (Avogadro's number is a constant that equals 6.022×10^{26} — obviously not a practical calculation in the laboratory). Again: Moles are the molecular weight of a substance expressed in grams.

For example, if the molecular weight of sodium chloride (NaCl) is 58.4430, then 58.4430 grams of NaCl equals one mole of NaCl. If 58.4430 of NaCl were put into 1 L of solution, that solution would be 1 molar NaCl, written 1M NaCl. (Assume water is the solvent unless otherwise specified.)

WAYS TO DESCRIBE CHEMICAL SOLUTIONS

The standard way to make or describe chemical solutions is using molarity. However, aqueous solutions (in which water is the solvent) are sometimes made using weight/volume (w/v) or volume/volume (v/v) calculations.

Weight/volume refers to the weight of the solute as a percentage of the volume. Weight is generally measured in grams and volume in milliliters. For aqueous solutions, weight/volume is the same as weight/weight, since one mL of water weighs one gram.

Let's say you need to make a solution of 30% sucrose in water. Since sucrose is a solid, and the solution liquid, this is a weight/volume calculation. If you were making 100 mL of 30% sucrose, you would use 30 grams of sucrose in a final volume of 100 mL of water. If you were making 1 L (1000 mL) of this solution, you would use 300 grams of sucrose in a final volume of 1 L.

Volume/volume is used to make a solute with two or more liquids, such as water, alcohols, acids, etc. The percentage describes the proportion of the solution contributed by the liquid solute (i.e., the non-water). So 70% ethanol means 70 parts ethanol to 30 parts water.

Note that many buffer solutions have multiple components — say, a buffer that contained NaCl, ethanol, and sucrose. The concentration of each component can be described in terms of molarity, w/v, or v/v, but it's important to remember that each concentration refers to the total volume of the entire solution.

HANDOUT: Molarity and Solutions (continued)

STOCK SOLUTIONS

When you're working in the lab, you will often make up reactions and buffers starting with stock solutions (not solid chemicals), which you dilute to your working concentration. Stock solutions can be described by molarity, w/v, or v/v.

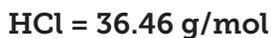
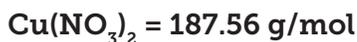
Their concentration is often described as the multiple of the working concentration: that is, a 10X buffer needs to be diluted by a factor of 10 to get the 1x working concentration. That means if the final volume of your reaction is 10 mL, you will use 1 mL stock solution.

To calculate dilutions you only need one formula:

(Concentration stock) X (volume of stock used) = (Concentration final) X (final volume)

But first you must convert units! The concentration of the stock must be expressed in the same units as the concentration of the final solution. The volume used will then be expressed in the same units as the final volume.

MOLECULAR WEIGHTS



Session One: Laboratory Math: WORKSHEET

Practice Problems**UNIT CONVERSION**

1) 2000 mg = _____ g

2) 5 L = _____ mL

3) 16 mm = _____ μm

4) 104 nm = _____ mm

5) 198 μg = _____ pg

6) 2500 mm = _____ nm

7) 480 μm = _____ m

8) 500 mM = _____ M

9) 75 μL = _____ L

10) 65 g = _____ mg

11) 0.9 nM = _____ μM

12) 5.6 g = _____ μg

13) 20 mM = _____ μM

Session One: Laboratory Math: WORKSHEET

Practice Problems**STOCK SOLUTIONS AND DILUTIONS**

$$C_1V_1=C_2V_2$$

1. How much 2.0 M NaCl solution would you need to make 250 mL of 0.15 M NaCl solution?
2. What would be the concentration of a solution made by diluting 45.0 mL of 4.2 M KOH to 250 mL?
3. What would be the concentration of a solution made by adding 250 mL of water to 45.0 mL of 4.2 M KOH?
4. How much 2% glucose solution can be made from 50 mL of 35% glucose solution?
5. A stock solution of 1.00 M NaCl is available. How many millilitres are needed to make 100.0 mL of 0.750 M
6. What volume of 0.250 M KCl is needed to make 100.0 mL of 0.100 M solution?
7. Concentrated H₂SO₄ is 18.0 M. What volume is needed to make 2.00 L of 1.00 M solution?
8. Concentrated HCl is 12.0 M. What volume is needed to make 2.00 L of 1.00 M solution?
9. A 0.500 M solution is to be diluted to 500.0 mL of a 0.150 M solution. How many mL of the 0.500 M solution are required?
10. A stock solution of 10.0 M NaOH is prepared. From this solution, you need to make 250.0 mL of 0.375 M solution. How many mL will be required?
11. 2.00 L of 0.800 M NaNO₃ must be prepared from a solution known to be 1.50 M in concentration. How many mL are required?

Session One: Laboratory Math: WORKSHEET

Practice Problems**MOLARITY – PART ONE**

- Determine the number of moles of solute to prepare these solutions:
 - 2.35 litres of a 2.00 M $\text{Cu}(\text{NO}_3)_2$ solution.
 - 16.00 mL of a 0.415-molar $\text{Pb}(\text{NO}_3)_2$ solution.
- Determine the grams of solute to prepare these solutions:
 - 0.289 litres of a 0.00300 M $\text{Cu}(\text{NO}_3)_2$ solution.
 - 16.00 millilitres of a 5.90-molar $\text{Pb}(\text{NO}_3)_2$ solution.
- Determine the final volume of these solutions:
 - 4.67 moles of Li_2SO_3 dissolved to make a 3.89 M solution.
 - 4.907 moles of Al_2O_3 to make a 0.500 M solution.
- Determine the molarity of these solutions:
 - 4.67 moles of Li_2SO_3 dissolved to make 2.04 litres of solution.
 - 0.629 moles of Al_2O_3 to make 1.500 litres of solution.
- How many moles of Na_2CO_3 are there in 10.0 L of 2.0 M solution?
- How many moles of Na_2CO_3 are in 10.0 mL of a 2.0 M solution?
- What is the molarity of 5.00 g of NaOH in 750.0 mL of solution?
- What is the molarity of 5.30 g of Na_2CO_3 dissolved in 400.0 mL solution?
- What volume (in mL) of 18.0 M H_2SO_4 is needed to contain 2.45 g H_2SO_4 ?

Session One: Laboratory Math: WORKSHEET

Practice Problems**MOLARITY – PART TWO**

10. What volume (in mL) of 12.0 M HCl is needed to contain 3.00 moles of HCl?
11. What weight (in grams) of H_2SO_4 would be needed to make 750.0 mL of 2.00 M solution?
12. How many grams of $\text{Ca}(\text{OH})_2$ are needed to make 100.0 mL of 0.250 M solution?
13. What is the molarity of 245.0 g of H_2SO_4 dissolved in 1.00 L of solution?
14. How many moles of NaCl are contained in 100.0 mL of a 0.20 M solution?
15. What weight (in grams) of NaCl would be contained in problem 10?
16. What is the molarity of a solution made by dissolving 20.0 g of H_3PO_4 in 50.0 mL of solution?
17. What weight (in grams) of KCl is there in 2.50 litres of 0.50 M KCl solution?
18. What is the molarity of a solution containing 12.0 g of NaOH in 250.0 mL of solution?
19. Sea water contains roughly 28.0 g of NaCl per litre. What is the molarity of sodium chloride in sea water?

Session Two: Pipetting and Making Solutions

LEARNING OBJECTIVES

Students should be able to calculate the appropriate amount of chemicals for solution making, make dilutions, and pipette accurately.

KEY TOPICS

- Solutions vs. dilutions
- Stock solutions
- Measurements: mass, volume, and micropipetting

CLASS OUTLINE

TIME	TOPIC	DESCRIPTION
15 minutes	Overview of Pipetting	Review proper use of pipettors; brief practice with both water and glycerine.
45 minutes	Pipetting Accuracy Test	Students choose a volume of water + glycerine to pipet into a 1.5 mL tube, and label the tube with their initials. Three other students measure the volume in each tube by pipetting. Finally, students write measurements on the board.
1 hour	Solution Making	Go over solutions to make: from solid salt, 35 mL of 2M NaCl stock solution (1/group); from stock solution, 50 mL of 150 mM working saline solution (2/group); and from 50X stock solution, 500 mL of TAE buffer. Label all solutions properly (concentration, solute, date, name).

MATERIALS

Micropipettors and tips, 1.5 ml tubes, mini centrifuges, colored water and colored glycerine in 1.5 ml tubes, graduated cylinders, NaCl, DI water, 50 mL tubes, Sharpies, 50X TAE, 500 mL-1L bottles, digital scale, weigh boats

PREPARATION

None

HOMEWORK

None

Session Three: DNA Extraction

LEARNING OBJECTIVES

Students should understand the principles of DNA purification, be able to pipette accurately, and use a centrifuge properly.

KEY TOPICS

- DNA extraction/purification

CLASS OUTLINE

TIME	TOPIC	DESCRIPTION
30 minutes	Wrap-up of Pipetting Accuracy	Go over results from pipetting accuracy test.
10 minutes	Laboratory Notebook Review	Discuss what should be recorded in a laboratory notebook, the purpose of a lab notebook.
1 hour 15 minutes	DNA Extraction	Give students protocol for Chelex cheek cell DNA extraction, go over as a class, let students perform on their own. Make sure to do the first spin as a class, and make sure they resuspend the Chelex prior to pipetting. Store extracted DNA in the freezer.
15 minutes	For Next Session: PCR Reaction Worksheet	Have students start the PCR reaction worksheet; finish as homework.

MATERIALS

- Chelex
- 1.5 ml tubes
- Micropipetters
- pipet tips
- microfuge
- gloves
- heat block

PREPARATION

Turn on heat block to 99C

HOMEWORK

Finish PCR reaction worksheet

Session Three: DNA Extraction: HANDOUT**Chelex DNA Isolation**

PROCEDURE

1. Pour ~8-10 mL saline solution into your mouth and vigorously rinse for at least 10 seconds.
2. Spit the saline solution back into a 50 mL tube.
3. Pour the saline solution into a 1.5ml microcentrifuge tube to fill.
4. Place your tube, along with the other student samples, in a balanced configuration in the centrifuge and spin it for 10 minutes at 500-1000 x g.
5. Carefully pour off the supernatant. Be careful not to disturb the cell pellet at the bottom of the tube.
6. Set the micropipette to 500 microliters. Draw the 10% Chelex suspension in and out of the pipet tip several times to suspend the resin beads. Before the resin settles, transfer 500 microliters of Chelex suspension to the tube containing your cell pellet.
7. Resuspend the cells by pipetting in and out several times. Examine the cell suspension against the light to confirm that no visible clumps of cells remain.
8. Place your sample in the heat block for 10 min. Use forceps to remove the tube and allow it to cool.
9. Place your sample tube, along with the others, in a balanced configuration in the centrifuge and spin for 30 seconds at full speed.
10. Use a fresh tip to transfer 200 micro liters of the clear supernatant into a clean 1.5ml tube. Be careful not to remove or disturb the Chelex and cell debris at the bottom of the tube.
11. Store your sample on ice or in the freezer until you are ready to begin the PCR procedure.

Session Three: DNA Extraction: WORKSHEET

PCR Reaction

COMPONENT / STOCK SOLUTION	VOLUME (FILL IN)	FINAL CONCENTRATION
10X PCR Buffer, minus Mg		1X
10 mM dNTP mixture (dATP, dCTP, dTTP, dGTP)		0.2 mM
50 mM MgCl ₂		1.5 mM
Primers (20 μM)		0.4 μM each primer
Template DNA (100 ng/μl)		2 ng/μl
Taq DNA Polymerase (5 units/μl)		1.0 unit in reaction
Water		NA

1. What information do you need before you can calculate the volume of each component?
2. You will run your PCR product on an agarose gel. To make a 2% agarose gel (w/v), how much agarose would you put into 50 mL of 1X TAE buffer?
3. If your stock solution of TAE is 50X, how much stock solution would you dilute in how much water to make the 50 mL of 1X buffer?
4. TAE buffer (1X, working dilution) is: 40 mM Tris base (short for 2-amino-2-hydroxymethylpropane-1,3-diol), 1.14% acetic acid (v/v), 1 mM EDTA. Calculate how much Tris, acetic acid, and EDTA stock solution you would need to make 1 L of 50X TAE. The molecular weight of Tris is 121, and your stock solution of EDTA is 0.5 M.

Session Four: PCR Amplification of mtDNA

LEARNING OBJECTIVES

Students should be able to calculate the volumes used in a PCR reaction and explain the mechanisms of PCR.

KEY TOPICS

- Polymerase chain reaction
- Solutions and dilutions

CLASS OUTLINE

TIME	TOPIC	DESCRIPTION
30 minutes	Lecture / Review	How does PCR work and what is it for? Short lecture using DNALC animation. Go over homework; determine proper volumes of reagents in PCR reaction. Discuss the function of each component in the reaction. http://www.dnalc.org/resources/animations/pcr.html
1 hour	PCR	Students set up PCR reactions; each student is responsible for setting up a reaction with his or her own DNA. Cycle parameters are: initial 5 min denaturation followed by 30 cycles of annealing at 56°C for 1 min, extension at 74°C for 1 min, and denaturation at 94°C for 45 s. Demonstrate PCR program to group before starting cycles.
30 minutes	PCR Worksheet	Students complete PCR worksheet.

MATERIALS

Micropipetters, tips, Taq/buffer/Mg, dNTPs, primers at 20 uM (L15996: CTC CAC CAT TAG CAC CCA AAG C; H408: CTG TTA AAA GTG CAT ACC GCC A), thin-walled PCR tubes, Thermocycler, ice buckets, ice, Sharpies, gloves

PREPARATION

Aliquot PCR reagents per group, program PCR machine

HOMEWORK

Finish PCR worksheet

Session Four: PCR Amplification of mtDNA: WORKSHEET**PCR**

You can refer to this website if necessary:

<http://www.dnalc.org/resources/animations/pcr.html>

(click on the amplification animation)

Set up a PCR reaction that includes the following DNA components:

- *Double-stranded template DNA with the sequence below*

5'- AGGTCGTAACGTACGCCTCACCAATATAGGCCGCCTAGCTA – 3'

3'- TCCAGCATTGCATGCGGTGTGGTTATATCCGGCGGATCGAT – 5'

- *And a primer pair with the following sequences (single-stranded)*

Primer 1: 5' –GTCGTAACGT– 3'

Primer 2: 5' –AGCTAGGCGGC–3'

1. Indicate the primer binding sites on the above template sequence. Make clear which template strand binds to which primer.
2. You put your reaction in the thermocycler, and it undergoes one cycle. Draw what the fragments of DNA in the reaction look like after each step. (Be sure to include the primers, the original template DNA, and reaction product in each step.)

95°C 30 sec

55°C 30 sec

72°C 1 min

Session Four: PCR Amplification of mtDNA: WORKSHEET - continued**PCR**

3. Now let the reaction go for a second full cycle (95°C, 55°C, 72°C). Draw the fragments that are in the reaction at the end.

Session Five: Restriction Digestion and Making an Agarose Gel

LEARNING OBJECTIVES

Students should understand the principal of restriction enzymes and gel electrophoresis.

KEY TOPICS

- Genotyping
- Restriction digestion (vs sequencing)
- Gel electrophoresis

CLASS OUTLINE

TIME	TOPIC	DESCRIPTION
15 minutes	Review – PCR	Review PCR worksheet as a group.
15 minutes	Setup – instructions for day	Discuss lab work for the day: <ul style="list-style-type: none"> • Each student sets up a restriction digest of his or her PCR product with MseI, 15 ul of PCR product. • Each group makes a 1% agarose gel in TAE with Sybrsafe, covers with foil.
1 hour, 30 minutes	Student lab work	Students complete assigned lab tasks (as above).

MATERIALS

MseI enzyme and buffer, agarose, student-made TAE, gel boxes, combs, and gates, 1.5 ml tubes, water bath, Sybrsafe, micropipettors, pipet tips, Sharpies, gloves, digital scale, weigh boats

PREP WORK

Turn on water bath @ 65°C

HOMEWORK

None

Session Six: Analysis of Results by Gel Electrophoresis

LEARNING OBJECTIVES

Students should be able to run and interpret a DNA gel.

KEY TOPICS

- Gel electrophoresis
- Analysis of RFLP results
- Genotyping

CLASS OUTLINE

TIME	TOPIC	DESCRIPTION
10 minutes	Review – Gel electrophoreses	Review gel electrophoresis, give precise instructions for preparing gel box, loading samples and ladder.
1 hour, 15 minutes	Gel running	Students load and run gels in groups. They should load entire digestion reaction mixed with loading dye, plus a pre-mixed ladder. Photograph results for discussion.
35 minutes	Analysis of results	Project gel results and discuss as a group. How many genotypes (RFLP patterns) are apparent in each group? (There should be no more than two.) If results are unexpected (no DNA product, digest didn't work, strange RFLP pattern), discuss possible explanations.

MATERIALS

Gel boxes and power sources, student-made gels and TAE buffer, loading dye, ladder (pre-mixed with loading dye), micropipetters, tips, gloves, UV light box and digital camera

PREP WORK

Aliquot dye and ladder

HOMEWORK

None

Session Seven: Working with raw sequence data

LEARNING OBJECTIVES

Students should be able to explain the method of cycle sequencing and interpret a sequence chromatogram.

KEY TOPICS

- DNA sequencing methods
- Working with sequence data

CLASS OUTLINE

TIME	TOPIC	DESCRIPTION
30 minutes	Lecture – DNA sequencing	Explore how cycle sequencing works.
1 hour	Analysis of raw sequence data	Students work in small groups to examine 4 sample chromatograms using Geneious. Demonstrate use of program, have students open 4 sample files, and ask them to answer the following questions: Which files are usable? Which are not? What do they think happened to the reaction in the unusable file(s)? (probably contaminated) Which sequences would need to be edited before being used? What would you do to identify these sequences?
30 minutes	Discussion	Discuss student answers; chromatogram interpretation.

MATERIALS

- Laptops with Geneious installed
- Sequence files

PREP WORK: **None**

HOMEWORK: **None**