PROVOST & WALLERT RESEARCH
MN STATE UNIVERSITY MOORHEAD
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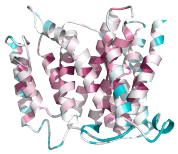
Molecular Biology

Intro and Prep Assignment



Print and start a lab binder of protocols for your time in the Provost lab. Start with this page printed in your binder!

Research Page - Read and Watch – every video, document and link from the lab research page. Read the lab math and NIH lab math Video. Read and watch all of the links and videos in the DNA and RNA protocols and the M-Bio Video Tutorials. You will need to do the lab math assignment.

When reading and watching the material – maintain a set of notes. Include your version of the important concepts. Also write out the terms that you are unfamiliar with. Look up the answers for these terms and write them down. We will review these pages with the group.

Use, materials, the videos, your instruction and our experienced researchers to learn how to do the following (Check off each item when done):

- use the autoclave for liquid and dry materials,
- visualize DNA using the biorad imager,
- pipetting, make bacterial culture media (liquid and plates),
- use the pH meter
- do Lab Math Practice Set
- Snapgene – load up a free full trial version. Learn and watch the following"
  - Watch each of the following videos under support. Introduction, working with features, working with translations, primers, PCR and mutagenesis, restriction cloning, Gibson cloning and aligning to a reference sequence
  - There is a practice sequence under the snapgene link on our webpage – one of a plasmid and one for a gene not in a plasmid. Create a new plasmid that uses the backbone and now includes the gene. Annotate this new gene for reading frame, MCS, antibiotic resistance… Use this sequence and try to do as many things as possible using snapgene. Make your own map, add in a primer for sequencing…

Both write out your answer and be prepared to discuss the following questions. Use OUR materials to answer these questions – DO NOT google the questions. These questions are meant to ensure you have learned the key points in the materials so you are ready for experiments. These questions are not all of the key points:

- What are the key components of a plasmid?
- What strain of bacterial cells should you use when making plasmid DNA vs protein?
- What are the key and most important steps when transforming competent cells?
- How do Amp, carbacillin and Kanamycin function? How are they prepared and where are they stored?
- How do you measure purity in a plasmid DNA preparation?
- How do you calculate concentration of a solution of double stranded DNA?
- What is the difference between Agar and Agarose. What are each used to do and the type of molecule of each?
- Do the practice lab math calculations linked from our page
- What is the difference between a gene and a plasmid?
- What is a supercoiled plasmid and how does it differ from a nicked or relaxed plasmid DNA? How do these run on an agarose gel?
- Describe the process in preparing a DNA gel (agarose gel) to run a plasmid.
- Read through a Qiagen plasmid miniprep spin instruction. Outline the process.
- What are the definitions of a plasmid, plasmid backbone, plasmid vector or plasmid construct?
- What is a restriction enzyme
- Outline a restriction enzyme digest
- What is the workflow for a subcloning project using a standard restriction enzyme approach?
- How does one clone in a gene into a plasmid using Gibson cloning? How would you create a fusion gene (half of gene A and half of gene B) into a plasmid vector using Gibson cloning?
- How does one validate that a cloning/subcloning/mutation is correct?
- Outline DNA sequencing of a plasmid. What are primers?

Create protocol pages within your lab archives (this can be done after you have learned the online notebook).

Pipetting. Pipette with a three different sized LTS and One of the EPD pipettors. Pipette 20 times for each pipettor set at a middle setting. Pipet into a small beaker on the analytical balance. No need to change the beaker each time, simply tare the balance after recording each pipetting result. Record the balance each time. Calculate the average and standard deviation. Look at the pipetting handout to determine if your pipetting and or pipettor is within standards.

DNA Gel – Learn how to run a DNA gel and test a few parameters. FIRST run gel 1. Then the other three gels. Don't try to do all at one time.

Prepare four 0.8% Agarose Gels.

- Gel one and two should include the proper amount of SyberSafe added to the agarose as it cools.
- Gel three should include no Sybersafe or other stain.
- Gel four should contain ethidium bromide.

STOCK DNA Preparation. See your instructor for which DNA preparation to use. MIX WELL AFTER EACH TUBE IS PREPARED! Save all stock solutions and Sample tubes at 4oC when done.

- Stock Solution 1 - Use CV=CV to prepare 100 μl of a 0.5 μg/μl solution of plasmid DNA in water
- Stock Solution 2 - Transfer 20 μl of 0.5 μg/μl stock solution and mix with 80 μl of water.
- Stock Solution 3 – Transfer 10 μl of stock solution 2 and mix with 90 μl of water
- Stock Solution 4 – Transfer 10 μl of stock solution 2 and mix with 90 μl of water
- Stock Solution 5 - Transfer 10 μl of stock solution 2 and mix with 90 μl of water

Sample Tubes	DNA Stock	μl of DNA 5 or 6X Sample Buffer added to tube	μl to load on gel
Tube 1	10 μl of Stock Solution 1	90 μl	10 μl
Tube 2	10 μl of Stock Solution 2	90 μl	10 μl
Tube 3	10 μl of Stock Solution 3	90 μl	10 μl
Tube 4	10 μl of Stock Solution 4	90 μl	10 μl
Tube 5	10 μl of Stock Solution 5	90 μl	10 μl

Each gel will be loaded with 5 μl of 1KB ladder in lane one. Lanes 2-6 will contain the indicated amounts of sample Tubes 1,2,3,4, or 5. Please don't load into the same well. Let the gel run until the dye is nearly at the end of the gel. Record the time for each gel.

Gel 1 – Run as a regular gel with SyberSafe in just the gel. Observe the intensity and background settings on the biorad imager. Do this each time. We want to see the sensitivity and background for each method.

Gel 2 – Run as gel 1, BUT include SyberSafe in the gel AND the running buffer.

Gel 3 – Run the gel without any SyberSafe. After the gel has run, transfer the gel to a solution of running buffer WITH sybersafe and incubate for 15 min, then transfer to a new container with running buffer but no syber safe for 2 min, then visualize.

Gel 4 – Run the gel but now with Ethidium Bromide.

LB Plates – prepare 10 LB plates with Amp and another 10 LB plates with Kan.

Transformation – Transform 50 μl of comp cells using 1 μl of stock solution 1. Plate on the appropriate LB-antibotic plate.

DNA MiniPrep. Perform 3 DNA miniprep purifications using your transformed cells!