

## Lab Recap: Miniprep (MP)

*This lab is almost more important than food.*

1

**Purpose:** To break open your bacteria and get your plasmid DNA.

### **Vocabulary/Terms**

**Plasmid DNA** - The duckweed DNA that you want to study has been inserted into plasmids. The plasmids have been put (transformed) into bacteria. The bacteria has two types of DNA: it's own chromosomal DNA and the plasmid DNA that someone put into it. The concept of extracting just that plasmid DNA from all other stuff in the bacterial cells is very important to this lab.

**Plasmid Preparation** - A plasmid prep is the general name for a lab procedure that isolates/purifies plasmid DNA by breaking open the bacteria to get the plasmids, which contain inserts--in our case duckweed DNA inserts. There are many types of plasmid preps depending on the size of the bacteria used or the amount of plasmid DNA (i.e. minipreps for small amounts all the way to gigapreps for large amounts).

**Miniprep (MP)** - A type of plasmid prep that involves relatively small amounts of plasmid DNA (small plasmid DNA yield). Note that different labs (universities, companies, etc.) have different miniprep procedures. If you get to work at a lab, it's good to get acquainted with its miniprep procedures. During the lab, we may refer to your "miniprep" as whatever is in the contents of the tubes you are dealing with. After the lab, we may refer to "your miniprep" as the contents that will end up in your yellow microfuge tube.

**Solution I** - Solution that acts as a **buffer** for cell suspension (regulates the pH of the cell suspension - limits DNA degradation). This is what you stored your cells in at the end of the PCR lab.

**Solution II** - Strong base that **denatures** DNA, RNA, proteins, and membranes. Contains NaOH (a strong base) and SDS (a detergent). Basically breaks open your cells to release your plasmid DNA plus a bunch of other cellular junk.

**Solution III** - Acid that **renatures** plasmid DNA and precipitates all other cellular material. Pretty much helps you get rid of all the annoying stuff that you don't need, because after all the only thing from the cell that you need for sequencing is the plasmid DNA.

**Spin Column** - **Smaller tube** used in the MP lab. Substances are put into it and then when centrifuged, parts of it flow out of small pores at the bottom (falling into the collection tube) and some stuff stays in the column.

**Collection Tube** - **Big 2ml tube** that you put your spin column in. In this lab, you will get 1 of these, which will contain unwanted cell parts in a liquid and you will throw out the liquid. Be careful not to mix this up with the yellow, slightly smaller microfuge tube, which will contain your plasmid DNA which you will send to be sequenced.

**Flow-Through** - The liquid at the bottom of the collection tube after centrifuging. Do not confuse this with supernatant, which is the liquid above a pellet after centrifugation.

## Lab Recap: Miniprep (MP)

*This lab is almost more important than food.*

2

**Wash Buffer** - Solution that you will use to remove additional RNA and proteins from your miniprep. This contains ethanol. You centrifuge your miniprep twice to make sure that all wash buffer is removed so that ethanol does not ruin your miniprep.

**Elution** - We leave the plasmids stuck to the spin column until we get rid of other cellular parts like DNA, RNA, proteins, etc. Then, we can take the plasmids off of the column or, *elute* the plasmid. You will be putting elution buffer (sterile water) into the spin column, then centrifuging it so that the water falls through the spin column, taking your plasmid DNA with it. As a result, you will be left with your plasmid DNA in sterile water at the bottom of your yellow microfuge tube.

### What you are doing and why you are doing it:

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- Adding various solutions to your bacterial cells and centrifuging your preps to separate your plasmid DNA from other cellular junk

#### Why you are doing it:

- We want to learn things about duckweed by studying its DNA.
- Your DNA needs to be sequenced so you can get the pretty waveform file that allows you to study the duckweed DNA using BLAST analysis and that fun stuff. However, everything other than your plasmid DNA (and the normal sequencing reagents) will get in the way of the sequencing reactions
- Therefore, you need to isolated the plasmid vectors, otherwise known as the plasmid DNA, so that the sequencing reactions work

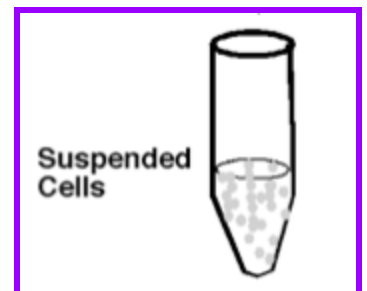
### IMPORTANT TO UNDERSTAND

In PCR, you added a bunch of stuff to a tube and a machine made all the PCR reactions happen. We gave you a bunch of notes explaining how the PCR process was going to happen on a molecular level, but you never saw any of the important reactions taking place. The one problem with the **PCR** lab is that **you did not see any of the important stuff happening.**

The Miniprep experience is much different because you will be able to know exactly which part of the process is happening at all times. For example, when you add solution II, you know that you are bursting all of the cells. Or, when you are centrifuging your miniprep with wash buffer, you know that you are clearing the miniprep of all unwanted proteins. The beauty of the **miniprep** lab is that **you are actually there to see the reactions happen.**

### Overview of Miniprep Lab (with picts b/c u ppl seem to love picts)

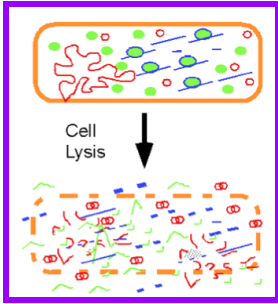
1. You actually already started the miniprep lab the same day as the PCR lab. You will receive your suspended bacterial cells in **Solution I**. Remember that those cells contain your duckweed DNA plus a bunch of other cellular junk from the bacteria hosts that you don't want.



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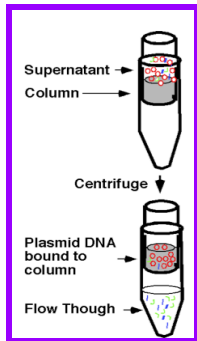
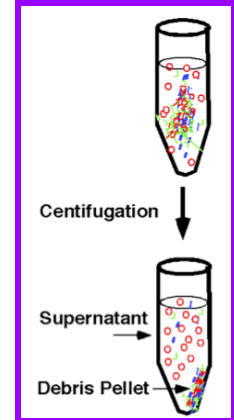
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3



2. Then you will add **Solution II**, which will *burst* open the bacterial cells and all of the cellular components (proteins, membranes, various nucleic acids) will flow out. Solution II will then denature mostly everything that is released from the cells. The one exception: Solution II CANNOT fully denature your plasmid DNA. However, all of the other cellular components are not so lucky. Solution II WON'T completely denature your plasmid DNA, but it WILL degrade other proteins and nucleic acids that you don't want.

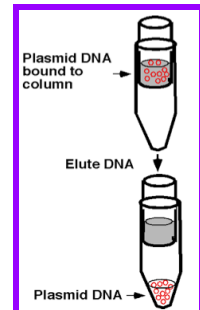
3. Then you will add **Solution III**, which creates optimal conditions to renature nucleic acids. Your plasmid DNA will renature because it has only been PARTIALLY denatured in the step before. Everything else will not renature. When you centrifuge it, the renatured plasmid DNA will stay in the solution while all the other cellular junk will fall to the bottom and concentrate into a white pellet (which is different than the pellet you saw a few labs ago).



4. So now you have you can just pour out the supernatant into another tube and call that your plasmid DNA right? WRONG. You might still have some cellular junk in your supernatant, so you have to find out a way to make sure that your miniprep is completely plasmid DNA. So, you will pour the **supernatant** into a spin column, making sure you do not pour out any of the white pellet. You will then place the **spin column** in the **collection tube**. When you spin it in a centrifuge, your plasmid DNA will stick to the column and everything else will come out as a liquid at the bottom in your **flow-through**.

5. At this point, you've isolated your plasmid DNA because it's all in the spin column right? WRONG. You still have to make sure you've removed EVERYTHING but the plasmid DNA. So, you will add **wash buffer** (which contains ethanol) to the spin column and centrifuge it to ensure that absolutely everything non-plasmid DNA gets removed.

6. Time for the finale! Now, you can safely say that you have just your plasmid DNA bound to the column. But where to put it? We are going to suspend it in sterile water. To do that, you just add **elution** buffer (sterile water) to the spin column. But instead of putting the spin column in a collection tube, you will put it in a yellow microfuge tube. You will spin everything in a centrifuge, the water will remove the plasmid DNA from the column, and you will be left with your **plasmid DNA** at the bottom of the yellow tube! Yay!!!!!!



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4

### **GOLDEN RULE FOR ALL LABS FROM THIS POINT ON:**

**Never** add anything to the yellow microfuge tube. You may only take things out of it.

### **Q & A**

**Q:** Why do I need to centrifuge twice in the “wash buffer” step?

A: Ethanol is a component of wash buffer and if you do not remove all of the wash buffer then the ethanol will contaminate your prep, cause problems with Restriction Digest, and will interfere with the sequencing reactions.

**Q:** Why do I need to invert gently after I add Solutions II and III?

A: If you shake too violently then the plasmid DNA will break and you won't get a good plasmid DNA yield. You wouldn't violently shake a baby, so don't violently shake your microfuge tubes.

**Q:** Why is it really important that I don't get the white precipitate into my spin column?

A: The white precipitate contains unwanted parts of your cell (cell debris, denatured chromosomal bacteria DNA, RNA, and proteins). The point of this lab is to get a sample that does not contain any of that stuff because the only thing you need for sequencing to work is plasmid DNA. Remember that any other cellular substance will contaminate your column and interfere with sequencing

**Q:** What's the point of all the buffers?

A: To make sure that your plasmid does not get harmed while you are isolating it. Having poor plasmid DNA will jeopardize your opportunity to have your DNA sequenced.

**VIDEO LINQUE** (use DSAP login information)

<https://wssp.rutgers.edu/WSSP-files/Videos/MP.mov>