

# Sequencing protocol

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## Introduction

Preparing sample to send to Genewiz for sequencing

**Sequencing Pick-up Times: 9:30am, 3:00 pm, 5:30 pm**

## Materials

- › DNA stock
- › Nuclease-free water
- › Primer (5uM working stock)
- › One thin-walled PCR tube, in strips
- ›

## Procedure

### Diluting the DNA

1. Label your tubes on the side **vertically** with "M1" and sample number (01, 02, 03, etc.). **Use image below as guideline.** Use "M1" instead of the "GW" in the picture.



2. Multiply the length of the plasmid *in kilobases* by 100. This is the number of nanograms of DNA required for the sequencing reaction.  
  
For instance, if my plasmid is 3 kb long, then I need 300 ng of DNA for the sequencing reaction (in a total volume of 10 uL).
3. Divide the number of micrograms from step (2) by the concentration of DNA in the miniprep tube. This is the volume of DNA required for the sequencing reaction.  
  
For instance, if I need 300 ng of DNA, and my miniprep concentration is 150 ng/ $\mu$ l, then I need 2  $\mu$ l of miniprep DNA.
4. Transfer the volume of DNA from step (3) to the strip tube.

**\*\*Make sure you have 2 tubes dedicated to the M13 forward and reverse primer. These will have the DNA and water, BUT DON'T ADD ANY PRIMERS. Genewiz will add the primers for you.**

5. Add sufficient water to bring the total volume to 10  $\mu$ l.

For example, if I added 2  $\mu$ l of miniprep DNA, then I will add 8  $\mu$ l of water.

6. Add 5  $\mu$ l of diluted primer to each tube.

\*\*Remember not to add any primers to the tubes meant for M13 Forward and M13 Reverse.

7. Log into genewiz. **Username:** igem-sequence@mit.edu **Password:** igem2014citrus

8. Click "create sequencing order"

9. Service Priority: click on "standard"

10. Create Order by: click on "online form"

11. Sample Type: click on "Pre-mixed"

12. Enter in how many samples you are sending in and click "Create new form"

13. Enter in descriptive names for your DNA (this is to help you remember when you get the results back), the DNA type, the DNA length (get this from your construct on benchling), the primer name (again, this is more for you). When you are done hit save and next.

14. It will give you an list of what you have entered and a price for each reaction. Hit "next step"

15. The payment information field should be autofilled. There has been an issue recently with the PO box number not being entered. If this is the case, click "credit card" (next to "payment info") and then click "PO" to bring you back to the original form. Now you should be able to enter in a PO number. Open up a previous submitted order (there should be some saved to the desktop called "sequencing" or something like that) and copy over the PO number. **PO Number: 5510061907**

16. Hit "next step". Once it gives you a submitted order form, print the form.

17. Put your PCR tubes in a ziploc baggie. Take the baggie out to the printer and staple the baggie to the newly printed order form.

18. Put the bag and order form in the Genewiz pickup box (located by the elevators, make sure you put in the **genewiz box**)

19. Open up the weiss lab orders spreadsheet (bookmarked on the computer). You need to request access to the spreadsheet if it is your first time ordering. Once you request access, **email Brian** so he knows that you have sent in a request.

20. Click the tab at the bottom called "genewiz".

21. Fill out the form. Make sure you indicate that the order is from iGEM so the right account can be charged.

22. You did it! YAY! Genewiz should get back to you pretty quickly, generally between 12-24 hrs.