

Sequencing Sample Submission

Do not submit any samples unless you have determined by miniprep and digest that an insert of the expected size is present. After that, you may submit up to three samples per BioBrick.

1. Sample Preparation

Your samples, or sequencing templates, must be between 100 – 200 ng/ul.

- Determine the concentration of your samples using the nano drop
- You need to submit at least 14 ul of template to be sequenced; you do NOT want to submit all of your miniprep unless absolutely necessary. Plan accordingly when doing your dilutions, and if need be, make absolutely sure you've got a backup of the same bacterial culture for later use
- Dilute enough of your sample to 100 – 200 ng/ul to obtain at least 14 ul of template at the proper concentration; an example of math is below
- You will turn in your template in a labeled eppendorf tube. *Write clearly.*

2. Naming your templates

- The name of your sample must be under 8 characters total
- Include your initials in the name so you can easily determine which sequences are yours
- I recommend format: Initials.BioBrick.Clone; for example, JD.14.1 would be Jessica Dymond, BioBrick BBa_K110014, clone 1
- Date and primer information is included by the sequencing facility and does not need to be included in the name you provide

3. Filling out the submission sheet

- On the front of the page:
 - Put your name in parenthesis next to “iGEM” on the top line
 - Write your email address on the proper line.
 - You may include your phone number if you'd like another method by which the sequencing facility can contact you in case of a problem
 - Everything else on the front has been filled out for you.
- On the back of the page:
 - Record the name, concentration, and volume being submitted for each sample

4. Turning in your samples:

- Room 372, Broadway Research Building at the School of Medicine – please see one of your teammates that works at the Medical School (James, Tejas, Alex, anyone else?) to ask if they can drop off your samples
- Samples turned in by 9 am will be processed that same day and results will usually be available the following morning. If you turn your samples in after 9 am, your sequences will be available two days later.

5. *Downloading and analyzing your sequencing results:*

- Direct your browser to Afp://10.16.101.251
- I will email you the user and password for access to the server
- After logging in, you will only be able to access the folder “Boeke”
- In the “Boeke” folder, look for the “iGEM” folder
- Identify your sequences and download them to your computer
 - You will have two sequences per clone (T7 and SP6)
 - ab1 and seq files will be available for both reads
 - Always look at your ab1 files! Seq files do not give you information about the status of your sequence
- Use FinchTV and ClustalW to compare your sequences to the sequence of your intended BioBrick; don't forget there may be some junk sequence (from the vector) flanking your insert sequence

Example of dilution math:

$$\frac{\text{Final concentration (ng/ul)}}{\text{Initial concentration (ng/ul)}} = \frac{1}{\text{Dilution Factor}}$$

$$\frac{\text{Final concentration (ng/ul)}}{\text{Initial concentration (ng/ul)}} = \frac{150 \text{ ng/ul}}{600 \text{ ng/ul}} = \frac{1}{4}$$

To dilute the template by 1/4: 5 ul template @ 600ng/ul
 + 15 ul H2O
 20 ul total volume @ 150ng/ul to submit

Explanation of the dilution:

5 ul template in a total final volume of 20ul
 5 ul / 20 ul = 1/4, the same dilution we calculated above
 1/4 dilution is the same as saying one part template in four parts total

For more information, see:

<http://www.wellesley.edu/Biology/Concepts/Html/volumetovolume.html>