

Bracken-Grissom Lab - Genewiz Online Order Protocol

1. If not already completed, fill out the reference plate map included with this guide in the PART 3 SEQUENCING YOUR AMPLICONS > Spreadsheets folder.
2. Fill out the Sample Map spreadsheet.
 - a. An easy way to do this is to copy the voucher #, genus, species, and gene columns, and paste that information into a text editor software. The BG Lab uses Text Wrangler.
 - b. Find and replace the spaces in each line of text with underscores.
 - i. Cmd+f > Find “\t” and Replace with “_” > Click Replace All
 - ii. If spaces still exist, Find “ ” (press the spacebar once), and leave Replace empty (No spaces either) > Click Replace All
 - c. Ensure each line is under 30 characters long. This necessary due to GeneWiz’s maximum name length policy
 - i. If the lines have the same Genus name, you can use find and replace function, as described above, to replace the genus name with only its first letter (i.e. 1001_Homo_sapiens_COI to 1001_H_sapiens_COI). Otherwise, the only way to do this is manually highlight and delete the genus names from each line yourself.
 - ii. If there are multiple genes on the same plate, the gene must be included in the name of each line. This will be crucial later to clean the sequence data.
 - iii. Ensure no line contains periods or commas. Only letters, numbers, and underscores.
 - d. Highlight and copy everything in text editor (cmd+A > cmd+C). Open the Sample Map spreadsheet, select the first cell in the “Sample Name” column, and paste (cmd+V). Each line should fill each cell in the column.
 - e. Save the Sample Map spreadsheet, changing the name to help you organize your sequencing submissions.
3. Fill out the Containers spreadsheet.
 - a. If you used M13 tails for every sample, you don’t have to change anything in the default spreadsheet. Otherwise, you need to add every primer used in the “My Primers per Container” row. Separate each primer name with a “;” and a space.
 - b. Save the Containers spreadsheet, changing the name to help you organize your sequencing submissions.

The following steps will require supervision from a BG Lab member if you use the BG Lab’s account information. It also assumes only M13-tailed primers. If you have to submit samples that didn’t utilize M13-tailed primers, contact the BG Lab Manager.

4. Navigate to www.genewiz.com and login.
5. Select “Sanger Sequencing” > “Flat Rate Value Plates.”
6. Fill out the top portion of the page
 - a. DNA Type = PCR Product – Un-Purified
 - b. Service Type = Custom
 - c. Number of Plates = 1 (Click Apply)
 - d. DNA Length = 501 – 1000 (Usually. If you’re sequencing a shorter fragment, select <500)
 - e. Difficult Template – leave blank
 - f. Layout = Horizontal
 - g. Connector = Underscore
 - h. Order Name = Whatever you want to name it

7. On the bottom portion of the page, click "Upload Container Template." Upload your Containers spreadsheet.
 - a. In the "My Primers per Cont" column, delete the n/a and leave blank.
 - b. Skip to Step 8, but return here after you've completed it. Under the column "Assign Sample Plate," Select the sample plate that you uploaded in step 8.
 - c. Skip to Step 9 if you've already completed Step 8.
8. On the right side of the page, under "Primer/Sample Plates" click "Upload Sample/Primer Plate." Upload your Sample Map spreadsheet.

Click "Edit." Your PCR samples should be displayed by the names you gave them in Step 2.

Type the name of the Sample Map in the "Plate Name" column.

(Return to step 7b)

9. Click "Save and Review at the very bottom of the window. This will progress you to the "Payment Methods" and "Order Finalization" sections of the order. Follow the prompts, entering in the necessary information.
 - a. If, for some reason, you can't progress to these sections of the order process, it is most likely because your Sample Map spreadsheet contains names with unacceptable symbols. Verify your samples have acceptable names.
 - b. If everything is done correctly, you should see your order contains two plate purchases (one for the forward strand sequences, and one for the reverse strand sequences). If you see you're purchasing more than two plates, make sure everything is submitted as described above.
 - c. If you still get errors trying to submit your order, contact GeneWiz customer support.
10. After the all steps have been completed, and the order has been submitted, print out the order confirmation pages. These will have to be submitted with the samples in the following protocol.

END