Lazzara Lab
Ashley Pandolf
Edits by Anne Campbell & Brooke McGirr, 09/07/20

Eurofins Sequencing

UPS shipping labels, envelopes, tube bags, barcodes, etc. are located in the bottom drawer under the microscope in the main lab. These materials are ordered from Eurofins and are provided at no charge.

Steps to Order

1. Go to Eurofins punchout in the UVA Integrated System. Under "Custom DNA Sequencing," click on "Tube Sequencing."
   Notes:
   - *Eurofins punchout can be problematic using the Safari browser on a Mac.*
   - *Other options are available if you have more than 24 samples. It's cheaper to send that many samples in a 96-well plate over Eppendorf tubes.*
2. Enter an email address for delivery of the results. Click submit.
3. Enter the number of samples to be sent for sequencing. Click next.
4. Fill out sample details.
   a. Click to indicate plasmid or PCR product. Indicate size and if pre-mixed with primers.
   b. Under "define sequencing reaction conditions," per sample tube, identify:
      i. Tube's barcode, found on the upper right corner of the label.
      ii. Template name (name of the sample/ future sequencing file name)
      iii. Sequencing primer supplied from Eurofins (not needed if pre-mixed)

   Notes:
   - *To add additional sequencing reactions for the same DNA template, click the boxed plus sign to add additional necessary primers.*
   - *If "difficult-to-read segments" are present in the DNA sample (i.e., shRNA constructs, AT-rich, GC/GT-rich, etc.), go to the advanced settings (the boxed star). Select "Power Read," and add the necessary specifications.*
5. Add to cart, and then return cart to Integrated System. Finalize and submit order. [Note that once the cart leaves the Eurofins punchout, to be transferred to Integrated Systems, those barcodes are counted as used and cannot be used again.]
6. Prepare samples (see below) and ship by placing in UPS dropbox (one located in the stairwell next to the mailroom in McKim Hall).

Steps to Prepare Samples

1. Ensure the sample and necessary primers meet the correct template concentration based on size and template type. Pay attention to units. Dilute if necessary.

Template Type	Size (kilo basepairs)	[Template] (ng/µL)	[Primer] (pmol/µL)
PCR Products, Purified	0.100–0.300	10–20	2–10
PCR Products, Purified	0.301–1.000	20–40	2–10
PCR Products, Purified	>1.000	40–60	2–10
Plasmid, Prepared	< 1 .000	30 - 60	2–10
Plasmid, Prepared	1.0 << 6 .0	60-150	2–10
Plasmid, Prepared	6.0 << 20.0	150-250	2–10
Large Contructs (BAC), Prepared	>20	250–500	10–15

2. Prepare samples in tubes:
   a. For 'template only' samples, add 10 µL of template DNA to tube.
      Notes: If > 1 sequencing reaction is desired, add an additional 5 µL per each additional reaction if using Standard Sequencing. Add an additional 10 µL per each additional reaction if using Power Read Sequencing.
   b. For 'pre-mixed' samples, add 8 µL of template DNA and 4 µL of primer to the tube.
3. Label tubes with stickers that include their respective barcodes as you ordered them online.
4. Bag them in DNA sequencing bags to include your name, email address, and phone #.