Sequencing Minipreps

Overview

This protocol covers sending minipreps for sequencing to Epoch Life Sciences.

Materials

- Miniprep Awaiting Confirmation
    - Ideally of high purity and high concentration.
- Tube Travel Rack
- .2mL Tubes
- 1.7mL Tubes
- Parafilm
- Fedex Envelope and Bill.
- NFW
- Sequencing Primers (100µM Stocks)

Procedure

1. Download the sample submission form from Epoch Life Sciences website. Fill in the quote number (as of 2018: nGS63198) and the purchase order number (as of 2018: 140127).
2. Create a number key corresponding to each sample you are sequencing
3. Fill out the form with the number key and sample name (eg. "1-WM18_AB_001 1C3 mp1). Include the concentration (ng/µL) and the sequencing primer (remember to make multiple reactions if needed)
    - Commonly, we will sequence with forward and reverse primers. Typically either with WM_Pad1/WM_Pad2 primers (for CD/longer 3G parts), VF2/VR primers (for short 3G parts) or UNS1/UNSX primers (for circuits).[i]
4. Key PCR tubes with your number key, then add miniprep to each tube.
    - For minipreps with concentration ≥80ng/µL send 7.5µL per sequencing reaction.
    - For minipreps with concentration <80ng/µL send 12.5µL per sequencing reaction.
    - Note that for minipreps with extremely low concentrations (<30ng/µL), sequencing is unlikely to work well (or at all).
5. In a labeled 1.7mL tube make 5µM primers. Each sequencing reaction needs 10µL of 5µM primer. For every 10µL of 5µM primer add 0.5µL of 100µM (stock) primer and 9.5µL of NFW.

[i] Remember that Sanger sequencing gets about 800-1000bp worth of sequence (assuming that your reaction is decent). The first 100-200 bp are fairly low quality, and the sequencing quality also tends to decline as the amplicon gets longer (around 1000 bps). This is why we typically sequence the way we do.

6. Place PCR tubes in tube travel rack. Cut a strip of parafilm and wrap them tightly, use tape if necessary. Remember these tubes will be shipped and we don't want them to spill.
7. Place the primer tubes on top of the parafilmed tube traveler, parafilm and or tape the tubes in place.
8. Place tube traveler in Fedex envelope and seal.
9. Fill out shipping bill. Include that the sender is iGEM and use the information for Epoch Life Sciences at the top of their sequencing form. Select standard overnight shipping.
10. Bring shipping bill and envelope to biology office and ask them to send it. If you ask **before 2pm** it will be picked up and arrive the next day (except for Friday).
11. Save your sequencing form in the format Sequencing Date (i.e. 180604 Sequencing). Place this sequencing form in the dropbox under orders/sequencing.
12. Fill out the sequencing cost excel sheet on the dropbox in the orders folder with the date, the number of sequence reactions and the price per reaction (typically $3).
13. Email the sequencing form to seq@epochlifescience.com and advise them that the tubes should arrive the next day.