GENEWIZ Protocol for Sanger Sequencing Unpurified PCR Product

Use this protocol if you will be submitting a sample of your PCR product to GENEWIZ for Sanger sequencing.

1). Confirm PCR product through electrophoresis and be sure to save an optimized image of the gel.

2). You will need an 8-well PCR tube strip (see Pedersen). We will try to coordinate with other groups needing to sequence to maximize use of the 8-well PCR tube strips and minimize shipping costs to GENEWIZ. We will also cut off empty tubes to save for next time.

3). Label the 8-well PCR tube strip. Write the labels (MP01, MP02, MP03…) on BOTH sides of your tubes using an ultra-fine point Sharpie. These labels should match your order receipt generated by Mr. Pedersen (see figure 1).

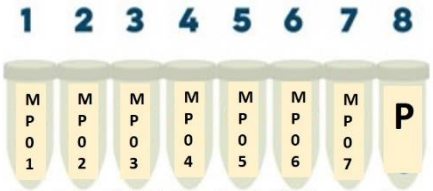

Figure 1: Correctly labeled tubes for submitting 7 reactions for Sanger sequencing. The eighth tube, labeled **P**, contains the forward primer. Because each reaction uses 5 µL, the tube contains at least 35 µL of 5 µM concentration primer. (7 x 5 µL = 35 µL)

4). Obtain the tube with the confirmed PCR product. If the annealing temperature of your PCR is within the range of 47 °C and 57 °C, obtain your forward primer (10 µM). This is the 10 µL primer that you used in the reaction and not the 100 µM primer that is in -20 °C. If your annealing temperature for PCR is not in this range, see Mr. Pedersen for alternative primer options. Lastly, obtain a tube of RNAse free water.

5). In each tube of the 8-well PCR tube strip, you need to carefully aliquot 10 µL of your PCR product. Make sure you are pipetting into the correctly labeled tube.

6). Each reaction for Sanger sequencing requires 5 µL of 5 µM primer. Thus, you will need to dilute your primer from 10 µM to 5 µM with RNase free water. You will add this diluted primer to a separate tube labeled with a large capital "P" on both sides (see figure 1). If you are submitting three samples, then you need 15 µL of 5 µM primer in the "P" tube.

7). In order to optimize the concentration of the sequencing reaction, GENEWIZ recommends submitting a representative gel image of your PCR products along with your samples. When you order with Mr. Pedersen, this image will be uploaded online.

8). I recommend inserted the gel image you saved (step 1) into PowerPoint. You can then label the amplicon clearly (e.g., MP01) with an arrow and the volume loaded into the well. Next, label the volume and type of ladder that was used (e.g., 10 µL of 100 bp ladder). Save this is a .jpg file to be uploaded to GENEWIZ.

9). Cut out the GENEWIZ mailing label below and tape onto a USPS Priority envelope. Wrap the 8-well PCR tube strip in bubble wrap. Obtain the order confirmation insert printed off by Mr. Pedersen. Place <u>both items</u> into a USPS Priority envelope. You are all set to send off the package. We should have results within a few days.

Mail to:

GENEWIZ, LLC

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From:

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