Quick Sheet:
Sanger Sequence Analysis using SnapGene Viewer and NCBI

Forward sequence only: Complete Step 1
Forward and Reverse sequences: Complete Steps 1-3

STEP 1: Edit the Forward Trace File

- ❑ Open SnapGene Viewer.
- ❑ Select Open >> Open Files >> YourFile_F.ab1.
- ❑ Select File >> Save As >> Mod.YourFile_F.scf to create a copy of your raw data file.
- ❑ Select "Show quality values" in the lower right-hand corner.
- ❑ Trim the 5'-end by identifying the contiguous sequence with ≥ 40 quality scores and highlighting ALL bases prior to this sequence. Hit 'Delete.'
- ❑ Repeat for the 3'-end.
- ❑ Scroll through the sequence. Are all quality scores ≥ 40? Adjust nucleotides to 'N' as needed. Never delete internal nucleotides.
- ❑ Save. Select File >> Export >> FASTA Format.

STEP 2: Edit the Reverse Trace File

- ❑ Repeat Step 1 with the reverse sequence.

STEP 3: Generate a Consensus Sequence

- ❑ Open NCBI in your web browser: https://www.ncbi.nlm.nih.gov/
- ❑ Select "BLAST" from the right-hand 'Popular Resources' menu
- ❑ Select "Nucleotide BLAST."
- ❑ *(optional)* Enter a Job Title.
- ❑ Click " Align two or more sequences" at the bottom of the first box.
- ❑ Load your forward FASTA file in the top box and the reverse FASTA file in the second box. Hit BLAST.
- ❑ Check the % Identity. It should be 100%. If not, refer back to the trace files and investigate the discrepancy.
- ❑ If your identity is 100%, select the Arrow next to "Download" and download FASTA (aligned sequences). Save. You have now generated a **Consensus Sequence**.