**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**.