A. Checking for Single Nucleotide Polymorphism (SNP)

1. Go to "https://digitalworldbiology.com/FinchTV"

2. Download and install the FinchTV DNA analysis software.

3. Run the software
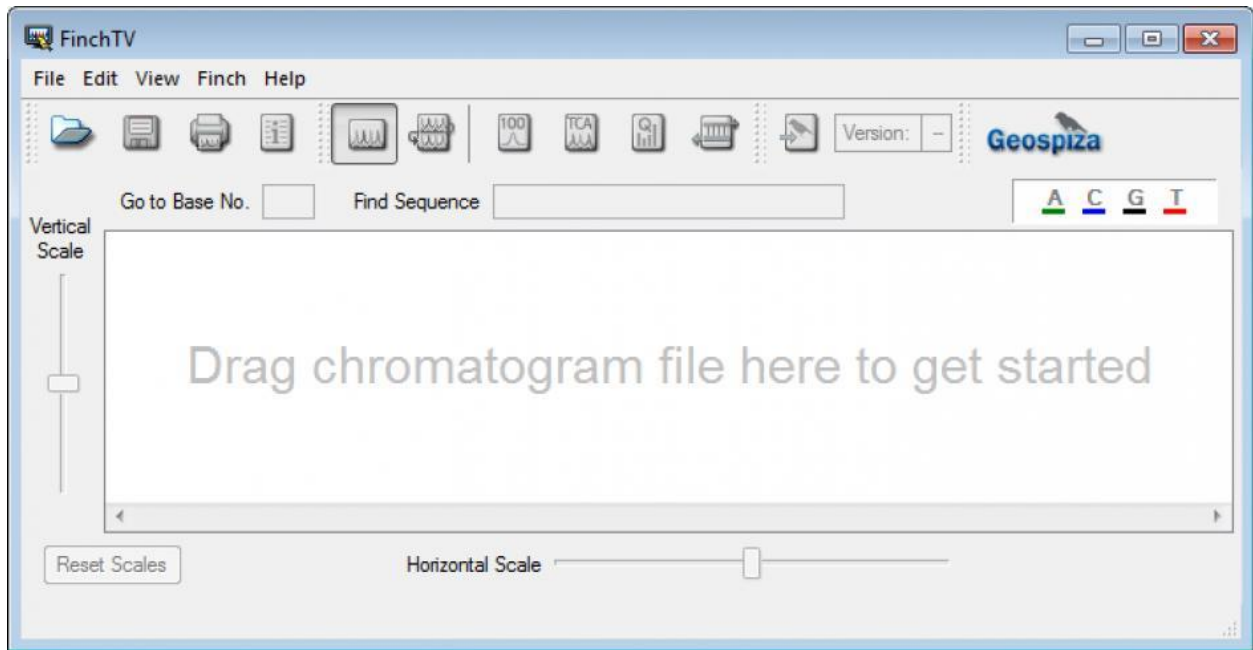
4. Drag and drop your sequence file (.abi) onto the software interface
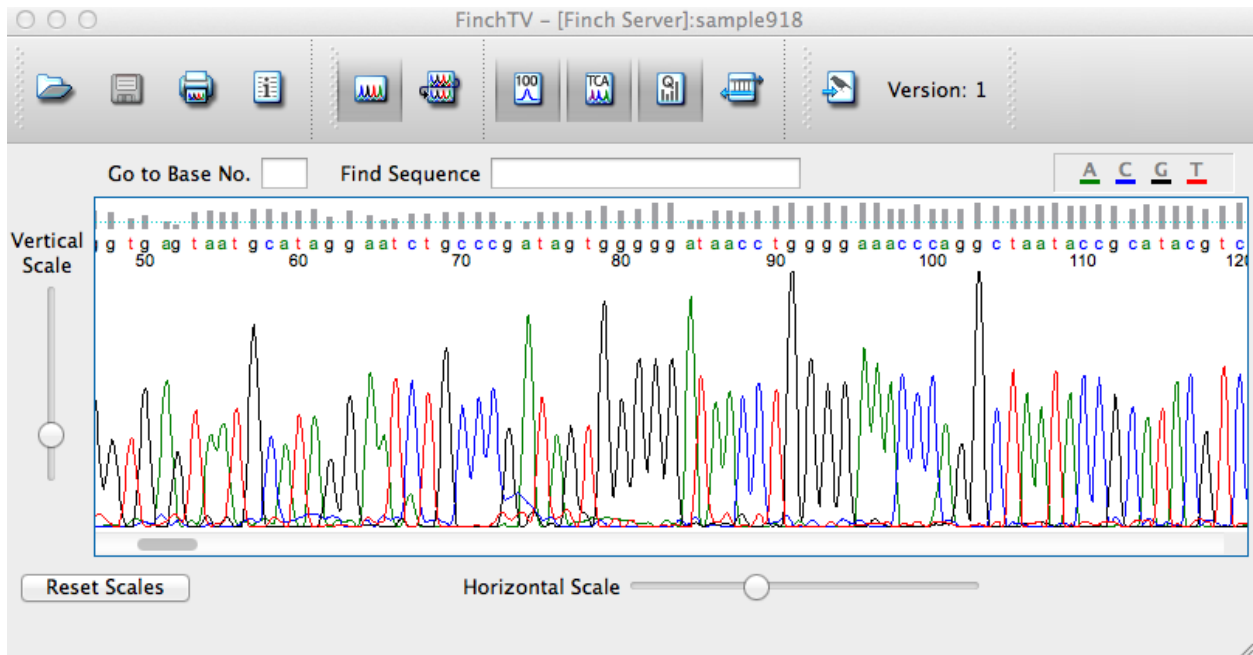
5. Go to edit, scroll down to 'Nucleotide, BLASTn'

6. You will be directed to NCBI website, copy all the FASTA sequence.
   No need to BLAST, you have done this in previous lab.

7. Go to http://www.attotron.com/cybertory/analysis/seqMassager.htm

8. Paste the sequence into the Sequence Massager program and perform the following:
   a. Remove Fasta comments
   b. Remove HTML tags
   c. Remove line breaks
   d. Remove numbers
   e. Remove white space

http://www.attotron.com/cybertory/analysis/seqMassager.htm
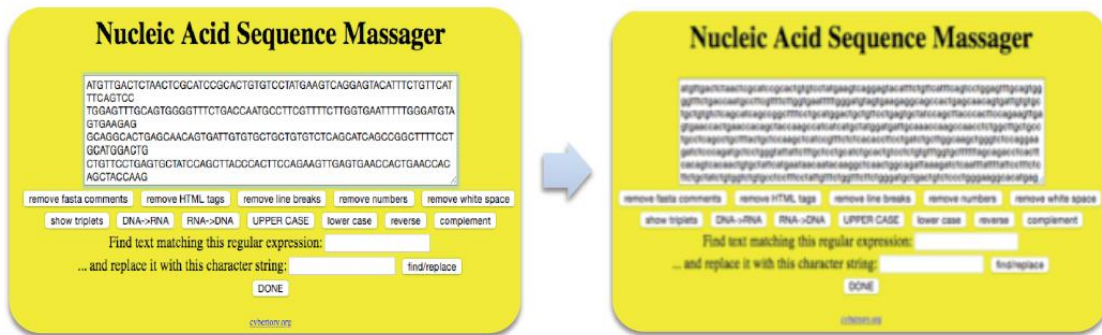
9. Copy and paste the massaged sequence into a word document.
   a. Name your document i.e. ARB60SCAR4 (Name should not be more than 10 characters)
   b. For example, write ">gi|ARB60SCAR4" before your gene sequence name:

>gi|ARB60SCAR4

aaacatagaacgattcgatagctgtcttgcacacacatcatggtaaaatattcatcagtacgaataatatgctgactaaagtttattaacactaaagga
caaaaagaccctctgaagctttatagggataaaacttgggtaacattctcaatcaaatattcttattgcatgccagctaaagtagtaaattattggttttc
tcctaagaccactgtttaattggggcgataaccttatcaacagccacctaaggtgaacaaagcgatacaaggatcctaaactttgaccaactaataa
gtgcaaagatacatgtttcgcttaatggcccaacaagtccttcccagtacttaaatgctatcttaatgagctttaaattaatttacccgagttaagctact
ctacggataacaggc

>gi|C_iso32

aaacatagaacgattcgatagctgtcttgcacacacatcatggtaaaatattcatcagtacgaataatatgctgactaaagtttattaacactaaagga
caaaaagaccctctgaagctttatagggataaaacttgggtaacattctcaatacaaatattcttattgcatgccagctaaagtagtaaattattggttt
tctcctaagaccactgtttaattggggcgataaccttatcaacagccacctaaggtgaacaaagcgatacaaggatcctaaactttgaccaactaata
agtgcaaagatacatgtttcgcttaatggcccaacaagtccttcccagtacttaaatgctatcttaatgagctttaaattaatttacccgagttaagctac
tctacggataacaggc

>gi|C_iso31

aaacatagaacgattcgatagctgtcttgcacacacatcatggtaaaatattcatcagtacgaataatatgctgactaaagtttattaacactaaagga
caaaaagaccctctgaagctttatagggataaaacttgggtaacattctcaatacaaatattcttattgcatgccagctaaagttagtaaattattggtt
ttctcctaagaccactgtttaattggggcgataaccttatcaacagccacctaaggtgaacaaagcgatacaaggatcctaaactttgaccaactaat
aagtgcaaagatacatgtttcgcttaatggcccaacaagtccttcccagtacttaaatgctatcttaatgagctttaaattaatttacccgagttaagcta
ctctacggataacaggc

10. Collect sequence data from other lab members that used similar marker. For example, if you worked with SCAR4, collect data from other ARB, MRC and PFB members that used SCAR4 in your lab.

11. Perform a multiple sequence alignment with the massaged FASTA file using MUSCLE: https://www.ebi.ac.uk/Tools/msa/muscle/
MUSCLE stands for **MU**ltiple **S**equence **C**omparison by **L**og- **E**xpectation.

12. Copy all the curated sequences and paste in the MUSCLE program as seen below:

13. Identify regions that are conserved and diverse.

14. Compare phylogeny by selecting 'phylogenetic tree' and interpret your result.

B. Phylogenetic analysis of *Symbiodinium* 23s

1. Download *Symbiodinium* 23s curated data from the lab website.

   This data contained 23s sequence fragments from the members of the family Symbiodinaceae.

2. Add the curated class data to this list as done in part A.

   Remember to write **>gi|** before your generic name.

3. Perform a multiple sequence alignment with the massaged FASTA file using MUSCLE:
   https://www.ebi.ac.uk/Tools/msa/muscle/

   Refer to step 11 above.

4. After submitting your query, compare phylogeny by selecting 'phylogenetic tree'

5. Identify which gene is closely related or diverse.