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

🖳 Fincl	hTV
File Ed	dit View Finch Help
۵	🔜 📾 🔢 📖 🖑 🕅 🕼 🕼 🖓 Version: - Geospiza
	Go to Base No. Find Sequence
Scale	Drag chromatogram file here to get started
Reset	t Scales Horizontal Scale

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:



- 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.
- Perform a multiple sequence alignment with the massaged FASTA file using MUSCLE: <u>https://www.ebi.ac.uk/Tools/msa/muscle/</u> MUSCLE stands for MUltiple Sequence Comparison by Log- Expectation.

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

		Input form	Web services	Help & Documentation	Bioinformatics Tools FAQ		Reedback	<share< th=""><th></th></share<>	
	,								
		Important no	ote: This tool can	align up to 500 sequenc	es or a maximum file size of	1 MB.			
		STEP 1 -	Enter your input	sequences					
		Enter or pas	ate a set of seque	nces in any supported fo	rmat:			_	
	•	>gilC_iso aaacatag tcaatcaaa agtgcaaa >gilC_iso aaacatag tcaatacaa	080 aacgattcgatagotg atattottattgoatgoo gatacatgtttogotta 032 aacgattogatagotg aatattottattgoatgoo	ytettge ac ac act at ggtaaa gge taaagtagtaaattattggttt atgge ce aac aagteette cea ytettge ac ac ac act at ggtaaa cage taaagtagtaaattattggt	aatattcatcagtacgaataatatgotgi totocotaagaccaotgtttaattggggo gtacttaaatgotatottaatgagotttaa aatattcatcagtacgaataatatgotgi tittotoctaagaccaotgtttaattgggg	ctaaagttattaacactaaaggacaaaaagacctctgaagc gataacttatcaacagccactaaggtgaacaaagcgatac attaatttacccgagttaagctactctacggataacaggc ctaaagttattaacactaaaggacaaaagaccctctgaagc gataacttatcaacggcacctaaggtgaacaaggcgata	tttatagggataaaacttgggtaac aaggatcctaaactttgaccaact tttatagggataaaacttgggtaac aaggatcctaaactttgaccaac	eatte aata	
		Or upload 8	a file: Choose File	No file chosen		Use a <u>example sequence</u> C	lear sequence I See more ex	ample inputs	
		STEP 2 -	Set your Parame	eters					
			DRMAT:						
		ClustalM	V					*	
		The defau	It settings will fulf	ill the needs of most use	rs.				
		More opti	ons) (Click her	re, if you want to view or	change the default settings.)				
		STEP 3 -	Submit your job						
		🔲 Be notifie	d by email (Tick th	is box if you want to be r	notified by email when the re	ults are available)			
	-	Submit							
		If you plop to	uno thono norvia	os durina o ocurso aloga	o contect un				

- 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: <u>https://www.ebi.ac.uk/Tools/msa/muscle/</u>

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.