Agenda

- Viewing FASTA files
- Downloading publicly available genomes
- Concatenating consensus genomes
- Sequence Alignment
- Tree inference

Viewing FASTA files

How to view FASTA files

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sample2.consensus.fasta	Mar 3,	2021 at 10:53 AM	31 KB	

- Open in your favorite text editor:
 - TextEdit (Mac)
 - Notepad++ (Windows)
 - gedit (Ubuntu)

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How to view FASTA files



- You can also open FASTA files in specialized software for working with genomic data
- e.g., AliView: https://ormbunkar.se/aliview/ (free software)
- Or view directly on command line using less

Downloading Publicly Available Data

Selecting background data for phylogenetics

- For pathogens with limited data, use all available sequences
- Selecting background data is not a trivial task
- Considerations include:
 - Geography
 - Time period
 - Hosts / sample source
 - Subsampling of highly similar sequences

There is a lack of broadly useful sampling guidance for phylogenetics

Downloading publicly available genomes



Downloading publicly available genomes



Downloading publicly available genomes

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Downloading publicly available reads

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Concatenating consensus genomes

Concatenating consensus genomes



- Two methods for concatenating sequences:
 - Command line using cat
 - Copying and pasting in a text editor
- Both methods produce a multi-fasta
- FASTA file format: (.fa, .fas, .fasta); sometimes .mfa is used to indicate multi-fasta

Concatenating consensus genomes

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Sequence Alignment

Multiple sequence alignment

Types of multiple sequence alignment



Alignment with a reference genome

- Faster and less computationally intensive
- Ensures consistent coordinates



Alignment without a reference genome

Selecting an appropriate reference genome

- Use the same reference genome as others working on the same outbreak
 - e.g., Wuhan-Hu-1 for SARS-CoV-2
- Use the earliest sequence from the outbreak, if available
- Use a sequence from a prior outbreak in the same location
- Use NCBI RefSeq (https://www.ncbi.nlm.nih.gov/refseq/)

The reference genome should always have a date **before** the earliest of your samples

MAFFT Server

MAFFT version 7 Multiple alignment program for amino acid or nucleotide sequences

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Download version Mac OS X Windows Linux Source Online version Alignment mafft --add Merge **Phylogeny** Rough tree Merits / limitations Algorithms Tips Benchmarks Feedback Follow

Hardware was upgraded, Jan 16, 2022. There should be no change in user interface. If you notice any unexpected changes, then please let us know. To avoid overload, try a light-weight option, for MSA of full-length SARS-CoV-2 genomes (2020/Apr). For a large number of short sequences, try an experimental service. Experimental service for aligning raw reads (2019/Aug) Multiple sequence alignment and NJ / UPGMA phylogeny Input: Paste protein or DNA sequences in fasta format. Example or upload a plain text file: Choose File No file chosen Use DASH to add homologous structures (protein only) New! 2018/Dec/23 Ouput original plus DASH sequences Output original sequences only Give structural alignment(s) externally prepared Allow unusual symbols (Selenocysteine "U", Inosine "i", non-alphabetical characters, etc.) Help

MAFFT Commandline

mafft --auto consensus.fasta > consensus_aln.fasta

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Phylogenetic Tree Inference

IQ-Tree Server

http://iqtree.cibiv.univie.ac.at/

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IQ-TREE web server: fas	t and accurate phylogenetic trees under maximum li	celihood			
Server load: 4%	Trifinopoulos J, Nguyen LT, von Haeseler A, Minh BQ	2016) Nucl. Acids Res. 44 (W1): W232-W235. <u>doi: 10.1093/nar/gkw256</u>		
Tree Inference Model Sel	ection Analysis Results				
For a quick start, take a lo Please visit the <u>IQ-TREE h</u> Data Privacy Statement: All yo will be automatically deleted a Input Data	ok at the <u>tutorial</u> for the IQ-TREE web server. <u>meneage for more information or if you want to download</u> ur personal data are strictly confidential and will not be shared wi fter 180 days.	the main software. th any third parties. Your	data		
Alignment file :	C:\fakepath\Study_and_Backgrot Browse Show example >		Select the multiple seque	nce alignment file	е
Use example alignment:	Tes	2			
Sequence type:	Auto-detect ONA OProtein OCodon DNA->AA Binary OMorphology	?			
Partition file:	This field is optional. Browse Show example >				
Partition type:	 Edge-linked Edge-unlinked 	7			
Substitution Model C	ptions				
Substitution model:	Auto	2			
FreeRate heterogeneity:	[]] Yes [+R]				
Rate heterogeneity:	Gamma [+G] Invar. sites [+I]	7			
#rate categories:	4				
State frequency:	Image: Sempirical (from data) A model (from matrix) ML-optimized Codon F1x4 Codon F3x4				
Ascertainment bias correction:	Types [+ASC]	?			
Branch Support Ana	lysis				
Bootstrap analysis:	None O Ultrafast Standard	?	 Select No Bootstraps 		
Number of bootstrap alignments:	1000 🗘				
Create .ufboot file:	Yes (write bootstrap trees to .ufboot file)				
Maximum iterations:	1000	2			

IQ-Tree Commandline

iqtree2 -s consensus_aln.fasta -T AUTO -m TEST -B 1000

What questions do you have?

