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General Assembly and Alignment in Geneious

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1 Works for me dx.doi.org/10.17504/protocols.io.bnvfme3n

Bark Beetle Mycobiome Research Coordination Network

ABSTRACT

The purpose of this protocol is to conduct general assembly and alignment of sequences in Geneious.

This protocol is part of the Bark Beetle Mycobiome (BBM) Research Coordination Network. For more information on the BBM international network: Hulcr J, Barnes I, De Beer ZW, Duong TA, Gazis R, Johnson AJ, Jusino MA, Kasson MT, Li Y, Lynch S, Mayers C, Musvuugwa T, Roets F, Seltmann KC, Six D, Vanderpool D, & Villari C. 2020. Bark beetle mycobiome: collaboratively defined research priorities on a widespread insect-fungus symbiosis. Symbiosis 81: 101–113 https://doi.org/10.1007/s13199-020-00686-9.

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1. Import .ab1 files to Geneious
2. Assemble - join sequences fwd and rev in one /de novo assembly
3. Check forward and reverse are ok (can be done during the alignment): rev is X primer, if not, RC button / save
4. Extract from Assemble consensus: right click on sequences / Generate Consensus Sequences
5. Export sequences as fasta file
6. Trim sequence - Trim with 0,01 limit of probability error. Also, add to trim out PCR primers on ends if sequenced.
7. Rename / batch rename

8. Group sequences into a list (right click) to export as fasta file
9. MAFFT alignment - automatic directions.
10. Check Frame to avoid Stopping Codons. Set: Invertebrate mitochondrial genome
11. Create Fast tree or NJ

To concatenate sequences: tools/concatenate, create separate alignments before and name then with the record number

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