

Botany 2013

Introduction to Next-Generation Sequencing Workshop

Please complete the following steps prior to attending the workshop. We do not anticipate having internet access during the workshop, so be sure to plan ahead.

Instructions for Downloading Software

1. Go to <http://www.geneious.com/web/geneious/download-trial>.
2. Download and install the free 2-week trial of the Geneious Pro software.
3. Download a copy of the Geneious manual at <http://www.geneious.com/assets/documentation/geneious/GeneiousManual.pdf>.
4. To familiarize yourself with the Geneious layout and basic options, go through parts 1-3 of the Geneious tutorial prior to the workshop. Much of this terminology will be used in the course of the workshop practical exercises.

Instructions for Downloading Data Files and Preparing a Directory Structure in Geneious Pro

1. Navigate to http://files.cgrb.oregonstate.edu/Botany/listonlab/Botany_2013_NGS_Workshop.
2. Download Botany_2013_NGS_workshop_files.tar.gz.
3. Uncompress the downloaded files to prepare them for import into Geneious. Note that you will need software installed capable of uncompressing files before attempting this step. Linux and mac users should already have programs for this. If you are a Windows user and do not have a program to uncompress the files, download 7-Zip (<http://www.7-zip.org>).
4. In Geneious, click on the Local documents directory in the Sources panel. Then under File, choose "New Folder..." and create a directory called "NGS_workshop."
5. Click on the newly created directory, and make two subdirectories: "read_mapping_and_reference_guided" and "de_novo_assembly." Then click on the first subdirectory and choose File -> Import -> From File... and move to the directory of the files downloaded for the workshop and import the files from the directory of the same name. Geneious may ask if the sequences are nucleotide or protein sequences. Choose nucleotide. Note that this process can take a long time for large files.
6. Repeat this process for the other directory.
7. Next you will need to create a custom BLAST database in Geneious because we will be unable to access GenBank during the workshop. To begin, choose "Tools" -> "Add/Remove Databases" -> "Set Up Search Services" and then select "Custom BLAST" from the Service drop-down box. Check "Let Geneious do the setup" and then click "OK."
8. Next add a BLAST database specifically tailored for this workshop by choosing "Tools" -> "Add Sequence Database" and selecting "Custom BLAST" from the Service drop-down

box. Choose "Create from file on disk" and select the directory where you have the downloaded files for the workshop and move to the "BLAST_database" directory and select the "selected_plant_genomes_database.fa". You will also want to make this the "Database Name." Check that the "Do not check file for duplicate names..." option is selected, and then click "Ok." Disregard any warning messages produced by Geneious relating to the content of this database.

9. Next allocate RAM to Geneious. Choose "Tools" from the Geneious menu and then select "Preferences." In the preferences dialog box, make sure that the max memory available to Geneious is at least 2000 Megabytes (2 GB) and choose "OK." If you do not have at least 2 GB of RAM to allocate to Geneious, that is ok, but your analyses will take longer.
10. Now you should be all set for the workshop. See you in New Orleans!

Questions or problems?

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