Botany 2012
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. To familiarize yourself with the Geneious layout and basic options, it may be helpful to go through parts 1-3 of the Geneious tutorial prior to the workshop. Some 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. Go to http://milkweedgenome.org/?q=taxonomy/term/9.
2. Download botany2012_NGS_workshop_files.tar_.gz.
3. Remove the underscore from between tar and .gz in the file name. Then 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 a free trial of WinZip (http://www.winzip.com/trialpay.htm).
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_de_novo_assembly" and "reference_guided_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.
6. Repeat this process for the "reference_guided_assembly" directory. Geneious will ask if the sequences are nucleotide or protein sequences. Choose nucleotide. Geneious will then ask if sequences containing gaps should be imported as separate sequences or as an alignment. Choose "Import as an alignment."
7. Next you will need to create a custom BLAST database in Geneious. 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, you can import partial results of the de novo assembly to use during the workshop (directions for this step will be provided during the workshop). You will be able to run the de novo assembly after the workshop, it will just take longer to run than the time we have allotted for the exercise.

10. Now you should be all set for the workshop. See you in Columbus!

Questions or problems?
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