

Botany 2015

Introduction to Next-Generation Sequencing Workshop

We are looking forward to your attendance at the upcoming workshop. Please complete the following steps **prior** to attending the workshop. We should have good internet access during the workshop, but many people trying to download large files simultaneously could cause a problem. Two of the files to download are 28 Mb, and may take some time over slower connections.

Instructions for Downloading Software

1. Install the Geneious R8 software on your laptop. You will need to request a 14-day trial from <http://www.geneious.com/request-trial> and then follow the directions in the confirmation email. In our experience, this arrives immediately. However, the website states that confirmation may take 48 hours, so please don't wait until the day of the workshop.
2. Download a copy of the Geneious manual at <http://www.geneious.com/assets/documentation/geneious/GeneiousManual.pdf>.
3. 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 R8

1. Navigate to http://files.cgrb.oregonstate.edu/Botany/listonlab/Botany_2015_NGS_Workshop/
2. Download the following:
 - Asclepias_euphorbiifolia_Hyb-Seq_reads_R1_001.fastq.gz
 - Asclepias_euphorbiifolia_Hyb-Seq_reads_R2_001.fastq.gz
 - Asclepias_euphorbiifolia_Hyb-Seq_reads_de_novo_Assembly.geneious
 - Asclepias_syriaca_chloroplast_sequence.gb
 - Asclepias_syriaca_single_copy_gene_exons.fasta
 - Matelea_chloroplast_sequence.gb
 - example_fasta.fa
 - example_fastq.fq
 - If you are on a slow connection or it is the day of the workshop, you can download the two "Subset..." files instead of the larger fastq files.

3. 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."
4. Select the new directory 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. If Geneious asks whether to "Keep sequences separate" or "Create sequence list," **choose list**. Note that this process can take a long time for large files.
5. 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.

Instructions for Obtaining an iPlant Account and Atmosphere Access

1. At the end of the hands-on portion of the workshop, we will offer an opportunity to complete a Hyb-Seq probe design exercise using Linux command line and iPlant resources for those that are interested. If you would like to take part, follow these instructions to prepare in advance.
2. Register for a free iPlant account at <http://user.iplantcollaborative.org>. Be sure to use an institutional email (e.g., *.edu) rather than a personal email (e.g., *gmail.com).
3. After receiving confirmation that your iPlant account has been set-up, go to <https://user.iplantcollaborative.org/dashboard>. Under "Available Services" select "Atmosphere." You will be asked for justification for accessing Atmosphere and it is acceptable to enter "workshop attendance/participation" here.
4. Once you have access to Atmosphere, log in to iPlant and go to the dashboard. Now Atmosphere should appear under "My Services." Click on it to launch. Then click on the "Launch New Instance" button when it appears.
5. Under "Select an Image" search for "Botany_Workshop." Click on it and then change the instance size to "tiny2 (1 CPUs, 8 GB memory, 60 GB root)" before clicking "Launch Instance."
6. It will take a while for the instance to build and deploy. Once your instance is active, click on the "Access via Shell" tab and enter your iPlant username and password.

7. Once you have checked to make sure you can log in successfully, you should suspend the instance until the workshop. If you do not suspend it, it is possible that you will use all of your resource allocation before the workshop begins.

You are all set. See you in Edmonton!

Questions or problems?

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