

# SEQUENCHER®

## Installation and Use of External Tools with Sequencher

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# Introduction

With the advent of Next Generation sequencing, several new external alignment algorithms have been integrated in Sequencher to align the large quantity of data produced by Next Generation sequencing machines. These algorithms, Maq and GSNAP, join Clustal that was the very first external algorithm to be added to Sequencher. The Maq and GSNAP algorithms were written to run on Unix-based systems. Mac OS X is a Unix-based operating system. Windows, on the other hand, will need to have Cygwin, a Unix emulation environment, installed in order to run the alignment tools. To take full advantage of these tools, you will also need to install Tablet—a third-party graphical viewer.

The instructions which follow show you how to use the all-in-one installers which will place almost everything you need to run these algorithms on your computer. The Tablet viewer is a separate installer but only takes moments to install. If you want to keep up with the very latest external tools releases, then use the more detailed step-by-step instructions starting on page 10 of this document.

## Quick and Easy Set Up for NGS Aligners

### INSTALLING MAQ AND GSNAP ON WINDOWS

The Windows self-extracting installer will place Cygwin, Maq, Maqview, and GSNAP and their related files where they need to be in order to work with Sequencher.

1. Download [ExternalToolsInstallerV1.zip](#) from the Gene Codes website at [www.genecodes.com/next-gen-seq-installer](http://www.genecodes.com/next-gen-seq-installer).
2. Double-click on the ZIP archive to open it. A window will open containing ExternalToolsInstallerV1.exe as well as src and Tablet folder.
3. Double-click on the *ExternalToolsInstallerV1.exe* and click the **Extract all** button. Accept the default settings provided by Windows for extracting all the files.
4. Double-click on the newly extracted *ExternalToolsInstallerV1.exe* file to start the installation process. A dialog will open asking for the location to Extract to:



5. You should see a path very much like the one in the picture above. *Do not accept this default path. Sequencher requires these files extracted directly to the root of your drive. Delete everything past the first \ character.* In most cases, your root drive location will be C:\ but you may have a different drive letter.
6. Click on the **Extract** button to continue.
7. A new window appears which shows the progress of the installer. When the screen disappears, the installation is complete and Maq and GSNAP are ready for use in Sequencher.

### INSTALLING THE TABLET VIEWER ON WINDOWS

To install the Tablet viewer on your computer, follow these steps:

1. Open the Tablet folder. There are two installers. The `tablet_windows_x64_1_11_05_03` installer is for 64-bit systems and the `tablet_windows_x86_1_11_05_03` installer is for 32-bit systems.

2. Tablet requires administrator privileges in order to install. If you are an Administrator user, double-click on the correct Tablet installer for your operating system. If you are a Standard user, right-click on the correct Tablet installer for your operating system and select 'Run as administrator'.
3. If you are presented with a dialog with a Run button on it, click on the Run button to continue.
4. A new window appears informing you that the installer is preparing the wizard to guide you through the setup.
5. The Tablet Setup Wizard dialog appears next. Click on the **Next** button on that dialog.
6. An installer license agreement window appears next. Accept the License Agreement and again click on the **Next** button.
7. You will be prompted for a destination location for the installation. The default location is Programs Files\Tablet. Don't change this location and click on the **Next** button.
8. You will be asked whether you want to create a Start Menu folder and create a shortcut. If you wish you may uncheck these checkboxes. Now click on the **Next** button to continue with the installation.
9. A final window appears informing you that the Setup has finished. Uncheck the **Run Tablet** checkbox and click on the **Finish** button. Tablet is now ready for use in Sequencher.

## INSTALLING MAQ AND GSNAP ON MAC OS X

You must be an Administrator or have an administrator's password in order to install the external tools on Mac. The Mac OS X installer will place Maq, Maqview, GSNAP, and their related files where they need to be in order to work with Sequencher.

1. Download [ExternalToolsInstallerV1.dmg](http://www.genecodes.com/next-gen-seq-installer) from the Gene Codes website at [www.genecodes.com/next-gen-seq-installer](http://www.genecodes.com/next-gen-seq-installer).
2. Double-click on the downloaded dmg file.
3. Double-click on the new volume icon named 'External Tools Installer V1' to open it.
4. Double-click on either the 32-bit or 64-bit installer package to begin installation. (Don't worry about choosing the wrong one—the installation will only proceed if it matches your machine.)
5. A welcome window will appear and you will be guided through the installation. Click on the **Continue** button.
6. An information screen appears telling you how much space is required for the installation. Click on the **Install** button.
7. You will be asked for your password. One is required in order to allow changes to be made to your system. Type in the password and click on the **OK** button.
8. The installer starts. You will see various status messages as the files are written to your system. Finally, a screen appears telling you that everything was installed successfully. Click on the **Close** button. The external tools Maq and GSNAP are now ready for use in Sequencher.
9. Eject the disk image icon from your desktop unless you are planning to install the Tablet viewer next.

## INSTALLING THE TABLET VIEWER ON MAC OS X

To install the Tablet viewer on your computer, follow these steps:

1. By default, Tablet will install in the Applications folder and that is where Sequencher expects to find it. Because installing in the Applications folder requires administrator privileges, you must be an administrator to install.
2. Double-click on the Tablet folder icon to open the folder.
3. Double-click on the tablet\_macos dmg file to open the installer.
4. Double-click on the Tablet Installer to start the installation.
5. A Welcome screen will appear. Click on the **Next** button.
6. An installer license agreement window appears next. Accept the License Agreement and again click on the **Next** button.

7. You are prompted for a destination location for the installation. The default location is Applications. Don't change this location. Next click on the **Next** button.
8. A final window appears informing you that the Setup has finished. Uncheck the **Run Tablet** checkbox and click on the **Finish** button. Tablet is now ready for use in Sequencher.
9. Eject the disk image icon from your desktop.

## Preparing Your Data for Sequence Alignment

Sequencher accepts many sequence formats. For Next Generation sequencing, the most relevant formats are FastA and FastQ. Although most sequencers have their own native formats, as long as the data can be converted into FastA or FastQ format, Sequencher will be able to align the reads.

When aligning sequences, you will be using a reference sequence that has been imported into Sequencher. The advantage of using a GenBank sequence is that it will usually carry annotations.

The alignment algorithms for Next Generation sequence alignment will work with single-end or paired-end data. Each algorithm has its own requirements for data input files that may require some modification to your files in advance of performing the alignment in order to succeed.

One thing to note is that, although it is possible to get FastQ format files from both Illumina and 454, the formats differ in how they encode confidence scores. Both represent confidence scores in single ASCII characters, but the key to decode them back into scores is different. 454 data conforms to what is popularly known as Sanger standard format. Illumina represents scores using a different range of ASCII characters. You will need to specify which FastQ variant you'll be using when aligning with GSNAP.

### DATA FILE REQUIREMENTS FOR MAQ:

- Must be in FastQ format. Maq expects Sanger standard encoded quality scores.
- Read lengths can be no greater than 127 bases and must be the same size for every read.
- Paired-ends data (2 FastQ files) and single-ends data (1 FastQ file) are supported.

### DATA FILE REQUIREMENTS FOR GSNAP:

- GSNAP supports both FastA and FastQ file formats (both Sanger standard and Illumina variants).
- Read lengths may vary in size and fall within the range of 14bp to 500bp. GSNAP may be configured for even longer read lengths though.
- 2 FastQ files will be treated as paired-ends reads and 1 FastQ file will be treated as single-ends reads. Paired-end data in FastQ format must list the reads in the same order in both files. Here is an example:

```
File 1:
@NC_014230.1|_1831264_1831446_0/1
TCTCCATAAGTTGAGATAAGTTAGAAACCAAGTGTT
+
&IIIIIII+IIIIIIIIBBII)$&IIII>.&+%E*I0
@NC_014230.1|_1066261_1066432_1/1
GCTGAACTTGCATAATAGTGGACCAATCATAAGAAT
+
D!"IIIIIII$!!*(&%.$IIIIIII3&III
File 2:
@NC_014230.1|_1831264_1831446_0/2
ATAGGATTCAAGGCAGATTTAAAATTGACGGCGCGC
+
III<IIIIIII' %IICII3/II>+=
@NC_014230.1|_1066261_1066432_1/2
AATCCTGGTAACAAAATGTTTTTACATTATAGCCTA
+
IIIIICIIIIIIADIIIIIIIIIIIIIIIIII
```


- FastA files may also represent both single-ends and paired-ends reads. GSNAP has specific requirements for modifying the basic FastA format for alignment.
  - The entire read must be on a single line—no line breaks in the DNA sections.
  - If you have paired-ends data, the second read must be on the next line. For example:

```
>name sequence header information
ATGAACAGGCGCGATCTTCTTTTACAAGAAATGGGCATTTCCCAGTGGGA
GAATGTAAGCAGCCTATTCGTTATTGGTTACTATCAGAAAATAGCGACCA
>next-sequence
CACTTTGCCATTTTGCAAGCAGGCTGAGCAGGTTTATCGC
TATCGCCCCGAGGTACTGCAAGGTTTCAGTAGGAATTAGTG
```

In this example, there are 4 DNA sequences—2 pairs, not 2 sequences.

## PREPARING A KNOWN SNPs DATA FILE

To perform a SNP-Tolerant alignment using GSNAP, you must provide a file containing the list of known SNPs. GSNAP performs SNP hunting in a different way than Maq. This text file has to list each SNP in a specific format—one SNP per line.

Name	Size	
 My Hflu Ref	1985832 BPs	>rs004341 My_Hflu_Ref:65..65 AT
		>rs004342 My_Hflu_Ref:154..154 AT
		>rs004343 My_Hflu_Ref:227..227 CG
		>rs004344 My_Hflu_Ref:632..632 AC
		>rs004345 My_Hflu_Ref:1396..1396 AG
		>rs004346 My_Hflu_Ref:1413..1413 GT

Each line must begin with the > character followed by a SNP identifier. In the example above, it is an rs number. The next pieces of data are reference and positional information in the format RefName:#..# followed by a major and minor allele. In real data, the reference name to use before the colon is the name of the sequence you select in the Project Window. If there are spaces in the name, these should be replaced with underscores. In the example above, 'My Hflu Ref' is selected for a SNP-tolerant GSNAP alignment. The reference identifier used in the known SNP file is therefore My\_Hflu\_Ref. The position information in this file always assumes that the first base of the reference sequence in Sequencher is 1, no matter what its actual numbering relative to its chromosomal or contig position is.

# Using External Tools with Sequencher

## USING GSNAP AND MAQ WITH SEQUENCHER

Sequencher's NGS data alignment using GSNAP or Maq takes data input files from the file system, performs the alignment to the selected reference sequence and adds the resulting contig's consensus to your Sequencher Project with links to detailed data results.

For more information on the Maq algorithm itself, see: *Li, et al, Genome Research, 2008 and Thompson, et al, Nucleic Acids Res., 1994 Nov 17*. Complete documentation for the Maq program can be found online at the Maq: Mapping and Assembly with Qualities web site <http://maq.sourceforge.net/index.shtml>. ~Sequencher has been tested with Maq version 0.6.8 through 0.7.1.

For more information on the GSNAP/GMAP alignment, see: Thomas D. Wu and Colin K. Watanabe, GMAP: a genomic mapping and alignment program for mRNA and EST sequence, *Bioinformatics 2005 21: 1859-1875*. More information regarding GSNAP and GMAP may be found on the web site <http://research-pub.gene.com/gmap/>. ~Sequencher has been tested with the 3-11-2011 release of GSNAP/GMAP

For more information on how to use all of the new NGS features of Sequencher please see the tutorial [Next Gen Sequence Alignment](#). The tutorial is available for download from the Gene Codes website at [www.genecodes.com/training/tutorials](http://www.genecodes.com/training/tutorials). It is also available in the Tutorials folder on the Sequencher 5.0 CD and installed with Sequencher on your computer.

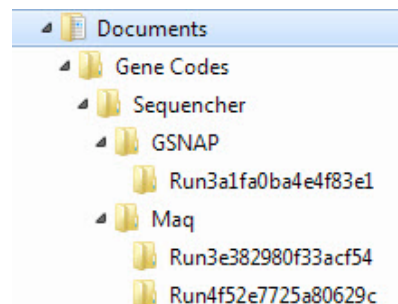
Sequencher expects GSNAP and Maq to be installed in the default location as described in the installation instructions section.

1. Select a sequence from the Project Window to act as a Reference Sequence. You may import GenBank sequences to take advantage of all the sequence annotation information.
2. From the **Assemble** menu, choose the **Align Data Files to Ref Using** command and choose **Maq...** or **GSNAP...**
3. Click the **Select File 1** button to choose the first sample reads file. Optionally, repeat with **Select File 2**.
4. The resulting contig of aligned reads will open for view in **Tablet**. If you prefer to use Maqview, or choose not to view the contig details immediately, select **Maqview** or **None** in the 'View Results Using' section.
5. You may choose Additional Analysis options. Maq supports SNP-calling, while GSNAP supports Methylation analysis for bisulfite-treated DNA and SNP-tolerant alignment given a file listing known SNPs (*formatted as >SNP-ID refname:2..2 AT*).
6. Click the **Align** button.

Regardless of whether you choose to view the aligned reads immediately, the consensus results aligned to the Reference Sequence will be added to your current project. All of the generated data files are saved and may be viewed later by selecting the consensus sequence or contig in your project and choosing the **Contig>Show NGS Data Using** command. You may open the associated SNP or Methylation Analysis files by choosing **Sequence>Analyses>Maq SNP Report**, **GSNAP Methylation Analysis**, or **GSNAP SNP Analysis**.

## CHOOSING THE EXTERNAL DATA LOCATION

Each invocation of a Maq or GSNAP alignment within Sequencher will create several data and log files saved in its own Run folder located in the Gene Codes Home Directory. Links to these unique locations are stored with Maq and GSNAP results in your project file. Although you may not change the Gene Codes directory structure without destroying these links, you may choose to change the location to save this data wherever you wish. By default, the Gene Codes Home directory is in your user Documents folder.



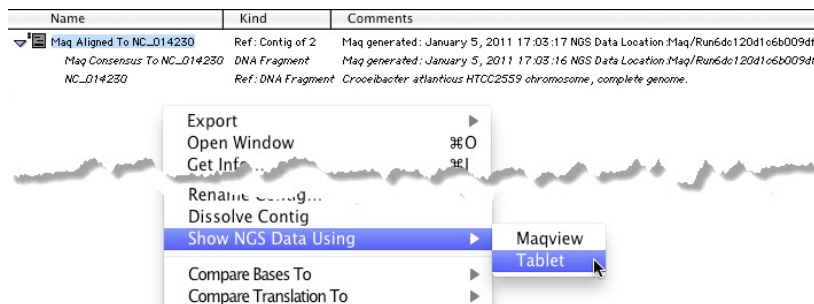
To change the file system location for Next Gen Sequence Data results, go to the **External Data** pane in Sequencher's **User Preferences** and click the **Browse...** button to select a new directory. From that point forward, all new alignment results will be located in the new Gene Codes Home location. If you wish to maintain links to previous results, you must move the old Gene Codes files to the new location.

## USING TABLET AND MAQVIEW VIEWERS WITH SEQUENCHER

Sequencher expects Tablet and Maq to be installed in default locations if you wish to use them.

Tablet requires Java SE 6 version 1.6 to install and run. To check which versions of Java are installed on your machine, open the Java Preferences application (located in /Applications/Utilities/Java Preferences). Java SE 6 cannot be installed on OS X 10.5 32bit machines.

Select a Maq generated contig or consensus sequence and choose **Show NGS Data Using > Tablet (or Maqview)** from the **Contig** menu or rt-click menu.



~Sequencher has been tested with Tablet version 1.11.02.18. ~Sequencher has been tested with Maqview version 0.2.3-0.2.5 on OS X.

## USING CLUSTAL WITH SEQUENCHER

If you are more interested in multiple sequence alignment than fragment assembly, you can download the latest executable of the ClustalW2 algorithm from the Clustal web site and run it from Sequencher on either Macintosh OS X or Windows.

Clustal algorithms and their implementations, including ClustalW2, are an example of multiple sequence alignment. One way to think of the difference between fragment assembly and multiple sequence alignment is to remember that the fragments on the far left and right ends of a large contig may not overlap with each other at all. Multiple sequence alignment assumes all sequences overlap each other and tries to find the best alignment amongst them. This is particularly valuable for Sequencher users involved in systematics research.

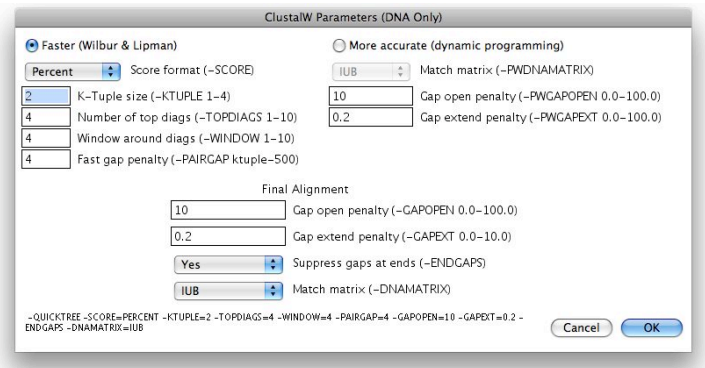
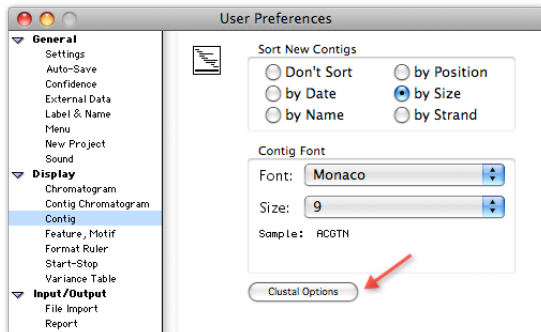
For more information on the algorithm itself, see: *Larkin, et al, Bioinformatics, 2007* and *Thompson, et al, Nucleic Acids Res., 1994 Nov 17*. Complete documentation for the Clustal program can be found at the official Clustal web site at [www.clustal.org](http://www.clustal.org).  
~Sequencher has been tested with ClustalW2 version 2.0.9 through 2.1.

### Invoking Clustal

Select your sequences as you normally would with Sequencher and choose Assemble->Align Using->Clustal to run the ClustalW program.

### Setting and changing Clustal parameters

To change the Clustal parameters, go to the Contig section of the Sequencher **User Preferences**. You will see a button that says "Clustal Options." This is where you can set various parameters used by ClustalW for DNA alignment.



For those of you who may be familiar with using Clustal from the command line, the command line parameters that will be sent to the algorithm are displayed at the bottom of the parameters window.

### Using Clustal with Assemble by Name

Assemble by Name using Clustal is also available. You can group your sequences using the standard naming handles and send them one-at-a-time to the Clustal assembly engine. The result will be a series of individual contigs, each of which was aligned using the Clustal algorithm.

# Build and Install External Tools on Windows

## REQUIREMENTS

Windows XP SP3 or later

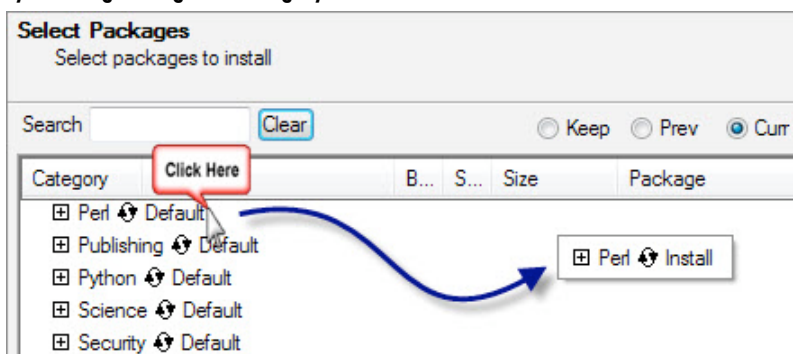
You are logged in as a user with Administrative privileges

## GETTING STARTED

This section contains step-by-step instructions for installing Cygwin, the environment on which the Next Gen applications will be installed and run. It also contains instructions for installing the applications Maq and Maqview, GMAP/GSNAP, Tablet and Clustalw2.

## INSTALL AND CONFIGURE CYGWIN (WINDOWS)

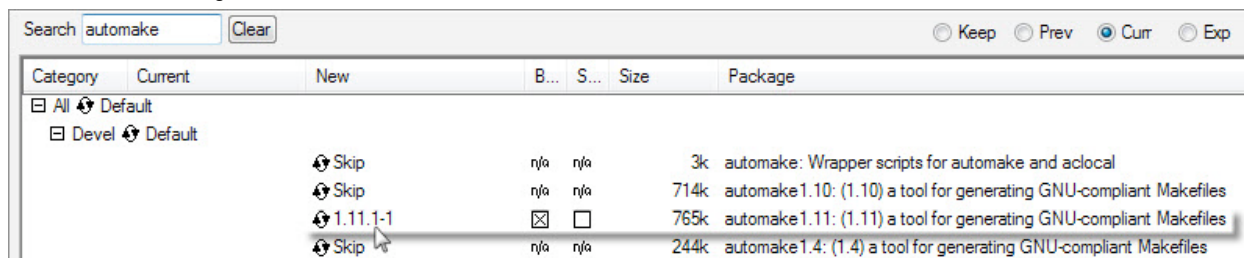
1. Launch your favorite browser and download the Cygwin installer from [www.cygwin.com](http://www.cygwin.com) by clicking the [setup.exe](#) link.
2. Double-click setup.exe to run the installer. Click the **Next** button to advance to the next screen.
3. Click the **Next** button on the next 4 screens, accepting the default options for Choose Installation Type, Choose Installation Directory, Select Local Package Directory, and Select Connection Type.
4. The next screen asks you to Choose A Download Site from the list of available mirrors. Choose one and click **Next**.  
(You may see a Setup Alert dialog warning that this is a major upgrade of Cygwin. Click OK to dismiss it.)
5. Now select the additional packages to install with Cygwin. You'll need to search for and choose 6 packages: Perl, automake, gcc-g++, make, OpenGL and zlib.
  - a. Select Perl by scrolling through the category list to find Perl. Click on the word 'Default' to change it to 'Install'.



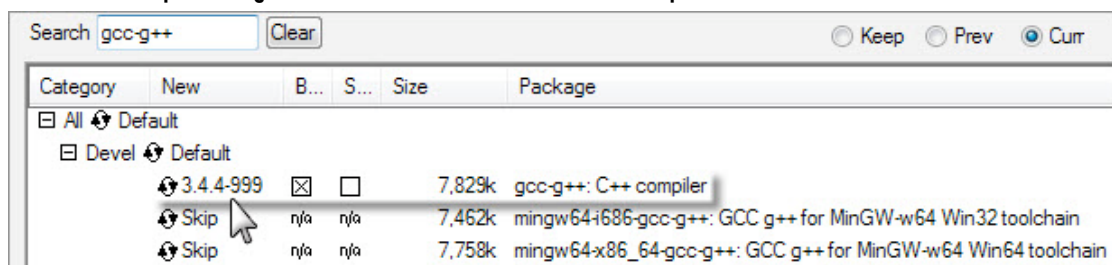
You'll need to search for the next 5 packages by name and expand the Devel category for each one by clicking the + toggle button.

*Don't hit the Enter button while searching or the installation will proceed before you've chosen all of the packages.*

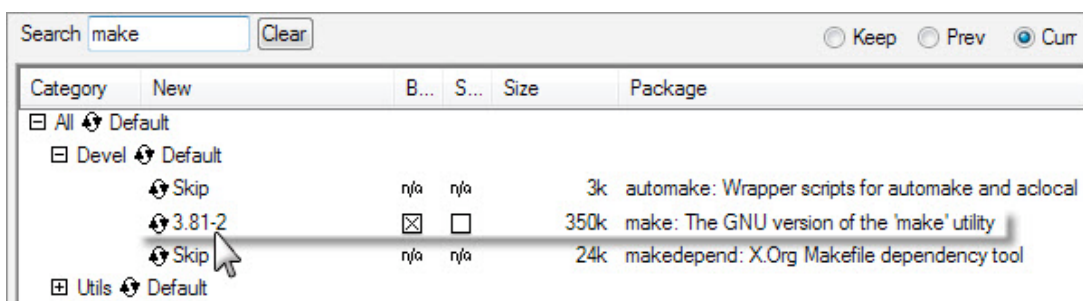
- b. Type "automake" into the Search field and toggle open the Devel category. There are quite a few choices for automake. Find **automake1.11: (1.11) a tool for generating GNU-compliant Makefiles** and click on the word Skip to change it to install the listed version of automake.



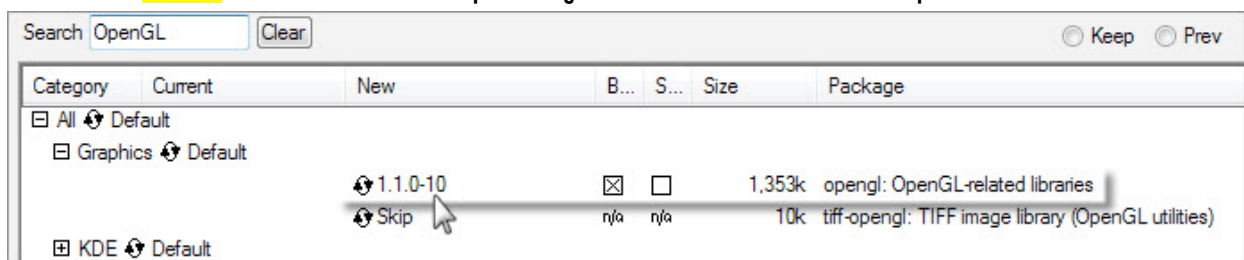
- c. Type “gcc-g++” into the Search field and toggle open the Devel category. Find **gcc-g++: C++ compiler** and click on the word Skip to change it to install the listed version of the compiler.



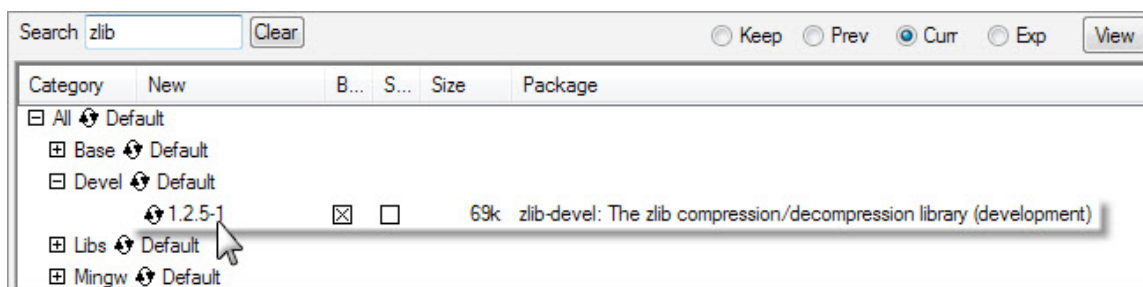
- d. Type “make” into the Search field and toggle open the Devel category. There are quite a few choices for make. Find **make: The GNU version of the ‘make’ utility** and click on the word Skip to change it to install the listed version of make.



- e. Type “OpenGL” into the Search field and toggle open the Graphics category. Find **opengl: OpenGL-related libraries** and click on the word Skip to change it to install the listed version of OpenGL.



- f. Type “zlib” into the Search field and toggle open the Devel category. Click on the word Skip to change it to install the listed version of zlib-devel.



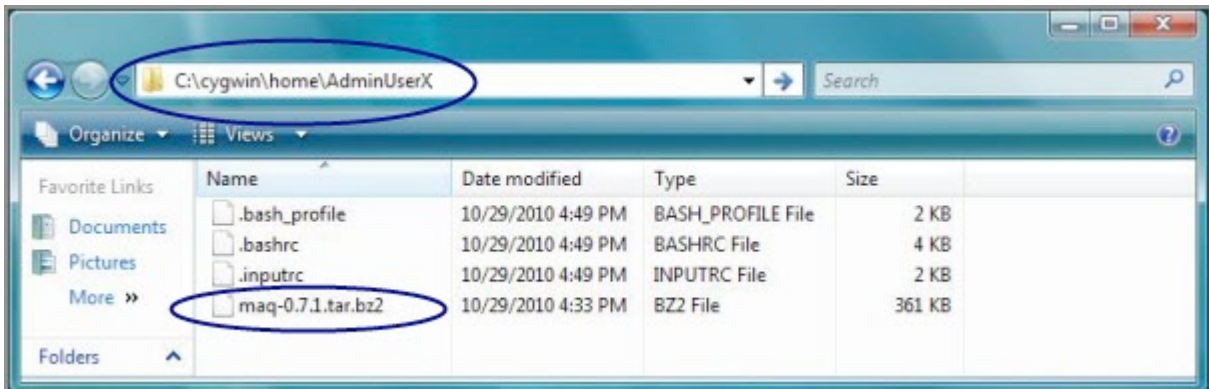
- Click **Next** after selecting the 6 packages to include with the default set. On the next dialog, make sure that the ‘**Select required packages**’ checkbox is checked on, and click the **Next** button to finish download and installation of all packages. *Note: if the download speed is particularly slow, it’s OK to cancel and start over from Step 2, choosing a different mirror.*
- Choose to create icons and click **Finish** to exit the installer. Windows 7 and Vista may present a warning dialog that the program did not install correctly. The installation likely went fine, just dismiss the dialog,

8. Double-click on the Cygwin icon to launch it for the first time. This will finish configuring Cygwin for use by creating your home folder and necessary files inside the cygwin directory, for instance: `C:\cygwin\home\AdminUserX`. Close Cygwin, its installation is complete.

## BUILD AND INSTALL MAQ (WINDOWS)

Installing Maq requires Cygwin installation as described in the Install and Configure Cygwin section.

1. Download the last version 0.7.1 of Maq: [maq-0.7.1.tar.bz2](http://sourceforge.net/projects/maq/files/maq/0.7.1/maq-0.7.1.tar.bz2/download)  
(<http://sourceforge.net/projects/maq/files/maq/0.7.1/maq-0.7.1.tar.bz2/download>)
2. Move the downloaded maq-0.7.1.tar.bz2 file into your cygwin/home/yourUserName folder on your hard drive.



3. Launch Cygwin from the Desktop or Start Menu icon.

This will create a new Cygwin shell window to type your commands into (press the Enter key after each command).

Remember that case matters when typing at the command line.

- Tip: you may Paste into this window by right-clicking on its title bar and choosing Edit => Paste from the context menu.
- Tip: press the tab key when typing out path names to auto-complete file paths and names.

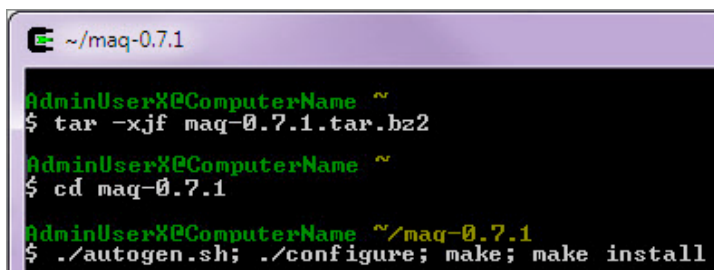
You'll be using Cygwin to unpack the maq-0.7.1 archive, change directories into the newly unpacked folder, build and install maq and its associated files into cygwin\usr\local\bin\.

4. maq-0.7.1.tar.bz2 is a compressed file archive that must be uncompressed before using. Uncompress the archive by typing the following command at the \$ command prompt and then press Enter:

```
tar -xjf maq-0.7.1.tar.bz2
```

5. The next \$ prompt will appear when the archive is unpacked. Change directories into the new maq-0.7.1 folder by typing the following command at this new prompt and press Enter:

```
cd maq-0.7.1
```

A screenshot of a Cygwin terminal window. The title bar shows the path '~/.maq-0.7.1'. The terminal output shows the user typing 'tar -xjf maq-0.7.1.tar.bz2' and 'cd maq-0.7.1'. The prompt changes from '\$' to '~' after each command.

6. Finally, build and install maq by typing the following command and press Enter:

```
./autogen.sh; ./configure; make; make install
```

The space between the semi-colon and the next word is important.

You will see messages being printed on the screen. This may take a few minutes. When the process is complete, another \$ command line prompt will appear.

7. Check that the maq program is installed properly by typing the following command and pressing Enter:

`which maq`

*If you see the following then maq has been installed properly and is ready to use:  
You can now start the Maq program by typing `maq` at the command line prompt.*

```
$ which maq
/usr/local/bin/maq
```

## 8. Exit Cygwin.

## BUILD AND INSTALL MAQVIEW (WINDOWS)

Installing Maqview requires Cygwin installation as described previously.

1. Download the last version 0.2.5 of Maqview: [maqview-0.2.5.tar.gz](http://sourceforge.net/projects/maq/files/maqview/maqview-0.2.5/maqview-0.2.5.tar.gz/download)  
(<http://sourceforge.net/projects/maq/files/maqview/maqview-0.2.5/maqview-0.2.5.tar.gz/download>)
2. Move the downloaded maqview-0.2.5.tar.gz file into your cygwin/home/yourUserName folder on your hard drive (just like Step2 in the Maq installation above).
3. Launch Cygwin from the Desktop or Start Menu icon.

*This will create a new Cygwin shell window to type your commands into (press the Enter key after each command). Remember that case matters when typing at the command line.*

- *Tip: you may Paste into this window by right-clicking on its title bar and choosing Edit => Paste from the context menu.*
- *Tip: press the tab key when typing out path names to auto-complete file paths and names.*

*You'll be using Cygwin to unpack the maqview-0.2.5 archive, change directories into the newly unpacked folder, build and install maqview and its associated files into cygwin\usr\local\bin\.*

4. maqview-0.2.5.tar.gz is a compressed file archive that must be uncompressed before using. Uncompress the archive typing the following command at the \$ command prompt and then press Enter:

```
tar -xzf maqview-0.2.5.tar.gz
```

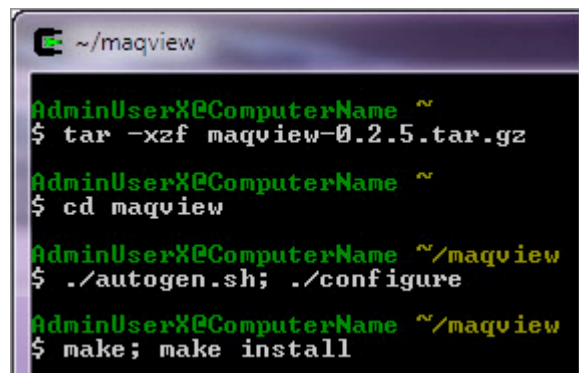
5. The next prompt will appear when the archive is unpacked. Change directories into the new maqview folder by typing the following command at this new prompt and pressing Enter:

```
cd maqview
```

6. Prepare to build Maqview by typing the following command and pressing Enter:

```
./autogen.sh; ./configure
```

You will see messages being printed on the screen. This may take a few minutes. When the process is complete, another \$ command line prompt will appear. There are several more commands to type in this window, so leave it open. You'll get back to it once you change one of the necessary files.



```
~/maqview
AdminUserX@ComputerName ~
$ tar -xzf maqview-0.2.5.tar.gz
AdminUserX@ComputerName ~
$ cd maqview
AdminUserX@ComputerName ~/maqview
$ ./autogen.sh; ./configure
AdminUserX@ComputerName ~/maqview
$ make; make install
```

7. Before continuing to build Maqview, one of the files needs to be modified. Go to the maqview folder inside your cygwin/home/user folder on your hard drive, and open the Makefile file in any text editor. It needs to be edited to include the OpenGL libraries. Do this by finding the "LIBS =" parameter (which should be empty) and modifying it to look like the following:

```
LIBS = -lglut32 -lglu32 -lopengl32 -lglui -lgluix
```

8. Save and exit out of the Makefile after the edit has been made.
9. Install Maqview by typing the following command:

```
make; make install
```

*The space between the semi-colon and the next word is important.*

*You will see messages being printed on the screen. This may take a few minutes. When the process is complete, another \$ command line prompt will appear.*

10. Check that the Maqview program is installed properly by typing the following command and pressing Enter:

`which maqview`

*If you see the following then Maqview has been installed properly and is ready to use:*

```
$ which maqview  
/usr/local/bin/maqview
```

11. You can now start the Maqview program by typing `maqview` at the command line prompt.
12. Exit Cygwin.

## BUILD AND INSTALL GMAP AND GSNAP (WINDOWS)

Installing GMAP/GSNAP requires Cygwin to have already been installed as described previously.

1. Download the latest GMAP/GSNAP from <http://research-pub.gene.com/gmap/>

*These instructions are written for the 2011-03-28 version, but should work for future versions as well.*

### **GMAP: A Genomic Mapping and Alignment Program for mRNA and EST Sequences, and GSNAP: Genomic Short-read Nucleotide Alignment Program**

---

#### **Program**

Source code for both GMAP and GSNAP:

- [Current version 2011-03-28](http://research-pub.gene.com/gmap/src/gmap-gsnap-2011-03-28.tar.gz) (<http://research-pub.gene.com/gmap/src/gmap-gsnap-2011-03-28.tar.gz>)
2. Move the downloaded `gmap-gsnap-2011-03-28.tar.gz` file into the `cygwin/home/yourUserName` folder on your hard drive or Save it there directly as the download location.



3. Launch Cygwin from the Desktop or Start Menu icon.

*This will create a new Cygwin shell window to type your commands into (press the Enter key after each command).*

- Remember that case matters when typing at the command line.
- Tip: you may Paste into this window by right-clicking on its title bar and choosing Edit => Paste from the context menu.
- Tip: press the tab key when typing out path names to auto-complete file paths and names.

*You'll be using Cygwin to unpack the `gmap-gsnap` archive, change directories into the newly unpacked folder, build and install `gmap` and `gsnap` and the associated files into `cygwin\usr\local\bin`.*

4. Unpack the downloaded archive by typing the following command at the \$ command prompt and then press Enter:

```
tar -xzf gmap-gsnap-2011-03-28.tar.gz
```

By default, GSNAP has a maximum read length setting that must be set before compiling the program. That limit can easily be increased to accommodate read lengths of 1000 or more allowing you to align sequence data from different sources. That limit must be changed before building the program. To increase the allowable read length, change the `MAX_QUERYLENGTH` in a text editor before running the build commands in step 6.

5. OPTIONAL: Navigate to your `cygwin\home\UserName` folder on your hard drive and open the `gmap-2011-03-28` folder. Inside the `src` folder, locate the `stage1hr.h` file and open it in any text editor. Change the `MAX_QUERYLENGTH` to the desired length, Save and Close the file.

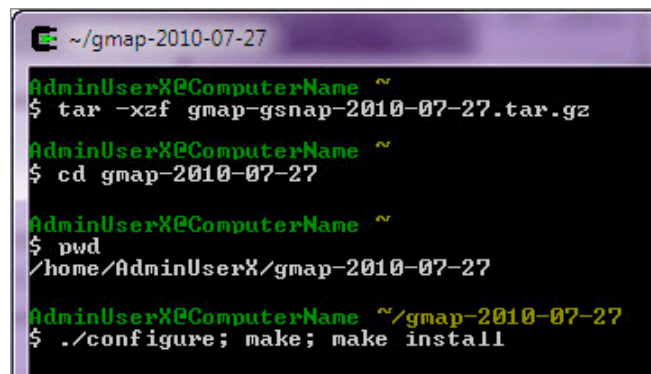
```
#define MAX_QUERYLENGTH 2000
```

6. Return to the cygwin window and change directories into the new folder by typing the following command at the new prompt:

```
cd gmap-2011-03-28
```

7. Finally, build and install GMAP and GSNAP by typing the following command:

```
./configure; make; make install
```



```
~/gmap-2010-07-27
AdminUserX@ComputerName ~
$ tar -xzf gmap-gsnap-2010-07-27.tar.gz
AdminUserX@ComputerName ~
$ cd gmap-2010-07-27
AdminUserX@ComputerName ~
$ pwd
/home/AdminUserX/gmap-2010-07-27
AdminUserX@ComputerName ~/gmap-2010-07-27
$ ./configure; make; make install
```

*The space between the semi-colon and the next word is important.*

You will see messages being printed on the screen. This may take a few minutes. When the process is complete, another command line prompt will appear.

8. Check that GMAP and GSNAP are installed properly by typing the following commands:

`which gsnap` (should match the picture below)

```
$ which gsnap
/usr/local/bin/gsnap
```

`which gmap` (should match the picture below)

```
$ which gmap
/usr/local/bin/gmap
```

*If both programs are properly installed in /usr/local/bin/ then you may start them by typing `gmap` or `gsnap` at the \$ prompt.*

9. Exit Cygwin.

## INSTALL TABLET (WINDOWS)

1. Download the latest version of Tablet from SCRI: [Tablet for Windows \(32 bit\) or Windows \(64 bit\)](#)

*(<http://bioinf.scri.ac.uk/tablet/download.shtml>)*

*Choose to download the version appropriate to your system. Tablet must be installed in the Program Files folder in order to work properly with Sequencher*

2. Save and open the downloaded file.
3. Double-click to run the Tablet Installer, accepting all the default settings.

## INSTALL CLUSTAL (WINDOWS)

1. Download the latest version of ClustalW from [clustal.org](http://www.clustal.org). The current clustalw win.msi installer: [clustalw-2.1-win.msi](#)

*(<http://www.clustal.org/download/current>)*

2. Save the clustalw msi download file.
3. Double-click on the clustalw msi file to launch the installer.
4. Accept all of the installation defaults.

# Build and Install External Tools on Macintosh OS X

## REQUIREMENTS

OS X 10.5.8 or later

You are logged in as a user with Administrative privileges

Developer tools have been installed on the computer

## GETTING STARTED

These instructions will work best for OS X 10.6 64bit Intel Macintosh machines. Installing on 32bit Intel or PPC Macintosh machines may require additional configuring for installation of Maq and Maqview. Tablet requires Java Virtual Machine 1.6, part of the Java SE 6 release which will not run natively on 10.5 32bit Intel or PPC.

### 1. Recommended:

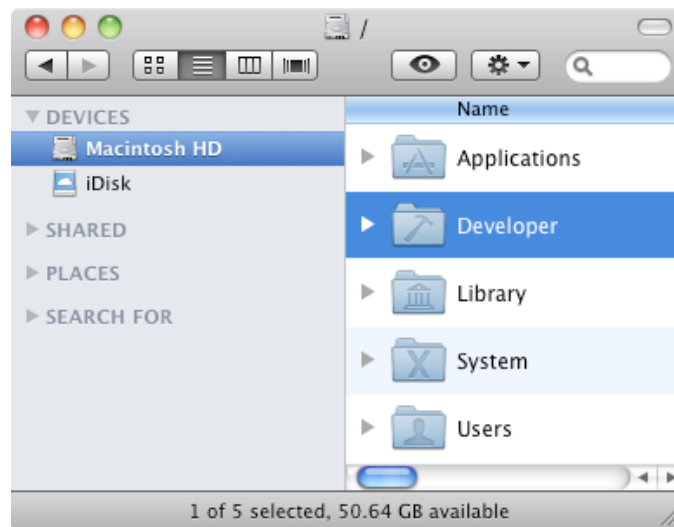
a. Install the latest updates with Software Update.

b. Repair Disk Permissions with Disk Utility.

### 2. Install Xcode if you don't have Developer tools already installed.

*If it's not possible to install the necessary tools, or if you prefer, you may try installing older, pre-built versions of Maq and Maqview and Maqindex. Please skip to the [Installing Pre-Built Binaries](#) section.*

a. If it's already installed then there will be a Developer folder as a peer to the Applications, Library, System, and Users folders on the hard drive.



b. If it's not installed, then insert your original Mac OS X Install DVD and open the Optional Installs folder.

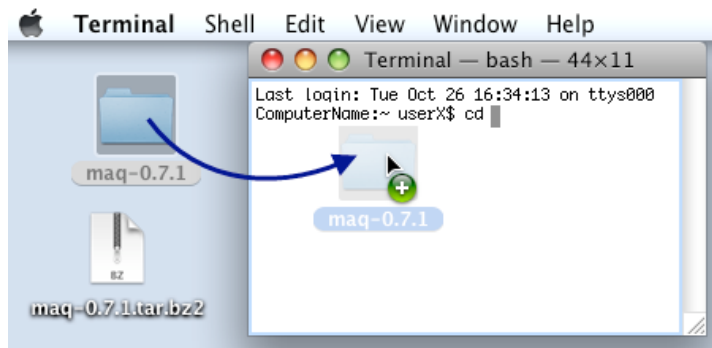
c. Double-click the Xcode.mpkg (or XcodeTools.mpkg inside the Xcode Tools folder for OS X 10.5) installer and install accepting all defaults.

d. Or, you may download the latest Xcode by registering as an Apple Developer at <http://developer.apple.com/programs/register/>.

### 3. Install any updates to Xcode using Software Update.

## BUILD AND INSTALL MAQ (OS X)

1. Download the last version 0.7.1 of Maq: [maq-0.7.1.tar.bz2](http://sourceforge.net/projects/maq/files/maq/0.7.1/maq-0.7.1.tar.bz2/download)  
(<http://sourceforge.net/projects/maq/files/maq/0.7.1/maq-0.7.1.tar.bz2/download>)
2. Double-click to extract the downloaded archive.  
*In the instructions below, the extracted folders are on the Desktop, but you may save them wherever you wish.*
3. Open the Terminal application (located in /Applications/Utilities/Terminal).  
*This will create a new Terminal shell window to type your commands into.*
4. Change directories to the maq-0.7.1 folder by typing `cd` followed by a space. Then drag the maq-0.7.1 folder onto the terminal window and hit Return to get the full file path into that folder (or you can type the full file path).



- a. It should look like this, where ComputerName and userX are replaced with your computer's name and your login account name:  

```
ComputerName:~ userX$ cd /Users/userX/Desktop/maq-0.7.1  
ComputerName:maq-0.7.1 userX$
```
5. Type the following command at the prompt: `./autogen.sh; ./configure` and Return.
  6. Type the following command at the prompt: `make; sudo make install` and Return.
  7. At the Password prompt, type in your user password (you won't see the characters that you type).  
*5 files will be created and installed in the /usr/local/bin directory.*
  8. Check that maq has been built and moved to its correct location by typing the following command:  
`which maq` and press Enter. The result should read `/usr/local/bin/maq`
  9. Confirm that maq has been built properly for your machine by typing `maq` and pressing Enter.  
Program information, including version and commands, should be listed\*:

\*If instead you see:

Bad CPU type in executable,

then you likely have a 32bit machine. You'll have to try the build and installation again with an additional step.

### Building Maq for 32bit Macintosh Computers

Some hardware and software combinations for Macintosh require additional modification of the distribution before Maq can be built properly.

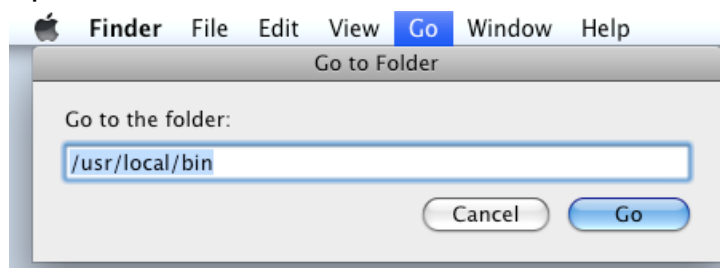
10. Delete the maq-0.7.1 folder and repeat steps 2 through 5.
11. After running the configure command in step 5, open the maq-0.7.1 folder and find the newly created file named 'Makefile'. Open Makefile in a text editor like Text Edit.
12. Search for and delete the term: `'-m64'` (there should be two instances). Save and close Makefile.
13. Continue from step 6.

## BUILD AND INSTALL MAQVIEW (OS X)

1. Download the last version 0.2.5 of Maqview: [maqview-0.2.5.tar.gz](http://sourceforge.net/projects/maq/files/maqview/maqview-0.2.5/maqview-0.2.5.tar.gz/download)  
(<http://sourceforge.net/projects/maq/files/maqview/maqview-0.2.5/maqview-0.2.5.tar.gz/download>)
2. Double-click to extract the downloaded archive.  
*In the instructions below, the extracted folders are on the Desktop, but you may save them wherever you wish.*
3. Open the Terminal application (located in /Applications/Utilities/Terminal).  
*This will create a new Terminal shell window to type your commands into.*
4. Change directories to the maqview folder by typing `cd` followed by a space. Then drag the maqview folder onto the terminal window and hit Return to get the full file path into that folder (or you can type the full file path).  
ComputerName:~ userX\$ `cd /Users/userX/Desktop/maqview`  
ComputerName:maqview userX\$
5. Type the following command: `./autogen.sh; ./configure` and Return.
6. Type the following command: `make; sudo make install` and Return.
7. At the Password prompt, type in your user password.  
*4 files will be created and installed in the /usr/local/bin directory.*
8. Check that Maqview has been built and moved to its correct location by typing the following command:  
`which maqview` and press Enter. The result should read `/usr/local/bin/maqview`
9. Confirm that Maqview has been built properly for your machine by typing `maqview` and pressing Enter. Program information, including version and commands, should be listed.

## INSTALLING PRE-BUILT BINARIES (IF YOU CANNOT BUILD THEM AS LISTED ABOVE)

1. Open the Terminal application (located in /Applications/Utilities/Terminal).  
*This will create a new Terminal shell window to type your commands into.*
2. Type the following command at the \$ prompt: `sudo mkdir -p /usr/local/bin` and Return.
3. At the Password prompt, type in your user password. This will create the /usr/local/bin folder if it does not already exist (nothing will happen if it's already there).
4. Open the /usr/local/bin folder by choosing Go to Folder... from the Go menu. Click the Go button to open the folder. You'll be copying the maq file into this location.



5. Download Maq version 0.7.0 darwin archive: [maq-0.7.0\\_i386-darwin.tar.bz2](http://sourceforge.net/projects/maq/files/maq/0.7.0/maq-0.7.0_i386-darwin.tar.bz2/download) and the Maqview version 0.2.3 [maqview-0.2.3\\_i386-darwin.tar.bz2](http://sourceforge.net/projects/maq/files/maqview/0.2.3/maqview-0.2.3_i386-darwin.tar.bz2/download)  
[http://sourceforge.net/projects/maq/files/maq/0.7.0/maq-0.7.0\\_i386-darwin.tar.bz2/download](http://sourceforge.net/projects/maq/files/maq/0.7.0/maq-0.7.0_i386-darwin.tar.bz2/download)  
[http://sourceforge.net/projects/maq/files/maqview/0.2.3/maqview-0.2.3\\_i386-darwin.tar.bz2/download](http://sourceforge.net/projects/maq/files/maqview/0.2.3/maqview-0.2.3_i386-darwin.tar.bz2/download)

6. Double-click the downloaded archive files and open the resulting darwin folders.
7. Drag the maq, maq.pl, maqindex and maqview files onto the open bin folder. You'll have to provide an Administrator's user name and password to modify this folder.
8. Click the Authenticate button and type in the user name and password. Click OK.

## BUILD AND INSTALL GMAP AND GSNAP (OS X)

1. Download the latest GMAP/GSNAP from <http://research-pub.gene.com/gmap/>

*These instructions are written for the 2011-03-28 version, but should work for future versions as well.*

### **GMAP: A Genomic Mapping and Alignment Program for mRNA and EST Sequences, and GSNAP: Genomic Short-read Nucleotide Alignment Program**

---

#### **Program**

Source code for both GMAP and GSNAP:

- [Current version 2011-03-28](http://research-pub.gene.com/gmap/src/gmap-gsnap-2011-03-28.tar.gz) (<http://research-pub.gene.com/gmap/src/gmap-gsnap-2011-03-28.tar.gz>)

2. Double-click to extract the downloaded archive.

*In the instructions below, the extracted folders are on the Desktop, but you may save them wherever you wish.*

By default, GSNAP has a maximum read length setting that must be set before compiling the program. That limit can easily be increased to accommodate read lengths of 1000 or more allowing you to align sequence data from different sources. That limit must be changed before building the program. To increase the allowable read length, change the `MAX_QUERYLENGTH` in a text editor before running the build commands in step 4.

3. **OPTIONAL:** Open the extracted `gsnap-2011-03-28` folder. Inside the `src` folder, locate the `stage1hr.h` file and open it in a text editor. Change the `MAX_QUERYLENGTH` to the desired length, Save and Close the file.

```
#define MAX_QUERYLENGTH 2000
```

4. Open the Terminal application (located in `/Applications/Utilities/Terminal`).

*This will create a new Terminal shell window to type your commands into.*

5. Change directories to the `gmap-2010-07-27` folder by typing `cd` followed by a space. Then drag the `gsnap-2010-07-27` folder onto the terminal window and hit Return to get the full file path into that folder (or you can type the full file path).

- a. It should look like this, where `ComputerName` and `userX` are replaced with your computer's name and your login account name:

```
ComputerName:~ userX$ cd /Users/userX/Desktop/gmap-2011-03-28
ComputerName: gmap-2011-03-28userX$
```

6. Build and install GMAP and GSNAP by typing the following: `./configure; make; sudo make install` and Return.
7. At the Password prompt, type in your user password. *GMAP and GSNAP tools will be installed in the `/usr/local/bin` directory.* You will see messages being printed on the screen. This may take a few minutes. When the process is complete, another command line prompt will appear.
8. Check that GMAP and GSNAP are installed properly by typing the following commands:

```
which gsnap should display /usr/local/bin/gsnap
```

```
which gmap should display /usr/local/bin/gmap
```

*If both programs are properly installed in `/usr/local/bin/` then you may start them by typing `gmap` or `gsnap` at the command line prompt.*

## **INSTALL TABLET (OS X)**

Tablet requires Java SE 6 version 1.6 to install and run. To check which versions of Java are installed on your machine, open the Java Preferences application (located in /Applications/Utilities/Java Preferences). Java SE 6 cannot be installed on OS X 10.5 32bit machines.

1. Download the latest version of Tablet from SCRI: [Tablet for Apple Mac OS X](http://bioinf.scri.ac.uk/tablet/download.shtml)  
(<http://bioinf.scri.ac.uk/tablet/download.shtml>)
2. Save and open the downloaded DMG file.
3. Double-click to run the Tablet Installer accepting all the default settings.

## **INSTALL CLUSTAL (OS X)**

1. Download the latest version of ClustalW from clustal.org. The current clustalw macosx.dmg: [clustalw-2.1-macosx.dmg](http://www.clustal.org/download/current)  
(<http://www.clustal.org/download/current>)
2. A window containing the clustalw folder will open. If you don't see this folder, please double-click on the downloaded disk image (.dmg) file.
3. Open the folder and drag the clustalw2 application to the Applications folder on your Macintosh Hard Drive.