

# SEQUENCHER®

## Installing DNA-Seq Tools for Sequencher

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## Introduction

With the advent of Next-Generation sequencing, several new external alignment algorithms have been integrated into **Sequencher** to align the large quantity of data produced by Next-Generation sequencing machines. These algorithms, **BWA-MEM**, **GSNAP**, **Maq**, and **Velvet**, join **Clustal** (the very first external algorithm to be added to **Sequencher**). The **MUSCLE** external algorithm, while not an NGS algorithm, has also been added to **Sequencher**. The **BWA-MEM**, **GSNAP**, **Maq**, **MUSCLE**, and **Velvet** algorithms were written to run on Unix-based systems. Mac OS X is a Unix-based operating system. We have built native binaries for Windows, so that there is no longer any 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. The **FastQC** installer is also a separate installer. Once **FastQC** is installed, you can generate FastQ Quality Control Reports and display them in **Sequencher**.

## Quick and Easy Set Up for NGS Aligners and Assemblers

### INSTALLING EXTERNAL APPLICATIONS ON WINDOWS

The Windows installer will place **BWA-MEM**, **GSNAP**, **Maq**, **Maqview**, **MUSCLE**, **Samtools**, and **Velvet** and their related files where they need to be. That location is Programs Files or Program Files (x86)/Gene Codes/Sequencher External Tools. **NOTE THAT GSNAP IS SUPPORTED AND INSTALLABLE ON 64-BIT SYSTEMS ONLY SO THIS INSTALLER WILL NOT INSTALL IT ON 32-BIT SYSTEMS.**

1. Download the DNA-Seq Tools Installer zip archive from the Gene Codes website at <http://www.genecodes.com/download>.
2. Extract/unzip the archive and open the installer folder if it is not opened for you. It will contain *DNA-SeqToolsInstaller.exe*, *Tablet*, *src*, *links*, and *ClustalW2* folders, and a *License.txt* file.
3. Double-click on *DNA-SeqToolsInstaller.exe*. Accept the installation defaults including the license agreement terms.
4. A new window appears which shows the progress of the installer. When the installation is complete, **BWA-MEM**, **GSNAP** (64-bit systems only), **Maq**, **Maqview**, **MUSCLE**, **Samtools**, and **Velvet** are ready for use in **Sequencher**.

### INSTALLING FASTQC ON WINDOWS

To install **FastQC** on your computer, follow these steps:

1. Download the FastQC Installer zip archive from the Gene Codes website at <http://www.genecodes.com/download>.

2. Extract/unzip the archive and open the installer folder if it is not opened for you. It will contain *FastQCInstaller.exe*.
3. Double-click on *FastQCInstaller.exe*. Accept the installation defaults including the license agreement terms.
4. A new window appears which shows the progress of the installer. When the installation is complete, **FastQC** is ready for use in **Sequencher**.
5. Note that Java version 1.7 or newer is needed in order to use **FastQC** with **Sequencher** on Windows 7. Version 1.8 or newer is needed on Windows 8.1. If Java is not installed on your computer, you can download it from <https://java.com/en/download>.

## 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` installer is for 64-bit systems and the `tablet_windows_x86` 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. If you are not an Administrator user, get assistance with getting administrator privileges so you can install.
3. Accept all of the installation defaults. You will be prompted for a destination location for the installation. The default location is `Program Files\Tablet`. Don't change this location.

## INSTALLING CLUSTAL ON WINDOWS

To install **Clustal** on your computer, follow these steps:

1. Open the **ClustalW2** folder.
2. **Clustal** requires administrator privileges in order to install. If you are an Administrator user, double-click on the **Clustal** installer. If you are not an Administrator user, get assistance with getting administrator privileges so you can install.
3. Accept all of the installation defaults. You will be prompted for a destination location for the installation. The default location is `Program Files\ClustalW2` for 32-bit systems or `Program Files (x86)\ClustalW2` for 64-bit systems. Don't change this location.

## INSTALLING EXTERNAL APPLICATIONS ON MAC OS X

You must be an Administrator or have an administrator's password in order to install the external tools on Mac. If you are installing on a computer with OS X 10.8 (Mountain Lion) or above, change your security setting in User Preferences to Allow applications downloaded from: Anywhere. **NOTE THAT GSNAP IS SUPPORTED AND INSTALLABLE ON 64-BIT SYSTEMS ONLY SO THIS INSTALLER WILL NOT INSTALL IT ON 32-BIT SYSTEMS.**

For a Standard Install, the Mac OS X installer will place **Maq, Maqview, MUSCLE, Samtools, Velvet**, and their related files where they need to be. That location is /usr/local/bin. For **BWA-MEM** and **GSNAP**, that location is /usr/local/GeneCodes. For a Custom install, the Mac OS X installer will place **Samtools** and any packages you select where they need to be. Again, that location is /usr/local/bin (or /usr/local/GeneCodes for **BWA-MEM** and **GSNAP**).

1. Download the DNA-Seq Tools installer disk image file (.dmg) from the Gene Codes website at <http://www.genecodes.com/download>.
2. Double-click on the downloaded dmg file.
3. Double-click on the new volume icon named 'DNA-Seq Tools Installer' to open it if it doesn't open automatically.
4. Double-click on the *DNA-Seq Tools* package to begin installation.
5. For a Standard install, accept installation defaults, including the license agreement. For a Custom install, again, accept installation defaults, except on the Installation Type page, click on the Customize button and select the specific packages you wish to install.
6. 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, **BWA-MEM, GSNAP** (64-bit systems only), **Maq, Maqview, MUSCLE, Samtools**, and **Velvet** are now ready for use in **Sequencher**.
7. Eject the disk image icon from your desktop unless you are planning to install **Clustal** or the **Tablet** viewer next.

## INSTALLING FASTQC ON MAC OS X

To install **FastQC** on your computer, follow these steps. The Mac OS X installer will place **FastQC** and its related files where they need to be. That location is /usr/local/GeneCodes.

1. Download the FastQC installer disk image file (.dmg) from the Gene Codes website at <http://www.genecodes.com/download>.
2. Double-click on the downloaded dmg file.
3. Double-click on the new volume icon named 'FastQC Installer' to open it if it doesn't open automatically.
4. Double-click on the *FastQC Installer* package to begin installation.
5. Accept installation defaults including the license agreement.
6. 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. **FastQC** is now ready for use in **Sequencher**.
7. Eject the disk image icon from your desktop unless you are planning to install **Clustal** or the **Tablet** viewer next.
8. Note that Java version 1.6 or newer is needed in order to use **FastQC** with **Sequencher** on Mac but the version may vary depending on your OS. If Java is not installed on your computer, you can download it from <https://java.com/en/download>.

## 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 Other Installers folder icon to open the folder.
3. If you are on Mountain Lion or above, change the security setting for 'Allow applications downloaded from:' to Anywhere.
4. Double-click on the *tablet.dmg* file to open the installer.
5. Double-click on the *Tablet Installer* to start the installation.
6. Accept all of the installation defaults. You are prompted for a destination location for the installation. The default location is Applications. Don't change this location.
7. Eject the disk image icon from your desktop if you are done.
8. If you are on Mountain Lion or above and you changed the 'Allow applications downloaded from:' security setting to Anywhere, change it back to 'Mac App Store and identified developers'.

## INSTALLING CLUSTAL ON MAC OS X

To install **Clustal** on your computer, follow these steps:

1. By default, **Clustalw2** needs to be in the Applications folder and that is where **Sequencher** expects to find it.
2. Double-click on the Other Installers folder icon to open the folder.
3. Double-click on the *clustalw2.dmg* file.
4. Double-click on the *clustalw-2.1-macosx* folder to open the folder.
5. Drag the clustalw2 application to the Applications folder on your Macintosh Hard Drive.
6. If you are on Mountain Lion or above, double-click on the clustalw2 application to run it once.
7. Eject the disk image icon from your desktop if you are done.

## MORE INFORMATION

If you would like more information on **BWA-MEM**, **FastQC**, **Maq**, **GSNAP**, **Velvet**, or working with MIDs, refer to the NGS for DNA and RNA-Seq chapter of the Sequencher User Manual, chapter 16. You will find this manual in the **Sequencher** installation folder. You can also find additional information in the following tutorials: "Next Gen Sequence Alignment", "Advanced Next Gen Sequence Alignment", "De Novo Sequence Assembly", "FastQ Quality Control Reports", and "Multiplex IDs with BWA, GSNAP, and Velvet".