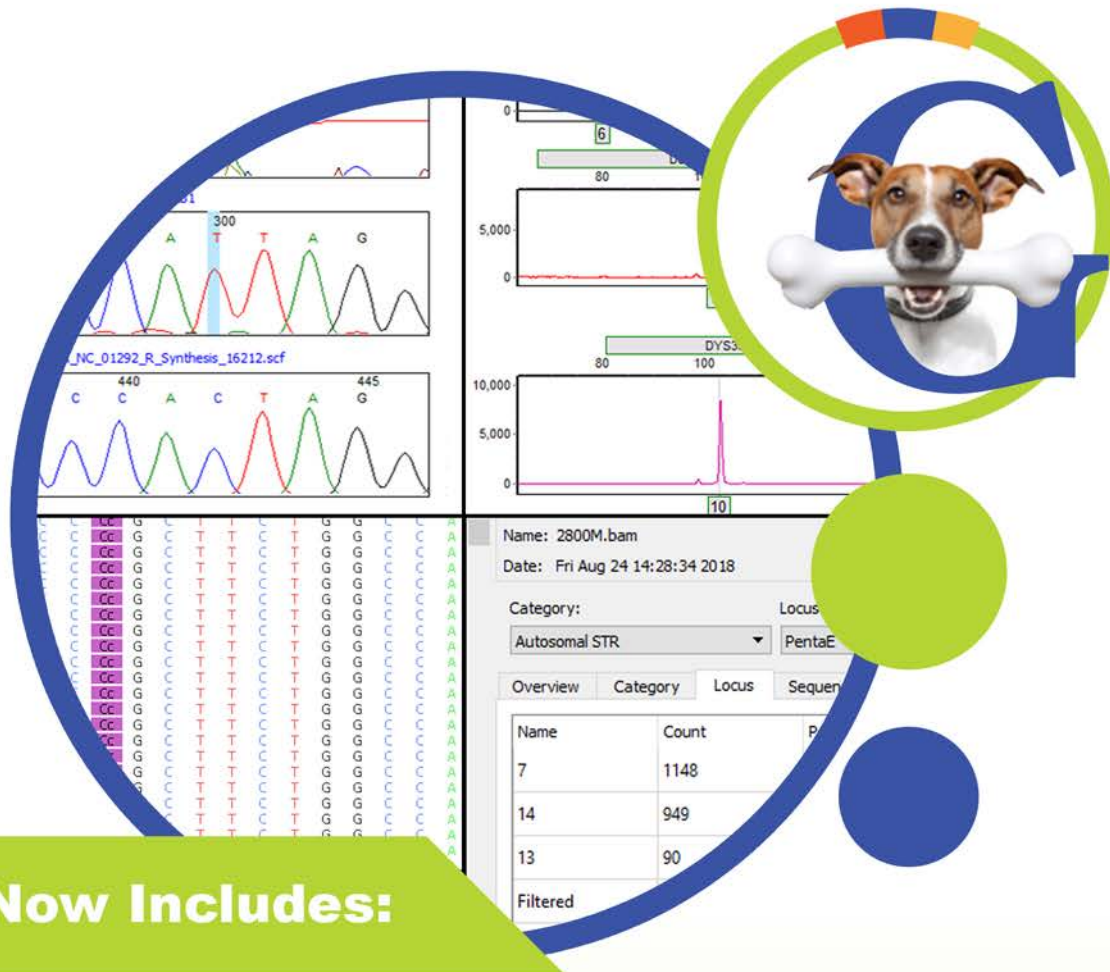


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SoftGenetics, LLC
100 Oakwood Ave, Suite 350
State College, PA 16803
814-237-9340
814-237-9343
888-791-1270
www.softgenetics.com

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Preface

Welcome to the *GeneMarkerHTS User's Manual*. The purpose of the *GeneMarkerHTS User's Manual* is to answer your questions and guide you through the procedures necessary to use the GeneMarkerHTS application efficiently and effectively.

Using the manual

You will find the *GeneMarkerHTS User's Manual* easy to use. You can simply look up the topic that you need in the table of contents or the index. Later, in this Preface, you will find a brief discussion of each chapter to further assist you in locating the information that you need.

Special information about the manual

The *GeneMarkerHTS User's Manual* has a dual purpose design. It can be distributed electronically and then printed on an as-needed basis, or it can be viewed online in its fully interactive capacity. If you print the document, for best results, it is recommended that you print it on a duplex printer; however, single-sided printing will also work. If you view the document online, a standard set of bookmarks appears in a frame on the left side of the document window for navigation through the document. For better viewing, decrease the size of the bookmark frame and use the magnification box to increase the magnification of the document to your viewing preference.

Conventions used in the manual

The *GeneMarkerHTS User's Manual* uses the following conventions:

- Information that can vary in a command—variable information—is indicated by alphanumeric characters enclosed in angle brackets; for example, <Project Name>. Do not type the angle brackets when you specify the variable information.
- A new term, or term that must be emphasized for clarity of procedures, is *italicized*.
- Page numbering is “online friendly.” Pages are numbered from 1 to x, *starting with the cover* and ending on the last page of the index.



Although numbering begins on the cover page, this number is not visible on the cover page or front matter pages. Page numbers are visible beginning with the first page of the table of contents.

Preface

- This manual is intended for both print and online viewing.
 - If information appears in [blue](#), it is a hyperlink. Table of Contents and Index entries are also hyperlinks. Click the hyperlink to advance to the referenced information.

Assumptions for the manual

The *GeneMarkerHTS User's Manual* assumes that:

- You are familiar with Windows-based applications and basic Windows functions, commands, and navigational elements.
- References to any third-party standards or third-party software functions were current as of the release of this version of GeneMarkerHTS, and might have already changed.

Organization of the manual

In addition to this Preface, the *GeneMarkerHTS User's Manual* contains the following chapters:

- [Chapter 1, "Getting Started with GeneMarkerHTS," on page 13](#) details the installation requirements for GeneMarkerHTS, installing GeneMarkerHTS, and activating your account. It also explains how to launch GeneMarkerHTS and provides an overview of the major navigational elements for GeneMarkerHTS.
- [Chapter 2, "GeneMarkerHTS Project Setup and Management," on page 49](#) details the New Project dialog box, which provides all the options for adding a new project in GeneMarkerHTS. It also explains how to open and work with existing projects in GeneMarkerHTS.
- [Chapter 3, "GeneMarkerHTS Viewers and Reports," on page 73](#) details the three viewers that are available in GeneMarkerHTS application for viewing and working with the results of an STR/mtDNA projects: the Mito Alignment Viewer, the STR Results Analysis Viewer, and the Comparison Viewer. It also details the different reports that are available for your mtDNA alignment results.
- [Appendix A, "GeneMarkerHTS Application Settings," on page 145](#) details the GeneMarkerHTS Application Settings dialog box, which provides a variety of settings for customizing an GeneMarkerHTS instance to best suit your working needs.
- The [Glossary on page 161](#) is an alphabetical list of the words or phrases that are relevant to the understanding and use of GeneMarkerHTS.

Chapter 1

Getting Started with GeneMarkerHTS

The GeneMarkerHTS software application provides a streamlined workflow for mtDNA data analysis from massively parallel sequencing (MPS) methods. The software also includes analysis of targeted amplicons from STRs and Y-STRs. This chapter details the installation requirements for GeneMarkerHTS, installing GeneMarkerHTS, and activating your account. It also explains how to launch GeneMarkerHTS and provides an overview of the major navigational elements for GeneMarkerHTS. It also details how to customize the layout and display of your GeneMarkerHTS instance.

This chapter covers the following topics:

- [“GeneMarkerHTS System Requirements” on page 15.](#)
- [“Installing GeneMarkerHTS” on page 16.](#)
- [“Installing the SoftGenetics License Server Manager” on page 20.](#)
- [“Registering a GeneMarkerHTS Network License” on page 25.](#)
- [“Registering a GeneMarkerHTS Local License” on page 37.](#)
- [“GeneMarkerHTS Registration Common Error Messages” on page 43.](#)
- [“Starting GeneMarkerHTS” on page 45.](#)
- [“The GeneMarkerHTS Main Window” on page 46.](#)

Chapter 1
Getting Started with GeneMarkerHTS

GeneMarkerHTS System Requirements

GeneMarkerHTS is currently available only for the Windows operating system. You must have Administrator rights for the computer on which you are installing GeneMarkerHTS.

Component	Description
OS	64-bit Windows 10 or greater that are within Windows Extended Support.
Processor	Intel Core i5 3rd generation (or equivalent) or better
Memory	16 GB RAM (Minimum)
Hard Drive	250 GB of free space (SSD recommended)
Monitor	1366 x 768 or higher resolution monitor

Installing GeneMarkerHTS

You must contact SoftGenetics directly to obtain the necessary executable file for installing GeneMarkerHTS. Note the following caveats when installing GeneMarkerHTS:

- You install GeneMarkerHTS as a standalone application on a Windows client.
- By default, when you install GeneMarkerHTS, the License Type is set to FullAligner, which is applicable for a commercial (purchased) installation.
 - A non-commercial installation of GeneMarkerHTS requires that you set the License Type to Trial. A Trial license is valid for 35 days. After 35 days, to continue using GeneMarkerHTS, you must set the License Type to FullAligner and purchase a network or local license.
 - A commercial installation of GeneMarkerHTS requires either a network license or a local license.
 - For a network license, you must also install the SoftGenetics License Server Manager (LSM). The network installation of GeneMarkerHTS is for an unlimited number of clients as long as the clients are all on the same network as the LSM.
 - For a local license, you can install GeneMarkerHTS on only a single client. The SoftGenetics LSM is not required. You simply register the product.
- A new version of GeneMarkerHTS does not replace the existing version of GeneMarkerHTS on a client during installation, even for point release/bug fix installations. Each version of GeneMarkerHTS is installed in its own unique directory. You can run multiple versions of GeneMarkerHTS on the same client.
- The GeneMarkerHTS installation requires the Microsoft Visual C++ 2015-2022 Redistributable. If this redistributable is already installed on the client on which you are installing GeneMarkerHTS, then no additional action is required during installation; otherwise, the GeneMarkerHTS Installation wizard installs the necessary redistributable.
- A default installation directory—C:\Program Files\SoftGenetics\GeneMarkerHTS—is specified for GeneMarkerHTS but you can select a different directory.
- A shortcut icon for the application is created and placed on the client desktop.
- The Start menu folder in which the shortcut to the application is created is set to SoftGenetics\GeneMarkerHTS v< >, but you have the option of creating a new folder, or you can decline the creation of the shortcut.

[Figure 1-1 on page 17](#) through [Figure 1-7 on page 19](#) show all the possible wizard pages for the installation of GeneMarkerHTS. The wizard page that is shown in [Figure 1-5 on page 18](#) opens only if the wizard must install the required Microsoft redistributable. After installation is complete, continue to one of the following as appropriate:

- For a Trial license: [“To set a Trial license” on page 150.](#)
- For a network license: [“Installing the SoftGenetics License Server Manager” on page 20.](#)
- For a local license: [“Registering a GeneMarkerHTS Local License” on page 37.](#)

Figure 1-1: GeneMarkerHTS Installation Wizard, Page 1

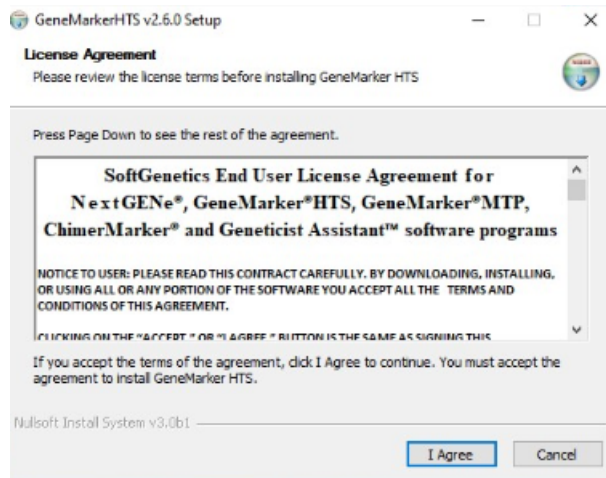


Figure 1-2: GeneMarkerHTS Installation Wizard, Page 2

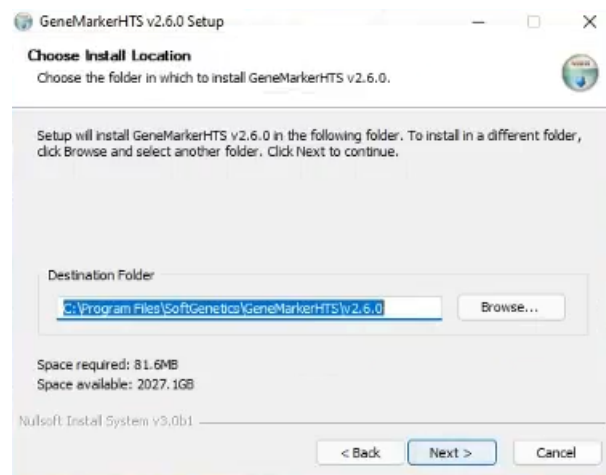
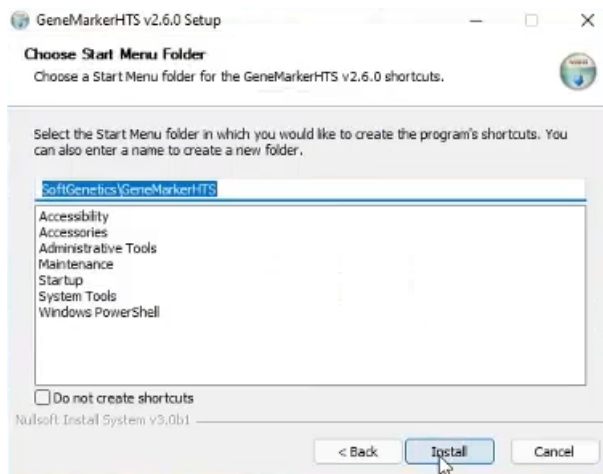


Figure 1-3: GeneMarkerHTS Installation Wizard, Page 3



Chapter 1
Getting Started with GeneMarkerHTS

Figure 1-4: GeneMarkerHTS Installation Wizard, Page 4

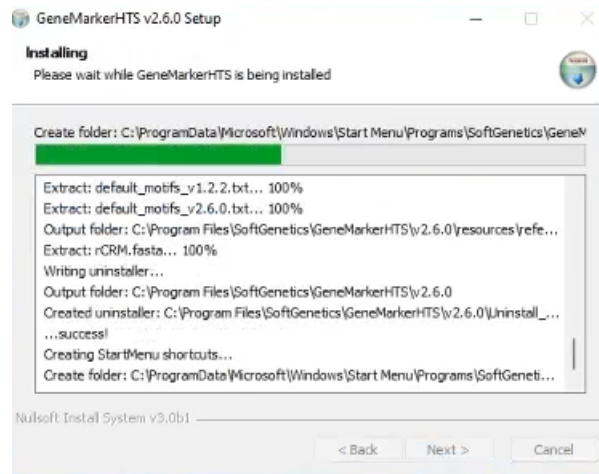


Figure 1-5: GeneMarkerHTS Installation Wizard, Page 5

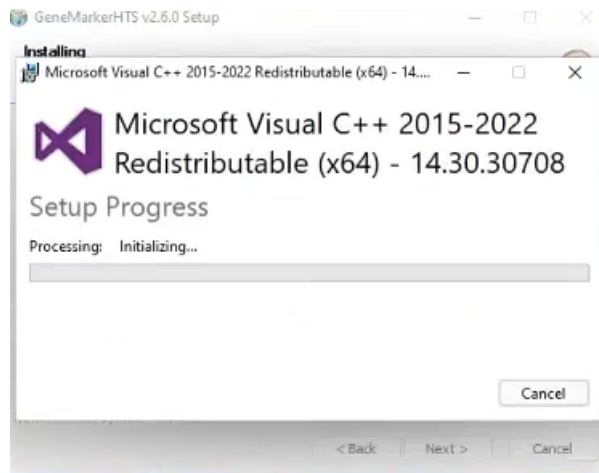


Figure 1-6: GeneMarkerHTS Installation Wizard, Page 6

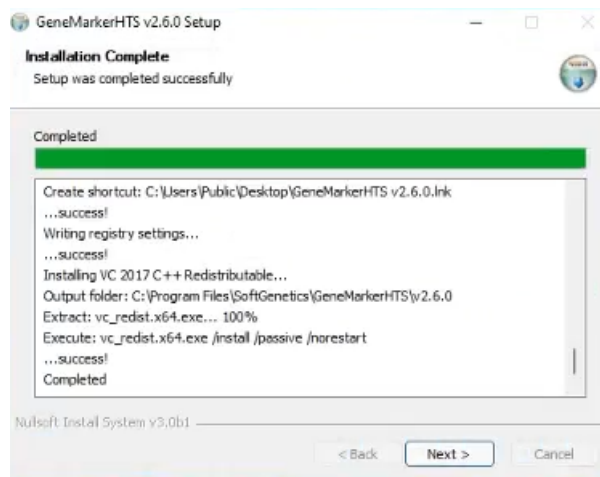


Figure 1-7: GeneMarkerHTS Installation Wizard, Page 7



To facilitate the registration of GeneMarkerHTS to the SoftGenetics LSM, SoftGenetics strongly recommends that you leave the default option of Start GeneMarkerHTS selected on the last page of the GeneMarkerHTS installation wizard.

Installing the SoftGenetics License Server Manager

If you have installed a *purchased* version of GeneMarkerHTS, and you have *not* installed any other SoftGenetics products that require the SoftGenetics License Server Manager (LSM), then you must also install the SoftGenetics LSM. The LSM is a central location either locally or on a network where software application licenses are stored and that GeneMarkerHTS users can access. The full installation of the SoftGenetics LSM consists of the following steps:

1. Installing the LSM.*
2. Registering GeneMarkerHTS for the LSM.
3. Configuring GeneMarkerHTS to connect to the LSM.



**If you have installed other SoftGenetics products that require the SoftGenetics LSM, then you must simply register GeneMarkerHTS with the LSM. See [“Registering a GeneMarkerHTS Network License” on page 25.](#)*

Before you install the SoftGenetics LSM, note the following:

- You must contact SoftGenetics for assistance in obtaining the executable to install the license server.
- You must install the LSM on a Windows client or server that is always on and running. You can install the LSM on the same client that is running GeneMarkerHTS.
- You must be an administrator of the Windows client or server on which you are installing the LSM. To satisfy the UAC settings on the client or server, you might also need to run the LSM setup file as an administrator. (After you download the file, you can right-click it, and then on the context menu that opens, select Run as Administrator.)
- To allow for online registration of the license server, the server/client on which you are installing the LSM must be connected to the Internet; otherwise, offline registration is required.
- The GeneMarkerHTS installation requires the Microsoft Visual C++ 2017 Redistributable. If this redistributable is already installed on the computer on which you are installing the LSM, then no additional action is required during installation; otherwise, the LSM Installation wizard installs this redistributable.
- The LSM is installed in the following directory, but you have the option of selecting a different directory: C:\Program Files (x86)\SoftGenetics\License Server.
- The Start menu folder in which the shortcut to the LSM is created is set to SoftGenetics\License Server, but you have the option of creating a new folder, or you can decline the creation of the shortcut.
- The network installation of GeneMarkerHTS is for an unlimited number of clients; however, you must purchase a license for each concurrent user.



SoftGenetics strongly recommends that you leave the default option of Start License Server selected on the last page of the installation wizard; otherwise, you must manually start the LSM before you can register a GeneMarkerHTS network license.

To install the SoftGenetics LSM

1. Download the SoftGenetics LSM Installation file, and then launch and run SrvSetup_<>_exe to install the LSM.

Figure 1-8 below through Figure 1-15 on page 23 show all the possible wizard pages that can open when you are installing the SoftGenetics LSM. The wizard page that is shown in Figure 1-12 on page 22 opens only if the wizard must install the required Microsoft redistributable.

Figure 1-8: SoftGenetics License Server Installation wizard, Wizard page 1

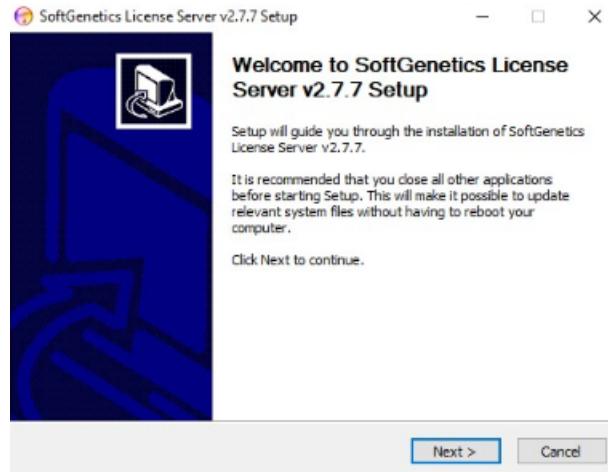


Figure 1-9: SoftGenetics License Server Installation wizard, Wizard page 2

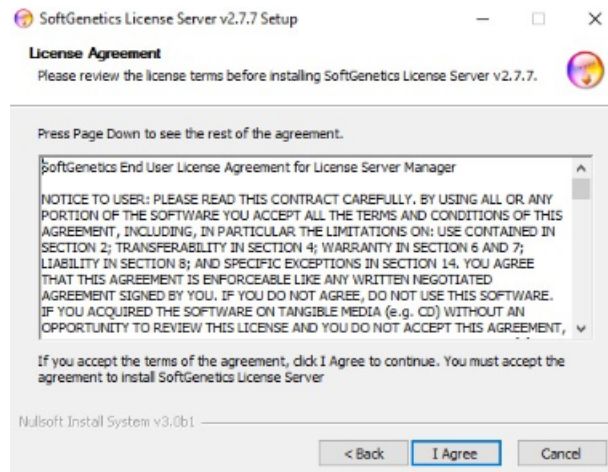


Figure 1-10: *SoftGenetics License Server Installation wizard, Wizard page 3*

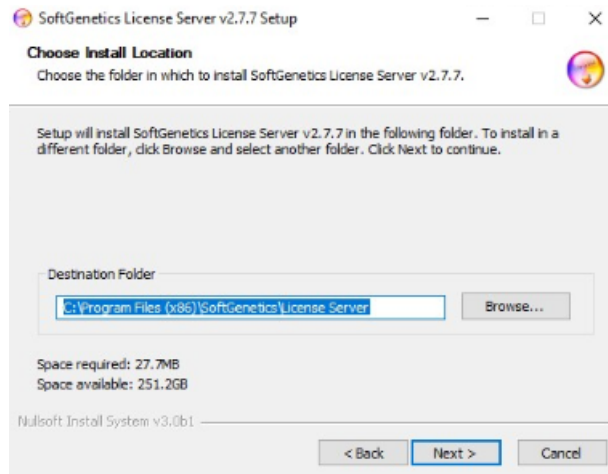


Figure 1-11: *SoftGenetics License Server Installation wizard, Wizard page 4*

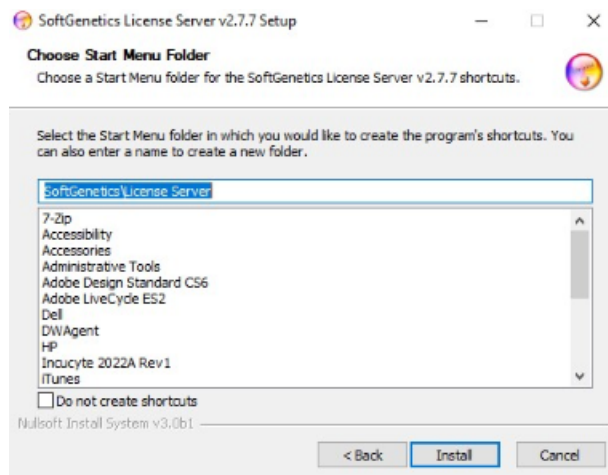


Figure 1-12: *SoftGenetics License Server Installation wizard, Wizard page 5*

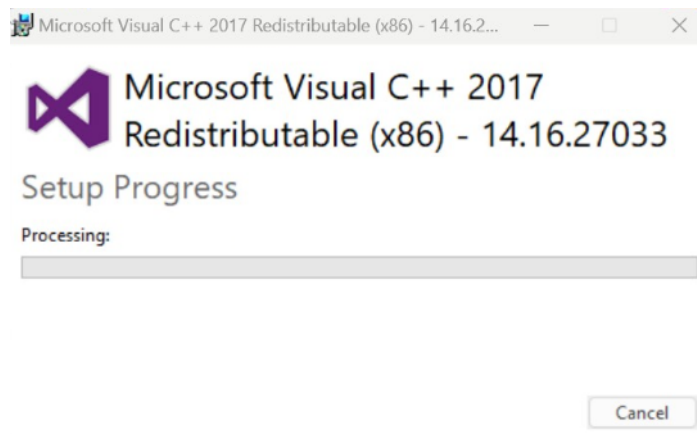


Figure 1-13: *SoftGenetics License Server Installation wizard, Wizard page 6*

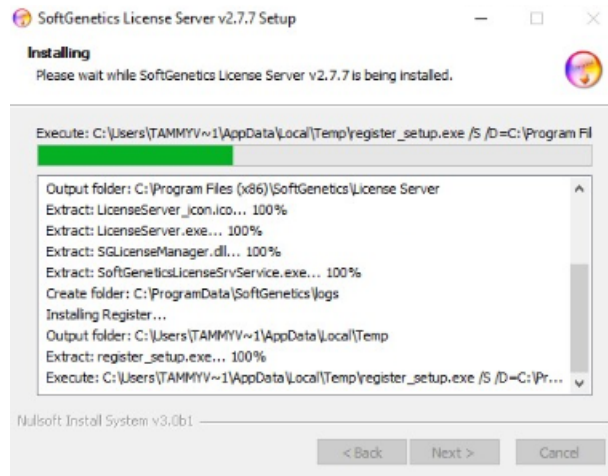


Figure 1-14: *SoftGenetics License Server Installation wizard, Wizard page 7*

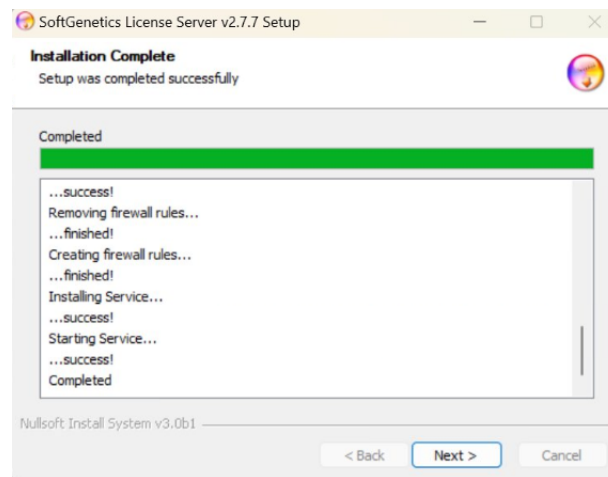
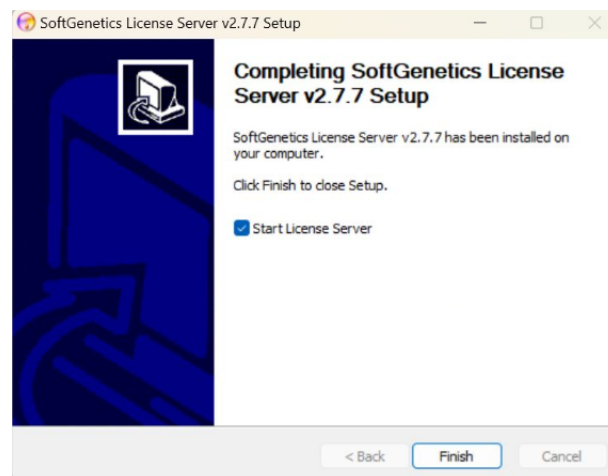


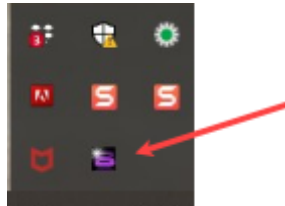
Figure 1-15: *SoftGenetics License Server Installation wizard, Wizard page 8*



Chapter 1
Getting Started with GeneMarkerHTS

2. After you click Finish on the last page of the SoftGenetics License Server Installation wizard, the following three actions occur:
 - The SoftGenetics License Server Installation wizard closes.
 - An icon for the License Server Manager (LSM) is placed in the system tray.

Figure 1-16: SoftGenetics LSM icon in system tray



- A Warning message opens indicating that no product has been registered with the LSM and instructing you how to register a product.

Click OK to close the message and open the LSM. The display is blank.

Figure 1-17: Warning message indicating that no product is currently registered

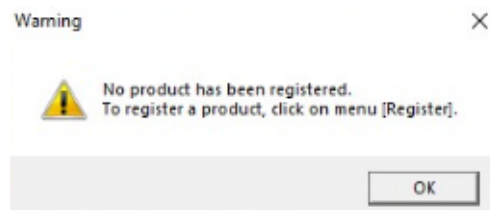
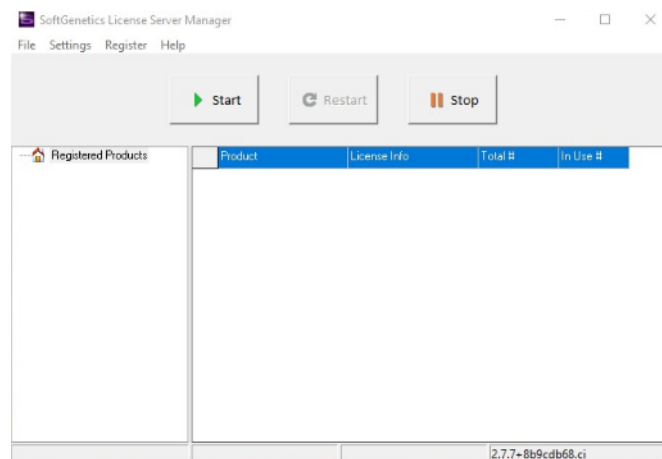


Figure 1-18: SoftGenetics LSM, No products registered



3. Continue to [“Registering a GeneMarkerHTS Network License”](#) on page 25.

Registering a GeneMarkerHTS Network License

Registering a GeneMarkerHTS network license is a two-step process. First, you must use the SoftGenetics LSM register the network license. Second, you must then configure GeneMarkerHTS to connect to the LSM.



The procedures in this section assume that you are able to carry them out error-free. For explanations of the most common error messages that might open during GeneMarkerHTS registration and their resolutions, see [“GeneMarkerHTS Registration Common Error Messages”](#) on page 43.

To register a GeneMarkerHTS network license

1. If the LSM is currently open, go to [Step 2](#); otherwise, in the system tray, click the LSM icon.

The LSM opens. If you have installed any other SoftGenetics products that require the LSM, then these products and their licensing information are displayed in the LSM; otherwise, the display is blank. See [Figure 1-20](#) on page 26.



*If the SoftGenetics LSM icon is not present in the system tray, then in the Windows Search feature, enter “license” to locate the LSM, and then click **Run as administrator** to open and start the SoftGenetics LSM. See [Figure 1-19](#) below.*

Figure 1-19: Optional launching of the SoftGenetics LSM

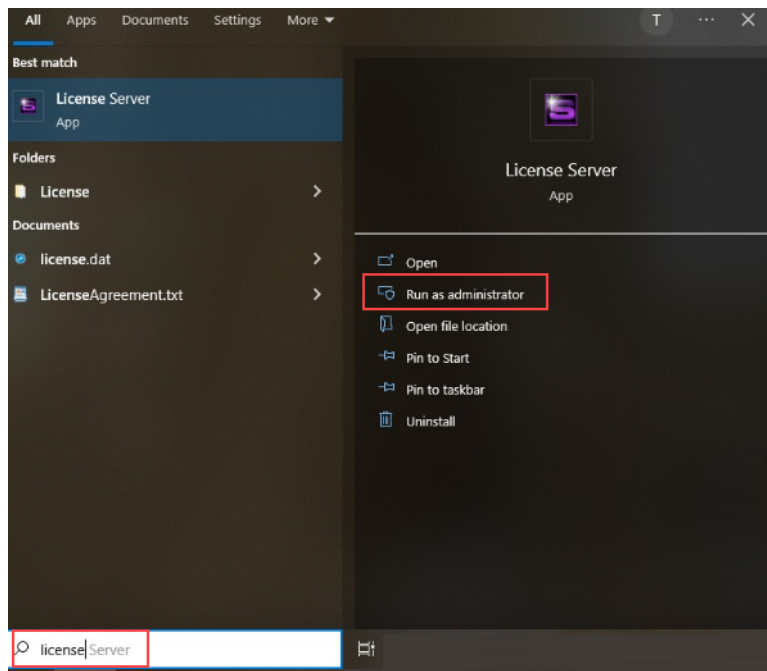
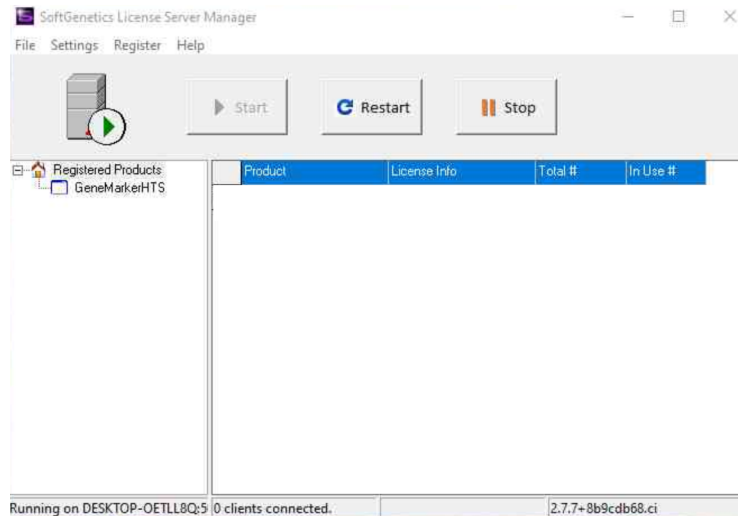


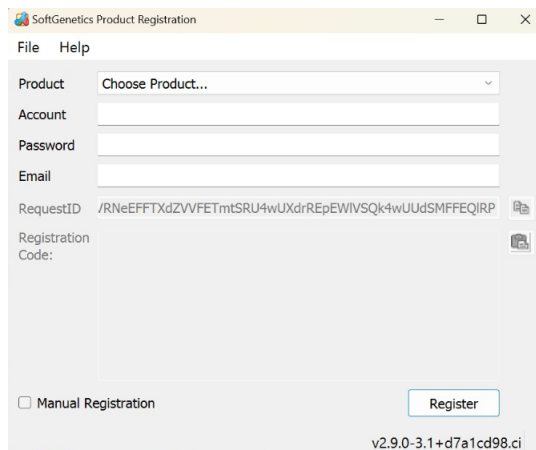
Figure 1-20: *SoftGenetics LSM, No products registered*



2. On the LSM main menu, click Register.

The SoftGenetics Product Registration dialog box opens. The Request ID field is pre-populated.

Figure 1-21: *SoftGenetics Product Registration dialog box*



3. Continue to one of the following:
 - If the computer on which you installed the LSM has an Internet connection and you do not use a proxy server, then continue to [“To register a GeneMarkerHTS network license with the LSM \(No Proxy server\)”](#) on page 27.
 - If the computer on which you installed the LSM has an Internet connection and you are using a Proxy server, then continue to [“To register a GeneMarkerHTS network license with the LSM using a Proxy server”](#) on page 29.
 - If the computer on which you installed the LSM does not have an Internet connection, then continue to [“To manually register a GeneMarkerHTS network license with the LSM”](#) on page 30.

To register a GeneMarkerHTS network license with the LSM (No Proxy server)

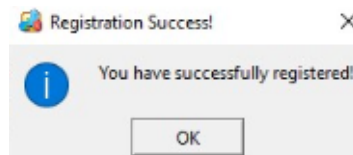
1. On the Product drop-down list, select GeneMarkerHTS.



Make sure to select GeneMarkerHTS and not GeneMarkerHTS Viewer.

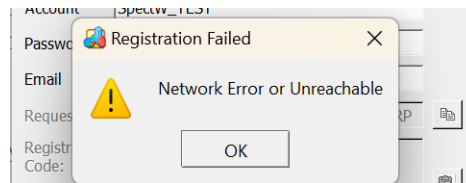
2. Enter the account credentials (Account and Password) that SoftGenetics supplied to you.
3. Enter the email address for the account.
4. Click Register. Two results are possible.
 - If the registration is successful, then a Registration Success dialog box opens. Continue to [Step 5](#).

Figure 1-22: Registration Success dialog box



- If the registration is not successful, then a Registration Failed dialog box opens. You must now manually register GeneMarkerHTS. Continue [“To manually register a GeneMarkerHTS network license with the LSM” on page 30](#).

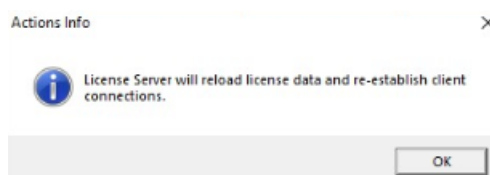
Figure 1-23: Registration Failed dialog box



5. Click OK in the Registration Success dialog box.

The Registration Success dialog box closes and an Actions Info dialog box opens, indicating that the license server will reload the license data and re-establish client connections.

Figure 1-24: Actions Info dialog box



Chapter 1
Getting Started with GeneMarkerHTS

6. Click OK.

The Actions Info dialog box closes and the LSM remains open.



After the Actions Info dialog box closes, a Message dialog box might open that prompts you to restart the LSM for the changes to take effect. Close the Message dialog box, and then click Restart Now to complete the registration process.

- The newly registered GeneMarkerHTS product is displayed in the LSM. See [Figure 1-25](#) below.
- A registration email is sent to the email address that you specified. The email acknowledges your successful registration and also details your account information. See [Figure 1-26](#) below.

Figure 1-25: License Server Manager

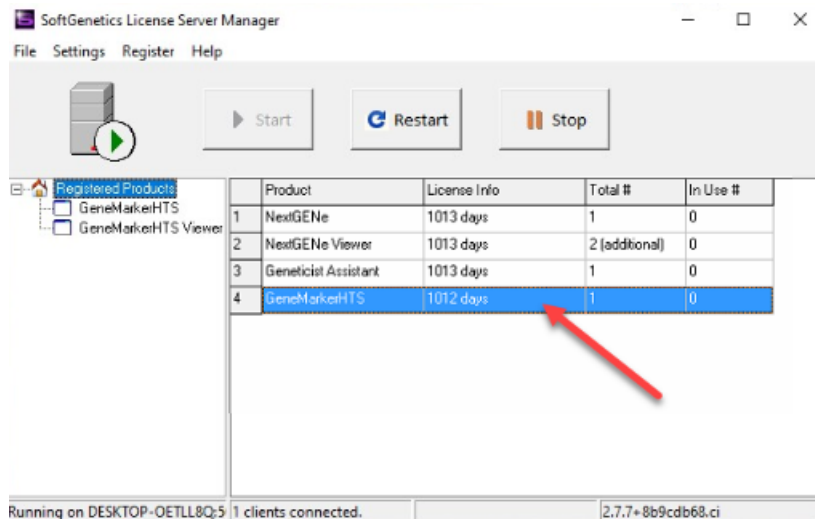
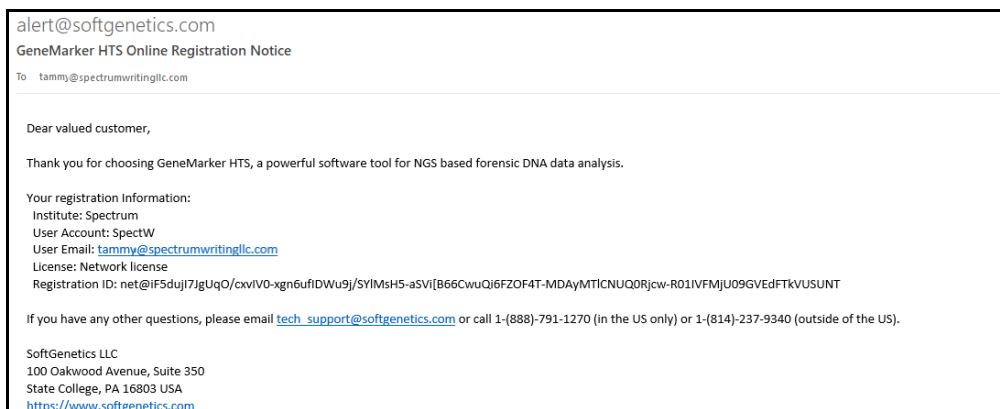


Figure 1-26: Registration email



7. Close the LSM, and then continue to “To configure the LSM” on page 34.

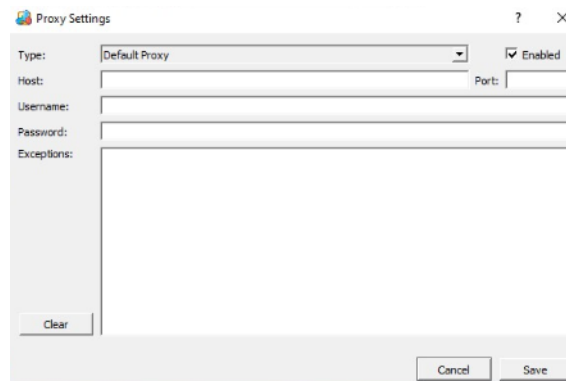
To register a GeneMarkerHTS network license with the LSM using a Proxy server

If you are using a Proxy server with your GeneMarkerHTS installation, then you must specify both your GeneMarkerHTS account settings and the Proxy server settings.

1. On the Product Name drop-down list, select GeneMarkerHTS.
2. Enter the account credentials (Account and Password) that SoftGenetics supplied to you.
3. Enter the email address for the account.
4. On the Product Registration dialog box main menu, click File > Proxy Settings.

The Proxy Settings dialog box opens.

Figure 1-27: Proxy Settings dialog box



5. On the Proxy Settings dialog box, click Enabled.
6. On the Type drop-down list, select the correct type for your Proxy Server.



If you select Default Proxy for Type, then either the proxy settings that are specified in Window Edge or the proxy settings that are specified in Windows System Settings are used for the connection and you do not need to specify any other settings on the Proxy Settings dialog box. Instead, continue to [Step 10](#).

7. Enter the Proxy server connection settings in the Host field and the Port field.
8. Enter the login credentials for the server in the Username field and Password field.
9. If applicable, enter any exceptions.
10. Click Save.

The Proxy Settings dialog box closes. The SoftGenetics Product Registration dialog box remains open.

11. Continue to [Step 4](#) of “[To register a GeneMarkerHTS network license with the LSM \(No Proxy server\)](#)” on page 27.

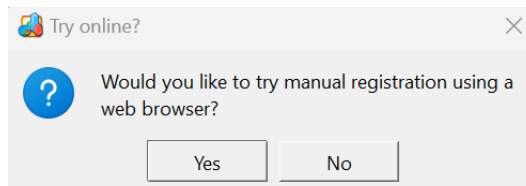
To manually register a GeneMarkerHTS network license with the LSM

If online registration for GeneMarkerHTS fails, then you must manually register GeneMarkerHTS with the LSM. Two manual registration options are available:

- If the computer on which you installed the LSM is connected to the Internet, then you can try manual registration using a web browser.
 - If the computer on which you installed the LSM is connected to the Internet, but you do not want to attempt manual registration using a web browser, or the computer is not connected to the Internet, then you must contact SoftGenetics to register the server.
1. In the Registration Failed dialog box, click OK.

The Registration Failed dialog box closes, and a Try Online dialog box opens, asking you if you want to attempt manual registration using a web browser.

Figure 1-28: Try online dialog box



2. Select either Yes or No.
 - If you select Yes, then the Try online dialog box closes, and you are redirected to a registration location on SoftGenetics’s website. Continue to [“To manually register GeneMarkerHTS using a web browser” on page 30.](#)
 - If you select No, then the Try online dialog box closes, and the SoftGenetics Product Registration dialog box remains open. Continue to [“To manually register GeneMarkerHTS offline” on page 33.](#)

To manually register GeneMarkerHTS using a web browser



The option to manually register GeneMarkerHTS using a web browser is also available on the Product Registration dialog box: **File > Open Website Registration.**

Figure 1-29: Manual registration of GeneMarkerHTS using a web browser

To register **Local or Network keyless secured (Text-based) products of SoftGenetics®**, please fill out the correct information in the following boxes and then you may register the software.
Required fields are marked with an asterisk(*)

Product: GeneMarkerHTS

Request ID: MUIUTXhFRFF3QWpSQk4wUUdSMFFEC *

Account: SpectW_TEST *

Password: ***** *

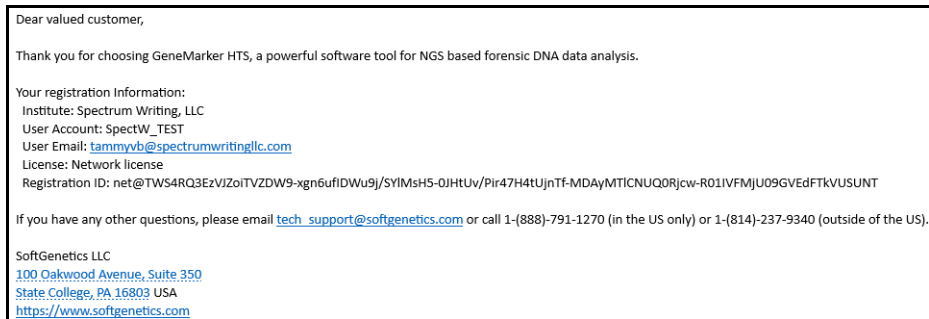
Email: tammyvb@spectrumwritingllc.com *

Register Reset

1. On the web browser page, click Register.

The web page is refreshed with a Registration message indicating that you have successfully registered GeneMarkerHTS and that you must complete the registration process. The page also displays the necessary Registration ID (the registration code) for completing the registration.

Figure 1-30: Manual registration of GeneMarkerHTS using a web browser



2. On the web page, below the Registration code, click Copy To Clipboard.
3. Return to the SoftGenetics Production Registration dialog box and paste the copied Registration ID (the registration code) into the Registration Code field.

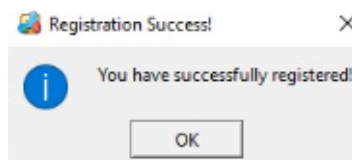


Manual Registration should already be selected on the SoftGenetics Product Registration dialog box, but before you continue to the next step, make sure that it is selected. If it is not selected, then you must select it.

4. Click Register.

A Registration Success dialog box opens.

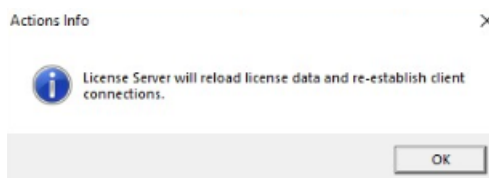
Figure 1-31: Registration Success dialog box



5. Click OK.

The Registration Success dialog box closes, and an Actions Info dialog box opens, indicating that the license server will reload the license data and re-establish client connections.


Figure 1-32: Actions Info dialog box



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6. Click OK.

The Actions Info dialog box closes and the LSM remains open.

 After the Actions Info dialog box closes, a Message dialog box might open that prompts you to restart the LSM for the changes to take effect. Close the dialog box, and then click Restart Now to complete the registration process.

- The newly registered GeneMarkerHTS product is displayed in the LSM. See [Figure 1-33](#) below.
- A registration email is sent to the email address that you specified. The email acknowledges your successful registration and also details your account information. See [Figure 1-34](#) below.

Figure 1-33: License Server Manager

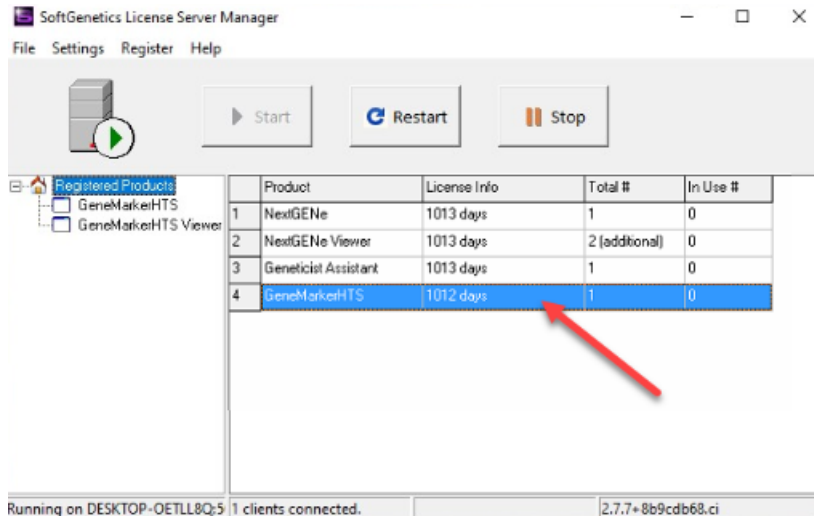
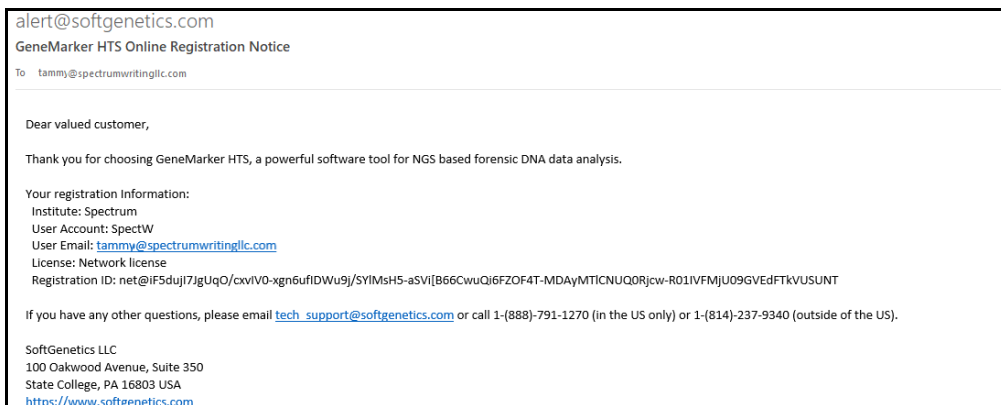


Figure 1-34: Registration email



7. Close the LSM, and then continue to “To configure the LSM” on page 34.

To manually register GeneMarkerHTS offline

1. Copy the entire Request ID string that is displayed in the Request ID field on the SoftGenetics Product Registration dialog box.
2. Paste the copied Request ID string into an email that includes your account credentials (Account, Password, and Email) and send the email to tech_support@softgenetics.com.
SoftGenetics emails you a new Registration ID (the registration code).
3. Copy the new Registration ID in this email to your client clipboard.
4. Return to the SoftGenetics Production Registration dialog box, ensure that your account credentials are still correctly entered (Name, Password, and Email), and then paste the copied Registration ID (the registration code) into the Registration Code field.

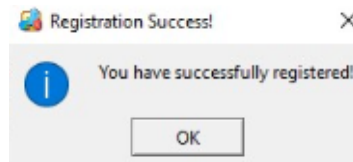


Manual Registration should already be selected on the SoftGenetics Product Registration dialog box, but before you continue to the next step, make sure that it is selected. If it is not selected, then you must select it.

5. Click Register.

A Registration Success dialog box opens.

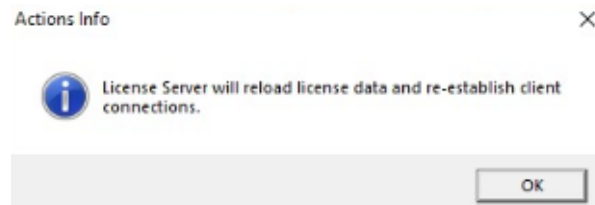
Figure 1-35: Registration Success dialog box



6. Click OK.

The Registration Success dialog box closes, and an Actions Info dialog box opens, indicating that the license server will reload the license data and re-establish client connections.

Figure 1-36: Actions Info dialog box



7. Click OK.

The Actions Info dialog box closes and the LSM remains open.



After the Actions Info dialog box closes, a Message dialog box might open that prompts you to restart the LSM for the changes to take effect. Close the dialog box, and then click Restart Now to complete the registration process.

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- The newly registered GeneMarkerHTS product is displayed in the LSM. See [Figure 1-37](#) below.
- A registration email is sent to the email address that you specified. The email acknowledges your successful registration and also details your account information. See [Figure 1-38](#) below.

Figure 1-37: License Server Manager

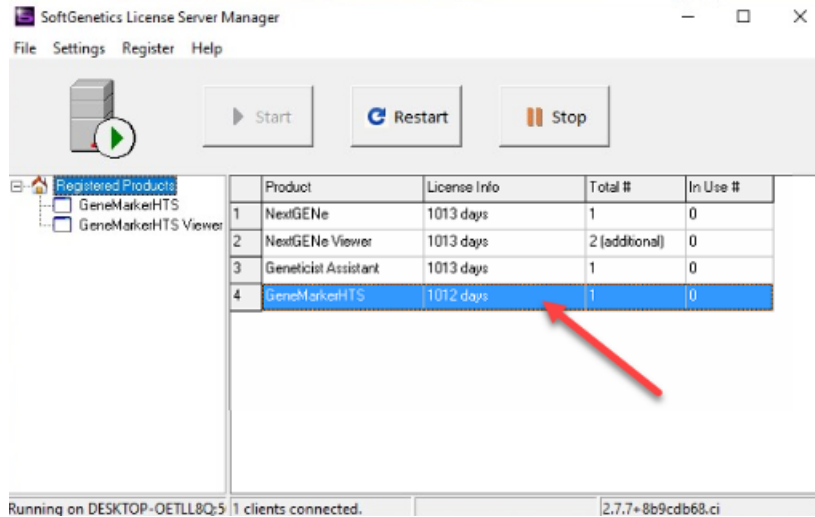
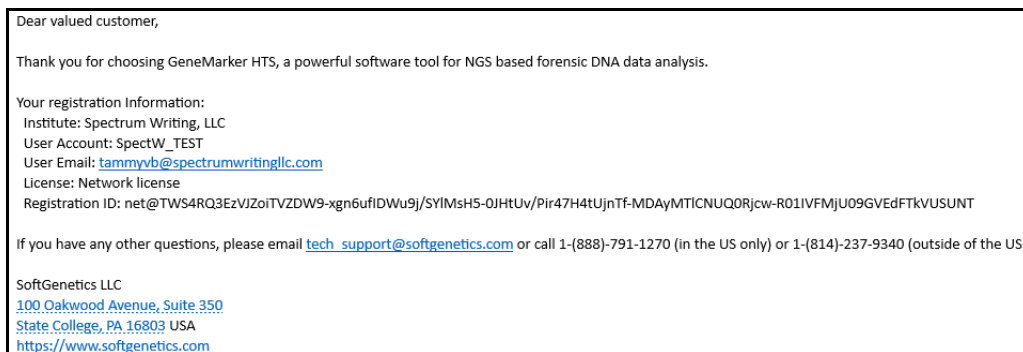


Figure 1-38: Registration email



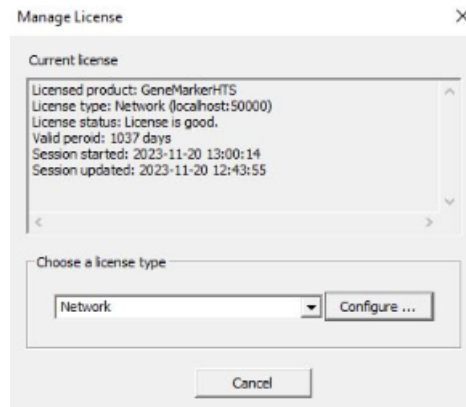
8. Close the LSM and then continue to ["To configure the LSM"](#) below.

To configure the LSM

1. If the Application Settings dialog box is not already open, then open it and ensure that the License pane is visible.
2. Next to the Type drop-down list, ensure that FullAligner is displayed, and then click Configure.

The Manage License dialog box opens. See [Figure 1-39 on page 35](#).

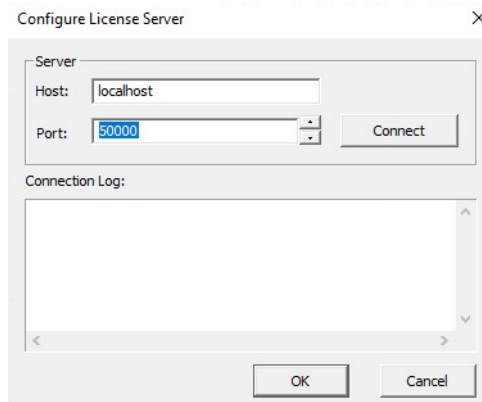
Figure 1-39: Manage License dialog box



3. Click Configure.

The Configure License Server dialog box opens.

Figure 1-40: Configure License Server dialog box



4. Do one of the following:
 - If the LSM and GeneMarkerHTS are installed on different computers, then in the Host field, enter the Fully Qualified Domain Name or IP address for the LSM.
 - If the LSM and GeneMarkerHTS are installed on the same computer, then leave Host set to localhost.

5. Leave the Port set to 50000.

6. Click Connect.

Messages are displayed in the Connection Log pane indicating that connection request was successful and that GeneMarkerHTS has accessed the commercial license.

7. Click OK.

The Configure License Server dialog box closes. The Application Settings dialog box remains open.

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8. Close the Application Settings dialog box.

GeneMarkerHTS is now ready for use.

Registering a GeneMarkerHTS Local License

You register a GeneMarkerHTS local license directly with SoftGenetics. If the client on which you installed GeneMarkerHTS is connected to the Internet, then online registration is possible; otherwise, if online registration fails, or if you do not want to register the GeneMarkerHTS license online, then you must manually register the GeneMarkerHTS license.



The procedures in this section assume that you are able to carry them out error-free. For explanations of the most common error messages that might open during GeneMarkerHTS registration and their resolutions, see [“GeneMarkerHTS Registration Common Error Messages”](#) on page 43.

To register a GeneMarkerHTS local license online

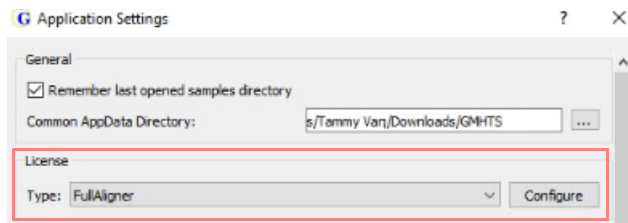
1. On the GeneMarkerHTS main window, click the Application Settings icon.

The Application Settings dialog box opens. From top to bottom, the dialog box has three panes: General, License, and Visuals.

2. Ensure that the License pane is visible.

The pane indicates that GeneMarkerHTS was installed with a FullAligner license.

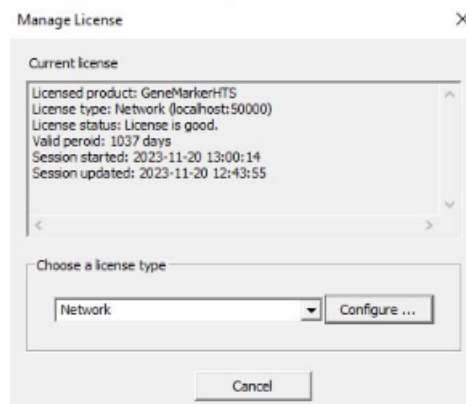
Figure 1-41: Application Settings dialog box, License pane with FullAligner license



3. Click Configure.

The Manage License dialog box opens. The License Type is set to Network.

Figure 1-42: Manage License dialog box

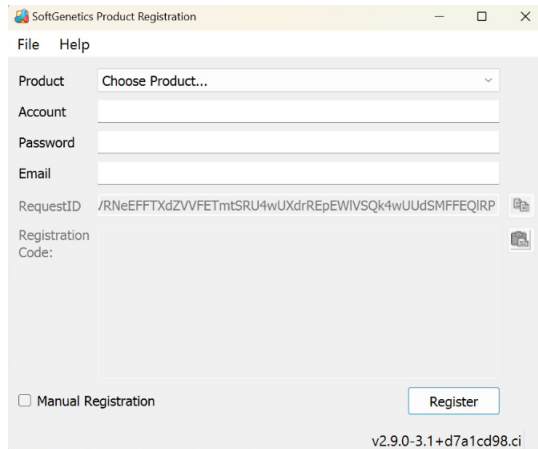


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4. On the Choose a License Type drop-down list, select Local (softkey).
5. Click Configure.

The SoftGenetics Product Registration dialog box opens. The Request ID field is pre-populated.

Figure 1-43: SoftGenetics Product Registration dialog box



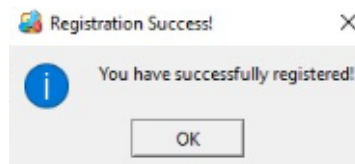
1. On the Product drop-down list, select GeneMarkerHTS.



Make sure to select GeneMarkerHTS and not GeneMarkerHTS Viewer.

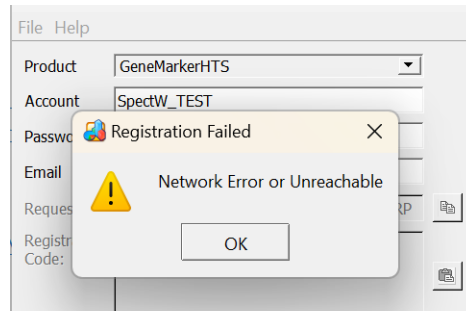
2. Enter the account credentials (Account and Password) that SoftGenetics supplied to you.
3. Enter the email address for the account.
4. Click Register. Two results are possible.
 - If the registration is successful, then a Registration Success dialog box opens. Continue to [Step 5](#).

Figure 1-44: Registration Successful message



- If the registration is not successful, then a Registration Failed dialog box opens. (See [Figure 1-45 on page 39](#).) You must now manually register GeneMarkerHTS. Continue [“To manually register GeneMarkerHTS offline” on page 41](#).

Figure 1-45: Registration Failed message



5. Click OK in the Registration Success message.

The Registration Success message closes. The Application Settings dialog box remains open. GeneMarkerHTS is now ready for use.

To manually register a GeneMarkerHTS local license

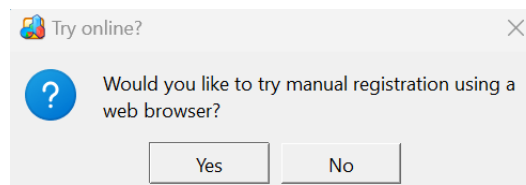
If online registration for your GeneMarkerHTS local license fails, then you must manually register GeneMarkerHTS with SoftGenetics. Two manual registration options are available:

- If the client on which you installed GeneMarkerHTS is connected to the Internet, then you can try manual registration using a web browser.
- If the client on which you installed GeneMarkerHTS is connected to the Internet, but you do not want to attempt manual registration using a web browser, or the client is not connected to the Internet, then you must contact SoftGenetics to register the application.

1. In the Registration Failed dialog box, click OK.

The Registration Failed dialog box closes, and a Try Online dialog box opens, asking you if you want to attempt manual registration using a web browser.

Figure 1-46: Try online dialog box



2. Select either Yes or No.
 - If you select Yes, then the Try online dialog box closes, and you are redirected to a registration location on SoftGenetics's website. Continue to ["To manually register GeneMarkerHTS using a web browser" on page 40.](#)
 - If you select No, then the Try online dialog box closes, and the SoftGenetics Product Registration dialog box remains open. Continue to ["To manually register GeneMarkerHTS offline" on page 33.](#)

To manually register GeneMarkerHTS using a web browser



The option to manually register GeneMarkerHTS using a web browser is also available on the Product Registration dialog box: File > Open Website Registration.

Figure 1-47: Manual registration of GeneMarkerHTS using a web browser

To register **Local or Network keyless secured (Text-based) products of SoftGenetics®**, please fill out the correct information in the following boxes and then you may register the software.
Required fields are marked with an asterisk(*)

Product: GeneMarkerHTS
Request ID: MUIUTXhFRFF3QWpSQk4wUUdSMFFEC *
Account: SpectW_TEST *
Password: ***** *
Email: tammyvb@spectrumwritingllc.com *
Register Reset

1. On the web browser page, click Register.

The web page is refreshed with a Registration message indicating that you have successfully registered GeneMarkerHTS and that you must complete the registration process. The page also displays the necessary Registration ID (the registration code) for completing the registration.

Figure 1-48: Manual registration of GeneMarkerHTS using a web browser

Dear valued customer,

Thank you for choosing GeneMarker HTS, a powerful software tool for NGS based forensic DNA data analysis.

Your registration information:
Institute: Spectrum Writing, LLC
User Account: SpectW_TEST
User Email: tammyvb@spectrumwritingllc.com
License: Network license
Registration ID: net@TWS4RQ3EzVIZoiTVZDW9-xgn6ufiDWu9j/SYIMsH5-OJHtUv/Pir47H4tUjnTf-MDAyMTICNUQ0Rjcw-R01IVFMJU09GVEDFTKVVUSUNT

If you have any other questions, please email tech_support@softgenetics.com or call 1-(888)-791-1270 (in the US only) or 1-(814)-237-9340 (outside of the US).

SoftGenetics LLC
[100 Oakwood Avenue, Suite 350](https://www.softgenetics.com)
State College, PA 16803 USA
<https://www.softgenetics.com>

2. On the web page, below the Registration code, click Copy To Clipboard.
3. Return to the SoftGenetics Production Registration dialog box and paste the copied Registration ID (the registration code) into the Registration Code field.

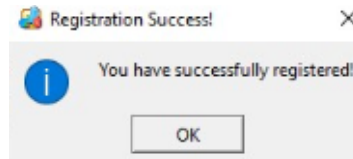


Manual Registration should already be selected on the SoftGenetics Product Registration dialog box, but before you continue to the next step, make sure that it is selected. If it is not selected, then you must select it.

4. Click Register.

A Registration Success dialog box opens. See [Figure 1-49 on page 41](#).

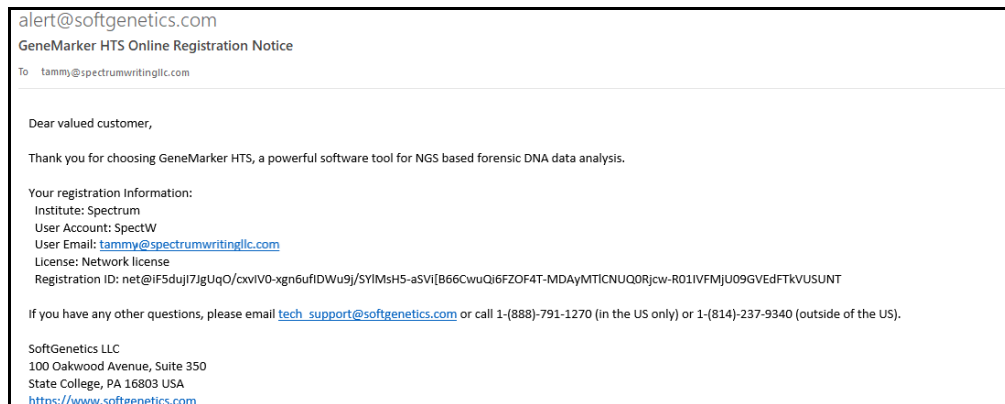
Figure 1-49: Registration Success dialog box



5. Click OK.

The Registration Success dialog box closes. A registration email is sent to the email address that you specified. The email acknowledges your successful registration and also details your account information. GeneMarkerHTS is now ready for use.

Figure 1-50: Registration email



To manually register GeneMarkerHTS offline

1. Copy the entire Request ID string that is displayed in the Request ID field on the SoftGenetics Product Registration dialog box.
2. Paste the copied Request ID string into an email that includes your account credentials (Account, Password, and Email) and send the email to tech_support@softgenetics.com. SoftGenetics emails you a new Registration ID (the registration code).
3. Copy the new Registration ID in this email to your client clipboard.
4. Return to the SoftGenetics Production Registration dialog box, ensure that your account credentials are still correctly entered (Name, Password, and Email), and then paste the copied Registration ID (the registration code) into the Registration Code field.



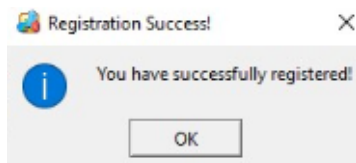
Manual Registration should already be selected on the SoftGenetics Product Registration dialog box, but before you continue to the next step, make sure that it is selected. If it is not selected, then you must select it.

5. Click Register.

A Registration Success dialog box opens. See [Figure 1-51 on page 42](#).

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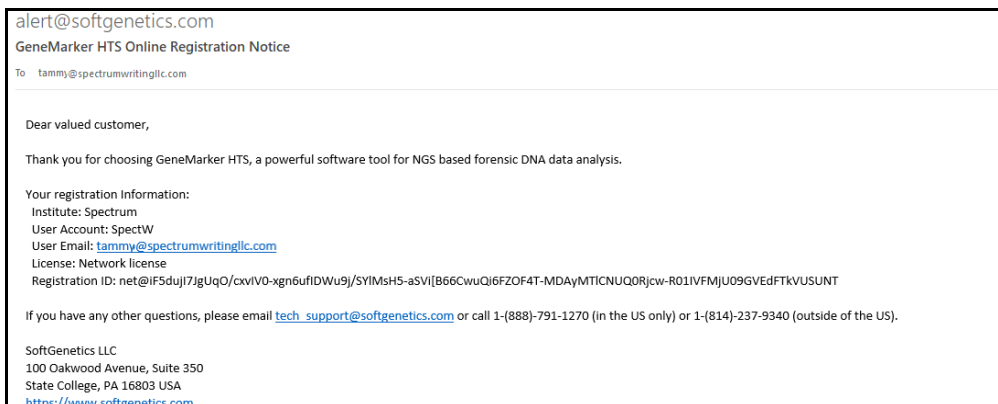
Figure 1-51: Registration Success dialog box



6. Click OK.

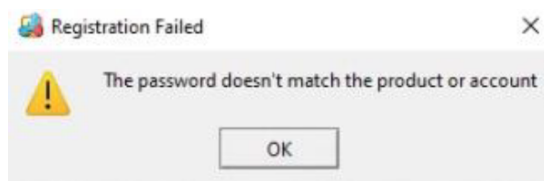
The Registration Success dialog box closes. A registration email is sent to the email address that you specified. The email acknowledges your successful registration and also details your account information. GeneMarkerHTS is now ready for use.

Figure 1-52: Registration email



GeneMarkerHTS Registration Common Error Messages

This section details the four most common error messages that you might encounter when you are registering your GeneMarkerHTS installation with the SoftGenetics LSM for a network license, or with SoftGenetics directly for a local license. It also describes the most appropriate resolution. If you cannot resolve an error message that opens during the registration of GeneMarkerHTS, or if you receive an error message that is not detailed here, then contact tech_support@softgenetics.com for assistance.

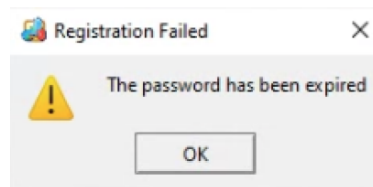


Meaning: The password that you entered on the Product Registration dialog box is not for the selected product or the indicated account.

Resolution:

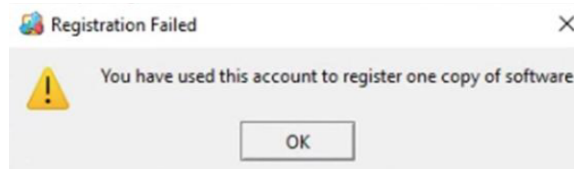
- Confirm that the correct product is selected on the Product drop-down list.
- Confirm that the account name and password have been entered correctly, remembering that both are case-sensitive and there should be no spaces for either registration variable.

Note: If you leave a space at the end of the registration password, this error message is triggered.



Meaning: Your GeneMarkerHTS license has expired and is no longer working.

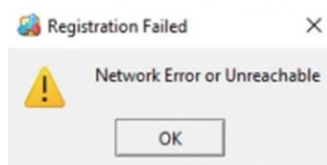
Resolution: Contact tech_support@softgenetics.com or your local distributor for assistance in updating your license.



Meaning: Your GeneMarkerHTS network license is already actively registered to another client.

Resolution: Contact tech_support@softgenetics.com for assistance.

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Meaning: The client on which you are registering GeneMarkerHTS was unable to access the SoftGenetics database through the Internet. Online registration has failed. This error is typically due to firewalls or other security settings.

Resolution: You must manually register your product. See ["To manually register a GeneMarkerHTS network license with the LSM" on page 30.](#)

Starting GeneMarkerHTS

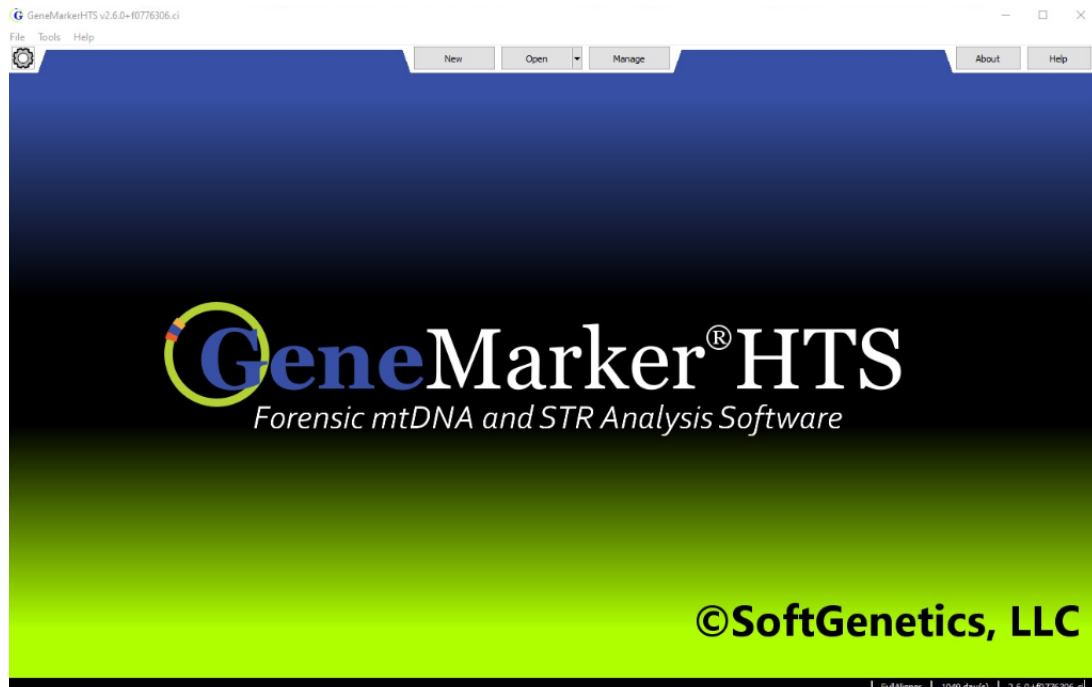
You can double-click the desktop icon to launch GeneMarkerHTS, or you can select the option from your Start menu, where the default value is: Start > SoftGenetics > GeneMarkerHTS v< >.

Figure 1-53: GeneMarkerHTS desktop icon



After you start GeneMarkerHTS, the GeneMarkerHTS main window opens. See [“The GeneMarkerHTS Main Window”](#) on page 46.

Figure 1-54: GeneMarkerHTS main window



The GeneMarkerHTS Main Window

The GeneMarkerHTS main window is your starting point for the GeneMarkerHTS application.

Figure 1-55: GeneMarkerHTS main window



The GeneMarkerHTS main window has seven major components, which are the:

- Title bar. See [“Title bar”](#) below.
- Main menu. See [“Main menu”](#) on page 47.
- Application Settings icon. See [“Application Settings icon”](#) on page 47.
- Project options. See [“Project options”](#) on page 47.
- About button. See [“About button”](#) on page 48.
- Help button. See [“Help button”](#) on page 48.
- Status bar. See [“Status bar”](#) on page 48.

Title bar

The name “GeneMarkerHTS” is displayed in the left corner of title bar at the top of the GeneMarkerHTS main window. The version of GeneMarkerHTS that you are running is displayed after the name.

Figure 1-56: GeneMarkerHTS Title bar



Main menu


The GeneMarkerHTS main menu is displayed below the title bar. The main menu is set up in a standard Windows menu format with menu commands grouped into menus (File, Tools, and Help) on the menu bar. Some commands are available in other areas of the application.

Figure 1-57: GeneMarkerHTS main menu

File Tools Help

Menu	Option
File	<ul style="list-style-type: none"> • New Project: Click to open the New Project dialog box, and set up a new analysis project in GeneMarkerHTS.
	<ul style="list-style-type: none"> • Open Project: Click to open the Open Project dialog box and browse to and select an existing GeneMarkerHTS project with which to work. <p>Note: By default, GeneMarkerHTS opens the folder in which the last project was <i>opened</i> for the GeneMarkerHTS instance.</p>
	<ul style="list-style-type: none"> • Open Recent: Opens a drop-down list of the ten most recent projects that were added or edited for the GeneMarkerHTS instance. • Open Recent > Clear Recent Projects: Click to clear all the recent projects that have been opened for the GeneMarkerHTS instance.
	<ul style="list-style-type: none"> • Settings: Click to open the Application Settings dialog box, which provides a variety of options for customizing your GeneMarkerHTS instance. See Appendix A, “GeneMarkerHTS Application Settings,” on page 145.
Tools	<ul style="list-style-type: none"> • Panel Manager: TBD. If you need assistance with creating or editing custom panels for your GeneMarkerHTS instance, contact tech_support@softgenetics.com.
	<ul style="list-style-type: none"> • Motif Editor: Click to open the Motif Editor dialog box, which provide the options (Add, Remove Motif, Remove Region) for managing the motifs for the GeneMarkerHTS instance. See “To create or edit a custom motif” on page 54.
Help	<ul style="list-style-type: none"> • Help: Click to open the Online Help for GeneMarkerHTS.
	<ul style="list-style-type: none"> • About: Click to open the About dialog box, which displays information about your GeneMarkerHTS installation, including the version.

Application Settings icon

The Application Settings icon () is displayed in the left corner of the main window underneath the main menu. Click the icon to open the Application Settings dialog box, which provides all the options for specifying the settings to customize your GeneMarkerHTS instance, including project directory information, license information, and visual settings, including colors. See [Appendix A, “GeneMarkerHTS Application Settings,”](#) on page 145.

Project options

The Project options—New, Open, and Manage—are displayed in the center of the main window underneath the title bar.

- Click New to open the New Project dialog box, and set up a new analysis project in GeneMarkerHTS.

- Click Open to open the Open Project dialog box and browse to and select an existing GeneMarkerHTS project with which to work.

See [Chapter 2, “GeneMarkerHTS Project Setup and Management,”](#) on page 49.

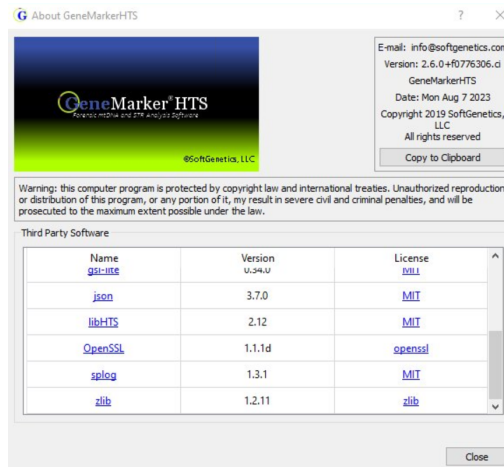


The full functionality of project management (Manage) in GeneMarkerHTS is currently under development.

About button

The About button is displayed in the top right corner of the main window underneath the title bar. Click About to open the About GeneMarkerHTS dialog box, which displays information about your GeneMarkerHTS installation, including the version. The About dialog box also contains a Copy to Clipboard button, which you can use to copy the version and build date information that is displayed in the dialog box to your client’s clipboard. You can then paste this information into a third-party application such as Microsoft Outlook email in the event that you must supply it to SoftGenetics.

Figure 1-58: About GeneMarkerHTS dialog box



Help button

The Help button is displayed in the top right corner of the main window underneath the title bar. Click the Help button to open the online help for GeneMarkerHTS.

Status bar

The Status bar is displayed in the lower right corner of the GeneMarkerHTS main window. From left to right, it displays the following information: The license type for the installation, the number of days that are remaining on the license, and the version number for the installation.

Figure 1-59: GeneMarkerHTS main window Status bar



Chapter 2

GeneMarkerHTS Project Setup and Management

GeneMarkerHTS provides a validated streamlined workflow for forensic mitochondrial, STR, and Y-STR casework as well as medical research of mitochondrial DNA from massively parallel sequencing platforms (MPS) such as the Illumina and Ion Torrent. The New Project dialog box provides all the options for adding a new project for mtDNA, STR, or Y-STR data analysis. You can also open and work with existing projects in GeneMarkerHTS. This chapter details all the options and functions that are available for setting up and managing your GeneMarkerHTS projects.

This chapter covers the following topics:

- [“New Project Dialog Box Settings” on page 51.](#)
- [“Working with a GeneMarkerHTS Project” on page 64.](#)
- [“GeneMarkerHTS Project Output Files” on page 70.](#)

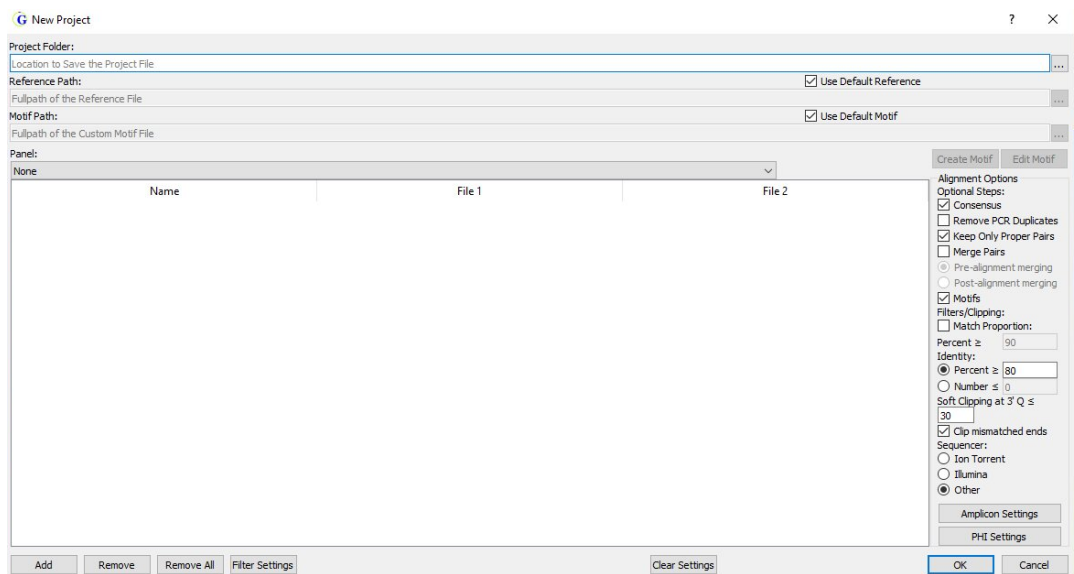
Chapter 2
GeneMarkerHTS Project Setup and Management

New Project Dialog Box Settings

You use the New Project dialog box to set up a new project for analyzing your data. You select the data that is to be analyzed for a project in the dialog box, and you also specify all the project settings in the dialog box. To open the New Project dialog box, do one of the following:

- At the top of the GeneMarkerHTS main window, click New.
- On the GeneMarkerHTS main menu, click File > New Project.

Figure 2-1: New Project dialog box



The following sections describe the settings on the New Project dialog box the first time that the dialog box is opened. Any selections that you make are persistent within your GeneMarkerHTS instance and across instances on the same client.

See:

- [“Folders and Paths” on page 52.](#)
- [“Motifs” on page 52.](#)
- [“Panel and Panel Options” on page 56.](#)
- [“Alignment Options: Optional Steps” on page 56.](#)
- [“Alignment Options: Filters/Clipping” on page 57.](#)
- [“Amplicon Settings” on page 59.](#)
- [“PHI Settings” on page 61.](#)

Folders and Paths

Setting	Description
Project Folder	<p>The folder in which to save the project file. The default location is the folder in which the project sample files are saved. For example, if the project sample files are saved in C:\GM_HTS\Project_A_SampleFiles, then the project file is also saved in \GM_HTS\Project_A_SampleFiles. To select a different location, next to the Project Folder field, click the Browse button to open the Project Save Location dialog box, and then browse to and select a different folder.</p> <p>Note: For additional project directory options, including remembering the last opened sample files directory, see "Configuring GeneMarkerHTS Project Directory Settings" on page 148.</p>
Reference Path Use Default Reference	<p>Do one of the following:</p> <ul style="list-style-type: none"> To use the revised Cambridge reference sequence (rCRS) that is supplied with GeneMarkerHTS, select Use Default Reference. To use a different reference, clear Use Default Reference, and then next to the Reference Path field, click the Browse button to open the Open Reference dialog box, and then browse to and select the reference. <p>Warning: If you select a different reference, then it must be in either FASTA or GBK format.</p>

Motifs

Setting	Description
<p>Note: Motif options are available only if Motifs is selected for an Alignment option. (See "Alignment Options: Optional Steps" on page 56.) If Motifs is not selected as an alignment option, then all Motif options are unavailable. SoftGenetics recommends the use of a motif for mtDNA analysis.</p>	
Motif Path Use Default Motif	<p>You can use a motif (a .txt file) to adjust the alignment to meet the expectations of your analysis. See "Overview of using a motif" on page 53. You can use the default motif that is supplied with GeneMarkerHTS or you can use a custom motif.</p> <ul style="list-style-type: none"> To use the default motif that is supplied with GeneMarkerHTS, leave Use Default Motif selected. To use the default motif as your starting point for creating a custom motif: <ol style="list-style-type: none"> Clear Use Default Motif. Click Create Motif, and then continue to "To create or edit a custom motif" on page 54. To use an existing custom motif as your starting point for creating another custom motif: <ol style="list-style-type: none"> Clear Use Default Motif. Click the Browse button that is displayed to the right of the Motif Path field, and browse to and select the motif. Click Edit Motif, and then continue "To create or edit a custom motif" on page 54.

Setting	Description
Create Motif	Available only if Use Default Motif is <i>not</i> selected. Click to open the Motif Editor dialog box and use the default motif that is supplied with GeneMarkerHTS as your starting point for creating a custom motif. See “To create or edit a custom motif” on page 54.
Edit Motif	Available only if Use Default Motif is <i>not</i> selected <i>and</i> you have selected an existing custom motif to use as your starting point for creating another custom motif. Click to open the Motif Editor dialog box and use the selected custom motif. See “To create or edit a custom motif” on page 54.

Overview of using a motif

Traditionally, alignment is focused on minimizing the number of mismatches between the reference and a sample read, or a “best fit” alignment; however, because forensic analysis has established conventions for the position of many common variants, this traditional approach to alignment is not always preferred when analyzing mtDNA. As a result, GeneMarkerHTS provides the option of using a motif to better meet your analysis requirements. A *motif* is a list of variant calls within a specified region that are translated into an expected sequence. You define this list of variant calls in a *motif file*, which is a text (.txt) file that has the following format:

- The first line for each motif definition in the file is preceded with a hash tag/pound symbol (#) and this line identifies the start and end points for the region.
- The variant calls in the motif file are listed with standard forensic nomenclature, for example, Deletion: 16191d, Insertion: 16193.1C, and SNP: 1184A.

Figure 2-2: Example of Best Fit alignment compared to Forensic alignment

	16180	16185	16190	16195
Reference	A A A A C C C C C T C C C C A T G			
Best Fit Alignment	A A - - C C C C C T C C C C CC A T G			
Forensic Alignment	A A C C C C C T C C C C - A T G			

Hash (#) mark indicates motif region.

```
#16179-16196
16181C, 16182C, 16183C, 16189C
16182C, 16183C, 16189C, 16191d, 16192d, 16193d
16182C, 16183C, 16189C, 16192d, 16193d
16182C, 16183C, 16189C, 16193d
16182C, 16183C, 16189C, 16193.1C
16182C, 16183C, 16189C, 16193.1C, 16193.2C
16182C, 16183C, 16187T, 16189C
16182C, 16183C, 16187T, 16189C, 16193d
16182C, 16183C, 16189C
```

When you are creating a motif file, note the following:

- Spacing of the variants is not absolute, but you must use commas to separate the variant calls.
- Regions cannot overlap.
- No two motifs in the same region can resolve to the same sequence.

Chapter 2
GeneMarkerHTS Project Setup and Management

If you elect to use motif alignment, then the alignments of reads that cross motif regions with expected sequences are adjusted to match the list of variants. Reads that start or end in motif regions are soft-clipped. You can use the default motif that is supplied with GeneMarkerHTS, or you can use an existing custom motif to create and/or edit your own custom motifs. See [“To create or edit a custom motif”](#) below.



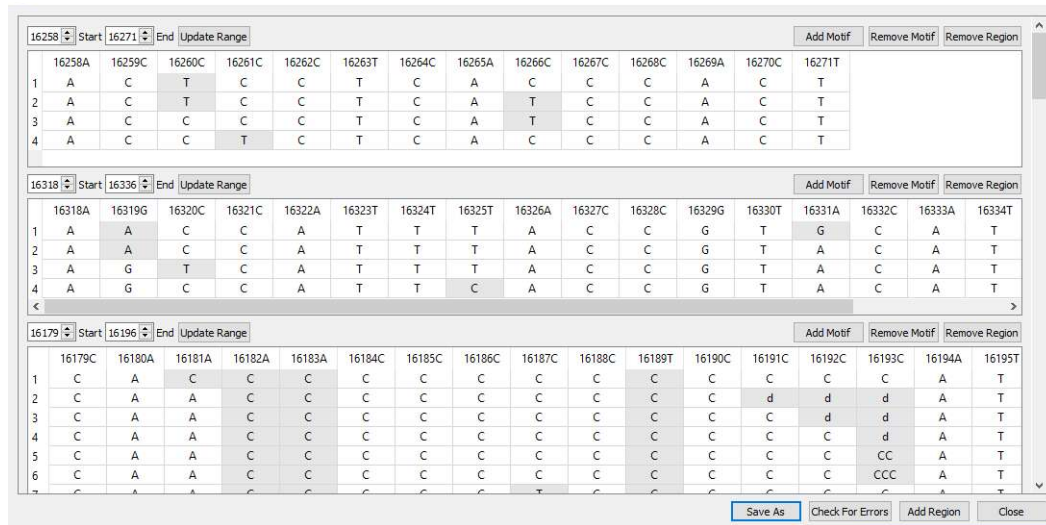
When creating a custom motif, you can use the default version that is supplied with Version 2.6 of GeneMarkerHTS as your starting point, or you can use a prior version. All prior versions of the default motif are found in the Resources folder in the installation directory for GeneMarkerHTS.

To create or edit a custom motif



Although you can manually edit a motif file, SoftGenetics strongly recommends that you use the built-in Motif Editor instead.

Figure 2-3: Motif Editor dialog box



1. For each motif region, do any or all of the following:

Option	Description
Update range	Adjust the Start and/or End values for a motif region (manually edit the values, or use the Up Arrow/Down Arrow buttons to adjust the value one base at a time), and then click Update Range. Tip: Because motifs require exact matches, shorter motifs are better for alignment.
Add Motif	Adds a new motif as the last in the list of displayed motifs for the region. The default sequence for a new motif is always set to the reference sequence.
Remove Motif	Select a motif, and then click to remove the selected motif from the motif region.
Remove Region	Click to remove a region entirely from the motif.

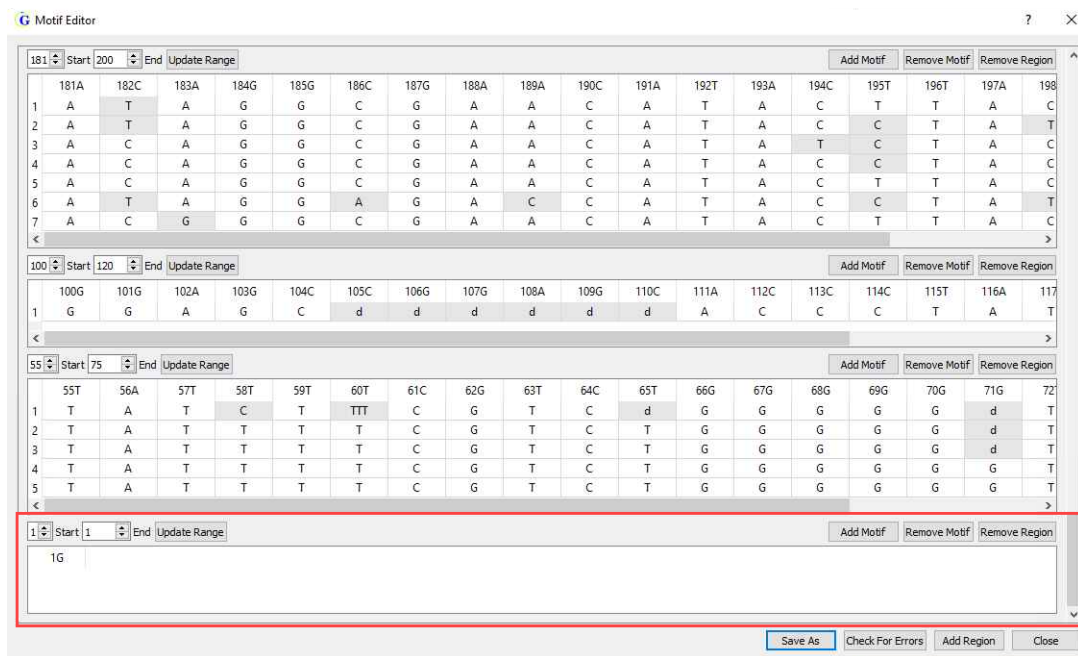
2. If you are done with customizing the motif, then continue to [Step 3](#); otherwise, optionally, to add one or more regions to the motif, do the following:
 - a. Click Add Region.

A blank new region placeholder is displayed at the bottom of the Motif Editor dialog box.



Use the scroll bar on the right side of the dialog box to scroll to the bottom of the dialog box and view the placeholder region.

Figure 2-4: Motif Editor dialog box with a new region placeholder



- b. Specify the start and end positions for the new region, and then click Update Range.
 - c. Create the needed motifs for the new range. (See [Step 1](#).)
3. Optionally, to check for errors before saving the customized motif, click Check For Errors; otherwise go to [Step 4](#).

If errors exist in the customized motif, then a Motif File Errors dialog box that details the errors in the motif file opens; otherwise, the Motif File Errors dialog box displays the message “No Errors.”

4. Click OK to close the Motif File Errors dialog box.
 - If the file did not contain any errors, then continue to [Step 5](#).
 - If the file did contain errors, then iteratively correct the file and click Check For Errors until you receive the message “No Errors,” and then continue to [Step 5](#).

- To save the new motif file, click Save As.

The Save Motif dialog box opens. The file format is set to .txt and you cannot edit this.

- Specify the name for the motif file, and then browse to and select the location in which to save the file, and then click Save.

The Save Motif dialog box closes. The Motif Editor dialog box remains open.

- Click “X” to close the Motif Editor dialog box.

You return to the New Project dialog box. The full path to the customized motif file, including the file name, is displayed in the Motif Path field. You can now use the motif for mtDNA analysis.

Panel and Panel Options

Setting	Description
Panel	<p>Required for STR data. Optional for mtDNA data. Leave Panel set to the default value of None or, on the Panel dropdown list, select from one of the four pre-supplied panels from Promega:</p> <ul style="list-style-type: none"> Promega_Powerseq_46GY_v1 (STRs and Y-STRs) Promega_Powerseq_CRM_v1 (mtDNA only) Promega_Powerseq_56GMY_v1 (STRs, Y-STRs, and mtDNA) Promega_Powerseq_WholeMito_v1 (mtDNA only) <p>Note: For a complete description of the available panels, see https://www.promega.com and search for the specific panel in which you are interested. If you need assistance in creating panels for other chemistries, contact tech_support@softgenetics.com.</p>
Allow Primer Mismatches	If selected, then a 1 bp difference in primer binding regions is allowed.

Alignment Options: Optional Steps

You can select one or more, or all of the following optional steps.

Setting	Description
Consensus	Selected by default. Do local indel realignment based on the consensus sequence. After variant calls are made based on the initial alignment, any reads that overlap indels but do not contain the indels are adjusted to include the indels if the new alignment is at least as good as the initial alignment.
Remove PCR Duplicates	<p>Reads are identified as PCR duplicates based on their start positions. If you are using paired-end data, then each pair of reads is considered together. If you leave the option unselected, then the PCR duplicates are not removed; otherwise, select the option to remove PCR duplicates.</p> <p>Note: If you are using targeted sequencing, then you typically do not select this value. If you are using whole genome sequencing, then you typically do select this value.</p>

Setting	Description
Keep Only Proper Pairs	<p>Ignore any reads that were not marked as being part of a proper pair, where a proper pair is defined as the following:</p> <ul style="list-style-type: none"> • Both reads from the pair must be aligned and the alignment must be on opposite strands. • The 5' end of the reverse read must be to the right of the 5' end of the forward read.
Merge Pairs	<p>Applicable only for mtDNA data that does <i>not</i> use a panel. If the reads are expected to fully overlap, then Merge Pairs is appropriate for mtDNA data. If you select Merge Pairs, then the overlapped portion of pairs is combined, which reduces coverage; however, because double counting is prevented, accuracy might be improved.</p> <p>If you select Merge Pairs, then the following options are enabled, and you can select one:</p> <ul style="list-style-type: none"> • Pre-alignment merging: Selected by default. Results in slower data analysis and is more prone to error, especially in repetitive regions, but for certain situations such as those that involve high quality data and/or sequences that are not highly repetitive, then selecting this option can improve alignment. • Post-alignment merging: Because the reads might already be correctly overlapped after alignment, selecting this option results in faster data analysis. <p>Tip: If you are not sure as to which option to select, analyze the data using both options, compare the results, and then make your selection.</p>
Motifs	<p>Selected by default. The alignments of reads that cross motif regions with expected sequences are adjusted to match the list of variants. See “Overview of using a motif” on page 53.</p> <p>Note: This is a default setting for all the Sequencer options (Ion Torrent, Illumina, and Other). If you do not want to use motifs, then you must manually clear this option.</p>

Alignment Options: Filters/Clipping

Setting	Description
Match Proportion	<p>Include the read if the proportion of the read that is aligned and not soft-clipped is greater than or equal to the indicated value, where the default value is Percent ≥ 90.</p> <p>Note: Soft-clipping can be carried out only if the proportion of the read that is aligned and not soft-clipped is greater than or equal to the indicated value.</p> <p>Tip: You should set this value to $< 90\%$ if you have a larger number of variants in the middle for the reads and you need more reads to align. Conversely, if your reads are of higher quality, particularly for the ends, then less reads need to align and you can set this to value to $>90\%$.</p>

Setting	Description
Identity	<p>Reads that are less similar to the reference than indicated value are not aligned. Select the way to identify the number of reads:</p> <ul style="list-style-type: none"> • Percent \geq: Selected by default. Default value is 90. If the percentage of reads for a sample that do not match the reference are greater than or equal to the indicated value, then reads are not aligned. • Number \leq: Default value is 0. If the total number of reads for a sample that align to reference are less than or equal to the indicated value, then the reads are not aligned.
Soft Clipping at 3' Q \leq	<p>When the base call quality is less than or equal to the indicated quality score, then the 3' ends of reads are trimmed accordingly. See “Soft clipping based on quality scores” below.</p>
<p>Note: GeneMarkerHTS automatically adjusts the default values for both Identity and Soft Clipping based on the selected sequencer. The default settings are based on using an Illumina or Ion Torrent sequencer. If you change the value for either one or both of the settings, then GeneMarkerHTS automatically selects “Other” for Sequencer.</p>	
Clip mismatched ends	<p>Selected by default. If mismatched bases are found near the end of the alignment, then soft clipping is also carried out on the 3' ends of reads.</p> <p>Tip: When carrying out panel analysis for mtDNA amplicons, you can clear this option to improve trimming if variants are at a boundary.</p> <p>See “Soft clipping based on quality scores” below.</p>

Soft clipping based on quality scores

If the base call quality is less than or equal to the quality score that is specified in the Alignment Settings for a project, then the 3' ends of reads are soft-clipped. If mismatched bases are found near the end of the alignment, then soft clipping is also carried out on the 3' ends of reads according to the following:

1. Starting at the end of the alignment sequence, move towards the middle of the alignment sequence, with a quality score for each nucleotide in the sequence calculated as follows. Add 1 for a matched base; otherwise, subtract 3 for a mismatched base. A quality score < -6 is not allowed.
2. Continue calculating a quality score for each base in the alignment sequence until a nucleotide with a quality score of 6 is found.
3. Move back from this position towards the end of the read until a mismatch is found.
4. Soft clip from this mismatch through the end of the read.

For example, if the alignment results in a CIGAR string of 100=2X10=1X3=. then:

- A score is calculated going back from the end: 0, 1, 2, 3, 0, 1, 2, 3, 4, 5, 6.
- Moving back to the end, soft clipping is started at the first mismatch through the end, which results in four bases total being soft-clipped: 100=2X10=4S.

Amplicon Settings

Available only if Panel is set to None. Opens the Amplicon Settings dialog box, which contains the settings for specifying which amplicon-sequenced regions were used for the mtDNA alignment project. See [“Specifying amplicon regions”](#) below.

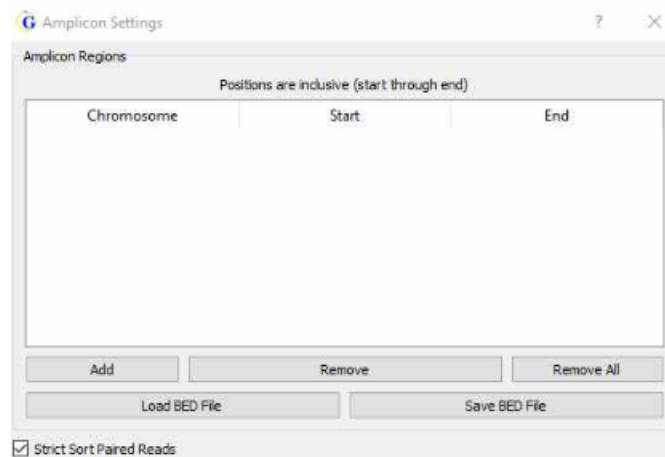
Specifying amplicon regions

The Amplicon Settings dialog box contains the settings for specifying which amplicon-sequenced regions were used for the mtDNA alignment project. If you select Amplicon Settings for an mtDNA alignment project, then you must do one of the following:

- You must load a BED file that includes the amplicon regions.
 - You must manually add the amplicon regions.
1. On the New Project dialog box, click Amplicon Settings.

The Amplicon Settings dialog box opens.

Figure 2-5: Amplicon Settings dialog box



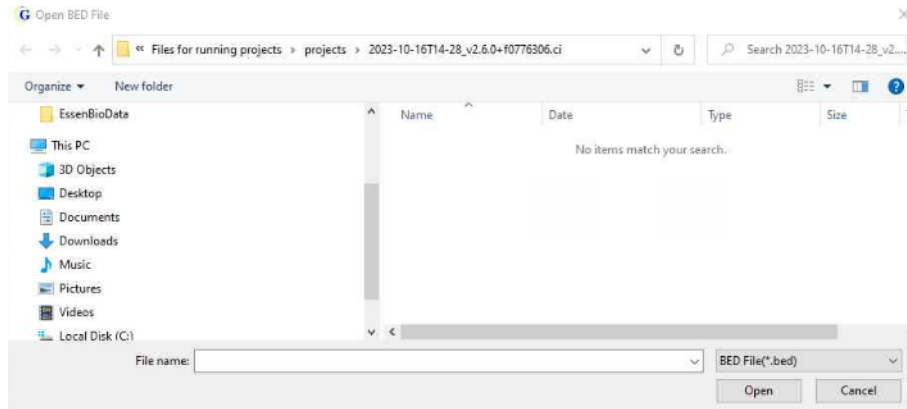
2. Continue to one of the following:
 - [“To load an amplicon BED file”](#) below.
 - [“To manually add amplicon regions”](#) on page 60.

To load an amplicon BED file

1. Click Load BED file.

The Open BED file dialog box opens. See [Figure 2-6 on page 60](#).

Figure 2-6: Open BED File dialog box



2. Browse to and select the existing amplicon BED file, and then click Open to select the BED file.
The Open BED File dialog box closes. The position for the BED file are displayed in the dialog box.
3. Leave Strict Sort Paired Reads selected, or optionally, clear the setting.



If Strict Sort Paired Reads is selected, then the paired reads sort to the same amplicon. If Strict Sort Paired Reads is not selected, then the two reads can sort to different amplicons. Each read, however, still sorts to only a single amplicon.

4. Click OK.
The Open BED File dialog box closes. The New Project dialog box remains open.
5. Continue with setting up the new GeneMarkerHTS project.

To manually add amplicon regions

You can manually add as many amplicon regions as needed.



For detailed information about the format for a BED file, see [“BED file” on page 161](#).

1. For every region that you must add to the BED file, click Add.
A new amplicon region is displayed in the Amplicon Settings dialog box. As you add a new region, it is numbered sequentially. By default, the chromosome is set to M, and both the Start and End positions are set to “1,” but you can edit any or all these values.

Figure 2-7: New amplicon region

148	22	29130361	29130739
149	chrM	1	1

2. As you manually add new amplicon regions, the following options are also available:
 - Remove: Click to remove a selected amplicon region. You can remove only a single amplicon region at a time. After you remove a region, the remaining regions are automatically re-numbered.



No confirmation message is displayed before you remove a region, so be cautious when doing so.

- Remove All: Click to remove all amplicon regions in a single step.
3. Leave Strict Sort Paired Reads selected, or optionally, clear the setting.



If Strict Sort Paired Reads is selected, then with paired reads, the start and end points for a paired read must match to the start and end points of the amplicon for the read to be assigned to the amplicon. In the event that a forward read that matches to Amplicon #1 extends into the reverse read that matches to Amplicon #2, this allows the assignment of the read to only one amplicon.

4. Click OK.

The Open BED File dialog box closes. The New Project dialog box remains open.
5. Continue with setting up the new GeneMarkerHTS project.

PHI Settings

Opens the PHI Settings dialog box. You use the options on this dialog box to hide regions in a subject's sample file from alignment if these regions could potentially reveal personal health information (PHI) about the subject. See ["Masking personal health information \(PHI\) for a subject" on page 61](#).

Masking personal health information (PHI) for a subject



You can specify which personal health information to mask for a subject only before carrying out the corresponding mtDNA alignment project.

When carrying out a forensic analysis, a lab technician must remain aware of the potential of exposing personal health information (PHI) about a subject that is not relevant to the investigation. For example, the alignment results could reveal that a subject is a carrier for an inherited disease such as Leigh's syndrome. To prevent this, you can hide regions from the alignment that could reveal PHI. You can elect to load default hidden regions, which hides the PHI regions that MITOMAP has identified, and/or hide the PHI regions that you explicitly define in a BED file.



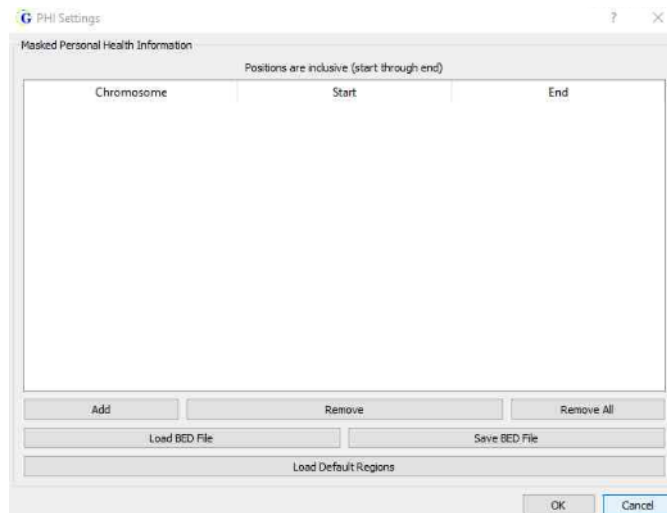
For detailed information about the format for a BED file, see “BED file” on page 161.

For a default or custom hidden region, the following is applicable:

- Nucleotides that are in the region are not displayed in the Pile-Up pane in the GeneMarkerHTS Viewer.
 - The CIGAR string is hidden in the tooltip for a read in the Pile-Up pane.
 - You cannot copy sequences for reads that cross the region.
 - Allele counts are hidden from all reports.
 - No variants are called within the region.
1. On the New Project dialog box, click PHI Settings.

The PHI Settings dialog box opens.

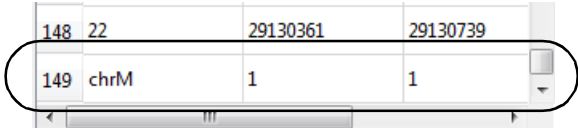
Figure 2-8: PHI Settings dialog box



2. Do one or both of the following:

Option	Description
Hide the PHI regions that MITOMAP has identified	Click Load Default Regions. Note: For a list of the MITOMAP locations that are the built in to PHI, see the following: <ul style="list-style-type: none"> • http://www.mitomap.org/foswiki/bin/view/MITOMAP/MutationsRNA • http://www.mitomap.org/foswiki/bin/view/MITOMAP/MutationsCodingControl
Hide the PHI regions that you have identified	Click Load BED File to open the Open BED File dialog box, and then browse to and select the appropriate BED file.

3. Optionally, after you have loaded the default and/or custom regions that are to be hidden, do any or all of the following as needed:

Option	Description
<p>Manually add a region</p> <p>Note: You can manually add as many regions as needed. You can add only a single region at a time.</p>	<p>Click Add.</p> <p>A new region is added. As you add a new region, it is numbered sequentially. By default, the chromosome is set to M, and the Start and End positions are both set to "1," but you can edit any or all of these values as needed.</p> <p><i>Figure 2-9: Manually added PHI region</i></p> 
<p>Remove one region at a time</p>	<p>Select the region, and then click Remove. After you remove a region, the remaining regions are automatically re-numbered.</p> <p>Warning: No confirmation message is displayed before you remove a region, so be cautious when doing so.</p>
<p>Remove all regions in a single step</p>	<p>Click Remove All.</p>

4. Click OK.
The PHI Settings dialog box closes. The New Project dialog box remains open. The selected regions will be hidden from alignment.
5. Continue with setting up the new project.

Working with a GeneMarkerHTS Project

The New Project dialog box provides all the options for adding a new project in GeneMarkerHTS. You can also open and work with existing projects in GeneMarkerHTS. See:

- “New GeneMarkerHTS project caveats” below.
- “To add a new GeneMarkerHTS project” on page 65.
- “To work with an existing GeneMarkerHTS project” on page 68.

New GeneMarkerHTS project caveats

- Every GeneMarkerHTS project is named project.pjt, and the project file as well as all the project output files are saved in a folder that, by default, is named as:

`<project creation date>T<project run time_v<GeneMarkerHTS version>+<f<n>).ci`

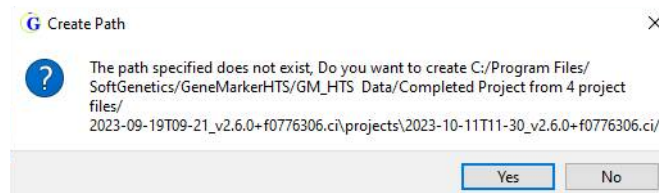
where:

- The date is in the following format: year_month_day.
- The time is in 24-hour time.
- The software version is three digit showing the major release, the minor release, and the point release.
- f<n> is developer-specific information that is used for debugging purposes.

For example, a project folder named 2023_09_12_T14-28_v2.6.0+ f07776306.ci indicates a project that was run on October 09, 2023 at 2:18 pm in the afternoon using version 2.0.6 of GeneMarkerHTS. This default naming convention allows you to save multiple projects in the same folder without overwriting an existing project.

- If you do not specify a folder in which to save the project, then GeneMarkerHTS informs you that the project folder was not set, and asks you if you want to use the default project folder, which is the folder that contains the project sample files.

Figure 2-10: Create Path prompt



- The default reference that is supplied in GeneMarkerHTS is the revised Cambridge Reference Sequence (rCRS). If you select a different reference, then it must be in either FASTA or GBK format.
- When you add a new project in GeneMarkerHTS, the sample files that you load must be in one of three formats: compressed FASTQ files (fastq.gz), uncompressed FASTQ files (.fastq), or BAM files (.BAM).

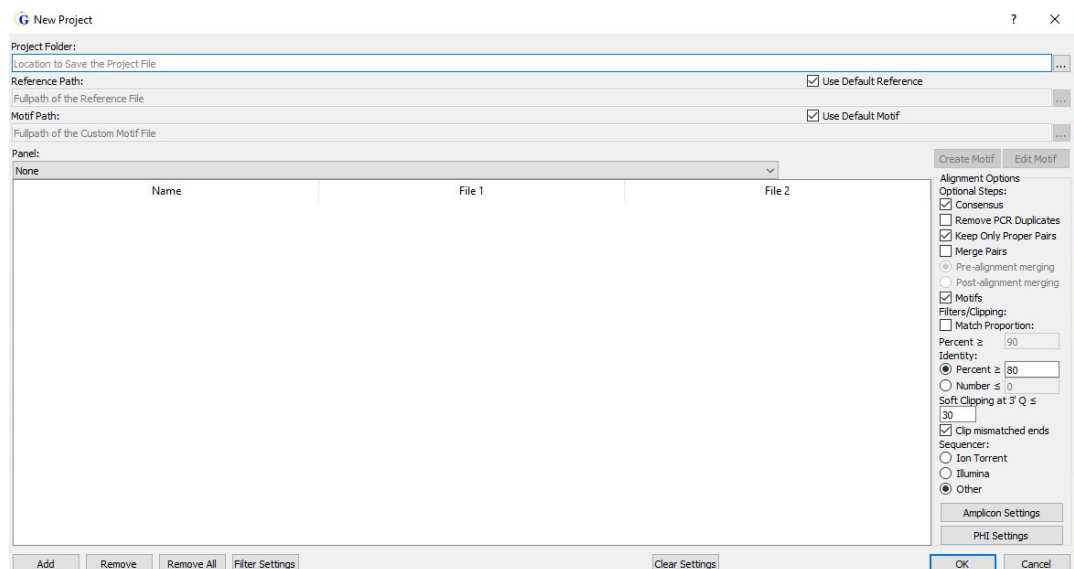
- The Name of each sample file and the names of all the corresponding output files for a project are automatically generated from the names of the added sample files. You can edit the Name for a sample file.
- GeneMarkerHTS automatically groups paired reads in a sample, but you can split a group of paired reads into individual samples.
- GeneMarkerHTS automatically adjusts the default values for both Identity and Soft Clipping based on the sequencer that you select. If you change the value for either one or both settings, then GeneMarkerHTS automatically selects “Other” for Sequencer, and you cannot select a different Sequencer value.
- Filter settings and table settings control the criteria for variant calling and for displaying project information in the Variants table in the Mito Alignment Viewer. Filter settings determine the alleles that are called for a project in the STR Analysis Viewer. You can adjust filter settings and table settings before or after alignment of the samples in a project.
- Amplicon settings define the amplicon-sequenced regions that were used for the mtDNA alignment project. You can adjust amplicon settings only *before* the alignment of the samples in a project.
- PHI settings control which personal health information to mask for a subject. You can adjust PHI settings only *before* the alignment of the samples in a project.

To add a new GeneMarkerHTS project

1. On the GeneMarkerHTS main window, click New.

The New Project dialog box opens. If this is the first project that is being run for the GeneMarkerHTS instance, then all the project settings are set to their default values; otherwise, by default, GeneMarkerHTS remembers the settings for the last run project.

Figure 2-11: New Project dialog box



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2. Optionally, if applicable, to reset all project settings to their default values, click Reset Settings, and then click Yes in the Reset Settings dialog box that opens.
3. Do one of the following to specify the location in which to save the project:
 - Leave the Project Folder blank, and then, by default, after you add the project sample files, the project folder is set to the same folder as the project sample files.
 - Next to the Project Folder field, click the Browse button to open the Project Save Location dialog box, and then browse to and select where the project (*.pjt) file is to be stored.
 - In the Project Folder field, manually enter the full path to the folder in which the project is to be saved.



If the specified folder does not exist, then GeneMarkerHTS prompts you to create the path before the project is created.

4. If the project is to use the default reference that is supplied with GeneMarkerHTS, then select Use Default Reference; otherwise, leave Use Default Reference blank, and then next to the Reference Filename field, click the Browse button to open the Open Reference dialog box, and then browse to and select the reference that the project is to use.
5. If the project is to use the built-in motif that is supplied with GeneMarkerHTS, then leave Use Default Motif selected; otherwise, to use a custom motif, clear Use Default Motif, and then do *one* of the following:
 - To use the default motif as your starting point for creating a custom motif:
 - a. Clear Use Default Motif.
 - b. Continue to [“To create or edit a custom motif” on page 54.](#)
 - To use an existing custom motif as your starting point for creating another custom motif:
 - a. Clear Use Default Motif.
 - b. Click the Browse button that is displayed to the right of the Motif Path field, and browse to and select the motif.
 - c. Continue to [“To create or edit a custom motif” on page 54.](#)
6. Specify all the options for the project, including the sequencer. See [“New Project Dialog Box Settings” on page 51.](#)
7. To add the samples for the project, click Add to open the Add Samples dialog box, and then select the sample files for the project.



After you add at least one sample, you can also right-click any sample and on the context menu that opens, click Add to add another sample.

The sample files are added to the project and are displayed in the Sample Files pane of the New Project dialog box. The Name for each sample file is automatically generated from the name of the added file.

8. Optionally, you can do any or all of the following for added sample files:
 - To change the name of a sample file, double-click the file to select it, and then change the name of the file.
 - To remove one or more sample files from a project, select the sample file (CTRL-click to select multiple files), and then click Remove. (You can also right-click any sample file and on the context menu that opens, click Remove.)
 - To split paired read sample files into separate sample entries, right-click any one of the paired read sample files, and on the context menu that opens, click Split.



To combine split paired read files, right-click any of the paired read sample files, and then on the context menu that opens, click Combine with <filename>.

- To remove all sample files in a single step, click Remove All.
9. Optionally, do any or all of the following:
 - To adjust the settings that affect variant calling and therefore, the variants that are displayed in the Variants table of the Mito Alignment Viewer, click Filter Settings to open the Filter Settings dialog box, and then edit any or all of the values on the Mito Variant tab and/or the Mito Alignment tab.



For detailed information about filter settings for an mtDNA alignment project, see ["Mito Alignment Viewer Filter Settings"](#) on page 93.

- To adjust the amplicon regions used for the alignment, click Amplicon Settings, and then add or remove regions as appropriate. See ["Specifying amplicon regions"](#) on page 59.
 - To adjust the settings that mask the personal health information (PHI) for the subject, click PHI Settings to open the PHI Settings dialog box, and then add and/or remove default and/or custom hidden regions as appropriate. See ["Masking personal health information \(PHI\) for a subject"](#) on page 61.
10. Click OK.

The Project Viewer dialog box opens. The samples that are being processed for the project are displayed on the left side of the dialog box in the form of "Sample buttons." The progress of processing a sample file is displayed on the right side of the dialog box. After a sample file is successfully processed for a project, the Sample button for the project is enabled. You can click the Sample button for any completed sample file to view its results in either the Mito Alignment Viewer (for an mtDNA only project) or the STR Analysis Viewer (for an STR analysis project), even if other sample files are being processed or are waiting to be processed. See [Figure 2-12 on page 68](#).


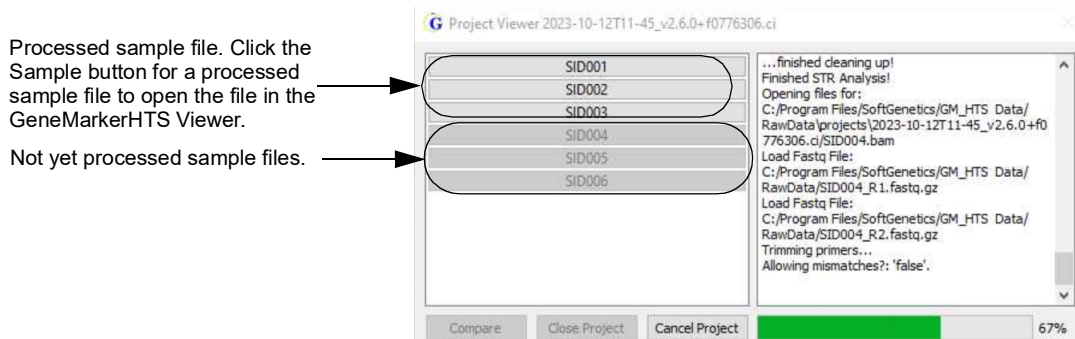
 Depending on your project setup, other messages might open before the Project Viewer dialog box opens, such as a message about creating a non-existent project folder. Answer all the prompts as needed to resolve any issues and open the Project Viewer dialog box.

Figure 2-12: Project Viewer dialog box with processed and unprocessed Sample files




 For a detailed list of the output files for a GeneMarkerHTS project, see [“GeneMarkerHTS Project Output Files” on page 70.](#)

11. Continue to [Chapter 3, “GeneMarkerHTS Viewers and Reports,” on page 73.](#)

To work with an existing GeneMarkerHTS project

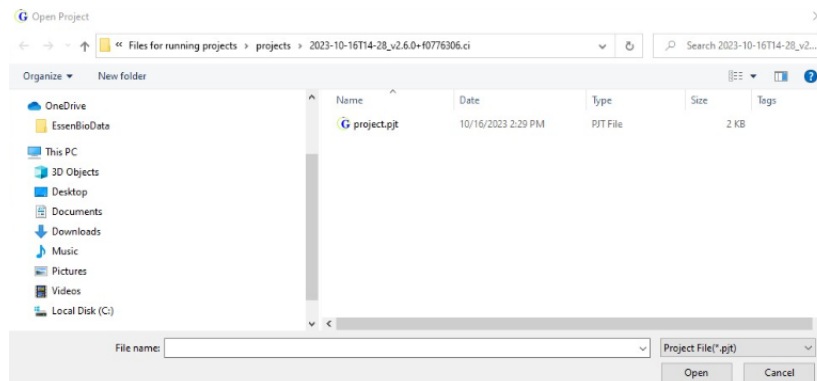
You can load a project that has been saved locally to the GeneMarkerHTS client.

 Remember, a GeneMarkerHTS project is named `project.pjt` and if you accepted the default project location when creating the project, the project file is located in the same directory as the project sample files.

1. On the GeneMarkerHTS main window, click Open.

The Open Project dialog box opens. By default, the Open Project dialog box is set to the directory for the project that was last opened for the GeneMarkerHTS instance.

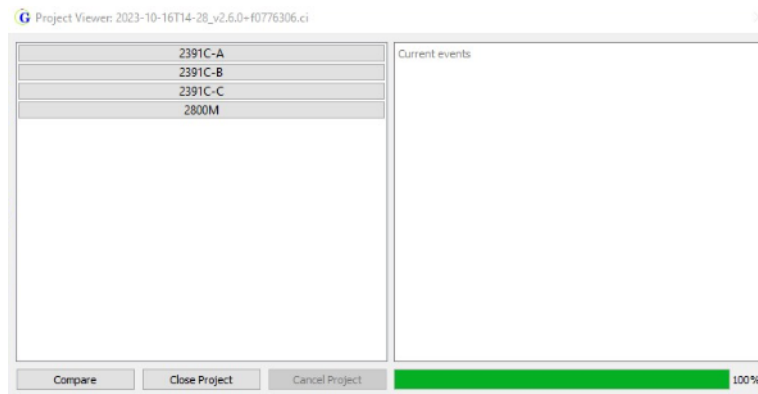
Figure 2-13: Open Project dialog box



2. Browse to and select the project with which you are working, and then click Open.

The Project Viewer dialog box opens. All the samples that were analyzed for the project are listed by name on the left side of the dialog box. The status bar at the bottom of the dialog box is set to 100%.

Figure 2-14: Project Viewer dialog box for a completed project



3. Do one the following:
 - To open the project in a viewer, double-click a Sample button.
 - If a panel was *not* used for the project, then the Mito Alignment Viewer opens, displaying the mtDNA alignment results for the sample. You can open multiple individual sample files at the same time.
 - If a panel was used for the project, then the STR Analysis Viewer opens, displaying the STR analysis results for the sample. You can open multiple individual sample files at the same time.
 - To compare the results for two or more samples in a Mito Alignment project in a single Comparison Viewer window, click Compare to open the Select Samples dialog box, select the sample to compare, and then click Compare.



For detailed information about the different project viewers that are available in GeneMarkerHTS, see [Chapter 3, “GeneMarkerHTS Viewers and Reports,”](#) on page 73.

GeneMarkerHTS Project Output Files

The table below lists the files that are generated for any GeneMarkerHTS project. All files are located in the project folder.

File	Description
Project Settings	The settings (alignment options, motif selection, and so on) for the project.
Trim Primer Log	Lists the read sorting results for all the amplicons.
Panel Primer Match Statistics	Lists the read sorting results for all the amplicons in a tab-separated values (*.tsv) format.
Analysis Log	Contains a summary of the alignment results in a standardized .json format.
Paired End Merge Report	Generated if Merge Pairs was selected for the project. Details how many reads were merged and how many reads could not be merged.

The table below lists the files and reports that are generated for each sample in an mtDNA alignment project. All the files are located in the project folder.

File	Description
BAM/BAI	Contains the fully detailed alignment results.
Alignment Statistic Log	Contains a summary of the alignment results.
Consensus Sequence	Details the consensus sequence with the major alleles in FASTA format, where a major allele is defined as the allele with the highest frequency at a given position. All major alleles are reported unless the allele is the same as the reference.
Consensus Statistics	Lists the number of reads with each allele at each position in the sample.
Major Allele Report	Variant report for all major alleles. Note: You can copy and paste a major allele string into EMPOP.
Minor Allele Report	Variant report for all minor alleles, where a minor allele is defined as any other allele with a frequency that is less than the allele with the highest frequency at a given position. Note: You can copy and paste a minor allele string into EMPOP.
Low Coverage Report	Lists all the regions with coverages that fell below the Total Coverage threshold that was specified for the project.
Motif	Lists all the motifs that were used for the project.
Amplicon	Lists all the amplicon regions that were used for the project along with the number of reads (forward, reverse, and total) per region.

File	Description
Variants	<p>A text file saved as <Sample Name>_variants.txt. The file details all the EMPOP variants for the project sample: Major, Minor, and Combined.</p> <p>Note: You can copy the variants that are detailed in this file and paste them into EMPOP to obtain the statistical information for the variants. An EMPOP direction option is also available on the main menu for the Mito Alignment viewer. See "Mito Alignment viewer main menu" on page 76.</p>

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GeneMarkerHTS Project Setup and Management

Chapter 3

GeneMarkerHTS Viewers and Reports

Three viewers are available in GeneMarkerHTS application for viewing the results of a project: the Mito Alignment viewer, the STR Analysis viewer, and the Comparison viewer.

- The Mito Alignment viewer is a viewing and editing tool that you use to view and edit the results of your mtDNA alignment projects and produce a variety of interactive reports that summarize the alignment and other analysis information.
- The STR Analysis viewer is a viewing and editing tool that you use to view the results of a simultaneous analysis of autosomal- and Y-STRs along with mtDNA data for your project samples.
- The Comparison viewer is an interactive viewing tool that you use to view a sample to sample comparison for an mtDNA project as well as a variant comparison of all the samples in the project at the same time.

This chapter details these viewers as well as the reporting options that are available for an STR project and for an mtDNA project.

This chapter covers the following topics:

- [“Mito Alignment Viewer Overview and Layout” on page 75.](#)
- [“Mito Alignment Viewer Navigation and Options” on page 85.](#)
- [“Mito Alignment Viewer Filter Settings” on page 93.](#)
- [“Mito Alignment Table Settings” on page 100.](#)
- [“Mito Alignment Viewer Reports” on page 102.](#)
- [“STR Analysis Viewer Overview and Layout” on page 106.](#)
- [“STR Analysis Viewer STR and STR Stutter Filter Settings” on page 123.](#)
- [“Comparison Viewer” on page 141.](#)



Remember, you can select project samples for viewing during project processing, or after project processing has completed. See [“Working with a GeneMarkerHTS Project” on page 64.](#)

Chapter 3
GeneMarkerHTS Viewers and Reports

Mito Alignment Viewer Overview and Layout

You use the Mito Alignment viewer to view the results of your mtDNA alignment projects. You can edit the display settings for the viewer and you can use viewer options to edit the results of the alignment project. You can load one or more sample files in their own Mito Alignment viewer windows while a project is being run, or you can load one or more sample files for a saved project. (See [“Working with a GeneMarkerHTS Project” on page 64.](#)) When you open a sample file for any project in the Mito Alignment viewer, the filter settings and table settings that were in place at the time that the project was saved determine the variants that were called and the information that is displayed in the Variants Table in the viewer, but you can always edit these settings as needed to better suit your working needs. See [“Mito Alignment Viewer Filter Settings” on page 93](#) and [“Mito Alignment Table Settings” on page 100.](#)

- If you load multiple sample files from *different* projects, then each project sample opens in its own separate Mito Alignment viewer window, and the viewer windows are *not* linked. You can independently navigate and edit the display settings for each sample file in its own viewer window.
- If you load multiple sample files from the *same* project, then each project sample opens in its own separate Mito Alignment viewer window, but the viewer windows *are* linked. All the viewer windows zoom and scroll *horizontally* across the reference in unison. Any changes that you make to the filter settings for one viewer window are applied to all opened viewer windows for the project’s sample files.

Figure 3-1: Mito Alignment viewer



From top to bottom, the Mito Alignment viewer has six major components:

- The title bar. See [“Mito Alignment viewer title bar”](#) on page 76.
- The main menu. See [“Mito Alignment viewer main menu”](#) below.
- The Global View pane. [“Global Viewer pane”](#) on page 78.
- The Panes display. See [“Pane displays”](#) on page 79.
- The Variants table. See [“Variants table”](#) on page 83.
- The Status bar. See [“Status Bar”](#) on page 84.

Mito Alignment viewer title bar

The Mito Alignment viewer title bar displays the name of the sample for which you are reviewing the mito alignment results.

Figure 3-2: Mito Alignment viewer title bar



Mito Alignment viewer main menu

The Mito Alignment viewer main menu is set up in a standard Windows menu format with menu commands grouped into menus across the menu bar.

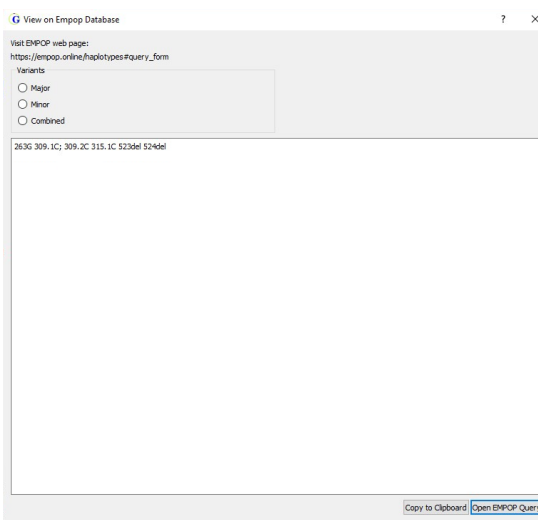
Menu Option	Description
Reports	Generate one of three reports for the project: the Coverage report, the Template Length report, or the Read Length report. See “Mito Alignment Viewer Reports” on page 102.
Filter Settings	Opens the Filter Settings dialog box, which contains options for changing the viewer display. See “Mito Alignment Viewer Filter Settings” on page 93.
Load Report	Opens the Load Report dialog box, in which you can select a sample report for display in the viewer. See “To save/load reports for an mtDNA project” on page 90.
Save BAM Edits	If you have made any edits to a BAM project file, then you can save the edited BAM file as a new BAM file.
Save Report	Opens the Save Report dialog box, in which you specify a folder for saving the information that is currently displayed in the Variants table as a report. See “To save/load reports for an mtDNA project” on page 90.
Table Settings	Opens the Table Settings dialog box, which lists all the data columns that are currently displayed in the Variants table of the Mito Alignment viewer. By default, all available columns are initially displayed in the Variants table. To remove a column from the Variants table display, clear its selection. To add a column to the display, select it. See “Mito Alignment Viewer Filter Settings” on page 93.
Toggle Table	Toggles the display (On or Off) of the Variants table in the Mito Alignment viewer. See “Variants table” on page 83.
Export to vcf	Exports all the variants that are displayed in the Pile-up pane to a .vcf file. By default, the .vcf file is named as <sample file name>.vcf and the file is saved in the project folder, but you can edit one or both these values.

Menu Option	Description
EMPOP	Opens the View on EMPOP Database dialog box, which lists all the called sample variants for the project by their classification (Major or Minor) on the EMPOP database. See “View on EMPOP Database dialog box” below. Note: Internet access is required for this option.

View on EMPOP Database dialog box

By default, when the View on EMPOP Database dialog box first opens, all the sample variants that are classified as Major in the EMPOP database are displayed first.

Figure 3-3: View on EMPOP Database dialog box



Do any of the following:

- To view only the sample variants classified as Minor on EMPOP, select Minor.
- To view the variants of both classifications at the same time, select Combined.

Major variants are listed first, separated by commas. Minor variants are listed second, separated by commas. A semi-colon delineates the major variants from the minor variants.

Figure 3-4: Viewing combined sample variants

263G 297M 304.1c; 304.2a 309.1c 309.1C; 309.2C 309.1c; 309.2c; 309.3c 310.1c 315.1C 316R 523del 524del 16519Y

- Copy to clipboard: Copies the currently displayed variant list to the GeneMarkerHTS client clipboard. You can then paste the list into third-party software such as Microsoft Excel.
- Open EMPOP Query: Opens an EMPOP online haplotype query form.

Global Viewer pane

The Global Viewer pane for the Mito Alignment viewer shows the coverage depth for the sample. By default, the forward read coverage is displayed in blue and the reverse read coverage in red. A solid black line/hollow black rectangle indicates the pile-up range. By default, green lines indicate the locations of major variants and orange lines indicate the locations of minor variants.


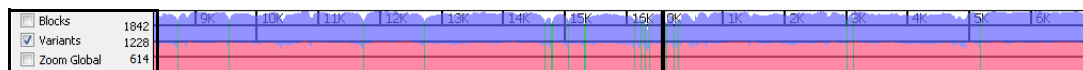
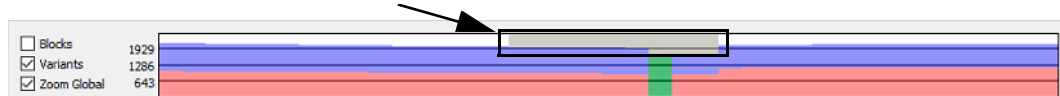
 The zoom level for the Pile-Up pane determines the appearance of the line/rectangle indicator in the Global Viewer pane. You can change all the default display colors for the Global Viewer pane. See [“Configuring GeneMarkerHTS Visuals Settings” on page 152.](#)


Figure 3-5: Global Viewer pane



By default, gray shading indicates areas of coverage that fall below the Total Coverage threshold that was specified for the filter settings for the project.

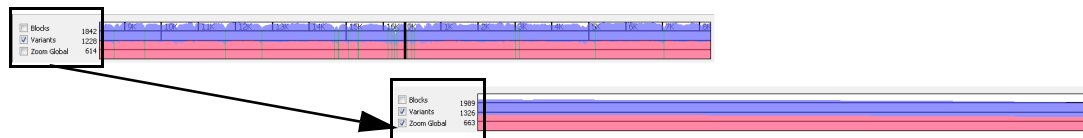
Figure 3-6: Shading alerts for low coverage regions in the Global View pane




 For detailed information about the Total Coverage threshold, see [“To specify the variant call settings for an mtDNA project” on page 93.](#) “N” is displayed for the consensus sequence in these areas of low coverage in the Consensus Sequence pane. See [“Reference pane” on page 80.](#)

To zoom the display of the Global Viewer pane to only that for the pile-up region, select Zoom Global.

Figure 3-7: Zoom Global selected



 For information about the Blocks option and the Variants option, see [“Pile-Up pane” on page 82.](#)

The following options are available for the Global Viewer pane. You can:

- Save the Global Viewer pane display as an image. See [“To save the Global Viewer pane display as an image” on page 79.](#)
- Change the default display colors. See [“To edit the Global Viewer pane colors” on page 157.](#)

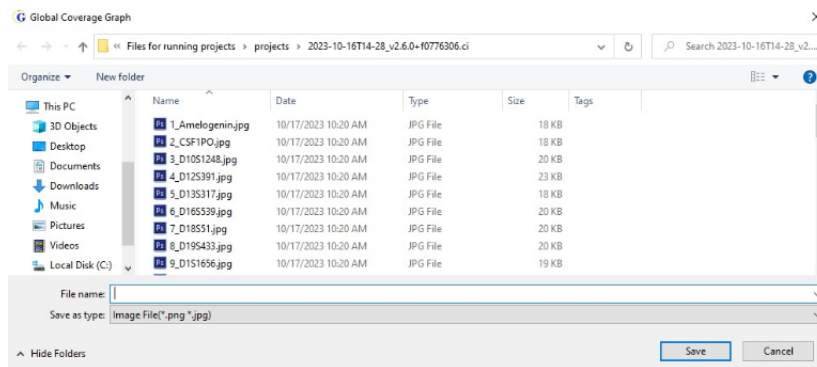
To save the Global Viewer pane display as an image

You can save the Global Viewer pane display as image with forward coverage stacked on top of reverse coverage. By default, the image is saved in two different formats (PNG/JPEG) in the project folder, but you can always select a different location. You must name the image when you save it.

1. Right-click in the Global Viewer pane, and on the context menu that opens, click Save Global Coverage Image.

The Global Coverage Graph dialog box opens. The default location is set to the project folder.

Figure 3-8: Global Coverage Graph dialog box



2. Enter a name for the image file.
3. Optionally, select a different location than the project folder for saving the file.
4. Click Save.

The Global Coverage dialog box closes. The Mito Alignment viewer remains open.

Pane displays

The Panes display for the Mito Alignment viewer consists of up to four separate panes. Reference Position numbers are displayed at the top of the Panes display. By definition, an mtDNA reference is circular, and this is reflected in the reference position numbers. As you scroll horizontally through a pane display, the position numbers increase up to the end position of the reference, and then the reference position numbering starts over again.


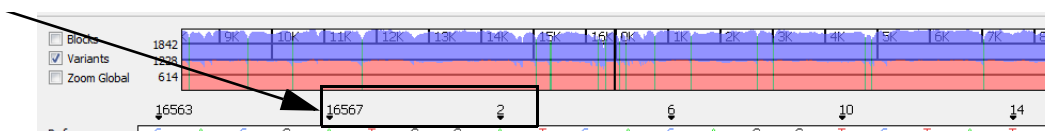
 For information about horizontally scrolling the display in the Mito Alignment viewer, see [“Mito Alignment Viewer Navigation and Options” on page 85.](#)

Figure 3-9: Circular reference reflected in reference position numbering in Panes display



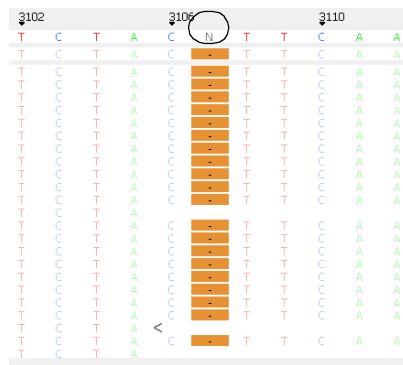
From top to bottom, the four panes in the Panes display are the following:

- The Reference pane. See [“Reference pane”](#) below.
- The Consensus pane. See [“Consensus pane”](#) below.
- The Motifs and Amplicons pane. See [“Motifs and Amplicons pane”](#) on page 81.
- The Pile-Up pane. See [“Pile-Up pane”](#) on page 82.

Reference pane

The Reference pane displays the sequence of the reference to which the samples were aligned. To maintain position numbering in an mtDNA alignment project, an “N” is sometimes used instead of a nucleotide read in the Reference sequence. Variant calls are not reported for reference positions with an “N.”

Figure 3-10: “N” for a reference position to maintain position numbering

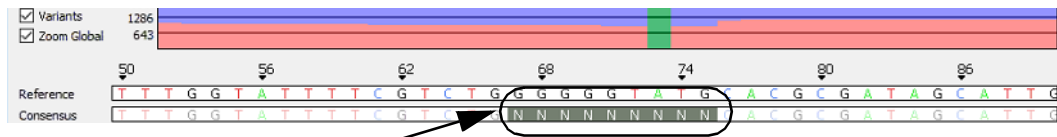


Position 3107 in the rCRS has an “N” to maintain position numbering. Bases that are aligned at this position are ignored.

Consensus pane

The Consensus pane displays the consensus sequence for the major alleles, where a major allele is defined as the allele with the highest frequency at a given position. If any areas of coverage fall below the Total Coverage threshold that was specified for the project filter settings, then the consensus sequence is set to “N” for these regions in the pane.

Figure 3-11: Low coverage regions in the Consensus Sequence pane

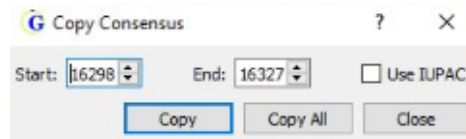


For detailed information about the Total Coverage threshold, see [“To specify the variant call settings for an mtDNA project”](#) on page 93. The corresponding areas of low coverage are highlighted in gray in the Global Viewer pane. See [“Global Viewer pane”](#) on page 78.

You can right-click anywhere in the Consensus pane to open a context menu with two options for copying the consensus sequence.

- Copy Consensus. Opens the Copy Consensus dialog box. The dialog box displays the start and end positions for the portion of the consensus sequence that is currently displayed in the Consensus pane.

Figure 3-12: Copy Consensus dialog box



The following options are available for the Copy Consensus dialog box:

- You can leave the Start and End positions as-is, or you can manually edit the values.
- You can copy the consensus sequence bases as displayed (G, A, etc.) or you can select Use IUPAC to copy the consensus sequence in its IUAPC nomenclature code instead.
- You can select Copy to copy the designated region for the sequence (start and end positions are included) to the client clipboard, or you can click Copy All to copy the entire consensus sequence, regardless of the Start and End positions that are indicated, to the client clipboard. You can now paste the copied information into a third-party application such as Microsoft Excel.
- Copy Entire Consensus. Copies the entire consensus sequence to the client clipboard. You can now paste the copied information into a third-party application such as Microsoft Excel.

Motifs and Amplicons pane

The Motifs and Amplicons pane is displayed only if one or both of the following variables were used for the project:

- A motif was used for the project. The pane displays the motif regions (green) that were used for the mtDNA alignment project. If you hold your mouse pointer for a motif region, a tooltip opens that displays the following information: the start and end points for the motif region and the variant calls in the region.
- Amplicon settings were specified for the project. The pane displays the amplicon-sequenced regions (blue) that were used for the mtDNA alignment project, whether a panel was used or not. If you hold your mouse pointer over an amplicon region, a tooltip opens that displays the following information: the chromosome, the chromosome start and end positions, and the number of reads that were assigned to the region.

See [Figure 3-13 on page 82](#).

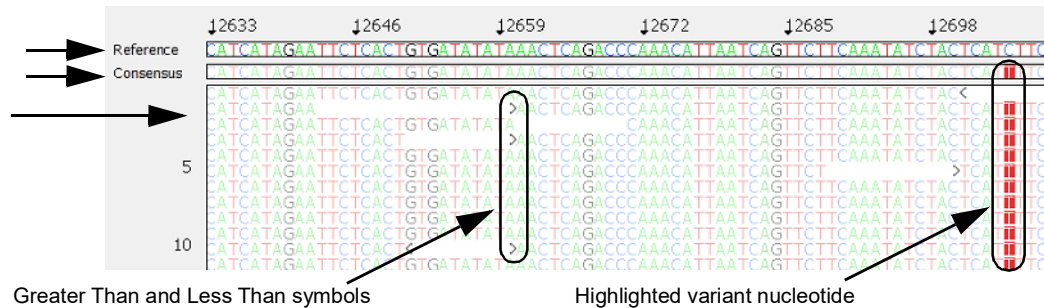
Figure 3-13: Motifs and Amplicons pane



Pile-Up pane

Unlike the other panes in the Mito Alignment viewer Panes display, the Pile-Up pane is not explicitly identified by name in the viewer; however, it is always located as the last pane in the display. The pane displays a view of all the reads for the sample as they align to the reference sequence. Greater Than and Less Than symbols point to the 5' end of the read, where the Greater Than (>) symbol is used for forward reads and the Less Than (<) symbol is used for reverse reads.

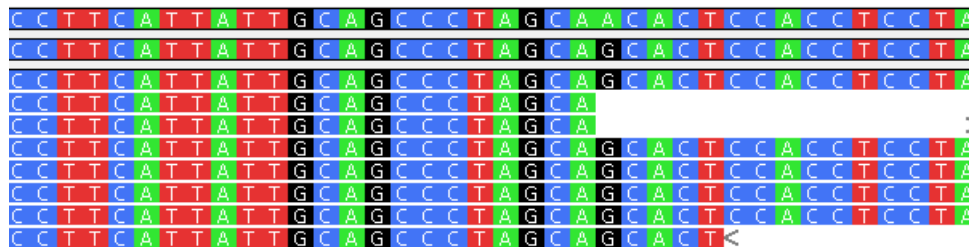
Figure 3-14: Variants selected for Pane displays



Two options that are displayed *next to the Global Viewer pane* are available for changing the Pile-up pane display:

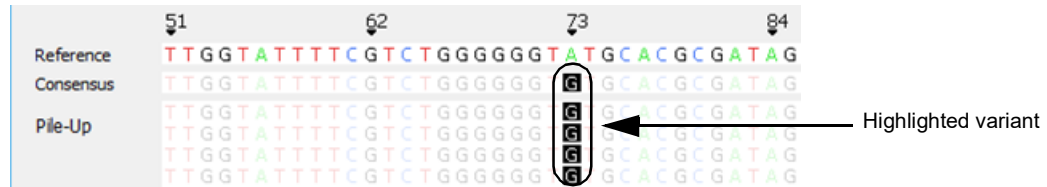
- Blocks – If Blocks is selected, then the background for each nucleotide is a color and the nucleotide (A, T, C, and G) is white.

Figure 3-15: Blocks selected for Pile-up pane display



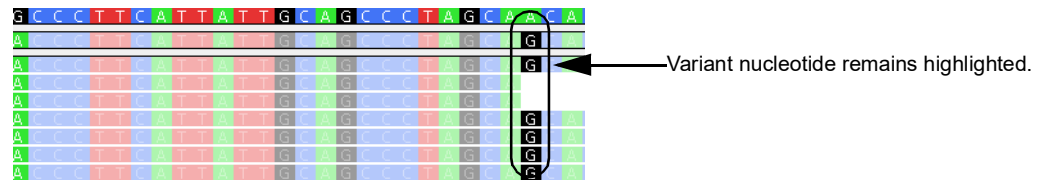
- Variants – Selected by default. If Variants is selected, then for all non-variant nucleotides, the background is white and the nucleotide (A, T, C, and G) is displayed in color. For variant nucleotides, the background is colored, and the nucleotide is displayed in white, which is referred to as a *highlighted* variant.

Figure 3-16: Variants selected for Pile-up pane display



You can select both Blocks and Variants for the Pile-up pane display. In this case, all nucleotides are still displayed in white on a colored background; however, all nucleotides other than variant nucleotides are dimmed and all variant nucleotides are highlighted to visually differentiate them from non-variant nucleotides. See [Figure 3-17](#) below. To edit the background colors, highlighting colors, nucleotide colors, and the nucleotide visibility for the Pile-up pane display, see [“To edit the background and highlighting colors in the Pile-up pane display”](#) on page 154 and [“To edit the visibility and default nucleotide colors for the Pile-up pane display”](#) on page 155.

Figure 3-17: Blocks and Variants selected for Pile-up pane display



Variants table

By default, the Variants table for the Mito Alignment viewer lists each variant call in increasing order of their sequence position, where:

- A *major* allele is defined as the allele with the highest frequency at a given position.
- All major alleles are reported unless the allele is the same as the reference.
- *Minor* alleles are any other allele at a given position with a frequency that is greater than the Sequence IT Count (the minimum threshold for a sequence to be flagged as a possible allele).
- Insertions are reported as major alleles if they occur at a frequency that is greater than or equal to 50% and as minor alleles if they occur at a frequency that is less than 50%.
- Because reference alleles can represent a secondary mutation of a major allele, reference alleles are reported as minor alleles if their frequencies occur at a rate that is greater than the minimum threshold for Allele Frequency.

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Also by default, the Variants table shows both (major and minor) allele types, but the following options are available:

- You can choose to show only major alleles or only minor alleles.
- The specific alleles and the information that is displayed for the alleles in the Variants table are determined by the filter settings and table settings that were in place at the time that the project was saved, but you can always edit these settings after the project is completed. [“Mito Alignment Viewer Filter Settings” on page 93.](#)
- You can click a column header to change the default sort order of the data. An icon in the column header indicates the sort direction. An Up arrow indicates an ascending sort and a Down arrow indicates a descending sort.

Figure 3-18: Variants table

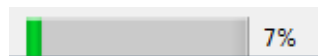
Indicates the type of allele to display in the Variants table

Index	Position	Ref	Alt	IUPAC	Variants	Variants %	Coverage	A(#F;#R)	C(#F;#R)	G(#F;#R)	T(#F;#R)	Del(#F;#R)	Ins(#F;#R)	A%	C%	G%	T%	Del%	Ins%	A Score	C Score	G Score	T
1	73	A	G	73G	A73G	99.82	1709	2;1	0;0	798;908	0;0	0;0	0;0	0.17	0.00	99.82	0.00	0.00	0.00	23;34	0;0	31;32	0;
2	195	T	C	195C	T195C	99.82	1722	1;0	825;894	0;0	1;1	0;0	0;0	0.05	99.82	0.00	0.11	0.00	0.00	28;0	32;32	0;0	2;
3	263	A	G	263G	A263G	99.77	1793	1;2	1;0	891;898	0;0	0;0	0;0	0.16	0.05	99.77	0.00	0.00	0.00	28;22	27;0	32;32	0;
4	489	T	C	489C	T489C	99.71	1781	0;0	896;880	1;0	0;4	0;0	0;0	0.00	99.71	0.05	0.22	0.00	0.00	0;0	32;32	27;0	0;
5	750	A	G	750G	A750G	99.56	1855	5;3	0;0	940;907	0;0	0;0	0;0	0.43	0.00	99.56	0.00	0.00	0.00	26;26	0;0	32;32	0;
6	1438	A	G	1438G	A1438G	99.78	1875	2;0	0;0	904;967	0;0	1;1	0;0	0.10	0.00	99.78	0.00	0.10	0.00	21;0	0;0	32;32	0;
7	2706	A	G	2706G	A2706G	99.83	1859	1;2	0;0	928;928	0;0	0;0	0;0	0.16	0.00	99.83	0.00	0.00	0.00	16;28	0;0	32;32	0;
8	3010	G	A	3010A	G3010A	99.88	1818	871;945	0;0	1;0	0;1	0;0	0;0	99.88	0.00	0.05	0.05	0.00	0.00	32;32	0;0	27;0	0;

Status Bar

After the Mito Alignment viewer opens, a temporary status bar is displayed in the lower left corner of the viewer below the Variants table. The status bar displays the progress of loading the project in the viewer. After the project is fully loaded, the status bar briefly displays 100%, and then it is closed and is no longer displayed in the viewer.

Figure 3-19: Status bar



You must wait until after the status for loading a project is 100% and the status bar closes before you can work with the project in the Mito Alignment viewer.

Mito Alignment Viewer Navigation and Options

You can easily navigate the Mito Alignment viewer using your mouse as well as some keyboard hotkeys. In the Global Viewer pane, the Pile-up pane, and the Variants table, you can use the mouse as well as some keyboard hotkeys to navigate the viewer display. In the Pile-up pane and the Variants table, you can also right-click to open a context menu that contains a list of options for working with and editing the project variant information. See:

- [“Mito Alignment viewer navigation”](#) below.
- [“Mito Alignment viewer variant-specific viewing and editing options”](#) on page 86.

Mito Alignment viewer navigation

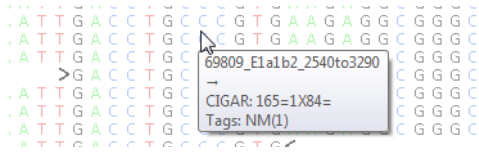
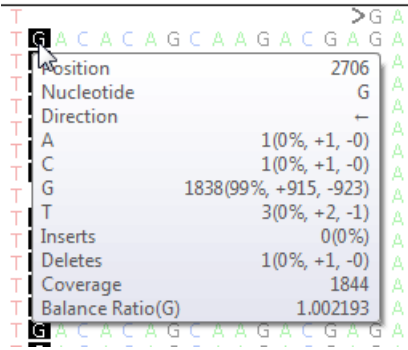
You can easily navigate the Mito Alignment viewer using your mouse as well as some keyboard hotkeys in the Global Viewer pane, the Pile-up pane, and the Variants table.

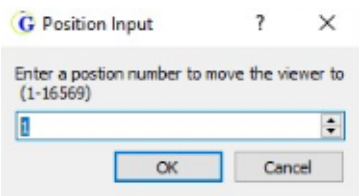
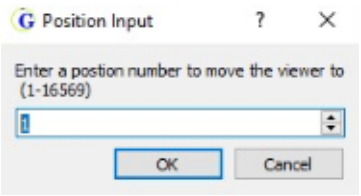
Option	Description
Global Viewer pane	
Double-click any region in the Global Viewer pane to move the Panes display to the same area.	
Pile-up pane display: When the Mito Alignment viewer first opens, the starting position for the Pile-Up pane is the first reference nucleotide position (Pos #1). Any of the following actions that you carry out for the Pile-up pane results in an automatic adjustment of the displays for the Reference pane, the Consensus pane, and the Motifs/Amplicon pane.	
Zoom In	Two options are available: <ul style="list-style-type: none"> • Hold down the left mouse button and draw a box from the upper left hand corner of the pane towards the lower right hand corner. A box is formed around the area that being reduced for viewing. • Hold down the CTRL key, and then roll the scroll wheel up on the client mouse.
Zoom Out	Two options are available: <ul style="list-style-type: none"> • Hold down the left mouse button and draw a box from the lower right hand corner of the pane towards the upper left hand corner. • Hold down the CTRL key, and then roll the scroll wheel down on the client mouse. <p>Note: The magnification for zooming out is always 100%.</p>
Horizontal Scroll	Hold down the <i>right</i> mouse button and then drag the cursor across the display from left to right, or right to left.
Vertical Scroll	Use the Vertical Scroll bar to the right of the Panes display, or use the Scroll wheel on your mouse.
Moving to each variant position in the pane	You can click the Pile-up pane, and then use the following keyboard shortcuts to move the display to each <i>variant</i> position: <ul style="list-style-type: none"> • CTRL + f moves the display forward one variant position at a time. • CTRL + b moves the display backward one variant position at a time.

Option	Description
Moving up and down in the pane	You can click in the Pile-up pane, and in lieu of using the scrollbar that is displayed at the far right side of the pane, you can use the following keyboard shortcuts to move up and down in the pane: <ul style="list-style-type: none"> • CTRL + d moves the pane display down. • CTRL + u moves the pane display up.
Move to a closest variant position in the Variants table	Right-click any position in the Pile-Up pane and on the context menu that opens, click Go to closest position in table. The variant that is closest to the selected position is highlighted in the Variants table.
Variants table	
Double-click any entry in the Variants table to move the Pile-up pane display to the corresponding variant position.	

Mito Alignment viewer variant-specific viewing and editing options

Options are available in both the Pile-up pane and the Variants table for viewing and editing variant-specific information. Some of these options require you to use your mouse and some keyboard hotkeys, while other options require you to right-click to open a context menu that has the options.

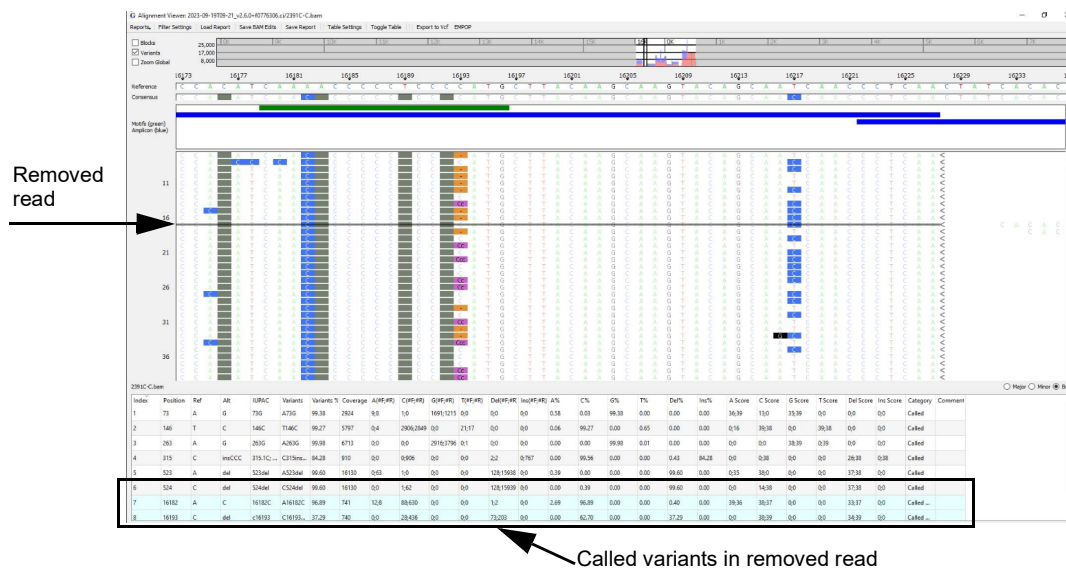
Option	Description
Pile-up pane	
Open a tooltip with the following information for a specific read (variant or non-variant): <ul style="list-style-type: none"> • Read name • Read direction • Read CIGAR string • Read Tags 	SHIFT + click the mouse scroll wheel, or CTRL +SHIFT, and then left mouse click. <i>Figure 3-20: Read tooltip</i> 
Open a tooltip with the following information for a specific variant: <ul style="list-style-type: none"> • The position number for the allele • The major allele at the position • The read direction for the allele • The counts for the different alleles at the position • The total coverage for the position • The Allele Balance Ratio • The Allele Balance Score 	Click the mouse scroll wheel or CTRL + left mouse click. <i>Figure 3-21: Variant tooltip</i> 
Add variant at position <n>	See "To add a variant at a given position" on page 89.

Option	Description
Jump to position	<p>Note: Jump to position moves the Pile-up pane left or right—you are moving the display to a particular variant position. The Reference position, therefore, changes accordingly in the Reference pane.</p> <p>Right-click any position, and on the context menu that opens, click Jump to Position. In the Position Input dialog box that opens, enter the position number to move the viewer to. The position is displayed in the middle of the viewer.</p> <p><i>Figure 3-22: Position Input dialog box</i></p> 
Jump to line	<p>Note: Jump to line moves the Pile-up pane up and down—you are moving the display to a particular read. The Reference position, therefore, does not change in Reference pane.</p> <p>Right-click any position, and on the context menu that opens, click Jump to line. In the Position Input dialog box that opens, enter the line number to move the viewer to. The read is displayed as the top of the Pile-up pane.</p> <p><i>Figure 3-23: Position Input dialog box</i></p> 
Go to closest position in table	<p>Right-click any position, and on the context menu that opens, select this option to highlight the entry in the Variants table for the variant that is closest to this selected position.</p>
Copy Read's Sequence	<p>To copy the sequence from any read to your clipboard, right-click any read, and on the context menu that opens, click Copy Read's Sequence. You can then use standard Paste commands to paste the copied information into a third-party product such as Microsoft Word or Notepad.</p>

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Option	Description
Remove Read/Undo Remove Read	<p>Right-click any position, and on the context menu that opens, select this option to remove the read from any consideration when analyzing or graphing the project results. A solid horizontal black line is displayed across the read to indicate that it has been removed. See Figure 3-24 below.</p> <p>To add a removed read back, right-click the read, and on the context menu that opens, click Undo Remove Read.</p> <p>Note: If the removed read contains any called variants, then these variants are highlighted in light blue in the Variants table. See "Variants table" on page 83.</p>
Variants table	
Copy selected	<p>Click and hold the left mouse button and then draw a box (square or rectangle) around the cells that are being copied. Right-click any of the copied cells, and on the context menu that opens, select Copy selected to copy the selected table cells to your clipboard. Use standard keyboard commands or menu commands to paste the copied cells into a third-party application such as Microsoft Word or Excel.</p>
Deleting a variant	See "To delete a variant at a given position" on page 89 .
Saving/Loading reports	See "To save/load reports for an mtDNA project" on page 90 .

Figure 3-24: Called variants in a removed read

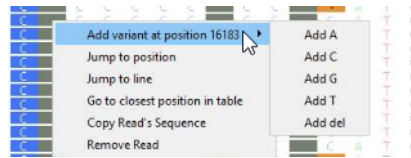


To add a variant at a given position

You can manually add a variant (insertion or deletion) at a position of your choice in the Pile-Up pane.

1. In the Pile-Up pane, right-click the position at which you are adding the variant.
A context menu opens with various options, including options for adding a variant.
2. Click Add Variant at position <n>.
A second context menu opens with different options for adding a variant at the given position, such as Add A or Add del.

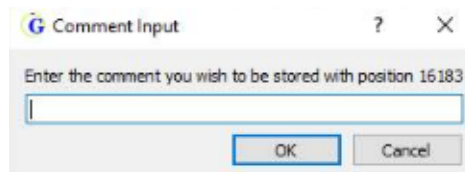
Figure 3-25: Pile-up context menu, Add variant at position <n> options



3. On the second context menu, select an Add variant at position option.

The context menu closes and Comment Input dialog box opens.

Figure 3-26: Comment Input dialog box



4. Optionally, enter the comment that is to be stored for the indicated position, and then click OK.

The Comment Input dialog box closes. A new entry that is highlighted in green is displayed for the added variant in the Variants table.

Figure 3-27: New variant entry in Variants table

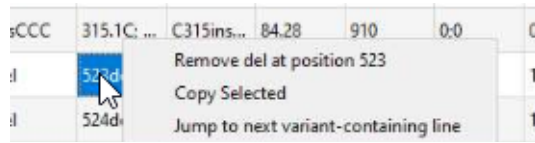
Index	Position	Ref	Alt	IUPAC	Variants	Variants %	Coverage	A(#F;#R)	C(#F;#R)	G(#F;#R)	T(#F;#R)	Del(#F;#R)	Ins(#F;#R)	A%	C%	G%	T%	Del%	Ins%	
9	2706	A	C	2706G	A2706C	0.05														
10	2706	A	G	2706G	A2706G	99.67														
11	2710	C	A	2710C	C2710A	0.00	2164	0;0	960;1201	0;1	1;0	0;1	0;0	0.00	99.86	0.04	0.04	0.04	0.00	
12	3027	T	C	3027C	T3027C	99.88	1800	0;0	900;898	0;0	2;0	0;0	0;0	0.00	99.88	0.00	0.11	0.00	0.00	

To delete a variant at a given position

You can manually delete a variant (insertion or deletion) from a project in the Variants table.

1. In the Variants table, right-click the variant that you are deleting.
A context menu opens with three options: Remove <variant> at position < >, Copy Selected, and Jump to next variant-containing line. See [Figure 3-28 on page 90](#).

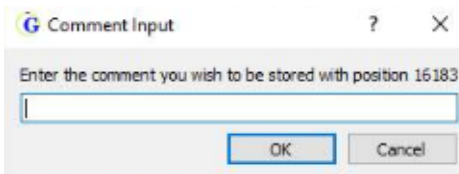
Figure 3-28: Variants table context menu



2. Select Remove <variant> at position < >.

The context menu closes and Comment Input dialog box opens.

Figure 3-29: Comment Input dialog box



3. Optionally, enter the comment that is to be stored for the indicated position, and then click OK.

The Comment Input dialog box closes. The variant remains displayed in the table, but it is now highlighted in red and the Category is set to removed.

Figure 3-30: New variant entry in Variants table

IUPAC	Variants	Variants %	Coverage	A(F:F:R)	C(F:F:R)	G(F:F:R)	T(F:F:R)	Del(F:F:R)	Ins(F:F:R)	A%	C%	G%	T%	Del%	Ins%	A Score	C Score	G Score	T Score	Del Score	Ins Score	Category	Comment
152C	T152C	99.93	8287	0:1	4506:3776	0:0	2:1	0:1	0:0	0.01	99.93	0.00	0.03	0.01	0.00	0.39	39.39	0:0	29.39	0:37	0:0	Called	
263G	A263G	99.96	17047	0:6	4550:12...	0:0	0:0	0:0	0:0	0.03	0.00	99.96	0.00	0.00	0.00	0.37	0:0	38.37	0:0	0:0	0:0	Removed	Testing

To save/load reports for an mtDNA project

After you generate an mtDNA project for the first time, the project settings (filter settings and table settings) are saved as Initial_Report at the Project Folder level. After you make any changes to the project settings and/or any manual edits to the project (for example, adding or deleting a variant), you can save these changes as another project report. Each report that you save is saved in a separate sub-folder under the primary project folder with a Change Log file that details the changes made to the project settings and project results. With a project opened in the Mito Alignment viewer, you can load any report that has been saved for the project to immediately update the information that is displayed for the project in the viewer. See:

- [“To save a report for an mtDNA project”](#) below.
- [“To load a report for an mtDNA project”](#) on page 91.

To save a report for an mtDNA project

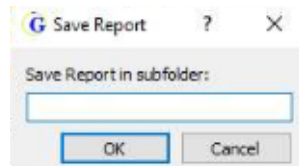
1. Do any or all of the following as needed:
 - On the Mito Alignment viewer main menu, click Filter Settings to open the Filter

Settings dialog box, make any needed edits to the settings, and then click OK to save the edited settings. See [“Mito Alignment Viewer Filter Settings” on page 93.](#)

- On the Mito Alignment viewer main menu, click Table Settings to open the Table Settings dialog box, make any needed edits to the settings, and then click OK to save the edited settings. See [“Mito Alignment Table Settings” on page 100.](#)
 - In the Pile-Up pane, remove a read, undo a removed read, add one or more variants, or do any combination of these three options. See [“Remove Read/Undo Remove Read” on page 88](#) or [“To add a variant at a given position” on page 89.](#)
 - In the Variants table, delete one or more variants. See [“To delete a variant at a given position” on page 89.](#)
2. On the Mito Alignment viewer main menu, click Save Report.

The Save Report dialog box opens.

Figure 3-31: Save Report dialog box



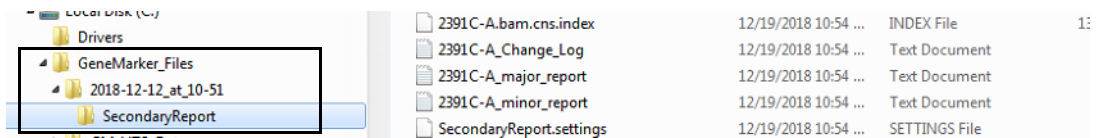
3. Enter a name for the sub-folder in which to save the report, and then click OK.



The sub-folder name cannot contain any spaces or special characters. Underscores (_) are allowed.

The Save Report dialog box closes. The report is saved in a separate sub-folder under the primary project folder with a Change Log file that details the changes made to the project results. You can now load this report for a project to immediately update the information that is displayed for the project in the viewer.

Figure 3-32: Saved Project report and folder structure



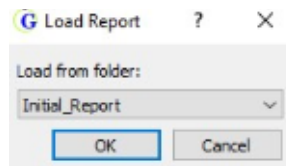
To load a report for an mtDNA project

1. On the Mito Alignment viewer main menu, click Load Report.

The Load Report dialog box opens. The location is initially set to Initial_Report. (Remember, this is the report that is automatically generated and saved after you run an mtDNA project for the first time.)

Figure 3-33: Load Report dialog box

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2. On the Load from folder drop-down list, leave Initial_Report selected, or select another folder that contains a secondary report for loading.
3. Click OK.

The Load Report dialog box closes, and a second dialog box, Loading, briefly opens and then closes as the selected report is loaded for the project. After the selected report is successfully loaded, the information that is displayed for the project is immediately updated in the Mito Alignment viewer.

Mito Alignment Viewer Filter Settings

Filter settings define the parameters for including a read in or excluding a read from an mtDNA analysis. When you open a sample in the Mito Alignment viewer, the filter settings that were in place at the time that the project was saved determine the results that are displayed in the Global Viewer and the Pane displays for the Mito Alignment viewer, but you can edit these settings to better suit your working requirements. You specify the variant call filter settings on the Mito Variant tab on the Filter Settings dialog box and you specify the reference region filter settings on the Mito Alignment tab. Four options are available for specifying the filter settings for an mtDNA project.

- You can apply the default filter settings as-is.
- You can edit the default values for the filter settings, and then apply the settings.
- You can load previously saved filter settings, and then apply the settings as-is.
- You can load previously saved filter settings, edit any of the values, and then apply the settings.

See:

- [“To specify the variant call settings for an mtDNA project”](#) below.
- [“To set the reference region settings for an mtDNA project”](#) on page 97.



Table settings define the information that is shown and the information that is hidden for a called variant in the Variants table in the Mito Alignment viewer. See [“Mito Alignment Table Settings”](#) on page 100.

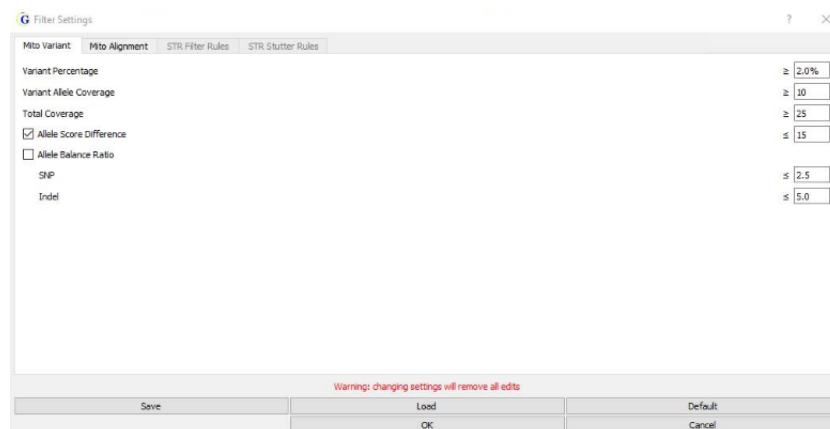
To specify the variant call settings for an mtDNA project

1. On the Mito Alignment viewer main menu, click Filter Settings.

The Filter Settings dialog box opens. The Mito Alignment tab is the open tab.

2. Open the Mito Variant tab

Figure 3-34: *Filter Settings dialog box, Mito Variant tab*



3. Do one of the following three options:
 - Review the default settings, and if you are satisfied that the Mito Variant filter settings are correct as-is for your project, then go to [Step 4](#).
 - Edit any of the default values for the Mito Variant filter settings. See [Table 3-1](#) below.



*If you edit any of the default values for the Mito Variant filter settings, you can click **Default**, and then click **Yes** in the **Default values** message that opens and all values are immediately reset to their default values.*

- Click **Load** to open the **Load Filter Settings** dialog box, and then browse to and select a **Filter Settings (.settings)** file.

*Both **Mito tabs** are populated with the values in the settings file. You can leave the values as-is on the **Mito Variant** tab, or you can edit any of the values before you apply the settings. See [Table 3-1](#) below.*

Figure 3-35: *Load Filter Settings dialog box*

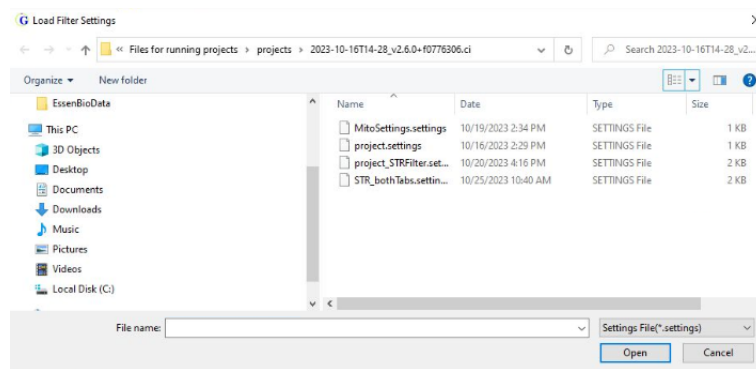
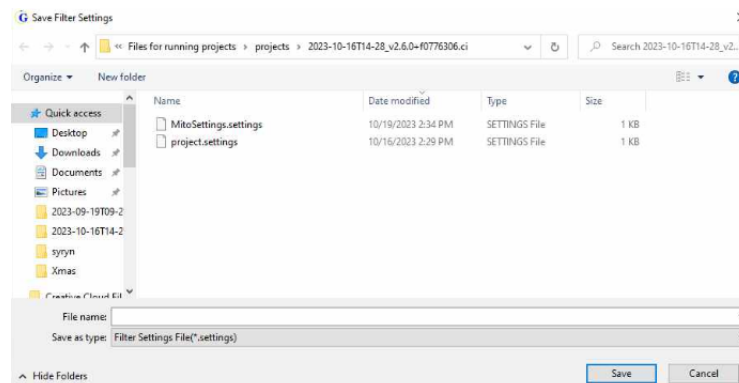


Table 3-1: *Filter Settings dialog box, Mito Variant tab*

Option	Description
Variant Percentage	The percent of reads that must contain the variant for the variant to be called at the indicated position.
Variant Allele Coverage	The total number of reads that must contain the variant at the indicated position for the variant to be called.
Total Coverage	The total number of reads that must occur at the indicated position for the variant to be called.
Allele Score Difference	A value that is calculated for each minor allele to determine if the allele is a true variant and therefore, is to be included in the analysis results, or if the allele is a false positive and therefore, is to be filtered from the results. See “Allele Score Difference” on page 95 .
Allele Balance Ratio	A value that is calculated for each allele to determine if the allele is a true variant and therefore, is to be included in the analysis results, or if the allele is a false positive and therefore, is to be filtered from the results. See “Allele Balance Ratio” on page 96 . SNP. Default value is ≤ 2.5 . Indel. Default value is ≤ 5.0 .

4. If you must change the reference region for the analysis, then go to [“To set the reference region settings for an mtDNA project” on page 97](#); otherwise, do one of the following:
 - Click OK to close the Filter Settings dialog box and immediately update the Mito Alignment viewer display based on the settings.
 - If you edited any of the filter settings and want to save these edits before applying them, then click Save to open the Save Filter Settings dialog box and save the filter settings to a Filter Settings (.settings) file.

Figure 3-36: Save Filter Settings dialog box



Note the following about saving a Filter Settings file:

- You must enter a name for the file.
- The file type is Filter Settings File (.settings) and you cannot change this.
- The default location for saving the Filter Settings file is the project folder, but you can always select a different location.
- You cannot save individual filter settings files for the Mito Variant Tab and the Mito Alignment tab. All settings on both Mito tabs are saved when you save a filter settings file for an mtDNA project.

After you save the filter settings file, you can click OK to close the Filter Settings dialog box and immediately update the Mito Alignment viewer display based on the applied settings.

Allele Score Difference

In GeneMarkerHTS, a quality score is calculated for every SNP or insertion allele as the median of the base Phred quality score from the original data. Because base quality scores are not possible for a deletion, the quality scores for the bases that surround a deletion are used instead. The Allele Score Difference filter, which is based on the base quality score for an allele, is applied individually *for each direction* as follows:

- The total number of reads for the *minor* allele is recorded.
- The base quality score for each minor allele is subtracted from the base quality score for the major allele to calculate the Allele Score Difference.

If the both of the following criteria are met, then the variant is assumed to be a false positive and the minor allele is filtered from the alignment results:

- The Allele Score Difference is greater than its set threshold.
- The total number of reads for the minor allele is greater than or equal to five.

If either of these criteria are not met, then the allele is assumed to be a true allele and it is not filtered from the results.

Allele Balance Ratio

The Allele Balance Ratio is another filter that you can use to determine if an allele is a true allele or a false positive. The Allele Balance ratio is defined as the maximum value for *one* of the following:

- Allele Forward %/Total Forward % or Total Forward %/Allele Forward %
- Allele Reverse %/Total Reverse % or Total Reverse %/Allele Reverse %

with different default thresholds specified for SNPs (2.5) and indels (5.0).

Consider the following scenario, where:

- Two forward reads and 79 reverse reads with the allele (a SNP), for 81 total *allele* reads.
- 1758 total forward reads and 6055 total reverse reads, for 7812 total reads.

This results in the following:

Value	Calculation
Total Forward %	$1758/7812 = 0.22501$
Total Reverse %	$6055/7812 = 0.77499$
Allele Forward %	$2/81 = 0.02469$
Allele Reverse %	$79/81 = 0.97531$
Allele Forward %/Total Forward %	$0.02469/0.22501 = 0.9113$
Total Forward %/Allele Forward %	$0.22501/0.02469 = 9.1134$
Allele Reverse %/Total Reverse %	$0.97531/0.77499 = 1.2585$
Total Reverse %/Allele Reverse %	$0.77499/0.97531 = 0.7946$
Determination: Because 9.1134 is > 2.5, this allele is filtered from the results.	



If an allele is determined to be one directional, then an Allele Balance Ratio is not calculated. Instead, if the allele is a major allele, then it is not filtered from the results.

Although SoftGenetics has supplied the default values for the calculation of the Allele Balance Ratio, you can always edit one or both of these thresholds. If you edit a value, you must try to maintain a balance of sensitivity versus specificity. For example, if you are concerned that variants are being called that are actually false positives and the variants

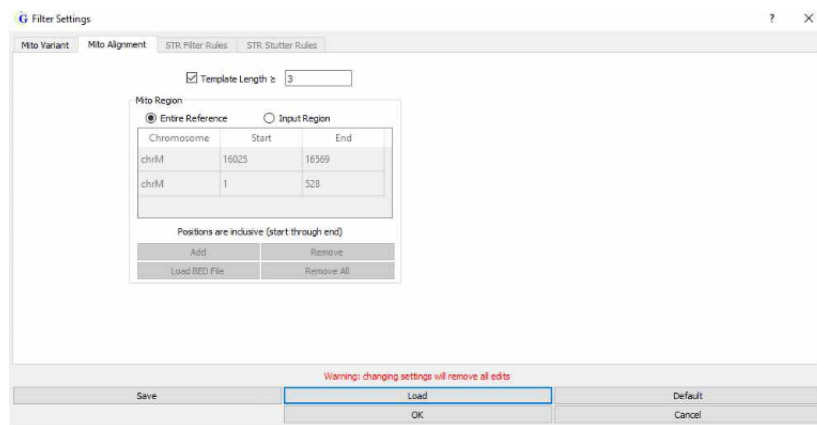
also appear to be unbalanced, then you might tighten the restrictions on variant calling and decrease the applicable threshold value. Conversely, if some expected variants are not being called, then you might loosen the restrictions on variant calling and increase the applicable threshold value.

To set the reference region settings for an mtDNA project

1. Do one of the following:
 - If the Filter Settings dialog box is already open, then open the Mito Alignment tab. Go to [Step 2](#).
 - On the Mito Alignment viewer main menu, click Filter Settings.

The Filter Settings dialog box opens. The Mito Alignment tab is the open tab.

Figure 3-37: Filter Settings dialog box, Mito Alignment tab



2. Do one of the following:
 - Review the default settings, and if you are satisfied that the Mito Alignment filter settings are appropriate as-is for your project, then go to [Step 3](#).
 - Edit any of the default values for the Mito Alignment filter settings. See [Table 3-2 on page 98](#).



If you edit any of the default values for the Mito Variant filter settings, you can click Default, and then click Yes in the Default values message that opens and all values are immediately reset to their default values.

- Click Load to open the Load Filter Settings dialog box, and then browse to and select a Filter Settings (.settings) file. See [Figure 3-38 on page 98](#).

Both Mito tabs are populated with the values in the settings file. You can leave the values as-is on the Mito Alignment tab, or you can edit any of the values before you apply the settings. See [Table 3-2 on page 98](#).

Figure 3-38: Load Filter Settings dialog box

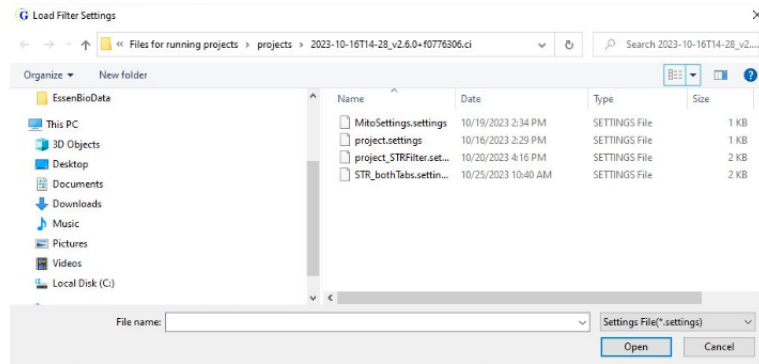
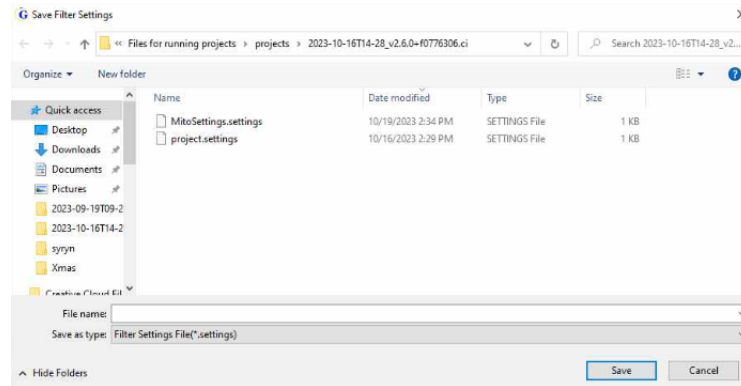


Table 3-2: Filter Settings dialog box, Mito Alignment tab

Option	Description
Template Length	Optional. Select to filter reads for paired data. The paired read is kept if the read length is \geq (TLEN) or \leq - (TLEN). You can leave the default value set to 100, or you can edit the value. Note: If the data is non-paired, then read length is used for filtering.
Variant Calling (You must select one option.)	
	<ul style="list-style-type: none"> Entire Reference: The default value. Select to call variants in any region. Input Region: Select to call variants in a single specific region. If you select this option, then you must adjust the Start and End values. Input Region: Select to call variants in multiple specific regions. If you select this option, then you can manually adjust the Start and End values for each region, or you can select Load BED File, and then in the Load BED Settings dialog box that opens, browse to and select the BED file that defines these multiple regions. <p>Note: For detailed information about the format for a BED file, see "BED file" on page 161. After you load a BED file, you can use the Add option and the Remove or Remove All options to add and remove regions for the file. If you add a region, you must specify the Start and End positions for it. Positions in a BED file are inclusive, start through end.</p>

- Do one of the following:
 - Click OK to close the Filter Settings dialog box and immediately update the Mito Alignment viewer display based on the applied settings.
 - If you edited any of the filter settings and want to save these edits before applying them, then click Save to open the Save Filter Settings dialog box and save the filter settings to a Filter Settings (.settings) file. See [Figure 3-39 on page 99](#).

Figure 3-39: Save Filter Settings dialog box



Note the following about saving a Filter Settings file:

- You must enter a name for the file.
- The file type is Filter Settings File (.settings) and you cannot change this.
- The default location for saving the Filter Settings file is the project folder, but you can always select a different location.
- You cannot save individual filter settings files for the Mito Variant tab and the Mito Alignment tab. All settings on both Mito tabs are saved when you save a filter settings file for an mtDNA project.

After you save the filter settings file, you can click OK to close the Filter Settings dialog box and immediately update the Mito Alignment viewer display based on the applied settings.

Mito Alignment Table Settings

Table settings define the data columns that are displayed and the data columns that are not displayed (hidden) for a called variant in the Variants table in the Mito Alignment viewer. By default, all available data columns are displayed for a called variant, and the data is categorized as one of the following: General, Coverage, Coverage Percent, Score, or Modification. When you open a sample for an mtDNA project in the Mito Alignment viewer, the table settings that were in place at the time that the project was saved determine the data columns that are displayed in the Variants table, but you can always edit these settings to better suit your working requirements.



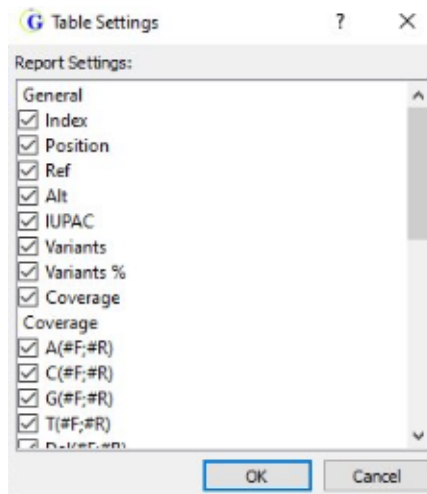
Filter settings define the parameters for including a read in the analysis or excluding a read from the analysis. See “Mito Alignment Viewer Filter Settings” on page 93.

To specify the table settings for an mtDNA project

1. On the Mito Alignment viewer main menu, click Table Settings.

The Table Settings dialog box opens. By default, all columns are selected for display in the Variants table.

Figure 3-40: Table Settings dialog box



2. Leave the columns that are to be displayed in the Variants table selected; otherwise, to hide a column from the display, clear its selection.

Column	Description
General	
Index	Unique system-generated identifier for the variant.
Position	The nucleotide position in the chromosome where the variant occurs.
Ref	The reference nucleotide at the variant position.

Column	Description
Alt	The alternate (variant) nucleotide at the variant position.
IUPAC	The IUPAC nucleotide code for the allele. If multiple alleles are called at a position, then the appropriate IUPAC ambiguity code is reported. Note: Mixtures of a deletion and other alleles use a lowercase letter.
Variants	The standard variant call.
Variant %	The variant frequency at the indicated position.
Coverage	The total number of reads (total coverage) at the variant position.
Coverage	
A #(F,R), C #(F,R), G #(F,R), T#(F,R)	The number of reads that show the indicated nucleotide at the variant location in the forward direction and the number of reads that show the indicated nucleotide at the variant location in the reverse direction.
Del #(F,R)	The number of reads that show a deletion at the variant location in the forward direction and the number of reads that show a deletion at the variant location in the reverse direction.
Ins #(F,R)	The number of reads that show an insertion at the variant location in the forward direction and the number of reads that show an insertion in the reverse direction at the variant location.
Coverage Percent	
A(%), C(%), G(%), T(%)	The percentage of reads that show the indicated nucleotide at the variant location.
Del(%)	The percentage of reads that show a deletion at the variant location.
Ins(%)	The percentage of reads that show an insertion at the variant location.
Score	
A Score, C Score, G Score, T Score	The median Phred quality score for the indicated allele (SNP) in both the forward and reverse directions.
Modifications	
Category	Indicates the variant status for the project in the Variants table - Called, Removed, Added,
Comment	Any comment that was entered for the added or deleted variant.

- Click OK to close the Table Settings dialog box and immediately update the display of the Variants table.

Mito Alignment Viewer Reports

Three reports are available from the Reports option on the Mito Alignment viewer main menu:

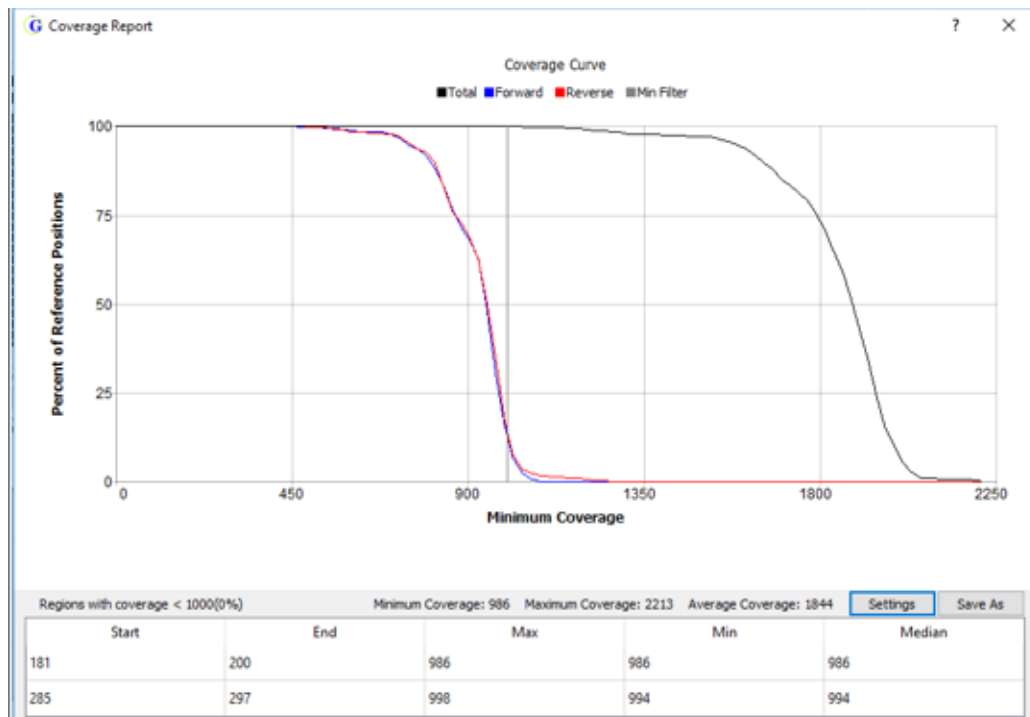
- Coverage report. See “Coverage report” below.
- Template Length Report. See “Template Length report” on page 104.
- Read Length report. See “Read Length report” on page 105.

To generate a report, simply select the report option on the Reports menu on the Mito Alignment viewer. For example, to generate the Coverage report, select Reports > Coverage Report. After a report is generated, it is displayed in a separate window that opens on top of the Mito Alignment viewer.

Coverage report

The Coverage report provides a high-level overview of the fraction of bases (the entire reference range, or the range of bases that was specified in the filter settings for the project) that have some level of coverage that is greater than or equal to the Total Coverage threshold that was specified for the project. The report displays the coverage distribution of sample reads without directional information (a solid black line) and individually for both the forward and reverse directions (blue and red lines, respectively). The report also details low coverage regions in a separate table below a Coverage Curve graph. The report is useful for identifying regions that were not adequately sequenced because of low coverage.

Figure 3-41: Coverage report



Optionally, after you generate a Coverage report, you can do one or both of the following for the report:

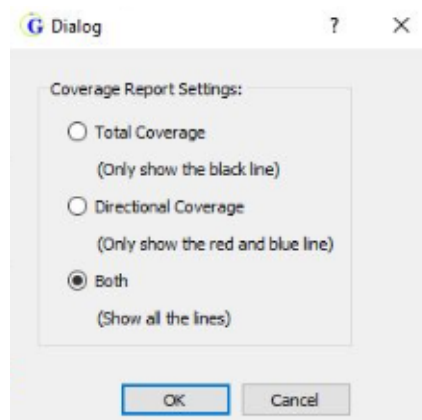
- Change the Coverage Report display. See [“To change the Coverage Report display”](#) below.
- Save the low coverage regions table as a text file. See [“To save the low coverage regions as a text file”](#) below.

To change the Coverage Report display

1. On the Coverage report window, click Settings.

The Coverage Report Settings dialog box opens. The dialog box shows the options for changing the display of the report. By default, Both is selected, which results in all lines (non-directional (black), forward (blue), and red (reverse)) being displayed on the report.

Figure 3-42: Coverage Report Settings dialog box



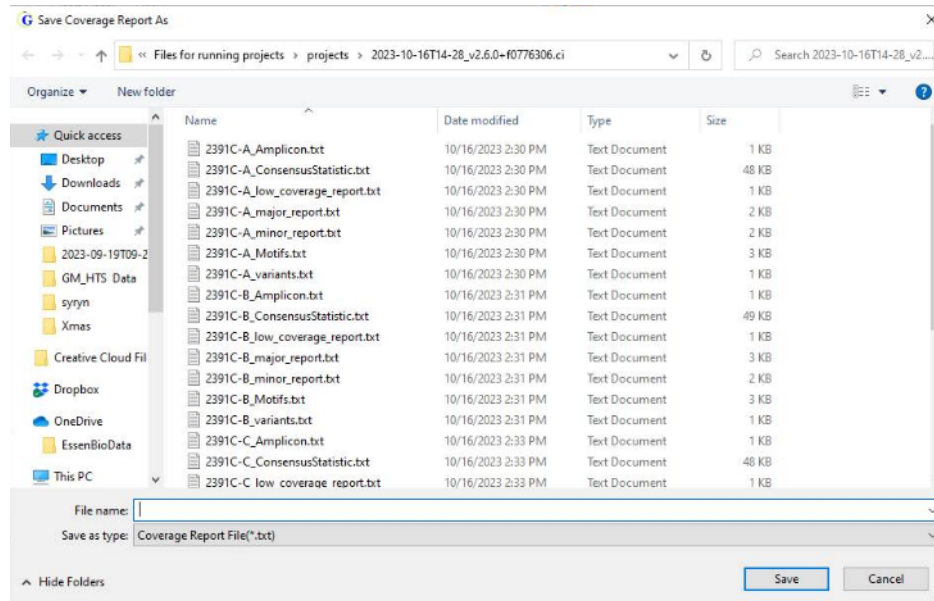
2. Do one of the following, and then click OK to close the dialog box and immediately apply the changes.
 - To display only the total coverage for all reads (non-directional black line), select Total Coverage.
 - To display only the forward and reverse reads (directional blue and red lines), click Directional Coverage.

To save the low coverage regions as a text file

1. On the Coverage report window, click Save As.

The Save Coverage Report dialog box opens. By default, the location in which to save the report is set to the top-level project folder, but you can always select a different location. The file type is set to Coverage Report File (*.txt) and you cannot change this. See [Figure 3-43 on page 104](#).

Figure 3-43: Save Coverage Report As dialog box



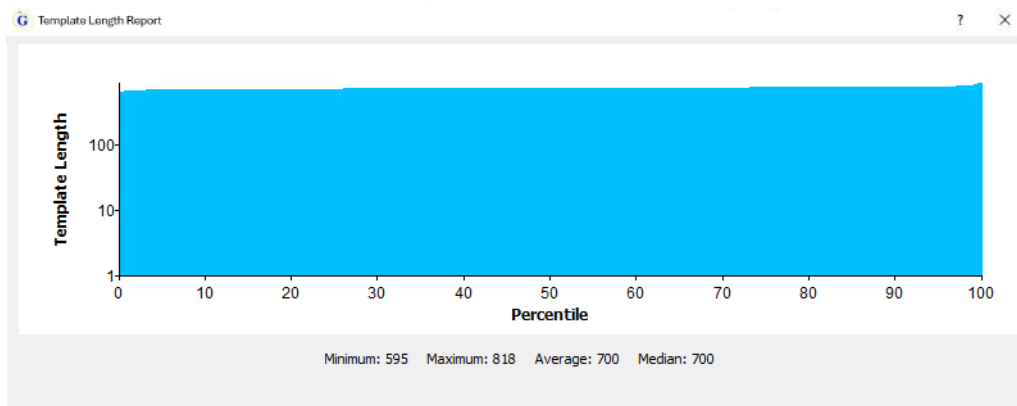
2. Enter a name for the report.
3. Optionally, browse to and select a different location for saving the report.
4. Click Save.

The Save Coverage Report dialog box closes. The Coverage report window remains opens.

Template Length report

The Template Length report shows a length distribution based on TLEN values in the BAM file for paired-end data. TLEN is a positive value in the BAM file for forward read data and the same value, but stored as a negative value, for the reverse read data.

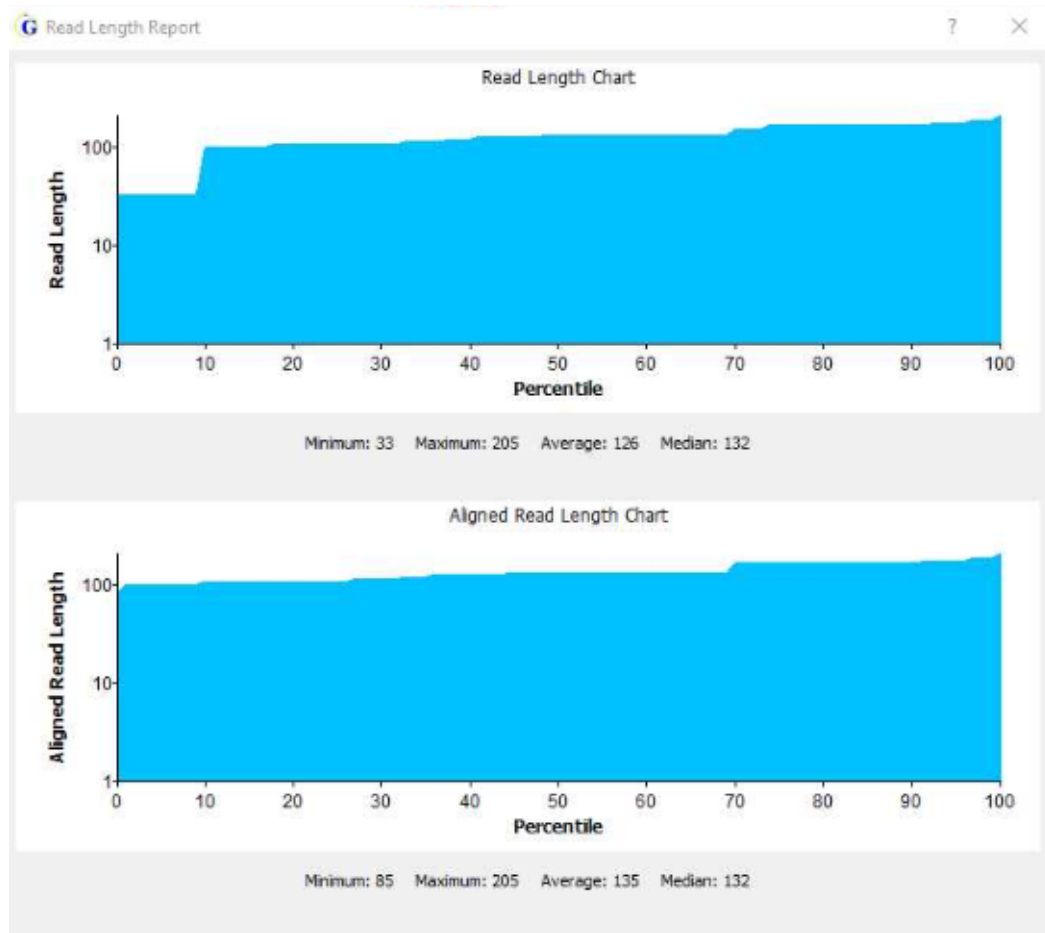
Figure 3-44: Template Length report



Read Length report

The Read Length report consists of two graphs. The Read Length (top) graph shows the length of the sequences in the BAM file. The Aligned Read Length (bottom) shows the length of the sequences, ignoring any soft-clipping in the CIGAR string.

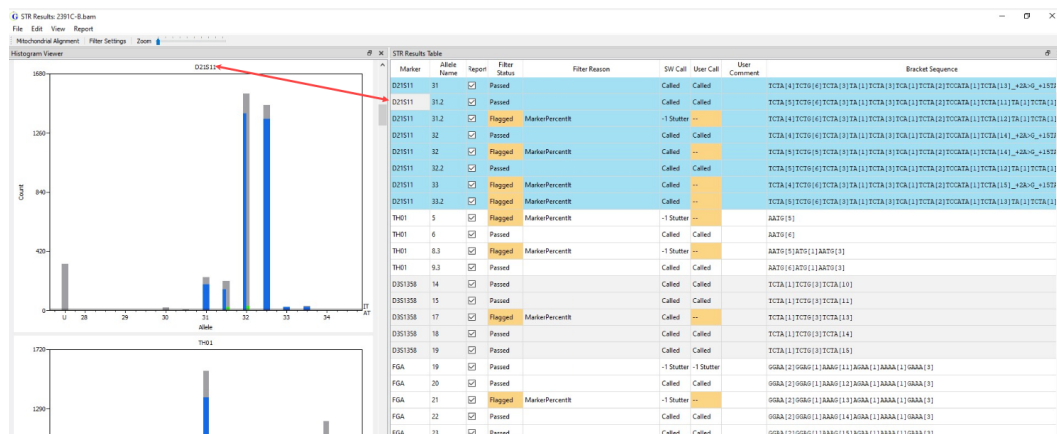
Figure 3-45: Read Length report



STR Analysis Viewer Overview and Layout

The STR Analysis viewer is an interactive view-only tool that you use to view the results of a simultaneous analysis of autosomal- and Y-STRs and the mitochondrial data for your project samples. You first use the STR Analysis viewer to view a summary of the autosomal- and Y-STRs analysis results for your project. If applicable, you can then open the Mito Alignment viewer directly from the STR Analysis viewer and view the mtDNA alignment data for the project. When you open a sample file for any project in the STR Analysis viewer, the filter settings that were in place at the time that the project was saved determine the alleles that were called for the project, but you can always edit these settings as needed to better suit your working needs. See [“STR Analysis Viewer STR and STR Stutter Filter Settings” on page 123.](#)

Figure 3-46: STR Analysis viewer



The STR Analysis viewer has two distinct but interactive panes: the Histogram Viewer pane and the STR Results pane. By default, when the viewer first opens, the Histogram Viewer pane is displayed on the left and the Results Table pane is displayed on the right. Both panes provide a way to navigate project results with the Histogram Viewer pane providing an image of the project results and the STR Results pane providing a tabulated display of the project results. As shown in [Figure 3-46](#) above, the pane displays are linked. If you double-click a histogram, then the focus in the STR Results Table pane is changed to the corresponding results, which are highlighted in light blue. Conversely, if you double-click any marker result in the STR Results Table pane, then the focus of the Histogram Viewer pane is set to that of the marker histogram and all the alleles for the marker are highlighted in light blue in the STR Results Table pane.

From top to bottom, the STR Results Analysis viewer has five major components:

- The title bar. See [“STR Analysis viewer title bar” on page 107.](#)
- The main menu. See [“STR Analysis viewer main menu” on page 107.](#)
- The Viewer Options menu. See [“Viewer Options menu” on page 110.](#)
- The Histogram Viewer pane. See [“Histogram Viewer pane” on page 110.](#)
- The STR Results Table pane. See [“STR Results Table pane” on page 114.](#)

STR Analysis viewer title bar

The STR Analysis viewer title bar is displayed at the top of the STR Analysis viewer. The title bar displays the name of the sample for which you are reviewing the STR analysis results.

Figure 3-47: STR Analysis viewer title bar



STR Analysis viewer main menu

The STR Analysis viewer main menu is displayed below the title bar. The menu is set up in a standard Windows menu format with menu commands grouped into menus across the menu bar. Some of the menu options are available in other areas of the viewer.

Menu Option	Description
File	<ul style="list-style-type: none"> Open Mitochondrial Alignment: Opens the Mito Alignment viewer. See "Mito Alignment Viewer Overview and Layout" on page 75.
	<ul style="list-style-type: none"> Export Table: Use this option to export the filtered STR results to a .csv file. By default, the file is named <sample file name>_stresults_filtered.csv, and the export location is the project folder, but you can edit one or both of these default values before you export the table.
	<ul style="list-style-type: none"> Export Histograms as Images: Use this option to export <i>all</i> the histograms in a single step that are displayed in the Histograms Viewer pane as .jpgs. Export Histograms as SVGs: Use this option to export <i>all</i> the histograms in a single step that are displayed in the Histograms Viewer pane as .svgs. Export Histograms as Single PDF: Use this option to export <i>all</i> the histograms in a single step that are displayed in the Histograms pane to a single PDF file.
<p>Note: By default, the default export location is the project folder, but you can select a different folder.</p>	
Edit	<p>Opens the Filter Settings dialog box, which contains options that affect the results that are displayed in the STR Results Table pane. See "STR Analysis Viewer STR and STR Stutter Filter Settings" on page 123.</p>
View	<ul style="list-style-type: none"> Stack Pane Display: Stacks the pane display so that both panes are displayed horizontally in the viewer, with the Histogram Viewer pane displayed above the STR Results Table pane. Side-by-Side Pane Display: The default display. Displays the panes side by side in the viewer, with the Histogram Viewer pane displayed on the left and the STR Results Table pane displayed on the right. Zoom In: Zooms in on the Histogram Viewer pane display. You can click this option as many times as needed to achieve a preferred zoom level, you can use the Zoom slider bar, or you can use the standard Windows keyboard command (CTRL ++). <p>Note: When the STR Analysis viewer first opens, the Histogram Viewer pane display is zoomed in at 100%.</p> <ul style="list-style-type: none"> Zoom Out: Zooms out the Histogram Viewer pane display. You can click this option as many times as needed to achieve a preferred zoom level, you can use the Zoom slider bar, or you can use the standard Windows keyboard command (CTRL + -).

Menu Option	Description
	<ul style="list-style-type: none"> Clear Marker Focus. Click to remove the highlighting that is displayed for markers that are in focus in the STR Results Table pane. Histogram Settings: Click to open the Histogram Settings dialog box, which contains options for customizing the display of the histograms in the Histogram Viewer pane. See “Histogram Settings dialog box” on page 112.
Report	<p>Click an option to generate the indicated report based on information in the STR Results Table pane.</p> <ul style="list-style-type: none"> NGS STR Allele Report (.tsv): The format is a tab-delimited file. NGS STR Allele Report (.fasta): The format is a FASTA report. CE STR Allele Report. The format is a tab-delimited file. CE STR Allele Report (GM). The format is a tab-delimited file. <p>See “STR Analysis viewer reports” below.</p>

STR Analysis viewer reports

Three reporting options are available under the Report menu command on the STR Analysis viewer main menu. If the Report option is selected for an allele in the STR Results Table pane, then the allele is included in the reports. By default, when you generate any STR Analysis viewer report, the report is named `<project sample name>_<report option>_report.<format>`, such as `2800M_ngs_str_allele_report.tsv`, and the project folder is selected as the folder for saving the generated report. You can edit the report name, but not the format, and you can select a different folder in which to save the report.

- NGS STR Allele Report. The NGS STR Allele report includes the following information for each allele: the Marker, the Allele Name, the Bracket Sequence, the Allele Total Count, the User Call, and the User Comment. You can generate this report in one of two formats: tab-separated values (*.tsv) or .fasta format.

Figure 3-48: NGS STR Allele report example, *.tsv format

Column1	Column2	Column3	Column4	Column5	Column6	Column7
#Report: NGS STR Allele Report						
#Format: 1						
#Version: 2.6.0+fd776306.ci						
#Datetime: 2023-10-25T20:39:52						
#User: tammy						
Sample	Marker	CE Allele	NGS Allele	Count	User Call	User Comm
2800M	Amelogenin	chrX		1216		
2800M	Amelogenin	chrY		1273		
2800M	PentaE	7	TCTTT[7]	1001	Called	
2800M	PentaE	14	TCTTT[14]	784	Called	
2800M	D18S51	15	AGAA[15]AAA[1]	136	--	
2800M	D18S51	16	AGAA[16]AAA[1]	1091	Called	
2800M	D18S51	17	AGAA[17]AAA[1]	186	--	
2800M	D18S51	18	AGAA[18]AAA[1]	990	Called	
2800M	D21S11	28	TCTA[4]TCTG[6]TCTA[3]TA[1]TCTA[3]TCA[1]TCTA[2]TCC...	144	--	
2800M	D21S11	29	TCTA[4]TCTG[6]TCTA[3]TA[1]TCTA[3]TCA[1]TCTA[2]TCC...	1159	Called	
2800M	D21S11	30.2	TCTA[5]TCTG[6]TCTA[3]TA[1]TCTA[3]TCA[1]TCTA[2]TCC...	103	--	
2800M	D21S11	31.2	TCTA[5]TCTG[6]TCTA[3]TA[1]TCTA[3]TCA[1]TCTA[2]TCC...	1138	Called	
2800M	TH01	6	AATG[6]	1313	Called	
2800M	TH01	9.3	AATG[6]ATG[1]AATG[3]	1233	Called	

- CE STR Allele Report. The CE STR Allele report includes the following information for each allele: the Marker, the Allele (which actually represents a particular sequence) and the corresponding “height” or read count for the allele sequence. Two options are available for this report: a “non-gm” report and a “gm” report. Both reports are a tab-separated values (*.tsv) file and both reports provide the same information, but in a different layout (organization of the file information), where:
 - a. The CE STR layout is easy to parse for downstream applications.
 - b. The CE STR GM layout is appropriate for exporting data to the MaSTR™ software application and the STRmix™ software application.

Figure 3-49: CE STR Allele report example

Column1	Column2	Column3	Column4
#Report: CE STR Allele Report			
#Format: 1			
#Version: 2.6.0+f0776306.ci			
#Datetime: 2023-10-25T20:03:48			
#User: tammy			
Sample	Marker	Allele	Height
2800M	Amelogenin	X	1216
2800M	Amelogenin	Y	1273
2800M	D8S1179	13	175
2800M	D8S1179	14	1612
2800M	D8S1179	15	1260
2800M	D12S391	17	180
2800M	D12S391	18	1372
2800M	D12S391	22	184
2800M	D12S391	23	1090
2800M	TPOX	10	178
2800M	TPOX	11	2869
2800M	TH01	6	1313
2800M	TH01	9.3	1233

Figure 3-50: CE STR GM Allele report example

Column1	Column2	Column3	Column4	Column5	Column6	Column7	Column8	Column9	Column10
#Report: CE STR GM Allele Report									
#Format: 1									
#Version: 2.6.0+f0776306.ci									
#Datetime: 2023-10-25T19:56:03									
#User: tammy									
Sample	Marker	Allele#1	Allele#2	Allele#3	Allele#4	Height#1	Height#2	Height#3	Height#4
2800M	Amelogenin	X	Y			1216	1273		
2800M	D8S1179	13	14	15		175	1612	1260	
2800M	D12S391	17	18	22	23	180	1372	184	1090
2800M	PentaE	7	14			1001	784		
2800M	TH01	6	9.3			1313	1233		
2800M	TPOX	10	11			178	2869		
2800M	DYS19	13	14			163	1857		
2800M	D21S11	28	29	30.2	31.2	144	1159	103	1138
2800M	FGA	19	20	22	23	131	1123	126	841
2800M	DYS389II	31				489			
2800M	D13S317	9	11			1015	895		
2800M	D18S51	15	16	17	18	136	1091	186	990
2800M	D3S1358	16	17	18		182	1584	811	
2800M	vWA	16	18	19		839	122	781	

Viewer Options menu

The Viewer Options menu is located below the STR Viewer main menu. It has three options:

- **Mitochondrial Alignment:** Opens the sample analysis results in the Mito Alignment viewer. This option is available only if an mtDNA alignment project was carried out for the sample. See [“Mito Alignment Viewer Overview and Layout” on page 75.](#)
- **Filter Settings:** Opens the Filter Settings dialog box, which contains options for editing the display of the STR Results Table pane. See [“STR Analysis Viewer STR and STR Stutter Filter Settings” on page 123.](#)
- **Zoom slider bar:** Slide the control on the Zoom slider bar to zoom in or zoom out the display of the Histogram Viewer pane.

Histogram Viewer pane

The Histogram Viewer pane displays a histogram for every STR marker that was analyzed, with each bar on the histogram corresponding to a marker allele. From top to bottom, the histograms follow the order of the markers in the panel that was used for the analysis. A marker histogram has the following characteristics:

- The X-axis variable is the allele name, with the histogram bars arranged in increasing order, from the smallest number of tandem repeats to the largest number of tandem repeats for all the marker alleles.
- The Y-axis variable is the total number of read counts (forward + reverse) for the allele.
- The colored part of a histogram bar contains the non-filtered allele counts with allele counts displayed blue and the iso-allele counts displayed in different colors. The number of different colors that are displayed is based on how many iso-alleles are called for a given allele.
- The gray part of a histogram bar contains the filtered out allele counts for both true allele positions and allele positions that do not have data.
- The “U” allele on the X-axis represents “unknown,” which are the reads that have the correct primers for the marker but GeneMarkerHTS cannot detect the number of repeats and therefore, cannot assign the read to a specific allele.

If you double-click a histogram in the Histogram Viewer pane, then the corresponding marker results are automatically highlighted in the STR Results Table pane.



For detailed information about the types of non-filtered allele counts that are contained in the colored portion of a histogram bar, see [“To specify the STR filter settings for an STR analysis project” on page 127.](#)

By default, when the Histogram Viewer pane first opens, the Zoom in level is set to 100%, where each histogram is displayed by itself in the pane. The following options are available for adjusting the zoom level of all the histograms at the same in the Histogram Viewer pane, where a zoom out level of 100% is the fully zoomed out view and all histograms for the project sample are displayed in the Histogram Viewer pane at the same time:

- You can use the Zoom slider bar that is displayed on the Viewer Options menu.
- You can use the View menu options of Zoom in and Zoom out:
- You also right-click any histogram that is displayed in the pane to open a context menu that has a Zoom in and a Zoom out option.

Figure 3-51: Fully zoomed out Histogram Viewer pane



Options for working with and adjusting the default display of the histograms in the Histogram Viewer pane are available from a histogram context menu and the Histogram Settings dialog box. See:

- [“Histogram context menu”](#) below.
- [“Histogram Settings dialog box”](#) below.

Histogram context menu

You can right-click any histogram in the Histogram Viewer pane to open a context menu with the following options:

Option	Description
Zoom in	Changes the Zoom in level for <i>all</i> the histograms that are displayed in the Histogram Viewer pane.
Zoom out	Changes the Zoom out level for <i>all</i> the histograms that are displayed in the Histogram viewer pane.
Save as Image	Exports the <i>individual</i> histogram as a .jpeg.
Save as SVG	Exports the <i>individual</i> histogram as an .svg.
Note: By default, the default location for both Export options is the project folder, but you can select a different folder.	

Histogram Settings dialog box

Options for adjusting the default display of the histograms in the Histogram Viewer pane are also available in the Histogram Settings dialog box. To open the dialog box, click View > Histogram Settings.

Figure 3-52: Histogram Settings dialog box

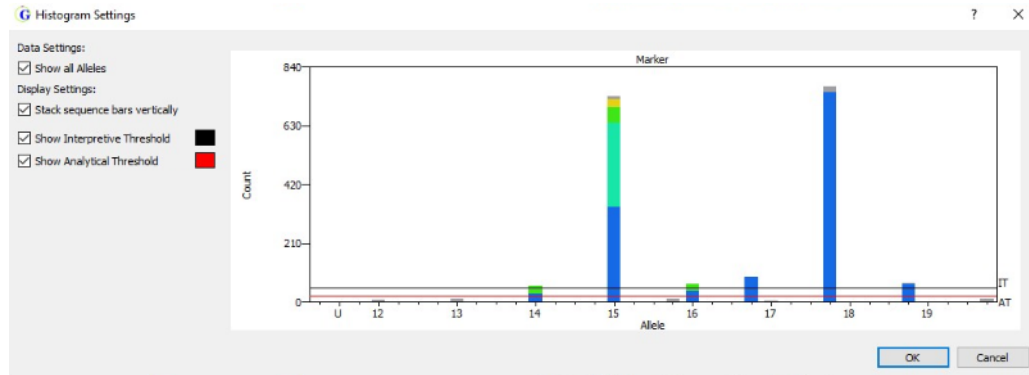
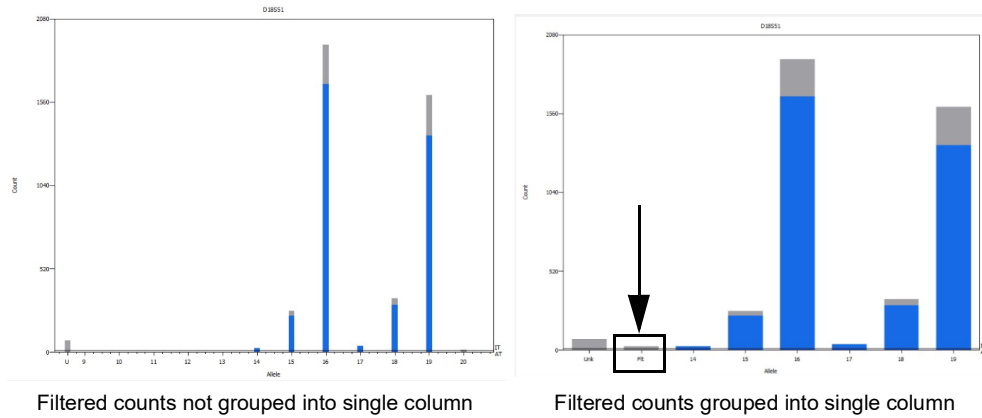


Table 3-3: Histogram Settings dialog box

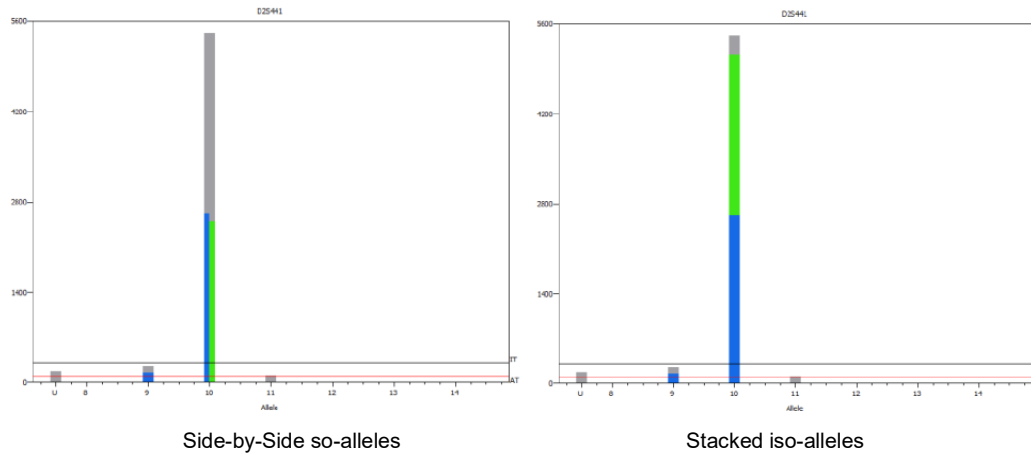
Setting	Description
Show All Alleles	<ul style="list-style-type: none"> Not selected: The default value. The filtered out counts for positions with data are stacked on top of the non-filtered allele counts. The filtered counts for positions without data are displayed as standalone histogram bars. Selected: The filtered counts for allele positions without data are combined into a single histogram bar labeled “Fit” that reflects the total number of filtered out counts. The histogram bar is displayed to the right of the Unknown (U) allele filtered counts on the far left side of the X-axis. <p>See Figure 3-53 on page 113.</p>
Stack sequence bars vertically	<ul style="list-style-type: none"> Not selected: The default value. The iso-allele count is stacked on top of the allele count. Selected. The iso-allele count is displayed side by side with the allele count at the same location on the X-axis. <p>See Figure 3-54 on page 113.</p>
Show Interpretive Threshold	<p>Selected by default. The Interpretive Threshold (IT) is displayed as a single horizontal black line that spans the X-axis of each histogram.</p> <p>Note: To change the default color of the line, double-click the Black switch to open the Select Color dialog box, and then select or specify a different color.</p> <p>See Figure 3-55 on page 114.</p>
Show Analytical Threshold	<p>Selected by default. The Analytical Threshold (AT) is displayed as a single horizontal red line that spans the X-axis of each histogram.</p> <p>Note: To change the default color of the line, double-click the Red switch to open the Select Color dialog box, and then select or specify a different color.</p> <p>See Figure 3-55 on page 114.</p>

Figure 3-53: Histogram Viewer pane: D21S11 allele filtered out allele counts displays



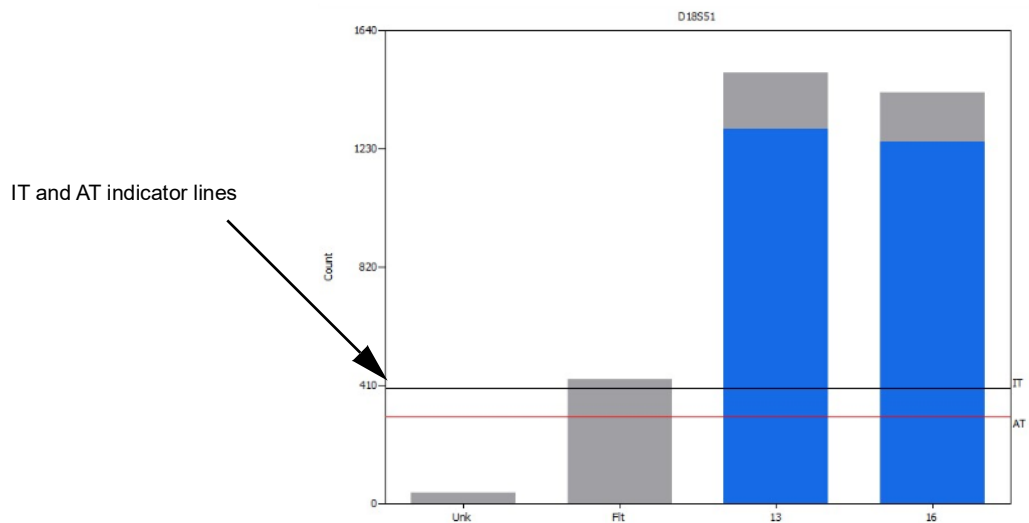
As shown in [Figure 3-53](#) above, for Marker D18551, the histogram on the left shows filtered counts stacked on top the following allele position with data: 14, 15, 16, 17, 18, and 19. Allele positions 9 through 13 have no data, and allele position 20 has no data but does show filtered counts. If you select Group Filtered out Alleles into single column, then the histogram display is updated to that shown for the histogram on the right. Allele positions 9 through 13, and 20 have been eliminated from the histogram, the histogram does not extend past the 19th allele position, and a single histogram bar labeled Fit is displayed to the right of the Unk histogram bar.

Figure 3-54: Histogram Viewer pane, D2S441 allele 9 and 10 iso-alleles displays



As shown in [Figure 3-54](#) above, for Marker D2S441, the histogram on the left shows the default display for iso-alleles with the iso-allele count (green) placed to the right of the allele count (blue) at the same location on the X-axis. If you select Stack sequence bars vertically, then the histogram display is updated to that shown for the histogram on the right, with the iso-allele count (green) stacked on top of the allele count (blue).

Figure 3-55: Histogram Viewer pane, IT and AT indicated on the histogram



STR Results Table pane

The STR Results Table pane (also referred to as the STR Results table) displays all the alleles that were identified for each marker along with critical analysis information. The marker order follows the order of the markers in the panel that was used for the analysis, with the background color alternating by marker between gray and white. Default colors are used for specific table cells to indicate critical information about the analysis results, such as the Filter Status or the User Call for a marker allele. (You can edit any or all of these default table cell colors. See [“To edit the STR Analysis viewer highlighting colors”](#) on page 160.)

Figure 3-56: STR Results Table pane

Marker	Allele Name	Report	Filter Status	Filter Reason	SW Call	User Call	User Comment	Bracket Sequence	Sequence Forward Count	Sequence Reverse Count	Sequence Total Count	Sequence Allele Percent	Sequence Marker Percent
DV5533	10	<input checked="" type="checkbox"/>	Passed		-1 Stutter	-1 Stutter		TATC[10]	65	66	131	94.93	7.39
DV5533	11	<input checked="" type="checkbox"/>	Passed		Called	Called		TATC[11]	683	751	1434	92.70	80.88
DV5533	12	<input checked="" type="checkbox"/>	Flagged	MarkerPercentit	Called	--		TATC[12]	9	6	15	100.00	0.85
DV5549	11	<input checked="" type="checkbox"/>	Passed		-1 Stutter	-1 Stutter		GATA[11]	125	112	237	95.56	7.77
DV5549	12	<input checked="" type="checkbox"/>	Passed		Called	Called		GATA[12]	1312	1223	2535	95.01	83.11
DV5549	13	<input checked="" type="checkbox"/>	Flagged	MarkerPercentit	Called	--		GATA[13]	23	24	47	100.00	1.54
DV5570	16	<input checked="" type="checkbox"/>	Flagged	MarkerPercentit	-1 Stutter	--		TTTC[16]	6	4	10	100.00	0.64
DV5570	17	<input checked="" type="checkbox"/>	Passed		-1 Stutter	-1 Stutter		TTTC[17]	63	63	126	94.74	8.11
DV5570	18	<input checked="" type="checkbox"/>	Passed		Called	Called		TTTC[18]	582	636	1218	95.83	78.38
DV5570	19	<input checked="" type="checkbox"/>	Flagged	MarkerPercentit	Called	--		TTTC[19]	7	10	17	94.44	1.09
DV5570	Unknown	<input checked="" type="checkbox"/>	Flagged	MarkerPercentit;BalanceRatioit	--	--			1	14	15	12.93	0.97
DV5570	Unknown	<input checked="" type="checkbox"/>	Flagged	MarkerPercentit;BalanceRatioit	--	--			1	13	14	12.07	0.90
DV5576	15	<input checked="" type="checkbox"/>	Flagged	MarkerPercentit	-1 Stutter	--		AAAG[15]	10	12	22	91.67	0.76
DV5576	16	<input checked="" type="checkbox"/>	Passed		Called	Called		AAAG[16]	159	175	334	94.35	11.55
DV5576	17	<input checked="" type="checkbox"/>	Passed		Called	Called		AAAG[17]	1118	1080	2198	94.50	76.00
DV5576	17	<input checked="" type="checkbox"/>	Flagged	MarkerPercentit;AllelePercentit	Called	--		AAAG[17]_15A-G	9	6	15	0.64	0.52
DV5576	18	<input checked="" type="checkbox"/>	Flagged	MarkerPercentit	Called	--		AAAG[18]	14	12	26	81.25	0.90
DV5635	19	<input checked="" type="checkbox"/>	Passed		-1 Stutter	-1 Stutter		TAGA[9]TACA[2]TAGA[2]TACA[2]TAGA[4]	74	78	152	89.41	5.64
DV5635	20	<input checked="" type="checkbox"/>	Passed		Called	Called		TAGA[10]TACA[2]TAGA[2]TACA[2]TAGA[4]	1161	1099	2260	97.54	83.83
DV5635	20	<input checked="" type="checkbox"/>	Flagged	MarkerPercentit;AllelePercentit	Called	--		TAGA[10]TACA[2]TAGA[2]TACA[3]TAGA[3]	12	6	18	0.78	0.67
DV5635	21	<input checked="" type="checkbox"/>	Flagged	MarkerPercentit	Called	--		TAGA[11]TACA[2]TAGA[2]TACA[2]TAGA[4]	8	6	14	93.33	0.52
DV5635	Unknown	<input checked="" type="checkbox"/>	Flagged	MarkerPercentit	--	--			8	8	16	8.79	0.59

When you are navigating the STR Results Table, note the following:

- If you double-click any cell in the table, then the focus is reset in both the Histogram

Viewer pane and the STR Results Table pane and all the rows for the corresponding marker are automatically highlighted in light blue.

- A selected cell is highlighted in dark blue. To clear all selected cells in a single step, including all automatically highlighted marker rows, click either the Histogram Viewer pane or the STR Results Table pane, and then press Esc on the client keyboard.

Table 3-4 below lists all the data columns that can be shown for each marker allele in the STR Results Table pane and whether the column is shown (visible) by default. Up to 24 data columns are available for display in the STR Results Table pane. To edit the table layout, including configuring the shown (visible) and hidden table data columns, the order in which the columns are displayed, or both, see [“To work with the STR Results Table pane configuration” on page 117.](#)

Table 3-4: STR Results Table, All available fields

Table Field	Default	Description
Category		One of three categories for the marker: Amelogenin, Autosomal STR, or ChrY STR.
Marker	X	The autosomal STR or Y-STR locus (marker) name.
Allele Name	X	The capillary electrophoresis (CE) name for a marker.
Report	X	Selected by default. If selected, the allele sequence is included in the NGS and CE reports. See “STR Analysis viewer reports” on page 108.
Filter Status	X	One of two statuses is possible based on the STR Filter settings and STR Stutter Filter settings that are specified for the project. <ul style="list-style-type: none"> • Passed: A read passed all specified STR settings. • Flagged: A read failed one or more specified STR settings.
Filter Reason	X	The STR filter settings and the STR Stutter filter settings that were triggered if a read was flagged.; otherwise, if a read passed, then the Filter Reason is set to > IT, which indicates that the allele counts were above the interpretative threshold.
SW Call	X	The software call based on the analysis parameters., where: <ul style="list-style-type: none"> • Called means all analysis parameters were met. • -1 Stutter (and any other stutter positions) are based on the following calculation: $\text{Sequence Total Count of potential stutter peak} / \text{Allele Total Count of true peak} = \text{Peak height ratio of potential stutter peak to True allele}$ If the value of this calculation falls below the STR Stutter filter settings, then the call is Stutter; otherwise, if the value falls above the STR Stutter filter settings, then the call is a True Peak. <p>Note: If multiple stutter calls are available for an allele, then the SW call differentiates between them.</p>
User Call	X	You can double-click the User Call for a flagged Filter Status to open the Edit User Call dialog box and enter a custom user call. See “To work with user calls and comments in the STR Results Table pane” on page 120.

Table 3-4: STR Results Table, All available fields (Continued)

Table Field	Default	Description
User Comment	X	Displays any optional user comments that were made in support of/explanation of the User Call. See “To work with user calls and comments in the STR Results Table pane” on page 120.
Bracket Sequence	X	The STR repeat sequence for the allele. The specified Bracket Sequence settings determine the format for the sequence display. See “To edit the bracket sequence settings” on page 153.
Sequence Group		A grouping method that was used prior to Bracket Sequences. Maintained only for backwards compatibility.
Sequence Forward Count	X	The total number of forward reads that contain the bracket sequence.
Sequence Reverse Count	X	The total number of reverse reads that contain the bracket sequence.
Sequence Total Count	X	The total number of reads (F+R) that contain the bracket sequence.
Sequence Allele Percent	X	The percentage of the reads that contain the bracket sequence for the allele, calculated as Sequence Total Count/Allele Total Count.
Sequence Marker Percent	X	The percentage of the reads that contain the bracket sequence for the marker, calculated as: Sequence Total Count/Marker Total Count.
Sequence Balance Ratio	X	The greatest value from all the following calculations: <ul style="list-style-type: none"> • Sequence Forward %/Marker Forward % • Marker Forward %/ Sequence Forward % • Sequence Reverse %/Marker Reverse % • Marker Reverse %/Sequence Reverse %
Allele Forward Count	X	The total number of reads for the allele in the forward direction, which includes filtered reads and sequence variants that have the same CE allele name.
Allele Reverse Count	X	The total number of reads for the allele in the reverse direction, which includes filtered reads and sequence variants that have the same CE allele name.
Allele Total Count	X	The total number of reads for the allele, which includes filtered reads and sequence variants that have the same CE allele name.
Allele Total Count Filtered	X	The number of reads for the allele that were filtered.
Allele Marker Percent	X	The ratio of the allele to all alleles for the marker.
Marker Forward Count		The total number of reads for the marker in the forward direction, including filtered reads.
Marker Reverse Count		The total number of reads for the marker in the reverse direction, including filtered reads.
Marker Total Count	X	The total number of reads (F+R) for the marker, including filtered reads.

Table 3-4: STR Results Table, All available fields (Continued)

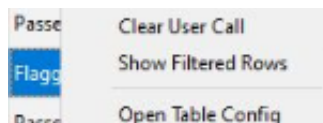
Table Field	Default	Description
Marker Total Count Filtered	X	The total number of reads for the marker that were filtered.
Sequence	X	The complete sequence for the read, with flanking sequences shown in lower case and repeat sequences shown in upper case.
Left Flank	X	The sequence for the left flank of the sequence read.
Repeat	X	The sequence for the repeat portion of the reads, but it is not shown in brackets.
Right Flank	X	The sequence for the right flank of the sequence read.

To work with the STR Results Table pane configuration

When the STR Results Table pane first opens, it opens with a default configuration, which means that pre-selected fields are displayed in a pre-set order. You can specify which fields to show in the table and which fields to hide from the table. You can also change the order in which the fields are displayed in the table. After you edit the table configuration, you can apply your changes immediately, or you can first save your changes to a configuration file, and then apply them. If you save the STR Results Table pane configuration to a file, you can select this file and apply the configuration to any other GeneMarkerHTS project. You can also include filtered rows in the STR Results Table pane.

1. Right-click the STR Results Table pane anywhere other than the Heading row to open a context menu with three options: Clear User Call, Show Filtered Rows, Open Table Config.

Figure 3-57: STR Results Table pane context menu



2. Continue to one of the following:
 - [“To include filtered rows in the STR Results Table pane”](#) below.
 - [“To manually edit the STR Results Table pane configuration”](#) on page 118.
 - [“To edit the STR Results Table pane configuration by loading a file”](#) on page 119.

To include filtered rows in the STR Results Table pane

To include filtered rows in the STR Results Table pane, click Show Filtered Rows. The context menu closes and the table configuration is updated accordingly.



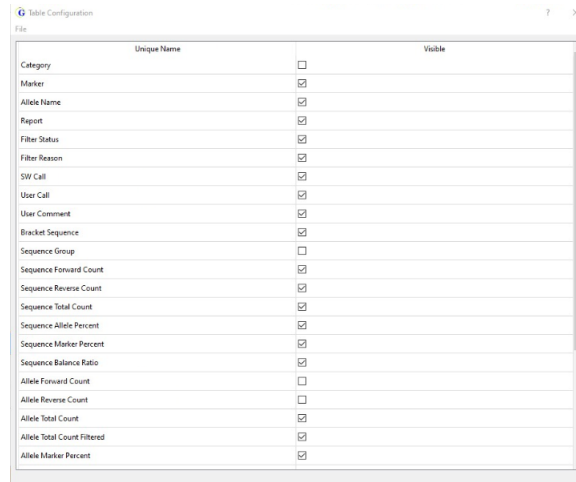
Show Filtered Rows is a toggle. To return the table configuration to its default configuration of showing only unfiltered rows, open the STR Results Table pane context menu, and then click Show Filtered Rows again.

To manually edit the STR Results Table pane configuration

1. Click Open Table Config.

The context menu closes. The Table Configuration dialog box opens on top of the STR Results Table pane. The dialog box displays all the fields that can be shown in the STR Results Table pane in a default order.

Figure 3-58: Table Configuration dialog box



2. Do any of the following:
 - To show a field in the results table, select the check box in the Visible column.
 - To hide a field from the results table, clear the check box in the Visible column.
 - To rearrange the order in which the fields are displayed in the results table, drag a field and drop it into its new location in the Unique Name (Fields) list.

As you edit the STR Results Table configuration, the display is dynamically updated.



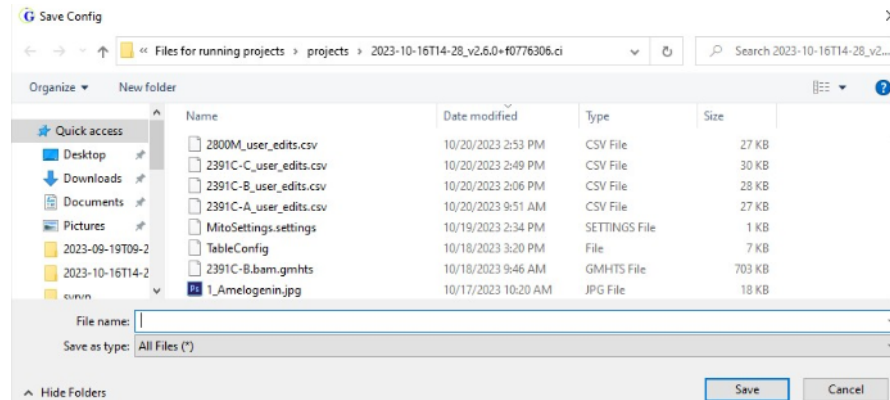
At any time that you are editing the STR Results Table pane configuration, if you rearrange the column order, change the visibility setting for one or more columns, or both, then you can click File > Reset Columns to return the columns to their default order and/or visibility.

3. After you are satisfied with the edited table configuration, do any of the following:
 - To immediately apply the changes to the results table and return to the STR Results Table pane, close the Table Configuration dialog box.
 - To save the configuration changes so that you can use them for other GeneMarkerHTS projects, on the menu for the Table Configuration dialog box:
 - a. Click File > Save Config As.

The Save Config dialog box opens. The File Type is set to All Files and you cannot change this. By default, the location for saving the Config file is set to the project folder, but you can select a different location.

- b. Continue to [Step 4](#).

Figure 3-59: Save Config dialog box



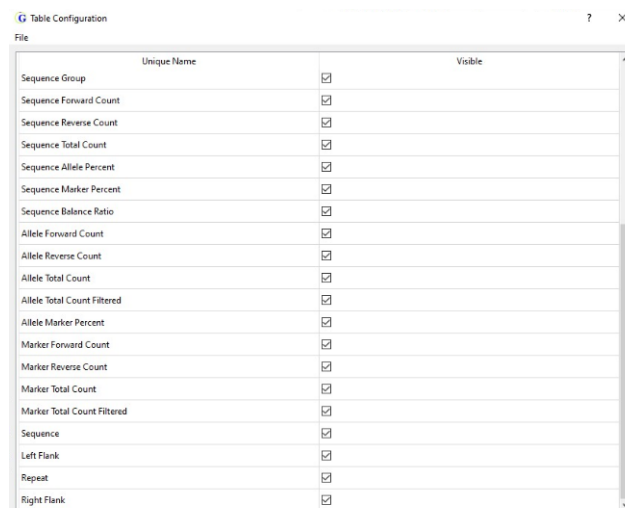
- Enter a name for the table configuration file, and then click Save.
The Save Config dialog box closes and the Table Configuration dialog box remains open.
- Close the Table Configuration dialog box.
The configuration changes are applied immediately to the STR Results Table pane.

To edit the STR Results Table pane configuration by loading a file

If a configuration that you prefer for the STR Results Table pane has been saved to a configuration file, then you can simply load this file to immediately change the table configuration.

- Click Open Table Config.
The context menu closes. The Table Configuration dialog box opens on top of the STR Results Table pane.

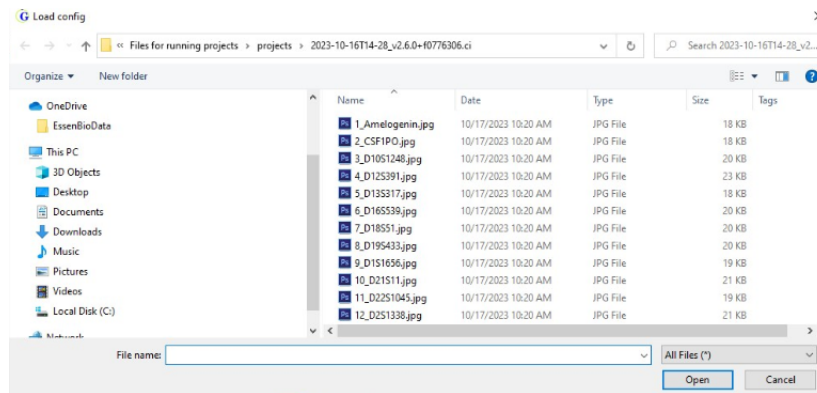
Figure 3-60: Table Configuration dialog box



2. On the Table Configuration dialog box, click File > Load Config.

The Load config dialog box opens. The file type is set to All Files. By default, the location for the configuration file is set to the project folder for the currently loaded project, but you can always browse to a different location to select the config file. For example, you can browse to the project folder for a different GeneMarkerHTS project.

Figure 3-61: Load config dialog box



3. Select the configuration file (the File Type is File), and then click Open.

The Load config dialog box closes. The loaded configuration is immediately applied to the STR Results Table pane.

4. Close the Table Configuration dialog box and continue working in the STR Analysis viewer.

To work with user calls and comments in the STR Results Table pane

You can do the following for custom user calls and comments in the STR Results Table:

- Enter or edit a custom user call. See [“To enter or edit a custom user call”](#) below.
- Enter a comment for any result in the STR Results Table pane. See [“To enter comments for a result in the STR Results Table pane”](#) on page 121,
- Clear a custom user call. See [“To clear a custom user call”](#) on page 122.

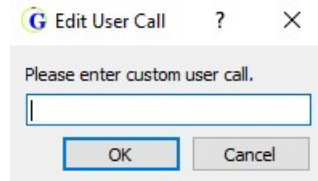
To enter or edit a custom user call

You can enter or edit a custom user call for any allele in the STR Results Table pane; however, you typically use this feature to enter a custom user call for an allele with a Filter Status of Flagged.

1. For an allele with a Filter Status of Flagged, double-click the User Call.

The Edit User Call dialog box opens. If this is the first time that you are entering a user call for the allele, then the dialog box is blank; otherwise, the dialog box displays the current value for the user call. See [Figure 3-62 on page 121](#).

Figure 3-62: Edit User Call dialog box



2. Enter a new custom user call, or edit the current custom user call, and then click OK.

The Edit User Call dialog box closes. The custom user call is now displayed in the User Call field. Initially, it is highlighted in the color that is specified for “Flagged” for the STR Viewer, with the default value being a golden yellow. After you click out of the cell, the cell is highlighted in the color that is specified for “Called” for the STR viewer, with the default color being a light green.



You can edit the color specified for Flagged, Called, or both in the STR Viewer. See [“To edit the STR Analysis viewer highlighting colors” on page 160..](#)

3. Optionally, you can now enter a comment/explanation in support of this custom user call. See [“To enter comments for a result in the STR Results Table pane”](#) below.

To enter comments for a result in the STR Results Table pane

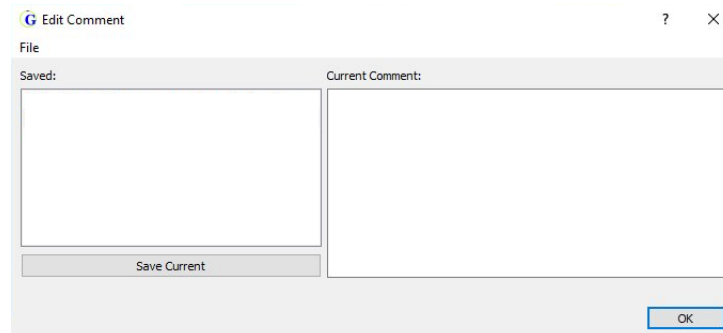
You can enter a comment for any result in the STR Results Table pane.

1. For any allele in the STR Results Table pane, double-click the User Comment field.

The Edit Comment dialog box opens. If this is the first time that you are entering a comment for a custom user call for the project, then both panes of the dialog box are blank; otherwise:

- Any comments that were previously entered and saved for any allele in the project are displayed in the Saved (left) pane.
- If the selected User Comment field already contains a comment, then the comment is displayed in the Current Comment (right) pane. Otherwise, the pane is blank.

Figure 3-63: Edit Comment dialog box



2. Do any of the following:

- To enter a new comment, click in the Current Comments field, and then enter a comment that explains or supports the custom user call.
 - If the comment is to be a “one-time” comment (used only for this allele and no others in the project), then click OK to close the Edit Comment dialog box and display the comment in the User Comment field.
 - If the comment is to be available for other alleles in the project, then click Save Current. The original comment remains displayed in the Current Comments pane and a copy of the comment is displayed in the Saved pane. Click OK to close the Edit Comment dialog box and display the comment in the User Comment field.
- To enter a previously saved comment, double-click the comment in the Saved pane. A copy of the selected comment is displayed in the Current Comment pane. Three options are now available. You can:
 - Click OK to close the Edit Comment dialog box and display the comment in the User Comment field.
 - Edit this comment (the source comment that is displayed in the Saved pane is not affected), and then click OK to close the Edit Comment dialog box and display the comment in the User Comment field.
 - Edit this comment (the source comment that is displayed in the Saved pane is not affected), and then click Save Current. The comment remains displayed in the Current Comments pane and a copy of the comment is displayed in Saved pane. Click OK to close the Edit Comment dialog box and display the comment in the User Comment field.

To clear a custom user call

If a custom user call has been entered for an allele in the project, you always have the option of clearing the call. When you clear a custom user call, the call is reset to the default value of blank (no call is displayed). To clear a custom user call, right-click in the appropriate User Call field, and on the context menu that opens, click Clear User Call. After you clear a custom user call, the cell is again highlighted in the color that is specified for “Flagged” for the STR Analysis viewer, with the default value being a golden yellow.

STR Analysis Viewer STR and STR Stutter Filter Settings

STR and STR filter setting determine the results that are displayed for a project sample in the STR Analysis viewer. Both types of settings can be applicable for all markers or alleles, or they can marker-specific or allele-specific.

- STR filter settings define the parameters for including a read in the analysis or excluding a read from the analysis.
- STR Stutter filter settings define the parameters for calling an allelic peak as either a true peak or a stutter (noise) peak.

When you open a sample in the STR Analysis viewer, the filter settings that were in place at the time that the project was saved determine the results that are initially displayed in the viewer, but you can edit these settings to better suit your working requirements. You specify the STR filter settings on the STR Filter tab on the Filter Settings dialog box and you specify the STR Stutter filter settings on the STR Stutter tab. Four options are available for specifying the filter settings for an STR analysis project.

- You can apply the default filter settings as-is.
- You can edit the default values for the filter settings, and then apply the settings.
- You can load previously saved filter settings, and then apply the settings as-is.
- You can load previously saved filter settings and edit any of the values, and then apply the settings.

When you load previously saved filter settings, you can load a settings file (*.settings) that affects the filter settings on *both* the STR Filter tab and the STR Stutter tab, or you can load a tab-specific settings file (*.csv) that affects the settings *on only one* tab. See:

- [“Filter Settings dialog box, STR Filter Tab”](#) below.
- [“Filter Settings dialog box, STR Stutter Tab”](#) on page 133.

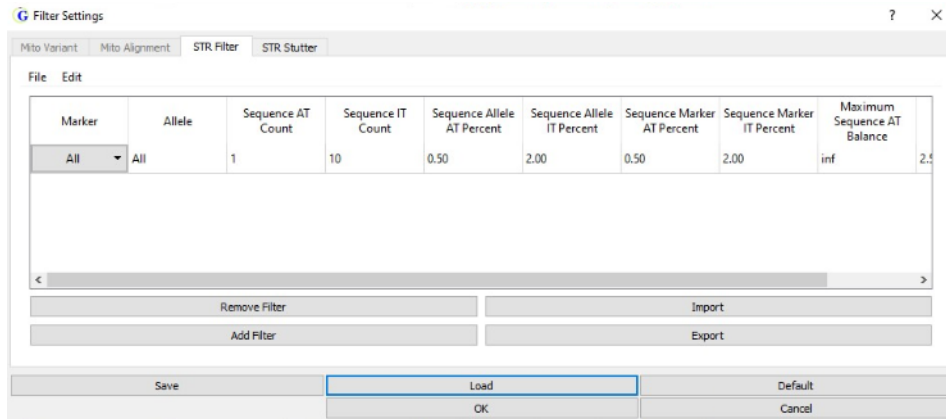
Filter Settings dialog box, STR Filter Tab

You specify the STR filter settings for an STR analysis project on the STR Filter tab of the Filter Settings dialog box. When the tab first opens, it has the following characteristics:

- The tab displays a single default filter setting that applies to all markers and all alleles.
- The tab opens in a default configuration which means that all available filter settings are displayed in a pre-set order. Default values are also provided for the filter settings.
- The four buttons that are displayed beneath the tab pane—Add Filter, Remove Filter, Import and Export—are tab-specific options.
 - Add Filter and Remove Filter are also available from the Edit menu that is displayed at the top of the tab.

- Import and Export are also available from the File menu that is displayed at the top of the tab.
- The buttons that are displayed at the bottom of the dialog box—Save, Load, Default, OK, and Cancel—act on the filter settings on *both* the STR Filter tab and the STR Stutter tab.
- Default is also available from the Edit menu that is displayed at the top of the tab.

Figure 3-64: Filter Settings dialog box, STR Filter tab



Optionally, before you specify the STR filter settings for an STR analysis project on the tab, you can also edit the configuration of the tab to better suit your working requirements, including configuring the shown (visible) and hidden table fields, the order in which the fields are displayed, or both. See:

- [“To edit the STR Filter tab configuration”](#) below.
- [“To specify the STR filter settings for an STR analysis project”](#) on page 127.

To edit the STR Filter tab configuration

1. Click Filter Settings.
The Filter Settings dialog box opens. The STR Stutter tab is the open tab.
2. Open the STR Filter tab.
3. Right-click anywhere in the tab pane and on the context menu that opens, click Open Table Config.

The context menu closes. The Table Configuration dialog box opens on top of the STR Filter tab. The dialog box displays all the fields that can be displayed on the tab in a default order. By default, all available fields are selected for display on the tab. [Figure 3-65 on page 125](#) shows the Table Configuration dialog box for the STR tab and [Table 3-5 on page 125](#) details all the fields (filter settings) that are available on the tab.

Figure 3-65: Table Configuration dialog box (STR Filter tab)

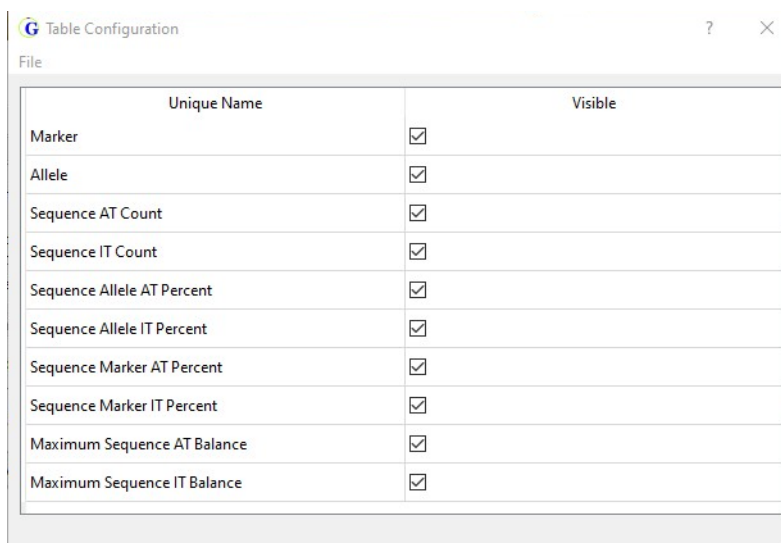


Table 3-5: Filter Settings dialog box, STR Filter tab

Filter Setting	Description
Marker	The default is All, which means that all the STR filter settings are applied to all the markers for a project sample.
Allele	The default is All, which means that all the STR filter settings are applied to all alleles for a project sample.
Sequence AT Count	The number of reads that are required to pass the analytical threshold (AT).
Sequence IT Count	The number of reads that are required to pass the Interpretive Threshold (IT), which results in the sequence being flagged as a possible allele.
Sequence Allele AT Percent	The percent of total reads for an allele that are required to pass the AT, calculated as Sequence AT Count/Allele Count.
Sequence Allele IT Percent	The percent of total reads for an allele that are required to pass the IT, calculated as Sequence IT Count/Allele Count.
Sequence Marker AT Percent	The percent of total reads for a marker that are required to pass the AT, calculated as Sequence AT Count/Marker Count.
Sequence Marker IT Percent	The percent of total reads for a marker that are required to pass the IT, calculated as Sequence IT Count/Marker Count.
Maximum Sequence AT Balance	<p>The balance is set to the greatest value out of the following calculations:</p> <ul style="list-style-type: none"> • SequenceForward%/MarkerForward% • MarkerForward%/SequenceForward% • SequenceReverse%/MarkerReverse% • MarkerReverse%/SequenceReverse% <p>Note: Any balance value that is greater than the balance value that is set here is automatically filtered out, which means that the sequence is not called as an allele. To disable filtering, set the value here to inf.</p>

Table 3-5: Filter Settings dialog box, STR Filter tab (Continued)

Filter Setting	Description
Maximum Sequence IT Balance	<p>The balance is set to the greatest value out of the following calculations:</p> <ul style="list-style-type: none"> • SequenceForward%/MarkerFoward% • MarkerForward%/SequenceForward% • SequenceReverse%/MarkerReverse% • MarkerReverse%/SequenceReverse% <p>Note: Any balance value that is greater than the balance value that is set here and that is less than the Maximum Sequence AT Balance is automatically flagged as a possible allele. To disable flagging, set the value here to inf.</p>

4. Do any of the following:

- To show a field on the tab, select the check box in the Visible column.
- To hide a field from the tab display, clear the check box in the Visible column.
- To rearrange the order in which the fields are displayed in the results table, drag a field and drop it into its new location in the Unique Name (Fields) list.

As you edit the STR Filter tab configuration, the display is dynamically updated.



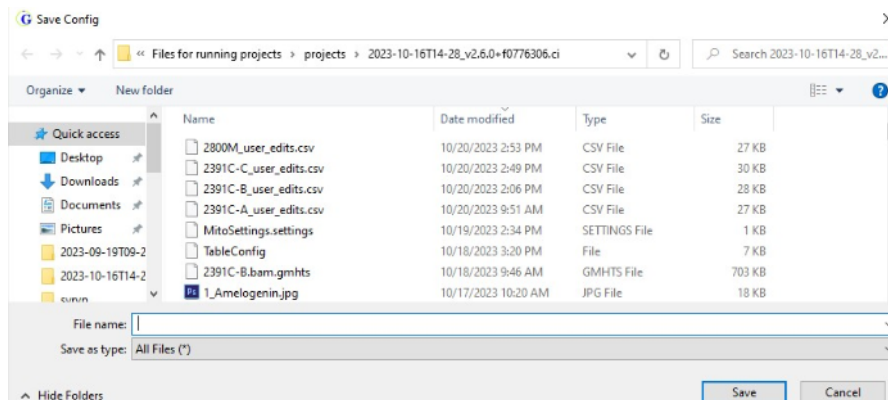
At any time that you are editing the STR Filter tab configuration, you can click File > Reset Columns to return the columns to their default order and/or visibility.

5. After you are satisfied with the edited tab configuration, do one of the following:

- To immediately apply the changes to the STR Filter tab and return to the tab, close the Table Configuration dialog box.
- To save the configuration changes so that you can use them for other GeneMarkerHTS projects, on the Table Configuration dialog box, do the following:
 - a. Click File > Save Config As.

The Save Config dialog box opens.

Figure 3-66: Save Config dialog box



- b. Enter a name for the configuration file.
- c. Optionally, select a different location in which to save the file. (By default, the location is set to the project folder.)
- d. Click Save.

The Save Config dialog box closes and the Table Configuration dialog box remains open.

- e. Close the Table Configuration dialog box.

The configuration changes are applied immediately to the STR Filter tab.

To specify the STR filter settings for an STR analysis project

1. Click Filter Settings.

The Filter Settings dialog box opens. The STR Stutter tab is the open tab.

2. Open the STR Filter tab.

A single default filter setting is displayed. The settings are applicable for all alleles for all project markers.

3. Review the default settings, noting the following:

- Any sequence/allele that has read counts/percentages that fall below the AT are not called and are filtered from the results. (The gray portion of the histogram bar contains these values.)
- Any sequence/allele that has read counts/percentages that fall in between the AT and the IT are flagged as a possible allele, and the user must enter a custom call. (The colored portion of the histogram bar contains these values, regardless of the user call.)
- Any sequence/allele that has read counts/percentages that are greater than the IT are called as an allele. (The colored portion of the histogram bar contains these values.)



If your lab does not use the Interpretive Threshold (IT), then you should set the IT values to match the Analytical Threshold (AT) values. See [Table 3-5 on page 125](#).

4. If you are satisfied that the default values are correct for your project, then go to [“To finish specifying the settings for an STR analysis project \(STR tab\)” on page 130](#); otherwise, go to one of the following:
 - [“To edit the STR filter settings” on page 128](#).
 - [“To load an STR/STR Stutter filter settings file” on page 129](#).
 - [“To import an STR Stutter tab-specific filter settings file” on page 138](#).

To edit the STR filter settings

1. Edit the filter settings as appropriate for the project, noting the following:
 - For a detailed explanation of the values for an STR filter setting, see [Table 3-5 on page 125](#).



To open a tooltip that provides an explanation of an STR filter setting, hold your mouse pointer over the column header for the setting.

- The default value of All which means that all the STR filter settings are applied to all the alleles for all the markers in a project sample.
- You can add more filter settings, delete any filter settings, or both.
 - If you add more filter settings, then marker-specific settings and allele-specific setting take precedence over “All” filter settings. See [“Marker-specific and Allele-specific settings”](#) below.
 - You can select only a single filter at a time for deletion.
- If you edit any of the default values for the STR filter settings, and want to return them to their default values, click Default, and then click Yes in the Default values message that opens. The values for *all* STR filter settings are immediately reset to their default values.

Marker-specific and Allele-specific settings

Marker-specific settings: To set marker-specific settings, you must carry out the following procedure in its entirety for every marker-specific filter setting that you are adding.

1. Click Add Filter.
A new row is displayed for adding a new filter setting.
2. On the Marker drop-down list, select the marker for which you are adding the marker-specific settings.
3. Edit any or all the STR settings other than the Allele default value of All for the marker. See [Table 3-5 on page 125](#).

Allele-specific settings: To set allele-specific settings, you must carry out the following procedure in its entirety for every allele-specific filter setting that you are adding.

1. Click Add Filter.
A new row is displayed for adding a new filter setting.
2. On the Marker drop-down list, select the marker for which you are adding the allele-specific setting.
3. In the Allele field, select the default value of “All,” and then edit the field to reflect the correct allele information.
4. Edit any or all the STR settings for the allele. See [Table 3-5 on page 125](#).

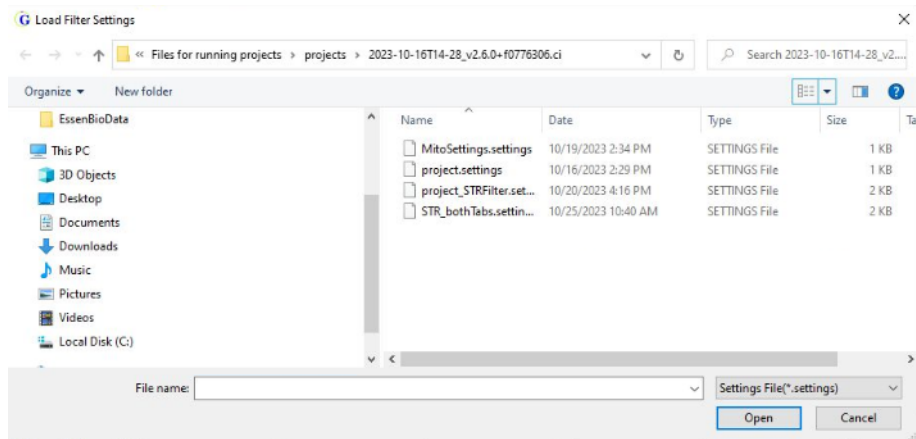
2. Continue to [“To finish specifying the settings for an STR analysis project \(STR tab\)” on page 130](#).

To load an STR/STR Stutter filter settings file

1. Click Load.

The Load Filter Settings dialog box opens.

Figure 3-67: Load Filter Settings dialog box



2. Browse to and select a Settings File (*.settings) file, and then click Open.

The Load Filter Settings dialog box closes. *Both* the STR Filter tab and the STR Stutter tab are populated with the values in the settings file.

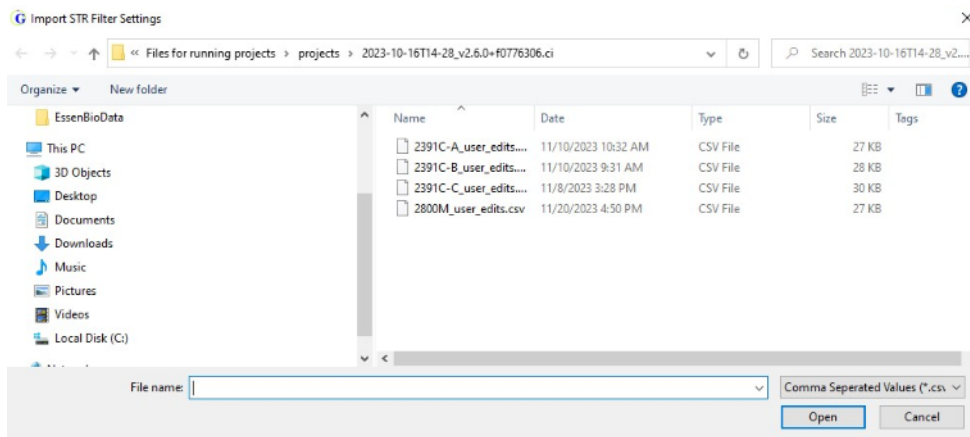
3. Review the loaded filter settings, and then do one of the following:
 - If you are satisfied that the loaded filter settings are correct as-is for your project, then go to [“To finish specifying the settings for an STR analysis project \(STR tab\)” on page 130.](#)
 - If you are not satisfied with the loaded filter settings, then go [“To edit the STR filter settings” on page 128.](#)

To import an STR Filter tab-specific filter settings file

1. Click Import.

The Import STR Filter Settings dialog box opens.

Figure 3-68: Import STR Filter Settings dialog box



2. Browse to and select the Comma Separated Values (*.csv) file, and then click Open.

The Import STR Filter Settings dialog box closes. *Only* the STR Filter tab is populated with the values in the settings file.

3. Review the loaded filter settings, and then do one of the following:
 - If you are satisfied that the imported filter settings are correct as-is for your project, then go to [“To finish specifying the settings for an STR analysis project \(STR tab\)”](#) below.
 - If you are not satisfied with the imported filter settings, then go [“To edit the STR filter settings”](#) on page 128.

To finish specifying the settings for an STR analysis project (STR tab)

Do one of the following:

- Immediately apply the filter settings. Click OK to close the Filter Settings dialog box and immediately apply the filter settings. The STR Analysis viewer display is dynamically updated based on the settings.
- Save the filter settings before applying them. See [“To save the STR filter settings before applying them”](#) on page 131.
- Export the filter settings to a tab-specific filter settings file before applying them. See [“To export the STR filter settings before applying them”](#) on page 132.

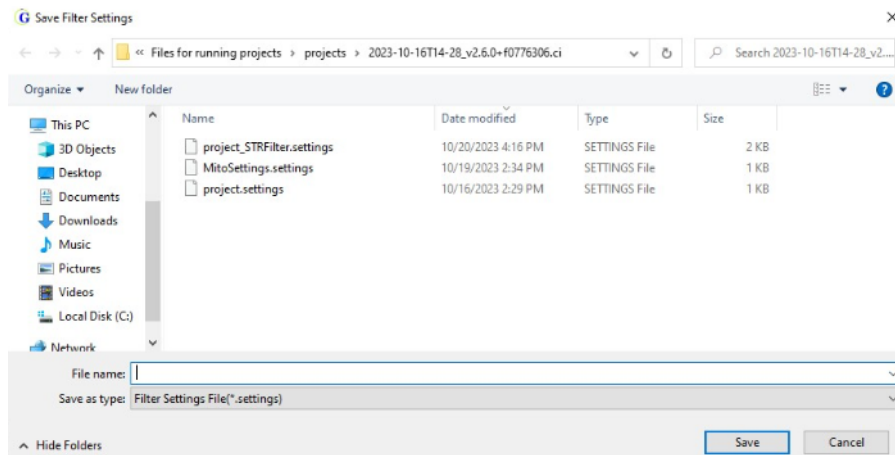
To save the STR filter settings before applying them

If you edited any of the STR filter settings, then to save these edits before applying them, you must save the settings to a Filter Settings (*.settings) file. When you save a filter settings file, the settings on *both* the STR Filter tab and STR Stutter tab are saved.

1. Click Save.

The Save Filter Settings dialog box opens.

Figure 3-69: Save Filter Settings dialog box



Note the following when you are saving a filter settings file:

- No default name is provided for the file.
- The file type is Filter Settings File(*.settings) and you cannot change this.
- The default location for saving the filter settings file is the project folder, but you can always select a different location.

2. Enter a name for the file.
3. Optionally, select a different location in which to save the file.
4. Click Save.

The Save Filter Settings dialog box closes.

5. Click OK.

The Filter Settings dialog box closes. The STR Analysis viewer display is immediately updated based on the applied settings.

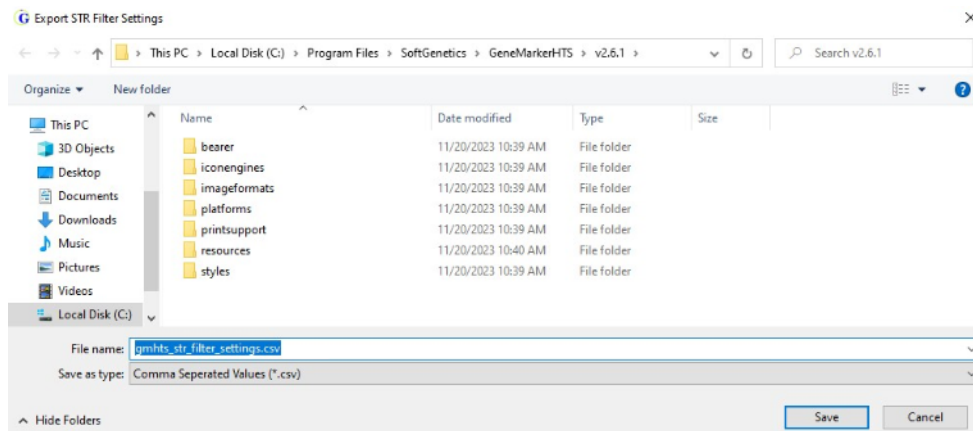
To export the STR filter settings before applying them

If you edited any of the filter settings and you want to save these edits only on the STR Filter tab before applying them, then you must export the filter settings to a Comma Separated Values (*.csv) file.

1. Click Export.

The Export STR Filter Settings dialog box opens.

Figure 3-70: Export STR Filter Settings dialog box



Note the following when you are exporting and saving a STR Filter tab-specific filter settings file:

- The default name is set to gmhts_str_filter_settings. You can leave the filename as-is, or you can edit it.
 - The file type is Comma Separated Values (.csv) and you cannot change this.
 - The default location for saving the tab-specific filter settings file is the project folder, but you can always select a different location.
2. Optionally, enter a different name for the file, select a different location in which to save the file, or both.
 3. Click Save.

The Export STR Filter Settings dialog box closes.

4. Click OK.

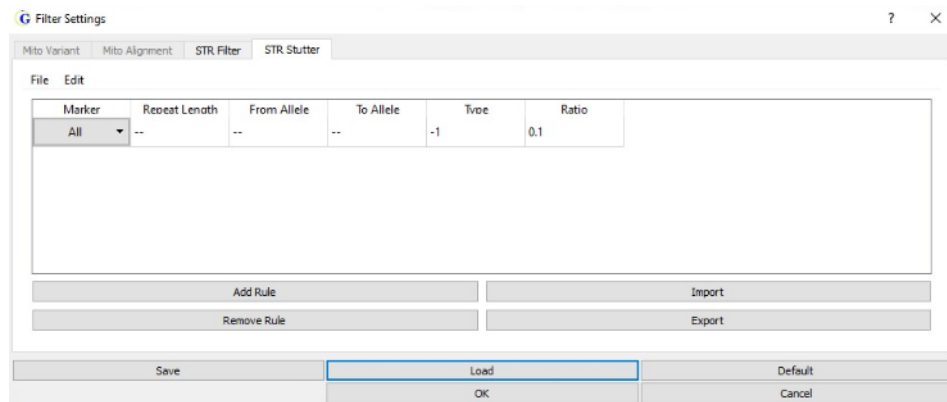
The Filter Settings dialog box closes. The STR Analysis viewer display is immediately updated based on the applied settings.

Filter Settings dialog box, STR Stutter Tab

You specify the STR Stutter filter settings on the STR Stutter tab of the Filter Settings dialog box. When the tab first opens, it has the following characteristics:

- The tab displays a single default filter setting that applies to all markers and all alleles.
- The tab opens in a default configuration which means that all available filter parameters are displayed in a pre-set order. Default values are also provided for the filter parameters.
- The four buttons that are displayed beneath the tab pane—Add Filter, Remove Filter, Import and Export—are tab-specific options.
 - Add Filter and Remove Filter are also available from the Edit menu that is displayed at the top of the tab.
 - Import and Export are also available from the File menu that is displayed at the top of the tab.
- The buttons that are displayed at the bottom of the dialog box—Save, Load, Default, OK, and Cancel—act on the filter settings on *both* tabs.
 - Default is also available from the Edit menu that is displayed at the top of the tab.

Figure 3-71: Filter Settings dialog box, STR Stutter tab



Optionally, before you specify the STR Stutter settings for an STR analysis project on the tab, you can also edit the configuration of the tab to better suit your working requirements, including configuring the shown (visible) and hidden table fields, the order in which the fields are displayed, or both. See:

- [“To edit the STR Stutter tab configuration”](#) below.
- [“To specify the STR Stutter filter settings for an STR analysis project”](#) on page 135.

To edit the STR Stutter tab configuration

1. Click Filter Settings.

The Filter Settings dialog box opens. The STR Stutter tab is the open tab.

2. Right-click anywhere in the tab pane and on the context menu that opens, click Open Table Config.

The context menu closes. The Table Configuration dialog box opens on top of the STR Stutter tab. The dialog box displays all the fields that can be displayed on the tab in a default order. By default, all available fields are selected for display on the tab. [Figure 3-72](#) below shows the Table Configuration dialog box for the STR Stutter tab and [Table 3-6](#) below details all the fields (filter settings) that are available on the tab.

Figure 3-72: Table Configuration dialog box (STR Stutter tab)

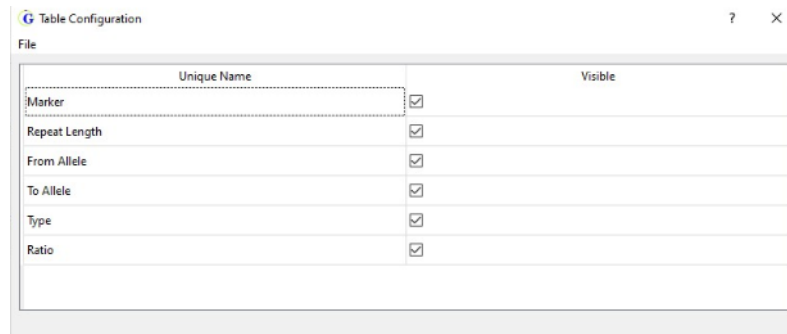


Table 3-6: Filter Settings dialog box, STR Stutter tab

Filter Setting	Description
Note: The descriptions provided here are for informational purposes only. For detailed information about editing the STR Stutter filter settings values for an STR analysis, “To specify the STR Stutter filter settings for an STR analysis project” on page 135.	
Marker	The available markers for the project. Automatically populated based on the loaded panel.
Repeat Length	Automatically populated based on the loaded panel.
From Allele To Allele	The default value for both parameters is All.
Type	The stutter position.
Ratio	The ratio of the total number of sequence counts in the stutter position to the total number of allele counts for the main peak.

3. Do any or all of the following:
 - To show a field on the tab, select the check box in the Visible column.
 - To hide a field from the tab display, clear the check box in the Visible column.
 - To rearrange the order in which the fields are displayed in the results table, drag a field and drop it into its new location in the Unique Name (Fields) list.

As you edit the STR Stutter tab configuration, the display is dynamically updated.

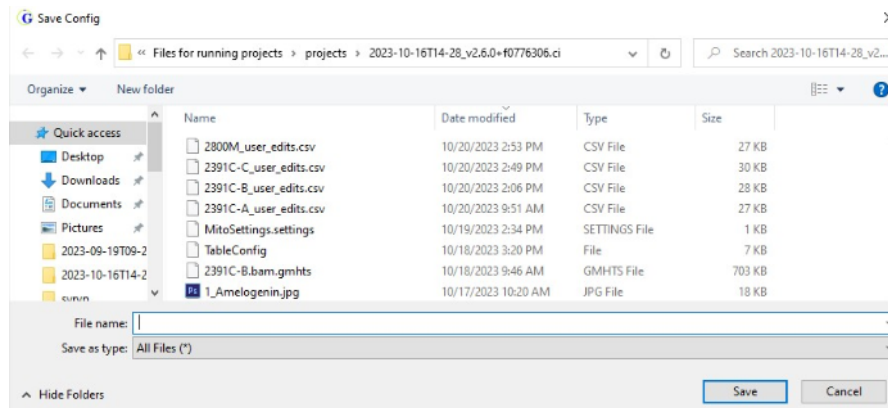


At any time that you are editing the STR Stutter tab configuration, you can click *File > Reset Columns* to return the columns to their default order and/or visibility.

4. After you are satisfied with the edited table configuration, do one of the following:
 - To immediately apply the changes to the STR Stutter tab and return to the tab, close the Table Configuration dialog box.
 - To save the configuration changes so that you can use them for other GeneMarkerHTS projects, on the Table Configuration dialog box, do the following:
 - a. Click File > Save Config As.

The Save Config dialog box opens.

Figure 3-73: Save Config dialog box



- b. Enter a name for the configuration file.
- c. Optionally, select a different location in which to save the file. (By default, the location is set to the project folder.)
- d. Click Save.

The Save Config dialog box closes and the Table Configuration dialog box remains open.

- e. Close the Table Configuration dialog box.

The configuration changes are applied immediately to the STR Stutter tab.

To specify the STR Stutter filter settings for an STR analysis project

1. Do one of the following:
 - If the Filter Settings dialog box is already open, then go to [Step 2](#).
 - On the Viewer Options menu, click Filter Settings.

The Filter Settings dialog box opens. The STR Stutter tab is the open tab.

2. Review the default settings, and if you are satisfied that the default values are correct for your project, then go to [“To finish specifying the settings for an STR analysis project \(STR Stutter tab\)” on page 138](#); otherwise, go to one of the following:
 - [“To edit the STR Stutter filter settings”](#) below.
 - [“To load an STR/STR Stutter filter settings file” on page 137](#).
 - [“To import an STR Stutter tab-specific filter settings file” on page 138](#).

To edit the STR Stutter filter settings

When you are editing the STR Stutter filter settings, note the following:

- The default value of All means that which means that all the STR filter settings are applied to all the alleles for all the markers in a project sample.
 - You can add more filter settings, delete any filter settings, or both.
 - If you add more filter settings, then marker-specific settings and allele-specific setting take precedence over “All” filter settings.
 - You can select only a single filter at a time for deletion.
 - If you edit any of the default values for the STR Stutter filter settings, and want to return them to their default values, click Default, and then click Yes in the Default values message that opens. The values for *all* STR Stutter filter settings are immediately reset to their default values.
1. Edit the settings as appropriate for the project.



To open a tooltip that provides an explanation of the STR Stutter filter setting, hold your mouse pointer over the column header for the setting.

Setting	Description
Marker	To add a marker-specific STR Stutter filter setting: <ol style="list-style-type: none"> 1. Click Add Filter. A new row is displayed for adding a stutter filter. 2. On the Marker drop-down list, select a marker. 3. Leave the From Allele value and the To Allele Value set to All.
Repeat Length	The marker repeat length. Automatically populated based on the loaded panel. You should not edit this value.
From Allele To Allele	The default value for both parameters is All. You must leave these values as-is for marker-specific stutter positions. If allele-specific stutter filters are appropriate, for example, the LUS stutter filter, then do the following: <ol style="list-style-type: none"> 1. Click Add Filter. A new row is displayed for adding a stutter filter. 2. On the Marker drop-down list, select a marker. 3. Edit the value for the From Allele parameter and then tab out of the field. The value for the To Allele parameter is automatically adjusted.

Setting	Description
Type	Set the stutter position: <ul style="list-style-type: none"> -1: One repeat smaller. -2: Two repeats smaller. -0.5: 1/2 repeat smaller, typically 2 bp smaller. +1: One repeat larger. +0.5: 1/2 repeat larger, typically 2 bp larger.
Ratio	The ratio of the total number of sequence counts in the stutter position to the total number of allele counts for the main peak.

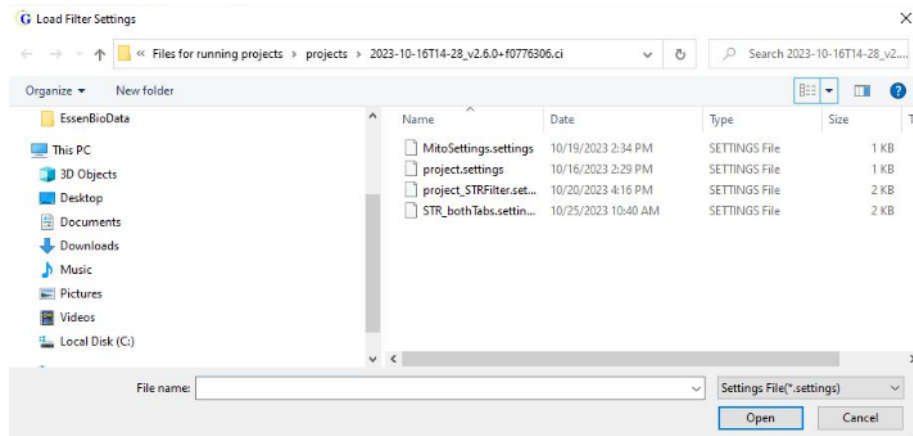
- Continue to [“To finish specifying the settings for an STR analysis project \(STR Stutter tab\)” on page 138.](#)

To load an STR/STR Stutter filter settings file

- Click Load.

The Load Filter Settings dialog box opens.

Figure 3-74: Filter Settings dialog box



- Browse to and select a Settings File (*.settings) file, and then click Open.

The Load Filter Settings dialog box closes. *Both* the STR Filter tab and the STR Stutter tab are populated with the values in the settings file.

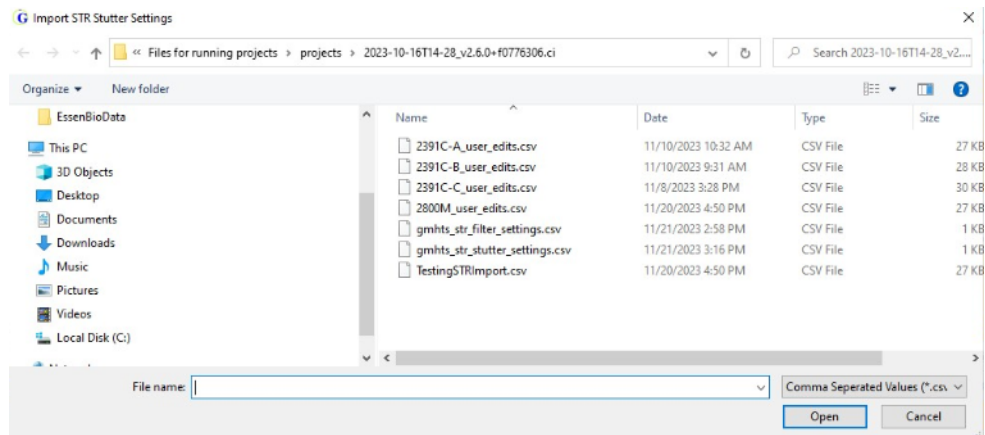
- Review the loaded filter settings, and do one of the following:
 - If you are satisfied that the loaded filter settings are correct as-is for your project, then go to [“To finish specifying the settings for an STR analysis project \(STR Stutter tab\)” on page 138.](#)
 - If you are not satisfied with the loaded filter settings for your project, then go to [“To edit the STR Stutter filter settings” on page 136.](#)

To import an STR Stutter tab-specific filter settings file

1. Click Import.

The Import STR Stutter Settings dialog box opens.

Figure 3-75: Import STR Stutter Settings dialog box



2. Browse to and select the STR Stutter tab-specific Comma Separated Values(*.csv) file, and then click Open.

The Import STR Stutter Settings dialog box closes. *Only* the STR Stutter tab is populated with the values in the settings file.

3. Review the imported filter settings, and do one of the following:
 - If you are satisfied that the imported filter settings are correct as-is for your project, then go to [“To finish specifying the settings for an STR analysis project \(STR Stutter tab\)” on page 138.](#)
 - If you are not satisfied with the imported filter settings for your project, then go to [“To edit the STR Stutter filter settings” on page 136.](#)

To finish specifying the settings for an STR analysis project (STR Stutter tab)

Do one of the following:

- Immediately apply the filter settings. Click OK to close the Filter Settings dialog box and immediately apply the settings. The STR Analysis viewer display is dynamically updated based on the settings
- Save the filter settings before applying them. See [“To save the STR Stutter filter settings before applying them” on page 139.](#)
- Export the filter settings to a tab-specific filter settings file before applying them. See [“To export the STR Stutter filter settings before applying them” on page 140.](#)

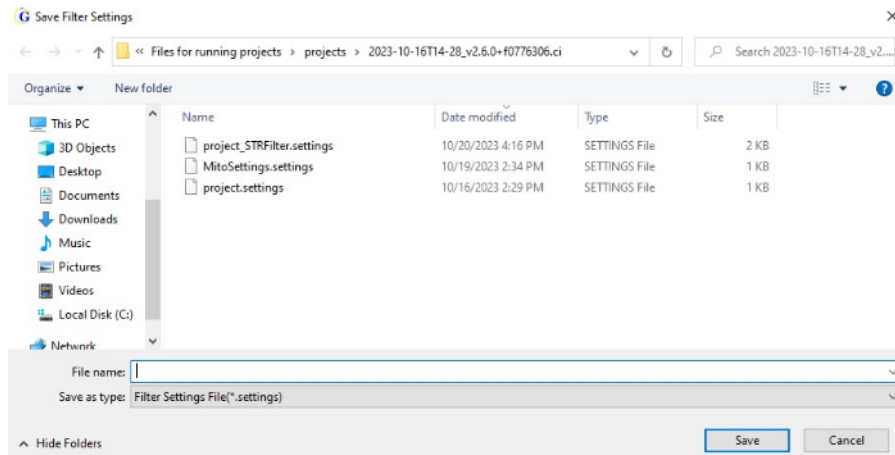
To save the STR Stutter filter settings before applying them

If you edited any of the STR Stutter filter settings and you want to save these edits before applying them, then you must save the settings to a Filter Settings (*.settings) file. When you save a filter settings file, the settings on *both* the STR Filter tab and STR Stutter tab are saved.

1. Click Save.

The Save Filter Settings dialog box opens.

Figure 3-76: Save Filter Settings dialog box



Note the following when you are saving a filter settings file:

- No default name is provided for the file.
- The file type is Filter Settings (*.settings) and you cannot change this.
- The default location for saving the filter settings file is the project folder, but you can always select a different location.

2. Enter a name for the file.
3. Optionally, select a different location in which to save the file.
4. Click Save.

The Save Filter Settings dialog box closes.

5. Click OK.

The Filter Settings dialog box closes. The STR Analysis viewer display is immediately updated based on the applied settings.

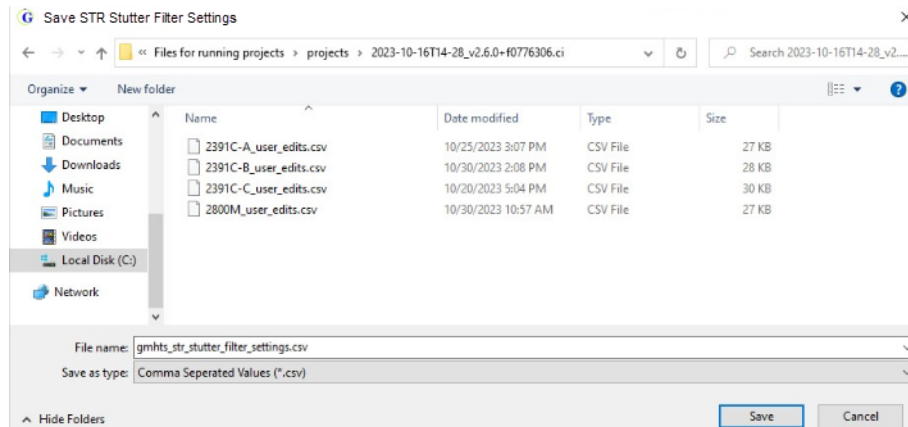
To export the STR Stutter filter settings before applying them

If you edited any of the filter settings and you want to save these edits only on the STR Stutter tab before applying them, then you must export save the filter settings to a Comma Separated Values (*.csv) file.

1. Click Export.

The Export STR Stutter Settings dialog box opens.

Figure 3-77: Save STR Stutter Filter Settings dialog box



Note the following when you are exporting and saving a tab-specific filter settings file:

- The default name is set to gmhts_str_stutter_settings. You can leave the filename as-is, or you can edit it.
 - The file type is Comma Separated Values (.csv) and you cannot change this.
 - The default location for saving the tab-specific filter settings file is the project folder, but you can always select a different location.
2. Optionally, enter a different name for the file, select a different location in which to save the file, or both.
 3. Click Save.

The Export STR Stutter Settings dialog box closes

4. Click OK.

The Filter Settings dialog box closes. The STR Analysis viewer display is immediately updated based on the applied settings.

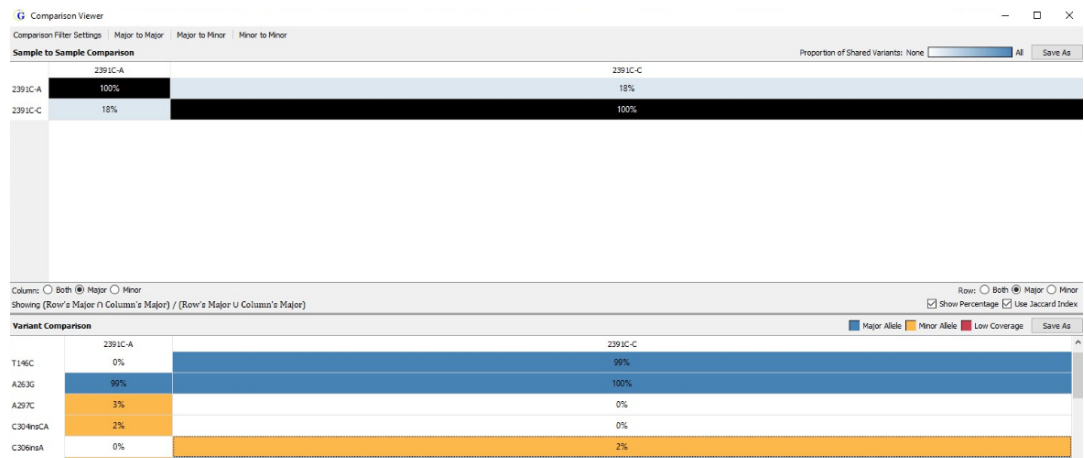
Comparison Viewer

You can compare the results for two or more samples or all samples in an mtDNA project in a single Comparison viewer window.



For detailed information about opening an existing project and selecting two or more samples to compare, see [“To work with an existing GeneMarkerHTS project” on page 68.](#)

Figure 3-78: Comparison viewer



From top to bottom, the Comparison viewer has three major components:

- The main menu. See [“Comparison viewer main menu”](#) below.
- The Comparison pane. See [“Sample to Sample Comparison pane” on page 142.](#)
- The Variant Comparison pane. See [“Variant Comparison pane” on page 143.](#)

Comparison viewer main menu

Figure 3-79: Comparison viewer main menu



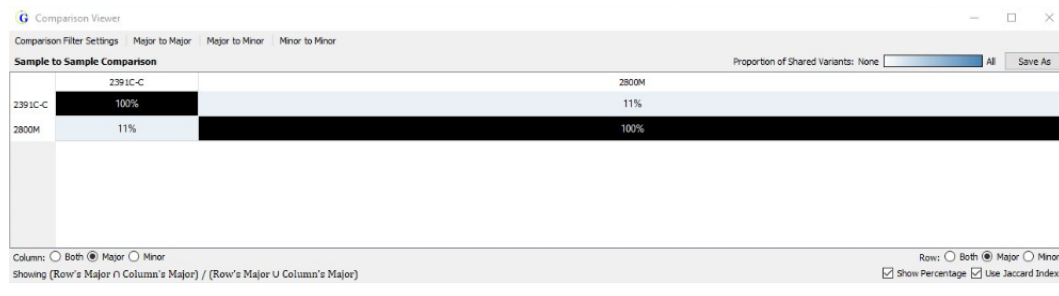
The Comparison viewer main menu has four unique options.

Option	Description
Comparison Filter Settings	Opens the Filter Settings dialog box. You use the options on this dialog box to adjust the filter settings for the project and dynamically update the Comparison viewer display. Note: Any edits that you make to the project filter settings here affect only the Comparison viewer display. They do not affect the Mito Alignment viewer display.
<Variant Comparison Type>	Three available options. Applicable only for the Sample to Sample Comparison pane. See “Sample to Sample Comparison pane” on page 142.

Sample to Sample Comparison pane

The Sample to Sample Comparison (top) pane for the Comparison viewer is a correlation table, that, by default, displays the percentage of major alleles that are shared among all the project samples, with the Jaccard Index measurement used to calculate the comparison. Diagonal cells in the correlation table compare a sample to itself and by default, are highlighted in black. All other cells in the correlation table compare one sample to another for the proportion of shared variants. By default, these cells are highlighted in a blue color gradient from 0 to 100%, where 0% cells are blank/white and 100% cells are the darkest blue in the gradient. The Proportion of Shared Variants, which is a read-only legend that is located above the pane, shows this gradient. See [Figure 3-80](#) below.

Figure 3-80: Comparison viewer, Sample to Sample Comparison pane

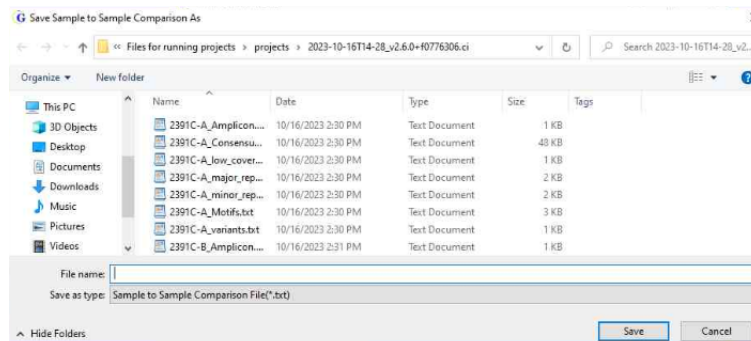


You can change the default highlighting colors that are used in the Sample to Sample Comparison pane. See [“To edit the Comparison viewer variant highlighting colors”](#) on page 158.

The following options are available for the Sample to Sample Comparison pane.

- **Save As:** You can click the Save As option that is displayed above the Comparison pane to open the Save Sample to Sample Comparison As dialog box and save the information that is displayed in the pane as text (.txt) file with a filename of your choosing. By default, the text file is saved in the same directory as the project, but you can always select a different location.

Figure 3-81: Save Sample to Sample Comparison As



- **Column variant type and Row variant type:** You can change the variant types that are used for calculating the correlation (Major, Minor, or Both). You can select the same or different variant types for the column and the row.



You can also use the options that are available on the Comparison viewer main menu (Major to Major, Major to Minor, and Minor to Minor) to quickly reset the variant types that are used for the calculation of the correlation.

- Show Percentage: Selected by default. Shows the percentage of shared variants for the samples that are being compared. If you clear this option, then the absolute number of shared variants is shown instead.
- Use Jacquard Index. Selected by default. The Jaccard Index measurement is used for the calculation, which results in the variant values in either the row or the column being used in the divisor. If you clear this option, then a Simple measurement is used for the calculation, which results in only the variant value in the column being used in the divisor.

As you change any of the options for the correlation calculation, both the correlation table display and the correlation calculation are updated accordingly. You can hold your mouse pointer over any entry in the correlation table to open a tooltip that displays the percentage or absolute number of matched variants, and if applicable, all the variants that are unique to each appropriate sample.

Figure 3-82: Correlation Table tooltip

Comparison Filter Settings		Major to Major	Major to Minor	Minor to Minor
Sample to Sample Comparison				
	D#3_1.fastq	L2c3_1.fastq		
D#3_1.fastq	100%	21%		
L2c3_1.fastq	21%	100%		
USb1e_1.fast	14%	23%		

Matched Variants:
A73G, 263G

Variants unique to USb1e_1.fast:

Variant Comparison pane

The Variant Comparison (bottom) pane lists all the variants that were found in at least one sample for the project, where each column is a different sample and the variant percentage for the sample is displayed. The display provides a quick way of visually determining which variants were shared among samples and which were not. Variants that can be classified as a major allele or as a minor allele are highlighted, by default, in blue or gold, respectively. If a variant was determined to be a Low Coverage variant, (the Total Coverage for the variant falls below the Total Coverage threshold that was set for the project), then by default, the variant is highlighted in red.

Figure 3-83: Variant Comparison pane

Variant	2391C-C	2800M
T146C	99%	0%
T152C	0%	99%
A263G	100%	99%
C304A	0%	3%
C306rsA	2%	0%
C309rsC	68%	1%
C315rsC	3%	99%
C315rsCCC	64%	0%
C315rsCCCC	2%	0%
G316A	0%	3%
G316C	7%	0%

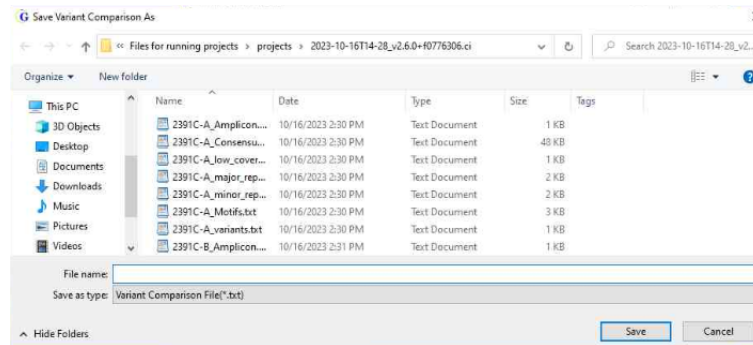


You can change the default highlighting colors that are used in the Variant Comparison pane. See [“To edit the Comparison viewer variant highlighting colors” on page 158.](#)

The following options are available for the Variant Comparison pane.

- **Save As:** You can click the Save As option that is displayed above the Comparison pane to open the Save Variant Comparison As dialog box and save the information that is displayed in the pane as text (.txt) file with a filename of your choosing. By default, the text file is saved in the same directory as the project, but you can always select a different location.

Figure 3-84: Save Variant Comparison As dialog box



- **Comparison Filter Settings:** The specific variants that are initially displayed in the Variant Comparison pane are determined by the filter settings, including the Total Coverage threshold, that were in place at the time that the mtDNA project was saved. To dynamically change the display, on the Comparison viewer main menu, click Comparison Filter Settings to open the Filter Settings dialog box, and make any needed edits. See [“To specify the variant call settings for an mtDNA project” on page 93.](#)

Appendix A

GeneMarkerHTS Application Settings

You use GeneMarkerHTS Application settings to quickly configure the application to provide a customized experience. You can use these settings to enable or disable features or configure feature behavior so that GeneMarkerHTS best fits your working needs. You configure GeneMarkerHTS application settings in the Application Settings dialog box. This appendix details the GeneMarkerHTS Application Settings dialog box including its layout, all the available settings, and how to manage and configure the different settings.

This appendix covers the following topics:


- [“GeneMarkerHTS Application Settings Dialog Box Layout” on page 147.](#)
- [“Configuring GeneMarkerHTS Project Directory Settings” on page 148.](#)
- [“Configuring GeneMarkerHTS License Settings” on page 150.](#)
- [“Configuring GeneMarkerHTS Visuals Settings” on page 152.](#)

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GeneMarkerHTS Application Settings

GeneMarkerHTS Application Settings Dialog Box Layout

You configure GeneMarkerHTS application settings in the Application Settings dialog box. The dialog box provides the options for configuring the SoftGenetics license settings, the GeneMarkerHTS project directory settings, and the Viewer and Report visualization settings. When you edit application setting, the change is persistent for the GeneMarkerHTS instance.

You can open the GeneMarkerHTS Application Settings dialog box from two locations in the application:

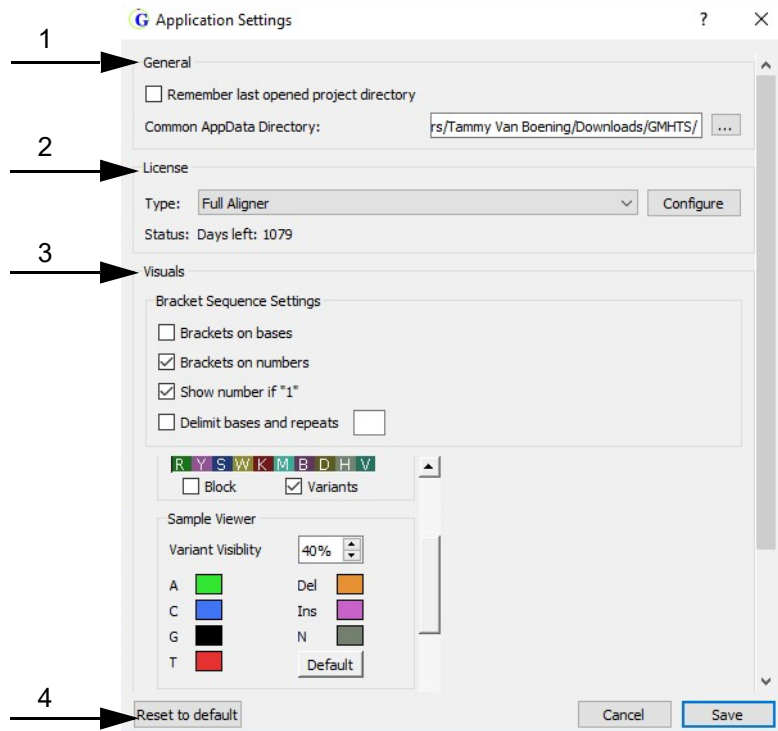
- From the GeneMarkerHTS main menu: File > Settings.
- On the GeneMarkerHTS main window, click the Application Settings icon .

From top to bottom, the dialog box has three panes:

- (1) General: Displays the options for setting project directory location and behavior.
- (2) License: Displays the options for configuring the SoftGenetics license settings.
- (3) Visuals: Displays the options for configuring the bracket sequence settings and the color settings for the different GeneMarkerHTS viewers and reports.

A (4) Reset to default button is displayed at the bottom of the dialog box. Click this button to reset *all* application setting values in *all* panes to their default values in a single step.

Figure A-1: GeneMarkerHTS Application Settings dialog box



Configuring GeneMarkerHTS Project Directory Settings

The General pane contains the GeneMarkerHTS project directory settings. There are two project directory settings that you can configure:

- Remember last opened samples directory. By default, GeneMarkerHTS remembers the last opened sample files directory, which means any time that you are adding sample files to a project, GeneMarkerHTS automatically points to the directory that was *last* used for adding sample files to a project. You can leave the default setting as-is, or you can clear this selection.
- Common AppData Directory. The Common Application Data Directory is the client directory in which custom project panels are saved. The directory is automatically created during installation, and by default, it is set to the following location:

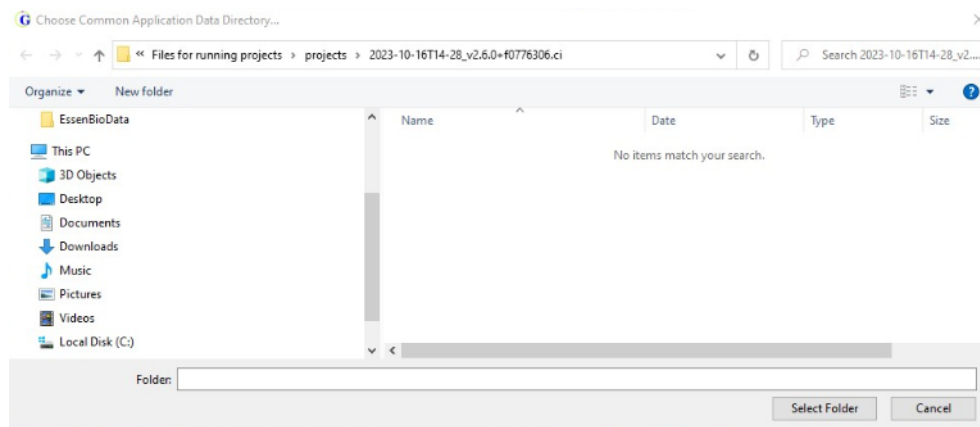
C:\Users*<user Name>*\Downloads\GMHTS.

You can leave this directory location as-is, or you can set a different default Common Application Data directory.

To configure GeneMarkerHTS project directory settings

1. On the Application Settings dialog box, ensure that the General pane is visible.
2. Do one or both of the following:
 - Clear Remember last opened samples directory.
If you clear this option, then when you are adding sample files to a project, GeneMarkerHTS opens the installation directory and you must browse to and select the sample files directory.
 - Next to the Common AppData Directory display field, click the Browse button to open the Choose Common Application Directory dialog box, and then select a different default Common Application Data directory.

Figure A-2: Choose Common Application Data Directory dialog box



3. Do one of the following:
 - If you are done specifying your settings, then click Save to close the Application Settings dialog box and return to the GeneMarkerHTS main window.
 - Continue to add and/or edit other application settings as needed.

Configuring GeneMarkerHTS License Settings

The License pane contains the GeneMarkerHTS license settings. There are two GeneMarkerHTS license settings that you can configure:

- The license type (Trial or FullAligner).
- The SoftGenetics LSM connection settings.

You must configure your license type of Trial or FullAligner (Network or Local) before you run your first GeneMarkerHTS project, noting the following:

- A Trial license is valid for 35 days. You are not required to register a Trial license; however, after your 35 days has expired, you must purchase a commercial license and then register the license properly before you can continue to use GeneMarkerHTS. See [“To set a Trial license”](#) below.
- After you purchase and configure a commercial license, you typically do not need to edit the license type and/or LSM connection settings again; however, if you must edit the license type and/or reconfigure the LSM connection settings, see the appropriate topic:
 - [“Registering a GeneMarkerHTS Network License”](#) on page 25.
 - [“Registering a GeneMarkerHTS Local License”](#) on page 37.

To set a Trial license

By default, when you install GeneMarkerHTS, the License Type is set to FullAligner, which is applicable for a commercial (purchased) installation. A non-commercial installation of GeneMarkerHTS requires that you set the License Type to Trial. A Trial license is valid for 35 days. After 35 days, to continue using GeneMarkerHTS, you must set the License Type to FullAligner and purchase and register a commercial (network or local) license.

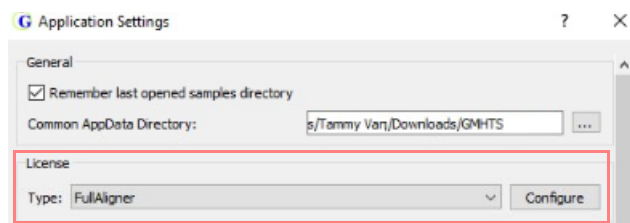
1. On the GeneMarkerHTS main window, click the Application Settings icon.

The Application Settings dialog box opens. From top to bottom, the dialog box has three panes: General, License, and Visuals.

2. Ensure that the License pane is visible.

The pane indicates that GeneMarkerHTS was installed with a FullAligner license.

Figure A-3: Application Settings dialog box, License pane with FullAligner license



3. On the License Type drop-down list, select Trial.
Configure is now disabled.

4. Close the Application Settings dialog box.

GeneMarkerHTS is now ready for use for 35 days. After 35 days, you can still open GeneMarkerHTS but it is not operable. You must purchase a commercial license to continue to use GeneMarkerHTS.

Configuring GeneMarkerHTS Visuals Settings

The Visuals pane contains the GeneMarkerHTS bracket sequence settings and color settings. The Visuals pane is divided into eight sub-panes for configuring these settings, which from top to bottom are the following.

- The Bracket Sequence Settings sub-pane. The bracket sequence settings determine how the STR results are displayed in the STR Results Table pane in the STR Viewer.
- The Color settings sub-panes, which from top to bottom are the following:
 - The Sample Viewer Preview sub-pane, which shows what the Pane displays currently look like in the Mito Alignment Viewer based on the colors and other settings that are specified in the Sample Viewer sub-pane.
 - The Sample Viewer sub-pane, which shows the currently selected colors and variant visibility for the Pane displays in the Mito Alignment Viewer.
 - The Global Viewer sub-pane, which shows the currently selected colors for the Global Viewer pane display in the Mito Alignment Viewer.
 - The Report Table Category pane, which shows the currently selected colors for the Variants Table pane in the Mito Alignment Viewer.
 - The Comparison Viewer Sample to Sample sub-pane, which shows the currently selected colors that indicate the overlap between two samples for the Sample to Sample pane of the Comparison Viewer.
 - The Comparison Viewer Variant sub-pane, which shows the currently selected colors for the different variant classifications (major or minor) and the color that indicates if the variant was a low coverage variant for the Variant pane of the Comparison Viewer.
 - The STR Viewer sub-pane, which shows the currently selected colors for the different STR statuses (Flagged, Passed, Filtered, and Called) in the STR Viewer.

To edit the Visuals application settings

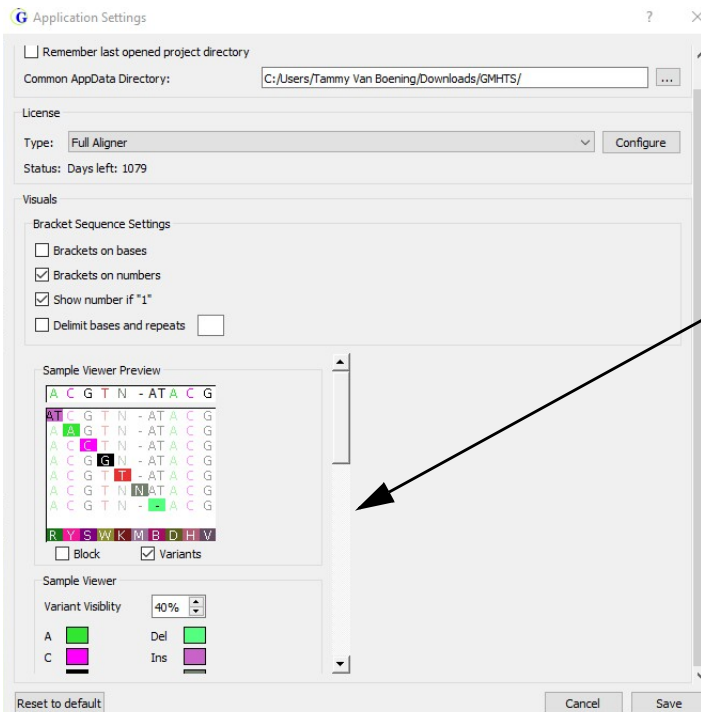
1. Open the Application Settings dialog box.
2. On the Application Settings dialog box, ensure that the Visuals pane is visible.

See [Figure A-4 on page 153](#).



To make it easier for you to edit any of the Visuals pane settings, use standard Windows functions to increase the size of the Application Settings dialog box. To view all the sub-panes that are displayed in the Visuals pane, use the scroll bar that is displayed to the right of the Visuals pane.

Figure A-4: Application Settings dialog box, Visuals pane



Use the scroll bar that is displayed to the right of the Visuals pane to move through all the available sub-panes. Use standard Windows controls to resize the Application Settings dialog box so that you can scroll completely through all the Visuals sub-panes.

3. Continue to any of the following:

- “To edit the bracket sequence settings” below.
- “To edit the background and highlighting colors in the Pile-up pane display” on page 154.
- “To edit the visibility and default nucleotide colors for the Pile-up pane display” on page 155.
- “To edit the Global Viewer pane colors” on page 157.
- “To edit the Variants table (Report Table) Category colors” on page 158.
- “To edit the Comparison viewer variant highlighting colors” on page 158.

To edit the bracket sequence settings

The bracket sequence settings determine how the STR results are displayed in the STR Results Table pane.

Figure A-5: STR Results Table pane, Bracket Sequence example

Marker	Allele Name	Report	Filter Status	Filter Reason	SW Call	User Call	User Comment	Bracket Sequence	Sequence Forward Count	St Rev
DY533	10	<input checked="" type="checkbox"/>	Passed		-1 Stutter	-1 Stutter		TATC[10]	65	66
DY533	11	<input checked="" type="checkbox"/>	Passed		Called	Called		TATC[11]	683	751
DY533	12	<input checked="" type="checkbox"/>	Flagged	MarkerPercentit	Called	--		TATC[12]	9	6
DY549	11	<input checked="" type="checkbox"/>	Passed		-1 Stutter	-1 Stutter		GATA[11]	125	112

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GeneMarkerHTS Application Settings

1. On the Application Settings dialog box, ensure that the Bracket Sequence Settings sub-pane is visible.
2. Edit the sequence settings as appropriate.

Setting	Description
Brackets on bases	Not selected by default. Select this setting to surround the bases in an STR in brackets. <ul style="list-style-type: none"> • The default format: TGAT[3] • The setting is selected: [TGAT][3]
Brackets on numbers	Selected by default. Repeat numbers for STRs are surrounded by brackets. <ul style="list-style-type: none"> • The default format: TGAT[3] • The setting is cleared: TGAT3
Show number if "1"	Selected by default. The number 1 is displayed for only a single repeat. <ul style="list-style-type: none"> • The default format: [TGAT][3]TGA[1][TGAT][3] • The setting is cleared: [TGAT][3]TGA[TGAT][3]
Delimit bases and repeats	Not selected by default. No delimiters are displayed between the bases in an STR and the repeat numbers. If you select this option, and then do not specify any delimiter, the default delimiter is space. <ul style="list-style-type: none"> • The default format: TGAT[3]_TGA_TGAT[3] • The setting is selected: TGAT_[3]_TGA_TGAT_[3] (The specified delimiter is an underscore.) <p>Note: You can designate any special character as a delimiter.</p>

3. Do one of the following:
 - If you are done specifying your settings, then click Save to close the Application Settings dialog box and return to the GeneMarkerHTS main window.
 - Continue to edit other application settings as needed.

To edit the background and highlighting colors in the Pile-up pane display

In the Sample Previewer sub-pane, you specify the colors to use for background/highlighting and the nucleotides in the Pile-up pane display for the Mito Alignment viewer. If you edit the display colors, then the Sample Viewer Previewer sub-pane is dynamically updated with your settings.

1. On the Application Settings dialog box, ensure that the Sample Previewer sub-pane is visible.
2. Edit the background/highlighting colors and/or the nucleotide colors, noting the following:
 - If Variants is selected (the default value), then all nucleotides *other* than variant nucleotides are displayed in color on a white background. Conversely, variant nucleotides are displayed in white on a colored background (referred to as a

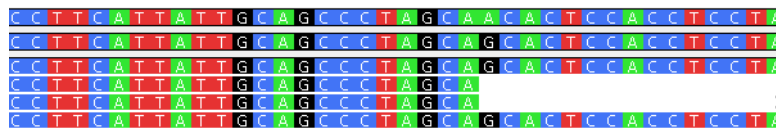
highlighted variant).

Figure A-6: Variants selected for Pane displays



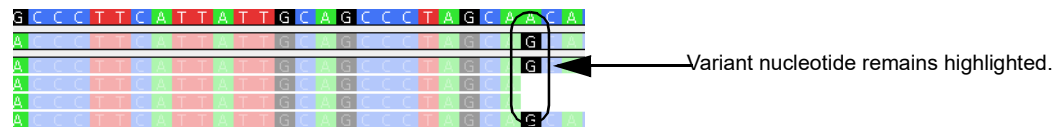
- If Blocks is selected, then *all* nucleotides are displayed in white on a colored background.

Figure A-7: Blocks selected for Pane displays



You can select both Variants and Blocks for the display. In this case, all nucleotides are still displayed in white on a colored background; however, all nucleotides other than variant nucleotides are dimmed and all variant nucleotides are highlighted to visually differentiate them from non-variant nucleotides. See Figure A-8 below.

Figure A-8: Variants and Blocks selected for Pane displays in the Mito Alignment Viewer



3. Do one of the following:
 - If you are done specifying your settings, then click Save to close the Application Settings dialog box and return to the GeneMarkerHTS main window.
 - Continue to edit other application settings as needed.

To edit the visibility and default nucleotide colors for the Pile-up pane display

In the Sample Viewer sub-pane, you specify the variant visibility and the nucleotide colors in the Pile-up pane display for the Mito Alignment viewer.

- Variant Visibility. Determines how dimmed non-variant nucleotides are when compared to the variant nucleotides when *both* Variants and Blocks are selected for the pane display in the Mito Alignment viewer. The default value is 40%.
- The sub-pane also displays the various colors that are to be used in the Pane displays where the *default* nucleotide colors are the following:
 - For non-variant nucleotides and SNPs: If Variants is selected, then Green, Blue, Black, and Red indicate the colors for the nucleotides A, C, G, and T, respectively. If

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GeneMarkerHTS Application Settings

Blocks is selected, then these indicate the colors for the nucleotide backgrounds.

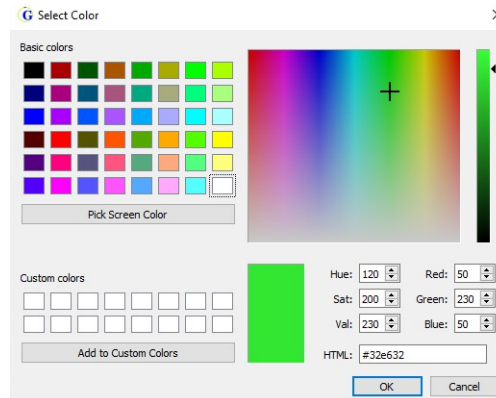
- For Deletions: No nucleotide is displayed. Instead, a burnt orange dash is displayed.
- For Insertions: If Variants is selected, then the nucleotide is displayed in purple. If Blocks is selected, then the nucleotide *background* is purple.
- If coverage areas fall below the Total Coverage value that is specified for the Filter Settings: If Variants is selected, then a gray “N” is displayed for the consensus sequence in these regions. If Blocks is selected, then the nucleotide background is displayed in gray and “N” is displayed in white.

Figure A-9: “N” indicating that coverage areas fall below the Total Coverage Value that is specified in the Filter Settings



1. On the Application Settings dialog box, ensure that the Global Viewer sub-pane is visible.
2. Do any or all of the following as needed to edit the variant visibility and/or change colors
 - Adjust the value for Variant Visibility.
 - To make the non-variant nucleotides more visible in the pane display when *both* Blocks and Variants are selected, increase the value.
 - Conversely, to make the non-variant nucleotides less visible in the pane display when both Blocks and Variants are selected, decrease the value. You can manually enter a new value, or you can use the Up/Down arrows.
 - Change one or more colors for the nucleotides and/or variants. To do so, double-click the color swatch that is displayed for a nucleotide or variant type to open the Select Color dialog box, select a different color, and then click OK.

Figure A-10: Select Color dialog box



The Select Color dialog box closes and the Application Settings dialog box remains open. The newly selected color is displayed for the selected nucleotide or variant type.

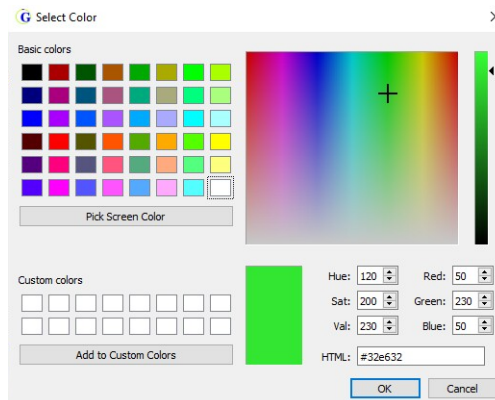
3. Do one of the following:
 - If you are done specifying your settings, then click Save to close the Application Settings dialog box and return to the GeneMarkerHTS main window.
 - Continue to edit other application settings as needed.

To edit the Global Viewer pane colors

You can specify the colors that indicate coverage (forward, reverse, and/or low) and variant type (major or minor) in the Global Viewer pane of the Mito Alignment Viewer, where the default colors are the following:

- Forward coverage: Blue
 - Reverse coverage: Red
 - Low coverage: Gray
 - Major variant: Green
 - Minor variant: Burnt orange
1. On the Application Settings dialog box, ensure that the Global Viewer sub-pane is visible.
 2. Double-click the color swatch that is displayed for a coverage type or variant type to open the Select Color dialog box, select a different color, and then click OK.

Figure A-11: Select Color dialog box



The Select Color dialog box closes and the Application Settings dialog box remains open. The newly selected color is displayed for the selected nucleotide or variant type.

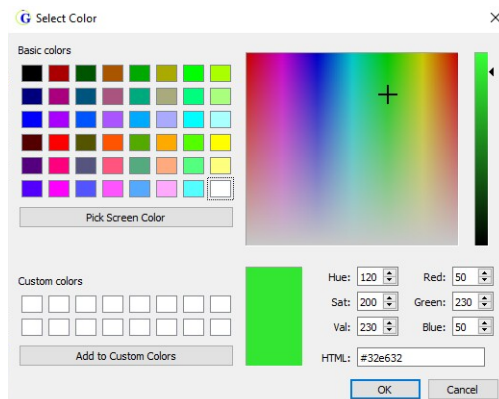
3. Do one of the following:
 - If you are done specifying your settings, then click Save to close the Application Settings dialog box and return to the GeneMarkerHTS main window.
 - Continue to edit other application settings as needed.

To edit the Variants table (Report Table) Category colors

You can specify the highlighting colors for the different statuses of edited variants (added, removed, and so on) in the Variants Table of the GeneMarkerHTS viewer, where the default colors are the following:

- Added and Edit Caused Add: Light green
 - Removed and Edit Caused Remove: Light red
 - Called and Edited: Light blue
1. On the Application Settings dialog box, ensure that the Report Table Category sub-pane is visible.
 2. Double-click the color swatch that is displayed for a variant status to open the Select Color dialog box, select a different color, and then click OK.

Figure A-12: Select Color dialog box



The Select Color dialog box closes and the Application Settings dialog box remains open. The newly selected color is displayed for the selected status.

3. Do one of the following:
 - If you are done specifying your settings, then click Save to close the Application Settings dialog box and return to the GeneMarkerHTS main window.
 - Continue to edit other application settings as needed.

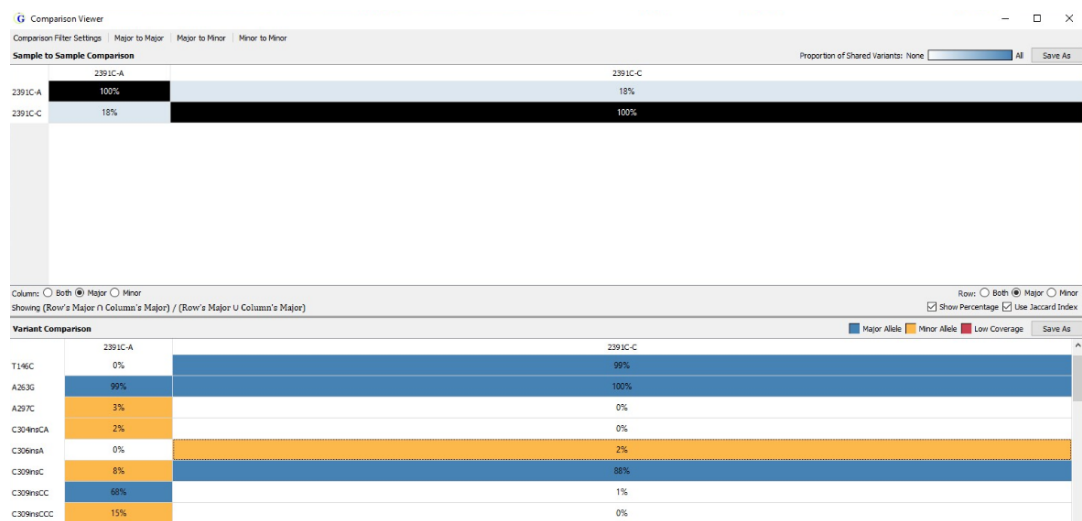
To edit the Comparison viewer variant highlighting colors

The Comparison viewer is divided into two panes: the Sample to Sample Comparison (top) pane and the Variant Comparison (bottom) pane.

- You can specify the variant highlighting colors in the Sample to Sample Comparison pane, where the default colors are the following:
 - Diagonal cells that compare a sample to itself are highlighted in black.

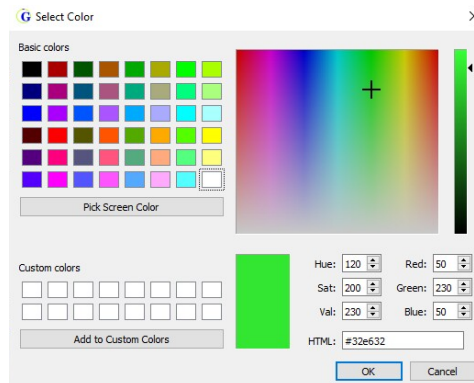
- All other cells in the correlation table compare one sample to another and are highlighted in a blue color gradient from 0 to 100%, where 0% cells are blank/white.
- You can specify the variant highlighting colors in the Variant Comparison pane, where the default colors are the following:
 - Variants that can be classified as a major allele are highlighted in blue.
 - Variants that can be classified as a minor allele are highlighted in gold.
 - If a variant was determined to be a Low Coverage variant, the variant is highlighted in red.

Figure A-13: Comparison viewer



1. On the Application Settings dialog box, ensure that the correct sub-pane, Comparison viewer: Sample to Sample or Comparison viewer: Variant, is visible.
2. Double-click the color swatch that is displayed for a variant highlighting color to open the Select Color dialog box, select a different color, and then click OK.

Figure A-14: Select Color dialog box



The Select Color dialog box closes and the Application Settings dialog box remains open. The newly selected color is displayed for the selected sample or variant type.

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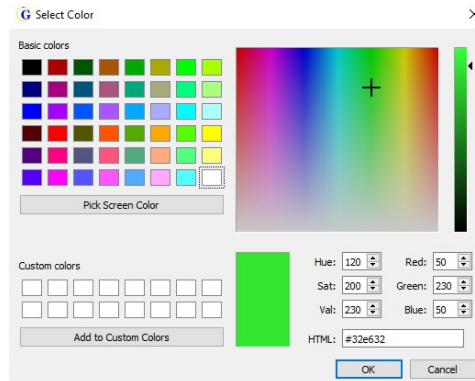
3. Do one of the following:
 - If you are done specifying your settings, then click Save to close the Application Settings dialog box and return to the GeneMarkerHTS main window.
 - Continue to edit other application settings as needed.

To edit the STR Analysis viewer highlighting colors

You can specify the highlighting colors for the filter status and analyst reason for an STR in the STR Results Table pane of the STR Analysis viewer, where the default colors are the following:

- Flagged: Orange
 - Passed: No highlighting (background is white)
 - Filtered: Orange
 - Called: Bright green
1. On the Application Settings dialog box, ensure that the STR Viewer sub-pane is visible.
 2. Double-click the color swatch that is displayed for a variant highlighting color to open the Select Color dialog box, select a different color, and then click OK.

Figure A-15: Select Color dialog box



The Select Color dialog box closes and the Application Settings dialog box remains open. The newly selected color is displayed for the selected filter status or analyst reason.

3. Do one of the following:
 - If you are done specifying your settings, then click Save to close the Application Settings dialog box and return to the GeneMarkerHTS main window.
 - Continue to edit other application settings as needed.

Glossary

B

BED file

Also known as Region of Interest (*.bed file). A BED file is a tab-delimited text file. You can upload a BED file only if the reference sequence contains chromosome information. Each row in the file contains a region of the reference that is to be used for the analysis, and at a minimum, the file must contain the following information:

- Field #1 - Chromosome number or name for the region (for example, chrM)
- Field #2 - Chromosome start position (for example, 300)
- Field #3 - Chromosome end position (for example, 305)
- Field #4 - Optional description column (for example, Region 102)

The positions are 0-based and open-ended. For example, a start of 10 and end of 15 would include reference positions 11, 12, 13, 14, and 15.

C

Consensus alignment

An optional alignment step. Because each read is considered on its own, indels that are near the end of reads are sometimes not correctly aligned. If this alignment option is selected, then an extra step is added after the initial alignment. In this extra step, indel positions are re-examined to possibly improve the alignment of these reads based on the alignment of other reads that have the indel.

M

Motif alignment

An optional alignment step. When equivalent sequences can be aligned in multiple ways (motifs), an analyst might not prefer the alignment method that the software selects. This step ensures that the selected motif is the motif that is defined by the motif file.

P

PCR duplicates

PCR duplicates are a set of pairs (paired-end data) or reads (non-paired-end data) that have been generated from the same original fragment. The "Remove PCR Duplicates" optional alignment step attempts to identify these reads and ignores all but one pair (or read) in each set.

Personal Health Information (PHI)

Personal health information can be inferred from some regions of whole mtDNA sequence. To maintain privacy during whole mtDNA analysis, a BED file can be used to specify the positions that must be hidden from the analyst.

Proper Pairs

A pair of aligned reads is referred to as a “proper pair” if the reads are aligned on opposite strands in the correct orientation. Non-proper pairs can result from misalignment, sequencing error, or off-target amplification.

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