

MaSTR



User Manual



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Release Information

<i>Document Version Number</i>	<i>MaSTR -1.9- UG001</i>
<i>Software Version</i>	<i>1.9</i>
<i>Document Status</i>	<i>Final</i>

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Customer Support

Customer support is available to organizations that purchase MaSTR and that have an annual support agreement. Contact SoftGenetics at:

SoftGenetics, LLC
100 Oakwood Ave, Suite 350
State College, PA 16803
(814) 237-9340
(888) 791-1270 (US Only)
tech_support@softgenetics.com
www.softgenetics.com

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Preface

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

Using the manual

You will find the *MaSTR 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 *MaSTR 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 *MaSTR 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, <Model 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.

- 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 *MaSTR User's Manual* assumes that:

- References to any third-party standards or third-party software functions were current as of this release version of MaSTR, and might have already changed.

Organization of the manual

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

- [Chapter 1, “Getting Started with MaSTR,” on page 13](#) details the software architecture for MaSTR, the minimum system requirements for installing MaSTR, installing MaSTR, and logging in to MaSTR. It also explains the different MaSTR modes that are available at login to a user.
- [Chapter 2, “MaSTR Primary Views,” on page 25](#) details the three primary views that are available in MaSTR, including their purposes and associated functions. This chapter also details the two types of files—a Profile file and a Signal file—that are required in various functional areas (views) of MaSTR for setting up a job analysis and carrying out the analysis, and the navigation tools that are common for all three views.
- [Chapter 3, “DNA Analysis in MaSTR,” on page 69](#) details how to run a forensic DNA analysis in MaSTR, how to validate a DNA analysis model in MaSTR, and finally, how to add a new DNA analysis model in MaSTR.
- [Chapter 4, “Reporting and Graphing DNA Analysis Results in MaSTR,” on page 81](#) details the various ways that you can report on and visualize your analysis results so that you can interpret the data and identify the keys areas of your assay that worked well as well as the areas that need attention or improvement.
- [Appendix A, “MaSTR Administration,” on page 97](#) guides you through the procedures that are necessary for administering your MaSTR installation efficiently and effectively, including viewing the audit trail, viewing and managing current MaSTR sessions, managing users and groups, backing and restoring the MaSTR database, and managing the settings for the MaSTR user interface (UI).
- [Appendix B, “Building a Custom MCMC Model for Probabilistic Mixture Analysis,” on page 121](#) provides a high-level overview of how MaSTR calculates peak heights and probability calculations, and where a custom model fits into this process. It also details *variables* and *expected peak height* calculations, which are the two required components

for building a custom MCMC model for probabilistic mixture analysis. Finally, it details how to define a model variable for a custom MCMC model.

- [Appendix C, “MaSTR Validation Study,” on page 133](#) provides a high-level overview and the conclusions that were reached by the preliminary validation of MaSTR, which indicated that MaSTR provides the rigor of a fully continuous probabilistic approach for evaluating genotypes in mixtures in a straight forward software program.
- The [Bibliography on page 145](#) is a list of citations to the sources (books, articles, and documents) that were used as references for the development and validation of MaSTR. The purpose of the annotation is to inform the reader of the relevance, accuracy, and quality of the sources cited.

Preface

Chapter 1

Getting Started with MaSTR

MaSTR is probabilistic genotyping software that provides fully continuous mixture analysis of STR results. This chapter details the software architecture for MaSTR, the minimum system requirements for installing MaSTR, installing MaSTR, and logging in to MaSTR. It also explains the different MaSTR modes that are available at login to a user.

This chapter covers the following topics:

- [“MaSTR Software Architecture” on page 15.](#)
- [“MaSTR System Requirements” on page 17.](#)
- [“Installing MaSTR” on page 18.](#)
- [“Starting the MaSTR Server and Logging in to MaSTR” on page 20.](#)
- [“Logging out of MaSTR” on page 23.](#)

Chapter 1

Getting Started with MaSTR

MaSTR Software Architecture

Figure 1-1 below shows the MaSTR software architecture. MaSTR consists of three primary components: a database, an API server, and a client. The database and the API server are contained within a virtual machine (VM) image, which means that the CPU for the computer that is running the VM must be enabled for VM support.

Figure 1-1: MaSTR software architecture

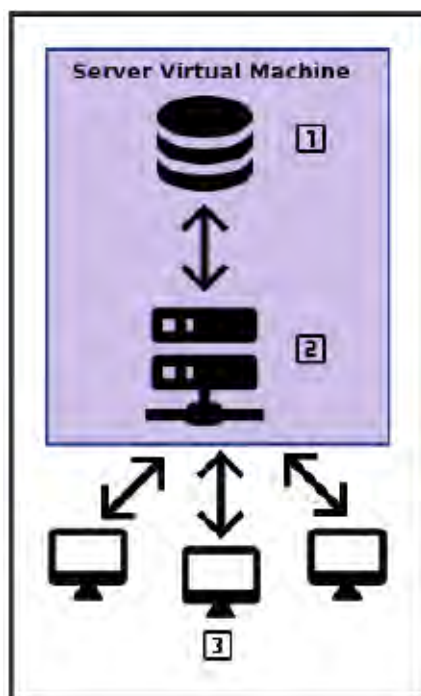


Table 1-1: MaSTR software architecture components

Component	Description
1	Database - Mongo database. Manages the job queues, stores required and critical supporting information for analysis jobs such as panels, models, protocol sets, and so on, and stores analysis job results.
2	API Server - Runs the analysis and communicates with the client to provide analysis results. The server must be running before a client can connect and run an analysis. See “The API server” on page 16 .
The database and API server are packaged together in a virtual machine (VM) that is run on a central computer.	
3	Client - One or more clients can connect to the central computer to submit jobs to the API server and review results. See “The MaSTR client” on page 16 .

The API server

The API server must be running before a client can connect and run an analysis. SoftGenetics provides the required open virtual appliance (.ova) file that describes the VM. You can use Oracle VirtualBox or any compatible virtualization program that allows you to create and provision VMs on desktop devices running an x86-based platform to import the .ova file. After you launch the server VM, a login window opens, but you do not need to log in to the server. Within a few seconds of the login window opening, the server is accessible to all the connected clients. By default, the 8000 port on the server is forwarded to the 8000 port on the computer that is hosting the server VM and this is the only port that must be exposed; however, if necessary, you can adjust the port forwarding settings in the virtualization program to expose a different port. Also, after the server machine is launched, if possible, you should increase the amount of RAM that is provided to the VM and you should also increase the number of virtual CPU processors. See [“Installing MaSTR” on page 18](#).

Figure 1-2: MaSTR API Server login window



The MaSTR client

You access the MaSTR client through a web browser, with Google Chrome being the preferred browser. The client accesses the server at port 8000 for the computer that is running the virtual machine, or whichever port is exposed from the virtual machine. If the client and server are running on the same computer, and port 8000 is the exposed port, then the location is <https://localhost:8000>. To trust the server's certificate, which encrypts the traffic between the client and the server, you must allow an exception. After a user logs in, a session is created.



To learn how to use the client, you should log in under “Demo” mode (no username and password are required), and then take the guided tour through the software. See [“Starting the MaSTR Server and Logging in to MaSTR” on page 20](#).

MaSTR System Requirements

Table 1-2 and Table 1-3 below detail the minimum, recommended, and required specifications for successfully installing and running MaSTR.

Table 1-2: Server specifications*

Server Component	Minimum Specifications*	Suggested Specifications
CPU	4-core	8-core or better
RAM	16 GB	32 GB or better
Hard Drive	1 TB	2 TB or better
Virtualization	Oracle VirtualBox or any compatible virtualization program that allows you to create and provision VMs on desktop devices running an x86-based platform to import the .ova file. Caution: *Complex mixtures might not be successfully analyzed.	Oracle VirtualBox or any compatible virtualization program that allows you to create and provision VMs on desktop devices running an x86-based platform to import the .ova file.

Table 1-3: Client specifications

Client Component	Specification
Connections	Connects to and interacts with the MaSTR server using a web browser: <ul style="list-style-type: none">• Google Chrome, v56 or higher (preferred)• Mozilla Firefox, v51 or higher
Supported OSs	64-bit OS enabled for VM support

Installing MaSTR

The MaSTR server is provided as a virtual machine (VM) image file, which is referred to as an open virtual appliance (.ova) file. To install the MaSTR server, you must have a virtualization program such as VMWare or Oracle VirtualBox available on the machine that is hosting the virtual server, and you must import the SoftGenetics-supplied .ova file that describes the VM.



The following instructions are based on using Oracle Virtualbox and are provided here for example purposes only. You can use any compatible virtualization program that allows you to create and provision VMs on a desktop device that is running an x86-based platform.

To install MaSTR

1. Download and install Oracle Virtualbox: <https://www.virtualbox.org/wiki/Downloads>.

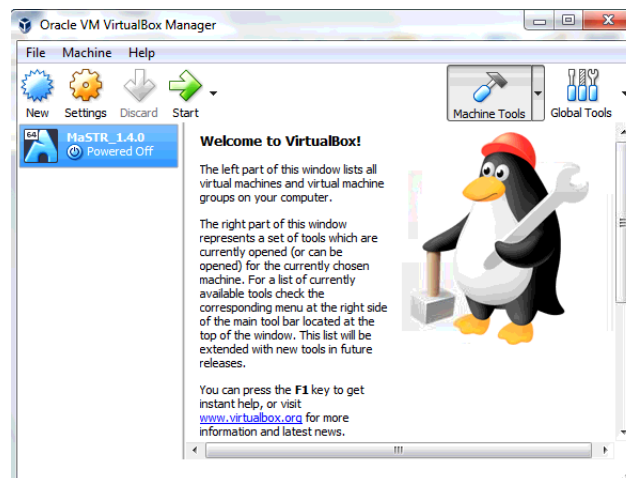


Make sure to select the correct host type (the OS of the computer that is running the VM).

2. Download and install the Oracle VM VirtualBox Extension pack.
3. Open VirtualBox.
4. On the main menu, click File > Import Appliance, and then browse to and select the .ova file that SoftGenetics supplied to you.
5. Click Next, and then click Import.

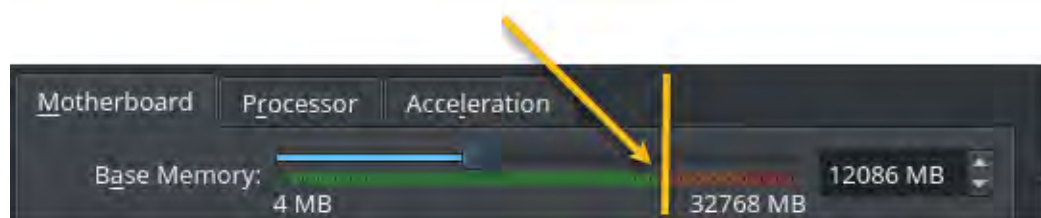
An entry for the MaSTR server is displayed in the left pane of the VirtualBox Manager main window. The server is powered Off.

Figure 1-3: Oracle VirtualBox Manager, main window with MaSTR server entry



6. Select the MaSTR server image, and on the main menu, click Settings.
The Settings dialog box opens.
7. In the left pane of the Settings dialog box, click System.
The right pane of the Settings dialog box displays the VM settings that can be adjusted.
The Motherboard tab is the open tab.
8. If possible, on the provided scale, increase the largest amount of RAM that is provided to the VM. Do not adjust the value past the green zone.

Figure 1-4: Adjusting the VM RAM setting



9. Open the Processor tab, and then on the provided scale, adjust the number of virtual CPU processors to highest possible value that remains within the green zone.

Figure 1-5: Adjusting the number of VM CPU processors



10. Close the Settings dialog box.
The VirtualBox Manager main window remains open.
11. Continue to [“Starting the MaSTR Server and Logging in to MaSTR”](#) on page 20.

Starting the MaSTR Server and Logging in to MaSTR

After you install MaSTR, you must power on the server VM. After you power on the server VM, you can log in and use MaSTR. You can use either Google Chrome or Mozilla Firefox to log in to MaSTR; however, Google Chrome is the preferred browser. When you log in to MaSTR, you must select a login mode, and then with the exception of the Demo mode, enter a username and password. Four login modes are available:

Table 1-4: MaSTR Login modes

Mode	Description
Admin	Provides access to the MaSTR administrator UI, including user management. See Chapter A, "MaSTR Administration," on page 97 .
Demo	Provides unrestricted access to a temporary database and guides you through the use of the software. Designed to help analysts understand and develop skills in the principles of routine mixture analysis. A username and password are not required.
Casework	Access to a permanent database that is to be used for routine application of validated models to casework samples.
Research/Validation	Access to a permanent database that is to be used for validation of existing models and development and testing of new models.

Five default user login accounts with case-sensitive credentials are provided with your MaSTR installation:

Table 1-5: MaSTR default login accounts

Account	Login credentials	Description
Root	root/changeThisPassword	Provides access not only to the four standard Admin mode functions (Log, Sessions, Users, and Settings) but also to the MaSTR database functions.
Admin	Admin/admin	Provides access to the four standard Admin mode functions (Log, Sessions, Users, and Settings).
Researcher	Researcher/researcher	Designed for a Researcher's work in the Research/Validation database.
Analyst	Analyst/analyst	Designed for a Researcher's work in the Research/Validation database.
Reviewer	Reviewer/reviewer	Designed for a reviewer's work in the Research/Validation database. Unlike the Researcher and Analyst logins, no special permissions are assigned to a reviewer for the Research/Validation database.

If you log in to MaSTR under any mode other than Admin, then when the MaSTR Login page opens, it displays the total number of licenses in use and the total number of licenses that are available. (If you log in under the Admin mode, then your login does not count against the total number licenses in use.) If all available licenses are in use, then you cannot

log in until at least one other user logs out.

Figure 1-6: MaSTR Login dialog box

The image shows the MaSTR login dialog box. At the top, the MaSTR logo is displayed in blue and black. Below the logo, there is a 'Mode' dropdown menu set to 'Demo'. Underneath, there are input fields for 'Username' and 'Password'. At the bottom left, there is a 'DOCS' link. In the center, a red-bordered box contains the text 'Trial expires in 24 days' and '1 of 2 Client Licenses in Use'. At the bottom right, there is a purple 'LOGIN' button.

After you log in to MaSTR, your login is active for eight hours. After your login expires, you are redirected to the MaSTR login page; however, none of your queued jobs or jobs that are already running on the server are affected.

To start and log in to MaSTR



The following procedure is a quick overview of starting and logging in to MaSTR using one of the MaSTR default user accounts. For a detailed discussion about the different MaSTR user accounts and managing these accounts, see [Appendix A, “MaSTR Administration,”](#) on page 97.

1. Open Oracle VM VirtualBox.
2. In the left panel of the VirtualBox Manager main window, make sure that the MaSTR server image is selected, and then on the main menu, click Start.

After the MaSTR server VM is successfully turned on, “Powered On” is displayed on the server image and a login window opens.

Figure 1-7: MaSTR API Server login window

```
Arch Linux 4.12.6-1-ARCH (tty1)
MaSTR_Server login:
```

3. Open a browser window. (Google Chrome is the preferred browser.)



The following step assumes that the exposed port is the default port of 8000. If your MaSTR administrator has exposed a different port to the MaSTR server, for example, 8080, then enter the value for this port instead.

4. In the Address field, enter the correct URL:
 - For local access to the MaSTR server, enter `https://localhost:8000/`.
 - For remote access to the MaSTR server, enter `https://<server address>:8000/`, where the server address is either the IP address or the fully qualified domain name (FQDN) for the MaSTR server.

The MaSTR Login dialog box opens. See [Figure 1-6 on page 21](#).

5. On the Login page, select the correct login Mode, for example, Research/Validation.
6. Do one of the following:
 - For Demo mode, click Login.
 - For any Mode other than Demo, enter your username and password, and then click Login.

For any mode other than Admin, the MaSTR software application opens in the Analysis view, which is the default view. The two other available views are Models and System. For the Admin mode, the Admin view opens.



After you log in to MaSTR, your login is active for eight hours. After your login expires, you are redirected to the MaSTR login page; however, none of your queued jobs or jobs that are already running on the server are affected.

7. Do one of the following:
 - If you logged in under Admin mode, then continue to [Appendix A, “MaSTR Administration,” on page 97](#).
 - If you logged in under any other mode other than Admin, then open the appropriate view and continue with your work in MaSTR.

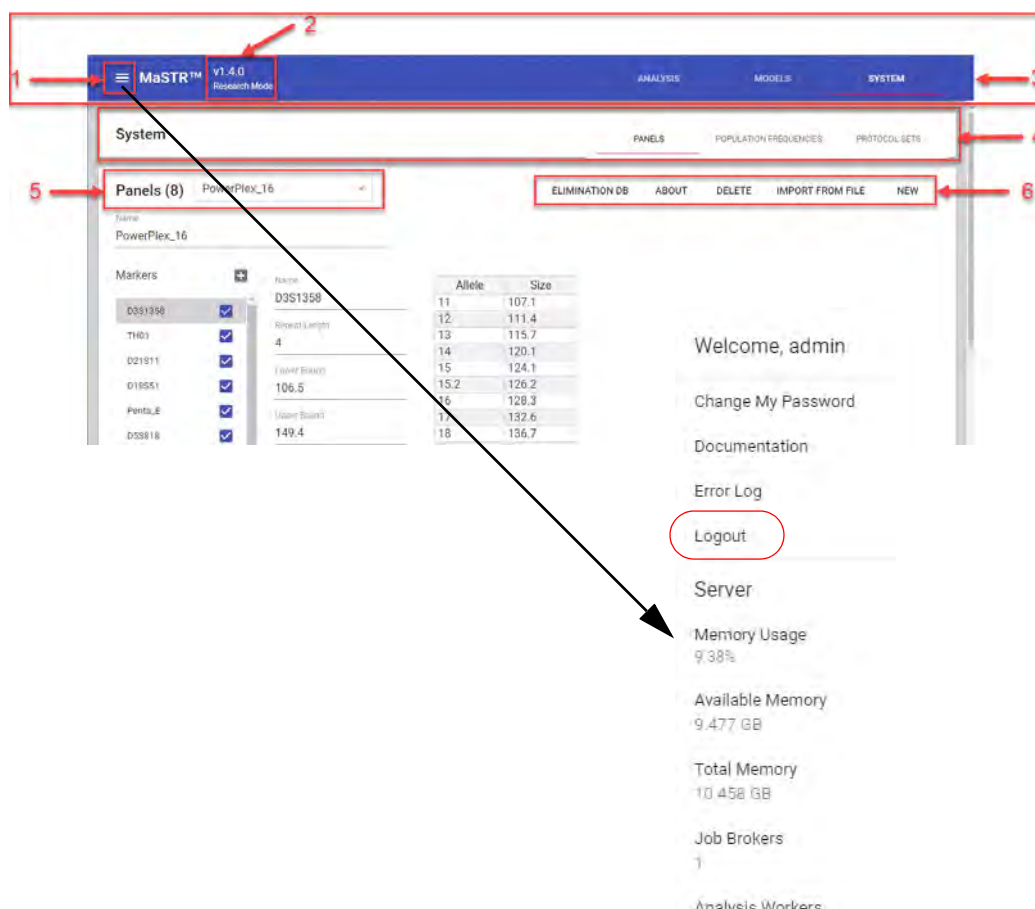


For a detailed discussion of the different primary views that are available in MaSTR, see [Chapter 2, “MaSTR Primary Views,” on page 25](#).

Logging out of MaSTR

After you log in to MaSTR, a main menu icon (the Hamburger icon, indicated as Item #1 in [Figure 1-8](#) below) is displayed in the upper left corner of the MaSTR title bar. You can click this open to open the MaSTR main menu, which contains a Logout option. Click Logout to immediately close and log out of MaSTR. When you log out of MaSTR, any unsaved work is lost; however, none of your queued jobs or jobs that are already running on the server are affected.

Figure 1-8: MaSTR main menu and Logout option



For detailed information about the items identified in [Figure 1-8](#) (the MaSTR common navigation tools), see [“MaSTR View Common Navigation Tools”](#) on page 30.

Chapter 1

Getting Started with MaSTR

Chapter 2

MaSTR Primary Views

If you log in to MaSTR under any mode other than Admin, then the application opens in the Analysis view, which is the default view. The other two primary views that are available in MaSTR are Models and System. This chapter details the three primary views that are available in MaSTR, including their purposes and associated functions. This chapter also details the two types of files—a Profile file and a Signal file—that are required in various functional areas (views) of MaSTR for setting up an analysis job and carrying out the analysis, and the navigation tools that are common for all three views.

This chapter covers the following topics:

- “MaSTR Profile Files and Signal Files” on page 27.
- “MaSTR View Common Navigation Tools” on page 30.
- “MaSTR Analysis View” on page 36.
- “MaSTR Models View” on page 38.
- “MaSTR System View” on page 46.



For detailed information about the Admin view, see [Chapter A, “MaSTR Administration,”](#) on page 97.

Chapter 2

MaSTR Primary Views

MaSTR Profile Files and Signal Files

Two types of files—a *Profile* file and a *Signal* file—are required in various functional areas (views) of MaSTR for setting up an analysis job and carrying out the job. Both file types have the following features in common:

- Both file types are tab-delimited text files that have a single header line and one row per marker.
- Both file types can include a sample name in each row of data with the following caveats:
 - If the file is for a single individual, then the Sample Name field is optional, and if you do not specify a name, then MaSTR automatically generates the name for the individual based on the file name. See [Figure 2-1](#) and [Figure 2-2](#) below.
 - If the file stores the information for multiple individuals, then you must give each individual a unique name. See [Figure 2-3 on page 28](#).

Figure 2-1: Sample file with a single sample name

Sample Name	Marker	Allele#1	Allele#2	Height#1	Height#2
Profile_GGC	AMEL	X	X	677	677
Profile_GGC	D3S1358	16	16	778	778
Profile_GGC	D1S1656	12	15	277	296
Profile_GGC	D2S441	12	14	213	264

Figure 2-2: Sample file with an automatically generated sample name

Elimination DB (PowerPlex_Fusion_6C_v1_2)

Profiles:

Elim_Profile_1

Name:

Elim_Profile_1

Marker	Allele 1	Allele 2	Height 1	Height 2
AMEL	X	X	0	0
D3S1358	14	17	0	0
D1S1656	15	15	0	0
D2S441	12	14	0	0
D10S1248	13	15	0	0

Figure 2-3: Sample file with multiple sample names

Sample Name	Marker	Allele#1	Allele#2
PersonA	D8S1179	13	12
PersonA	D21S11	28	31
PersonA	D7S820	11	11
PersonA	CSF1PO	10	10
PersonA	D3S1358	15	18
PersonA	TH01	9	11
PersonB	D8S1179	12	12
PersonB	D21S11	30	31
PersonB	D7S820	9	11
PersonB	CSF1PO	10	12
PersonB	D3S1358	15	18
PersonB	TH01	7	8
PersonC	D8S1179	13	16
PersonC	D21S11	28	32.2
PersonC	D7S820	7	11
PersonC	CSF1PO	7	11
PersonC	D3S1358	16	16
PersonC	TH01	7	7

Profile files

A *profile* file, also referred to as a *genotype* file, lists the alleles for specific individuals at the loci that are being tested. Optionally, a profile file can also include the signal (peak) height for each allele.

Figure 2-4: Example Profile file

Marker	Allele#1	Allele#2
AMEL	X	X
D3S1358	16	17
D1S1656	13	16
D2S441	11	11

In MaSTR, there are five profile types to consider:

- Reference - The profile that the job is analyzing. A Reference Profile file can contain only a single profile.
- Known - A profile that is assumed to be in the mixture that is being analyzed. A Known Profile file can contain only a single profile.
- Alternate - An alternate profile is calculated as if it were the reference profile. An Alternate Profile file is useful when there is a chance that a different individual might have contributed to the mixture that is being analyzed. An alternate profile is also useful when determining the LR's (Likelihood Ratios) for known mixtures. An Alternate Profile file can contain only a single profile.



Collectively, Reference, Known, and Alternate Profile types are referred to as Analysis Profile types. If you load an Analysis Profile file that contains more than one sample name for a job, then MaSTR loads only the data from the first sample.

- **Elimination** - The profile for a laboratory individual or other individual whose DNA could potentially contaminate a sample. Elimination profiles are stored in the MaSTR Elimination database. MaSTR uses elimination profiles to calculate Likelihood Ratios against the mixture that is being analyzed and reports them if their results exceed set thresholds. An Elimination Profile file can contain a single profile, or it can contain multiple profiles.
- **Protocol Set** - Also referred to as the System profile. The profile that is required for establishing the parameters for a lab-specific analysis protocol, or standard operating procedure (SOP). A System profile requires multiple profiles in a single file.

Signal file

A *signal file* lists the alleles for specific individuals at the loci that are being tested *and* the signal (peak) height for each allele. A signal file must contain at least one non-OB allele for each marker where OB is a flag that indicates “out of bounds.”

Figure 2-5: Example Signal file

Marker	Allele#1		Allele#2		Allele#3		Allele#4		Height#1
	Height#2		Height#3		Height#4				
AMEL	X	Y			2544	510			
D3S1358	15	16	17	18	189	881	665	356	
D1S1656	11	14	16		945	218	320		
D2S441	10	11	13	14	237	531	300	282	

MaSTR View Common Navigation Tools

If you log in to MaSTR under any mode other than Admin, then the application opens in the Analysis view, which is the default view, and is referred to as a *primary* view. The two other primary views that are available are Models and System. The Analysis View has a single tab - the Analysis Jobs tab. The Models view and the System view have multiple tabs.

If you log in to MaSTR under the Admin mode, then the application opens in the Audit view, which is the default view, and is referred to as a *secondary* view. The other secondary views that are always available are Sessions, User Management, Settings. If you log in as root under the Admin mode, then another secondary view, the Database view, is also available.

Regardless of the mode that you are logged in under, and the view and tab that are open, the following navigation tools remain the same.

Figure 2-6: MaSTR application views, common navigation tools

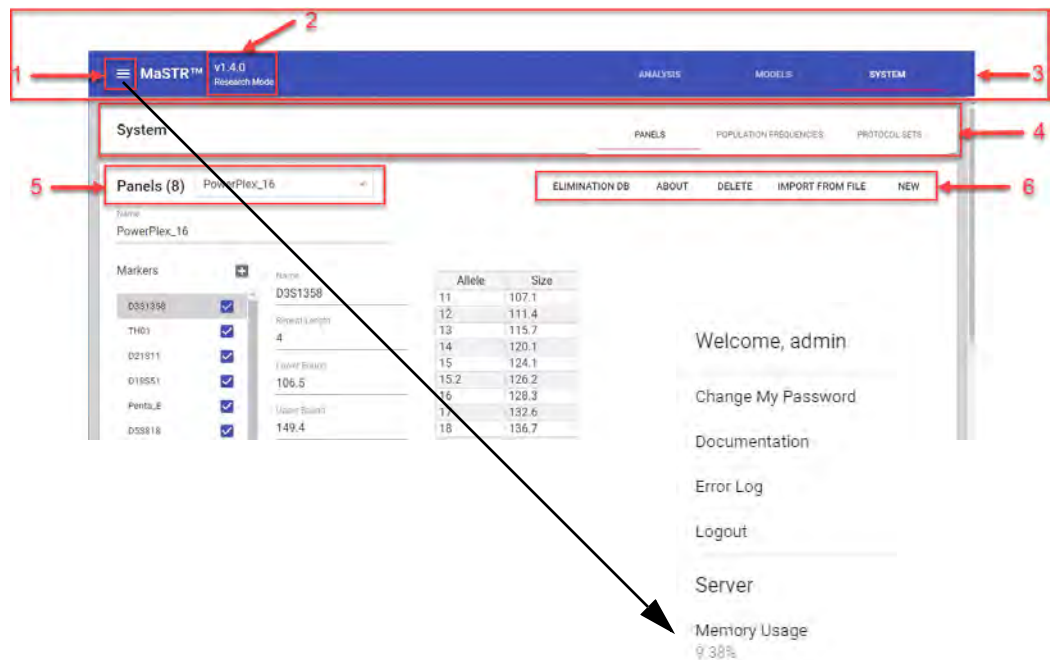


Table 2-1: MaSTR View common navigation tools

Item	Description
Note: Collectively, Items 1, 2, and 3 make up the MaSTR title bar.	
1	Main menu icon. Click this icon to open the MaSTR main menu. Regardless of the view, the main menu options are the same. See "Main menu" on page 31 . Note: All MaSTR main menu options are always displayed to you; however, the availability (functioning) of some options depends on the permissions that your MaSTR administrator has assigned to your user account.
2	Version/Mode information. The version of MaSTR that you are currently logged in to and the mode (Admin, Casework, Demo, or Research/Validation) that you used to log in.

Table 2-1: MaSTR View common navigation tools (Continued)

Item	Description
3	View options. The view options that are available in MaSTR, which are determined by the mode that you are currently logged in to. A horizontal line (View Indicator line) is displayed underneath the name of the currently opened view. To open a specific view, click the appropriate view option.
4	View Tab bar. The name of the view that is currently opened is displayed on the left side of the View Tab bar. The different tabs that are available for the currently opened view are displayed on the right side of the bar. A horizontal line is displayed underneath the name of the currently opened tab (the Tab Indicator line). To open a different tab for a view, click the appropriate tab.
5	Tab name. The name of the tab that is currently opened for a view is displayed at the top of the tab on the left side of the tab.
6	Tab menu. The menu options for a tab are displayed at the top of the tab on the right side of the tab. Note: All tab menu options are always displayed to you; however, the availability (functioning) of some options depends on the permissions that your MaSTR administrator has assigned to your user account.



To change the default color of the MaSTR title bar, and/or the different indicator lines, see [“Customizing the Default Report and View Displays” on page 118](#).

In addition to the common navigation tools found for every view, some tabs also have the following features in common:

- A number of rows displayed per page feature. See [“Number of rows displayed per page feature” on page 32](#).
- A filter feature. See [“Filter feature” on page 33](#).
- A sort feature. See [“Sort feature” on page 33](#).
- A dropdown list filter feature. See [“Dropdown list filter feature and total number of items in database” on page 34](#).
- A non-modal popup feature. See [“Non-modal popup feature” on page 35](#).

Main menu

You click the main menu icon (the Hamburger icon) in MaSTR to open the MaSTR main menu. At the top of the main menu, a Welcome message is displayed, along with the username (the name that you used to log in to MaSTR). After the Welcome message, regardless of the view, the same menu options are displayed from top to bottom. See [Table 2-2 on page 32](#).

Table 2-2: MaSTR view main menu options

Option	Description
Change My Password	Click this option to open the Change Password popup, and then change the password that you use to log in to MaSTR. Note: Your MaSTR password cannot contain any spaces and it must be a minimum of 12 characters. You should also ensure that your new password adheres to your organization's standards.
Documentation	Click this option to open the full MaSTR documentation set. The documentation opens in a non-modal dialog box, which you can close by clicking anywhere else in the application.
Error Log	If an error occurs while carrying out an action on a tab (for example, importing a panel file in to the MaSTR database on the Panels tab), then an Error pane that details the error opens automatically at the bottom of the tab. If you close the Error pane, then at any time during the same session of MaSTR, you can click Error Log to open the pane and view the details again. Note: If multiple errors occur during a MaSTR session, then when you click Error Log, the Error pane shows the most recent error.
Logout	Click this option to immediately close and log out of MaSTR. Note: Any unsaved work is lost; however, none of your queued jobs or jobs that are already running on the server are affected.
Server	Read-only information about the MaSTR server that you are currently logged in to, including the total memory for the server, the amount of memory that is currently available, the current memory usage (shown as a percentage), and the number and types of users who are currently logged in to the MaSTR server.

Number of rows displayed per page feature

When some view tabs first open, by default, they display 10 rows per page. For example, when the Analysis Jobs tab opens, it displays the ten most recent analysis jobs that are queued on the server, currently running on the server, or have finished running on the server in reverse chronological order. You can use the Previous and Next features at the bottom of each page to scroll through all the analysis jobs that have ever been carried out on the server. To change the number of rows that are displayed per page, select a different value on the Rows per page dropdown list.

Figure 2-7: Viewing and scrolling page rows features



Filter feature

On some view tabs, a Filter feature is displayed. You can use the Filter feature to reduce the number of items that are displayed on a tab and exclude those items that do not match specific filtering criteria.

Figure 2-8: Filter feature



The screenshot shows the MaSTR v1.4.0 Demo Mode interface. The top navigation bar is purple with a hamburger menu icon, the text 'MaSTR™ v1.4.0 Demo Mode', and three tabs: 'ANALYSIS' (selected), 'MODELS', and 'SYSTEM'. Below the navigation bar, the 'Analysis Jobs' tab is active. A red rectangle highlights the 'Filter' input field next to the 'Analysis Jobs' label. To the right of the filter field are 'RELOAD' and 'NEW' buttons. Below the filter field is a table with the following columns: 'User', 'Queue Time ↓', 'Name', 'Reference', 'Contributors', and 'Stage'. The table contains two rows of data.

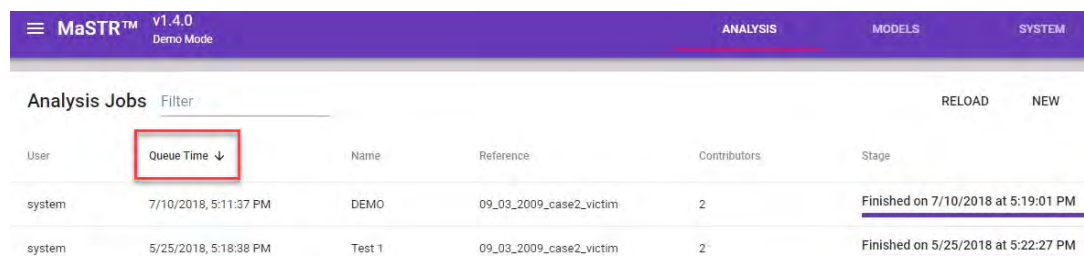
User	Queue Time ↓	Name	Reference	Contributors	Stage
system	7/10/2018, 5:11:37 PM	DEMO	09_03_2009_case2_victim	2	Finished on 7/10/2018 at 5:19:01 PM
system	5/25/2018, 5:18:38 PM	Test 1	09_03_2009_case2_victim	2	Finished on 5/25/2018 at 5:22:27 PM

For example, when the Analysis Jobs tab opens, it displays the ten most recent analysis jobs that are queued on the server, currently running on the server, or have finished running on the server in reverse chronological order. To filter the display on a tab, click in the Filter field, enter the filter criteria, and then press Enter. Any items (in this example, any analysis jobs) that do not match the filter criteria are excluded from the display and the tab display is updated accordingly. The filtering is based on the exact order of the characters in the filter string, but the string is not case-sensitive and it can appear anywhere in the search results. The filtering might or might not be carried out against all columns on the page. For example, if you enter **Test** as the filter criteria on the Analysis Jobs tab, then the filtering is carried out against the User column, the Name column and the Reference column. Any jobs that do *not* have this string in at least one of these columns are excluded from the display. (Only those jobs that have this string in at least one of these columns (in this example, the Name column), such as **test1** or **BlankTest**, are displayed on the tab. To have all items displayed again on a tab, you can click the Clear Field icon (the “x”) in the Filter field, or you can clear the Filter field (make sure that the field is blank), and then press Enter.

Sort feature

On some view tabs, you can sort the default display on the tab by any characteristic or property (column) of the items on the tab. If sorting is possible on the tab, then when the tab opens, a Sort Order icon (an up or down arrow), is displayed next to the default sorting property.

Figure 2-9: Sort feature



The screenshot shows the MaSTR v1.4.0 Demo Mode interface, similar to Figure 2-8. The 'Analysis Jobs' tab is active. A red rectangle highlights the 'Queue Time ↓' sort icon in the 'Queue Time' column header. The table structure and data are identical to the one in Figure 2-8.

User	Queue Time ↓	Name	Reference	Contributors	Stage
system	7/10/2018, 5:11:37 PM	DEMO	09_03_2009_case2_victim	2	Finished on 7/10/2018 at 5:19:01 PM
system	5/25/2018, 5:18:38 PM	Test 1	09_03_2009_case2_victim	2	Finished on 5/25/2018 at 5:22:27 PM

For example, when the Analysis Jobs tab opens, it displays the ten most recent analysis jobs that are queued on the server, currently running on the server, or have finished running on the server in reverse chronological order and a reverse Order Icon is displayed next to the Queue time column. To sort the job display on the tab by User, click the User column header. An ascending Sort Order icon is displayed next to the User column header. To reset the sort order, click the User column header again. A descending Sort Order icon is now displayed next to the User column header.

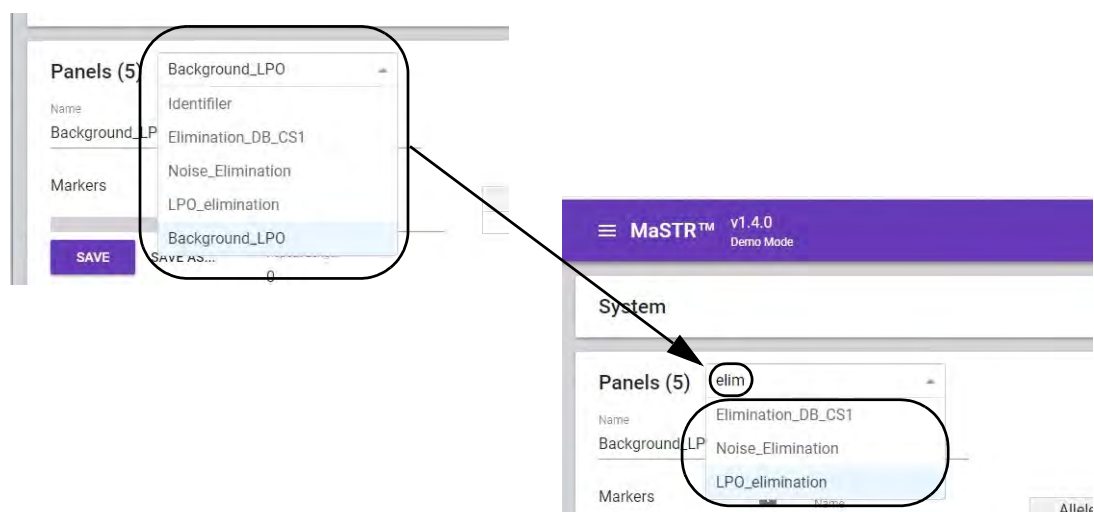


The Sort feature is not persistent. If you change the sort order of the display on a tab, exit the tab, and then return to the tab, the sort order is returned to its default order.

Dropdown list filter feature and total number of items in database

Some view tabs display the items that are available in the MaSTR database in a dropdown list. The total number of these items that are available in the MaSTR database is displayed in parentheses after the tab name. For example, on the Panels tab, all the panels that are available in the database for your MaSTR installation are displayed on the Panels dropdown list and the total number of panels that are available in the MaSTR database is displayed in parenthesis after the tab name. When a tab that has a dropdown list opens, the name of the item (for example, a panel or a model) that is first in the list is displayed. You can open the dropdown list and scroll to another item to select it, or you can enter a filter string. As you enter the filter string, the list of items that meet the criteria is dynamically updated. The string is limited to the exact order of the characters in the string, but the filter is not case-sensitive and the filter string can be found anywhere in the results. For example, as shown in [Figure 2-10](#) below, entering the string of **elim** in the Panels field limits the display of the Panels dropdown list to only those panels with the **elim** string found somewhere in their names - **E**limination_DB_CS1, Noise_**E**limination, and IPO_**e**limination. To have all items displayed again on a dropdown list, you can click the Clear Field icon (the “x”) in the Filter field, or you can clear the Filter field (make sure that the field is blank), and then press Enter.

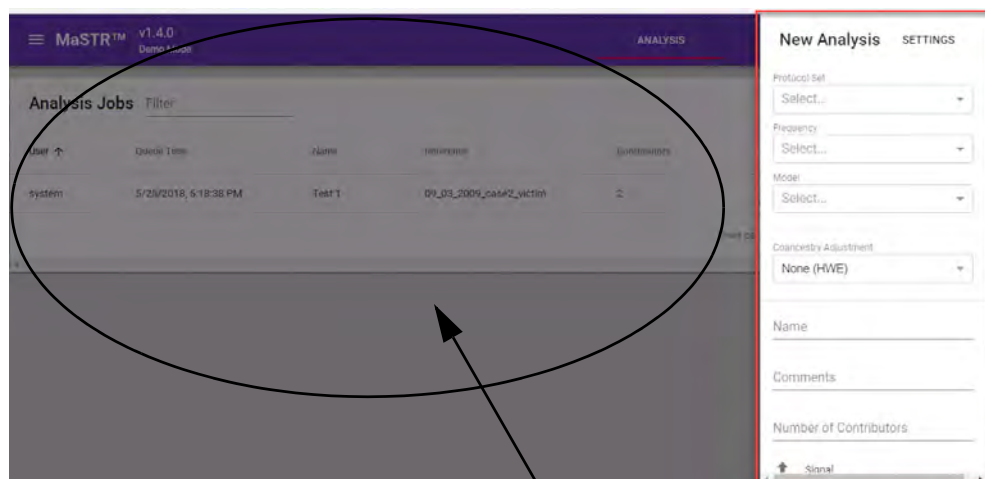
Figure 2-10: Dropdown dynamic list feature



Non-modal popup feature

On some view tabs, after you click a button to initiate an action, a non-modal popup opens. A non-modal popup is an active popup that has focus; however, although the application behind the popup is dimmed, the application remains active.

Figure 2-11: Non-modal popup



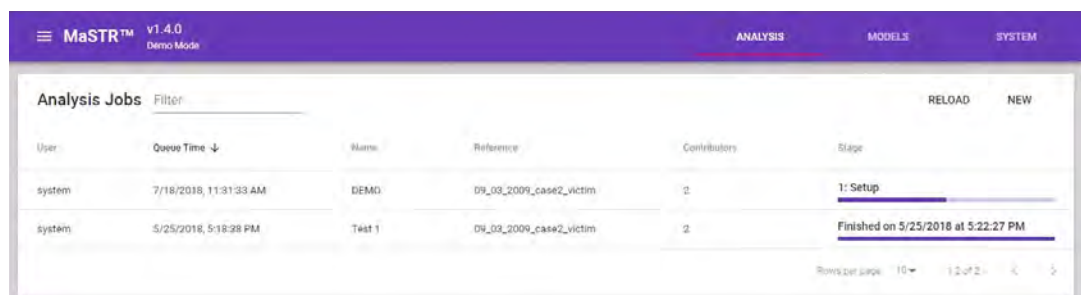
Click anywhere other than the non-modal New Analysis Settings popup to close the popup and return to the MaSTR application.

You do not have to interact with a non-modal popup to close it. Instead, you can click anywhere in MaSTR other than on the non-modal popup itself to close the popup and return to MaSTR. For example, in [Figure 2-11](#) above, the New Analysis Settings popup is open and the Analysis Jobs tab is dimmed. You can click anywhere on the Analysis Jobs tab to close the New Analysis Settings popup and return to the Analysis Jobs tab and access the full functionality of MaSTR.

MaSTR Analysis View

If you log in to MaSTR under any mode other than Admin, then the application opens in the Analysis view, which is the default view. The view has a single tab, the Analysis Jobs tab. The tab lists all the jobs that are queued or currently running on the server, or that have finished running on the server, in reverse chronological order, with a default value of 10 jobs per tab page.

Figure 2-12: MaSTR Analysis view, Analysis Jobs tab



User	Queue Time ↓	Name	Reference	Contributors	Stage
system	7/18/2018, 11:31:33 AM	DEMO	09_03_2009_case2_victim	1	1: Setup
system	5/25/2018, 5:18:28 PM	Test 1	09_03_2009_case2_victim	2	Finished on 5/25/2018 at 5:22:27 PM

- If an analysis job is queued on the server when the Analysis Jobs tab opens, then Queued is displayed in the Stage column for the job.
- If an analysis job is currently running when the Analysis Jobs tab opens, then one of four stages is in the Stage column for the job:
 - Setup - Initiating the job.
 - MCMC - Carrying out the MCMC calculations. During this stage, the step number and the estimated remaining time is displayed.

Figure 2-13: MCMC stage information displayed for a running job on the Analysis Jobs tab

2: MCMC - Step 100 of 5000 (Estimating time remaining...)

- Calculate LR - Calculating the likelihood ratio. See [“Likelihood Ratios report” on page 89](#).
- Collect and Save Results - See [Chapter 4, “Reporting and Graphing DNA Analysis Results in MaSTR,” on page 81](#).
- If analysis job has finished, then **Finished** is displayed in the Stage column for the job, along with the date and time that the job was finished.

Figure 2-14: Finished job on the Analysis Jobs tab

Finished on 5/25/2018 at 5:22:27 PM



To refresh the display on the Analysis Jobs tab at any time, click Reload.

You can do the following on the Analysis Jobs tab:

- Click anywhere for a finished job to open a table that summarizes the job results. Likelihood Ratio (LR) values greater than one are highlighted in green while LR values less than one highlighted are in red. The darker the green shading, the greater the LR value while the darker the red shading, the lesser the LR value.

Two options are also displayed: Download Summary Report and Open. You can click Download Summary report to view the Summary tables for the populations in the mixture and you can click Open to open and view the Likelihood Ratios report. See [“Likelihood Ratios report” on page 89](#).

Figure 2-15: Job results summary



	Known (ContribC.txt)	Known (ContribD.txt)	Known (ContribE.txt)	Unknown Contributor 1	Unknown Contributor 2
ContribH.txt	n/a	n/a	n/a	0.17e+15	6.27e+15
ContribA.txt	n/a	n/a	n/a	2.10e+15	6.20e+17
Median	0.13	0.25	0.05	0.48	0.10

- Double-click a finished job to open the corresponding Results page and view the results of the job. See [“Generating and Downloading an Analysis Report” on page 83](#).
- Right-click on any job in the list and on the context menu that opens, select the appropriate option:

Option	Description
Open	If the job is finished, then this option opens the Results view for the job, which details the results for the job on six different tabs. When the Results view opens, the Likelihood Ratio tab is the open tab. See “Generating and Downloading an Analysis Report” on page 83 .
Delete	If your user account has the correct permissions, then click this option to delete the analysis job from the MaSTR database.
Copy to New Analysis	Opens a New Analysis popup, with the settings pre-populated from the selected job. Tip: This option is useful for setting up new analyses when you have multiple similar jobs to run. Rather than setting up multiple new analysis jobs, you can simply edit the existing settings as needed, and then save these modified settings as a new analysis job.
Download Report	Create and download the job results in report that is saved as a PDF. See “Generating and Downloading an Analysis Report” on page 83 .
Cancel	Cancel a job that is queued or currently running. You can cancel only those jobs that you created. You cannot cancel another user's jobs.

MaSTR Models View

The underlying concept behind MaSTR probabilistic genotyping software is that of a *model*. A model is a set of parameters that govern the Markov Chain Monte Carlo (MCMC) process and specify how the software calculates the expected signal (peak) heights in a sample. You manage models for your MaSTR installation in the MaSTR Models view.



This section discusses models only from an application perspective. For an annotated list of reference documentation about Markov Chain Monte Carlo (MCMC) and the theory behind continuous probabilistic mixture analysis, see [Bibliography on page 145](#).

To open the MaSTR Models view, on the MaSTR title bar, click Models. The view has a single tab, the Models tab. The tab displays the information for the model that is first on the Models dropdown list.

Figure 2-16: MaSTR Models View, Models tab

MaSTR comes populated with eight standard models that have been optimized for probabilistic mixture analysis. You can use any of these standard models as-is, or you can edit a model as needed. You can also import external models. See [“To edit a model” on page 44](#) and [“To import a model” on page 42](#).

Table 2-3: Pre-defined models in MaSTR

Model	Description
Noise	A model that is used for illustrating the addition of variables to account for additional parameters in the mixture analysis.
Demo	A basic model with a small number of iterations. Provides rapid analysis of the demo data when operating MaSTR software in Demo mode.
Standard	A basic model for use in training with simple mixture signal data.
1-Person	A basic model with a small number of iterations for use with suspected single source signal data.

Table 2-3: Pre-defined models in MaSTR

Model	Description
2-Person	A basic model with more iterations typically used to start evaluating a model for use with 2-person mixtures.
3-Person	A basic model with additional iterations, burn in, and number of chains that is typically used to start evaluating a model for use with 3-person mixtures.
4-Person	A basic model with additional iterations, burn in, and number of chains that is typically used to start evaluating a model for use with 4-person mixtures.
5-Person	A basic model with additional iterations, burn in, and number of chains that is typically used to start evaluating a model for use with 5-person mixtures.

You can do the following on the Models tab:

- View the information for a model. See [“To view the information for a model”](#) below.
- Import a model. See [“To import a model”](#) on page 42.
- Edit a model. See [“To edit a model”](#) on page 44.
- Delete a model. See [“To delete a model”](#) on page 45.

To view the information for a model

1. If you have not done so already, open the Models tab for the System view. (System > Models).
2. On the Models dropdown list, select the name of the model for which to view the information.

The Models tab displays the properties for the selected model.

Table 2-4: Models tab, Model properties

Option	Description
Name	The model name.
MCMC Settings	
• Iterations	The number of iterations that MaSTR runs and records during the MCMC process. As you increase the number of iterations, more of the parameter is explored; however, processing time is increased as a result. Enter an even whole number (integer) for the number of iterations in the thousands, for example, 10 for 10,000 iterations.
• Burn In	The number of <i>non-recorded</i> iterations that MaSTR runs before the recorded iterations. You typically set the Burn In value to 20% of the recorded Iterations value. Enter an even whole number (integer) for the number of iterations in the thousands, for example, 2 for 2000 if the number of iterations is set to 10 for 10,000.

Table 2-4: *Models tab, Model properties (Continued)*

Option	Description
<ul style="list-style-type: none"> Thin N 	<p>Specifies the in-series step that is to be ignored, for example, every 3rd or 4th step for the defined iteration period. Specifying a thinning value reduces the total amount of data that is analyzed and potentially improves results by reducing autocorrelation. Thinning also improves the “mixing” of the Markov chain (how much movement occurs).</p> <p>Caution: If you specify a thinning value, then you must also increase the total number of iterations.</p>
<ul style="list-style-type: none"> Chains 	<p>The number of starting points (the initial values for the iterated parameters) for the in-series steps in the MCMC phase of job analysis. For example, if Iterations = 50,000 and Chains = 8, then 400,000 iterations of the in-series steps are being carried out during the MCMC process to calculate the probability values. If the server has the capacity, then you can increase the number of chains, which essentially repeats the iteration process in parallel.</p> <p>Note: If Preprocessing Steps is enabled and multiple chains are running in the main MCMC process, then each chain uses the same starting values for allele selections.</p>
Analysis Settings	
<ul style="list-style-type: none"> Drop-in Coefficient (N) 	<p>Models the probability of drop-in. Increasing this value means decreasing the probability of genotype sets with drop-in alleles and conversely, decreasing this value means increasing the probability of genotype sets with drop-in alleles. “1” is typically a good starting value and changes typically are not observed if the value is < 0.1 or > 5.</p>
<ul style="list-style-type: none"> Preprocessing Steps 	<p>Works in conjunction with Sampler Warmup. A quick, simplified MCM process is run before the full process to find reasonable starting points. Enter an even whole number (integer) for the number of iterations in the thousands, for example, 10 for 10,000 iterations.</p> <p>As the number is increased, so is the processing time, but this potentially produces more accurate results. (Without the warmup process, the primary MCMC process might not finish because of poor values for some parameters.) To disable this function, clear Sampler Warmup.</p> <p>Note: The only unknown variable in this simplified process is the contributor allele section. The ratio is divided evenly among the contributors and the other variables are held at their default starting values.</p>
<ul style="list-style-type: none"> Apply Stutter 	<p>Selected by default. The model applies stutter during calculations of the probability values. Unless your data does not have good stutter estimates, SoftGenetics recommends that you leave Apply Stutter selected.</p> <p>Caution: If Apply Stutter is selected, then Stutter must be calculated for the protocol set that the model uses. See “Protocol Sets tab” on page 59.</p>

Table 2-4: *Models tab, Model properties (Continued)*

Option	Description
<ul style="list-style-type: none"> • Sampler Warmup 	Works in conjunction with Preprocessing Steps . Must be selected for Preprocessing Steps to be enabled. If cleared, then Preprocessing Steps is disabled.
<ul style="list-style-type: none"> • Save All Iteration Data 	Not selected by default. Save all the data for each iteration. Leave this option cleared to decrease processing time.
Advanced Settings - Specifies the sampling methods, which determine how MaSTR chooses the alleles or variable values that are to be tested.	
<ul style="list-style-type: none"> • Allele Step Method 	<p>Sampling alleles during the MCMC process. Two options are available:</p> <ul style="list-style-type: none"> • Subset Merge - An MCMC method that attempts to move to a new genotype list each iteration by editing the current genotype list. This method is almost as fast as the Continuous Step method, and therefore, is recommended for most use cases. • Uniform - An MCM method that attempts to move to a new genotype list each iteration by assigning each allele in every contributor. Although this method can be relatively fast, if there are many contributors, then it has the potential of being slowed significantly as most of the genotype lists will have a very low probability. Ideally used for a maximum of three contributors.
<ul style="list-style-type: none"> • Continuous Step 	<p>Sampling variables during the MCMC process. Two options are available:</p> <ul style="list-style-type: none"> • Metropolis - A simple, fast MCMC method for the continuous variables. Recommended for most use cases. • Slice - A more advanced MCMC method for the continuous variables that allows for more movement during each iteration. This increased movement, however, is at the expense of more computation time, which makes this method slower than Metropolis.
<ul style="list-style-type: none"> • Degradation 	<p>If a sample is degraded, then the template DNA is fragmented, which has a deleterious effect on the amplification of larger fragments. You can specify a Degradation setting to account for degradation. Three options are available:</p> <ul style="list-style-type: none"> • No Adjustment - Degradation is not considered during the MCMC process when calculating probability values. • Linear - Degradation is assumed to occur with a linear relationship to allele size. • Exponential - Degradation is assumed to occur with an exponential relationship to allele size. <p>Caution: If Linear or Exponential is selected, then the Variance Factor must be calculated for the protocol set that the model uses. See "Protocol Sets tab" on page 59.</p>
Variables	
Advanced settings for building a model. See Appendix B, "Building a Custom MCMC Model for Probabilistic Mixture Analysis," on page 121 .	

Table 2-4: Models tab, Model properties (Continued)

Option	Description
Expected Height Calculations	
Advanced settings for building a model. See Appendix B, “Building a Custom MCMC Model for Probabilistic Mixture Analysis,” on page 121.	

3. You can also do the following:

- To view information about another model, select it on the Models dropdown list.
- To view additional information about a selected model, on the Models tab menu, click About.

The About popup opens. The popup displays the date and time that the model was uploaded to the MaSTR database, the date and time that the model was last modified, and if applicable, any notes that were added for the model.

Figure 2-17: About Model popup



To import a model

Although you typically add a model directly to the MaSTR database, you also have the option of importing a model. If you import a model, *the file must be in .json format*. After you import a model, it is available immediately for use in MaSTR. [Figure 2-18 on page 43](#) shows an example of a JSON-formatted model file.



The model name must be unique in the MaSTR database.

Figure 2-18: Example model file, .json format

```
{
  "name": "Marker-Contrib Adjustment",
  "mcmc_options": {
    "burn_in": 2000,
    "iterations": 10000,
    "thin_n": 2,
    "chains": 10
  },
  "analysis_options": {
    "drop_in_coefficient": 1.0,
    "stutter": true,
    "sampler_warmup": true,
    "preprocessing_steps": 10000
  },
  "allele_step_method": "Subset Merge",
  "continuous_step": "Metropolis",
  "degradation": "Linear",
  "variables": [
    {
      "params": {
        "value": 1
      },
      "description": "Set a value of 1 for each marker to shape a child variable as marker-contributor",
      "shape": "marker",
      "dist": "Fixed",
      "name": "Marker_Fixed_1"
    },
    {
      "params": {
        "mean": "Marker_Fixed_1",
        "sd": 0.2,
        "lower": 0.5,
        "upper": 1.5
      },
      "description": "",
      "shape": "contributor",
      "dist": "Bounded_Normal",
      "name": "Contrib-Marker"
    }
  ],
  "expected_height_calc": [
    [
      "Multiply",
      "Contrib-Marker"
    ]
  ]
}
```



For assistance with formatting a model that is to be imported, contact tech_support@softgenetics.com.

1. If you have not done so already, open the Models view.
2. On the Models tab menu, click Import From File.
The Open dialog box opens.
3. In the Open dialog box, browse to and select the appropriate model file, and then click Open.

The Open dialog box closes and the Models tab remains open. The selected model is imported in to the MaSTR database.

To add a new model

Every model must have a unique name to be added to the MaSTR database.



The following procedure describes adding an entirely new model to the MaSTR database. To quickly add a model that is similar to an existing model, you can edit the existing model, and then use Save As to save the model with a unique name. See “To edit a model” on page 44.

1. If you have not done so already, open the Models view.
2. On the Models tab menu, click New.

The Models tab displays the options for adding a new model to the MaSTR database.

Figure 2-19: Blank Models tab

3. In the Name field, enter a name for the new model.



Every model must have a unique name to be added to the MaSTR database.

4. Define the model properties. See [Table 2-4 on page 39](#).
5. Click Save.

To edit a model

Because the information for a model that is used in an analysis job is saved with the job, you can edit a model without impacting any jobs that were run with a prior version of the model.

1. If you have not done so already, open the Models view.
2. On the Models dropdown list, select the model that you are editing.
3. Edit the model properties as appropriate. See [Table 2-4 on page 39](#).

4. Do one of the following:
 - To save the modified model as-is in the MaSTR database, click Save.
 - To save the modified model as a new model in the MaSTR database, click Save As, and then in the Save As dialog box that opens, enter a unique name for the model.

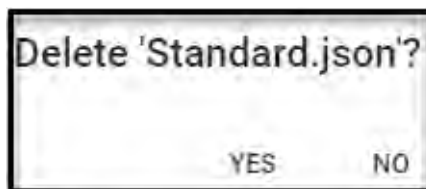
To delete a model

Because the information for a model that is used in an analysis job is saved with the job, you can delete a model without impacting any jobs that were previously run with the model.

1. If you have not done so already, open the Models view.
2. On the Models dropdown list, select the model that you are deleting.
3. On the Models tab menu, click Delete.

A Delete <Model Name> popup opens, prompting you to confirm the deletion.

Figure 2-20: Delete Model confirmation popup



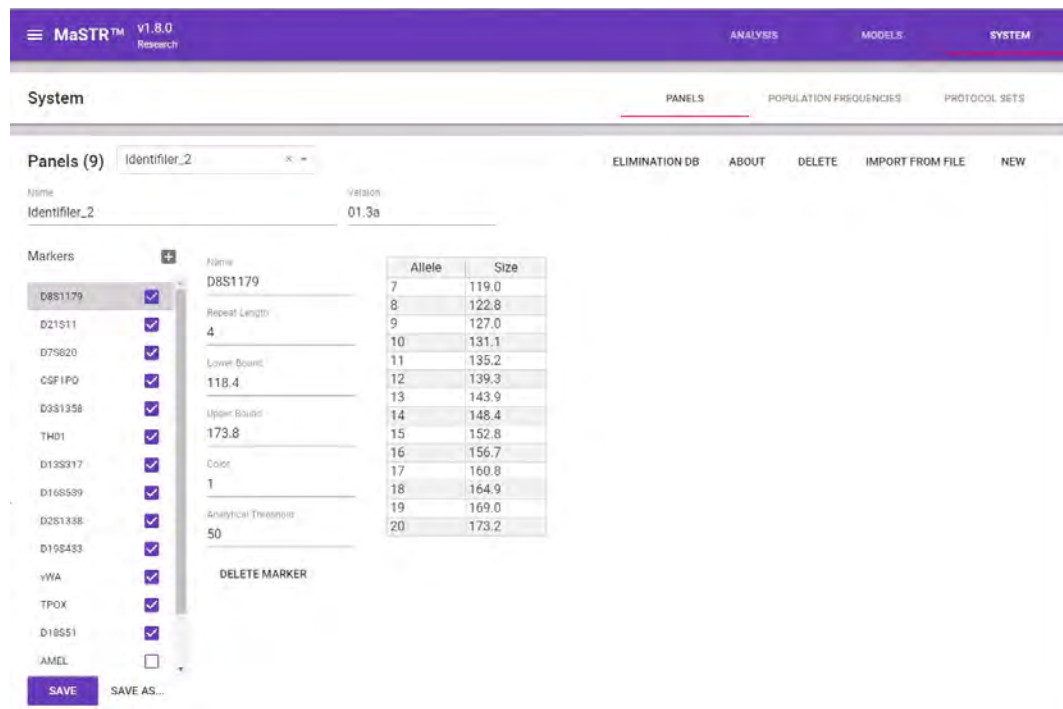
4. Click Yes.

The model is deleted from the MaSTR database, and the Delete Model confirmation popup closes after the model is deleted. The Models tab displays the information for the next model on the Models dropdown list.

MaSTR System View

To open the MaSTR System view, on the MaSTR title bar, click System. When the System view opens, the Panels tab is the open tab. The other tabs are the Population Frequencies tab and the Protocol Sets tab.

Figure 2-21: System View, Panels tab



In the System view, you work with panels, frequencies, and protocol sets. See:

- [“Panels tab”](#) below.
- [“Population Frequencies tab”](#) on page 55.
- [“Protocol Sets tab”](#) on page 59.

Panels tab

You work with panels on the Panels tab in the System view. In MaSTR, a *panel* contains information about the loci that were amplified for an STR analysis job, including the marker name, the markers that are present and enabled in the panel, the alleles that are possible in each marker, the marker repeat length, the lower and upper bounds for each marker, and the analytical threshold for each marker. Panels are listed in ascending order by name, and then within the name, by version number, on the Panels dropdown list on the tab. When the System view opens, the Panels tab is the open tab, and the tab displays the information for the panel that is first on the Panels dropdown list. See [Figure 2-21](#) above.

You can do the following on the Panels tab:

- View the information for a panel. See [“To view the information for a panel”](#) below.
- Import a panel file. See [“To import a panel file”](#) on page 48.
- Add a new panel. See [“To add a new panel”](#) on page 49.
- Edit a panel. See [“To edit a panel”](#) on page 51.
- Delete a panel. See [“To delete a panel”](#) on page 51.
- Import or delete elimination profiles. See [“To work with elimination profiles for a panel”](#) on page 52.

To view the information for a panel

1. If you have not done so already, open the Panels tab for the System view. (System > Panels).
2. On the Panels dropdown list, select the name of the panel for which to view the information.

The Panels tab displays the information for the selected panel. The Markers list on the left side of the tab displays all the markers for the selected panel. By default, the first marker in the panel is selected. To the right of the selected marker, the tab displays information about the marker. If a marker is enabled for a panel, then a checkmark is displayed next to the marker. To the right of the Markers list, the tab displays the possible alleles and their sizes for the currently selected marker.

3. You can also do the following:
 - To view information about another marker, select it on the Markers list.
 - To view information about another panel, select it on the Panels dropdown list.
 - To view additional information about a selected panel, on the Panels tab menu, click About.

The About popup opens. The popup displays the date and time that the panel was uploaded to the MaSTR database, the date and time that the panel was last modified, and if applicable, any notes that were added for the panel.

Figure 2-22: About Panel popup



To import a panel file

You can import standard xml-formatted panel files in to the MaSTR database, and these panel files will be available immediately for use. [Figure 2-23](#) below shows a partial example of an xml-formatted panel file.



You can import a panel file as-is from GeneMarker HID or from other software applications as long as the imported file has the correct format. Contact tech_support@softgenetics.com for assistance.



The panel name and version number must be unique in the MaSTR database.

Figure 2-23: Sample panel file

```
<?xml version="1.0"?>
<Panel>
  <Vendor>GeneMarker</Vendor>
  <FileVersion>01.3a</FileVersion>
  <PanelName>PowerPlex_16</PanelName>
  <n_Ploidy>2</n_Ploidy>
  <Chemistry></Chemistry>
  <Lot_Number></Lot_Number>
  <Fragment_Enabled>0</Fragment_Enabled>
  <Loci>
    <Locus>
      <MarkerTitle>D3S1358</MarkerTitle>
      <DyeIndex>1</DyeIndex>
      <n_NucleotideRepeats>4</n_NucleotideRepeats>
      <Enabled>1</Enabled>
      <LowerBoundary>106.5</LowerBoundary>
      <UpperBoundary>149.4</UpperBoundary>
      <MarkerComments></MarkerComments>
      <LocusFilter>
        MinIntensity=50 HomoInconclusive=200
        MinHeteroIntensity=50 HeteroInconclusive=100 LocalPercent=20
        DecimalLocalPercent=0 StutterPer_N_L4=13
        DecimalStutterPer_N_L4=0 StutterPer_N_L8=1
        DecimalStutterPer_N_L8=0 StutterPer_N_R4=1
        DecimalStutterPer_N_R4=0 HetroImbalancePer=60
        DecimalHetroImbalancePer=0
      </LocusFilter>
      <Allele>
        Label=11 DefSize=106.20 Size=107.08
        Left_Binning=0.50 Right_Binning=0.50 Control=0
        Distance=0.00 Chrom_Pos= Recom_Freq=-1.00
        Height=90 Comments=
      </Allele>
      <Allele>
        Label=12 DefSize=110.42 Size=111.40
        Left_Binning=0.50 Right_Binning=0.50 Control=1
        Distance=0.00 Chrom_Pos= Recom_Freq=-1.00
        Height=126 Comments=
      </Allele>
    </Locus>
  </Loci>
</Panel>
```

1. If you have not done so already, open the Panels tab for the System view. (System > Panels).

The Panels tab opens. The tab displays the information for the panel that is listed first on the Panels tab dropdown list.

2. On the Panels tab menu, click Import From File.

The Open dialog box opens.

3. In the Open dialog box, browse to and select the appropriate panel file, and then click Open.

The Open dialog box closes and the Panels tab remains open. The selected panel is imported in to the MaSTR database.

To add a new panel

Every panel must have a unique combination of name and version number to be added to the MaSTR database.



The following procedure describes adding an entirely new panel to the MaSTR database. To add multiple similar panels quickly, you can edit an existing panel, and then use Save As to save the panel with a unique combination of name and version number. See [“To edit a panel” on page 51](#).

1. If you have not done so already, open the Panels tab for the System view. (System > Panels).
2. On the Panels tab menu, click New.

The Panels tab displays the options for adding a new panel to the MaSTR database.

Figure 2-24: Blank Panel tab

3. In the Name field, enter a name for the new panel.
4. In the Version number field, enter a version number for the new panel.



The combination of name and panel number must be unique for each panel that you add to the MaSTR database. For example, you can name a panel identically to a panel that already exists in the MaSTR database as long the panel has a different version number.

5. For every marker that you are adding to the panel, do the following:

- a. Next to Markers, click the Add (+) icon.

A blank field opens as a placeholder for the new marker. By default, the marker is automatically enabled for the panel. (A checkmark is displayed in the blank field.)

- b. Leave the marker enabled, or clear it.



Even if you enable an amelogenin marker, MaSTR automatically disables this type of marker. MaSTR checks for the string “amel” in a marker name, and if it finds this string, then it automatically disables the marker.

- c. Enter the name for the marker.



MaSTR automatically replaces any space in a marker name with an underscore. For example, Penta D is automatically renamed to Penta_D.

- d. Enter the marker properties.

Table 2-5: STR marker properties

Property	Description
Repeat Length	Integer value. The length (in number of bp) of the repeat: 2 for a dinucleotide (for example, AC), 3 for a trinucleotide (for example, AAC), 4 for a tetranucleotide (for example, AGAT), and so on.
Lower Bound	The low molecular weight boundary of the marker.
Upper Bound	The high molecular weight boundary of the marker.
Color	The dye that is linked to the marker in the given PCR chemistry/kit.
Analytical Threshold	The peak height (RFU) lower limit to call a peak. Peaks that fall below the analytical threshold are not used in the analysis.

- e. In the blank Allele table, enter the allele information (allele number and size) for the marker.



To assist you in efficiently adding the alleles for a marker, right-click on the Allele table for a marker to open a context menu with options such as Copy, Paste, and Insert.

6. Optionally, on the Panels tab menu, click About to open the About dialog box, and then enter any pertinent notes for the panel.
7. Click Save.

To edit a panel

Because panel information is saved with a job, you can edit a panel without impacting any jobs that were run with a prior version of the panel.

1. If you have not done so already, open the Panels tab for the System view. (System > Panels).
2. On the Panels dropdown list, select the panel that you are editing.
3. Do any or all of the following as needed to edit the panel:
 - Add another marker to the panel. See [Step 5 of “To add a new panel” on page 49](#).
 - To enable a marker, click in the blank checkbox for the marker. Conversely, to disable a marker, click in the checkbox to clear it.
 - Edit any of the properties for a marker as appropriate. See [Table 2-5 on page 50](#).
 - To delete a marker, select it in the Markers list, and then below the marker properties, click Delete Marker.
 - Add, edit, or delete the possible alleles for a marker.



To assist you in efficiently adding, editing, or deleting the alleles for a marker, right-click on the Allele table for a marker to open a context menu with options such as Copy, Paste, and Insert.

- On the Panels tab menu, click About to open the About dialog box, and then add or edit any pertinent notes for the panel.
4. Do one of the following:
 - To save the modified panel as-is in the MaSTR database, click Save.
 - To save the modified panel as a new panel in the MaSTR database, click Save As, and then enter a unique combination of Name and Version Number for the panel.

To delete a panel

Because panel information is saved with a job, you can delete a panel without impacting any jobs that were previously run with the panel.

1. If you have not done so already, open the Panels tab for the System view. (System > Panels).
2. On the Panels dropdown list, select the panel that you are deleting.
3. On the Panels tab menu, click Delete.

A Delete <Panel Name> popup opens, prompting you to confirm the deletion. See [Figure 2-25 on page 52](#).

Figure 2-25: Delete Panel confirmation popup



4. Click Yes.

The panel is deleted from the MaSTR database, and the Delete Panel confirmation popup closes after the panel is deleted. The tab displays the information for the next panel on the Panels dropdown list.

To work with elimination profiles for a panel

In MaSTR, you can associate one or more *elimination profiles* with a panel. Elimination profiles are stored in the MaSTR Elimination database. MaSTR uses elimination profiles to calculate Likelihood Ratios against the mixture and reports them if their results exceed set thresholds. You populate the Elimination database by importing the necessary elimination profile files. You can associate elimination profiles with a panel on the Panels tab, you can edit an elimination profile for a panel, and you can delete an elimination profile for a panel.



For a review of the different types of Profile files in MaSTR, and their various requirements, see [“MaSTR Profile Files and Signal Files” on page 27](#).



Profile names must be unique in the Elimination database for the associated panel.

1. If you have not done so already, open the Panels tab for the System view. (System > Panels).
2. On the Panels dropdown list, select the panel with which you are associating the elimination profiles.
3. On the Panels tab menu, click Elimination DB.

The Elimination DB (Panel) popup opens. The Profiles dropdown list displays all the elimination profiles that have already been associated with the panel. If no elimination profiles have been associated with the panel, then the list is blank. See [Figure 2-26 on page 53](#).

Figure 2-26: Elimination DB popup

The screenshot shows a web-based popup window titled "Elimination DB (Confirmation)". Inside the window, there is a section labeled "Profiles" containing a dropdown menu with the text "Select...". Below this, there is a text input field labeled "Name". At the bottom of the window, there are three buttons: "DELETE", "IMPORT FROM FILE", and "SAVE". The "SAVE" button is highlighted in blue.

4. Continue to one of the following:
 - [“To import an elimination file for a panel”](#) below.
 - [“To edit an elimination profile for a panel”](#) on page 54.
 - [“To delete an elimination profile from a panel”](#) on page 54.

To import an elimination file for a panel

1. Click Import From File.

The Open dialog box opens.

2. In the Open dialog box, browse to and select the appropriate profile file, and then click Open.

The Open dialog box closes and the Elimination DB popup remains open. The selected profile file is imported in to the Elimination database. The Name field displays the name of the imported file, and the profile information is displayed below the file name.

3. Click Save.

To edit an elimination profile for a panel

1. On the Profiles dropdown list, select the elimination profile that you are editing.
2. Do any of the following as appropriate:
 - To add another elimination profile to the panel, click Import from File and in the Open dialog box, browse to and select the appropriate file.
 - Add, edit, or delete any of the information (Marker, Allele, Height) for the selected elimination profile.



To assist you in efficiently editing an elimination profile, right-click on the profile to open a context menu with options such as Copy, Paste, and Insert.

3. Click Save.

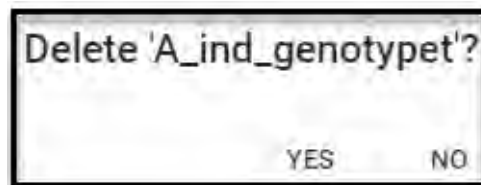
The Elimination DB popup remains open.

To delete an elimination profile from a panel

1. On the Profiles dropdown list, select the elimination profile that you are deleting.
2. Click Delete.

A Delete (Elimination Profile) popup opens, prompting you to confirm the deletion.

Figure 2-27: Delete Profile confirmation popup



3. Click Yes.

The profile is deleted from the MaSTR elimination database, and the Delete Profile confirmation popup closes after the profile is deleted. The Elimination DB popup displays the information for the next profile in the Panels dropdown list.

Population Frequencies tab

You work with frequencies on the Population Frequencies tab in the System view. In MaSTR, a *frequency* contains population frequencies for alleles in a specific marker. Frequencies are listed alphabetically by author on the Population Frequencies dropdown list on the tab. When the Population Frequencies tab opens, it displays the information for the population frequency that is first on the Population Frequencies dropdown list.



Although Population Frequency is found in the system view, population frequency is independent of both panels and protocol sets. The allele frequencies are used solely for the purpose of calculating the Likelihood Ratio. See “Likelihood Ratios report” on page 89.

Figure 2-28: System View, Population Frequencies tab

MaSTR™

v1.4.0

Demo Mode

ANALYSIS

MODELS

SYSTEM

System

PANELS

FREQUENCIES

PROTOCOL SETS

Population Frequencies (2)

HILL_et_al 2013

ABOUT

DELETE

IMPORT FROM FILE

Source: HILL_et_al

Year: 2013

Reference: (No Reference)

Frequency

Marker	Allele	Population 1	Population 2	Population 3	Population 4	Population 5
CSF1PO	12	0.34459	0.29532	0.38011	0.375	0.3866
CSF1PO	13	0.06564	0.04678	0.08172	0.05932	0.08763
CSF1PO	14	0.00917	0.00877	0.0097	0.00636	0.01546
CSF1PO	15	0.00097	0.00731	0.00693	0.00212	0.00515
D10S1248	8	0.00097	0.00292	0.00693	0.01059	0.02577
D10S1248	9	0.00097	0.00292	0.00693	0.01059	0.02577
D10S1248	10	0.0029	0.00731	0.00693	0.01059	0.00515
D10S1248	11	0.01303	0.03509	0.00139	0.00424	0.02577
D10S1248	12	0.07191	0.13012	0.03186	0.04237	0.08763
D10S1248	13	0.27654	0.23392	0.30748	0.27331	0.31959
D10S1248	14	0.29585	0.27632	0.29778	0.33898	0.25258
D10S1248	15	0.20125	0.19737	0.19668	0.21186	0.20619
D10S1248	16	0.10714	0.08772	0.13296	0.09958	0.09794
D10S1248	17	0.02654	0.02485	0.0227	0.02542	0.03093
D10S1248	18	0.00145	0.00146	0.00139	0.00212	0.02577
D10S1248	19	0.00145	0.00731	0.00277	0.00212	0.02577
D12S391	14	0.00048	0.00146	0.00693	0.01059	0.02577
D12S391	15	0.05068	0.07749	0.03186	0.04449	0.04124
D12S391	16	0.04054	0.06725	0.02216	0.04237	0.01031
D12S391	17	0.12452	0.16667	0.12742	0.07627	0.08247

Sample Size

Marker	Population 1	Population 2	Population 3	Population 4	Population 5
	All data	AFAm	Cauc	Hisp	Asian
AMEL	1036	342	361	236	97
CSF1PO	1036	342	361	236	97
D10S1248	1036	342	361	236	97
D12S391	1036	342	361	236	97
D13S317	1036	342	361	236	97
D16S539	1036	342	361	236	97
D18S51	1036	342	361	236	97
D19S433	1036	342	361	236	97
D151656	1036	342	361	236	97
D21S11	1036	342	361	236	97
D22S1045	1036	342	361	236	97
D251338	1036	342	361	236	97
D25441	1036	342	361	236	97
D3S1358	1036	342	361	236	97
D5S818	1036	342	361	236	97
D6S1043	1036	342	361	236	97
D7S820	1036	342	361	236	97
D8S1179	1036	342	361	236	97
F13A01	1036	342	361	236	97
F13B	1036	342	361	236	97
FESFPS	1036	342	361	236	97
FGA	1036	342	361	236	97
LPL	1036	342	361	236	97
Penta_C	1036	342	361	236	97
Penta_D	1036	342	361	236	97
Penta_E	1036	342	361	236	97
SE33	1036	342	361	236	97
TH01	1036	342	361	236	97
TPDX	1036	342	361	236	97
vWA	1036	342	361	236	97

SAVE

You can do the following on the Population Frequencies tab:

- View the information for a population frequency. See [“To view the information for a population frequency”](#) below.
- Import a population frequency file. See [“To import a population frequency”](#) on page 57.
- Edit a population frequency. See [“To edit a population frequency”](#) on page 58.
- Delete a population frequency. See [“To delete a population frequency”](#) on page 58.

To view the information for a population frequency

1. If you have not done so already, open the Population Frequencies tab for the System view. (System > Population Frequencies).
2. On the Population Frequencies dropdown list, select the name of the population frequency for which to view the information.

The Population Frequencies tab displays the information for the selected frequency in two tables.

- The Frequency table (top table) details the frequency author (publication) name, the year that the frequency information was published, and any references for the publication. The tab also lists the markers, organized by allele, that are detailed in the publication, the specific populations, organized by ethnicity, for which the marker frequencies were calculated, and the frequency of each marker/allele combination for each ethnicity.
- The Sample Size table (bottom table) details the number of samples by population that were used to calculate the frequency information.

See [Figure 2-28 on page 55](#).

3. You can also do the following:
 - To view information about another population frequency, select it on the Population Frequencies dropdown list.
 - To view additional information about a selected population frequency, on the Population Frequencies tab menu, click About.

The About popup opens. The popup displays the date and time that the population frequency was uploaded to the MaSTR database, the date and time that the frequency was last modified, and if applicable, any notes that were added for the frequency. See [Figure 2-29 on page 57](#).

Figure 2-29: About Population Frequency popup

About 'Hill_et_al 2013'

Uploaded by	Uploaded on
system	1/3/2018, 3:56:36 PM
Last modified by	Last modified on
system	1/3/2018, 3:56:36 PM

Notes

To import a population frequency

You can import standard tab-delimited text file that details the population frequencies in to the MaSTR database, and these frequencies files will be available immediately for use. The file must have the following format:

- Three distinct header lines in the following order: Author, Year, and Reference Information.



The author name and (publication) year must be unique in the MaSTR database and the decimal designator must be a period.

- Tab-delimited listing of marker name, allele name, and population frequency.
- For *each* marker, the first allele must be listed as “N” and instead of frequency, the file must specify the sample number.

Figure 2-30 below shows a partial example of a tab-delimited population frequency file.

Figure 2-30: Sample population frequency file

```
#Author Hill_et_al
#Year 2013
#Reference (No Reference)
Marker Allele All data AfAm Cauc Hisp Asian
CSF1PO N 1036 342 361 236 97
CSF1PO 7 0.02317 0.05556 0.00693 0.01271 0.02062
CSF1PO 8 0.02124 0.05556 0.00554 0.00424 0.02577
CSF1PO 9 0.02944 0.03947 0.01385 0.02331 0.06701
CSF1PO 10 0.23214 0.25000 0.22022 0.23729 0.20103
CSF1PO 11 0.27365 0.24854 0.30886 0.27966 0.21649
CSF1PO 12 0.34459 0.29532 0.36011 0.37500 0.38660
CSF1PO 13 0.06564 0.04678 0.08172 0.05932 0.08763
CSF1PO 14 0.00917 0.00877 0.00970 0.00636 0.01546
CSF1PO 15 0.00097 0.00731 0.00693 0.00212 0.00515
D10S1248 N 1036 342 361 236 97
D10S1248 8 0.00097 0.00292 0.00693 0.01059 0.02577
```

1. If you have not done so already, open the Population Frequencies tab for the System view. (System > Population Frequencies).

The Population Frequencies tab opens. The tab displays the information for the population frequency that is listed first on the Population Frequencies tab dropdown list.

2. On the Population Frequencies tab menu, click Import From File.

The Open dialog box opens.

3. In the Open dialog box, browse to and select the appropriate population frequency file, and then click Open.

The Open dialog box closes and the Population Frequencies tab remains open. The selected population frequency is imported in to the MaSTR database.

To edit a population frequency

Because frequency information is saved with a job, you can edit a frequency without impacting any jobs that were run with a previous version of the frequency.

1. If you have not done so already, open the Population Frequencies tab for the System view. (System > Population Frequencies).
2. On the Population Frequencies dropdown list, select the frequency that you are editing.
3. Edit the frequency as needed, including:
 - Editing the Author, Year, and/or Reference information for the frequency.
 - Adding another marker to the frequency.
 - Adding or deleting alleles for a marker in the frequency.
 - Editing the frequency for an allele in one or more populations.
 - Edit the sample information for a frequency.



To assist you in efficiently editing a population frequency, right-click on either the Frequency table or Sample Size table to open a context menu with options such as Copy, Paste, and Insert.

4. Optionally, click About to open the About dialog box, and then add or edit any pertinent notes for the frequency.
5. Click Save.

To delete a population frequency

Because frequency information is saved with a job, you can delete a frequency without impacting any jobs that were previously run with the frequency.

1. If you have not done so already, open the Population Frequencies tab for the System view. (System > Population Frequencies).

2. On the Population Frequencies dropdown list, select the frequency that you are deleting.
3. On the Population Frequencies tab menu, click Delete.

A Delete <Population Frequency> popup opens, prompting you to confirm the deletion.

Figure 2-31: Delete Population Frequency confirmation popup



4. Click Yes.

The frequency is deleted from the MaSTR database, and the Delete Population Frequency confirmation popup closes after the frequency is deleted. The tab displays the information for the next frequency on the Population Frequencies dropdown list.

Protocol Sets tab

You work with protocol sets on the Protocol Sets tab in the System view. In MaSTR, a *protocol set* (also referred to as a standard operating procedure or SOP) is a lab-specific file serves that two purposes:

- Defines the variables that the lab uses for a specific STR analysis chemistry (the commercial kit used), including instrument, injection time, PCR cycle, and so on. This is the SOP portion of the protocol set.
- Establishes the expected parameters, based on single-source data, for stutter expectations and peak height variation when analyzing samples according to this SOP.

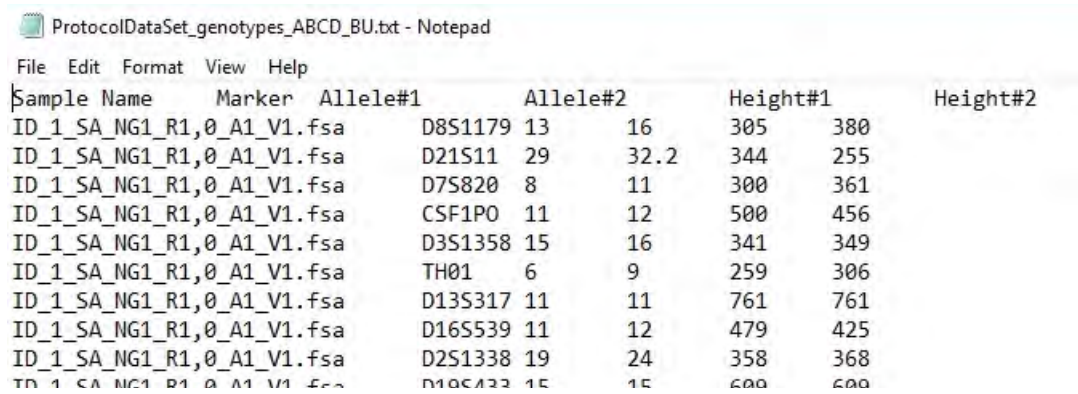


At least one protocol set is required per chemistry and SOP combination that a lab uses for STR analysis.

The following files are always associated with a protocol set:

- Panel file - Details the information about the markers and alleles that are to be analyzed for a job. A single Panel file is always associated with a protocol set.
- Genotype file - Details the true genotypes of the samples that are listed in the Signal file. A genotype file requires multiple profiles in a single file. See [Figure 2-32 on page 60](#).

Figure 2-32: Protocol set file, required genotype information

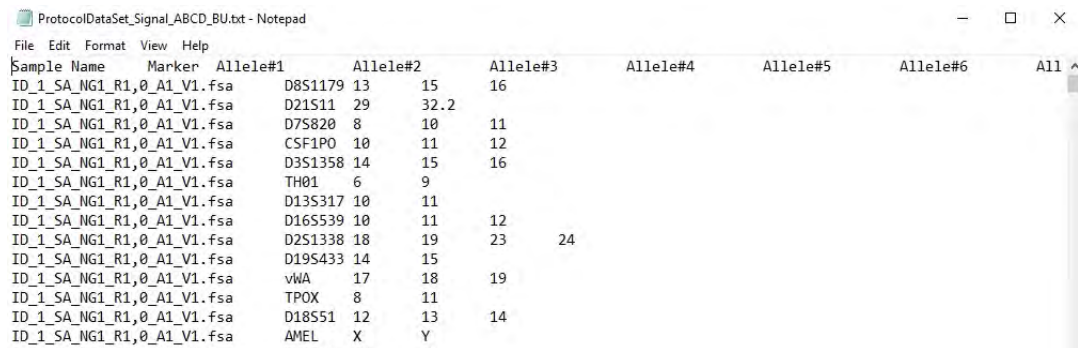


ProtocolDataSet_genotypes_ABCD_BU.txt - Notepad

Sample Name	Marker	Allele#1	Allele#2	Height#1	Height#2
ID_1_SA_NG1_R1,0_A1_V1.fsa		D8S1179 13	16	305	380
ID_1_SA_NG1_R1,0_A1_V1.fsa		D21S11 29	32.2	344	255
ID_1_SA_NG1_R1,0_A1_V1.fsa		D7S820 8	11	300	361
ID_1_SA_NG1_R1,0_A1_V1.fsa		CSF1PO 11	12	500	456
ID_1_SA_NG1_R1,0_A1_V1.fsa		D3S1358 15	16	341	349
ID_1_SA_NG1_R1,0_A1_V1.fsa		TH01 6	9	259	306
ID_1_SA_NG1_R1,0_A1_V1.fsa		D13S317 11	11	761	761
ID_1_SA_NG1_R1,0_A1_V1.fsa		D16S539 11	12	479	425
ID_1_SA_NG1_R1,0_A1_V1.fsa		D2S1338 19	24	358	368
ID_1_SA_NG1_R1,0_A1_V1.fsa		D19S433 15	15	600	600

- Signal file - Details the alleles for specific individuals at the loci that are being tested *and* the signal (peak) height for each allele.

Figure 2-33: Protocol set, required single-source data signal (peak) height information

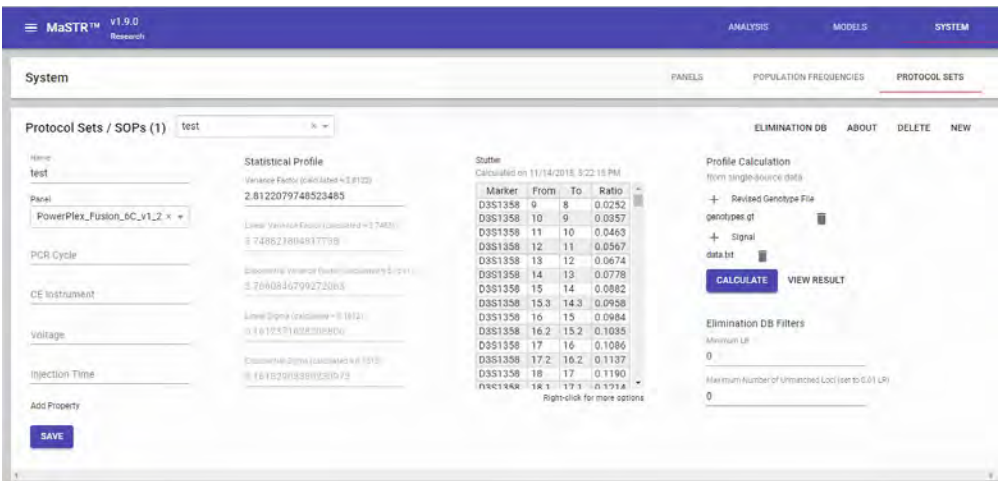


ProtocolDataSet_Signal_ABCD_BU.txt - Notepad

Sample Name	Marker	Allele#1	Allele#2	Allele#3	Allele#4	Allele#5	Allele#6	Allele#7
ID_1_SA_NG1_R1,0_A1_V1.fsa		D8S1179 13	15	16				
ID_1_SA_NG1_R1,0_A1_V1.fsa		D21S11 29	32.2					
ID_1_SA_NG1_R1,0_A1_V1.fsa		D7S820 8	10	11				
ID_1_SA_NG1_R1,0_A1_V1.fsa		CSF1PO 10	11	12				
ID_1_SA_NG1_R1,0_A1_V1.fsa		D3S1358 14	15	16				
ID_1_SA_NG1_R1,0_A1_V1.fsa		TH01 6	9					
ID_1_SA_NG1_R1,0_A1_V1.fsa		D13S317 10	11					
ID_1_SA_NG1_R1,0_A1_V1.fsa		D16S539 10	11	12				
ID_1_SA_NG1_R1,0_A1_V1.fsa		D2S1338 18	19	23	24			
ID_1_SA_NG1_R1,0_A1_V1.fsa		D19S433 14	15					
ID_1_SA_NG1_R1,0_A1_V1.fsa		vWA 17	18	19				
ID_1_SA_NG1_R1,0_A1_V1.fsa		TPOX 8	11					
ID_1_SA_NG1_R1,0_A1_V1.fsa		D18S51 12	13	14				
ID_1_SA_NG1_R1,0_A1_V1.fsa		AMEL X	Y					

When the Protocol Sets tab opens, it displays the information for the protocol set that is first on the Protocol Set dropdown list.

Figure 2-34: System View, Protocol Sets tab



The screenshot shows the MaSTR v1.9.0 System View, Protocol Sets tab. The interface includes a sidebar with 'System' and 'Protocol Sets / SOPs (1)' sections. The main area displays a 'test' protocol set with a 'Statistical Profile' and a 'Sutter' table. The 'Statistical Profile' shows a 'Peak' of 2.8122079748523405 and a 'PCR Cycle' of 27. The 'Sutter' table lists markers and their ratios. The right sidebar contains 'Profile Calculation' and 'Elimination DB Filters' sections.

Marker	From	To	Ratio
D3S1358	9	8	0.0252
D3S1358	10	9	0.0357
D3S1358	11	10	0.0403
D3S1358	12	11	0.0567
D3S1358	13	12	0.0674
D3S1358	14	13	0.0778
D3S1358	15	14	0.0882
D3S1358	15.3	14.3	0.0958
D3S1358	16	15	0.0984
D3S1358	16.2	15.2	0.1035
D3S1358	17	16	0.1086
D3S1358	17.2	16.2	0.1137
D3S1358	18	17	0.1190
D3S1358	18.3	17.3	0.1241

You can do the following on the Protocol Sets tab:

- View the information for a protocol set. See [“To view the information for a protocol set”](#) below.
- Add a new protocol set. See [“To add a new protocol set”](#) on page 62.
- Edit a protocol set. See [“To edit a protocol set”](#) on page 66.
- Delete a protocol set. See [“To delete a protocol set”](#) on page 67.

To view the information for a protocol set

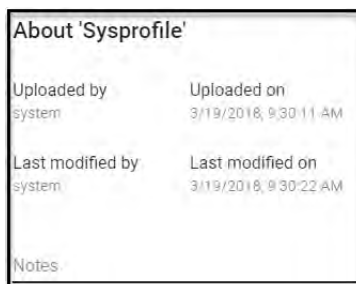
1. If you have not done so already, open the Protocol Sets tab for the System view. (System > Protocol Sets).
2. On the Protocol Sets dropdown list, select the name of the protocol set for which you are viewing the information.

The Protocol Sets tab displays the information for the selected protocol set. From left to right, the tab displays the following information for the selected protocol set:

- The name of the protocol set and all its properties.
 - The statistical profile for the set, including the Variance Factor, which accounts for the Drop-In Coefficient, Degradation, and other parameters.
 - The expected stutter values for the indicated markers when samples are analyzed according to the protocol.
 - The Elimination DB filters for the set’s assigned panel.
3. You can also do the following:
 - To view information about another protocol set, select it on the Protocol Sets dropdown list.
 - To view additional information about a selected protocol set, on the Protocol Sets tab menu, click About.

The About popup opens. The popup displays the date and time that the protocol set was uploaded to the MaSTR database, the date and time that the set was last modified, and if applicable, any notes that were added for the protocol set. See

Figure 2-35: About Protocol Set popup



1. If you have not done so already, open the Protocol Sets tab for the System view. (System > Protocol Sets).

2. On the Protocol Sets tab menu, click Import From File.

The Open dialog box opens.

3. In the Open dialog box, browse to and select the appropriate protocol set file, and then click Open.

The Open dialog box closes and the Protocol Sets tab remains open. The selected protocol set is imported in to the MaSTR database.

To add a new protocol set

Before you can add a new protocol set to the MaSTR database, you must do the following:

- On the Panels dropdown list, confirm that the correct chemistry-specific panel is available.



The Panels dropdown list is pre-populated with the commercially available megaplexes that forensic labs most commonly use. If the correct panel is not present, then before you can continue, you must either import the required panel (see “[To import a panel file](#)” on page 48), or contact SoftGenetics for the panel (tech_support@softgenetics.com).

- Open the Population Frequencies tab, and on the Population Frequencies dropdown list, select a publication, and then confirm that the selected publication contains the correct population frequency (allele frequencies) table.



If the correct population frequency table is not present in the selected publication, then you can import the necessary table. See “[To import a population frequency](#)” on page 57.



Remember, a population frequency is independent of both the panels and protocol sets. The allele frequencies are used solely for the purpose of calculating the Likelihood Ratio. See “[Likelihood Ratios report](#)” on page 89.

Every protocol set must have a unique name to be added to the MaSTR database.

1. If you have not done so already, open the Protocol Sets tab for the System view. (System > Protocol Sets).

2. On the Protocol Sets tab menu, click New.

The Protocol Sets tab displays the options for adding a new protocol set to the MaSTR database. See [Figure 2-36 on page 63](#).

Figure 2-36: Blank Protocol Set tab

3. Enter the information for the new protocol set.



Only the name and panel are required. All other information is optional and can be added or modified at a later date.

Table 2-6: Protocol Set information

Option	Description
Name	The protocol set name. A protocol set name must be unique in the MaSTR database
Panel	A single panel must be associated with a protocol set. A panel is the chemistry that was used to amplify the DNA samples prior to analysis. A panel details the information about the markers and alleles that should be analyzed for a job.
PCR Cycle	The number of PCR cycles used for the amplification of the samples.
CE Instrument	The instrument on which the amplified samples were separated.
Voltage	The voltage of the instrument run as reported in the .fsa or .hid files from the CE instrument.
Injection Time	Injection time of the samples as reported in the .fsa or .hid files from the CE instrument.

4. Optionally, for each additional property to define for the protocol set, do the following:
 - a. Click Add Property.

The Add a property popup opens.

Figure 2-37: Add a property popup

- b. Enter the name and value for the property.
 - c. Click Add.
5. Under Profile Calculation, do the following:
 - Click Revised Genotype File to open the Open dialog box, and then browse to and select the appropriate genotype file (a .txt file) for the single-sourced data.
 - Click Signal to open the Open dialog box, and then browse to and select the appropriate data (peak height) file (a .txt file) for the single-sourced data.



These files are the standard validation and sensitivity files that your lab uses. These files must contain data for a number of individuals with replicates and a known dilution series. For information about the correct file format and information, see [“MaSTR Profile Files and Signal Files” on page 27.](#)

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 6. Optionally, to have MaSTR check an attached Elimination database for a project, under Elimination DB Filters, enter the values for Minimum LR (the minimum Likelihood Ratio) and Maximum Number of Loci with 0.01 LR (Likelihood Ratio markers).

MaSTR flags the profiles in the Elimination database that is attached to the protocol set's associated panel if the profiles exceed the threshold for the Minimum LR *and* have fewer than the Maximum Number of LR markers.



If you do not enter values for the Elimination DB filters, then MaSTR does not check an attached Elimination database for a job.

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 7. Click Calculate.

MaSTR calculates the parameters for variance (which accounts for allele drop-out), stutter expectations, and peak height variation that will be used when analyzing samples according to the SOP. After the calculations are complete, the results, Stutter Ratio (for each allele in every marker) and Variance, are graphed and automatically displayed for viewing. See [Figure 2-38](#) and [Figure 2-39 on page 65.](#)

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 8. If you are satisfied with the results, close the graphs, and then click Save to save the protocol; otherwise, continue to edit the protocol set and calculate the results until you are satisfied with the results, and then you can save the protocol. See [“To edit a protocol set” on page 66.](#)



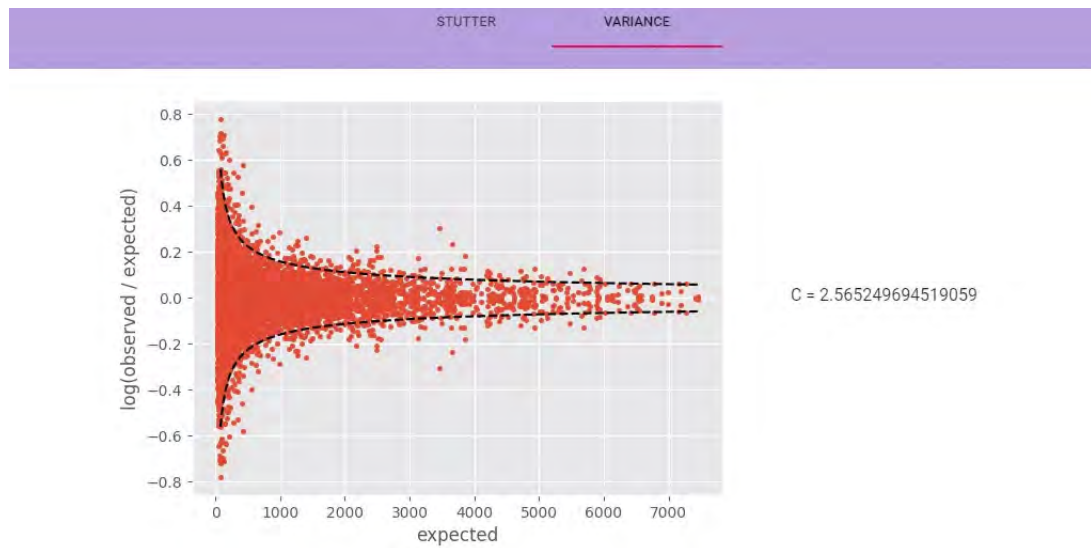
At any time after you close the graphs, you can click Results to open and view the graphs again.

Figure 2-38: Stutter Ratio graph



To view the Stutter Ratio graph for each marker, use the scroll feature at the right side of the display.

Figure 2-39: Variance graph



To edit a protocol set

The information for a panel that is used in an analysis job is saved with the job. As a result, any modifications that you make to a panel do not impact any jobs that were run with a prior version of the panel.



If you are adding a new protocol set by modifying an existing set, and the existing protocol set has one or more associated Elimination databases, then you must reattach these databases to the modified set.

1. If you have not done so already, open the Protocol Sets tab for the System view. (System > Protocol Sets).
2. On the Protocol Sets dropdown list, select the protocol set that you are editing.
3. Do any or all of the following as needed to edit the panel:
 - To associate a different panel with the protocol set, select the panel on the Panel dropdown list.
 - To edit the Elimination DB that is attached to the associated panel, on the Protocol Sets tab menu, click Elimination DB to open the Elimination DB popup, and then do any of the following as appropriate:
 - Add another elimination profile. See [“To import an elimination file for a panel” on page 53.](#)
 - Add, edit, or delete the information for a selected profile. See [“To edit an elimination profile for a panel” on page 54.](#)
 - Delete a selected profile. See [“To delete an elimination profile from a panel” on page 54.](#)
 - Revise and/or replace the genotype text file and/or the signal file used to establish the parameters for the protocol set.



You can click the Delete icon that is displayed next to a file to delete the currently loaded file, and then upload a new file, or you can simply select another file to upload and the currently loaded file is automatically replaced.

- To modify the Stutter values, right-click the Stutter table to open a context menu with options such as Copy, Paste, and Insert.
- Edit the Minimum LR and/or Maximum Number of Loci with 0.01 LR Elimination DB Filters.
- On the Protocol Sets tab menu, click About to open the About dialog box, and then enter or edit any notes for the protocol set.

4. Click Calculate.

MaSTR calculates the parameters for variance (which accounts for allele drop-out), stutter expectations, and peak height variation that will be used when analyzing samples according to the SOP. After the calculations are complete, the results, Stutter Ratio (for each allele in every marker) and Variance, are graphed and automatically displayed for viewing. See [Figure 2-38](#) and [Figure 2-39](#) on page 65.

5. If you are satisfied with the results, close the graphs, and then click Save to save the modified protocol; otherwise, continue to edit the protocol set and calculate the results (see [“To edit a protocol set”](#) on page 66) until you are satisfied with the results, and then click Save.



At any time after you close the graphs, you can click Results to open and view the graphs again.

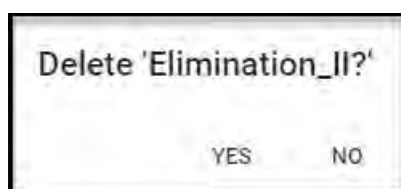
To delete a protocol set

Because the information for a protocol set that is used in an analysis job is saved with the job, you can delete a protocol set without impacting any jobs that were previously run with the set.

1. If you have not done so already, open the Protocol sets tab for the System view. (System > Protocol Sets).
2. On the Protocol Set dropdown list, select the protocol set that you are deleting.
3. On the Protocol Sets tab menu, click Delete.

A Delete <Protocol Set> popup opens, prompting you to confirm the deletion.

Figure 2-40: Delete Population Frequency confirmation popup



4. Click Yes.

The protocol set is deleted from the MaSTR database, and the Delete Protocol Set confirmation popup closes after the frequency is deleted. The tab displays the information for the next protocol set on the Protocol Set dropdown list.

Chapter 2

MaSTR Primary Views

Chapter 3

DNA Analysis in MaSTR

Now that you have learned about the fundamental components for a DNA analysis in MaSTR— Protocol Sets, Population Frequencies, and Models—you can carry out an actual analysis. You carry out an analysis in MaSTR for one of three purposes: for running a forensic DNA analysis, for validating an analysis model, or for researching and developing a new analysis model. This chapter details how to run a forensic DNA analysis in MaSTR, how to validate a DNA analysis model in MaSTR, and finally, how to add a new DNA analysis model in MaSTR.

This chapter covers the following topics:

- [“Running a DNA Analysis in MaSTR” on page 71.](#)
- [“Validating a DNA Analysis Model” on page 76.](#)
- [“Adding a New DNA Analysis Model” on page 78.](#)

Chapter 3

DNA Analysis in MaSTR

Running a DNA Analysis in MaSTR

To run a forensic DNA analysis in MaSTR, you apply a validated model to casework samples. The three fundamental components that are required for a DNA analysis are a protocol set, a population frequency (allele frequency) table, and a model. The following procedure describes how to run a forensic DNA analysis in MaSTR. You can set up and run an entirely new analysis according to an SOP, you can rerun an existing analysis as-is, or you can copy an existing analysis, and then modify it to create a new analysis, and then run this new analysis.

To run a new DNA analysis in MaSTR

1. Open and log in to MaSTR.



Typically, the mode that you would use to log in to MaSTR for analyzing casework samples is Casework, but if you have any questions about your login credentials, contact your MaSTR system administrator.

The Analysis view is the open view and the Analysis Jobs tab is the open tab.

2. Click New.

The New Analysis popup opens.

Figure 3-1: New Analysis popup

3. Select the analysis components - Protocol Sets, Population Frequency, and Model.



If you are a member of Research/Validation, then to quickly test without editing and saving an existing model, you can click Settings to open the Job Settings popup, and then simply override MCMC Settings and/or Analysis Settings for the model, such as changing the number of iterations.

4. Select the Coancestry Adjustment method, and then enter the adjustment value.



Most US-based labs use NRCII Recommendation 4.1 with an Adjustment Value of 0.01, but make sure to adhere to the method and value that the SOP specifies.

5. Enter the name for the analysis.
6. Optionally, enter any comments for the analysis.
7. Select the expected number of contributors.
8. Upload the appropriate Signal and Reference files. If applicable, include Known and/or Alternate files.
9. Click Submit Job.

The New Analysis popup closes and the Analysis Jobs tab remains open. The tab displays the process of the submitted job and after the job is finished, **Finished** is displayed in the Stage column for the job, along with the date and time that the job was finished.

Figure 3-2: Finished job on the Analysis Jobs tab

Finished on 5/25/2018 at 5:22:27 PM



To review the results of the analysis before generating any reports, or carrying out any other work for the analysis, you can click anywhere for the finished job to open a table that summarizes the job results.

To rerun an existing DNA analysis as-is

1. Open and log in to MaSTR.



Typically, the mode that you would use to log in to MaSTR for analyzing casework samples is Casework, but if you have any questions about your login credentials, contact your MaSTR system administrator.

The Analysis view is the open view and the Analysis Jobs tab is the open tab. See [Figure 2-12 on page 36](#).

2. On the open Analysis Jobs tab, find the analysis that you are rerunning.



Remember, you can filter the list of displayed jobs. See [“Filter feature” on page 33](#).

3. Right-click the selected job, and on the context menu that opens, click Copy to New Analysis.

The New Analysis popup opens, populated with all the information for the selected analysis job. The Name field is flagged and underlined in red.

Figure 3-3: New Analysis popup populated with information for a selected analysis job

4. Enter a new name for the analysis, and then click Submit Job.



Although not required, you should enter pertinent comments as well to indicate that this is a rerun of an existing analysis; for example, something as simple as Rerun of <original job name>.

The New Analysis popup closes and the Analysis Jobs tab remains open. The tab displays the process of the submitted job and after the job is finished, **Finished** is displayed in the Stage column for the job, along with the date and time that the job was finished.

Figure 3-4: Finished job on the Analysis Jobs tab

Finished on 5/25/2018 at 5:22:27 PM



To review the results of the analysis before generating any reports, or carrying out any other work for the analysis, you can click anywhere for the finished job to open a table that summarizes the job results.

To modify and run an existing DNA analysis in MaSTR

1. Open and log in to MaSTR.



Typically, the mode that you would use to log in to MaSTR for analyzing casework samples is Casework, but if you have any questions about your login credentials, contact your MaSTR system administrator.

The Analysis view is the open view and the Analysis Jobs tab is the open tab. See [Figure 2-12 on page 36](#).

2. On the open Analysis Jobs tab, find the analysis that you are rerunning.



Remember, you can filter the list of displayed jobs. See [“Filter feature” on page 33](#).

3. Right-click the selected job, and on the context menu that opens, click Copy to New Analysis.

The New Analysis popup opens, populated with all the information for the selected analysis job. The Name field is flagged and underlined in red.

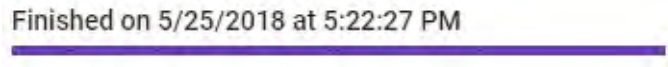
Figure 3-5: New Analysis popup populated with information for a selected analysis job

4. Modify any or all of the analysis information as needed - Protocol Set, Population Frequency, Model, Coancestry Adjustment method and/or adjustment value, and the expected number of contributors.
5. Enter the name for the analysis.
6. Optionally, enter any comments for the analysis.
7. Upload the appropriate Signal and Reference files. If applicable, include Known and/or Alternate files.

8. Click Submit Job.

The New Analysis popup closes and the Analysis Jobs tab remains open. The tab displays the process of the submitted job and after the job is finished, **Finished** is displayed in the Stage column for the job, along with the date and time that the job was finished.

Figure 3-6: *Finished job on the Analysis Jobs tab*



Finished on 5/25/2018 at 5:22:27 PM



To review the results of the analysis before generating any reports, or carrying out any other work for the analysis, you can click anywhere for the finished job to open a table that summarizes the job results.

Validating a DNA Analysis Model

Before an analyst runs any DNA analysis, the analysis model must be *validated*. Validation of a DNA analysis model ensures that the analysis meets or exceeds minimum analytical quality for a specific and known data set. It is important that you validate your different analysis models by understanding their quality and characteristics before you deploy them to a production environment.



The following procedure assumes that the appropriate protocol set has already been added to the MaSTR database. See [“To add a new protocol set” on page 62](#).

To validate a DNA analysis model

1. Open and log in to MaSTR.



Typically, the mode that you would use to log in to MaSTR for validating an analysis model is Research/Validation, but if you have any questions about your login credentials, contact your MaSTR system administrator.

The Analysis view is the open view and the Analysis Jobs tab is the open tab. See [Figure 2-12 on page 36](#).

2. Click New.

The New Analysis popup opens.

Figure 3-7: New Analysis popup

3. Select the analysis components - Protocol Set, Population Frequency, and Model.



When you select a model, make sure to select a standard model that SoftGenetics has supplied.

4. Select the Coancestry Adjustment method, and then enter the adjustment value.

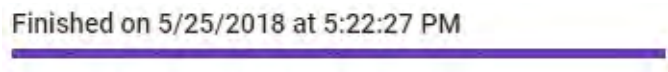


Most US-based labs use NRCII Recommendation 4.1 with an Adjustment Value of 0.01, but make sure to adhere to the method and value that the SOP specifies.

5. Enter the name for the analysis.
6. Optionally, enter any comments for the analysis.
7. Enter the expected number of contributors.
8. Upload the appropriate Signal and Reference files. If applicable, include Known and/or Alternate files.
9. Click Submit Job.

The New Analysis popup closes and the Analysis Jobs tab remains open. The tab displays the process of the submitted job and after the job is finished, **Finished** is displayed in the Stage column for the job, along with the date and time that the job was finished.

Figure 3-8: Finished job on the Analysis Jobs tab



Finished on 5/25/2018 at 5:22:27 PM

10. Review the analysis results to determine if the selected model provides the expected results and therefore, the model validation is successful; otherwise, you must iteratively select a different standard model and/or modify the model properties (other than Variables, all properties such as Iterations or Burn In) and run the assay until the expected results are achieved.



For detailed information about reporting and viewing analysis results, see [Chapter 4, "Reporting and Graphing DNA Analysis Results in MaSTR,"](#) on page 81.

For example, if you initially selected a Standard_30k_8chains model and you determine that this model did not provide enough stabilization to interpret the analysis results, then you might select a Standard_60k_8chains model and rerun the analysis. The results of this second analysis run, however, indicate that the Standard_60k_8chains model greatly exceeds the stabilization requirements. Therefore, you would select either the Standard_30k_8chains model or the Standard_60k_8chains model, and then iteratively modify the appropriate model properties (in this example, you might modify the MCMC Iterations value to achieve 45k iterations) to create a new model and run the analysis until the expected results are achieved.



For detailed information about the properties that you can modify for a model, see [Table 2-4](#) on page 39.

Adding a New DNA Analysis Model

If none of the standard models meet your DNA analysis needs either as-is, or with modification, then you can always develop new DNA analysis models. When you develop a new DNA analysis model, you can modify all the model properties, including Variables. As with any DNA analysis model, after you develop it, you must validate it before you deploy it to a production environment.



The following procedure assumes that the new DNA analysis and appropriate protocol set has already been added to the MaSTR database. See [Chapter B](#), “Building a Custom MCMC Model for Probabilistic Mixture Analysis,” on page 121 and “To add a new protocol set” on page 62.

To add a new DNA analysis model

1. Open and log in to MaSTR.



Typically, the mode that you would use to log in to MaSTR for developing a new analysis model is Research/Validation, but if you have any questions about your login credentials, contact your MaSTR system administrator.

The Analysis view is the open view and the Analysis Jobs tab is the open tab. See [Figure 2-12 on page 36](#).

2. Click New.

The New Analysis popup opens.

Figure 3-9: New Analysis popup

3. Select the analysis components - Protocol Set, Population Frequency, and Model.



When you select a model, make sure to select the new model that you are testing and validating.

4. Select the Coancestry Adjustment method, and then enter the adjustment value.

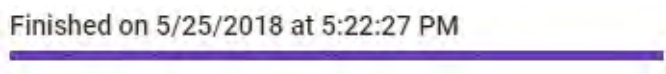


Most US-based labs use NRCII Recommendation 4.1 with an Adjustment Value of 0.01, but make sure to adhere to the method and value that the SOP specifies.

5. Enter the name for the analysis.
6. Optionally, enter any comments for the analysis.
7. Enter the expected number of contributors.
8. Upload the appropriate Signal and Reference files. If applicable, include Known and/or Alternate files.
9. Click Submit Job.

The New Analysis popup closes and the Analysis Jobs tab remains open. The tab displays the process of the submitted job and after the job is finished, **Finished** is displayed in the Stage column for the job, along with the date and time that the job was finished.

Figure 3-10: Finished job on the Analysis Jobs tab



Finished on 5/25/2018 at 5:22:27 PM

10. Review the analysis results to determine if the new analysis model provides the expected results and therefore, the model validation is successful; otherwise, you must iteratively modify the model properties (all properties, including Variables) and run the assay until the expected results are achieved.



For detailed information about reporting and viewing analysis results, see [Chapter 4, "Reporting and Graphing DNA Analysis Results in MaSTR,"](#) on page 81.

Chapter 3

DNA Analysis in MaSTR

Chapter 4

Reporting and Graphing DNA Analysis Results in MaSTR

After your analysis is complete, MaSTR provides numerous ways for visualization and investigation of your results, including reporting and the graphing of your analysis results. This chapter details the various ways that you can report on and visualize your analysis results so that you can interpret the data and identify the keys areas of your assay that worked well as well as the areas that need attention or improvement.

This chapter covers the following topics:

- [“Generating and Downloading an Analysis Report” on page 83.](#)
- [“Reviewing Analysis Project Results” on page 87.](#)

Chapter 4

Reporting and Graphing DNA Analysis Results in MaSTR

Generating and Downloading an Analysis Report

After an analysis job is completed, **Finished** is displayed in the Stage column on the Analysis Jobs tab for the job, along with the date and time that the job was finished. If you do not intend to review the data, you can simply generate and download the Analysis report. When you generate and download the Analysis report, you can specify the sections that are to be included in the report and the populations for which you are generating the report. The report is downloaded as a PDF.



To review the results of an analysis project before generating the Analysis report, see [“Reviewing Analysis Project Results” on page 87](#).

To generate and download an Analysis report

1. Open and log in to MaSTR.



The mode that you would use to log in to MaSTR for reporting analysis results can be either Casework or Research/Validation, but if you have any questions about your login credentials, contact your MaSTR system administrator.

The Analysis view is the open view and the Analysis Jobs tab is the open tab. See [Figure 2-12 on page 36](#).

2. On the open Analysis Jobs tab, find the analysis for which you are generating the report.



Remember, you can filter the list of displayed jobs. See [“Filter feature” on page 33](#).

3. Right-click the selected job, and on the context menu that opens, click Download Report.

The Analysis Report for <analysis job> popup opens. The popup indicates the name of the analysis for which you are generating and downloading the report. The popup also lists all the sections that can be included in the report and all the populations that can be reported on. By default, all sections and all populations are selected. See [Figure 4-1 on page 84](#).



[Figure 4-1 on page 84](#) shows the report popup for a in-house version of MaSTR. If you are an administrator, then you can customize the report display to show your organization name and/or logo that is displayed on the report popup and the analysis report. See [“Customizing the Default Report and View Displays” on page 118](#).

Figure 4-1: Analysis Report popup

Organization	Sections	Populations
[Organization Name]	Likelihood Ratios <input checked="" type="checkbox"/>	All data <input checked="" type="checkbox"/>
MaSTR™	Genotype Set Results <input checked="" type="checkbox"/>	AfAm <input checked="" type="checkbox"/>
	Ratio Plots <input checked="" type="checkbox"/>	Cauc <input checked="" type="checkbox"/>
	Degradation Plots <input checked="" type="checkbox"/>	Hisp <input checked="" type="checkbox"/>
	Protocol Set / SOP <input checked="" type="checkbox"/>	Asian <input checked="" type="checkbox"/>
	Stutter <input checked="" type="checkbox"/>	
	Variance <input checked="" type="checkbox"/>	
	Panel <input checked="" type="checkbox"/>	
	Frequency <input checked="" type="checkbox"/>	
	Model <input checked="" type="checkbox"/>	
	Signal <input checked="" type="checkbox"/>	
	Reference <input checked="" type="checkbox"/>	
	Known <input checked="" type="checkbox"/>	
	Alternate <input checked="" type="checkbox"/>	
	DOWNLOAD	

4. Clear the options for the sections and populations that are *not* to be included in the report.



You should download only the report sections that are relevant to your analysis as the size of the downloaded report can increase exponentially the more information that you select.

Table 4-1: Analysis report

Sections	Description
Note: Report sections are hard-coded and the same sections are displayed for every analysis.	
Likelihood Ratios	The report Likelihood ratios that were calculated from the weighted genotypes. See “Likelihood Ratios report” on page 89.
Genotype Set Results	A heat map and a table of weight genotypes for each locus. See “Genotype Results report” on page 90.
Ratio Plots	Summary of contributor ratios from the MCMC process. See “Interactive reports (Contributor Ratio, Degradation Variables, and Model Variables)” on page 90.

Table 4-1: Analysis report (Continued)

Sections	Description
Degradation Plots	Plots the values of degradation variables. See “Interactive reports (Contributor Ratio, Degradation Variables, and Model Variables)” on page 90.
Model Variable Plots	Displayed as an option only if the model contains variables that were sampled as part of the MCMC process. See “Interactive reports (Contributor Ratio, Degradation Variables, and Model Variables)” on page 90.
Protocol Set/SOP	Detailed information about the protocol set/SOP that was used for the analysis.
Stutter	The Stutter Ratio graph for the protocol set. See Figure 2-38 on page 65.
Variance	The Variance graph for the protocol set. See Figure 2-39 on page 65.
Panel	Detailed information about the panel that was used for the analysis. See “Panels tab” on page 46.
Frequency	Detailed information about the population frequencies (all the allele frequency tables) that were used for the analysis. “Population Frequencies tab” on page 55.
Model	Detailed information about the model that was used for the analysis.
Signal	A histogram of the Signal.text file that the protocol set used. See “Signal plot” on page 88.
Reference	Detailed information about the Reference profile. See “Profile files” on page 28.
Known	Detailed information about the Known profiles. See “Profile files” on page 28.
Alternate	Detailed information about the Alternate Profiles. See “Profile files” on page 28.
Note: If the analysis did not include a profile type, for example, an Alternate profile, then the section is still displayed on the Analysis Report popup, but it is not enabled/available for selection.	
Populations	Description
Note: Population options are soft-coded and the options that are displayed for a selected analysis depend on the populations that were contained in the Population Frequency tables that were used for the analysis. For example, in Figure 4-2 on page 86 , four populations were present in the Population Frequency tables and were coded as AfAm for African American, Cauc for Caucasian, Hisp for Hispanic, and Asian. By default, all populations are always selected for reporting.	
AfAm	The results (all selected report sections) for the indicated population (African American).
Cauc	The results (all selected report sections) for the indicated population (Caucasian).
Hisp	The results (all selected report sections) for the indicated population (Hispanic).
Asian	The results (all selected report sections) for the indicated population (Asian).

Table 4-1: Analysis report (Continued)

Sections	Description
Tip:	You should select only the relevant populations for the analysis. For example, if the mixture is known to have only Caucasian and Hispanic contributors, then in this example, you would clear the AfAm and Asian selections.

5. Click Download.

The report is generated and downloaded. By default the report is named as Results of <analysis name>, and the report is timestamped with the date that the analysis was run. The first page of the report is always the Run Overview. The report options that you selected determine the contents of the remainder of the report. You can save the report in a location of your choosing, and you can change the default name when you save the report.

Figure 4-2: MaSTR Analysis report example

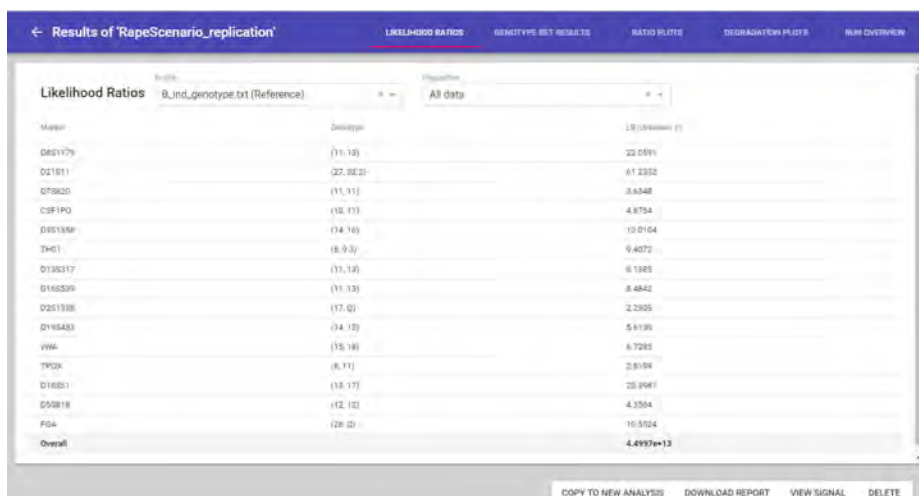


Reviewing Analysis Project Results

After an analysis job is completed, **Finished** is displayed in the Stage column on the Analysis Jobs tab for the job, along with the date and time that the job was finished. Before you generate and download the Analysis report, you always have the option of first opening the Results view for the analysis (double-click the finished analysis), and then reviewing the project results. See:

- Likelihood Ratios. See [“Likelihood Ratios report” on page 89](#).
- Genotype Results. See [“Genotype Results report” on page 90](#).
- Interactive plots. See [“Interactive reports \(Contributor Ratio, Degradation Variables, and Model Variables\)” on page 90](#).
- Run Overview. See [“Run Overview report” on page 95](#).

Figure 4-3: Results view for a selected analysis



In addition to reviewing specific analysis results, the following four options are always displayed at the bottom of every analysis Results tab:

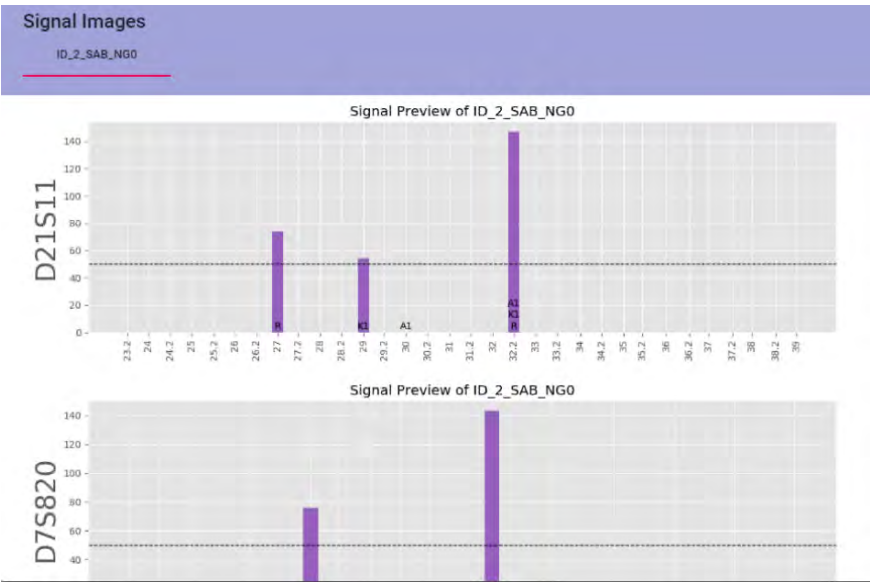
Option	Description
Copy to New Analysis	<p>Opens a New Analysis popup, with the settings pre-populated from the selected job.</p> <p>Tip: This option is useful for setting up new analyses when you have multiple similar jobs to run. Rather than setting up multiple new analysis jobs, you can simply edit the existing settings as needed, and then save these modified settings as a new analysis job.</p>

Option	Description
Download Report	Create and download the job results in report that is saved as a PDF. See Figure 4-2 on page 86 . Note: Unlike generating and downloading the report from the context menu for an analysis job, you do not have the option of selecting the information and populations that are to be included in the report. Instead, all report sections and all populations that are contained in the Population Frequency tables that were used for the analysis are included in the report. To select only specific information and/or populations that are to be included in the Analysis report, see “Generating and Downloading an Analysis Report” on page 83 .
View Signal	A signal file lists the alleles for specific individuals at the loci that are being tested and the signal (peak) height for each allele. The Signal plot shows the signal height for the alleles as a histogram. See “Signal plot” below.
Delete	Enabled only if your administrator has assigned the correct privileges to your user account. Click this option to immediately delete the analysis job from the MaSTR database.

Signal plot

A signal file lists the alleles for specific individuals at the loci that are being tested and the signal (peak) height for each allele. The Signal plot shows the signal height for the alleles as a histogram. Each peak is labeled with the genotypes that have the allele, where K# = Known, A# = Alternate, and R = Reference. The dotted line in the histogram indicates the analytical threshold that was set in the associated panel. Any peaks that fall below the analytical threshold are displayed in red.

Figure 4-4: Signal plot

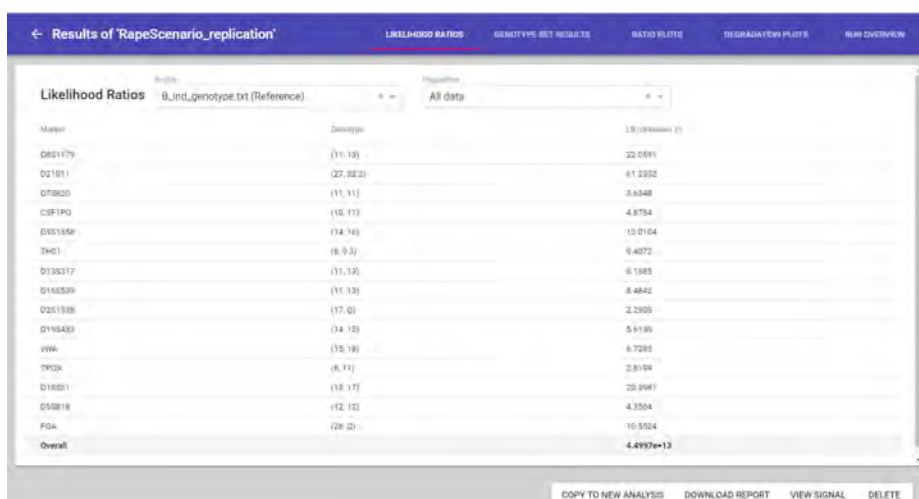


Likelihood Ratios report

When the Results view first opens for a selected analysis, the Likelihood Ratios tab is the open tab. The tab displays the reported likelihood ratio that was calculated from the weighted genotypes for each marker and the overall value. You must specify two variables to calculate the Likelihood Ratios report for a marker:

- Profile - Choose the profile for which to calculate the L/R ratio, either the Reference profile, any Alternate profile, or any detected profile from the Elimination database.
- Population - The population for which to calculate the L/R ratio.

Figure 4-5: Likelihood Ratios report for a selected profile and population



Marker	Genotype	LR (Hypothesis)
D8S1179	(11, 13)	22.0591
D21S11	(22, 23, 2)	61.2332
D7S820	(11, 11)	3.6348
CSF1PO	(10, 11)	4.8754
DSS18BP	(14, 14)	10.0164
TH01	(8, 9.3)	9.4072
D19S117	(11, 13)	6.1985
D16S539	(11, 13)	8.4842
D2S1338	(17, 2)	2.2905
D19S433	(14, 12)	5.9195
VWA	(15, 16)	6.7285
TP20X	(8, 11)	2.8198
D16S51	(13, 17)	20.9987
D5S818	(12, 12)	4.3504
FGA	(28, 2)	10.5524
Overall		4.4997e+13

If you select LR Plot vs Random for the profile, then the report displays the range for the overall LR for random profiles from each population, which are indicated by circles. The overall LR from any tested profile is shown as a box plot. The plot shows the expected range from randomly generated individuals for the population, with different colors for each population database.

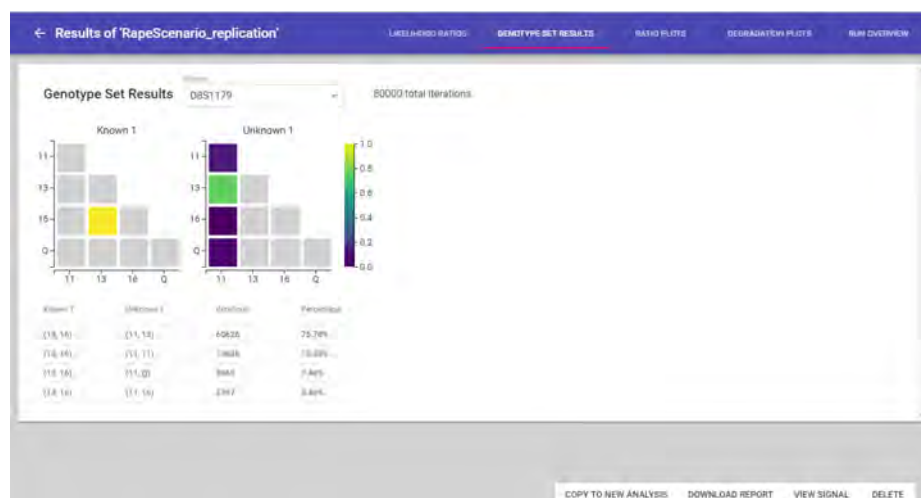
Figure 4-6: Likelihood Ratios report for random profiles from each population



Genotype Results report

The Genotype Results report is a heat map and a table of weighted genotypes for each locus. The report shows the relative probability of different genotype combinations based on how frequently these combinations were accepted during the MCMC process.

Figure 4-7: Genotype Set Results report



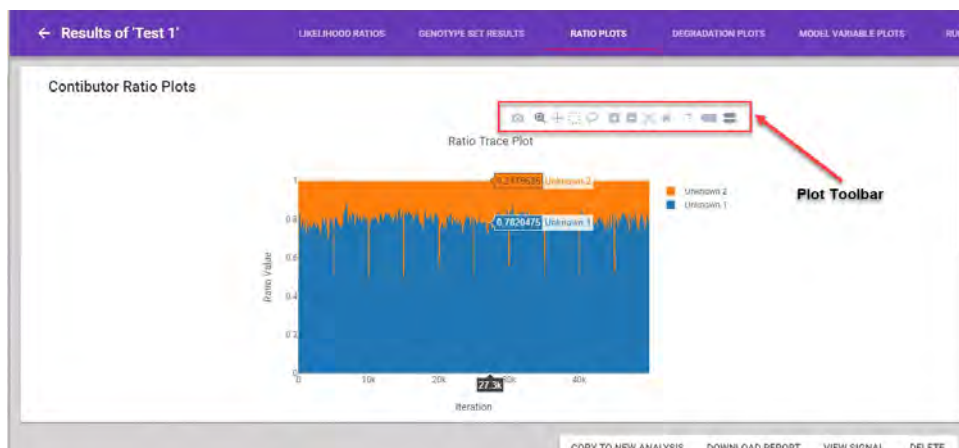
Interactive reports (Contributor Ratio, Degradation Variables, and Model Variables)

Up to three different interactive analysis reports are available for a finished analysis: a Contributor Ratio Plots report, a Degradation Variables Plots report, and a Model Variables Plots report. All three interactive reports have the following characteristics in common:

- All three reports display their relevant information in two plot types - a trace plot and a histogram plot. For example, the (Contributor) Ratio Plots report displays its information in the Ratio Trace plot, which shows the plot of all the individual contributor ratio values in each trace and the Ratio Histogram plot, which shows a histogram for each individual contributor ratio value. See [Figure 4-9 on page 93](#).
- Both plot types (Trace and Histogram) display 1,000 data points that have been evenly sampled from the trace. All the original data is stored in the MaSTR database, and these 1000 data points are used for efficiency when the plots are generated.
- If a plot legend is displayed to the right of a plot, then you can click on a symbol/key in the plot legend to show/hide specific information in the plot. For example, by default, when the Contributor Ratio plots are first generated and displayed, the plots display the ratio information for *every* individual contributor in the mix. To exclude the ratio information for a specific individual contributor in a plot, click the symbol/key for the contributor in the plot's legend. Click the symbol/key again to include the information.

- Both plot types (Trace and Histogram) have a toolbar. If you hold your pointer over any x/y point in a plot, then a toolbar is displayed above the plot and relevant information is displayed for the selected x/y point. For example, as shown in Figure 4-8 below, if you hold your pointer over any Contributor Ratio Trace plot, then a toolbar is displayed above the report and *all* individual contributor ratio values at the selected x/y point are displayed on the report.

Figure 4-8: Interactive Contributor Ratio Trace Plot report



- Some of or all the following icons are displayed on a Interactive report plot toolbar:

Table 4-2: Interactive report plot toolbar

















Icon	Description
	Download icon - Download the plot in .png format. The file is always downloaded as newplot.png. You can select a location in which to save the file and change the name of the file when you save it.
	Zoom icon - Zoom in or Zoom out the entire plot display: <ul style="list-style-type: none"> Zoom in - Hold down the left mouse button and draw a box from the upper left hand corner of a plot towards the lower right hand corner. A box is formed around the area that is being reduced for viewing. Zoom out - Hold down the left mouse button and draw a box from the lower right hand corner of a plot towards the upper left hand corner.
	Pan icon - Shifts the view of a plot without changing the viewing direction or magnification. Place your pointer at the start location, press the left mouse button down, and then drag the pointer to the new location.
	Box Select icon - Select a box-shaped area of a plot for focus. Hold down the left mouse button and drag the pointer from any point on a plot towards another point on the plot. A box is formed around the area that is being selected and the focus is placed on the selected area.
	Lasso Select icon - Select a free-form area of a plot for focus. Hold down the left mouse button and drag the pointer from any point on a plot towards another point on the plot. A free-form outline is displayed around the area that is being selected and the focus is placed on the selected area.

Table 4-2: Interactive report plot toolbar (Continued)

Icon	Description
	Zoom In icon - Click this icon repeatedly to zoom in on the plot display.
	Zoom Out icon - Click this icon repeatedly to zoom in on the plot display.
	Autoscale icon - Click this icon and then click and hold your pointer on a plot. The pointer changes to a Move pointer  . Move the pointer to drag the plot in any direction. As you move the plot, the axes are automatically rescaled to reflect the area of the plot that you have dragged in to focus.
	Toggle Spike Lines icon - Turned off by default. To turn on Toggle Spike Lines, click the icon. When you hold the pointer on a plot, the pointer changes to a Move pointer  . As you move the pointer around the plot, Spike Lines that delineate the information that is in focus are displayed. A tooltip also opens that shows the relevant value at the farthest x/y point for the in focus area. For example, for a Contributor Ratio plot, the tooltip shows the individual contributor ratio value at the farthest x/y point for the area that is in focus. To turn off Spike Lines, click this icon again.
	Show Closest Data on Hover icon - Click this icon and then hold the pointer on a plot. When you hold the pointer on a plot, the pointer changes to a Move pointer  . As you move the pointer around the plot, a tooltip opens that shows a relevant value at the nearest x/y point on the plot. For example, for a Contributor Ratio plot, the tooltip shows a single individual contributor ratio value at the nearest x/y point on the plot.
	Compare Data on Hover icon - Click this icon and then hold the pointer on a plot. When you hold the pointer on a plot, the pointer changes to a Move pointer  . As you move the pointer around the plot, multiple tooltips open, with each tooltip showing the relevant value at the nearest x/y point on the plot. For example, for a Contributor Ratio plot, each tooltip shows an individual contributor ratio value at the nearest x/y point on the plot.

Note the following about a plot toolbar and its icons:

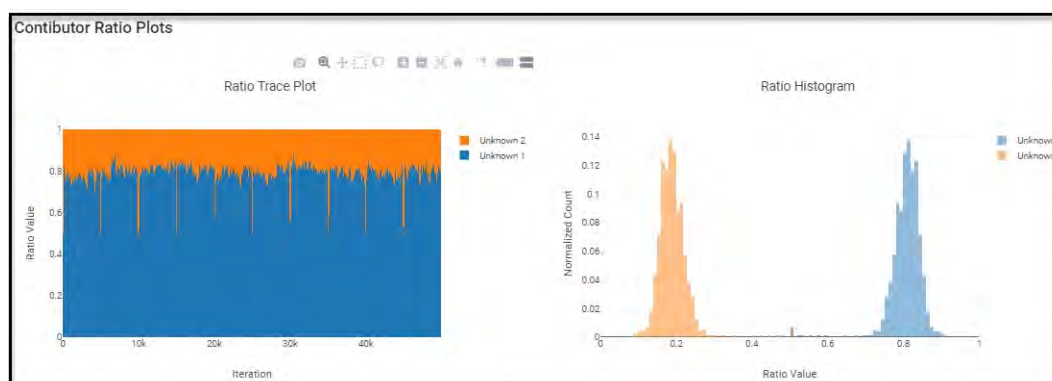
- If the plot toolbar is not displayed, then to display it again, hold the pointer (or you might have to click the pointer) in the location that you would expect to see it displayed.
- You can hold the pointer over an icon on the toolbar to open a tooltip that displays the icon name.
- For some plots, you can use the icon functions in conjunction with each other, but you must be aware of the dependencies for some of these features. For example, for a Contributor Ratio plot:
 - You can use the Lasso icon and the Zoom In icon to zoom in on a selected area of a plot.
 - You can turn on Spike Lines in conjunction with the Show Closest Data on Hover feature or the Compare Data on Hover feature, but the selected data feature takes precedence over the Spike Lines feature. (A tooltip shows the data for the nearest x/y point on the plot, not the farthest point x/y point.)

- If you change any of the default values for a plot, such as the zoom level or focus, then to reset the plot to its default display values at any time, click the Reset  icon.

Ratio Plots report

The (Contributor) Ratio Plots report is a summary of the sampled ratio values for each contributor.

Figure 4-9: Contributor Ratio plots



Degradation Variable Plots report

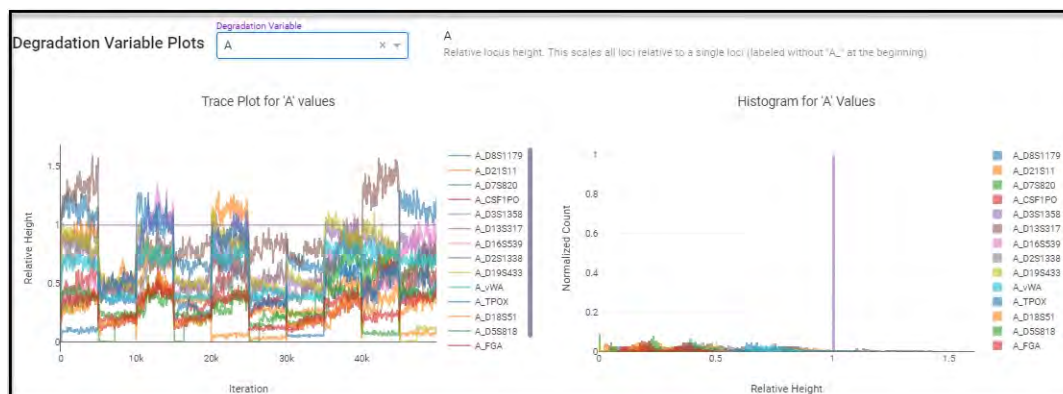
If degradation was considered during the MCMC process when calculating probability values, then the Degradation Variable plots show the values for the degradation variables. The four variable that can be plotted are the following:

Variable	Definition
amount	The amount Variable plot scales all peak (signal) heights for a locus from a relative height to an absolute height. All expected peak heights therefore have real values (645, 992, 82, and so on) instead of relative values (0.5375, 0.8267, 00725, and so on).
mu_A	The mu_A Variable plot displays the average value of A, where “A” is the relative locus height.
A	The Relative Locus Height Variable plot scales all the loci <i>relative</i> to a single loci. This single loci is designated without the “A” prefix in the plot legend. Note: One value for each marker. The value for one marker is set to 1.0 and the values for all other markers are set relative to this maker. Because some markers can amplify more efficiently or less efficiently than other markers, the “A” variable allows for each marker to have a different peak height, given some number of copies of an allele and all other factors being equal.
D	The Degradation Factor Variable plot scales down the peak (signal) heights of larger alleles, with a different amount of scaling per contributor. The D variable has one value per contributor, and in the calculations, linear or exponential degradation is dependent on molecular weight.
See Figure 4-10 on page 94 for an example Degradation Variable plot.	

Chapter 4

Reporting and Graphing DNA Analysis Results in MaSTR

Figure 4-10: Degradation Variable plot example (A variable)



- Model Variable Plots report - The Model Variable plots tab is displayed for the Results view only if the model that was used for the MCMC process contained custom model variables that were sampled. (See [Chapter B, “Building a Custom MCMC Model for Probabilistic Mixture Analysis,”](#) on page 121.) At the top of the Model Variables tab, you can select the variable for which to generate the plots. You can limit the plots to specific contributors, markers, and/or alleles. For example, in [Figure 4-11](#) below, the plot is for a model variable that has a different value for each contributor and marker, and the results have been filtered to show the results only for D1S1656.

Figure 4-11: Model Variable plot



Run Overview report

The Run tab displays the Run Overview report. The Run Overview report summarizes key points about the finished analysis, including the Protocol Set, Panel, Frequency, and Model that were used, the run name and any run comments, the files (Signal, Reference, Known, and/or Alternate) that were analyzed, the number of contributors, and other information.



If you generate and download the Analysis report, then the Run Overview is always the first page of the report and you cannot exclude this information from the report. See [Figure 4-2 on page 86](#).

Figure 4-12: Run Overview report



Chapter 4

Reporting and Graphing DNA Analysis Results in MaSTR

Appendix A

MaSTR Administration

This appendix guides you through the procedures that are necessary for administering your MaSTR installation efficiently and effectively, including viewing the audit trail, viewing and managing current MaSTR sessions, managing users and groups, backing and restoring the MaSTR database, and managing the settings for the MaSTR user interface (UI).

This appendix covers the following topics:

- [“Admin Mode Default User Accounts” on page 99.](#)
- [“Viewing and Downloading the MaSTR Audit Trail” on page 100.](#)
- [“Viewing and Managing Active Sessions” on page 103.](#)
- [“Managing Groups in MaSTR” on page 105.](#)
- [“Managing Users in MaSTR” on page 110.](#)
- [“Managing the MaSTR Databases” on page 115.](#)
- [“Customizing the Default Report and View Displays” on page 118.](#)

Appendix A
MaSTR Administration

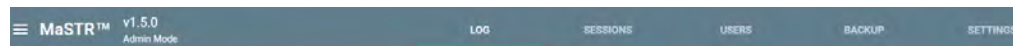
Admin Mode Default User Accounts

The Admin mode is the only mode in MaSTR that provides the access to all the functions that are necessary for administering and maintaining your MaSTR installation, including audit trails, user management, and database management. Two default user accounts that can log in to the Admin mode are provided as part of the MaSTR installation: root and admin.

- root account - The root account provides access not only to the four standard Admin mode functions (Log, Sessions, Users, and Settings) but also to the MaSTR Database functions, including backing up and restoring the MaSTR database. The root account is the *only* admin account with access to the MaSTR database functions. The root account also has the ability to access any APIs. Further, although the root account has access to user management functions, the account itself is not visible in user management, which means that other users cannot edit its permissions or password, or delete the account.

The default username and password for the root account are root and changeThisPassword. As the MaSTR administrator, as soon as it is feasibly possible for you to do so, you should change the password for the root account. When you change this password, make sure to store it in a secure place, as the password is not recoverable.

Figure A-1: MaSTR title bar and tabs for root account login



- admin account - The admin account provides access to the four standard Admin mode functions (Log, Sessions, Users, and Settings). The default username and password for the admin account are Admin and admin. As the MaSTR administrator, as soon as it is feasibly possible for you to do so, you should either change the password for the account, or after you set up accounts for your actual users, at a minimum, change the account password, or delete the account.

Figure A-2: MaSTR title bar and tabs for admin account login



Viewing and Downloading the MaSTR Audit Trail

In MaSTR, a *session* is a temporary and interactive information interchange between the MaSTR server computer and a user. MaSTR records the following information for every API request during every MaSTR session that resulted in a change to the information in a MaSTR database:

- Date/timestamp of the request.
- Host name - The name of MaSTR server on which the request was made.
- IP address - The local IP address of the machine from which the request was made.
- Username and password for the user who made the request.
- Login mode for the user - Admin, Research, Casework, Review.
- Session ID - The ID for the session during which the request was made.

Collectively, this information is referred to as an *audit trail*. You can view the entire audit trail up and to and including the current day's date and time for a MaSTR installation in the Audit Trail view. You can also download the same audit trail from this tab to a tab-separated values file that, by default, is named MaSTR_Audit_Trail<download date> (for example, MaSTR_Audit_Trail_8_6_2018), and then work with this file independently as appropriate.

To view and download the MaSTR audit trail

1. Open and log in to MaSTR under the Admin mode.
2. On the MaSTR title bar, click Audit.

The Audit Trail view opens. The view has a single tab, the Audit Trail tab. The tab displays the audit information for every API request during every session for the MaSTR installation that resulted in a change to the information in a MaSTR database. The information is sorted by session date/time in reverse chronological order.

Figure A-3: Audit Trail view, Audit Trail tab

Datetime ↓	Host	IP	User	Action	Mode	Session
11/9/2018, 5:38:47 PM	localhost	10.0.2.2	Admin	User Login	admin	b822ee4-9ead-44bd-68b5-072d081b3be9
11/9/2018, 3:47:29 PM	localhost	10.0.2.2	Admin	User Login	research	02662e9a-234c-4523-be84-5d3c7e2b25d0
11/9/2018, 3:47:16 PM	localhost	10.0.2.2	Admin	POST /sys/settings	admin	3d045749-9411-4f87-8b1d-515cae450750
11/9/2018, 3:44:08 PM	localhost	10.0.2.2	Admin	POST /sys/settings	admin	3d045749-9411-4f87-8b1d-515cae450750
11/9/2018, 3:43:33 PM	localhost	10.0.2.2	Admin	User Login	admin	3d045749-9411-4f87-8b1d-515cae450750
11/9/2018, 3:43:14 PM	localhost	10.0.2.2	Admin	User Login	research	ce2edc2f-4a23-4501-8ed0-b252028cc2dc
11/9/2018, 3:43:01 PM	localhost	10.0.2.2	Admin	POST /sys/settings	admin	77b011cc-337c-46b7-b706-78f1822c149a
11/9/2018, 3:42:02 PM	localhost	10.0.2.2	Admin	User Login	admin	77b011cc-337c-46b7-b706-78f1822c149a
11/9/2018, 3:41:46 PM	localhost	10.0.2.2	Admin	User Login	research	44fb4835-b0b5-4d0a-b429-65b1b7c3df33
11/9/2018, 3:41:30 PM	localhost	10.0.2.2	Admin	User Login	admin	6d837c22-9d32-4acc-8995-2ae732170d5e



By default, 10 rows per page are displayed, but remember, you can always select a different value. See [“Number of rows displayed per page feature” on page 32](#).

3. Optionally, to filter the Audit view, in the Filter field, enter a filter string.

The string is sensitive to User, Action, and mode.



For information about filtering a view, see [“Filter feature” on page 33](#).

4. Optionally, to download the audit trail, do the following:

- a. Click Download.

The Download Audit Trail popup opens.

Figure A-4: Download Audit Trail popup

- b. Optionally, to filter the results in the tab-separated values file, enter a filter string in the Filter field. See [Figure A-5 on page 102](#).
- c. Do one of the following:
 - i To download the *entire* audit trail up and to and including the current day’s date and time to a tab-separated values file, click Download.
 - i To download only specific information for the audit trail to a tab-separated values file, specify the appropriate information and then click Download. For example, to download all the audit records up and to and including the current day’s date and time only for Admin users, clear all users other than Admin, and then click Download.

Appendix A
MaSTR Administration

Figure A-5: Filtered Audit Trail file

The screenshot displays the MaSTR interface with the 'Download Audit Trail' dialog box open. The dialog box has a 'Date Range' section with 'Begin' set to 10/28/2018 and 'End' set to 11/28/2018. A red box highlights the 'Add a job' button in the top right corner of the dialog. A red arrow points from this button to the 'Action' column of the audit trail file in the background Excel spreadsheet.

The background Excel spreadsheet, titled 'MaSTR_Audit_Trail_11_28_2018 (1).tsv', shows a filtered audit trail file with the following data:

Datetime	Host	IP	User	Action	Mode	Session
11/14/201	emma	10.0.2.2	Admin	Add a Job	research	dcaf7775-ed9d-4122-ada9-35f012ea1555
11/14/201	emma	10.0.2.2	Admin	Add a Job	research	dcaf7775-ed9d-4122-ada9-35f012ea1555
11/14/201	emma	10.0.2.2	Admin	Add a Job	research	dcaf7775-ed9d-4122-ada9-35f012ea1555
11/14/201	emma	10.0.2.2	Admin	Add a Job	research	dcaf7775-ed9d-4122-ada9-35f012ea1555
11/14/201	emma	10.0.2.2	Admin	Add a Job	research	dcaf7775-ed9d-4122-ada9-35f012ea1555
11/14/201	emma	10.0.2.2	Admin	Add a Job	research	dcaf7775-ed9d-4122-ada9-35f012ea1555
11/14/201	emma	10.0.2.2	Admin	Add a Job	research	dcaf7775-ed9d-4122-ada9-35f012ea1555
11/14/201	emma	10.0.2.2	Admin	Add a Job	research	dcaf7775-ed9d-4122-ada9-35f012ea1555
11/14/201	emma	10.0.2.2	Admin	Add a Job	research	dcaf7775-ed9d-4122-ada9-35f012ea1555
11/14/201	emma	10.0.2.2	Admin	Add a Job	research	dcaf7775-ed9d-4122-ada9-35f012ea1555
11/14/201	emma	10.0.2.2	Admin	Add a Job	research	dcaf7775-ed9d-4122-ada9-35f012ea1555
11/14/201	emma	10.0.2.2	Admin	Add a Job	research	dcaf7775-ed9d-4122-ada9-35f012ea1555
11/14/201	emma	10.0.2.2	Admin	Add a Job	research	dcaf7775-ed9d-4122-ada9-35f012ea1555
11/14/201	emma	10.0.2.2	Admin	Add a Job	research	dcaf7775-ed9d-4122-ada9-35f012ea1555

Viewing and Managing Active Sessions

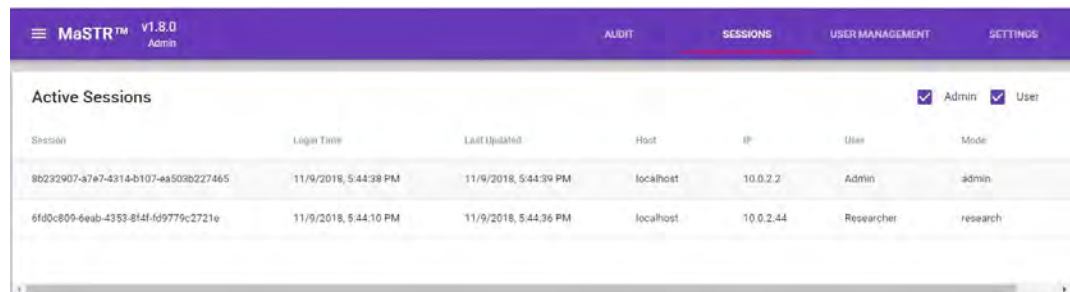
In MaSTR, a *session* is a temporary and interactive information interchange between the MaSTR server computer and a user. You can view all the currently active sessions for all users (admin or user) for a MaSTR installation on the Sessions tab in the Admin view. Optionally, from this tab you can filter the view to limit the display to sessions only for admin users or only for “standard” users, and you can also “kill” an active session for any user. If you “kill” an active session for a user, then the user is immediately logged out of MaSTR. Any work that user has *not* saved is lost; however, none of the user’s queued jobs or jobs that were already running on the MaSTR server are affected.

To view and manage active sessions

1. Open and log in to MaSTR under the Admin mode.
2. On the MaSTR title bar, click Sessions.

The Sessions tab opens. The tab displays all the currently active sessions for all users (admin or user) in reverse chronological order (based on the user login time) for the MaSTR installation.

Figure A-6: Admin view, Active Sessions tab



Session	Login Time	Last Update	Host	IP	User	Mode
8b292907-a7e7-4314-b107-aa509b227465	11/9/2018, 5:44:39 PM	11/9/2018, 5:44:39 PM	localhost	10.0.2.2	Admin	admin
6f00c809-6eab-4353-8f4f-499779c2721e	11/9/2018, 5:44:10 PM	11/9/2018, 5:44:36 PM	localhost	10.0.2.44	Researcher	research



Figure A-6 shows two active sessions: an admin session from the current computer and a “Researcher” user who is logged in to the research database from another computer. By default, 20 rows per page are displayed, but remember, you can always select a different value. See “Number of rows displayed per page feature” on page 32.

3. Optionally, do any of the following as appropriate:
 - To filter the display to only active sessions for Admin users, clear the User option.
 - To filter the display to only active sessions for “standard” users, clear the Admin option.
 - To kill an active session for any user, click the “x” that is displayed to the far right of the session entry, and then in the Kill session popup that opens, click Yes. See [Figure A-7 on page 104](#).

Figure A-7: Kill Session popup



The Kill session dialog box closes, and the Sessions tab display is immediately updated to reflect the killing of the session. (The session entry is removed from the tab.)

Managing Groups in MaSTR

Users are the people who log into MaSTR, whether they are adding and reviewing content, or just using MaSTR in a read-only capacity. User management in MaSTR is based on the concept of every user being assigned to a *group*, where a group is a collection of users who have the same permissions. Four default users, three default groups, and three login modes* are provided with your MaSTR installation. The Admin login mode determines the admin permissions that be assigned to a user on an individual basis (regardless of any groups to which the user belongs), while the Research and Casework login modes determine the databases—Research or Casework—to which a group, and therefore the users in a group, have access. All three default groups—Analyst, Manager, and Researcher—are available for assignment to the Research or Casework login mode.

- Analyst - Grants access to all the permissions that are required to run and view jobs; however, does not grant access to the permissions for updating or deleting jobs or the permissions for adding, updating, or deleting the associated job files (panels, frequencies, models, and SOPs).
- Manager - Grants access to all permissions in the database (Research and/or Casework) for which it is enabled, including User Administrator, which grants permission to the Admin UI for adding, editing, and deleting groups and users in MaSTR.
- Researcher - With the exception of deleting jobs, grants access to all permissions in the database (Research and/or Casework) for which it is enabled, including User Administrator, which grants permission to the Admin UI for adding, editing, and deleting groups and users in MaSTR.

[Table A-1](#) details these default MaSTR group permissions.

Table A-1: Default MaSTR group permissions

Default Group	Default Permissions					
	Add Permissions		Update Permissions		Delete Permissions	
Researcher	x	Add an Elimination Profile	x	Update an Elimination Profile	x	Delete an Elimination Profile
	x	Add a Frequency	x	Update a Frequency	x	Delete a Frequency
	x	Add a Model	x	Update a Model	x	Delete a Model
	x	Add a Panel	x	Update a Panel	x	Delete a Panel
	x	Add a Protocol Set	x	Update a Protocol Set	x	Delete a Protocol Set
	x	Add a Job	x	Update a Job		Delete a Job
Manager	x	Add an Elimination Profile	x	Update an Elimination Profile	x	Delete an Elimination Profile
	x	Add a Frequency	x	Update a Frequency	x	Delete a Frequency
	x	Add a Model	x	Update a Model	x	Delete a Model
	x	Add a Panel	x	Update a Panel	x	Delete a Panel
	x	Add a Protocol Set	x	Update a Protocol Set	x	Delete a Protocol Set

Table A-1: Default MaSTR group permissions (Continued)

Default Group	Default Permissions					
	Add Permissions		Update Permissions		Delete Permissions	
	x	Add a Job	x	Update a Job	x	Delete a Job
Analyst		Add an Elimination Profile		Update an Elimination Profile		Delete an Elimination Profile
		Add a Frequency		Update a Frequency		Delete a Frequency
		Add a Model		Update a Model		Delete a Model
		Add a Panel		Update a Panel		Delete a Panel
		Add a Protocol Set		Update a Protocol Set		Delete a Protocol Set
	x	Add a Job		Update a Job		Delete a Job



**A fourth login mode, Demo, is also provided and all permissions are granted in this mode; however, the Demo database is not a permanent database. Instead, it is immediately cleared after a user logs out.*

A user can be assigned to one or more groups for both the Research database and the Casework database. Because the different groups have different permissions, and a user can belong to more than one group for both the Research database and the Casework database, a user's potential access to a given MaSTR database is the sum of the database access for all the groups to which the user belongs. [Table A-2](#) summarizes this information.

Table A-2: Default MaSTR user permissions and database access

Default User	Default Admin Permissions and Default Database Access (Login mode)					
	Admin		Research		Casework	
Admin	x	Change Own Password	x	Analyst	x	Analyst
		Change Server Settings	x	Manager	x	Manager
		User Administrator		Researcher		Researcher
Analyst	x	Change Own Password	x	Analyst	x	Analyst
	x	Change Server Settings		Manager		Manager
		User Administrator		Researcher		Researcher
Researcher	x	Change Own Password		Analyst		Analyst
	x	Change Server Settings		Manager		Manager
		User Administrator	x	Researcher	x	Researcher
Reviewer	x	Change Own Password		Analyst		Analyst

Table A-2: Default MaSTR user permissions and database access (Continued)

Default User	Default Admin Permissions and Default Database Access (Login mode)					
	Admin		Research		Casework	
		Change Server Settings		Manager		Manager
		User Administrator		Researcher		Researcher

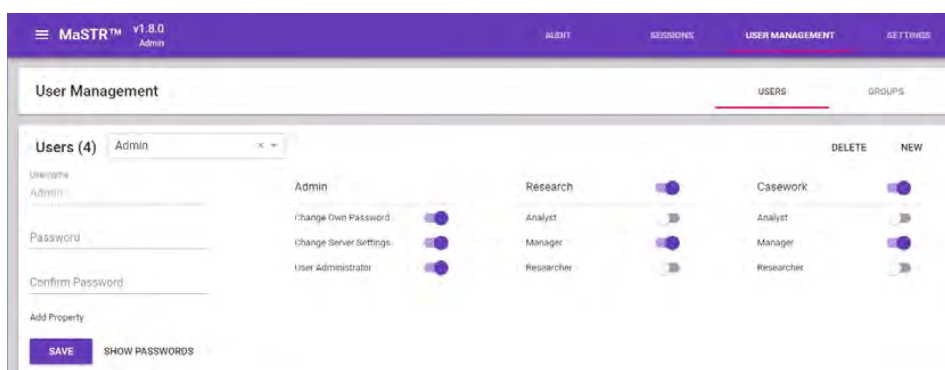
If you are the root user, or a user with the correct admin privileges (admin user) assigned, then you are responsible for managing groups for your MaSTR installation. Managing groups in MaSTR consists of adding new groups, editing existing groups, and deleting groups.

To manage groups in MaSTR

1. Open and log in to MaSTR under the Admin mode.
2. On the MaSTR title bar, click User Management.

The User Management view opens. The view has two tabs - the Users tab, which is the open tab, and the Groups tab.

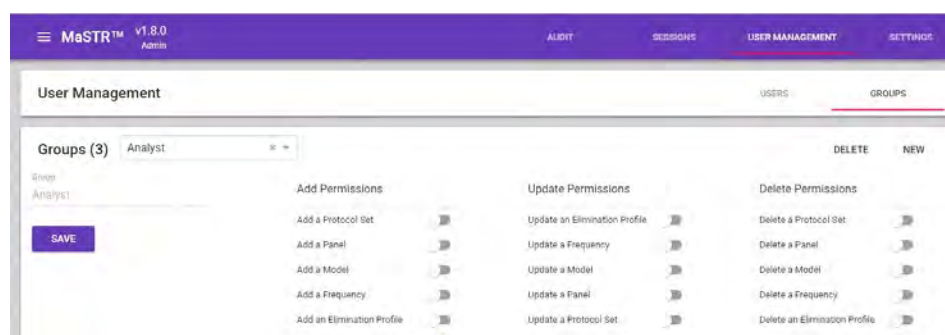
Figure A-8: User Management view, Users tab



3. Click Groups.

The Groups tab opens.

Figure A-9: User Management view, Groups tab



4. Continue to one of the following:
 - [“To add a group”](#) below.
 - [“To edit a group”](#) below.
 - [“To delete a group”](#) on page 109.

To add a group

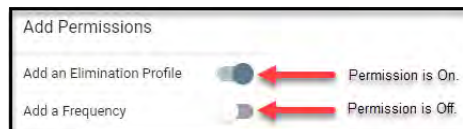
1. Click New.

All fields and permissions on the Groups tab are cleared.
2. Enter the information for the new group.
 - Click in the Group field, and then enter the group name.
 - Set the permissions for the group.



All group permissions are toggles. Click a permission once to turn on the permission for a group (the toggle filled with a solid color). Click the toggle again to turn off the permission for the group (the toggle is white.) See [Figure A-10](#) below.

Figure A-10: Toggling a permission on and off



3. Click Save.

To edit a group

You can edit only the permissions for a group and the group can be a default group, or any other group.



You cannot edit a group name. To “edit” a group name, you must delete the group, and then add the group with its new name. See [“To delete a group”](#) below.

1. On the Groups dropdown list, select the group for which you are editing the permissions.
2. Edit the permissions for the group as needed.



Remember, all group permissions are toggles. Click a permission once to turn on the permission for a group (the toggle is filled with a solid color). Click the toggle again to turn off the permission for the group (the toggle is white.) See [Figure A-10 on page 108](#).

3. Click Save.

To delete a group

You can delete any default group or other group MaSTR. If you delete all the groups to which a user belongs, then unless the user's access to a database has been completely disabled, the user will still have basic login and viewing permissions as part of the built-in User group.

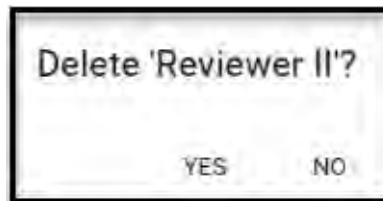


To edit the name for a group, you must delete the group, and then create a new group with a different group name. See [“To add a group” on page 108](#).

1. On the Groups dropdown list, select the group that you are deleting.
2. Click Delete.

A Delete <Group> popup opens, prompting you to confirm the deletion.

Figure A-11: Delete Group confirmation popup



3. Click Yes.

The group is deleted from the MaSTR database, and the Delete Group confirmation popup closes after the group is deleted. The Groups tab displays the information for the next group on the Groups dropdown list.

Managing Users in MaSTR

Users are the people who log into MaSTR, whether they are adding and reviewing content, or just using MaSTR in a read-only capacity. If you are the root user, or a user with the correct admin privileges (User Administrator) granted, then you are responsible for managing the users for your MaSTR installation. Managing users in MaSTR consists of adding new users, editing existing users, and deleting users.



Remember, you can also view the activity for all your MaSTR users on the Audit tab. See [“Viewing and Downloading the MaSTR Audit Trail” on page 100](#).

To add a new user

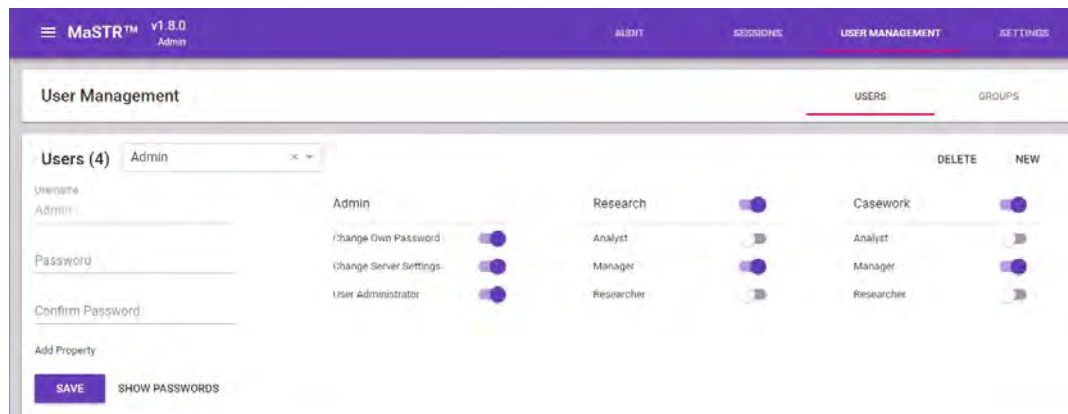
When you add a new user, you must specify the login credentials (username and password) for the user. When you are adding a new user, you have the option of adding miscellaneous properties for the user, for example, Full Name and Title. These miscellaneous properties are displayed for reference purposes only. MaSTR does not use these properties in any way when determining a user's access and permissions.

You can grant the permissions for the user only after you specify the username and password and then save the user. When you save the new user, by default, no Admin permissions are granted, and the Research and Casework login modes are selected, but no groups are selected for these login modes. If you do not assign the user to any group, then unless you disable the user's access to a database (Research or Casework), the user will still have basic login and viewing permissions as part of the built-in User group.

1. Open and log in to MaSTR under the Admin mode.
2. On the MaSTR title bar, click User Management.

The User Management view opens. The view has two tabs - the Users tab, which is the open tab, and the Groups tab.

Figure A-12: *User Management view, Users tab*



3. Click New.

All fields and permissions on the Users tab are cleared.

4. Enter the information for the new user.

- a. Click in the Username field, and then enter the login name for the user.
- b. In the Password field, enter the login password for the user.



The password must have a minimum of 12 characters and cannot contain any spaces. Ideally, however, you should also ensure that the password adheres to any password standards that your organization requires such as the number of uppercase or special characters, and so on.

- c. In the Confirm Password field, enter the user password again, exactly as you entered it in the Password field.



When you are adding a new user, you should click Show Passwords to view the passwords and ensure that the passwords match in both fields. After you save the user, you can click Hide Passwords to ensure the security of the password.

5. Optionally, to add miscellaneous properties for the user, do the following:

- a. Click Add Property.

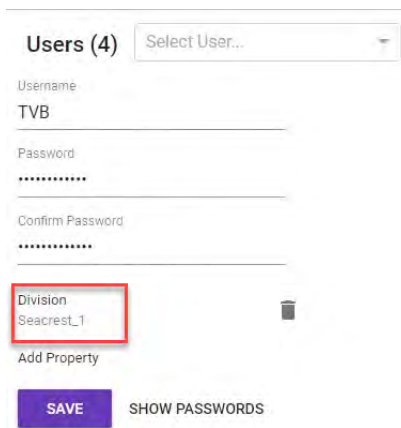
The Add a Property popup opens.

Figure A-13: Add a Property popup

- b. In the Name field, enter a name for the property.
- c. In the Value field, enter the property value,
- d. Click Add.

The Add a Property popup closes. The property is displayed below the Confirm Password field. See [Figure A-14 on page 112](#).

Figure A-14: User with a miscellaneous property



The screenshot shows a user management form. At the top, there is a dropdown menu labeled 'Users (4)' with a 'Select User...' option. Below this are input fields for 'Username' (containing 'TVB'), 'Password' (masked with dots), and 'Confirm Password' (also masked). A red rectangle highlights the 'Division' dropdown menu, which currently shows 'Seacrest_1'. Below the dropdown is an 'Add Property' button. At the bottom of the form are two buttons: a purple 'SAVE' button and a 'SHOW PASSWORDS' link.



At any time, you can click the Delete icon that is displayed next to a user property to delete the property without affecting any of the permissions or groups for the user.

6. Click Save.

The user is saved and the user is displayed on the Users dropdown list. By default, no Admin permissions are granted, and the Research and Casework login modes are selected, but no groups are selected for these login modes, and therefore, no permissions have been granted to the user.

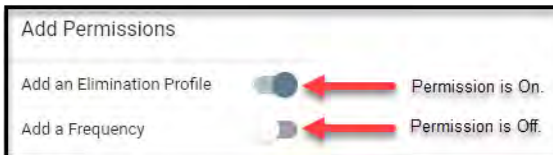
7. Grant the permissions for the user.

- For the Admin login mode, grant the appropriate admin permissions for the user. If you grant the User Administrator permission, then the user has full access to the Admin interface, including modifying the permissions and passwords not only for his/her own account but also for the accounts for all other users with the exception of the root account. See [“Admin Mode Default User Accounts” on page 99](#).
- For the Research login mode - To grant additional Research database privileges, turn on the appropriate roles. [Table A-1 on page 105](#) details these default MaSTR group permissions.
- For the Casework login mode - To grant additional Casework database privileges, turn on the appropriate roles. [Table A-1 on page 105](#) details these default MaSTR group permissions.



All user permissions are toggles. Click a permission once to turn on the permission for a user (the toggle is filled with a solid color). Click the toggle again to turn off the permission for the user (the toggle is white.) See [Figure A-15 on page 113](#).

Figure A-15: Toggling a permission on and off



8. Click Save.

To edit a user

You can edit only the permissions for a user and the user can be one of the default users, or any other user.



You cannot edit a user name. To “edit” a user name, you must delete the user, and then add the user with a new name. See [“To delete a user” on page 113](#).

1. Open and log in to MaSTR under the Admin mode.
2. On the MaSTR title bar, click User Management.

The User Management view opens. The view has two tabs - the Users tab, which is the open tab, and the Groups tab.

3. On the Users dropdown list, select the user for which you are editing the permissions.
4. Edit the permissions for the user as needed.



Remember, all group permissions are toggles. Click a permission once to turn on the permission for a group (the toggle is filled with a solid color). Click the toggle again to turn off the permission for the group (the toggle is white.) See [Figure A-10 on page 108](#).

5. Click Save.

To delete a user

You can delete any default user or other user in MaSTR.



To edit the name for a user, you must delete the user, and then create a new user with a different user name. See [“To add a new user” on page 110](#).

1. Open and log in to MaSTR under the Admin mode.
2. On the MaSTR title bar, click User Management.

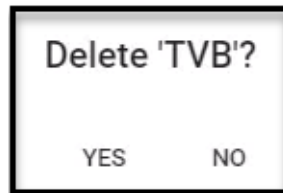
The User Management view opens. The view has two tabs - the Users tab, which is the open tab, and the Groups tab.

Appendix A
MaSTR Administration

3. On the Users dropdown list, select the user whom you are deleting.
4. Click Delete.

A Delete <User> popup opens, prompting you to confirm the deletion.

Figure A-16: Delete User confirmation popup



5. Click Yes.

The user is deleted from the MaSTR database, and the Delete User confirmation popup closes after the group is deleted. The Users tab displays the information for the next user on the Users dropdown list.

Managing the MaSTR Databases

The root user is the only user with the ability to manage the MaSTR databases, which includes backing up the full MaSTR database, validating an archived database before restoring it, and restoring an archived database.

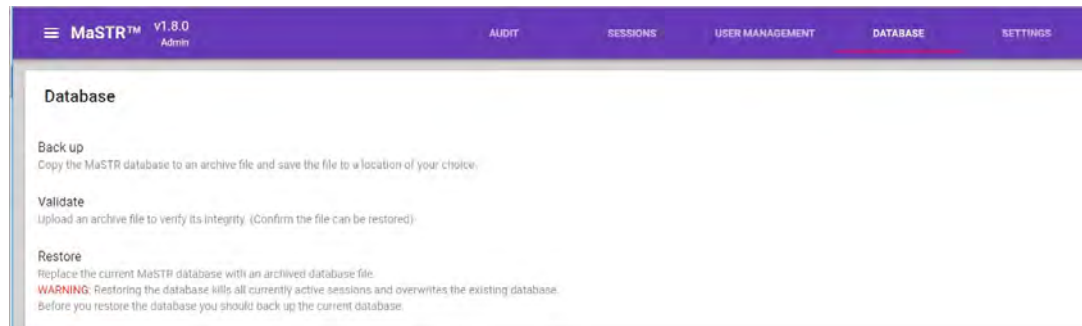
To back up the MaSTR database

When you back up the MaSTR database, it is a full backup. You do not have the option of incremental or partial backups. Creating an appropriate backup schedule should not hamper daily activities for MaSTR database users while ensuring that the backup file holds the most current information. An ideal backup schedule would for the full database would be during the time when the database is the most inactive.

1. Open and log in to MaSTR under the Admin mode as root.
2. On the MaSTR title bar, click Database.

The Database view opens. The view has three options: Back Up, Validate, and Restore.

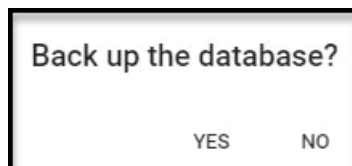
Figure A-17: Database view



3. Click Back Up.

A Back up the database popup opens.

Figure A-18: Back up the database popup



4. Click Yes.

The popup closes and the backup is started. When the backup is complete, an archived file named MaSTR_<backup date>_<backup time> is generated and downloaded. You should store this file in a safe but accessible location in the event that you must use it to restore the MaSTR database.

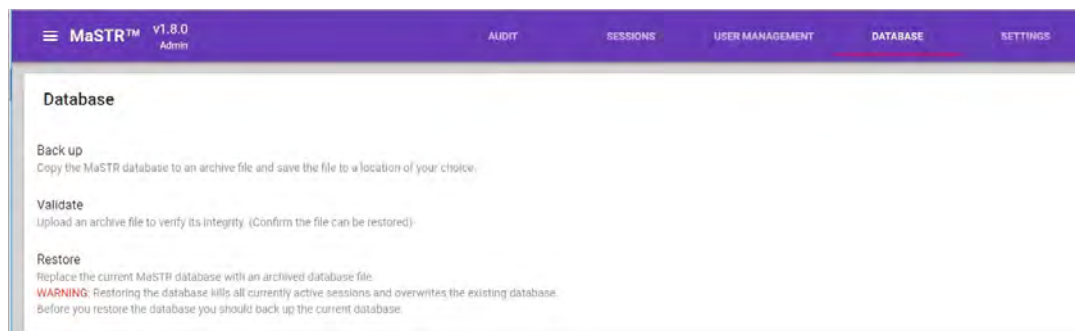
To validate an archived database

Before you restore the MaSTR database from an archived file, you should always validate the file (verify its integrity) to ensure that it can be restored without any errors.

1. Open and log in to MaSTR under the Admin mode as root.
2. On the MaSTR title bar, click Database.

The Database view opens. The view has three options: Back Up, Validate, and Restore.

Figure A-19: Database view



3. Click Validate.
- The Open dialog box opens.
4. In the Open dialog box, browse to and select the archived file that you are validating, and then click Open.

The Open dialog box closes, and MaSTR validates the selected file. If the file is valid, then the message “No Errors detected in the <filename>.archive” are detected. You can now use this file to safely restore the MaSTR database. See [“To restore the MaSTR database”](#) below.

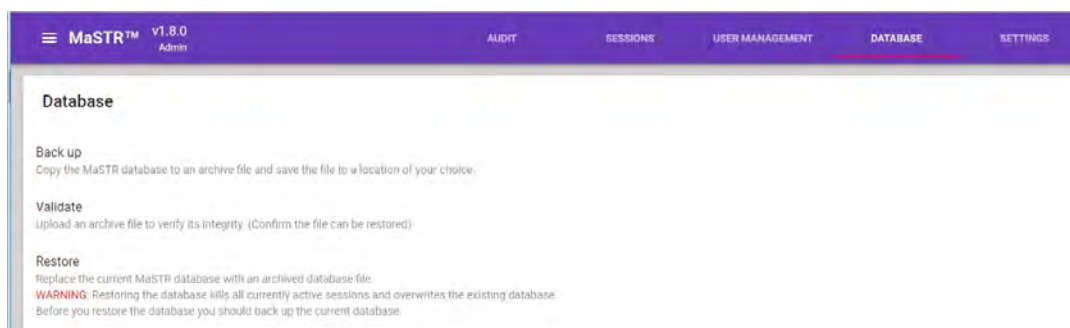
To restore the MaSTR database

When you restore the database, all active sessions are immediately stopped and any current data is overwritten by the restored data. Restoring a database should not hamper daily activities for your MaSTR database users while ensuring that the backup file holds the most current information possible. You should always restore the MaSTR database only after your users have logged off, and only from a validated archived file. (See [“To validate an archived database”](#) on page 116.)

1. Open and log in to MaSTR under the Admin mode as root.
2. On the MaSTR title bar, click Database.

The Database view opens. The view has three options: Back Up, Validate, and Restore. See [Figure A-20 on page 117](#).

Figure A-20: Database view



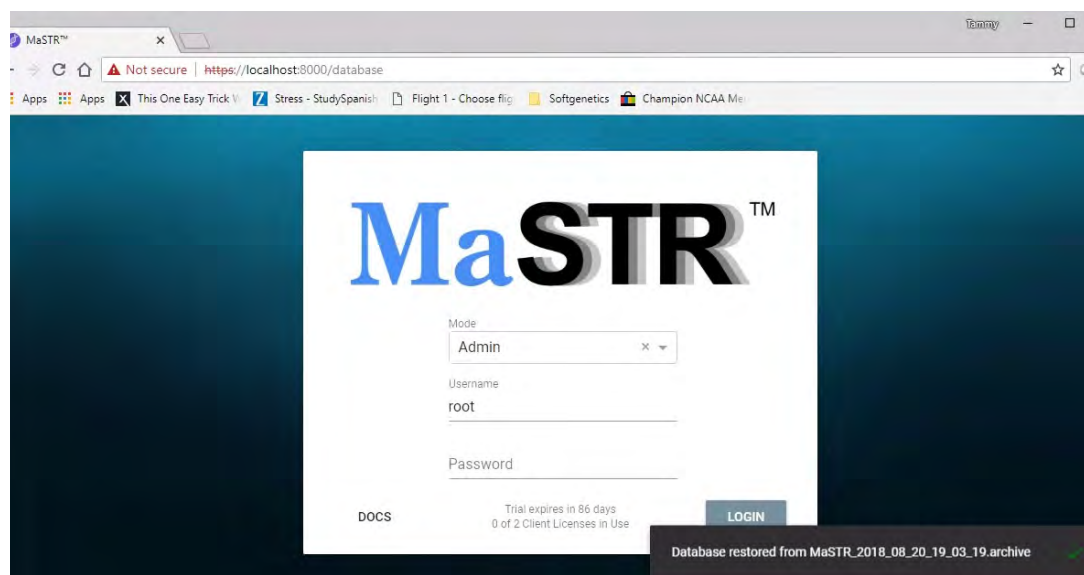
3. Click Restore.

The Open dialog box opens.

4. In the Open dialog box, browse to and select the archived file that you are restoring, and then click Open.

The Open dialog box closes, and MaSTR begins to restore the database based on the selected file. During the Restore process, a process bar is displayed at the bottom of the Database view. After the restoration is complete, the Database view closes, and you are automatically logged out of MaSTR. You return to the Login window with a message about the successful restoration displayed at the bottom of the Home page.

Figure A-21: Successful MaSTR database restore



Customizing the Default Report and View Displays

When MaSTR is first installed, it is installed with default settings for the following:

- The number of jobs that can be carried out consecutively on the MaSTR server. All other jobs are queued. By default, the value is set to two.
- The logo and the name of the organization that is displayed on the Analysis Report popup and on the first page of an analysis report. The logo is also displayed on the MaSTR login dialog box. By default, the logo is set to the MaSTR logo and the organization name is set to a placeholder of [Organization Name]. See [Figure 4-2 on page 86](#).
- The Primary color, which is the color for the MaSTR title bar and components such as buttons, toggles, and checkboxes on a View tab.
- The Secondary color, which is the color for the View Indicator line, the Tab Indicator line, the buttons on the MaSTR login dialog box, and other components.

You can customize any of these settings to better reflect your organization or business requirements.




To customize the view display

1. Open and log in to MaSTR under the Admin mode.
2. On the MaSTR title bar, click Settings.

The Settings view opens. The view has a single tab, the Settings tab. By default, when the tab first opens, Admin is the selected mode.

Figure A-22: Settings view, Settings tab

Settings

Server	Organization	Colors
Workers (Jobs Processed Consecutively) 2	Logo  Name [Organization Name]	Mode Admin Primary Color  Secondary Color 

SAVE **IMPORT LOGO**

3. Optionally, do any or all of the following as required:
 - For Number of Consecutive Jobs, leave the default value set to two, or enter a new value.
 - To customize the report display, do one or both of the following:
 - To change the report logo, click Import Logo, and then browse to and select the image. The logo can be in one of the following formats: .png, or jpeg/.jpeg, and it can be no bigger than 5 MB.
 - To have your organization name displayed on the report popup and analysis report, select the default/current value, and then edit it accordingly.
 - To customize the display for a selected mode, do the following:
 - i On the Mode dropdown list, select the mode for which you are applying the selected colors.
 - ii. Click the current Primary color to open a color palette, and then click to select a different primary color.
 - iii. Click the current Secondary color to open a color palette, and then click to select a different secondary color.

The web color code and RGB color scheme for a selected color is displayed in a read-only field below the color palette. See [Figure A-23](#) below.

Figure A-23: Selected colors for the Admin mode



4. Click Save.

The customizations are effective immediately.

Appendix B

Building a Custom MCMC Model for Probabilistic Mixture Analysis

MaSTR comes pre-populated with eight standard models that SoftGenetics has determined produce the best analysis results with the least amount of data bias. Moreover, if you are a member of Research/Validation, then to quickly test without editing and saving an existing model, you can simply override MCMC Settings and/or Analysis Settings for an existing model, such as changing the number of iterations. As member of Research/Validation, however, if you want to consider additional factors during an analysis, or you want to reconsider a given calculation for your data, then you can build a custom MCMC model for probabilistic mixture analysis. This appendix provides a high-level overview of how MaSTR calculates peak heights and probability calculations, and where a custom model fits into this process. It also details *variables* and *expected peak height* calculations, which are the two required components for building a custom MCMC model for probabilistic mixture analysis. Finally, it details how to define a model variable for a custom MCMC model.

This appendix covers the following topics:

- “Peak Heights and Probability Calculations from MCMC Results” on page 123.
- “Defining Model Variables for Custom MCMC Models” on page 127.



This appendix provides high-level information about variables and expected peak height calculations for a custom MCMC model. For detailed information about these components and/or assistance with building a custom MCMC model, contact tech_support@softgenetics.com.

Appendix B

Building a Custom MCMC Model for Probabilistic Mixture Analysis

Peak Heights and Probability Calculations from MCMC Results

MaSTR comes pre-populated with eight standard models that SoftGenetics has determined produce the best analysis results with the least amount of data bias. Each of these standard models provides an explanation of how the analysis data is generated starting with the DNA and then ending ultimately with the quantitative data (expected peak heights and probability calculations) that MaSTR produces.

Peak heights

Peak heights in MaSTR are calculated according to the following steps:



All assumptions detailed below are for example purposes only.

1. Begin with a suitable mixture of genotypes, with a value of 1 per allele.
Assumptions: Contributors are 14,13 and 14,10 with 14=2, 13=1, 10=1 and other alleles in the panel =0.
2. Apply a contributor ratio.
Assumptions: 75%:25% and 14 = 1, 13 = 0.75, 10 = 0.25.
3. Apply the amount values, where:
 - One overall degradation *amount* multiplied by “A” for each marker. See [“Degradation Variable Plots report” on page 93.](#)
 - Calculate 4000 total peak height (2000 per allele) with an “A” value of 0.9 for this marker, which equates to 1800 per allele.
 - 14 = 1800, 13 = 1350, 10 = 450.
4. Apply degradation, where the “D” value for each contributor lowers the expected height of larger alleles for the contributor. See [“Degradation Variable Plots report” on page 93.](#)
5. Optionally, apply any custom addition, subtraction, multiplication, or division of custom models variables as defined in the model. A variable value is added to the Expected Peak Height calculation through either addition, subtraction, multiplication, or division of the value from the calculated pre-stutter signal. See [“Defining Model Variables for Custom MCMC Models” on page 127.](#)
6. Apply any stutter that is specified in the protocol set. See [“Stutter calculation” on page 124.](#)
 - $14 > 13 = 0.1$, $13 > 12 = 0.08$
 - 14 = 1620, 13 = 1422, 10 = 450.

Stutter calculation

Stutter ratios are calculated as part of the protocol set using single-source data. If stutter is enabled for a protocol set, then for every allele, stutter ratios are used to move some of the expected peak height from an allele to its stutter position. See “Protocol Sets tab” on page 59.

1. Collect stutter ratio values.

For each sample and each marker in the panel, the stutter ratios are measured as follows: the height of the stutter peak at the minus-1 repeat position is divided by the height of the peak from the contributor’s alleles.

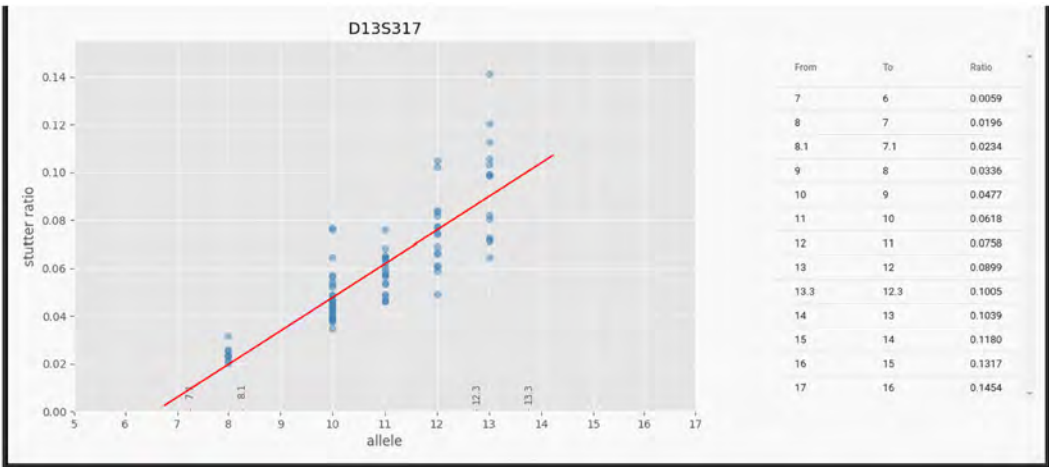


If the genotype for a contributor is (A-1, A) at a marker, then the A-1 peak is partially due to stutter and partially due to the genotype. As a result, the contributor samples are not used for calculating stutter at the marker.

2. Apply a liner regression.

To estimate the stutter ratio as a function of allele length, a linear regression is applied to the stutter data for each marker. The stutter ratio for any allele, therefore, can be estimated.

Figure B-1: Linear fit of stutter for D13S317. Blue circles show the raw values and a red line shows the fitted equation



Probability Calculations

After MaSTR calculates the expected peak heights, MaSTR then compares these heights to the observed peak heights to calculate a probability. MaSTR calculates the overall probability as the product of the probability of each peak height comparison. Three comparison calculations are possible:

- Expected and Observed: When a peak is both expected and observed, the heights of each are compared and a probability is calculated. The variance (as calculated in the Protocol Set) is an important part of this calculation.

Figure B-2: Variance graph



Even with identical conditions, the peak heights for a sample are not identical across multiple runs. The variance is a measurement of how much noise is expected from run to run. After MaSTR calculates the expected peak heights for a sample, the variance is used to judge the relative weight of this peak height prediction. As the peak height increases, a lower log-fold change is expected between the expected peak height and the actual peak height.

- Drop-out: Drop-out occurs when a peak is expected but is not observed. When generating genotypes in MaSTR, an expected Q allele that represents any allele that was not called in the signal data is included. Because no single height was observed, the peak height is integrated over all values from zero to the analytical threshold.



When MaSTR generates potential sets of genotypes, it does not consider drop-out of two alleles in the same genotype as a possibility.

- Drop-in: Drop-in occurs when a peak is not expected, but is observed. Models in MaSTR include a drop-in coefficient, Λ , which determines the likelihood of a drop-in peak based on the peak height. Increasing this value means a decreasing the probability of genotype

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sets with drop-in alleles and conversely, decreasing this value means increasing the probability genotype sets with drop-in alleles. “1” is typically a good starting value and changes typically are not observed if the value is < 0.1 or > 5 .

Defining Model Variables for Custom MCMC Models

As member of Research/Validation, if you want additional factors considered during an analysis, or you want to reconsider a given calculation for the data, then you can build a custom MCMC model for probabilistic mixture analysis. All the standard MCMC models and any custom MCMC model for probabilistic mixture analysis have the following components in common: Name, MCMC Settings, Analysis Settings, and Advanced Settings. (See “[MaSTR Models View](#)” on page 38.) For a custom MCMC model, you can also define one or more custom *model variables* as part of the model. A model variable provides a way of better representing the underlying process that generates allele peak height measurements from DNA. Values are randomly selected from a specified distribution and the MCMC process finds an optimal value for each variable. A model variable has the following components for its complete definition:

Table B-1: Model variable definition components

Component	Description
Name	Each model variable must have a unique name.
Description	Optional. A description of what the value that the variable represents.
Distribution	MaSTR is delivered with the following ten built-in distributions and you must select one of these values for a variable:
	<ul style="list-style-type: none"> • Fixed* • Normal • Uniform • Bounded Normal • Symmetric Dirichilet
	<ul style="list-style-type: none"> • Beta • Gamma • Bernoulli • Lognormal • Exponential
	Note: Fixed is the only Distribution value that uses a single value instead of sampling from a distribution. This option is particularly useful for creating hierarchical variables that have different shapes. Because model variables with a Fixed distribution do not change throughout a trace, they are not shown in the final analysis results.
Shape	<p>The number of values that are sampled from the specified distribution. You must select one of the following options:</p> <ul style="list-style-type: none"> • Marker - One value per marker. Every contributor and every allele in the marker use the same value. • Model - One value for the entire model. • Allele - One value per allele. Every contributor for this individual allele uses the same value. • Contributor - One value per contributor. Every allele from the same contributor uses the same value.

When you are defining a model variable, you can define it as an *independent* variable or as a *hierarchical* variable. An independent variable has its own distinct input parameters and its value does not rely on the value of another model variable. A hierarchical model variable uses the value for a previously defined model variable as one of its input parameters, which creates hierarchical models. In hierarchical models, shapes are automatically broadcast as needed. For example, an `allele` variable with a `marker` prior variable generates a value for each allele using the prior value for the corresponding marker.



Although you can improve the accuracy of a model by adding model variables, there is a trade-off between this accuracy and model complexity (more model variables result in slower run times and more memory usage), so be aware of this as you define your variables.

To define a model variable

1. If you have not done so already, open the Models tab for the System view. (System > Models).

2. On the Models tab, click New.

All fields on the Models tab are cleared in preparation for adding the new model.

3. Name the model and define the common model properties (MCMC Settings, Analysis Settings, and Advanced Settings). See [“To view the information for a model” on page 39](#).

4. Under Variables, click Add.

The Edit Variable dialog box opens. You use the options on this dialog box to define the custom model variable. The input values for the variable depend on the selected Distribution value and whether the variable is an independent variable or a hierarchical variable.

5. Define the model variable.



When specifying the input values for a model variable, ideally, to avoid bias in the model, you should make the input values as broad as is realistically possible.

[Figure B-3](#) below through [Figure B-5 on page 130](#) provide some representative examples of defining a custom model variable.

Figure B-3: Independent model variable, Fixed distribution, single input value

Edit Variable

Distribution: Fixed x Shape: Model x

Name: _____

Description: _____

Parameters

Value: Input Value 1

DELETE SAVE

Figure B-4: Independent model variable, Distribution other than fixed, multiple input values

Edit Variable

Distribution: Normal

Shape: Model

Name:

Description:

Parameters

Mean: 1

Standard Deviation: 1

Figure B-5: Hierarchical model variable

The figure consists of two screenshots of the 'Edit Variable' interface, illustrating a hierarchical model variable setup. The top screenshot shows the 'Marker_Fixed_1' variable with a 'Fixed' distribution and 'Marker' shape. The bottom screenshot shows the 'Contrib-Marker' variable with a 'Normal' distribution and 'Contributor' shape. An arrow points from the 'Marker_Fixed_1' variable in the top screenshot to the 'Marker_Fixed_1' mean parameter in the bottom screenshot.

Edit Variable

Distribution: Fixed Shape: Marker

Name: Marker_Fixed_1

Description: Set a value of 1 for each marker to shape a child variable as marker-contributor

Parameters

Value: Input Value 1

DELETE SAVE

Edit Variable

Distribution: Normal Shape: Contributor

Name: Contrib-Marker

Description:

Parameters

Mean: Marker_Fixed_1 Marker_Fixed_1

Standard Deviation: Input Value 1

DELETE SAVE

In this example: Regardless of the number of markers in the panel, start with a value of “1” for each marker for the prior variable, the Marker_Fixed_1 model variable. For each contributor, sample a value. The value for the prior variable is used as the Input mean value for this second variable, the Contrib-Marker variable. This hierarchical model variable creates a distribution from which to sample for each contributor, but the distribution is different depending on the marker. The final result is one value per contributor per marker. As example, for three contributors and ten markers, the Marker_Fixed_1 model variable has 10 unique values, with one value per marker, and the Contrib-Maker model variable has 30 unique values.

6. Click Save.

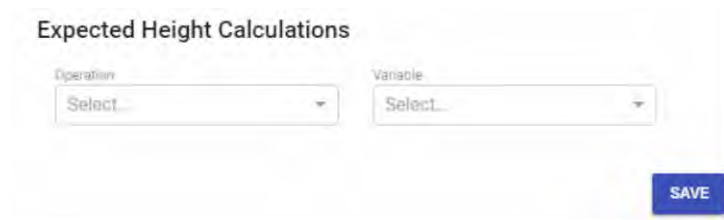
The Edit Variable dialog box closes. The Model tab remains open. The newly added custom model variable is displayed by name under the Variables heading on the tab.

Figure B-6: Newly added custom model variable displayed on the Models tab

7. Next to the newly added variable, under Expected Height Calculations, click Add.

The Expected Height Calculations dialog box opens.

Figure B-7: Expected Height Calculations dialog box

The image shows a dialog box titled "Expected Height Calculations". It contains two dropdown menus. The first is labeled "Operation" and has a "Select..." option. The second is labeled "Variable" and also has a "Select..." option. A blue "SAVE" button is located at the bottom right of the dialog box.

8. On the Operation dropdown list, select the operation (addition, subtraction, multiplication, or division) by which the variable value is to be added to the Expected Peak Height calculation.
9. On the Variable dropdown list, select the variable.
10. Click Save.

The Expected Height Calculations dialog box closes. The Model tab remains open. The newly added custom model variable is displayed by name under the Variables heading on the tab and its corresponding operation is displayed under the Expected Height Calculations header.

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Appendix C

MaSTR Validation Study

This appendix provides a high-level overview and the conclusions that were reached by the preliminary validation of MaSTR, which indicated that MaSTR provides the rigor of a fully continuous probabilistic approach for evaluating genotypes in mixtures in a straight forward software program.

This appendix covers the following topics:

- “Abstract” on page 135.
- “Methods” on page 137.
- “Models” on page 139.
- “Results” on page 140.
- “Summary” on page 142.
- “Acknowledgments and References” on page 143.



For the full article and poster that detail the initial MaSTR validation study, see <https://softgenetics.com/MaSTR.php> and click the Validation Study Link at the top of the page.

Appendix C
MaSTR Validation Study

Abstract

Probabilistic genotyping (PG) for evaluation of mixtures provides many advantages compared to historical (CPI, binary likelihood ratio) approaches. Fully continuous approaches utilize more of the information from the evidentiary profile such as peak heights, stutter percentages, mixture ratio, and the probability of drop-in/out to provide weighted genotypes from the mixture profile. The weighted genotypes are used to calculate a likelihood ratio that a profile is included, or not included, in the mixture. Mixture profiles are compared to hypothetical profiles created based on these parameters and hypothetical combinations of genotypes present in the sample in order to calculate how probable it is that a given individual contributed to the mixture. Integration over a large number of interrelated parameters is required to calculate relative probabilities but doing so directly is not feasible. Markov Chain Monte Carlo (MCMC) is a widely used sampling method to approximate such complex integrals with high accuracy.^{1,3} Statistically sound, rigorous software tools are essential for forensic interpretation of complex mixtures that often have multiple contributors, low-level DNA of one or more of the contributors, shared alleles, minor contributor alleles in stutter positions to major contributors, and drop-out. Thorough validation of methodologies is required for forensic laboratories. Conclusions of a preliminary evaluation of MaSTR software indicated MaSTR software provides the rigor of a fully continuous probabilistic approach¹ in a straight-forward software program. A full validation was proposed as the results of the preliminary study were concordant with the known mixture contributors. The validation study of MaSTR software provides the forensic community with a detailed report of the capabilities and reliability of this newly available tool to assist the forensic analyst in applying their expertise to evaluate mixture profiles.

The validation study was designed in accordance with the Scientific Working Group on DNA Analysis Methods (SWGDM) and Organization of Scientific Area Committees (OSAC) guidelines. Purified, de-identified DNA from 40 donors was obtained from the Nebraska Biobank (University of Nebraska Medical Center), quantified using the Quantifiler Human DNA Quantification kit (Thermo Fisher), amplified with PowerPlex™ Fusion (Promega Corporation), and analyzed on an ABI PRISM® 3130 Genetic Analyzer (Thermo Fisher). Replicates of single source samples were genotyped (GeneMarker HID software). Files were selected to provide a range of allele calls and stutter information. Results of these replicates and their dilutions were imported into MaSTR software to calculate the variance coefficient and stutter ratios required for the protocol data set. The protocol data set provides the software with context of expected peak height variation over the range of potential RFU values and stutter during the evaluation of mixture profiles. Mixtures ranged from simple, two-contributors with major/minor components with few shared alleles to complex four and five-contributor mixtures with a range of mixture ratios and shared alleles. Mixture samples were also diluted to examine low-template effects across the range of contributors.

The study utilized the standard model within the software. This model utilizes peak heights, drop-in/out, degradation, stutter, co-ancestry (NRC I and NRC II) and allele frequencies from USA populations.⁵ Tests included evaluation of mixtures with: 1) a known contributor profile and no person of interest (POI) profile(s), 2) a known contributor profile and POI profile(s), 3) no known and no POI profile(s) to evaluate deconvolution capabilities and 4) contributor and non-contributor samples to evaluate exclusion capabilities. Additional

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parameters included use of the elimination database, containing some of the known contributor genotypes, to mimic detection of staff profiles in a mixture in order to evaluate MaSTR's capability to detect contamination. The number of MCMC iterations and chains required increased with complexity of the mixtures. Results were consistent with known contributors and exclusions. Methodology and tabulated results are presented here.

Methods

Keywords: DNA Quantification, Mixture Construction, Amplification, Capillary Electrophoresis

All human DNA samples used in this study were purchased as de-identified extracts from the Nebraska BioBank, and were used according to the University of Nebraska-Lincoln's Institutional Review Board's (IRB) approved policies for ethical standards and methods for human testing. Sample collection and experimentation commenced only after the IRB review process was completed.

DNA was extracted at the Nebraska BioBank from 40 donor samples using the Qiagen QIAcube and the QIAamp 96 DNA QIAcube HT kit optimized for downstream genomic assays. Addition of proK and AL buffer and incubation at 56°C resulted in lysis of the cells. The samples were added to the spin columns in the Qiacube for washing and elution of DNA. The DNA was washed via a salt wash (AW1) followed by a salt eliminating wash (AW2) then an ethanol wash. DNA eluted from the column via a low ionic elution buffer. The resulting DNA was quantified using the NanoDrop™ One microvolume UV-Vis spectrophotometer at the Nebraska BioBank.

In order to further determine the concentration of DNA in each sample after they were delivered, the extracts were quantified using an Applied Biosystems 7500 real-time PCR system and the Applied Biosystems Quantifiler™ Human DNA Quantification Kit according to the manufacturer's recommendations.

Prior to the analysis of mixed samples, a protocol data set was created for the MaSTR software to use in the assessment of stutter peaks and the calculation of a variance factor. Ten samples were chosen that demonstrated the greatest level of allelic variation available. Each sample was amplified using a n initial quantity of 0.5 ng with five replicates. The samples were then diluted 1:2 and 1:4 and amplified again, each with five replicates, yielding a total of 150 analysis samples with 10 distinct genotypes and a broad range of peak heights for the MaSTR software to use.

Mixture samples were created using two, three, four, or five of the single source samples. Two different strategies were used in creating the two and three-person mixtures. After each single source sample was genotyped, an appropriate number of samples (two or three) were selected that showed minimal or maximal allele sharing amongst the available genotypes, in order to test the MaSTR software under these different conditions. Two different two-person mixture combinations were created, one in which the samples shared a total of five alleles amongst five loci (low share) and one in which the samples shared 19 alleles amongst 18 loci (high share). The same process was used for the three-person mixtures, resulting in one combination with 16 alleles shared amongst 15 loci between the three contributors (low share) and a different combination of contributors showing 31 alleles shared amongst 21 loci (high share). The four- and five- person mixtures were created by randomly selecting previously unused samples and combining them without regard to allele sharing. This gave a total of 19 different single source genotypes used to create 6 mixture genotypes, no genotype was used in more than one combination. Two-person mixtures were made in the following ratios: 1:1, 1:2, 1:3, 1:5, and 1:10. Three-person mixtures were made in the following ratios: 1:1:1, 1:1:2, 1:3:5, and 1:2:10. Four-person mixtures were made in the following ratios:

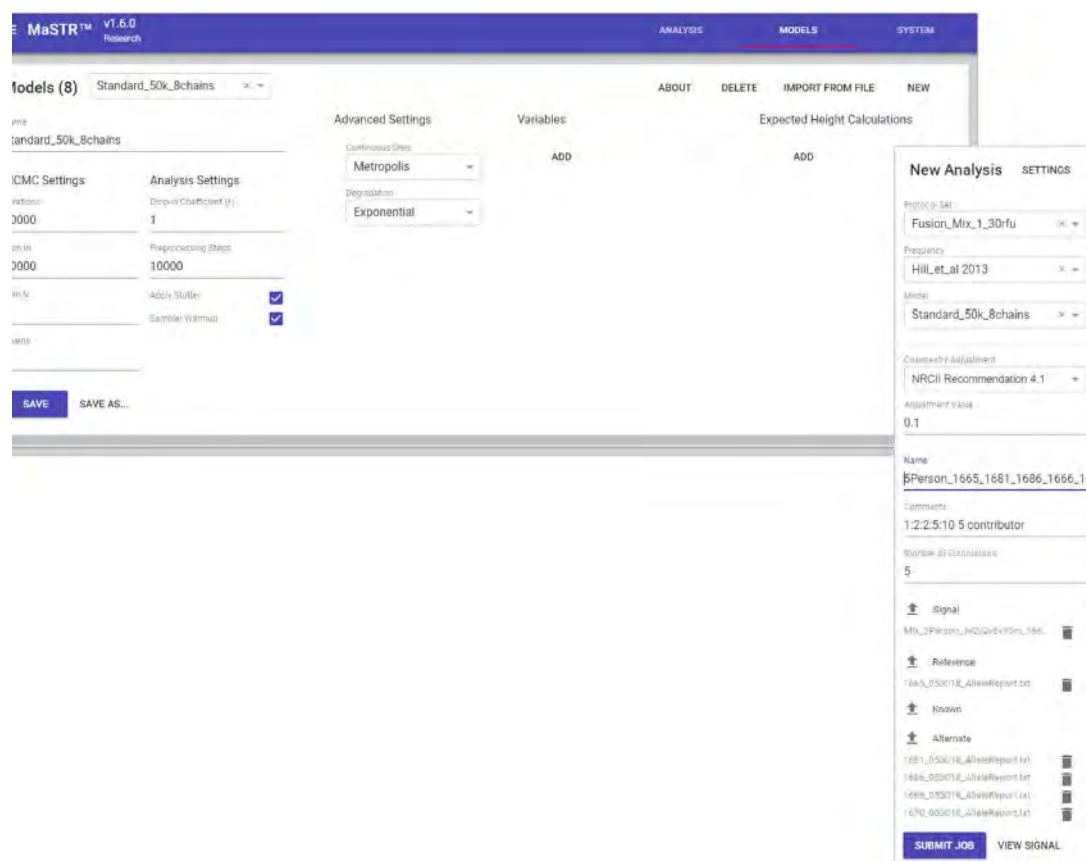
1:1:1:1, 1:2:2:5, and 1:1:3:10. Five-person mixtures were made in the following ratios: 1:1:1:1:1, 1:2:2:5:10, and 1:1:5:5:10. The mixture ratio balance for each sample was in the planned mixture. The amount of input DNA for each contributor was then adjusted so that the peak heights in the mixture should correspond to the desired ratio. Test mixture samples were then assembled and run in triplicate to assess if the amount of DNA in the respective amplifications gave acceptable peak height ratios or needed further adjustment. Once the test mixture samples demonstrated peak heights that fit expectations for the desired mixture ratio, those quantities of DNA were used to create the experimental samples. Each mixture sample was also serially diluted 1:2, 1:4, and 1:8 in order to examine the same contributor ratios with and without stochastic amplification effects. Every final mixture/dilution combination sample was run in triplicate.

All samples were amplified using the Promega PowerPlex® Fusion 5C kit following the manufacturer's protocols. The target initial quantity of DNA for amplification was 0.5 ng and was subsequently adjusted according to the requirements of the specific experimental conditions. The amplification reaction final volume was 25 µL for all samples and thermal cycling was performed in an Applied Biosystems GeneAmp PCR System 9700 using maximum ramp speed for 30 cycles. All amplification sets included a positive control (cell line 2800M DNA, included in kit) and a negative control (deionized water used for sample dilutions).

Separation and detection of the PCR products was performed using an Applied Biosystems 3130 Genetic Analyzer with Foundation Data Collection software v3.0, following all manufacture recommended protocols. All injections were performed at 3 kV for 5 seconds. Separation occurred with Applied Biosystems Hi-Di™ formamide in Applied Biosystems POP4 Polymer. Analysis of all the injection products was performed using SoftGenetics GeneMarker HID v2.9.0 software. All of the two-person mixture samples were analyzed with an analytical threshold of 50 rfu, as were the three-person mixtures except for those that were diluted 1:8, which used an analytical threshold of 30 rfu. All the four- and five person mixtures also used an analytical threshold of 30 rfu. The differing analytical thresholds reflected the prevalence of low level peaks in each condition and the desire to include allele dropout in some of the combinations in order to further challenge the MaSTR software at each mixture ratio.

Models

Figure C-1: Example of model and submitted jobs



Models were consistent with the exception of varying the number of MCMC iterations. Models with increasing numbers of iterations were used to provide information on minimum and optimal number of MCMC iterations for 2, 3, 4 and 5 contributor mixtures.

The jobs submitted included the mixture sample text file and text files of single source genotypes to be evaluated, without specifying any known single source genotypes. These settings we re-selected in order to mimic the most difficult cases where a known contributor swab is not available. The analyses included simple 2-person mixtures with very limited allele sharing, as well as complex 3 - 5-person mixtures. Two-person mixtures with up to 70% allele sharing were used to mimic related individuals in a mixture. The NR C II 4.1 approach for co-ancestry was selected and coefficient was 0.1 Fifteen mixture analyses included non-contributor single source samples to evaluate exclusion capabilities.

Results

Accuracy of inclusion and exclusion: Seventy-seven analyses of 2-5 person mixtures resulted in accurate inclusion and positive likelihood ratio for all tested samples. Fifteen of the analyses included non-contributor samples. All of the non-contributor samples were excluded, having negative likelihood ratio results.

Figure C-2: Ratio plot showing the correlation between the ratio of a contributor to a mixture and the average ratio value reported by MaSTR when the contributor's profile was tested

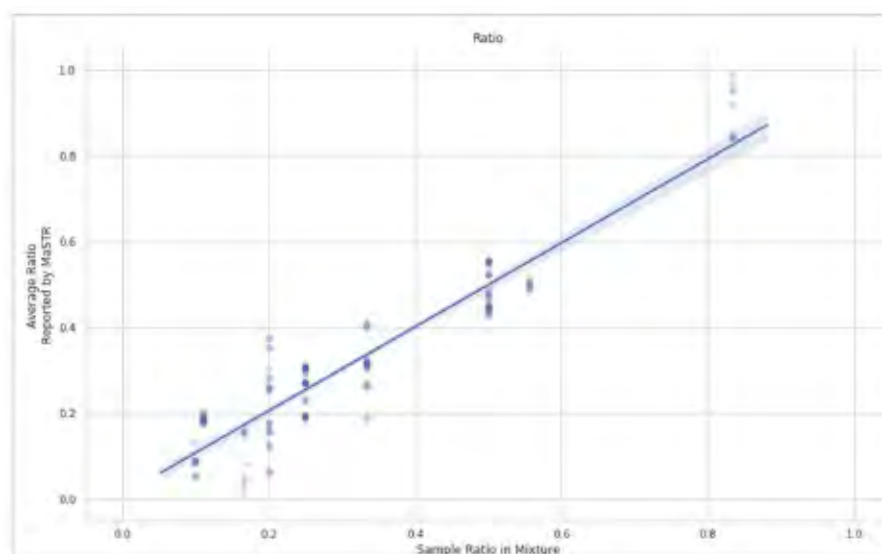


Figure C-3: Likelihood Ratio plot showing the highest LR reported for contributor profiles that were tested as unknowns, showing each contributor's known ratio in the mixture, the total number of contributors, and the amount of allele sharing amongst the contributors in the sample.

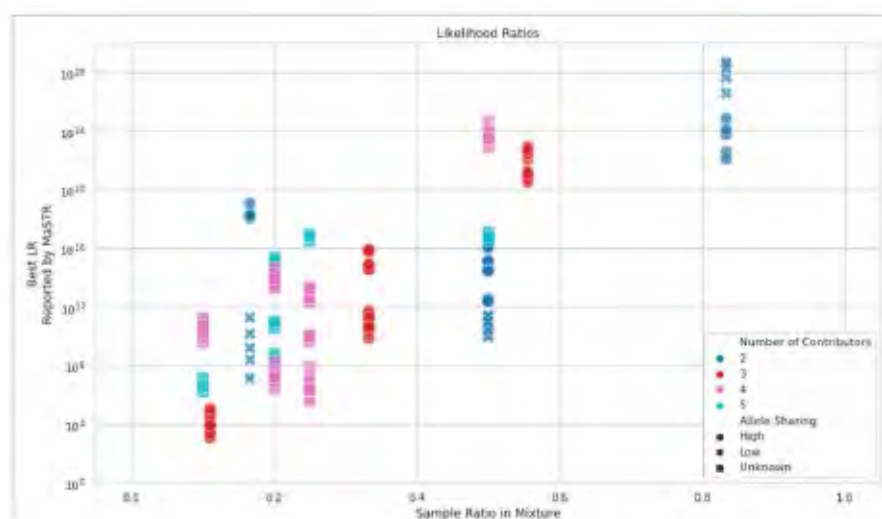


Figure C-4: Likelihood Ratio results report from a two-person 1:1 mixture without a known contributor specified. Sample 1653 (left) has a strong likelihood corresponding to the deconvoluted unknown 2. Sample 1678 (right) has a strong LR corresponding to the deconvoluted unknown 1. Likelihood ratios are calculated from the weighted genotype results.

1653_053016_AlleleReport.txt (Alternate)				1678_053018_AlleleReport.txt (Reference)			
Marker	Genotype	1:1 (Unknown 1)	1:1 (Unknown 2)	Marker	Genotype	1:1 (Unknown 1)	1:1 (Unknown 2)
ABG1508	(15, 18)	0.0000	0.0000	ABG1508	(15, 18)	0.0000	0.0000
ABG1509	(15.1, 15.2)	0.0000	0.0000	ABG1509	(14, 15)	0.0000	0.0000
ABG1510	(15, 18)	0.0000	0.0000	ABG1510	(15, 18)	0.0000	0.0000
ABG1511	(15, 18)	0.0000	0.0000	ABG1511	(15, 18)	0.0000	0.0000
ABG1512	(15, 18)	0.0000	0.0000	ABG1512	(15, 18)	0.0000	0.0000
ABG1513	(15, 18)	0.0000	0.0000	ABG1513	(15, 18)	0.0000	0.0000
ABG1514	(15, 18)	0.0000	0.0000	ABG1514	(15, 18)	0.0000	0.0000
ABG1515	(15, 18)	0.0000	0.0000	ABG1515	(15, 18)	0.0000	0.0000
ABG1516	(15, 18)	0.0000	0.0000	ABG1516	(15, 18)	0.0000	0.0000
ABG1517	(15, 18)	0.0000	0.0000	ABG1517	(15, 18)	0.0000	0.0000
ABG1518	(15, 18)	0.0000	0.0000	ABG1518	(15, 18)	0.0000	0.0000
ABG1519	(15, 18)	0.0000	0.0000	ABG1519	(15, 18)	0.0000	0.0000
ABG1520	(15, 18)	0.0000	0.0000	ABG1520	(15, 18)	0.0000	0.0000
ABG1521	(15, 18)	0.0000	0.0000	ABG1521	(15, 18)	0.0000	0.0000
ABG1522	(15, 18)	0.0000	0.0000	ABG1522	(15, 18)	0.0000	0.0000
ABG1523	(15, 18)	0.0000	0.0000	ABG1523	(15, 18)	0.0000	0.0000
ABG1524	(15, 18)	0.0000	0.0000	ABG1524	(15, 18)	0.0000	0.0000
ABG1525	(15, 18)	0.0000	0.0000	ABG1525	(15, 18)	0.0000	0.0000
ABG1526	(15, 18)	0.0000	0.0000	ABG1526	(15, 18)	0.0000	0.0000
ABG1527	(15, 18)	0.0000	0.0000	ABG1527	(15, 18)	0.0000	0.0000
ABG1528	(15, 18)	0.0000	0.0000	ABG1528	(15, 18)	0.0000	0.0000
ABG1529	(15, 18)	0.0000	0.0000	ABG1529	(15, 18)	0.0000	0.0000
ABG1530	(15, 18)	0.0000	0.0000	ABG1530	(15, 18)	0.0000	0.0000
ABG1531	(15, 18)	0.0000	0.0000	ABG1531	(15, 18)	0.0000	0.0000
ABG1532	(15, 18)	0.0000	0.0000	ABG1532	(15, 18)	0.0000	0.0000
ABG1533	(15, 18)	0.0000	0.0000	ABG1533	(15, 18)	0.0000	0.0000
ABG1534	(15, 18)	0.0000	0.0000	ABG1534	(15, 18)	0.0000	0.0000
ABG1535	(15, 18)	0.0000	0.0000	ABG1535	(15, 18)	0.0000	0.0000
ABG1536	(15, 18)	0.0000	0.0000	ABG1536	(15, 18)	0.0000	0.0000
ABG1537	(15, 18)	0.0000	0.0000	ABG1537	(15, 18)	0.0000	0.0000
ABG1538	(15, 18)	0.0000	0.0000	ABG1538	(15, 18)	0.0000	0.0000
ABG1539	(15, 18)	0.0000	0.0000	ABG1539	(15, 18)	0.0000	0.0000
ABG1540	(15, 18)	0.0000	0.0000	ABG1540	(15, 18)	0.0000	0.0000
ABG1541	(15, 18)	0.0000	0.0000	ABG1541	(15, 18)	0.0000	0.0000
ABG1542	(15, 18)	0.0000	0.0000	ABG1542	(15, 18)	0.0000	0.0000
ABG1543	(15, 18)	0.0000	0.0000	ABG1543	(15, 18)	0.0000	0.0000
ABG1544	(15, 18)	0.0000	0.0000	ABG1544	(15, 18)	0.0000	0.0000
ABG1545	(15, 18)	0.0000	0.0000	ABG1545	(15, 18)	0.0000	0.0000
ABG1546	(15, 18)	0.0000	0.0000	ABG1546	(15, 18)	0.0000	0.0000
ABG1547	(15, 18)	0.0000	0.0000	ABG1547	(15, 18)	0.0000	0.0000
ABG1548	(15, 18)	0.0000	0.0000	ABG1548	(15, 18)	0.0000	0.0000
ABG1549	(15, 18)	0.0000	0.0000	ABG1549	(15, 18)	0.0000	0.0000
ABG1550	(15, 18)	0.0000	0.0000	ABG1550	(15, 18)	0.0000	0.0000
ABG1551	(15, 18)	0.0000	0.0000	ABG1551	(15, 18)	0.0000	0.0000
ABG1552	(15, 18)	0.0000	0.0000	ABG1552	(15, 18)	0.0000	0.0000
ABG1553	(15, 18)	0.0000	0.0000	ABG1553	(15, 18)	0.0000	0.0000
ABG1554	(15, 18)	0.0000	0.0000	ABG1554	(15, 18)	0.0000	0.0000
ABG1555	(15, 18)	0.0000	0.0000	ABG1555	(15, 18)	0.0000	0.0000
ABG1556	(15, 18)	0.0000	0.0000	ABG1556	(15, 18)	0.0000	0.0000
ABG1557	(15, 18)	0.0000	0.0000	ABG1557	(15, 18)	0.0000	0.0000
ABG1558	(15, 18)	0.0000	0.0000	ABG1558	(15, 18)	0.0000	0.0000
ABG1559	(15, 18)	0.0000	0.0000	ABG1559	(15, 18)	0.0000	0.0000
ABG1560	(15, 18)	0.0000	0.0000	ABG1560	(15, 18)	0.0000	0.0000
ABG1561	(15, 18)	0.0000	0.0000	ABG1561	(15, 18)	0.0000	0.0000
ABG1562	(15, 18)	0.0000	0.0000	ABG1562	(15, 18)	0.0000	0.0000
ABG1563	(15, 18)	0.0000	0.0000	ABG1563	(15, 18)	0.0000	0.0000
ABG1564	(15, 18)	0.0000	0.0000	ABG1564	(15, 18)	0.0000	0.0000
ABG1565	(15, 18)	0.0000	0.0000	ABG1565	(15, 18)	0.0000	0.0000
ABG1566	(15, 18)	0.0000	0.0000	ABG1566	(15, 18)	0.0000	0.0000
ABG1567	(15, 18)	0.0000	0.0000	ABG1567	(15, 18)	0.0000	0.0000
ABG1568	(15, 18)	0.0000	0.0000	ABG1568	(15, 18)	0.0000	0.0000
ABG1569	(15, 18)	0.0000	0.0000	ABG1569	(15, 18)	0.0000	0.0000
ABG1570	(15, 18)	0.0000	0.0000	ABG1570	(15, 18)	0.0000	0.0000
ABG1571	(15, 18)	0.0000	0.0000	ABG1571	(15, 18)	0.0000	0.0000
ABG1572	(15, 18)	0.0000	0.0000	ABG1572	(15, 18)	0.0000	0.0000
ABG1573	(15, 18)	0.0000	0.0000	ABG1573	(15, 18)	0.0000	0.0000
ABG1574	(15, 18)	0.0000	0.0000	ABG1574	(15, 18)	0.0000	0.0000
ABG1575	(15, 18)	0.0000	0.0000	ABG1575	(15, 18)	0.0000	0.0000
ABG1576	(15, 18)	0.0000	0.0000	ABG1576	(15, 18)	0.0000	0.0000
ABG1577	(15, 18)	0.0000	0.0000	ABG1577	(15, 18)	0.0000	0.0000
ABG1578	(15, 18)	0.0000	0.0000	ABG1578	(15, 18)	0.0000	0.0000
ABG1579	(15, 18)	0.0000	0.0000	ABG1579	(15, 18)	0.0000	0.0000
ABG1580	(15, 18)	0.0000	0.0000	ABG1580	(15, 18)	0.0000	0.0000
ABG1581	(15, 18)	0.0000	0.0000	ABG1581	(15, 18)	0.0000	0.0000
ABG1582	(15, 18)	0.0000	0.0000	ABG1582	(15, 18)	0.0000	0.0000
ABG1583	(15, 18)	0.0000	0.0000	ABG1583	(15, 18)	0.0000	0.0000
ABG1584	(15, 18)	0.0000	0.0000	ABG1584	(15, 18)	0.0000	0.0000
ABG1585	(15, 18)	0.0000	0.0000	ABG1585	(15, 18)	0.0000	0.0000
ABG1586	(15, 18)	0.0000	0.0000	ABG1586	(15, 18)	0.0000	0.0000
ABG1587	(15, 18)	0.0000	0.0000	ABG1587	(15, 18)	0.0000	0.0000
ABG1588	(15, 18)	0.0000	0.0000	ABG1588	(15, 18)	0.0000	0.0000
ABG1589	(15, 18)	0.0000	0.0000	ABG1589	(15, 18)	0.0000	0.0000
ABG1590	(15, 18)	0.0000	0.0000	ABG1590	(15, 18)	0.0000	0.0000
ABG1591	(15, 18)	0.0000	0.0000	ABG1591	(15, 18)	0.0000	0.0000
ABG1592	(15, 18)	0.0000	0.0000	ABG1592	(15, 18)	0.0000	0.0000
ABG1593	(15, 18)	0.0000	0.0000	ABG1593	(15, 18)	0.0000	0.0000
ABG1594	(15, 18)	0.0000	0.0000	ABG1594	(15, 18)	0.0000	0.0000
ABG1595	(15, 18)	0.0000	0.0000	ABG1595	(15, 18)	0.0000	0.0000
ABG1596	(15, 18)	0.0000	0.0000	ABG1596	(15, 18)	0.0000	0.0000
ABG1597	(15, 18)	0.0000	0.0000	ABG1597	(15, 18)	0.0000	0.0000
ABG1598	(15, 18)	0.0000	0.0000	ABG1598	(15, 18)	0.0000	0.0000
ABG1599	(15, 18)	0.0000	0.0000	ABG1599	(15, 18)	0.0000	0.0000
ABG1600	(15, 18)	0.0000	0.0000	ABG1600	(15, 18)	0.0000	0.0000

Figure C-5: Genotype Set Results. A heat map indicates the most likely genotype combination of the test samples on a scale of 0-1, with the least likely being 0 (shown in dark purple) and the most likely being 1 (shown in yellow).



Summary

Experimental data have shown that the MaSTR software gives accurate Likelihood Ratio results when used with 2-, 3-, and 4-person mixtures. The calculated values for each contributor LR fit well with the true identities and mixture ratios of the synthesized samples. All true-exclusion known samples gave negative (un-supporting) LRs for the appropriate unknown “position” in MaSTR.

The “Major” and “Minor” contributors (mixture ratios of at least 3:1) were clearly and accurately distinguishable via their calculated LRs. Mixture contributors that were at or close to a 1:1 ratio were accurately distinguishable in 2-person mixtures with no identified known sample in mixtures with a high degree of allele overlap. In 3- and 4-person mixtures with a high degree of allele overlap, contributors present in near equal amounts with no identified known samples showed LRs accurately supporting both samples being part of the mixture, but were sometimes difficult to tell apart.

Preliminary work shows that using 10,000 MCMC iterations with eight chains works well for 2-person mixtures. 3-person mixtures, especially with minor contributors, worked well using 20,000 MCMC iterations with eight chains and 4+ person mixtures showed the most consistent results running 50,000 MCMC iterations with eight chains. Increasing the number of MCMC iterations gave more consistent LR values across the replicate MaSTR runs, especially with samples containing minor contributors, allele dropout, shared alleles, and close ratio contributor data.

MaSTR software runs using less than 100K total MCMC iterations were resolved within 15 minutes or less, while longer runs (400K) were completed within 45 minutes using a desktop computer (Intel® Core™ i7-7700 cpu, 3.60 GHz, 4 core, 8 logical processors) with 8 GB RAM operating with Windows 10.

Further interrogation of the data set is planned to evaluate likelihood ratio results and analysis time when known contributor(s) are included in the analysis procedure, as well as peak height variation in replicate samples.

Acknowledgments and References

We thank Promega Human Identity Group for providing the PowerPlex Fusion and matrix kits.

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Appendix C
MaSTR Validation Study

Bibliography

Using continuous DNA interpretation methods to revisit likelihood ratio behaviour

Taylor, Duncan. Forensic Science International: Genetics 11 (2014) 144-153.

Keywords: DNA profile, interpretation Mixtures, Likelihood ratio, Assumed contributors, STRmix, Reliability

Abstract: Continuous DNA interpretation systems make use of more information from DNA profiles than analysts have previously been able to with binary, threshold based systems. With these new continuous DNA interpretation systems and a new, more powerful, DNA profiling kit (GlobalFiler) there is an opportunity to re-examine the behaviour of a commonly used statistic in forensic science, the likelihood ratio (LR). The theoretical behaviour of the LR has been known for some time, although in many instances the behaviour has not been able to be thoroughly demonstrated due to limitations of the biological and mathematical models being used. In this paper the effects of profile complexity, replicate amplifications, assuming contributors, adding incorrect information, and adding irrelevant information to the calculation of the LR are explored. The empirical results are compared to theoretical expectations and explained. The work finishes with the results being used to dispel common misconceptions around reliability, accuracy, informativeness and reproducibility. Crown Copyright 2014. Published by Elsevier Ireland Ltd. All rights reserved.

Internal validation of STRmixTM for the interpretation of single source and mixed DNA profiles

Tamya R. Morettia, Rebecca S. Justa, Susannah C. Kehl, Leah E. Willisa, John S. Buckleton, Jo-Anne Bright, Duncan A. Taylor, Anthony J. Onorato. Forensic Science International: Genetics 29 (2017) 126-144.

Keywords: STRs, DNA Mixtures, Probabilistic Genotyping, Likelihood Ratios

Abstract: The interpretation of DNA evidence can entail analysis of challenging STR typing results. Genotypes inferred from low quality or quantity specimens, or mixed DNA samples originating from multiple contributors, can result in weak or inconclusive match probabilities when a binary interpretation method and necessary thresholds (such as a stochastic threshold) are employed. Probabilistic genotyping approaches, such as fully continuous methods that incorporate empirically determined biological parameter models, enable usage of more of the profile information and reduce subjectivity in interpretation. As a result, software-based probabilistic analyses tend to produce more consistent and more informative results regarding potential contributors to DNA evidence. Studies to assess and internally validate the probabilistic genotyping software STRmixTM for casework usage at the Federal Bureau of Investigation Laboratory were conducted using lab-specific parameters and more than 300 single-source and mixed contributor profiles. Simulated forensic specimens, including constructed mixtures that included DNA from two to five donors across a broad range of template amounts and contributor proportions, were used to

examine the sensitivity and specificity of the system via more than 60,000 tests comparing hundreds of known contributors and non-contributors to the specimens. Conditioned analyses, concurrent interpretation of amplification replicates, and application of an incorrect contributor number were also performed to further investigate software performance and probe the limitations of the system. In addition, the results from manual and probabilistic interpretation of both prepared and evidentiary mixtures were compared. The findings support that STRmix™ is sufficiently robust for implementation in forensic laboratories, offering numerous advantages over historical methods of DNA profile analysis and greater statistical power for the estimation of evidentiary weight, and can be used reliably in human identification testing. With few exceptions, likelihood ratio results reflected intuitively correct estimates of the weight of the genotype possibilities and known contributor genotypes. This comprehensive evaluation provides a model in accordance with SWGDAM recommendations for internal validation of a probabilistic genotyping system for DNA evidence interpretation. © 2017 Published by Elsevier Ireland Ltd.

Characterisation of artefacts and drop-in events using STR-validator and single-cell analysis

Oskar Hansson, Peter Gill. Forensic Science International: Genetics 30 (2017) 57-65.

Keywords: Drop-in, Spurious alleles, Contamination Artefact, STR-validator, DEPAArray

Abstract: There is a need to understand the occurrence of drop-in and their peak height distributions to further improve existing models to interpret complex DNA results. The necessary data is usually very time-consuming to collect. Here we present a practical approach for simultaneous characterisation of reproducible artefacts and drop-in contamination from negative controls. As an alternative to the traditional approach we demonstrate the use of the DEPAArray system as a source of single molecules for amplification to infer the peak height distribution of drop-in events. Though signal detection is often confounded by noise and artefact, single molecule contamination, or allele drop-in, is also regularly detected as demonstrated by the overlap in signal intensities between suspected drop-in events and signal obtained from single-cells. © 2017 Published by Elsevier Ireland Ltd.

A fully continuous system of DNA profile evidence evaluation that can utilise STR profile data produced under different conditions within a single analysis

Duncan Taylor, Jo-Anne Bright, Hannah Kelly, Meng-Han Lin, John Buckleton. Forensic Science International: Genetics 31 (2017) 149-154A.

Keywords: Multiple DNA profiling kits, Continuous DNA interpretation, STRmix Modelling, DNA mixtures

Abstract: The introduction of probabilistic DNA interpretation systems has made it possible to evaluate many profiles that previously (under a manual interpretation system) were not. These probabilistic systems have been around for a number of years and it is becoming more common that their use within a laboratory has spanned at least one technology change. This may be a change in laboratory hardware, the DNA profiling kit used, or the manner in which the profile is generated. Up until this point, when replicates DNA profiles are generated, that span a technological change, the ability to utilise all the information in all replicates has been

limited or non-existent. In this work we explain and derive the models required to evaluate (what we term) multi-kit analysis problems. We demonstrate the use of the multi-kit feature on a number of scenarios where such an analysis would be desired within a laboratory. Allowing the combination of profiling data that spans a technological change will further increase the amount of DNA profile information produced in a laboratory that can be evaluated.

Validation Standards for Probabilistic Genotyping Systems

Organization of Scientific Area Committees for Forensic Science (OSAC) ASB Standard 018, First Edition 2017. Editor: Brian Higgins.

Draft Working Group Members: Joel Sutton, Steven Myers, Michael Coble, Ph.D., Simone Gittelsohn, Ph.D., Sandy Zabell, Ph.D., Kathleen Corrado, Jeffrey Nye, Susan Greenspoon, Michael Adamowicz, James Curran, Ph.D., Adele Michell, Ph.D., Mark Powell.

Keywords: validation, probabilistic genotyping, statistics, likelihood ratio

Abstract: These standards were designed to provide direction and guidance to laboratories for the validation of probabilistic genotyping systems as they relate to the interpretation of autosomal short tandem repeat analysis.

Scientific Working Group on DNA Analysis Methods (SWGDAM) Interpretation Guidelines for Autosomal STR Typing by Forensic DNA Testing Laboratories 2017

<https://www.swgdam.org/publications>

Validating multiplexes for use in conjunction with modern interpretation strategies

DuncanTaylor, Jo-AnneBright, CatherineMcGoven, ChristopherHefford, TimKalafut, JohnBuckleton. Science International: Genetics Volume 20, January 2016, Pages 6-19

<https://doi.org/10.1016/j.fsigen.2015.09.011>

Abstract: In response to requests from the forensic community, commercial companies are generating larger, more sensitive, and more discriminating STR multiplexes. These multiplexes are now applied to a wider range of samples including complex multi-person mixtures. In parallel there is an overdue reappraisal of profile interpretation methodology. Aspects of this reappraisal include:

1. The need for a quantitative understanding of allele and stutter peak heights and their variability.
2. An interest in reassessing the utility of smaller peaks below the often used analytical threshold.
3. A need to understand not just the occurrence of peak drop-in but also the height distribution of such peaks, and
4. A need to understand the limitations of the multiplex-interpretation strategy pair implemented.

In this work we present a full scheme for validation of a new multiplex that is suitable for

informing modern interpretation practice. We predominantly use GlobalFiler™ as an example multiplex but we suggest that the aspects investigated here are fundamental to introducing any multiplex in the modern interpretation environment.

Analysis and interpretation of mixed forensic stains using DNA STR profiling

Clayton, T., Whitaker, J.P., Sparkes, R.L., and Gill, P. (1998). *Forensic Sci. Int.* 91:55-70.

Abstract: The use of multiplex PCR and fluorescent dye technology in the automated detection and analysis of short tandem repeat loci provides not only qualitative information about the profile--i.e. which alleles are present--but can also provide quantitative information on the relative intensities of the bands, and is therefore a measure of the amount of amplified DNA. The availability of this quantitative information allows for the interpretation of mixtures in a detailed way which has not been previously possible with many other human identification systems. In this paper we present a simple approach to the resolution and analysis of mixed STR profiles resulting from the testing of mixed biological stains in forensic casework and highlight factors which can affect it. This approach requires a detailed knowledge--gained through a mixture of experiments and validation studies--of the behaviour of each locus within the multiplex systems described. We summarise the available data from previously published experimental work and validation studies to examine the general principles underlying this approach.

The interpretation of single source and mixed DNA profiles

Taylor D., Bright J.-A., Buckleton J.S. 2013. *Genetics* 7:516-528.

Keywords: Continuous models, Forensic DNA interpretation, Low template DNA, Mixtures

Abstract: A method for interpreting autosomal mixed DNA profiles based on continuous modelling of peak heights is described. MCMC is applied with a model for allelic and stutter heights to produce a probability for the data given a specified genotype combination. The theory extends to handle any number of contributors and replicates, although practical implementation limits analyses to four contributors. The probability of the peak data given a genotype combination has proven to be a highly intuitive probability that may be assessed subjectively by experienced caseworkers. Whilst caseworkers will not assess the probabilities per se, they can broadly judge genotypes that fit the observed data well, and those that fit relatively less well. These probabilities are used when calculating a subsequent likelihood ratio. The method has been trialled on a number of mixed DNA profiles constructed from known contributors. The results have been assessed against a binary approach and also compared with the subjective judgement of an analyst.

DNA commission of the International Society of Forensic Genetics: Recommendations on the evaluation of STR typing results that may include drop-out and/or drop-in using probabilistic methods

Gill P., Gusmão L., Haned H., Mayr W.R., Morling N., Parson W., et al. *Forensic Sci. Int:Genetics* 6:679-688.

Abstract: DNA profiling of biological material from scenes of crimes is often complicated because the amount of DNA is limited and the quality of the DNA may be compromised.

Furthermore, the sensitivity of STR typing kits has been continuously improved to detect low level DNA traces. This may lead to (1) partial DNA profiles and (2) detection of additional alleles. There are two key phenomena to consider: allelic or locus 'drop-out', i.e. 'missing' alleles at one or more genetic loci, while 'drop-in' may explain alleles in the DNA profile that are additional to the assumed main contributor(s). The drop-in phenomenon is restricted to 1 or 2 alleles per profile. If multiple alleles are observed at more than two loci then these are considered as alleles from an extra contributor and analysis can proceed as a mixture of two or more contributors. Here, we give recommendations on how to estimate probabilities considering drop-out, $Pr(D)$, and drop-in, $Pr(C)$. For reasons of clarity, we have deliberately restricted the current recommendations considering drop-out and/or drop-in at only one locus. Furthermore, we offer recommendations on how to use $Pr(D)$ and $Pr(C)$ with the likelihood ratio principles that are generally recommended by the International Society of Forensic Genetics (ISFG) as measure of the weight of the evidence in forensic genetics. Examples of calculations are included. An Excel spreadsheet is provided so that scientists and laboratories may explore the models and input their own data.

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