# TrueAllele® VUIer™

## Analyze Module

Cybergenetics

**TrueAllele® VUIer™: Analyze Module**



Document prepared on August 30, 2018 for VUIer™ software release 2018.



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# 1 Overview

The purpose of the Analyze module is to transform raw sequencer data into quality-checked, quantitated peaks. The output of the Analyze process is a data *.gel* file containing all signal and peak information from the original raw data. The *.gel* file is uploaded to the TrueAllele server in the Data module, making the data available for TrueAllele interpretation.

The Analyze module uses *DataDisks* to organize the data as it moves through the process. A *DataDisk* is a folder that contains all of the original electropherogram data, auditing information, and output. A *DataDisk* is self-contained and structures the data for the software.

The general flow of processing requires the computer to perform a quality check on the data, and then an analyst reviews the computer's quality check. The computer will direct the analyst to any potential data issues as part of the review process.

To open the Analyze module, the analyst selects the Analyze icon in the *Module Chooser* window (Figure 1).

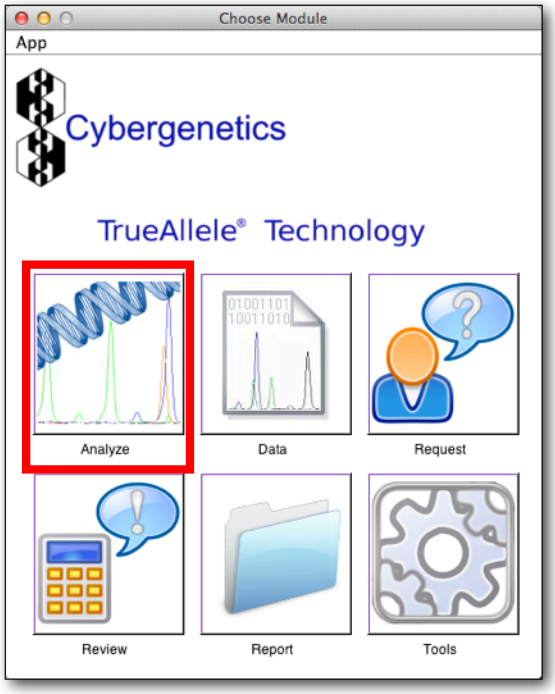


**Figure 1.** *Module Chooser* window. The Analyze module is indicated in the red box.

This action opens the *Command Window* of the Analyze program (Figure 2), which is independent of the VUIer™ software. The *Command Window* is the starting point of Analyze and where each computer process, followed by human review, is driven.

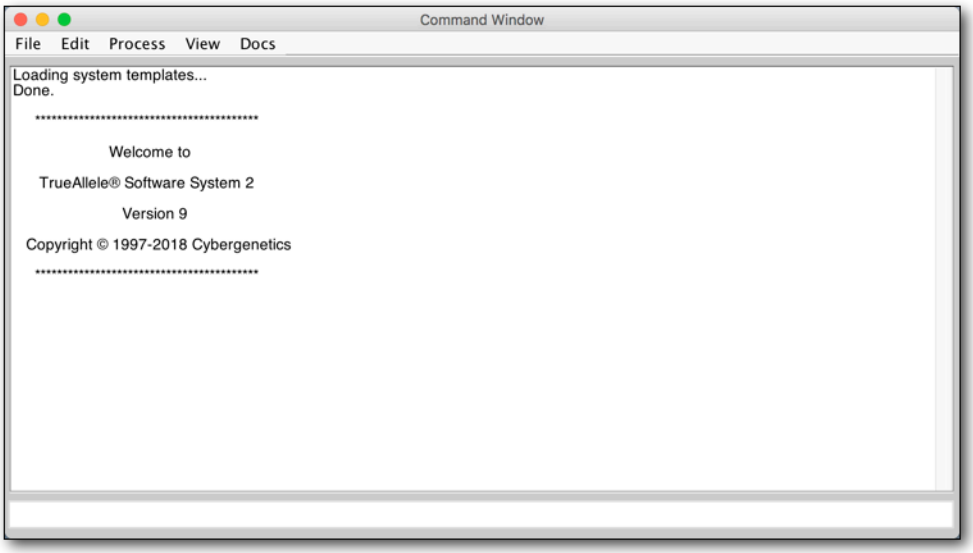


**Figure 2.** *Command Window.*

# 2 Preferences

Before processing data in Analyze, the analyst must set the initial preferences, specific to the data, and create a *DataDisk*, where all of the information is stored. Once this step is complete, the analyst continues the Analyze process by checking the ILS sizing and controls. Note: The ABI 3500 genetic analyzer data may have peak heights three times higher than previous genetic analyzer models. This data may need to be rescaled before TrueAllele processing. The **Appendix ▸ 3500 Rescaling** describes this process.

## 2.1 Initial Preferences

In order for the data to be correctly analyzed, the initial preferences must be set for the specific type of sequencer data. These preferences tell the program information about the type of data being used, such as the kit, size standard, and sequencer format. These settings can be viewed under **Edit ▸ Preferences ▸ Init**.... Once initially set, the preferences are retained between Analyze sessions.

The **Appendix ▸ Initial Settings** contains detailed information about the initial preference settings. If further information is needed, please contact Cybergenetics for assistance.

After the program is told what type of data to expect, a *DataDisk* can be created. A *DataDisk* contains all of the information necessary for the program to process the data.

## 2.2 Creating a *DataDisk*

A *DataDisk* is used to proceed through the Analyze process, storing the quality-checked data and quantitative information for the STR data prior to upload to a TrueAllele World.

Before creating a *DataDisk*, the analyst must ensure that the folder layout for the STR sequencer files on is correct. For *DataDisk* creation, the STR data files (*.fsa* or *.hid* files) must be in a run folder. That run folder must be inside a parent folder. This folder-in-folder structure is necessary for success in the Analyze module (Figure 3). Additional information on the layout of the folder is detailed in the **Appendix ▸ Sequencer Format**.

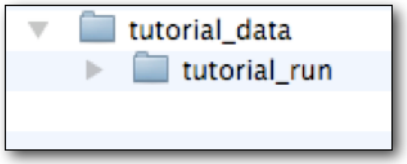


**Figure 3.** *Folder-in-folder structure.*

From the *Command Window*, an analyst directs the computer to the location of the STR data folder. Selecting **File ▸ New** from the toolbar allows the analyst to point to the parent folder in which the STR data sequencer file run folder is located.

Upon opening a folder of STR data, a *DataDisk* is automatically created using the raw data, template, and preferences selected. Each *DataDisk* folder stores all of the information generated during the Analyze process. This folder is designated by a "_DD" and automatically saved in the same directory as the original data folder.

Upon creation of a *DataDisk* for a specific set of data, the *DataDisk* is automatically opened in the *Command Window*. To return to a *DataDisk* that was previously created, the analyst can reopen that *DataDisk* in Analyze by selecting **File ▸ Open**.

# 3 Sizing

The first step of the Analyze process is *Image Call*, where the program tracks the internal lane size standard. Following this computer step, the analyst reviews and verifies this tracking in the *Cap View* interface before proceeding to the next step.

## 3.1 Image Call

In *Image Call*, the computer tracks and checks the ILS size standard sizing for each lane of data. This computer step is initiated by selecting **Process ▸ Image Call**. The computer informs the analyst when tracking is complete.

## 3.2 Cap View

Once *Image Call* is complete, the first human review step is *Cap View*, where an analyst reviews the computer's size standard tracking. To start the review process, the analyst selects **View ▸ Cap View**. When first entering an interface, the analyst is prompted to log in with his or her initials. A default login of "user" is available. If needed, an analyst can add their login initials to the 'operators.txt' file located in the **trueallele ▸ user** folder in Documents.

In *Cap View*, the analyst is presented with a "virtual gel" view showing the size standard dye (Figure 4). In this view, each injection is shown as a single column. The size standard peaks are shown as bands. The horizontal axis shows the lane numbers, and the vertical axis the pixel values.

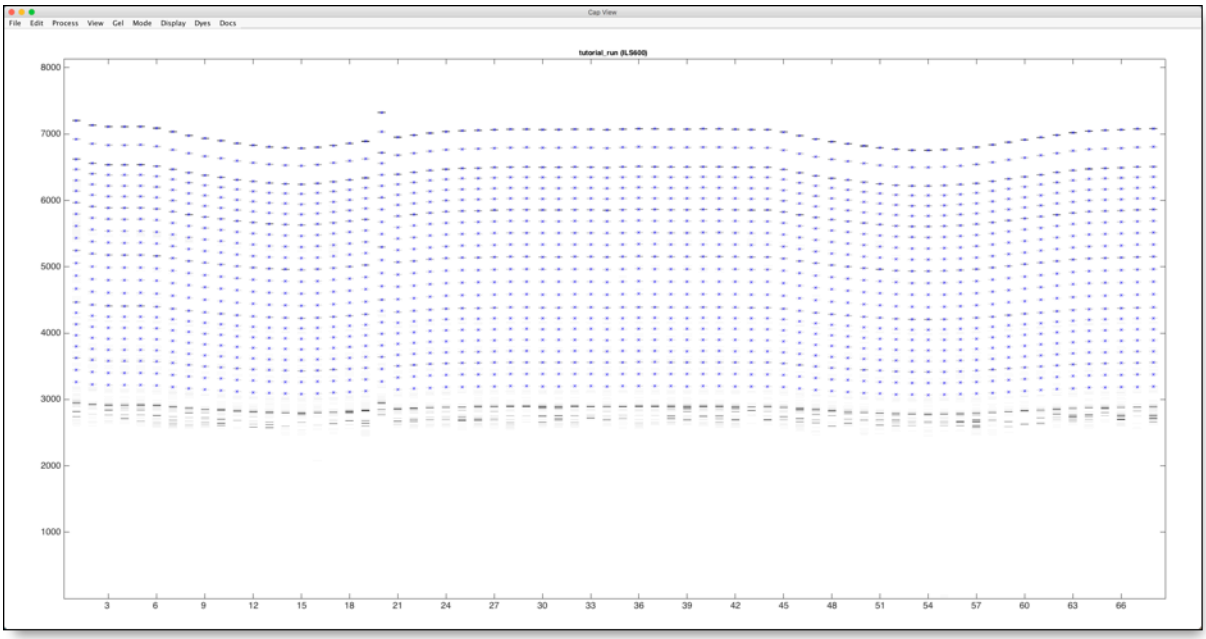


**Figure 4.** *Cap View.* The Grid tracking is displayed.

This interface provides a global overview of the sizing, allowing for efficient review to ensure the computer properly tracked the size standard for each data lane. The computer automatically checks the ILS tracking and flags lanes with possible issues with a red **X**. The analyst reviews the lanes with the red **X**'s to determine the next action. Figure 5 shows *Cap View* with lane 23 flagged with a red **X.**

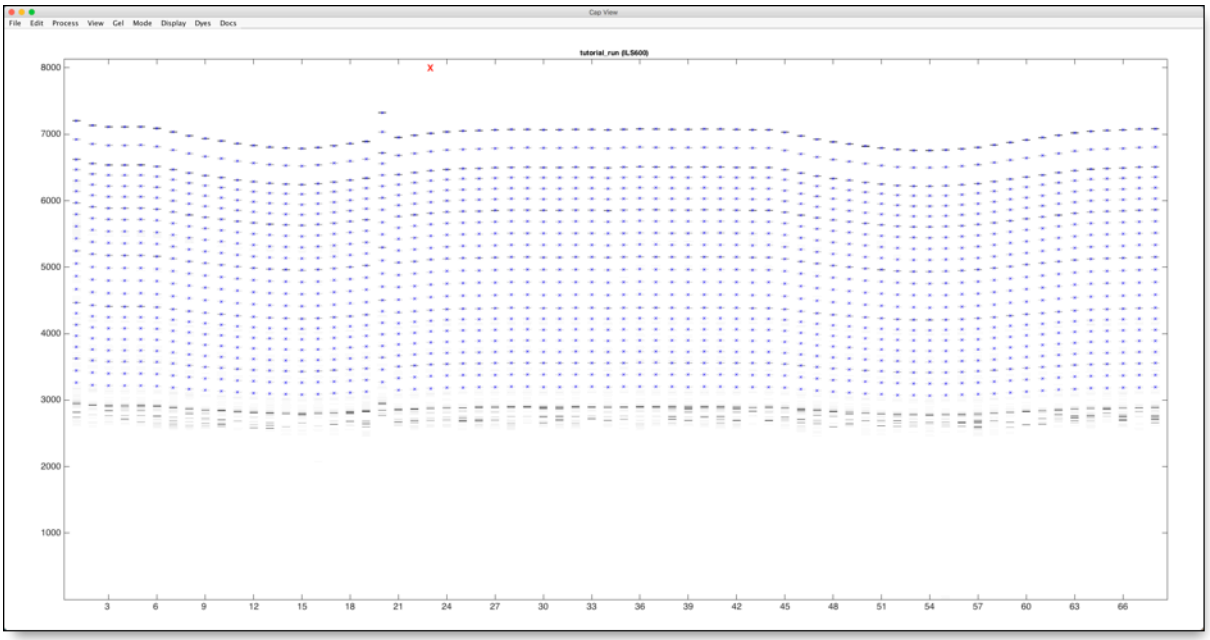


**Figure 5.** *Cap View* with lane 23 flagged with a red **X**.

## Modify Mode

When the analyst sees an injection flagged with a red **X**, the analyst uses Modify Mode to update the ILS tracking. From the *Cap View* window, the analyst selects **Mode ▸ Modify** and selects the mis-tracked capillary using the Modify cursor. This action opens the *AutoEdit Sizing* window for that capillary (Figure 6).

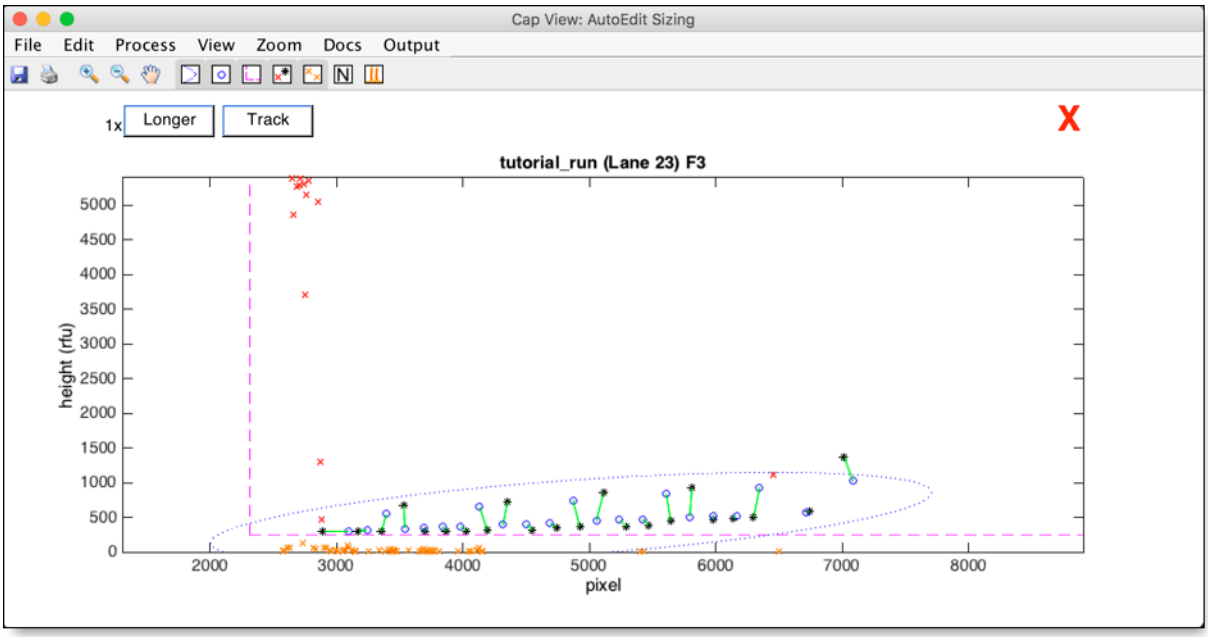


**Figure 6.** *AutoEdit Sizing* window.

The *AutoEdit Sizing* window allows an analyst to modify the ILS tracking for the selected capillary. The window shows data points, the model, and how the computer framed the lane data. The x-axis displays the pixel coordinates, and the y-axis displays the peak height in *rfu*. The gel name, lane number, and sample name are indicated above the active region.

Additional options and views are available using the toolbar buttons. From left to right, the analyst can save the *AutoEdit Sizing* window image, print the window image, and zoom and pan across the data. The next five toolbar buttons allow the analyst to toggle the ellipse (expected data region), the data model, the frame, and peaks within and outside the frame region. The analyst also can display the size standard labels (N button). Active labels are connected to the data points with dashed lines. Inactive labels, or labels not used in sizing are not connected to the data. In addition, the analyst can view the ILS electropherogram trace to orient the analyst to the data points (right-most button).

Underneath the toolbar are the Longer and Track buttons. The analyst uses these buttons when modifying the tracking. The Longer button, when selected, repeatedly doubles the number of cycles used when re-tracking the ILS. The Track button starts the re-tracking process.

The analyst modifies the tracking by adjusting the data frame. To do this, the analyst drags the vertical frame to the right of the primer region and the horizontal frame up or down to include the data peaks (Figure 7). This frame adjustment gives the computer additional information to better track the ILS. Note: The computer uses the frame position only as a starting point. Therefore, the analyst only needs to move the frame to the general area of the data.

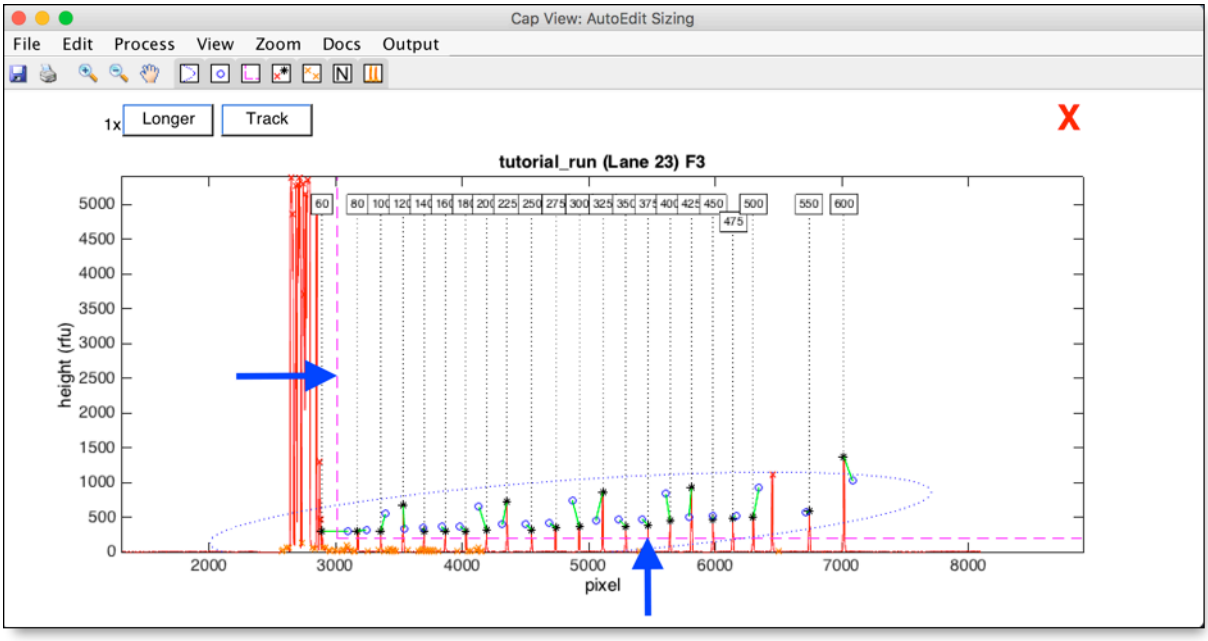


**Figure 7.** Adjusting the frame in the *AutoEdit Sizing* window.

Additionally, an analyst can remove labels from consideration (e.g., if they run off the virtual gel, spikes, etc.). First, the analyst uses the toolbar button to display the labels (N button). Then, the analyst clicks on the label where there is no peak present in the data. The label box changes from white to gray for that label and the

labels to the right of it, indicating that they are also disabled. The analyst can enable data labels by clicking the label again, changing it from gray to white.

After the analyst adjusts the frame and/or peak labels available for tracking, the analyst clicks the Track button (or keyboard shortcut ⌘-t or ctrl-t). The computer re-tracks the lane ILS using the updated parameters provided by the analyst. Once it finishes, the computer displays the new ILS tracking results (Figure 8). When the model and data are close, the computer removes the red **X** flag from the interfaces (*Cap View* and *AutoEdit Sizing),* indicating that the ILS is successfully tracked.

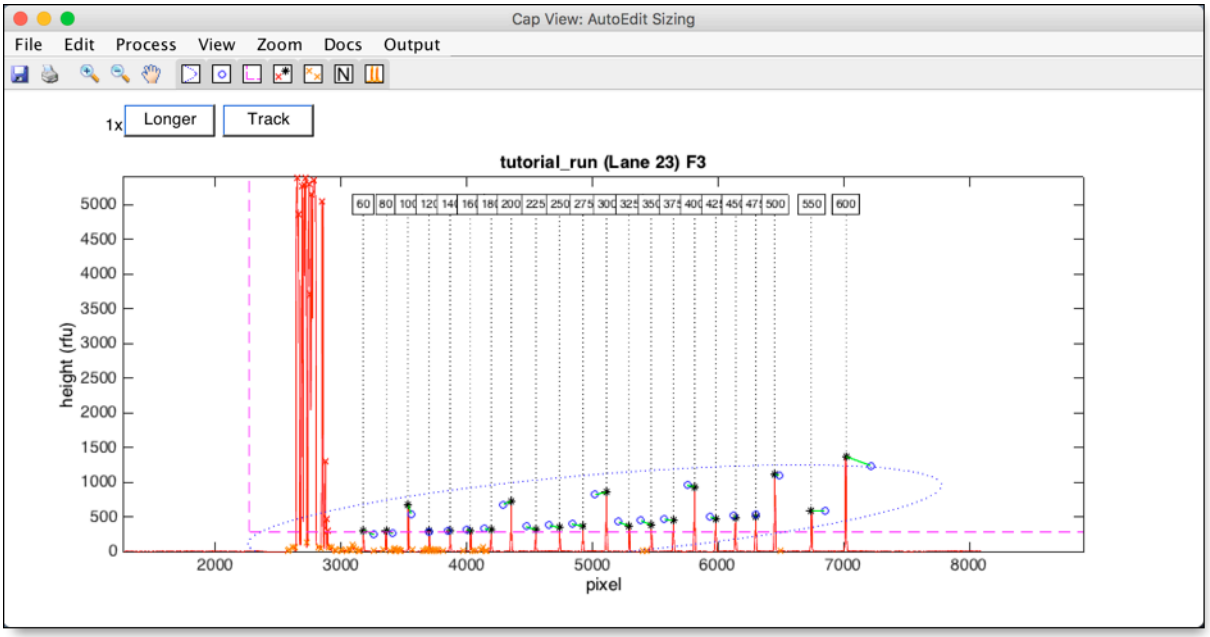


**Figure 8.** Adjusting the frame in the *AutoEdit Sizing* window.

After the injection has been re-tracked, the analyst can close the *AutoEdit Sizing* window.

The analyst now sees that the blue x's in the main interface are updated as well. For more challenging data, additional rounds of re-tracking may be required, including using the 'Longer' button.

Image Call may flag lanes with a red **X** that are tracked accurately when the diagnostic parameters exceed expectations (e.g., sig2x, sig2y, probability). An analyst can verify the tracking and manually accept the lane by changing the red **X** to an orange **O**. To change the **X** to an **O**, the analyst opens the *AutoEdit Sizing* window and clicks on the red **X** at the top right of the window. This action changes the **X** to an **O** in both the *AutoEdit Sizing* window and the main *Cap View* interface (Figures 9 and 10). The orange **O** indicates that the analyst reviewed the tracking for that lane and verified that it is accurate.

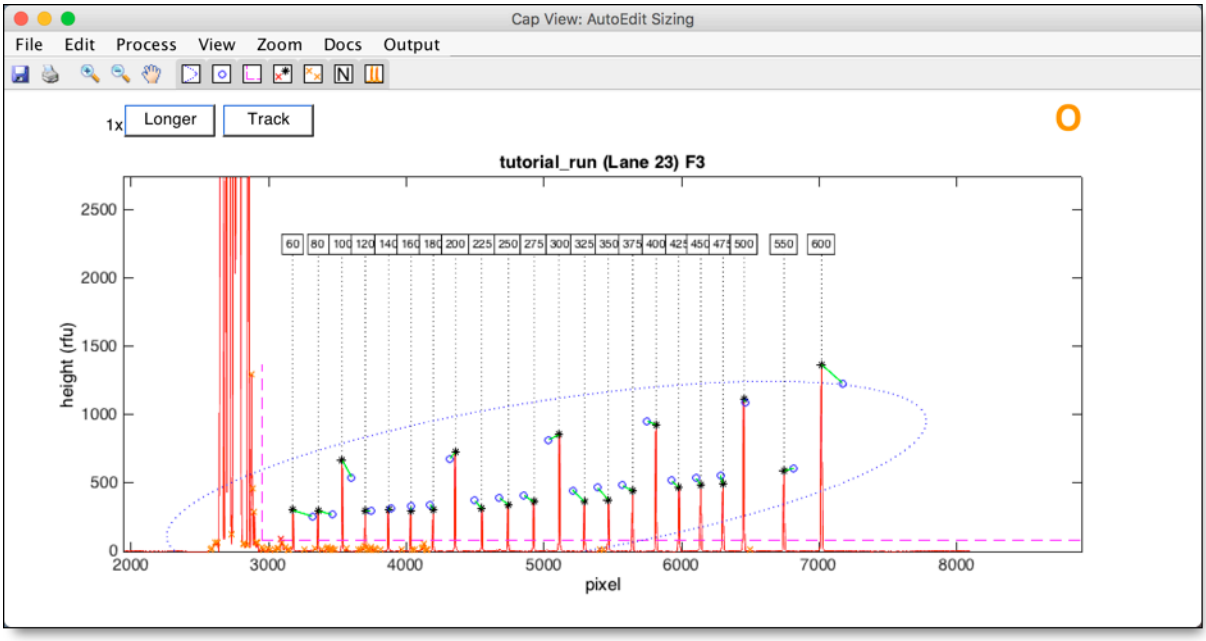


**Figure 9.** *AutoEdit Sizing* with the orange **O**.

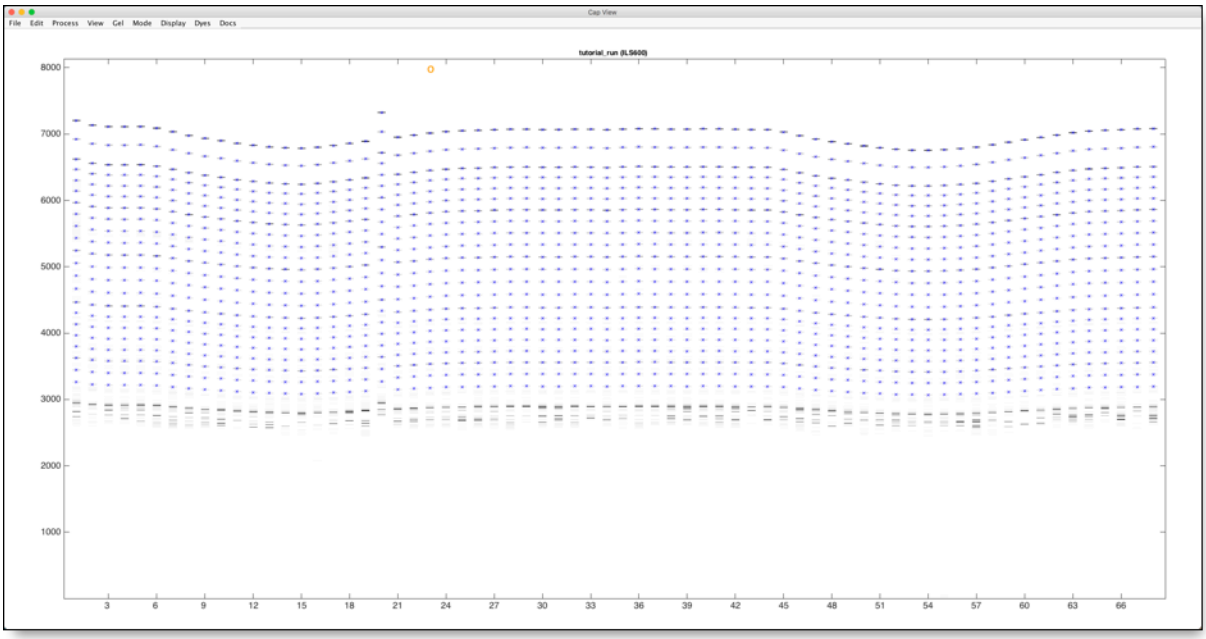


**Figure 10.** *Cap View* with the orange **O**.

A record of the modified capillaries is logged in the gel's **Edit ▸ track_edits** file, and is available with the Databank software.

### Select Mode

If an analyst sees a capillary where the sizing peaks appear to be low quality, the analyst can remove it from further processing. To do this, the analyst selects **Mode ▸ Select**, which enables Select mode. From here, the analyst selects the capillary to be removed. A dialog box prompts the analyst is to provide a reason for removing the lane (Figure 11).

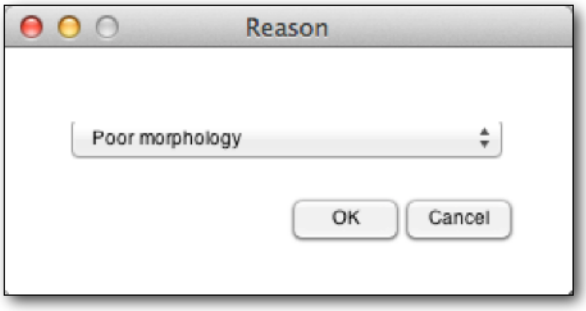


**Figure 11.** *Reason* window.

In the typical process, the analyst scans the injections and removes any injections with sizing issues. Additional information on troubleshooting for problematic data is found in the **Appendix ▸ Troubleshooting**.

Once the analyst is satisfied with the ILS tracking, the analyst moves on to the next step of Analyze, which involves checking the ladders and controls.

# 4 Controls

The next step of the Analyze process involves quality-checking the controls and allelic ladders. The program does this process in *Marker Call*. The analyst then reviews the results of these checks in *Control Check*, where rules guide the process.

## 4.1 Marker Call

*Marker Call* is the step where Analyze calls the allelic ladder peaks as well as verifies the quality of the allelic ladders, positive controls, and negative controls. Analyze also looks for other possible data issues. The analyst initiates this step by selecting **Process ▸ Marker Call**. This step takes 1 or 2 minutes for the computer to complete.

## 4.2 Control Check

Once *Marker Call* is complete, an analyst reviews the computer results in the *Control Check* interface. This process typically takes about 30 seconds per plate, since the Analyze rules direct the review. If no rules fire, then no further action is required. More information regarding these rules is found in the **Appendix ▸ Analyze Rules**.

The analyst begins the review by selecting **View ▸ Control Check**. Again, the analyst will be prompted to log in with initials or the default entry of “user”.

Upon entering *Control Check*, there is a main window and a smaller *Rules* window (Figure 12). The main window displays a ‘virtual gel’ view of a specific locus for

each data lane. The *Rules* window displays a list of the Analyze rules and check boxes.

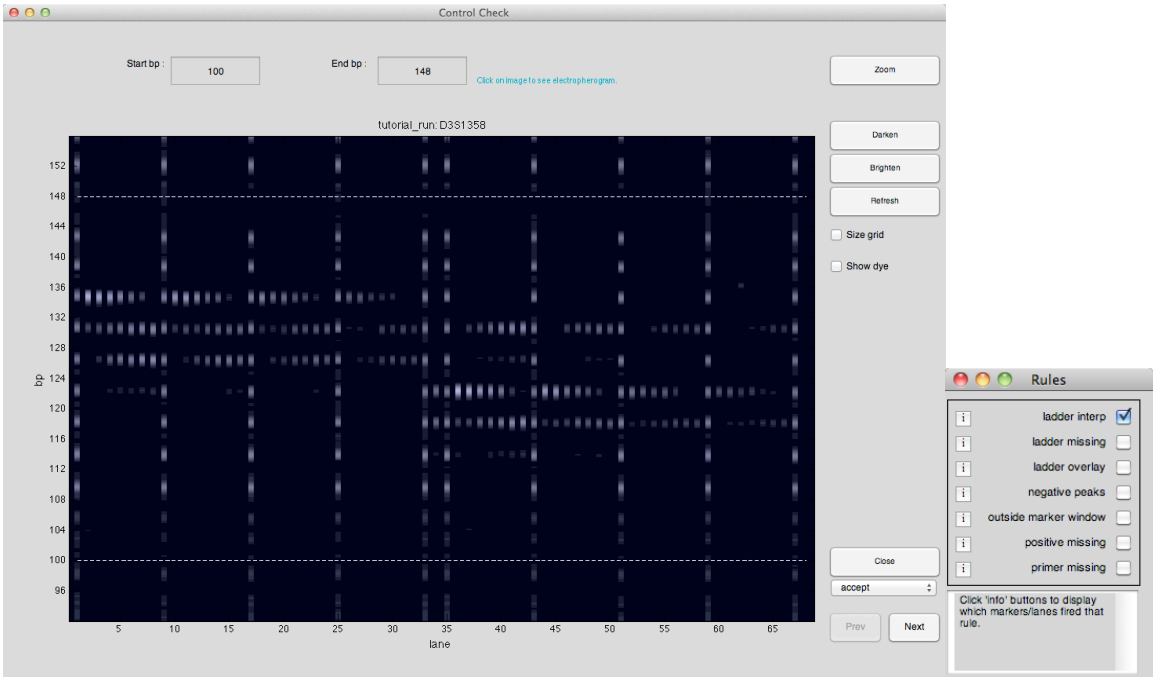


**Figure 12.** *Control Check* and *Rules* windows.

In the typical process, if none of the boxes are checked, then the analyst is assured of the data's quality, and no further action is required. The analyst can then leave this interface and proceed to *Allele Call*.

If a rule has fired, Analyze directs the analyst to review the possible issue. Each rule has associated interfaces to diagnose these issues. A brief example is provided here, while the full details of the diagnostic interfaces are provided in the **Appendix ▸ Analyze Rules** and **Appendix ▸ Troubleshooting**.

In this example, the 'ladder interp' rule has fired (Figure 13). This rule fires when the Analyze module interpolates at least one peak in an allelic ladder. The program uses this rule to let the analyst know that, for the indicated loci, the expected sizing

of allelic ladder peaks for one or more ladders is shifted from what is expected. This may or may not be problematic.

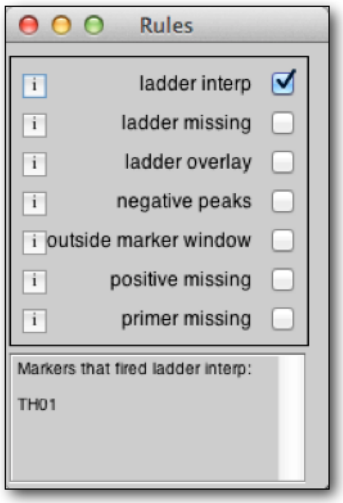


**Figure 13.** *Rules window.* The Ladder interp rule fired at TH01.

Selecting the 'i' box next to the rule provides the analyst with some additional information. Here, the analyst is directed to the TH01 ladder. When this rule fires, the analyst verifies the ladder tracking for the indicated locus in the *Ladder Check* interface. To get to this interface, the analyst selects **View ▸ Ladder Check**.

*Ladder Check* opens with a view showing all of the allelic ladders overlain on top of one another (Figure 14). The analyst switches to the TH01 marker by selecting **Markers ▸ TH01**. Vertical lines show the ladder peaks that Analyze has designated.

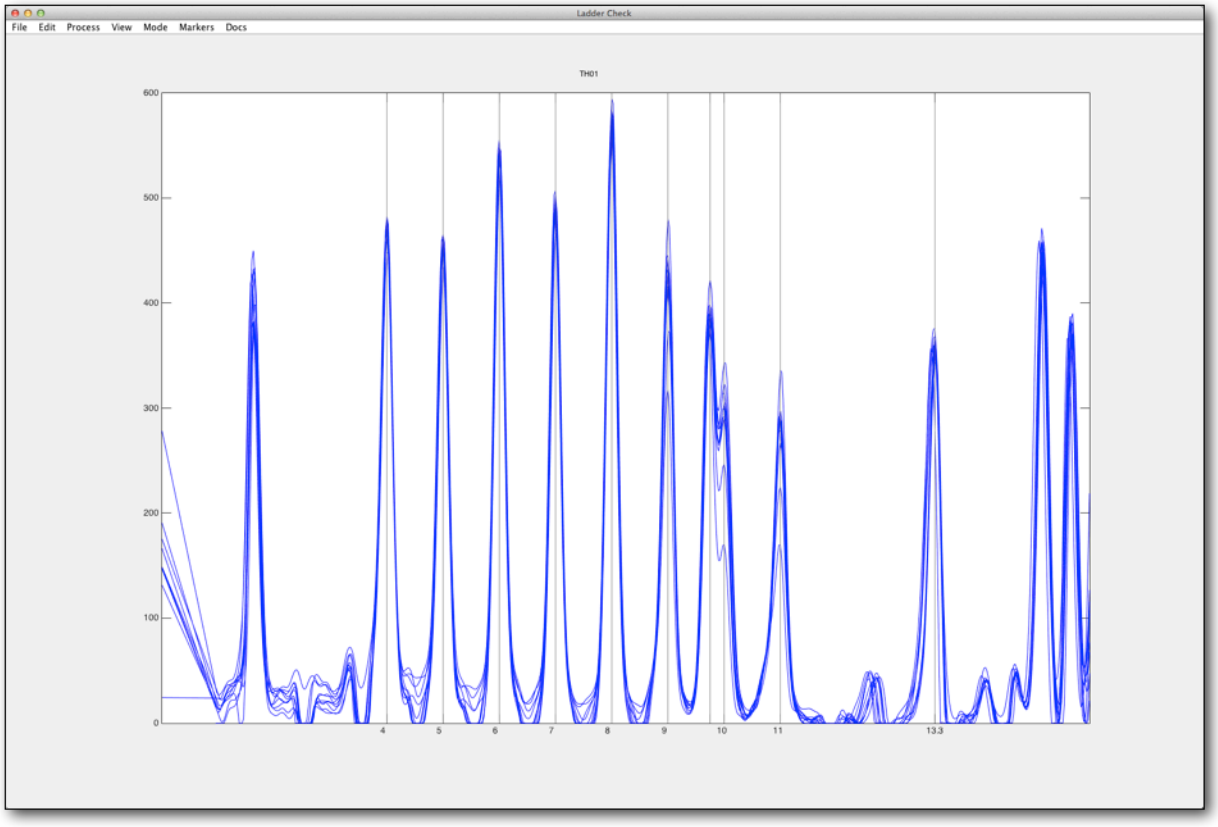


**Figure 14.** *Ladder Check* at the TH01 locus.

Clicking on the electropherogram separates each ladder injection into its own row (Figure 15). In the fourth row, it appears that the vertical designation line is not through the center of the peak designated 13.3. The analyst selects this lane to reject this specific ladder lane. All data dependent on this ladder lane will be automatically reassigned to a new allelic ladder.

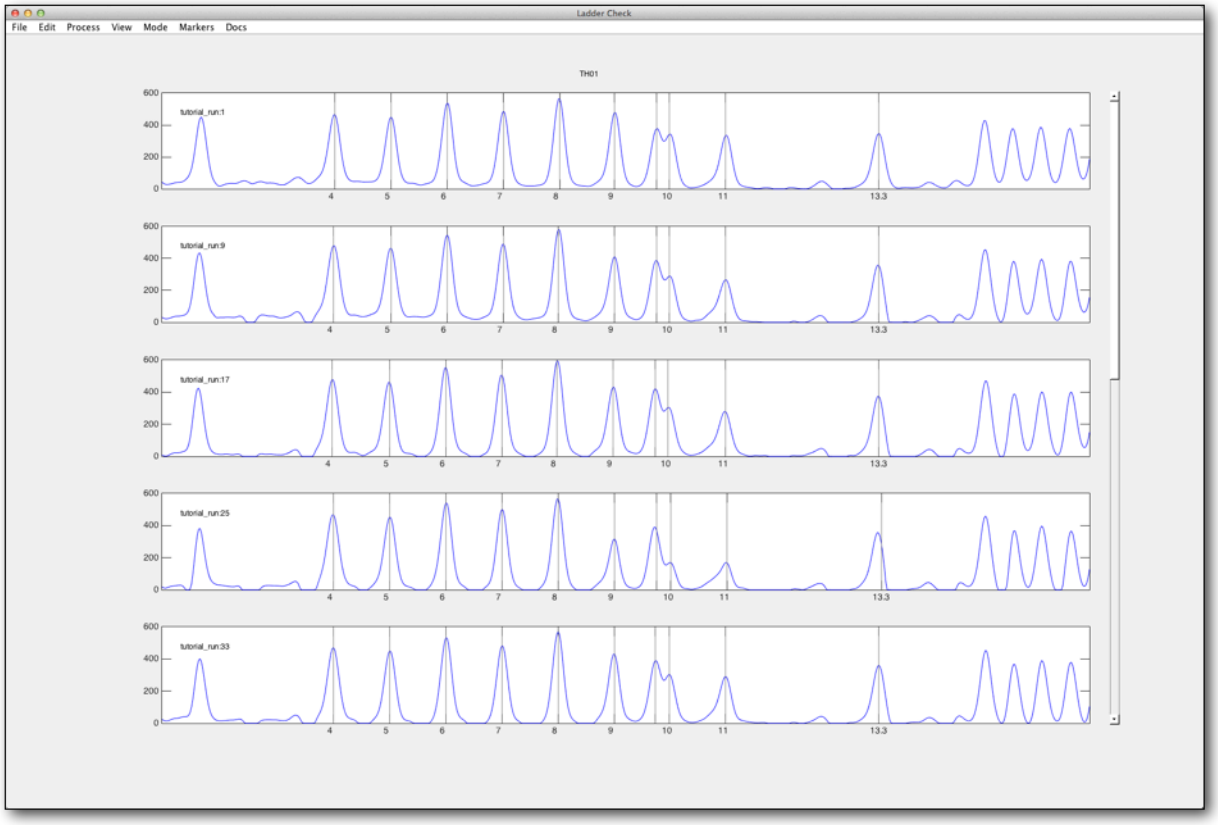


**Figure 15.** *Ladder Check.* Viewing the individual ladders for TH01.

Having addressed the rule firing, the analyst is now assured of the run quality. The analyst now continues to the last step of the Analyze module, where the data is quantitated prior to upload to a TrueAllele World.

# 5 Output

Now that the data quality is verified, the Analyze process is nearly complete. In *Allele Call*, the program performs peak quantitation and produces the data *.gel* file that stores this information. There is no further human review step following *Allele Call*.

## 5.1 Allele Call

To initiate the final step of the Analyze process, the analyst selects **Process ▸ Allele Call.** In *Allele Call*, the computer completes the process of transforming the raw electropherogram data into quality-checked, quantitated peaks for use in TrueAllele interpretation. This step involves quantitation of each data peak. *Allele Call* takes about a minute of processing time per locus per plate to complete.

## 5.2 Data *.gel* File

The final output of the Analyze process is a data *.gel* file. This file contains the original data signals and all of the quality-checked, quantitative peak information. This file will be uploaded to a TrueAllele database for genotype separation.

The *.gel* file is located in the *DataDisk* folder inside the data folder for each individual run (Figure 16). If more than one run was processed in the DataDisk, each run will have its own folder and data *.gel* file. Additionally, the analyst can direct Analyze to place all of the gel files in a specific folder, such as /Documents/gels, to allow for more efficient collation. Contact Cybergenetics for more information.

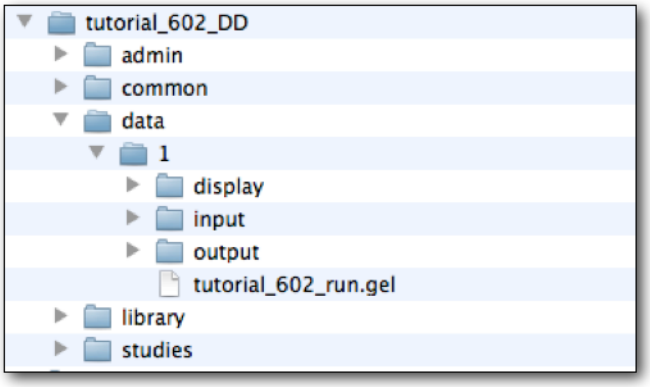


**Figure 16.** Data *.gel file location*.

The data *.gel* file is used in the Data module upload the data to the TrueAllele World. Additionally, the *.gel* file is used in batch request processing for a more efficient workflow. The "TrueAllele® VUIer™: Data Module" manual discusses these applications further.

## 5.3 Completing Analyze

Once *Allele Call* finishes, the data *.gel* file is automatically created, and the Analyze process is complete. To proceed to the *Data* module, the analyst selects **File ▸ Open Module Chooser**. This action closes the *Command Window* of Analyze and returns the analyst to the *Module Chooser* window.

# Appendix

## Initial Settings

A brief overview of the initial preference settings is provided in this section. An analyst accesses these settings by selecting **Edit ▸ Preferences ▸ Init…** from the *Command Window* (Figure 17).

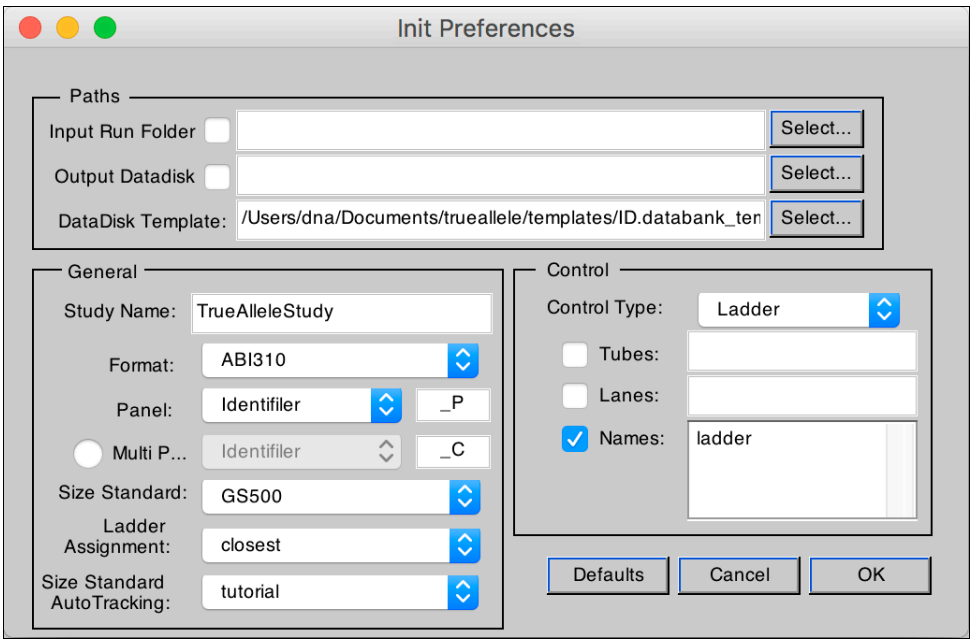


**Figure 17.** *Initial Preferences.*

### Paths

The Paths section indicates to the program where to find the data, where to create the *DataDisk*, and the type of data to expect. By default, the Input and Output are left blank, allowing the analyst to specify a different location each time a *DataDisk* is created.

*Input Run Folder:* This setting allows the analyst to specify the location of the data input. It is typically left blank, as this allows the program to prompt the analyst upon *DataDisk* creation.

*Output DataDisk:* This setting allows the analyst to specify a location for the newly created *DataDisks*. By default, leaving this blank will create the *DataDisk* in the same location as the input folder.

*DataDisk Template:* The *DataDisk* template provides the Analyze module with the information required to run different types of data. The analyst should ensure that the *DataDisk* template selected corresponds to the type of data being processed. Casework templates for the commonly used kits are provided with the software. A list of the kits currently supported is found below. If an additional kit is used for which a template is not provided, please contact Cybergenetics for assistance.

*Supported kits*

PowerPlex® 16
PowerPlex® 21
PowerPlex® ESI-17
PowerPlex® Fusion
PowerPlex® Fusion 6C
Profiler®, Profiler Plus®, and COfiler®
Identifiler®, Identifiler® Plus, and Identifiler® Direct
GlobalFiler™
NGM™ and NGM SElect™
SGM plus®
MiniFiler™
IDplex
Investigator 24plex QS

**General**

The General section is where the analyst provides Analyze with some background information on the type of data to expect.

*Study Name:* The study name is used to name a batch of *.gel* files. The default name for the study is *TrueAlleleStudy* and can be changed as desired.

*Format:* This preference indicates the sequencer format. See additional notes on supported sequencer formats in the **Appendix ▸ Sequencer Formats**.

*Panel:* This preference indicates the panel or kit used to amplify the data. The text box following this entry is only necessary if the Multi Panel feature is being used.

*Multi Panel:* For data where multiple panels are processed within one run folder, Analyze can determine the panel using flags in the sample name. Checking the Multi Panel button enables this feature. The text boxes following the Panel and Multi Panel entries indicate the text in the sample names used to differentiate the different panels.

*Size Standard:* This preference indicates the internal lane standard (ILS) used to size the data.

*Ladder Assignment:* This preference sets the ladder assignment that is used with the data. By default, the setting is 'closest' for all capillary data. Staggered sample loading in gel data may require the 'loading' setting.

*Size Standard AutoTracking:* This preference sets the values used with the Bayesian tracking method. Each dropdown entry corresponds to a row in the *'size_standard_autotracking.txt'* preference file.

### Control

The Control section allows an analyst to set the position and name of the allelic ladders as well as the negative and positive controls. Important: when filling in information for a positive control, the exact control must be selected from the dropdown menu; selecting “Positives” will prompt the user to select a specific control.

*Control Type:* This menu indicates the control that is currently being addressed.

*Tubes:* The Tubes entry specifies the chosen control’s location by tube or well number.

*Lanes:* The Lanes entry specifies the chosen control’s location by lane / injection number.

*Names:* The string name match feature allows the analyst to indicate a text within a sample name to find and designate the controls. For example, an analyst may provide the text ‘lad’ to allow Analyze to identify and designate allelic ladders with sample names of ‘ladder.’

## Sequencer Formats

### Available formats

The Analyze module supports the automatic setup (“*AutoSetup*”) of capillary data from the following sources:

ABI 310

ABI 3100 (standard and plate formats), ABI 3100-Avant

ABI 3130, ABI 3130xl

ABI 3500, ABI 3500xl

ABI 3700

ABI 3730

RapidHIT

SpectruMedix

MegaBACE

WhiteheadInstitute

### Format specifications

The following sections describe the specifications for running Analyze using the indicated sequencer formats.

### ABI 310

To create a *DataDisk* with ABI 310 data, the analyst places the capillary run folder inside another ‘data’ folder. Additional run folders can be added as desired. The analyst then sets the initial preferences and points to the capillary data folder as the input. AutoSetup will then automatically create the *DataDisk*.

### ABI 3100/3130/3130xl/3130-Avant

TrueAllele software supports different data formats for the ABI 3100 family: standard and plate. The standard format has a set of 96 capillary files in each separate run, whereas the plate format has six sets of 16 capillary files grouped together in one run (Figure 18). Both formats result in one ‘run’ of 96 capillaries.

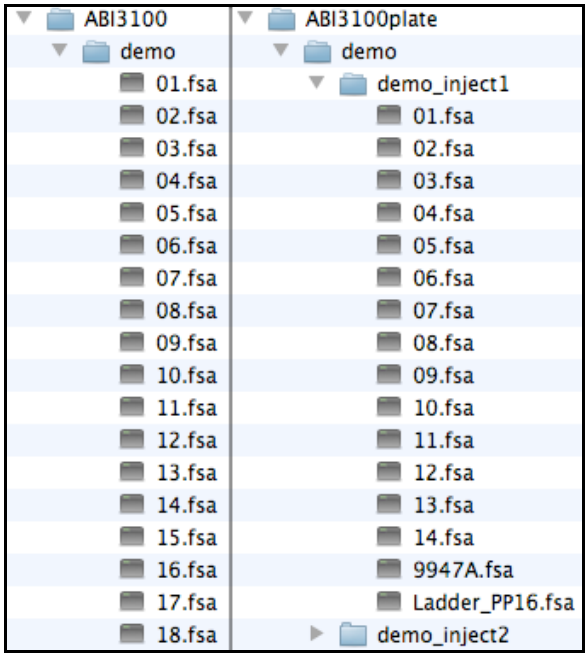


**Figure 18.** ABI3100 – Standard format (left) and Plate format (right).

### ABI 3500/3500xl/3700/3730

ABI 3500, ABI 3500xl, ABI 3700, and ABI 3730 format follow the ABI 3100 specification.

## 3500 Rescaling

The ABI 3500 genetic analyzer produces peak heights about three times higher than previous genetic analyzer models. Data from this instrument may need to be rescaled before TrueAllele processing. The *custom_thresholds.txt* file (located in the Documents > trueallele > templates > specific template > common folder) is edited to rescale the data. This file contains a *rescale* field that can be filled in to rescale the data as needed based on validation.

## Data Acquisition Settings

The order in which the data lanes are acquired from the sequencer files can be based on sequencer plate position (auto) or read in by file name. This setting must be changed prior to the creation of a *DataDisk*.

Regardless of which setting is used, the system reads in all of the sequencer files for a plate and then orders them based on the *newdd_prefs* settings (described below). This initial step allows the setup to exclude those injections/samples/files that are missing from the plate.

| Field | Value | Behavior |
|---|---|---|
| *newdd_acquire* | auto | The data is acquired and ordered data as per sequencer used. |
| | file | The data is acquired and ordered alphabetically by sequencer file name (as determined by OS). |
| *newdd_injection* | [\._-]\d.fsa | When multiple injections of the same well are indicated by a period, an underscore, or a dash and a number before the *.fsa* or *.hid* extension, the data is acquired and ordered where subsequent injections from a well follow the initial injection. |

The *newdd_prefs* settings can be changed in the associated Tab-delimited text (*.txt*) file. This file is located at **Documents ▸ TrueAllele ▸ user ▸ newdd_prefs.txt** on a Macintosh (**My Documents ▸ TrueAllele ▸ user ▸ newdd_prefs.txt**). To change the data acquisition from “auto” to “file”, the analyst opens the ‘newdd_prefs.txt’ file using a text editor application (TextEdit, Notepad). Next to the preference named “newdd_acquire”, the analyst can replace “auto” with “file”. Once the changes are saved, any new *DataDisks* will collect the data by the order of the file names.

The system continues to acquire the data by file until the 'newdd_prefs.txt' file is again changed. The analyst is able to reset the data acquisition preference back to the default setting when desired.

The data acquisition method used to generate the *DataDisk* affects the lane number attached to the data.

## Troubleshooting

### Sizing

This section provides some additional details and actions for a range of possible sizing issues. These actions all relate to the human review performed in *Cap View*.

*The labels presented in Cap View do not appear to correspond to the size standard peaks that are observed.*

When the analyst sees that the computer tracking is drastically different from the expected sizing, the analyst should confirm the size standard selected in *Initial Preferences*. It is possible that the incorrect size standard was used during *DataDisk* creation. If that is the case, the analyst adjusts the size standard setting, recreates the *DataDisk*, and reruns the *Image Call* process.

*An injection is missing some or all labels.*

In some cases, a data issue may result in some or all labels missing for an injection. The tracking can usually be adjusted in the *AutoEdit Sizing* window by moving the frame (discussed in the Modify Mode in **Section 3.2**). Consult the "TrueAllele® Databank Reference" manual for more information regarding size standard tracking settings.

### Controls

This section summarizes the detailed information presented in the Analyze Rules section. For each rule, the associated interfaces and actions are listed in the summary table.

*Summary Table*

| Rule | Interface | Action |
|---|---|---|
| Ladder Interp | Ladder Check | Verify tracking, reject if necessary. |
| Ladder Missing | Ladder Failures | Confirm at least one ladder is present. |
| Ladder Overlay | Ladder Check | Confirm ladder sizing. |
| Negative Peaks | Electropherogram | Verify absence of contamination. |
| Outside Marker Window | Electropherogram | Adjust marker windows if necessary. |
| Positive Missing | Positive Control Check | Verify positive controls. |
| Primer Missing | Cap View | Verify primer presence in negative. |

## Analyze Rules

This section provides detailed descriptions of the Analyze rules that are used in *Control Check*. Rule descriptions and corresponding actions to perform when rules fire are presented here. A summary table is provided in the **Appendix ▸ Troubleshooting**.

### Analyze Rule Descriptions

The *Control Check: Rules* window displays the results of the data quality assurance checks (Figure 19). The analyst can open the window by selecting **View ▸ Rules** in the Control Check window. Rule firings are indicated by a checked box to the right of the rule name in the Rules window. For additional information, such as which loci or lane(s) fired that particular rule, the analyst can click on the 'i' button to the left of the rule name.

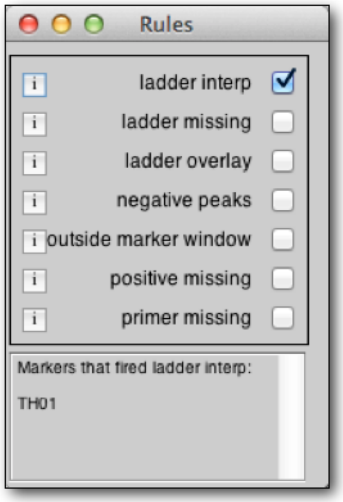


**Figure 19.** *Control Check Rules* window.

### Ladder interp/interpolated

This rule fires when the Analyze module interpolates at least one peak in an allelic ladder. The rule firing tells the analyst that the software could not find all of the expected allelic ladder peaks for the indicated loci or that the expected sizing of allelic ladder peaks for one or more ladders is shifted from what is expected. This issue may or may not be problematic. Ladder tracking for the indicated locus is verified in the *Ladder Check* interface found by selecting **View ▸ Ladder Check**.

### Ladder missing

Allelic ladders should be found in the designated lanes. When the Ladder Missing rule fires, allelic ladders for the indicated loci could not be found. The analyst must check to ensure that the allelic ladder lanes were indicated correctly in the initial setup of the *DataDisk*, and that the allelic ladder peak heights are above threshold.

To see which allelic ladder(s) are missing for each locus of a particular run, the analyst clicks on the 'i' button to the left of the 'ladder missing' rule name. In the text box at the bottom of the Analyze *Rule* interface, the loci that have missing ladders appear.

To see the missing (failed) ladders visually, the analyst can go to **View ▸ Ladder Failures** in the menu bar. The *Ladder Failures* window appears (Figure 20). In this particular example, there are ten allelic ladders in lanes 1, 9, 17, 25, 33, 35, 43, 51, 59, and 67. These allelic ladders passed for all loci.

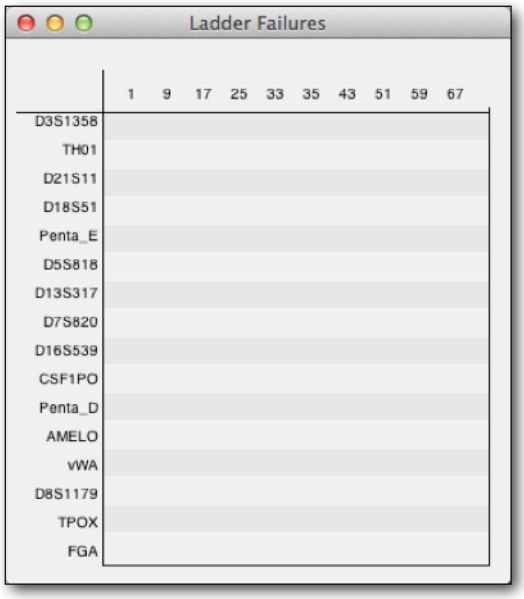


**Figure 20.** *Ladder Failures* window.

If an allelic ladder fails, the Ladder Failures window indicates the failed allelic ladder with an 'X' next to the locus in the specific allelic ladder column.

### Ladder overlay

Allelic ladder peaks should not vary in length between lanes. When this rule fires, the lengths of at least one of the allelic ladder peaks in two different lanes differ by at least 0.5 bp for the indicated loci. Therefore, one (or more) of the allelic ladders may be sized incorrectly. The ladder overlay problems can be viewed by selecting **View ▸ Ladder Check** in the menu bar for the specific marker (refer to Figures 14 & 15).

**Negative peaks**

The designated negative control lanes should not contain any peaks. This rule fires when peaks were found in the negative control lanes in the indicated marker regions. These peaks could indicate contamination of some type. If the rule fires, the analyst clicks on the 'i' button to see which negative control lane is flagged. Clicking on the lane on the virtual gel opens the *Control Check Electropherogram* view for further review.

**Outside marker window**

True allelic peaks may be observed outside the analyst-defined marker ranges. When this rule fires, one or more peaks (greater than the analyst-defined threshold) were found outside of the marker ranges. The specific lane, dye, and size are indicated for each peak.

The analyst can indicate the range within which the Analyze module should look for peaks for a marker. For example, a peak could be found at 255 bp in the green dye, but is not inside the D7S820 or D16S539 PowerPlex® 16 marker windows. When the peak is found, the specific lane, the dye, base pair size, and height are displayed in the text box when the 'i' button is selected. If a true allelic peak appears outside a marker window, the marker range can be adjusted in the main *Control Check* interface by altering the marker window 'start bp' and 'end bp' (Figure 21, red box). The base pair range of each marker can be viewed in **View ▸ Panel Info**.

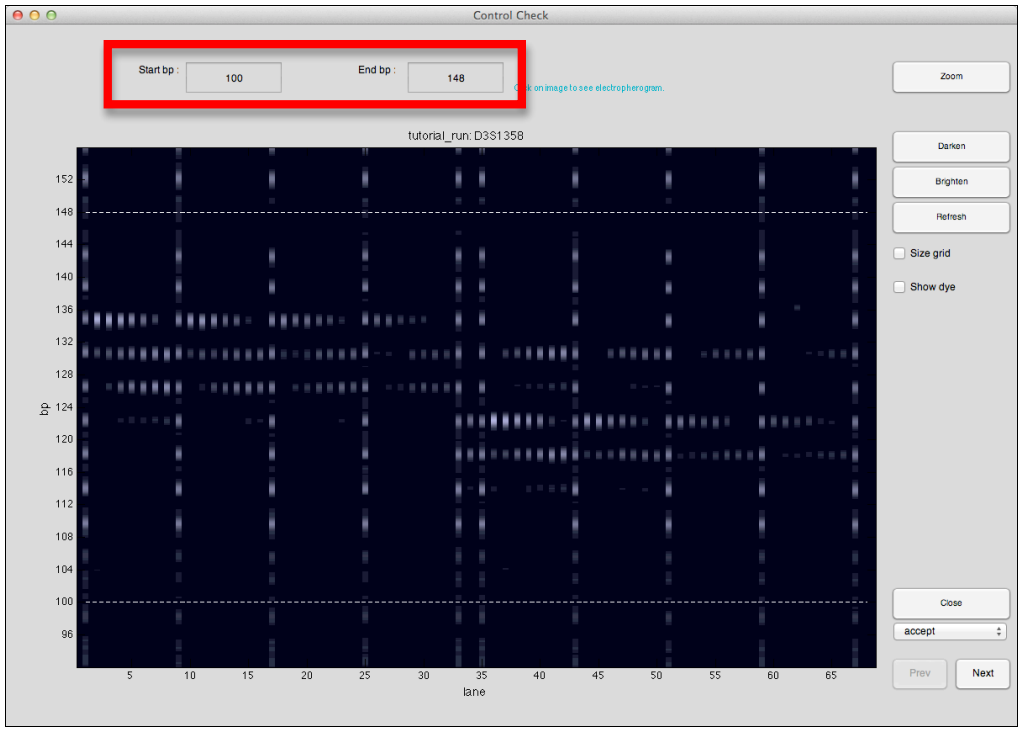


**Figure 21.** Editing the marker range.

### Positive missing

The analyst-designated positive control lanes should contain a specific trace pattern for each dye plane. When the software cannot find adequate peaks or the correct pattern for one or more positive control lanes, it fires this rule. This could indicate either failure of the positive control(s) or additional peaks appearing in the control. If the rule fires, click on the 'i' button to see which positive control lane(s) fired the rule.

To view the positive control flags, the analyst selects **View ▸ Positive Control Check**. This interface shows all of the designated positive controls, the lane numbers, and whether the controls passed or were flagged by the rigorous quality assurance check (Figure 22).

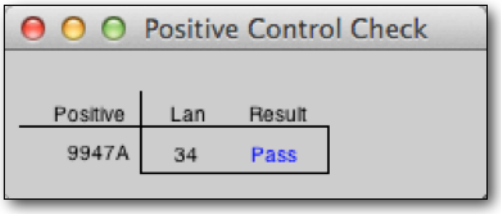


**Figure 22.** *Positive Control Check* window.

The *Positive Control Check* window also flags lanes that appear to be positive controls (indicated by the string 'Check'), which can be useful for diagnosing mis-loading and mis-designation problems.

### Primer missing

A true negative control lane should contain primer peaks. The primer missing rule reviews the primer region for each negative control. Lanes with insignificant or missing primer peaks fire the rule and can be viewed in *Cap View*.