

ChimerMarker™

Automated Chimerism Analysis Software

Frequently Asked Questions (FAQ)

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(1) What is a CHM Panel? - The CHM panel, short for “Chimertyping Panel”, is a project specific panel created during data processing. The CHM panel is required for chimerism analysis.

Run Wizard

Data Process - Chimerism Analysis

Set data process options

Give the Chimertyping Panel a name using the field below:

Raw Data Analysis

- Auto Range (frame)
- Start: End:
- Smooth Enhanced Smooth
- Peak Saturation Baseline Subtraction
- Enhanced Baseline Subtraction
- Pull-up Correction Spike Removal

Allele Call

- Auto Range (bps)
- Start: End:
- Peak Detection Threshold: ?
- Min Intensity: Max Intensity:
- Percentage > Global Max
- Please Enter Grouping File Path
- Only Call Alleles Present in CHM Panel
- Auto Create CHM Panel
- Customize Marker Parameters

Chimerism Sample Identification

CHM Panel Name:

Fixed Bin Width:

No.	Type	Sample Name
1	R	Recipient
2	D1	Donor
3		Sample1
4		Sample2
5		Sample3
6		Sample4
7		Sample5

Size Call

- Local Southern Cubic Spline

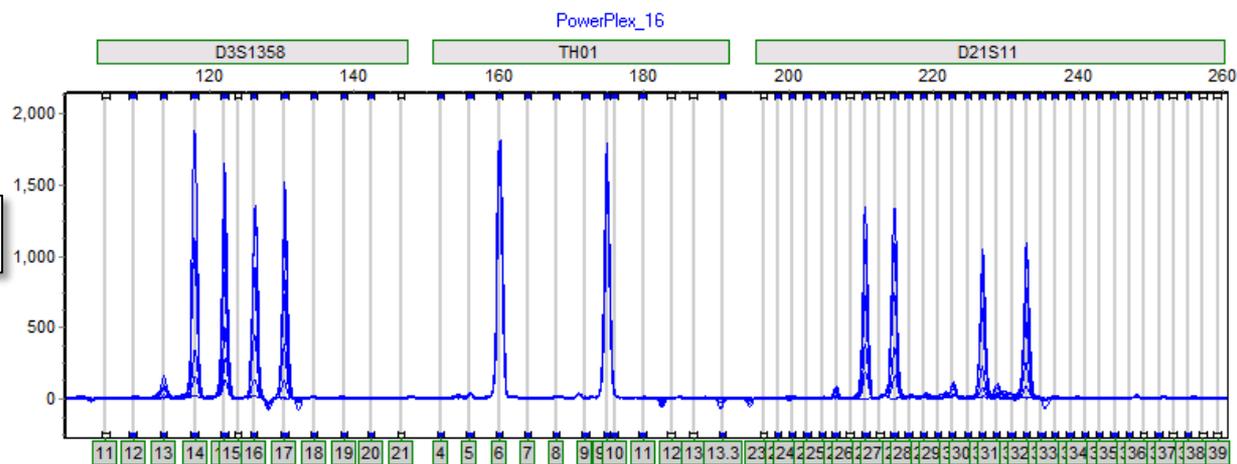
Load Default Save Default

Next >> Cancel

To create a CHM panel (and perform Chimerism analysis), select these two options.

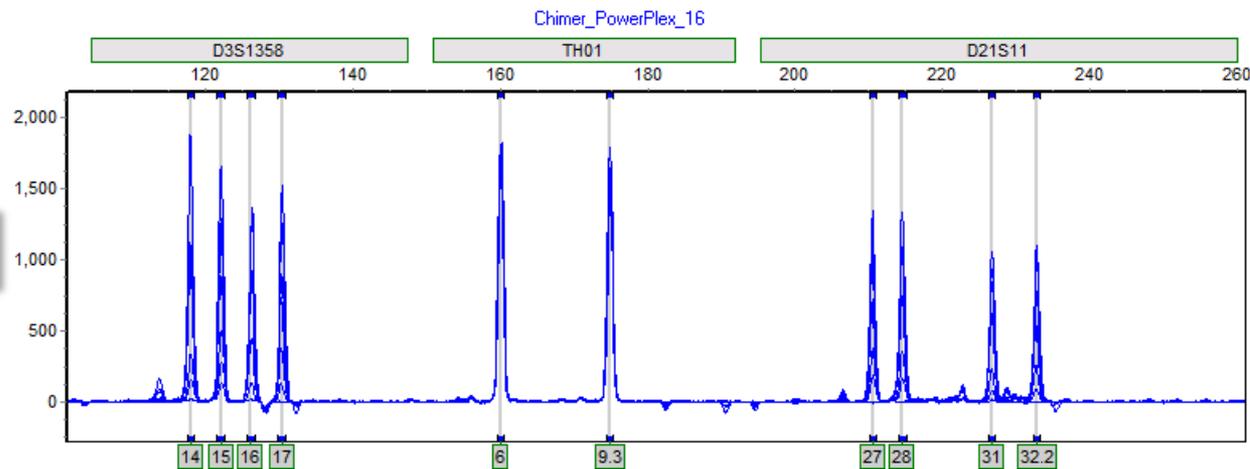
The Chimertyping Panel is derived from the Genotyping Panel. Only bins shared by either the donor or recipient are saved, the others are discarded.

Genotyping Panel

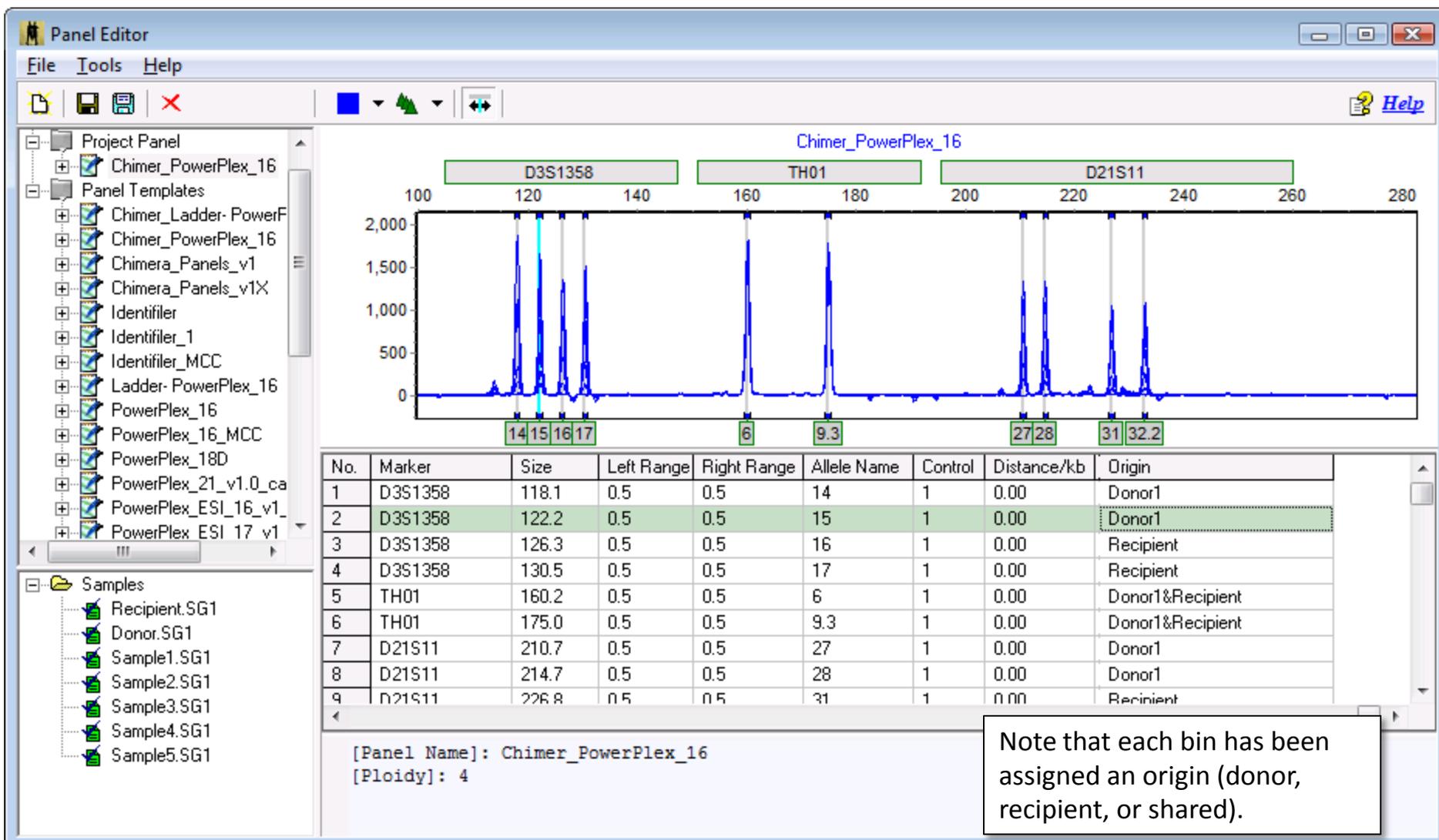


↓ (data processing)

Chimertyping Panel



The Chimertyping panel can be viewed in the panel editor (Tools > Panel Editor). The Chimertyping panel for your current project will be listed under “Project Panel”.



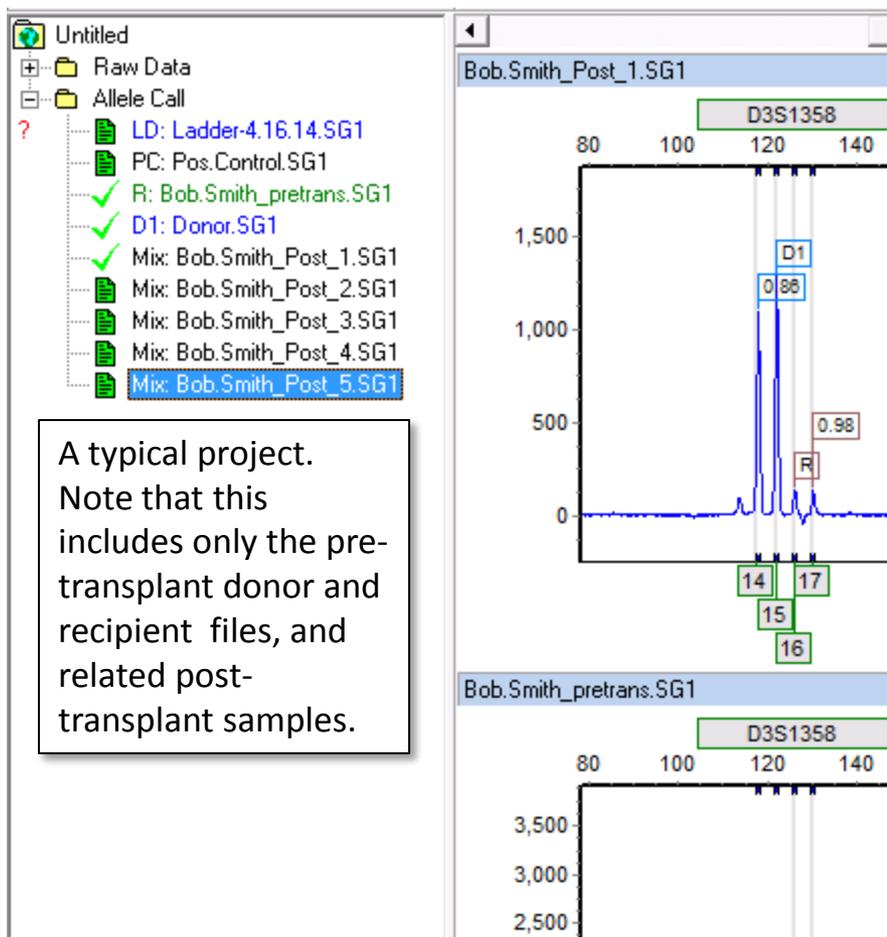
The screenshot shows the Panel Editor window with the following components:

- Project Panel:** A tree view on the left showing the current project structure, including 'Chimer_PowerPlex_16' and various templates and samples.
- Chromatogram:** A plot titled 'Chimer_PowerPlex_16' showing fluorescence peaks for markers D3S1358, TH01, and D21S11. The x-axis represents size in base pairs (100-280), and the y-axis represents intensity (0-2,000). Peaks are labeled with allele names: 14, 15, 16, 17 for D3S1358; 6, 9.3 for TH01; and 27, 28, 31, 32.2 for D21S11.
- Data Table:** A table below the chromatogram listing the detected alleles and their origins.

No.	Marker	Size	Left Range	Right Range	Allele Name	Control	Distance/kb	Origin
1	D3S1358	118.1	0.5	0.5	14	1	0.00	Donor1
2	D3S1358	122.2	0.5	0.5	15	1	0.00	Donor1
3	D3S1358	126.3	0.5	0.5	16	1	0.00	Recipient
4	D3S1358	130.5	0.5	0.5	17	1	0.00	Recipient
5	TH01	160.2	0.5	0.5	6	1	0.00	Donor1&Recipient
6	TH01	175.0	0.5	0.5	9.3	1	0.00	Donor1&Recipient
7	D21S11	210.7	0.5	0.5	27	1	0.00	Donor1
8	D21S11	214.7	0.5	0.5	28	1	0.00	Donor1
9	D21S11	226.8	0.5	0.5	31	1	0.00	Recipient
- Summary:** At the bottom of the window, it displays '[Panel Name]: Chimer_PowerPlex_16' and '[Ploidy]: 4'.

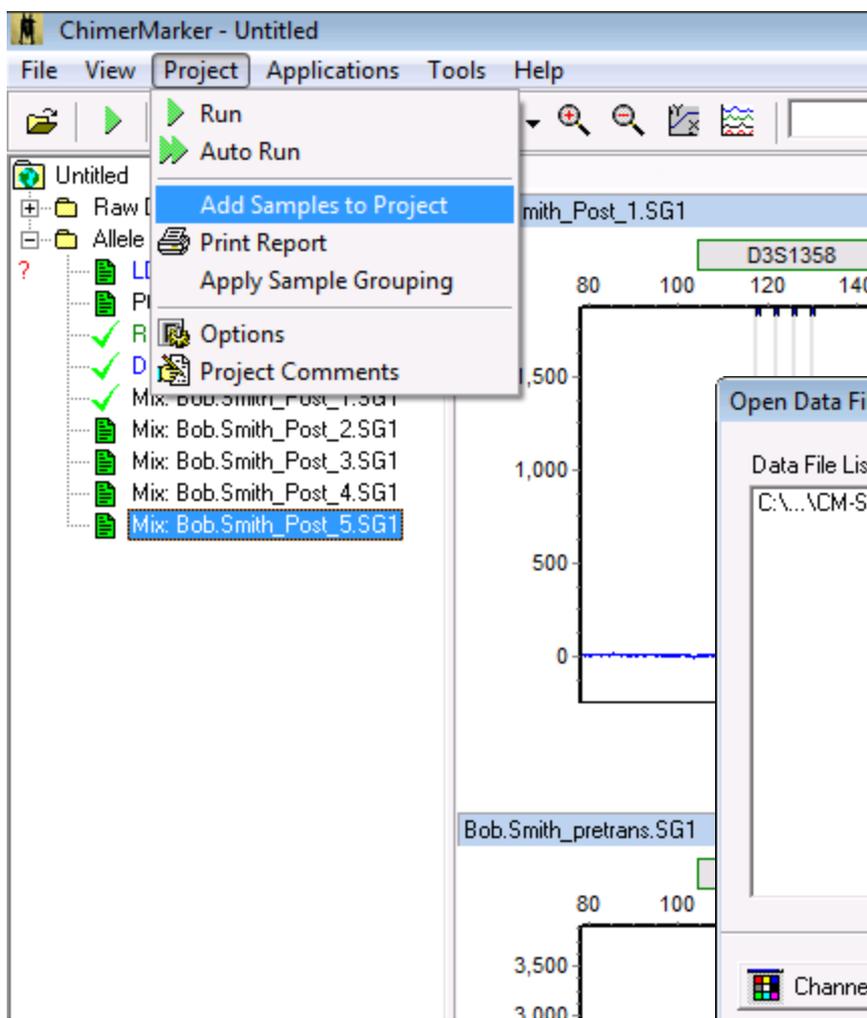
Note that each bin has been assigned an origin (donor, recipient, or shared).

(2) How many recipients can I analyze at a time? – One. Because the Chimertyping panel is *project specific*, only one donor/recipient group may be analyzed at a time. This includes the pre-transplant donor and recipient files, and any number of post transplant (mixture) samples.



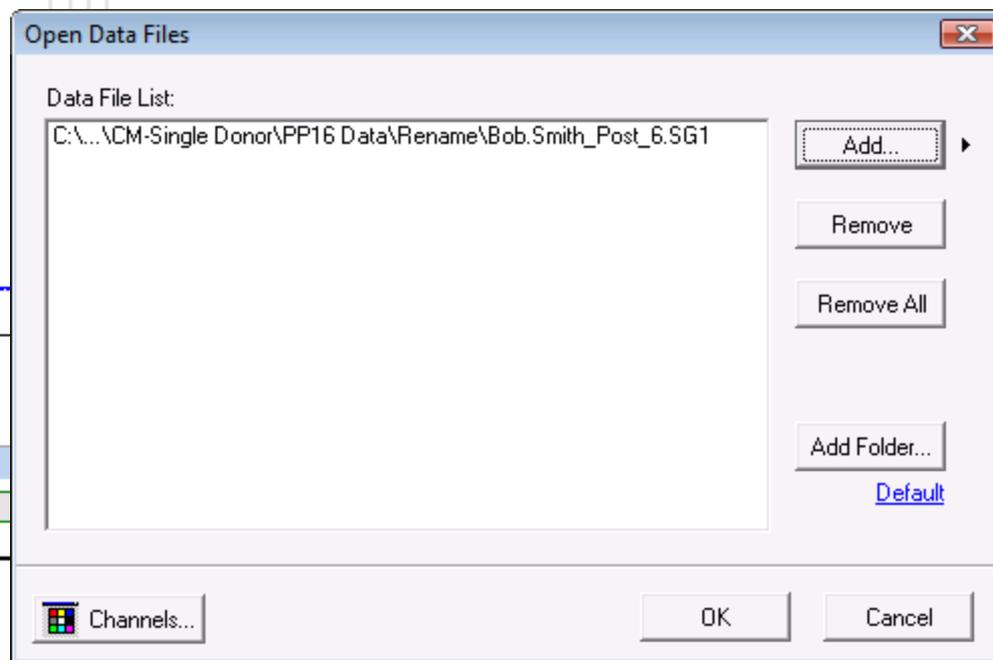
You may also include positive controls, negative controls, and ladder samples.

(3) How do I add samples to an existing project? – Simply open the project (file > open project) and click Project > Add Samples to Project.



Click Add, and then navigate to the sample(s) that you would like to add.

After clicking OK, the program will analyze the new samples using the analysis parameters saved in your project.

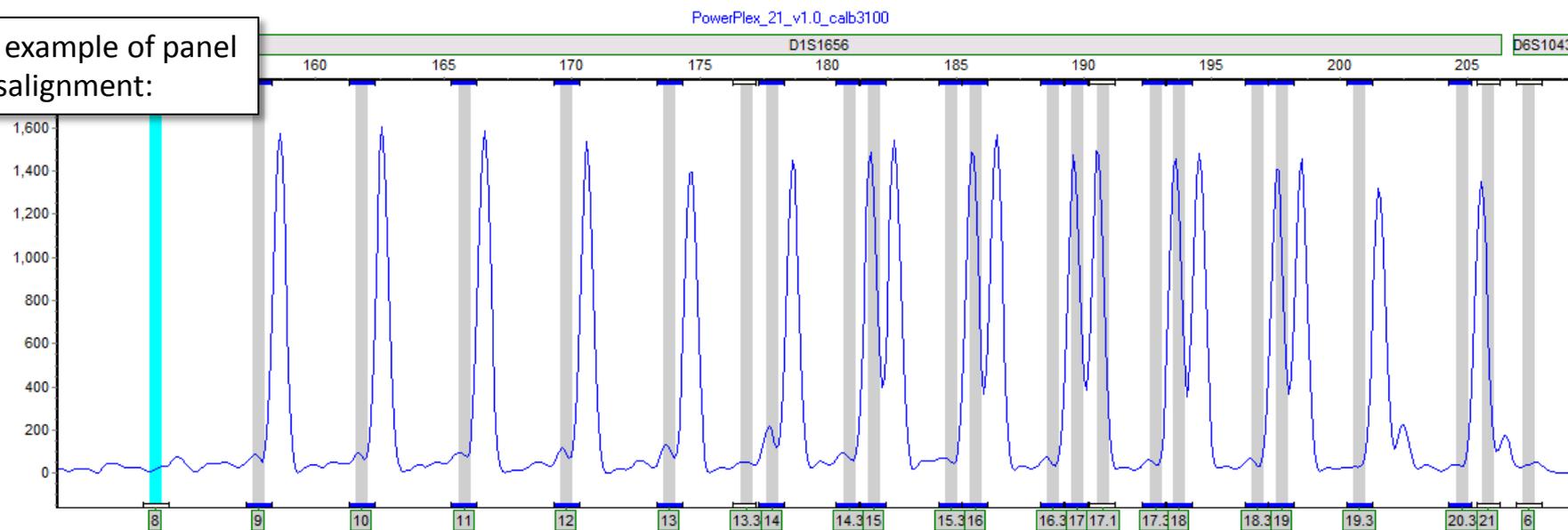


(4) How do I calibrate my panel? – Sometimes, particularly after running a new chemistry or maintaining your CE instrument, you may notice some amount of misalignment between your genotyping panel and your CE files.

This can be an issue because when processing data, peaks may not “fall” into their correct bins, or they may be missed entirely.

However, ChimerMarker provides a series of easy steps that allow the user to automatically calibrate their genotyping panel using an Allelic Ladder sample.

An example of panel misalignment:



Begin by reprocessing your samples. This time, in the second page of the Run Wizard, **deselect** “Only Call Alleles Present in CHM Panel” and “Auto Create CHM Panel”.

Run Wizard
Data Process - Chimerism Analysis
Set data process options

Raw Data Analysis

- Auto Range (frame)
- Start: End:
- Smooth Enhanced Smooth
- Peak Saturation Baseline Subtraction
- Enhanced Baseline Subtraction
- Pull-up Correction Spike Removal

Allele Call

- Auto Range (bps)
- Start: End:
- Peak Detection Threshold:
- Min Intensity: Max Intensity:
- Percentage > Global Max
- Please Enter Grouping File Path
- Only Call Alleles Present in CHM Panel
- Auto Create CHM Panel
- Customize Marker Parameters

Size Call

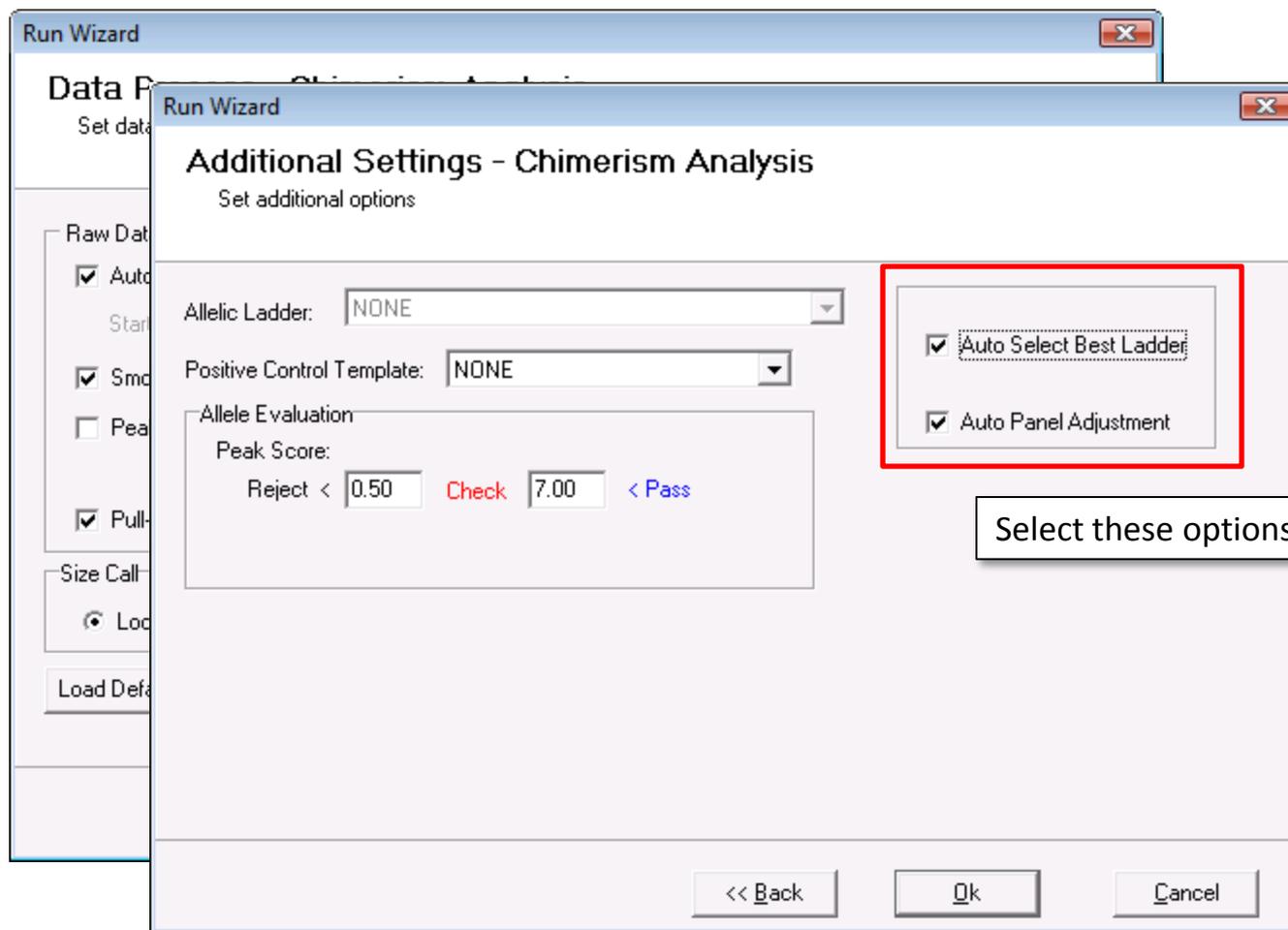
- Local Source

Load Default Save Default

<< Back Next >> Cancel

Leave these options unselected.

In the last page of the Run Wizard, select “Auto Select Best Ladder” and “Auto Panel Adjustment”.



After Processing your samples, you should observe that your Ladder sample is now in bold lettering. This indicates that the program was able to successfully calibrate your panel using the ladder sample.

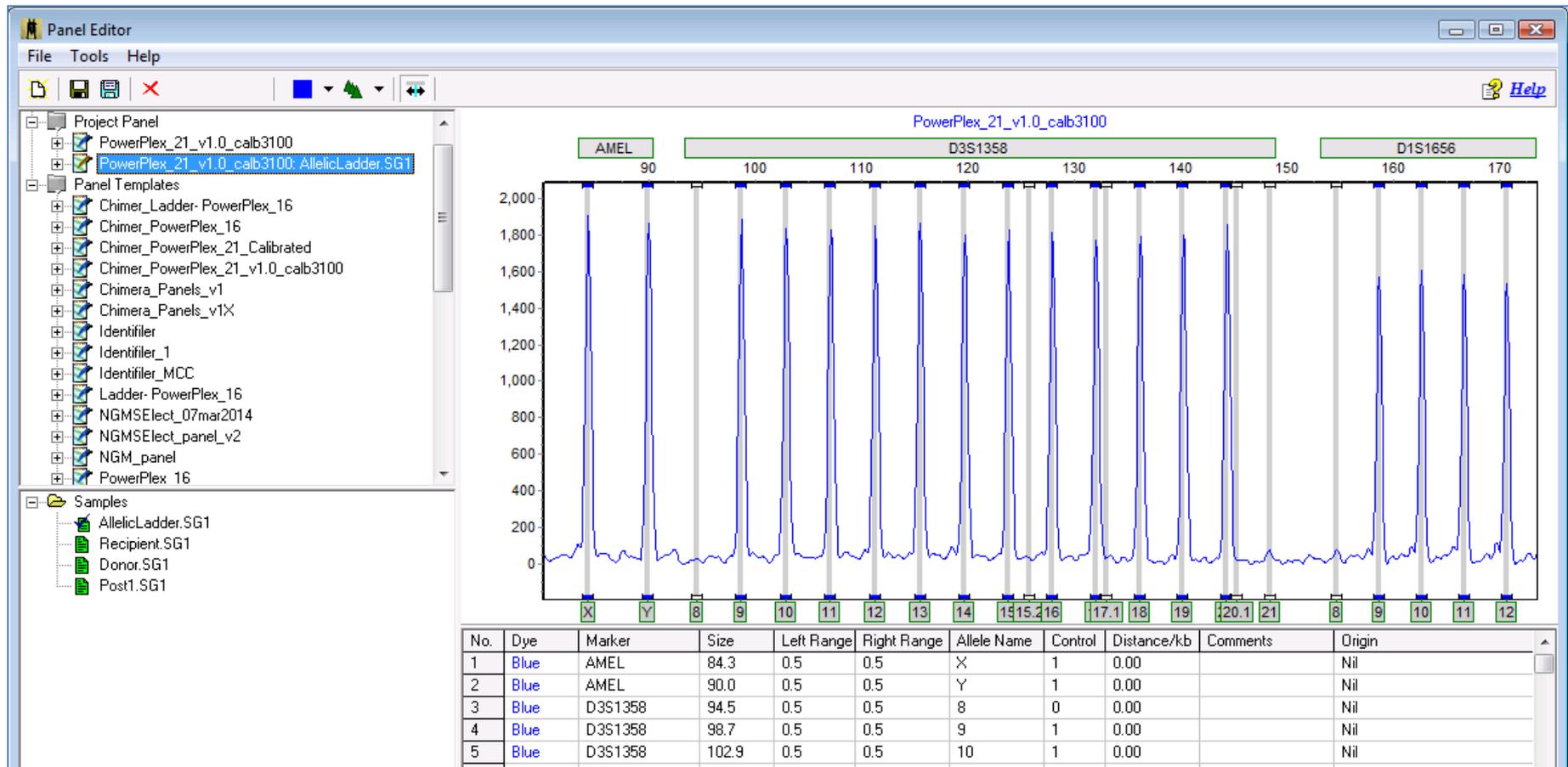
Next, navigate to the Panel Editor (Tools > Panel Editor).

The screenshot shows the ChimerMarker software interface. The 'Tools' menu is open, with 'Panel Editor' selected. Below the menu, two electropherogram panels are visible: 'AllelicLadder.SG1' and 'Donor.SG1'. The 'AllelicLadder.SG1' panel shows a trace with peaks labeled D3S1358, D1S1656, D6S1043, D13S317, and Penta E. The 'Donor.SG1' panel shows a similar trace. To the right, a table displays sample results for various markers across different samples.

8	Donor.SG1	D3S1358	16	17
9	Donor.SG1	D1S1656	15	16
10	Donor.SG1	D6S1043	13	14
11	Donor.SG1	D13S317	9	9
12	Donor.SG1	Penta E	12	15
? 13	Post1.SG1	AMEL	X	X
? 14	Post1.SG1	D3S1358	16	17
? 15	Post1.SG1	D1S1656	X	16
? 16	Post1.SG1	D6S1043	13	14
? 17	Post1.SG1	D13S317	9	9
? 18	Post1.SG1	Penta E	12	15

At the bottom of the software window, a status bar shows: New 4 samples PC error: 0/0 NC error: 0/0 Ladder error: 1/1 #Failed=0 #Flagged=1

The panel editor contains all of the panel files saved in ChimerMarker. In the file tree to the left, you should see a section called **Project Panels** – these are the panels used to process your current project.



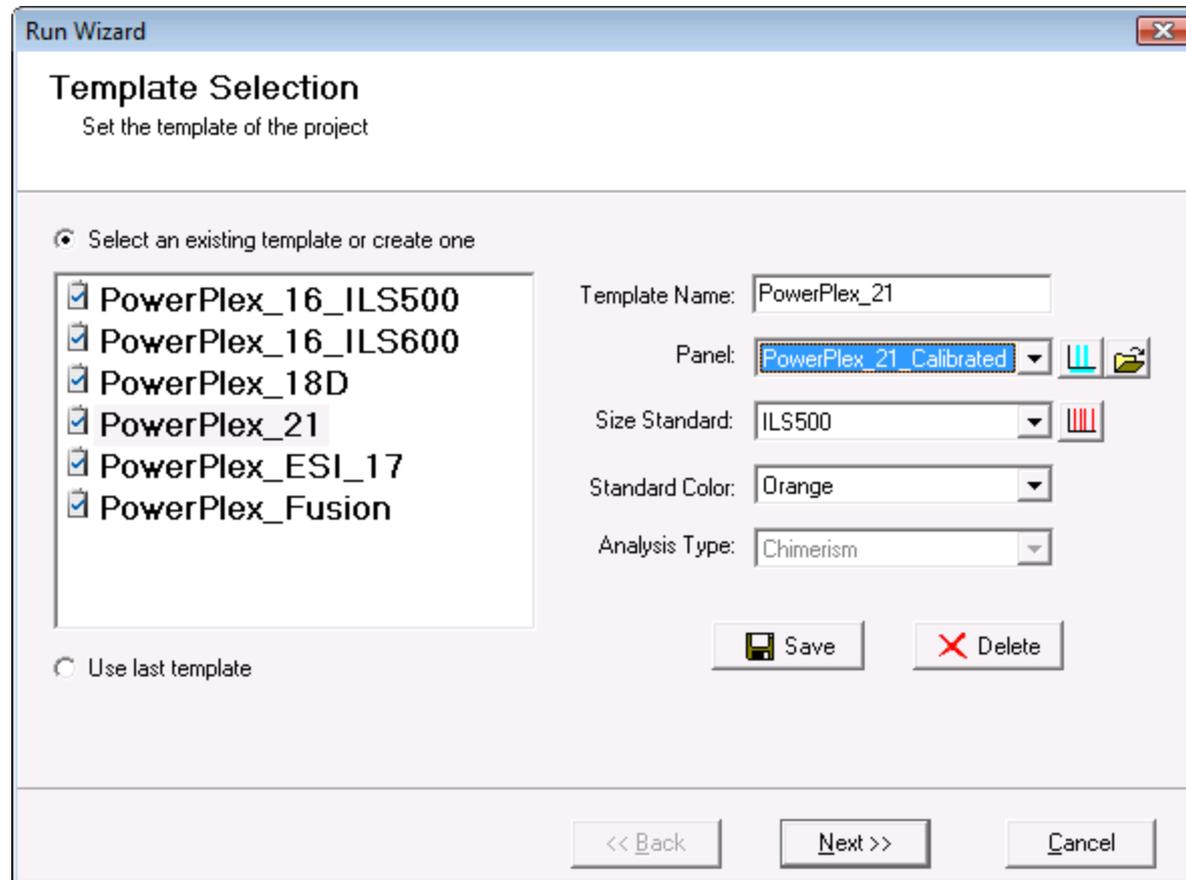
You should see two or more project panels – the original panel you used to genotype your samples, and another panel, appended with your ladder name.

This second panel is the calibrated panel. To save it, select it and Navigate to File > Save as New Panel.

The screenshot shows the Panel Editor software interface. The 'File' menu is open, and the 'Save As New Panel' option is selected. An 'Input Dialog' box is displayed in the center, prompting for a 'New panel name' with the text 'PowerPlex_21_Calibrated' entered. A callout box points to the dialog with the text: 'Give the panel an informative name, and then click OK.' The background shows a DNA profile plot for 'PowerPlex_21_v1.0_calb3100' with markers AMEL, D3S1358, and D1S1656. The plot shows peaks for various alleles across chromosomes X, Y, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 8, 9, 10, 11, 12. A table at the bottom of the interface provides details for the first marker:

No.	Dye	Marker	Size	Left Range	Right Range	Allele Name	Control	Distance/kb	Comments	Origin
1	Blue	AMEL	84.3	0.5	0.5	X	1	0.00		Nil

To reprocess your samples with this new calibrated ladder, simply reopen the Run Wizard (project > run) and be sure to select your new calibrated panel from the panel dropdown menu.



In the final page of the Run Wizard, do not select either “Auto Select Best Ladder” or “Auto Panel Adjustment”, as we have already done this.

Run Wizard

Additional Settings - Chimerism Analysis

Set additional options

Allelic Ladder: NONE

Positive Control Template: NONE

Allele Evaluation

Peak Score:

Reject < 0.50 Check 7.00 < Pass

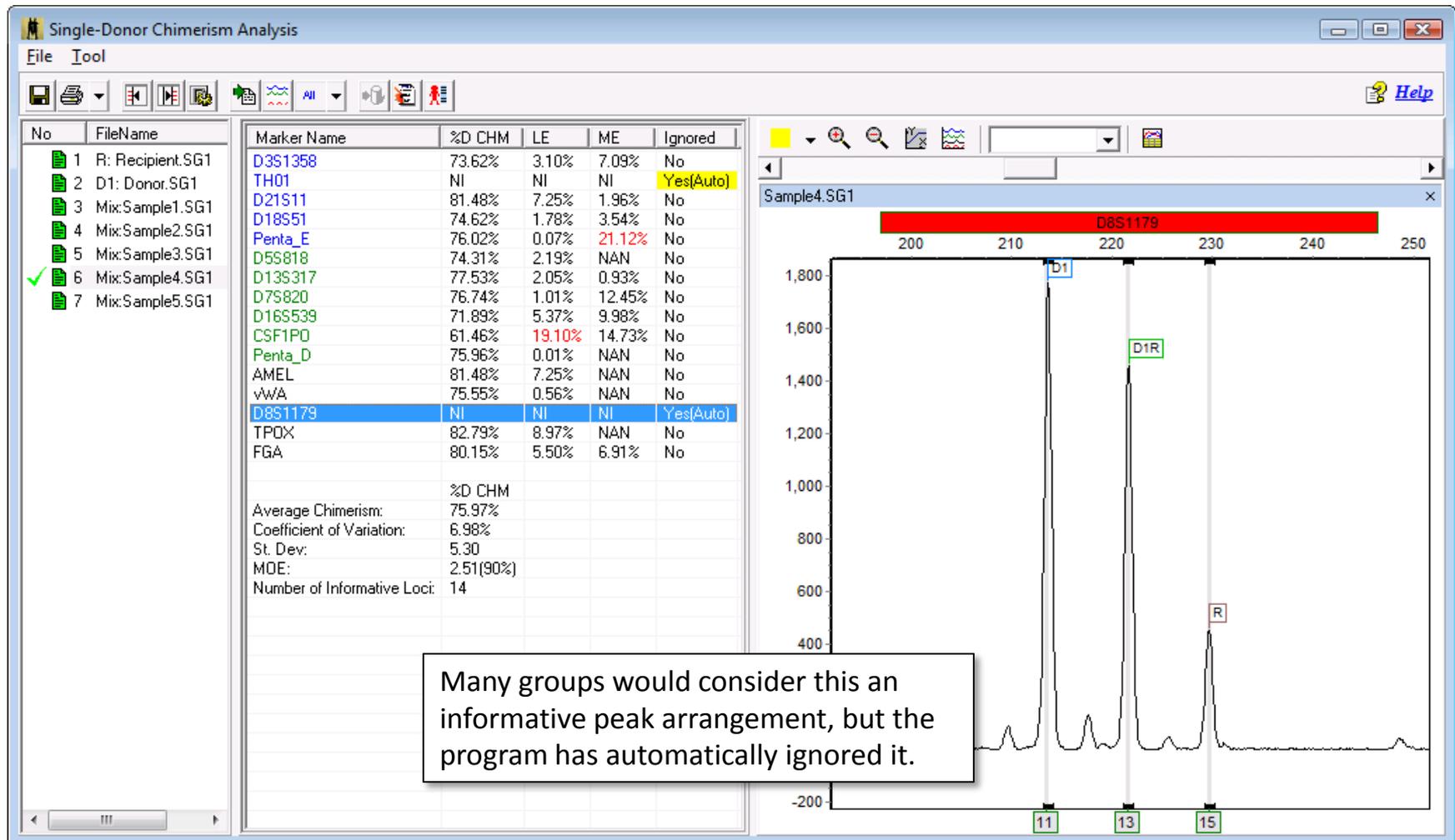
Auto Select Best Ladder

Auto Panel Adjustment

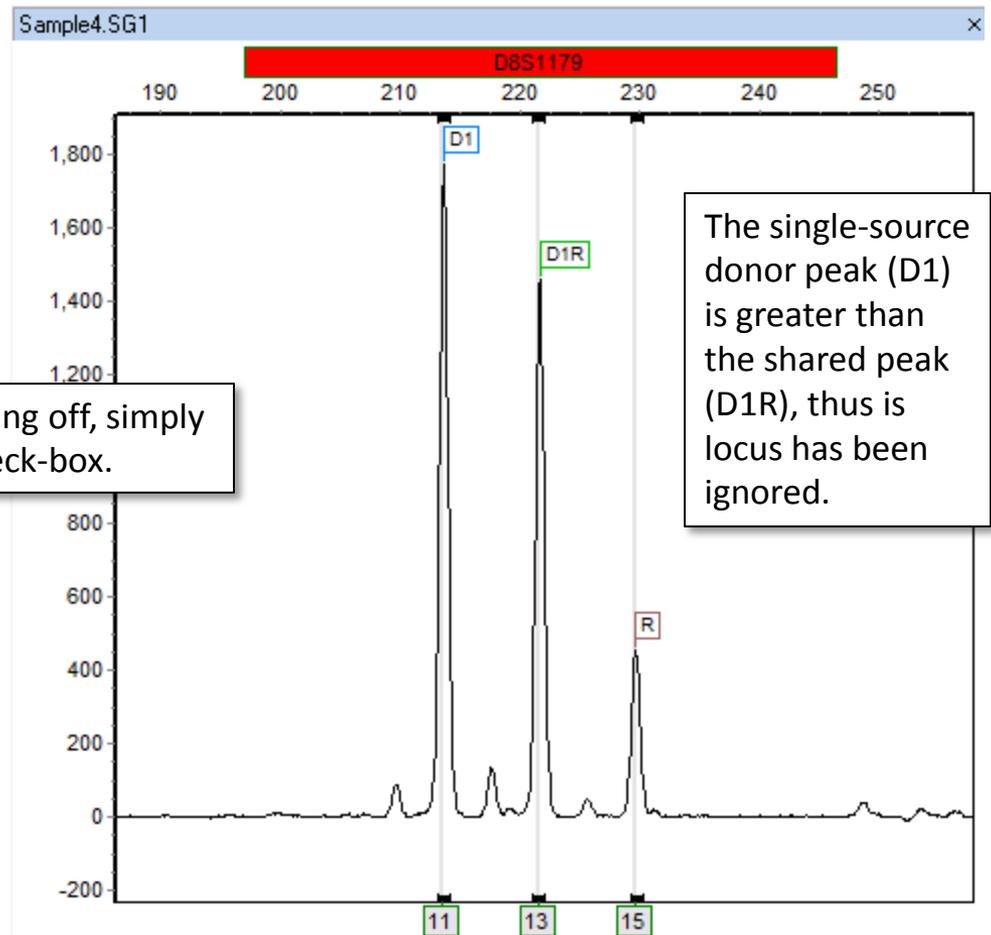
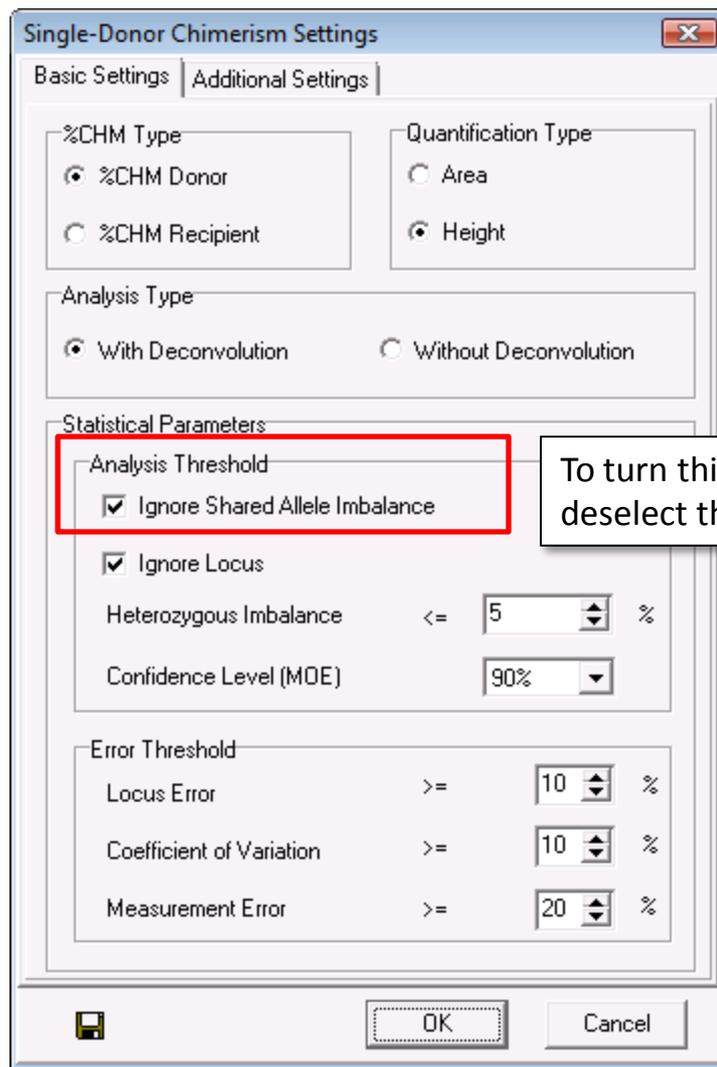
Ok Cancel

With a calibrated panel, you should see improved peak calling and more accurate results. Re-calibrating is recommended after analyzing a new chemistry, or after maintenance has been performed on your Genetic analyzer.

(5) Why are some informative loci being ignored? Sometimes, markers that appear informative may be ignored. This is usually related to the settings that were chosen in the Chimerism Settings window. An example is shown below:

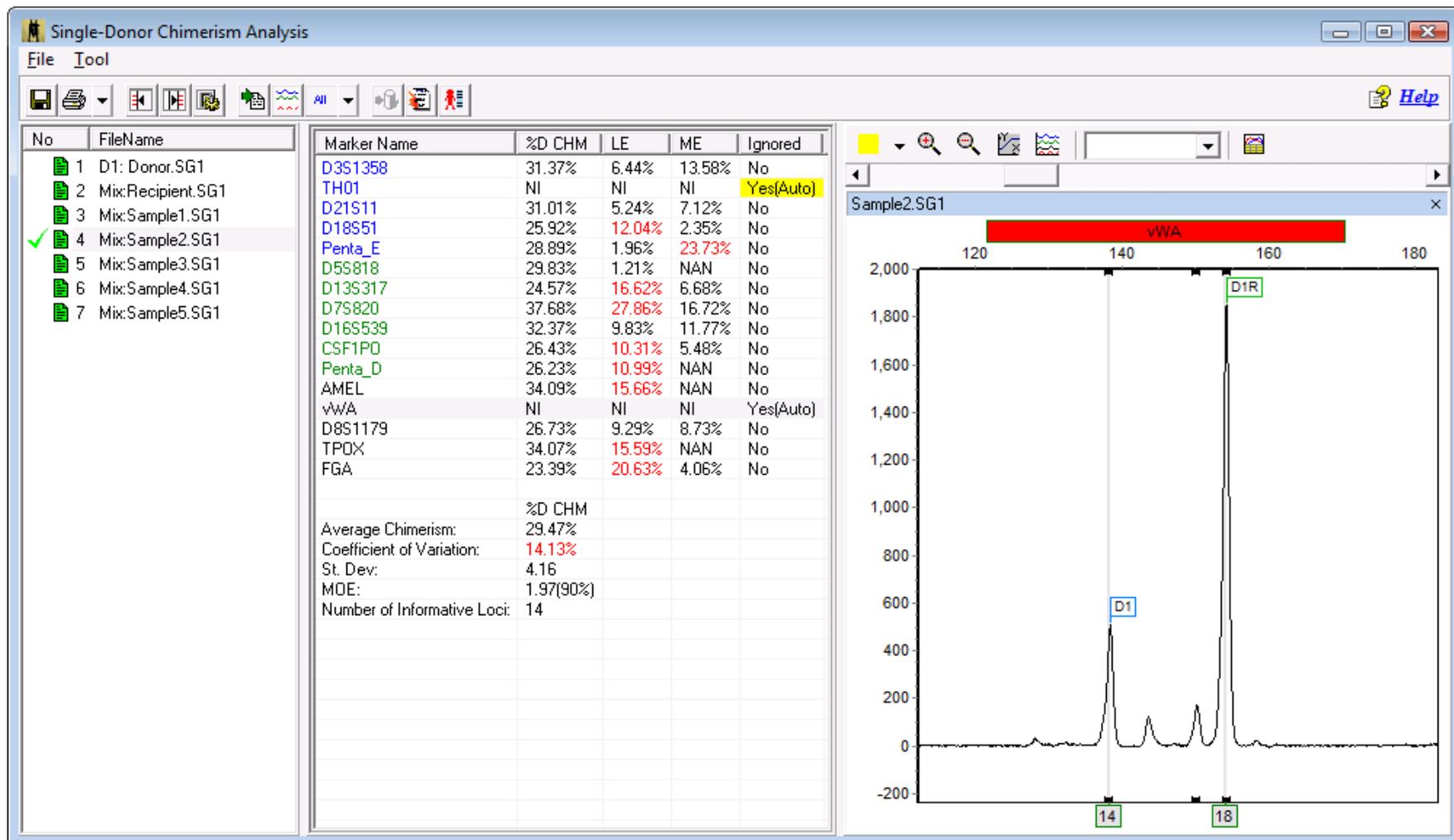


The “Ignore Shared Allele Imbalance” filter will ignore any locus in which a single source peak has greater RFU than a shared peak. Thus the marker pictured below was ignored.



The single-source donor peak (D1) is greater than the shared peak (D1R), thus this locus has been ignored.

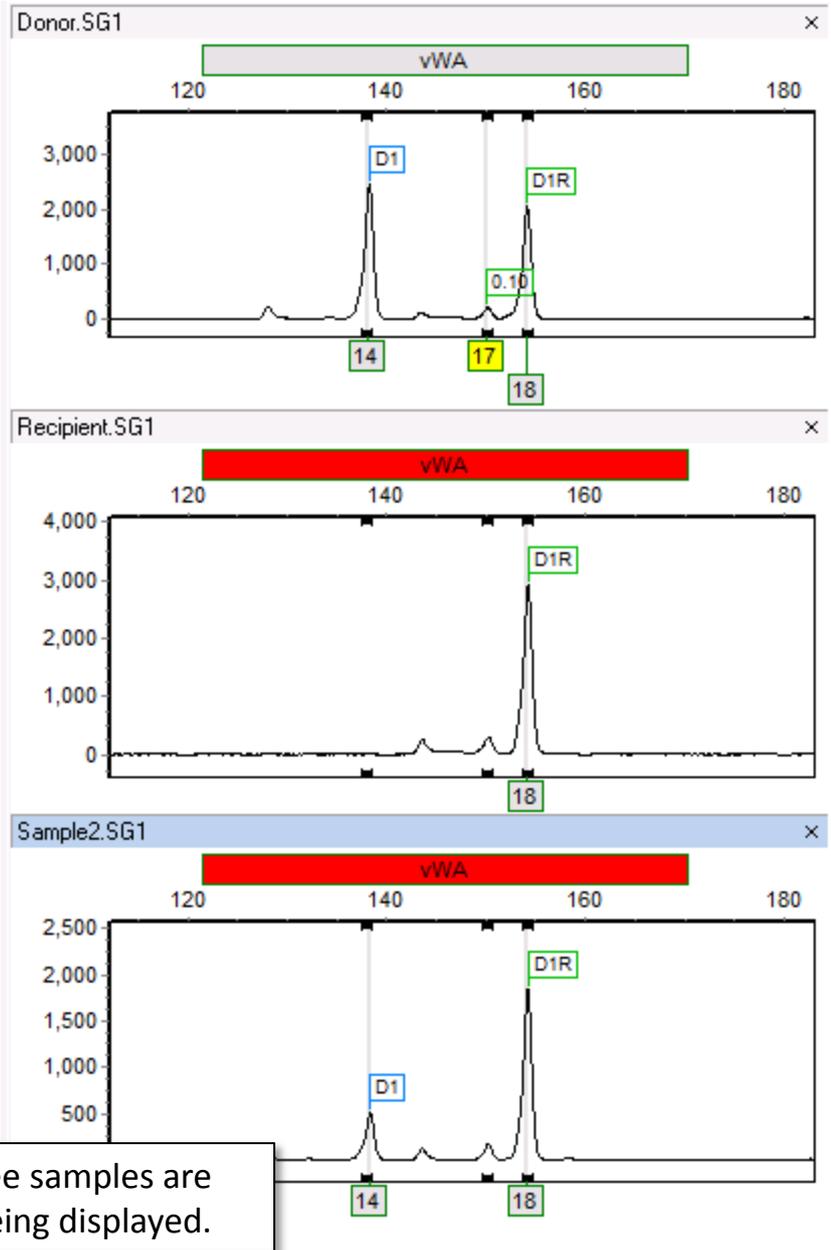
Here is another marker that is being ignored for no obvious reason:



Why are informative loci being ignored? – Part 4

You can navigate back to the main analysis screen to look at this marker in more detail, or while in the chimerism analysis screen, you can use the “Multi Sample View” icon.

No	FileName	Marker Name	%D CHM	LE
1	D1		6.44%	
2	Mix		NI	
3	Mix		5.24%	
4	Mix		12.04%	
5	Mix			
6	Mix			
7	Mix			



All three samples are now being displayed.

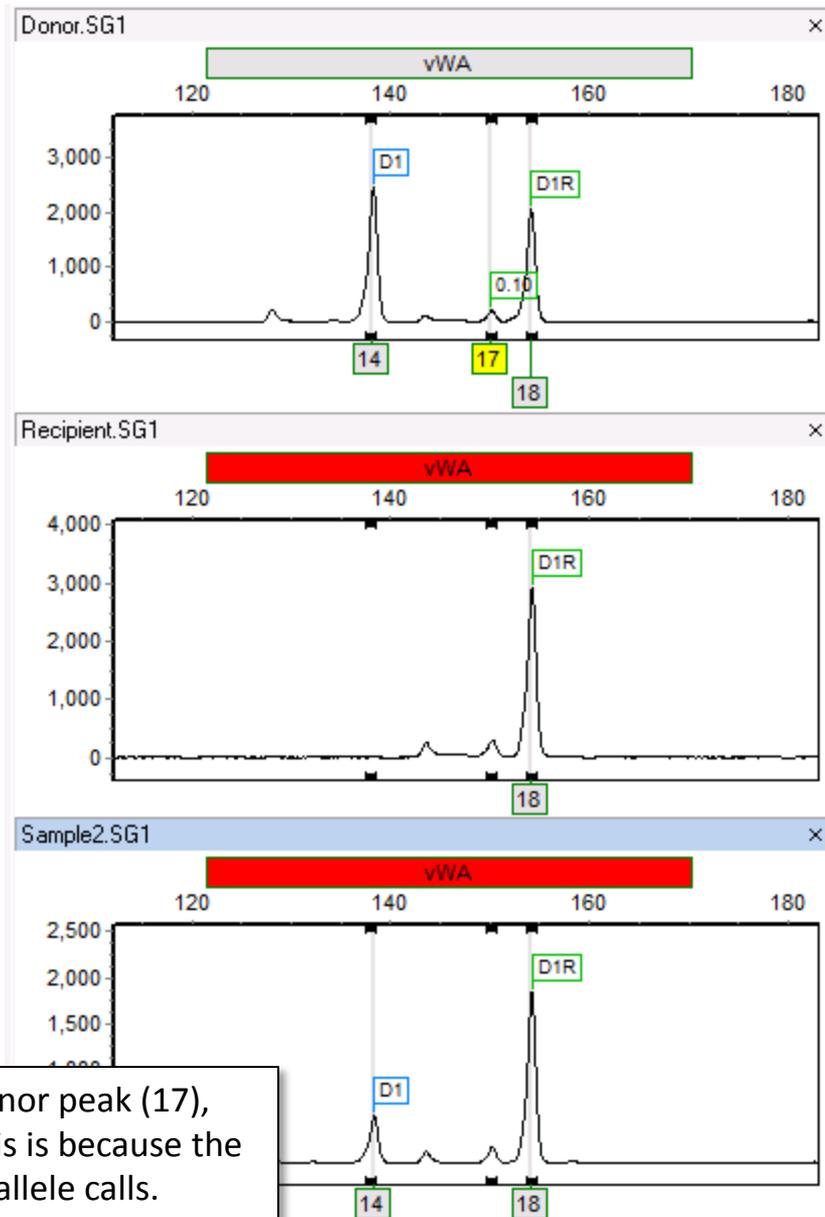
With all three traces displayed, the issue becomes more clear: the donor sample has three peaks!

ChimerMarker cannot use this marker because it is expecting only one or two peaks from the donor (and recipient).

It may be tempting to simply delete this peak in the main analysis window. However this will not solve the problem.

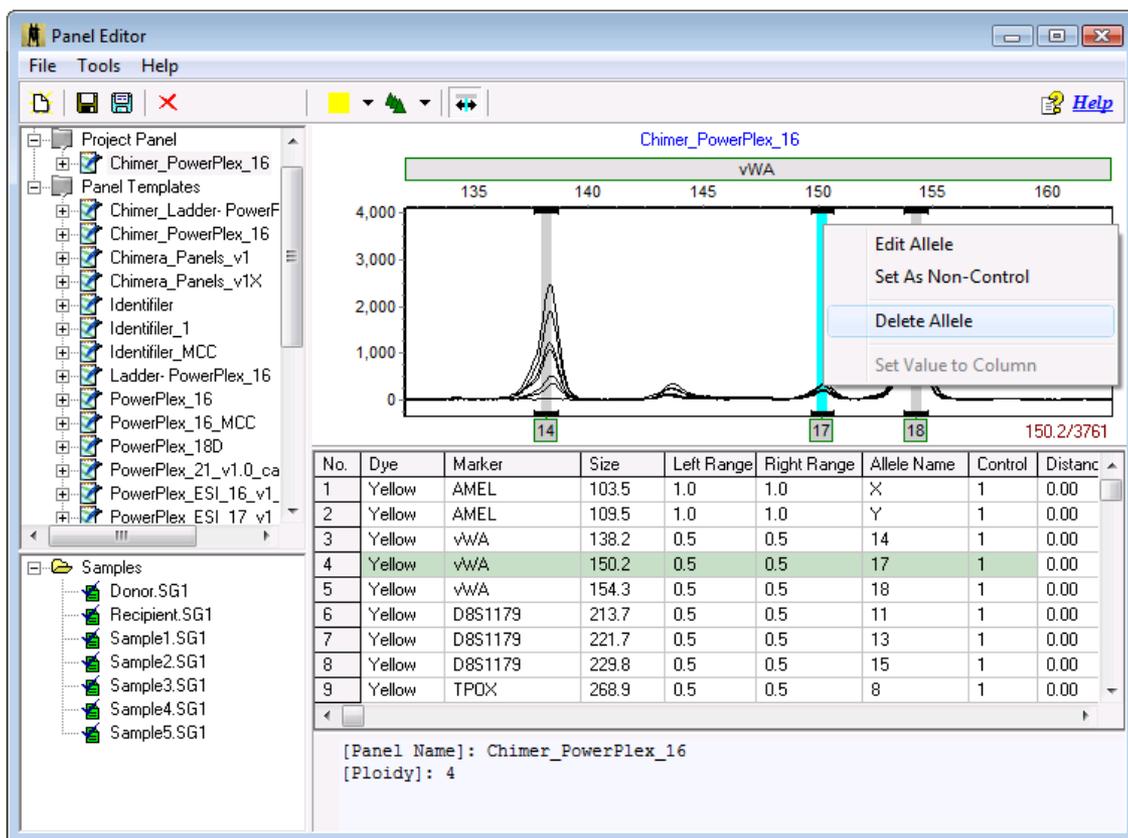
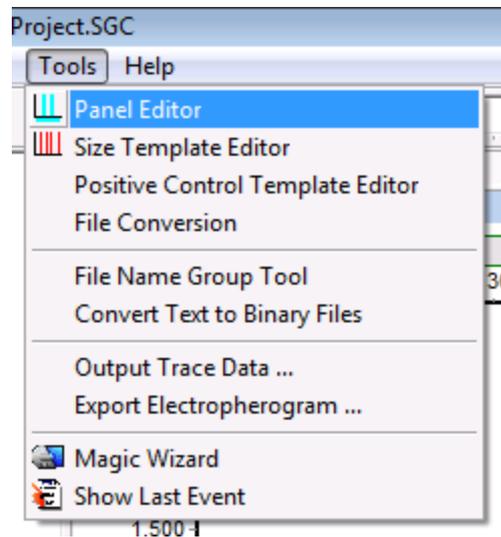
The main issue is that the program has created a Donor bin at the site of this peak. Even if we delete the peak, the bin will remain. Thus, you must delete the peak from the panel.

Note that the program has not only called a third donor peak (17), but it has also added a donor bin at this location. This is because the CHM panel is created based on donor and recipient allele calls.



Why are informative loci being ignored? – Part 6

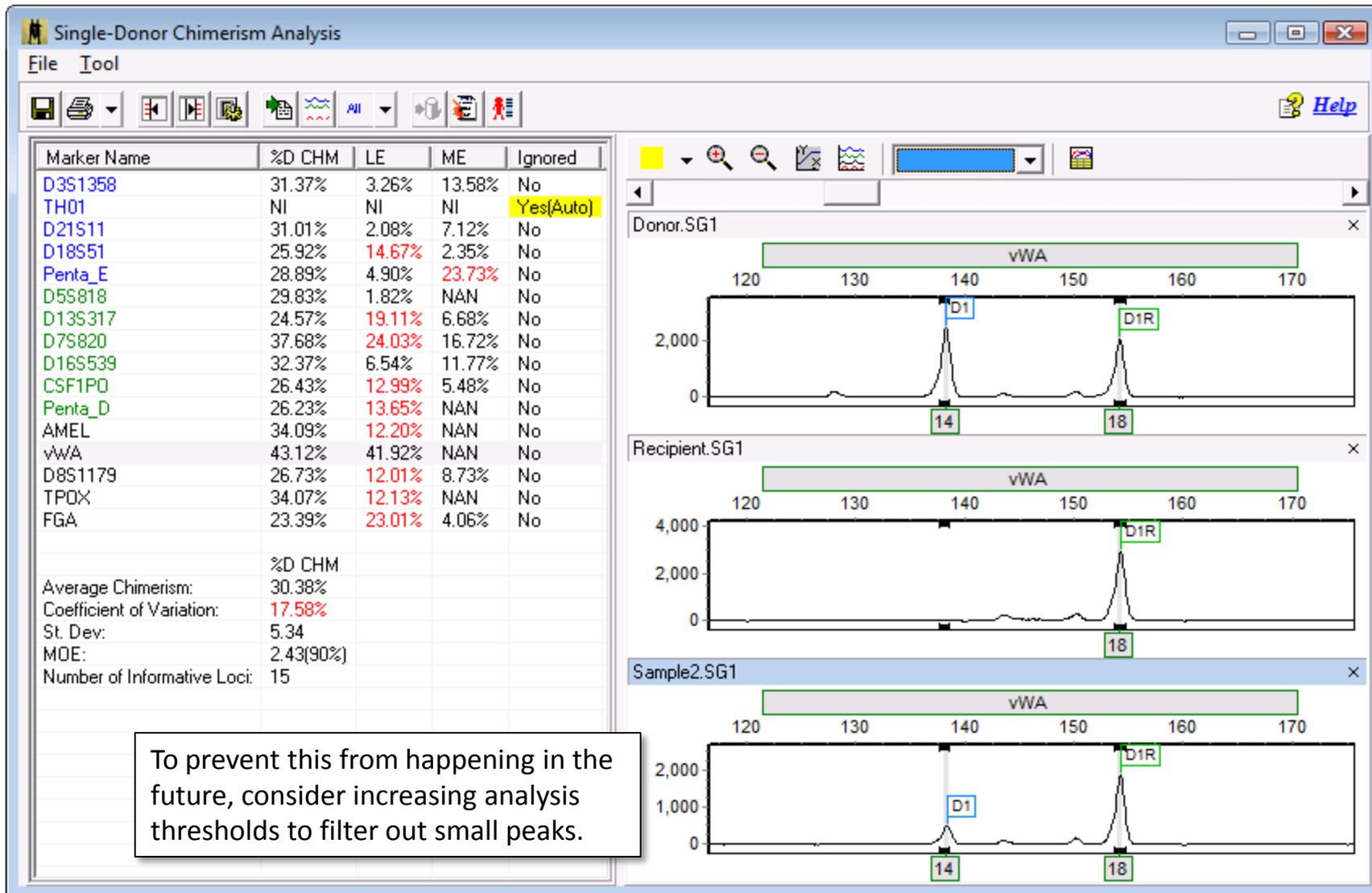
The Chimertyping panel can be viewed in the panel editor (Tools > Panel Editor). The Chimertyping panel for your current project will be listed under “Project Panel”.



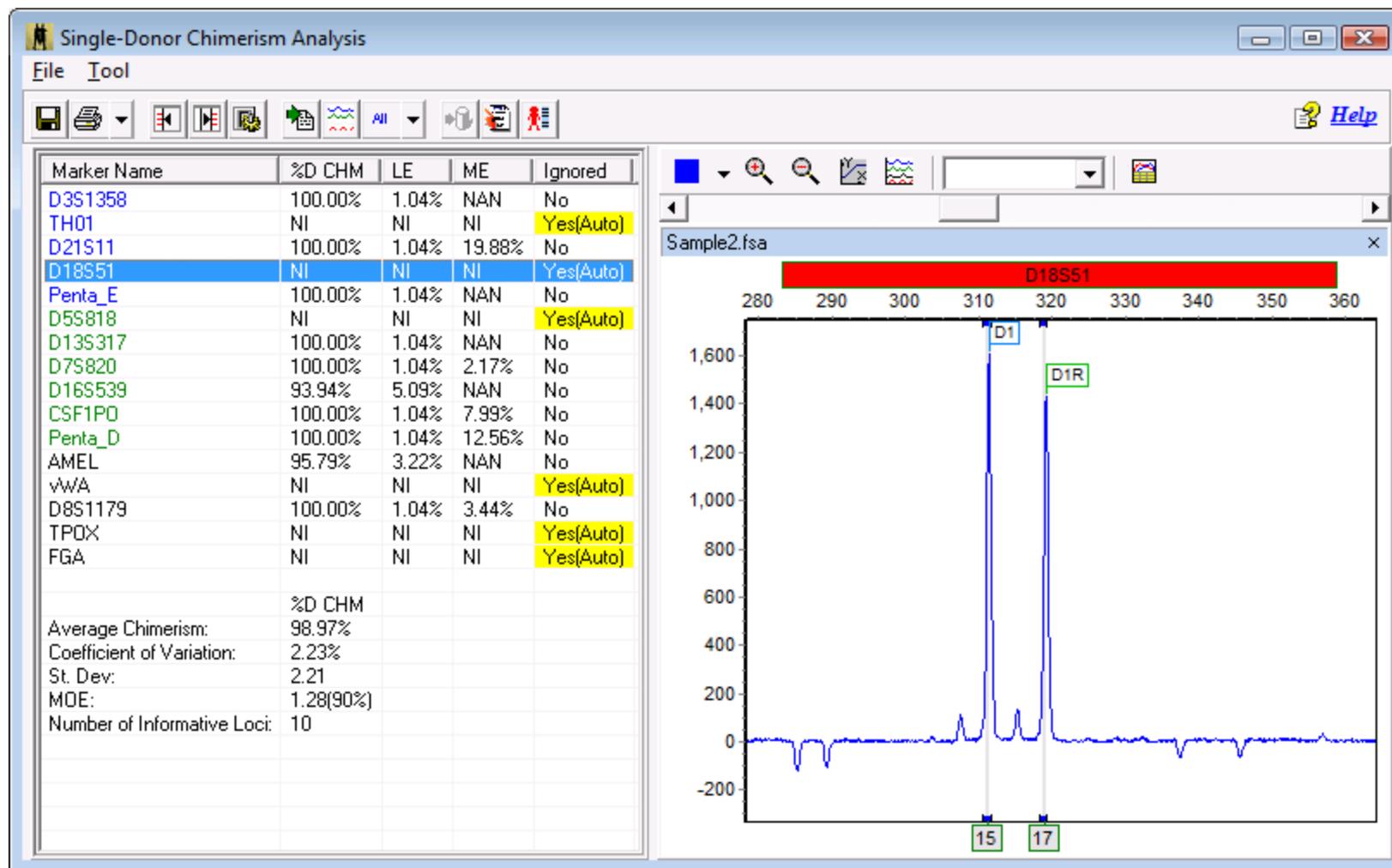
Simply right-click on the extra bin, and select Delete Allele. Save the changes by clicking the save icon

Why are informative loci being ignored? – Part 7

After deleting the peak, saving the changes, and exiting the panel editor, the results will be immediately updated. Now the marker is no longer ignored by the program:



Here is a third common example of a marker being ignored. In this case, the only alleles that were called were 15 and 17.

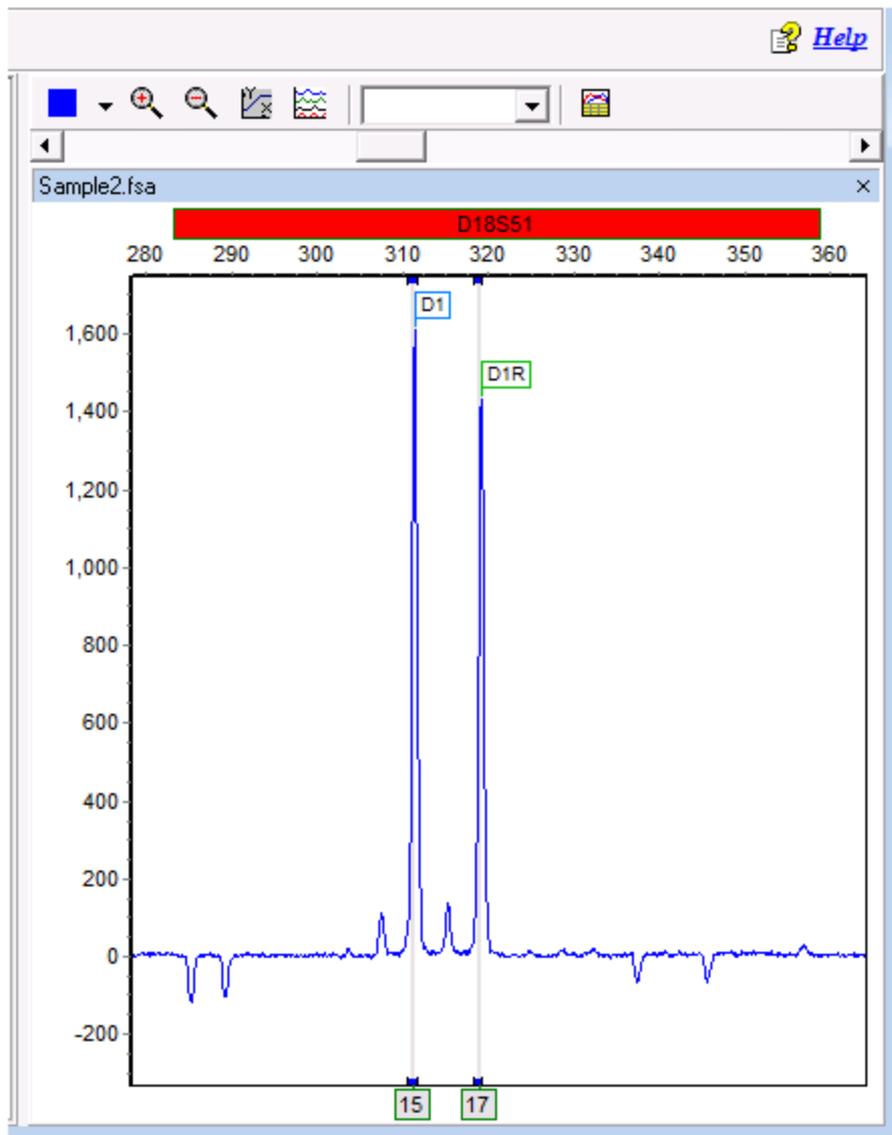


Our first impulse may be to blame the shared allele imbalance filter discussed on slide 17. However, even with this option deactivated, the marker will still be ignored.

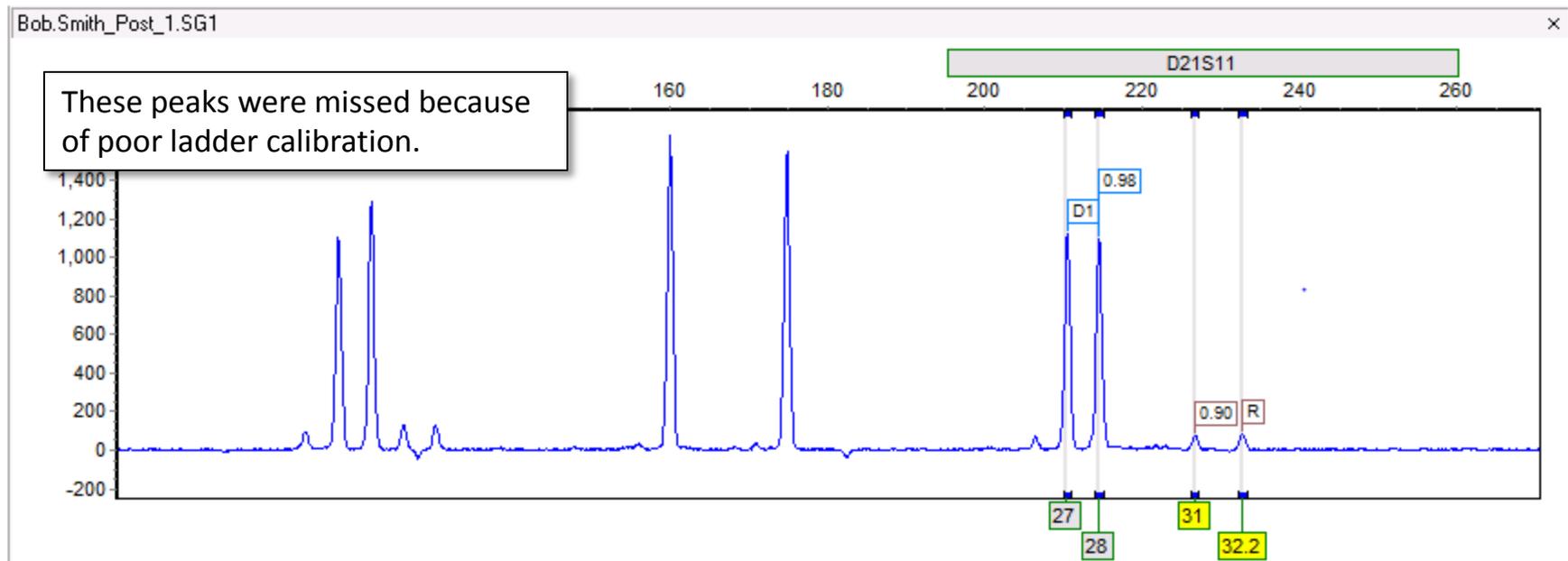
In this special case, only two alleles were called. Thus, when the program attempts to deconvolute the shared peak, it can only use the height of D1 as a reference.

However, D1 is taller than D1R! Because of this, the deconvolution results in a negative number – and the calculation fails.

The result is that markers with peak orientations specifically like that shown cannot be used by the program.



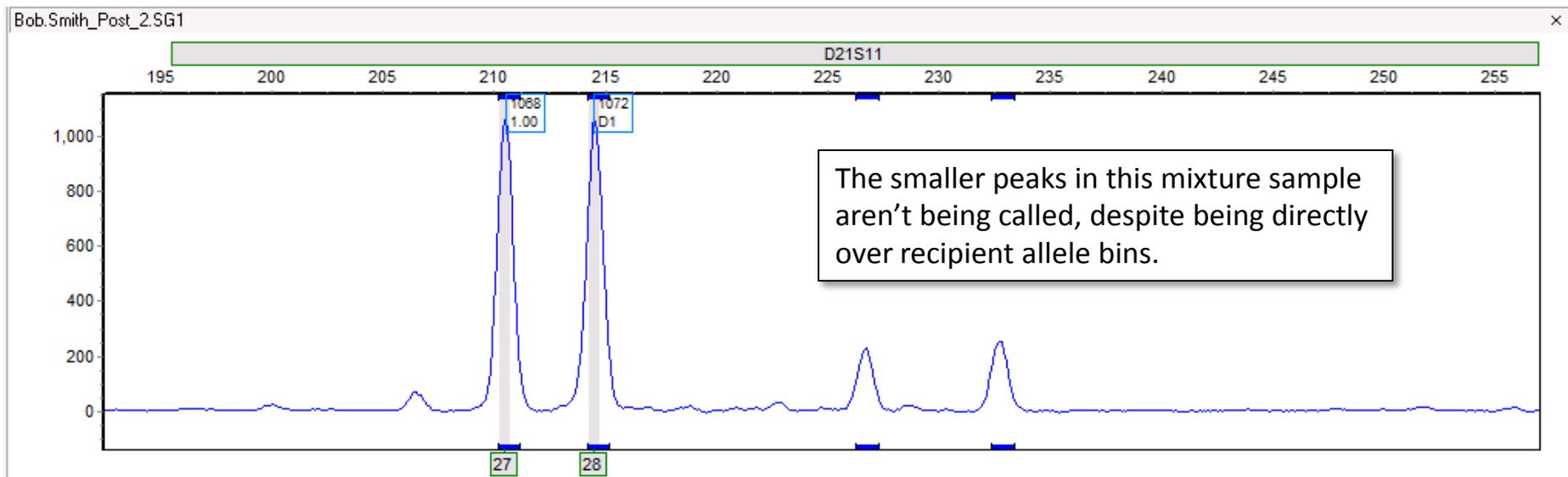
(6) Why aren't some peaks being called? - Occasionally you may notice that certain peaks, or even entire markers aren't being called by the program:



Most commonly, this is because your panel needs to be **Calibrated**. Please see the section titled "How do I calibrate my panel" (slides 7-15) for more information.

If you have confirmed that your panel *is* calibrated and peaks are still being missed, it could be that your analysis filters are set too high.

For example, the two right-most peaks below aren't being called, despite the obvious presence of allele bins directly underneath them:



Thus, it is likely our analysis settings that are filtering them out. These peaks are within a marker – D21S11 – therefore we must check the marker specific settings for D21S11. These can be found in the panel editor (Tools > Panel Editor).

Click on your panel from the file tree to the left. Right click on a marker label (grey rectangle) and select **Edit Marker** to open the marker specific settings.

The screenshot shows the 'Panel Editor' window. On the left is a file tree with 'Project Panel' and 'Panel Templates'. The main area displays a DNA profile plot for 'Chimer_PowerPlex_16'. The plot has a scale from 0 to 3,000 and a horizontal axis from 100 to 480. Markers are labeled at the top: D3S1358, TH01, D21S11, D18S51, and Penta_E. A context menu is open over the D21S11 marker label, showing options: 'Edit Marker...', 'Update Alleles', 'Delete Marker', and 'Set Value to Column'. Below the plot is a table with the following data:

No.	Dye	Marker	Size	Left Range	Right Range	Allele Name	Control	Distance/kb	Comments
1	Blue	D3S1358	118.1	0.5	0.5	14	1	0.00	
2	Blue	D3S1358	122.2	0.5	0.5	15	1	0.00	
3	Blue	D3S1358	126.3	0.5	0.5	16	1	0.00	F
4	Blue	D3S1358	130.5	0.5	0.5	17	1	0.00	F
5	Blue	TH01	160.2	0.5	0.5	6	1	0.00	C
6	Blue	TH01	175.0	0.5	0.5	9.3	1	0.00	
7	Blue	D21S11	210.7	0.5	0.5	27	1	0.00	C
8	Blue	D21S11	214.7	0.5	0.5	28	1	0.00	C
9	Blue	D21S11	226.8	0.5	0.5	31	1	0.00	F

At the bottom of the window, the following text is displayed:

```
[Panel Name]: Chimer_PowerPlex_16
[Ploidy]: 4
```

Here we can see the marker-specific settings for D21S11. In this case, the Min Heterozygote intensity is set to 350 RFU, which is well above the two smaller peaks.

Edit Marker [X]

Marker Parameters

Marker Name:

Nucleotide Repeats (x):

Boundary: To

Min Homozygote Intensity:

< = Inconclusive < =

Min Heterozygote Intensity:

< = Inconclusive < =

Max Heterozygote Imbalance: %

Min Heterozygote Imbalance: %

Apply Homo/Hetero Settings to All Markers

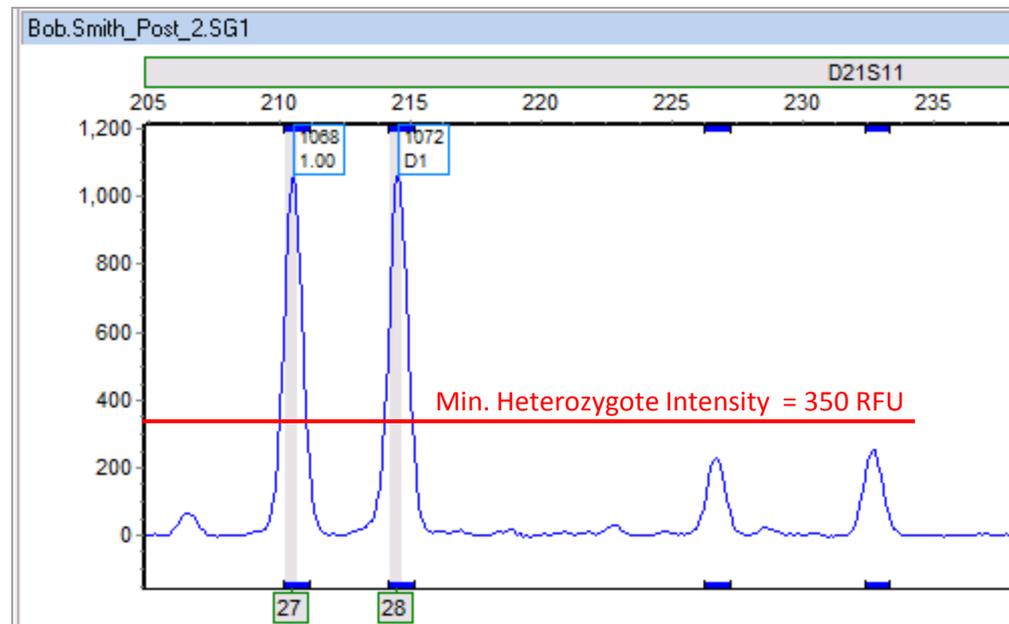
Stutter Filter: N - x %

N - 2x %

N + x %

Apply Stutter Settings to All Markers

OK Cancel



After lowering the threshold to 200 and saving the results, we can see that the peaks are now called, as expected.

Edit Marker ✕

Marker Parameters

Marker Name:

Nucleotide Repeats (x):

Boundary: To

Min Homozygote Intensity:

< = Inconclusive < =

Min Heterozygote Intensity:

< = Inconclusive < =

Max Heterozygote Imbalance: %

Min Heterozygote Imbalance: %

Apply Homo/Hetero Settings to All Markers

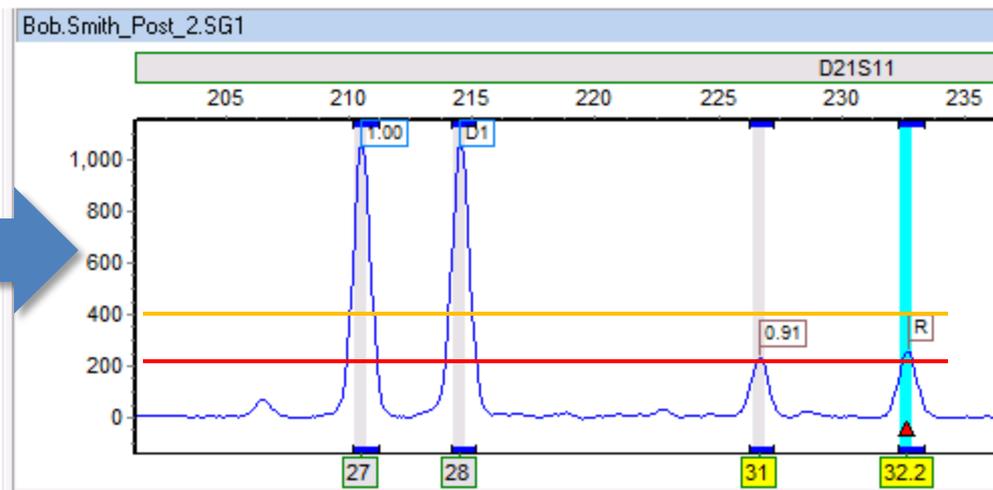
Use the "Apply to All" box to apply the changes to all markers.

%

%

%

Apply Stutter Settings to All Markers

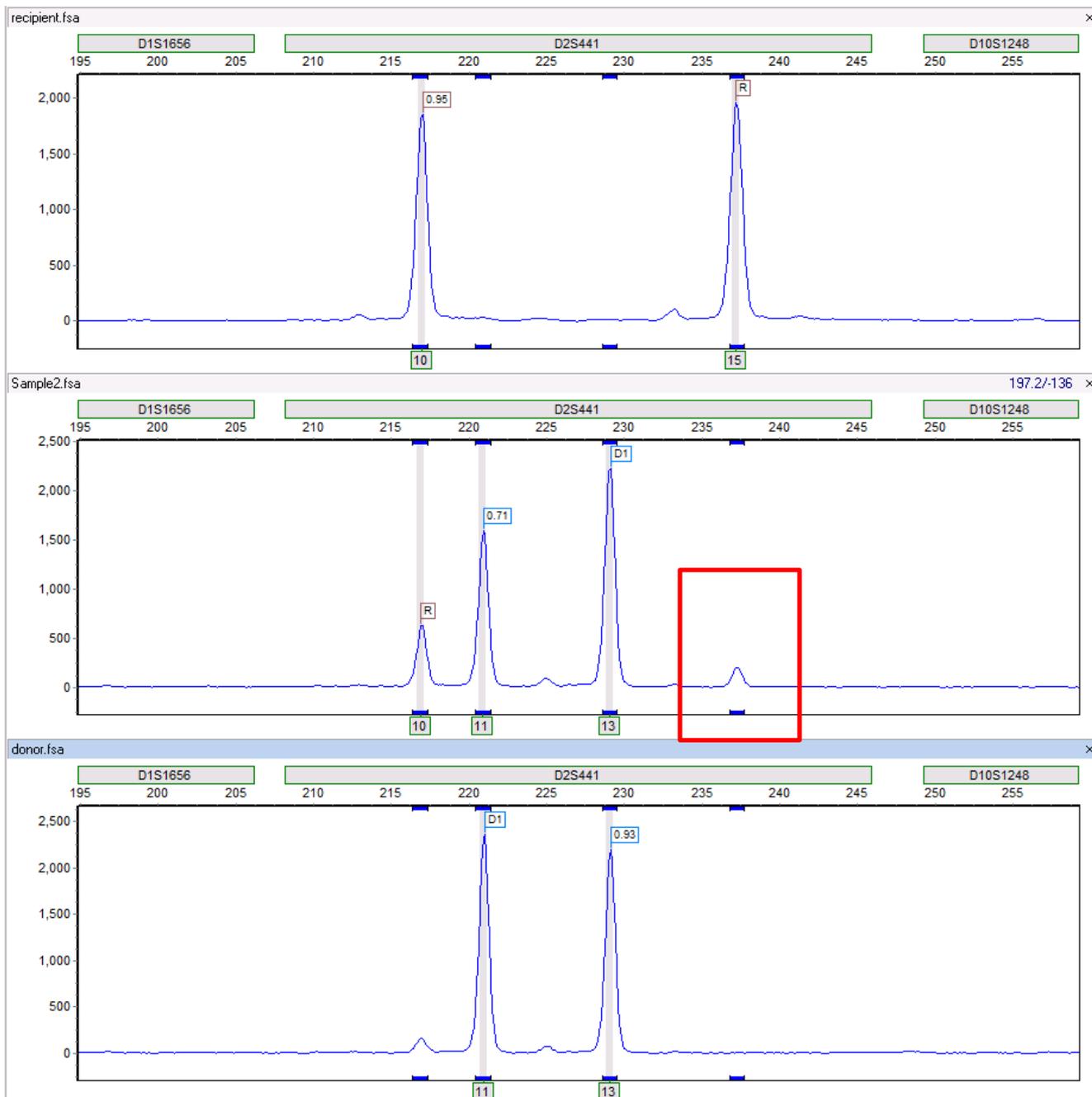


Note however that these peaks are flagged yellow. This is because despite being above the Min Heterozygote Intensity, the peaks are still below the upper range of 400 RFU, and are therefore still in the inconclusive range.

Here is a second example.
Why isn't the small peak
in Sample2 being called?

We can clearly see a
recipient bin directly
under the peak.

Again, we should check
the marker-specific panel
settings for this locus.



In this case, we can see that the peak is above the 50 RFU threshold of the Min Heterozygote Intensity filter. However, we may observe that this recipient peak is significantly shorter than the other recipient peak.

Here, it is the Min Heterozygote Imbalance filter, set currently at 40%, which is preventing this peak from being called.

Edit Marker [X]

Marker Parameters

Marker Name:

Nucleotide Repeats (x):

Boundary: To

Min Homozygote Intensity:

< = Inconclusive < =

Min Heterozygote Intensity:

< = Inconclusive < =

Max Heterozygote Imbalance: %

Min Heterozygote Imbalance: %

Apply Homo/Hetero Settings to All Markers

Stutter Filter:

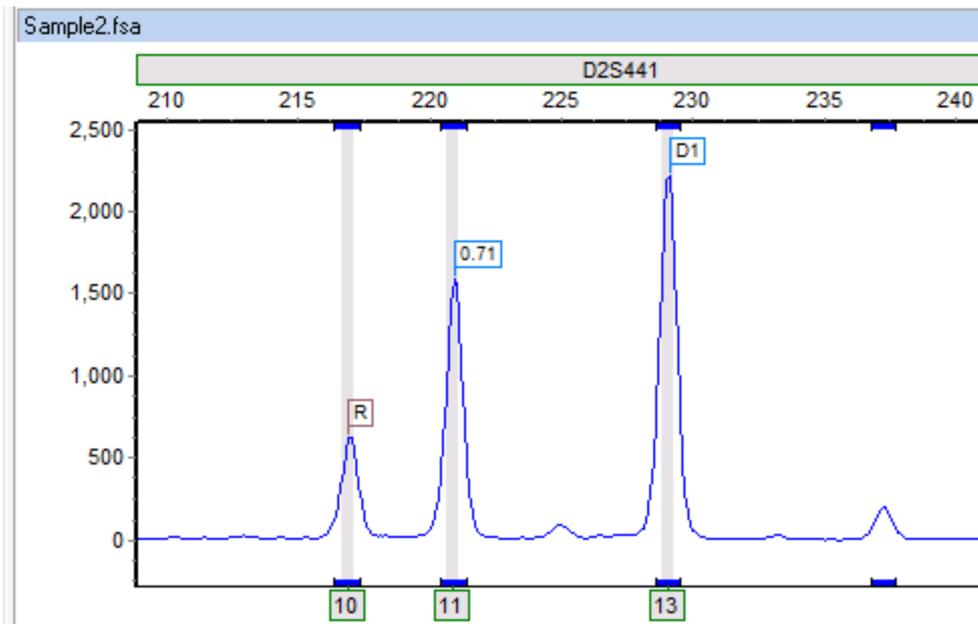
N - x %

N - 2x %

N + x %

Apply Stutter Settings to All Markers

OK Cancel



After lowering the Min Heterozygote Imbalance filter to 20%, saving the changes, and reprocessing the data, we can see that the peak is now called. Furthermore, we can see that the smaller peak is actually 32% the height of the other recipient peak, as indicated by the 0.32 flag.

Edit Marker [X]

Marker Parameters

Marker Name:

Nucleotide Repeats (x):

Boundary: To

Min Homozygote Intensity:

< = Inconclusive < =

Min Heterozygote Intensity:

< = Inconclusive < =

Max Heterozygote Imbalance: %

Min Heterozygote Imbalance: %

Apply Homo/Hetero Settings to All Markers

Stutter Filter: N - x %

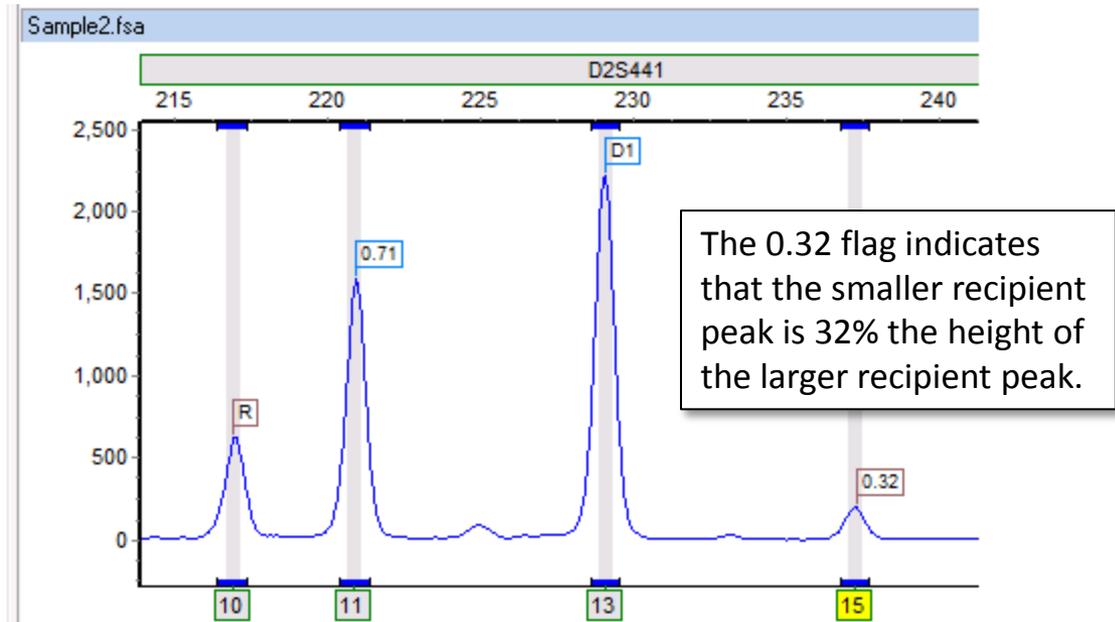
N - 2x %

N + x %

Apply Stutter Settings to All Markers

OK Cancel

Note that the peak is flagged yellow, because 32% still puts the peak in the inconclusive range – defined here as being lower than 60%.

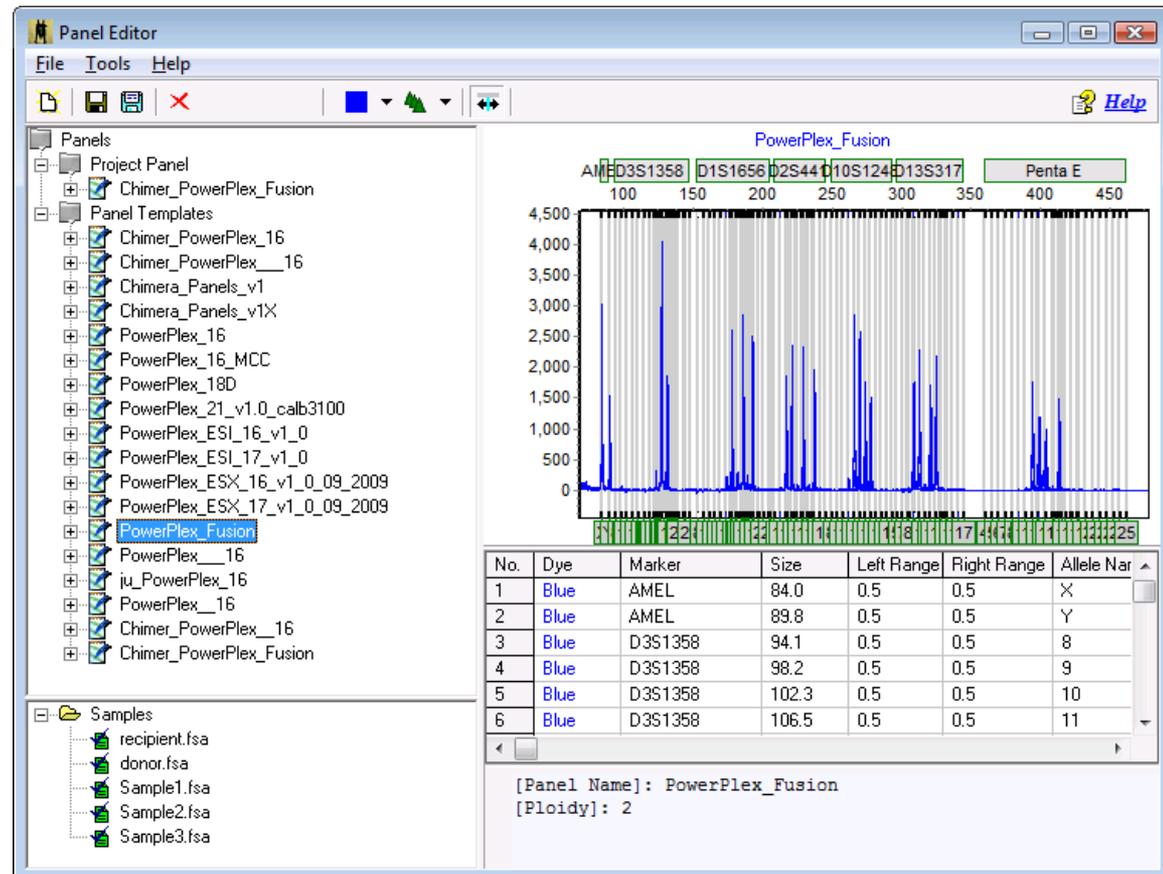


One final note about modifying panels: As mentioned in slides 2-4, The *Chimertyping panel* (prefix: Chimer) is a project specific panel derived from the original genotyping panel.

If you do choose to modify panel settings we recommend modifying the original genotyping panel, saving the changes, and then reprocessing your samples.

By modifying the genotyping panel directly, all derivative Chimertyping panels will reflect the changes.

However, if you instead modify the Chimertyping panel, you will only see improvements in that specific project.



(7) What is Deconvolution? “With deconvolution” and “Without deconvolution” are both options in the Chimerism analysis settings window.

Single-Donor Chimerism Settings

Basic Settings | Additional Settings

%CHM Type

%CHM Donor

%CHM Recipient

Quantification Type

Area

Height

Analysis Type

With Deconvolution

Without Deconvolution

Statistical Parameters

Analysis Threshold

Ignore Shared Allele Imbalance

Ignore Locus

Heterozygous Imbalance <= 5 %

Confidence Level (MOE) 90%

Error Threshold

Locus Error >= 10 %

Coefficient of Variation >= 10 %

Measurement Error >= 20 %

OK Cancel

When “With Deconvolution” is selected, the program will use shared peaks (D1R) in its chimerism calculations.

When “Without Deconvolution” is selected, shared peaks will not be considered when calculating the chimerism percentage. This may lead to fewer informative markers.