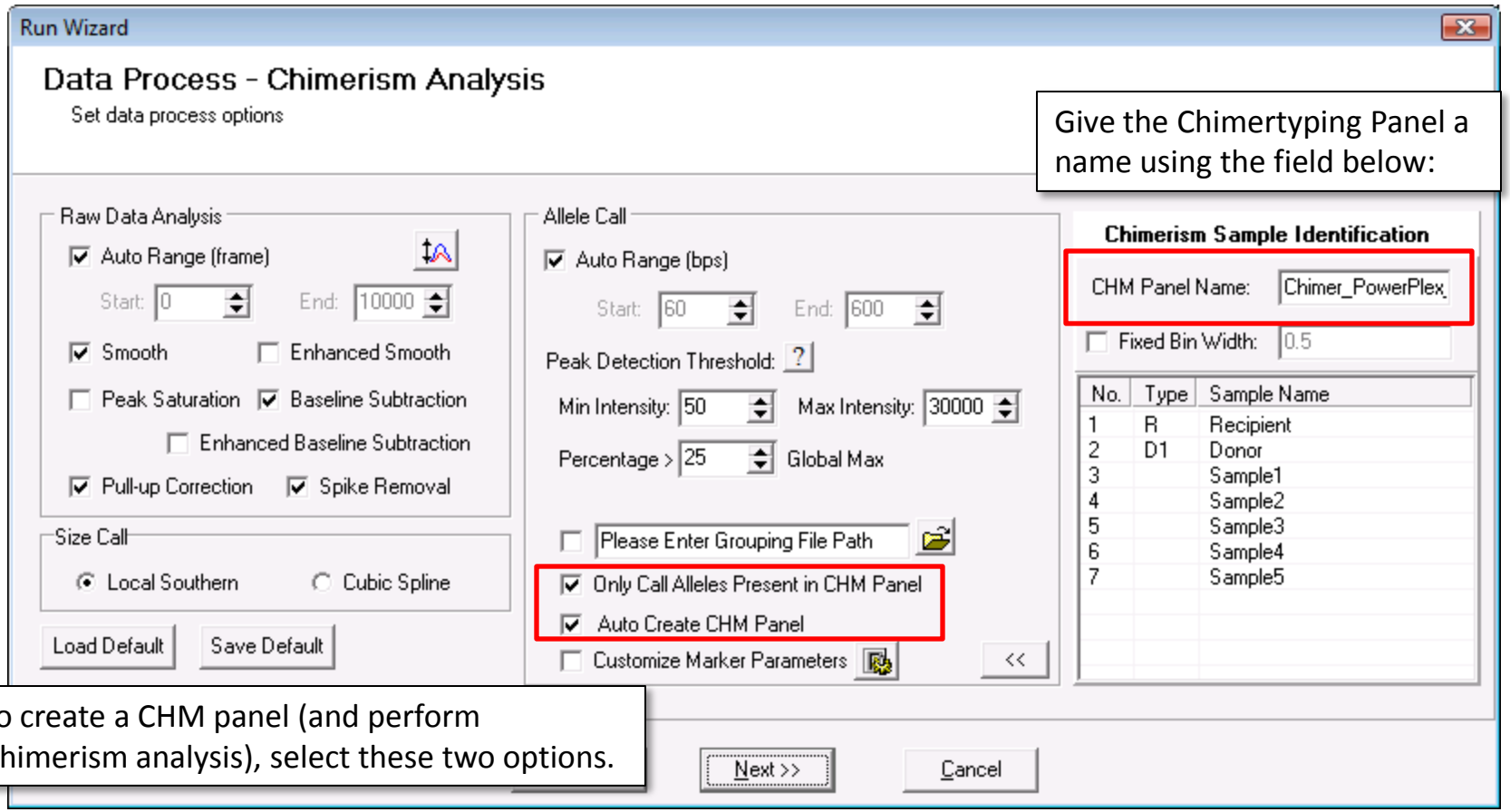
ChimerMarker™

Automated Chimerism Analysis Software

Frequently Asked Questions (FAQ)

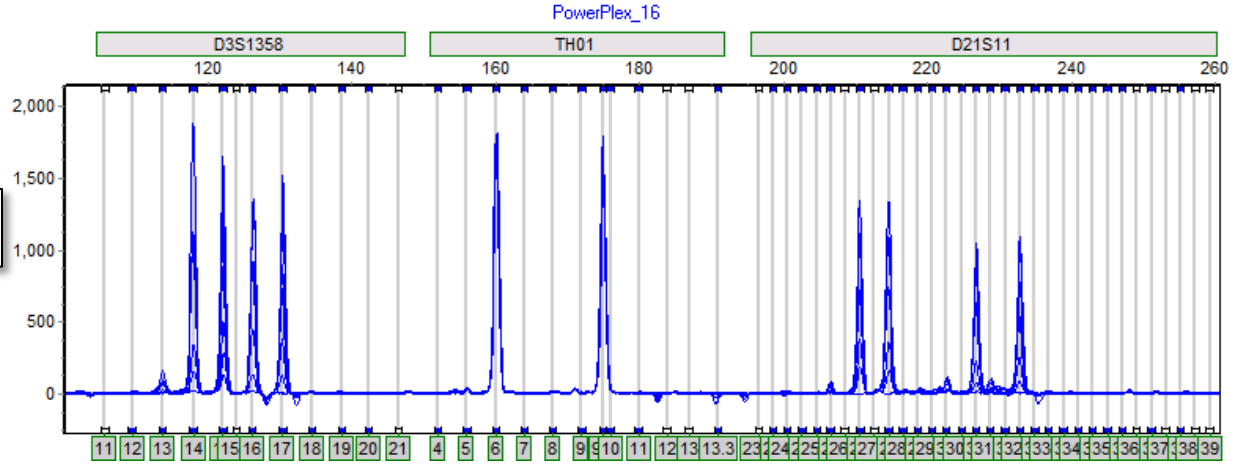
Question: Slide(s)

(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.



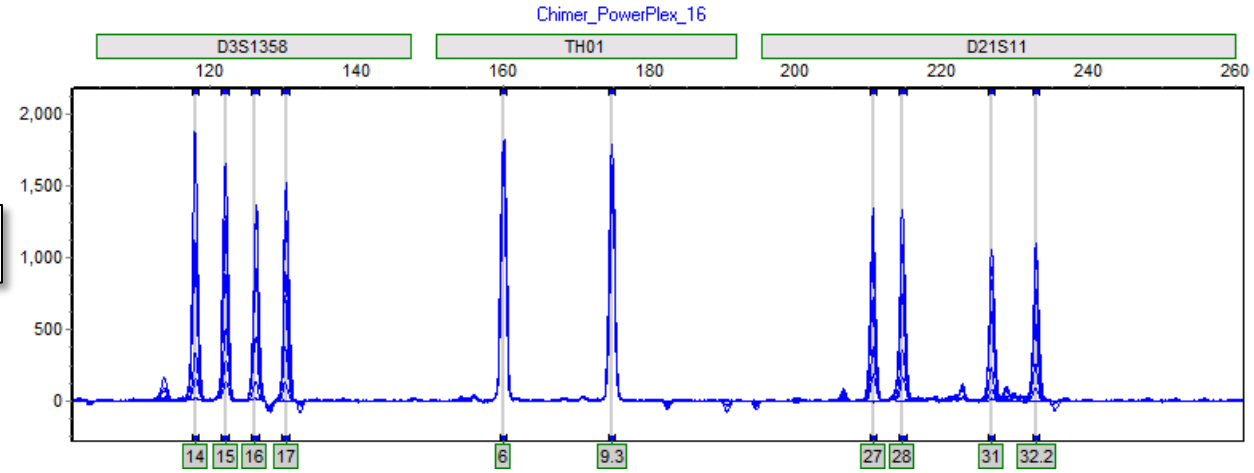
The Chimertyping Panel is derived from the Genotyping Panel. Only bins shared by either the donor or recipient are saved, the others are discarded.

Genotyping Panel

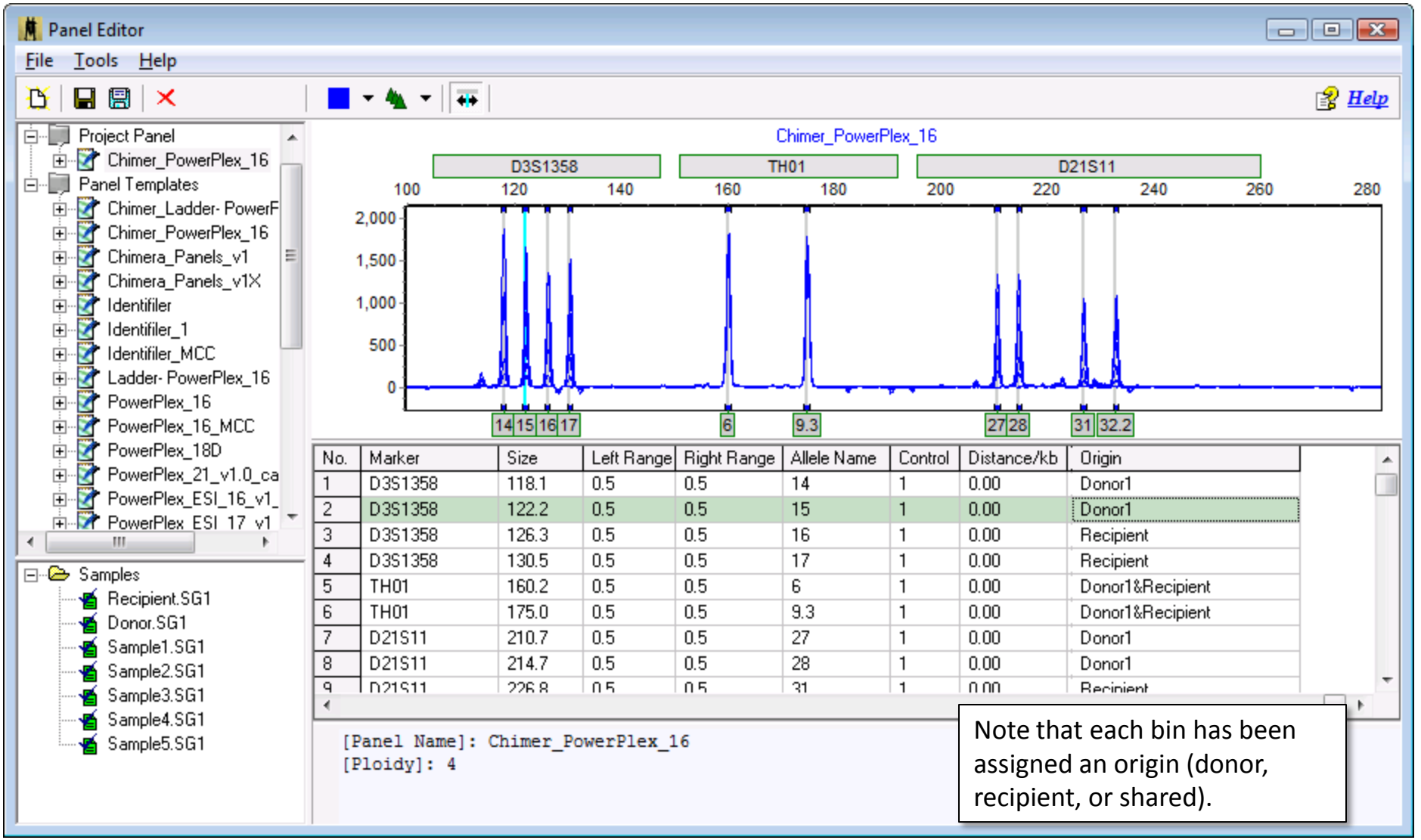


(data processing)

Chimertyping Panel

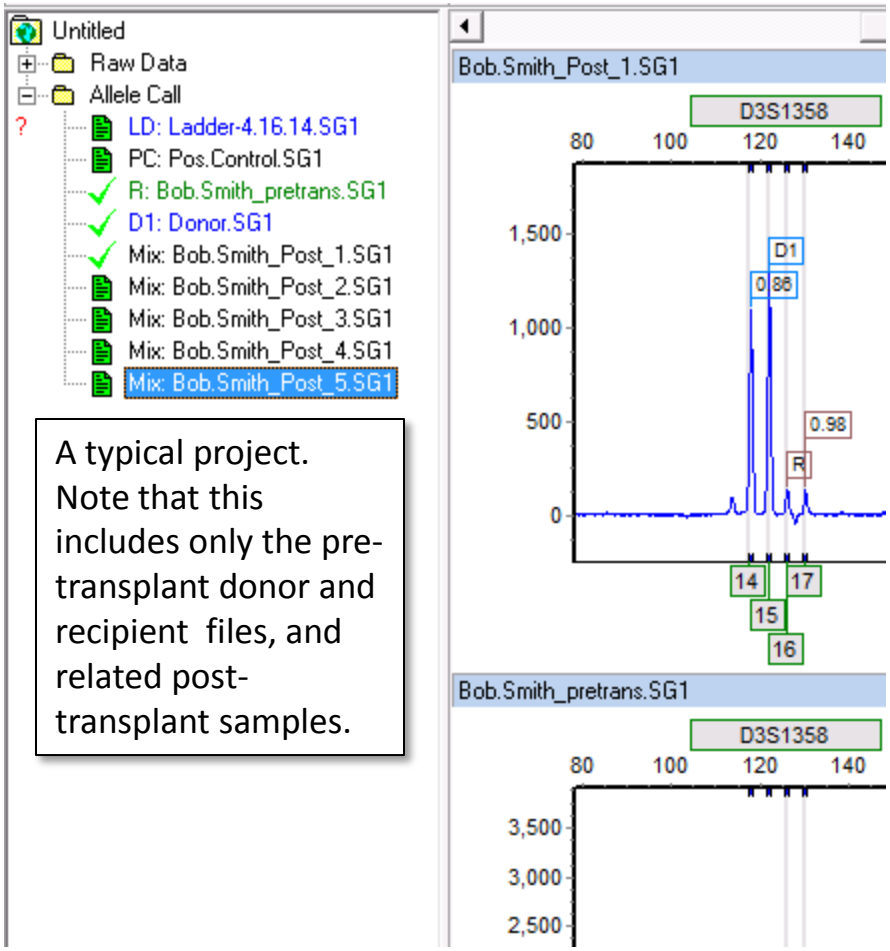


The Chimertyping panel can be viewed in the panel editor (Tools > Panel Editor). The Chimertyping panel for your current project will be listed under “Project Panel”.

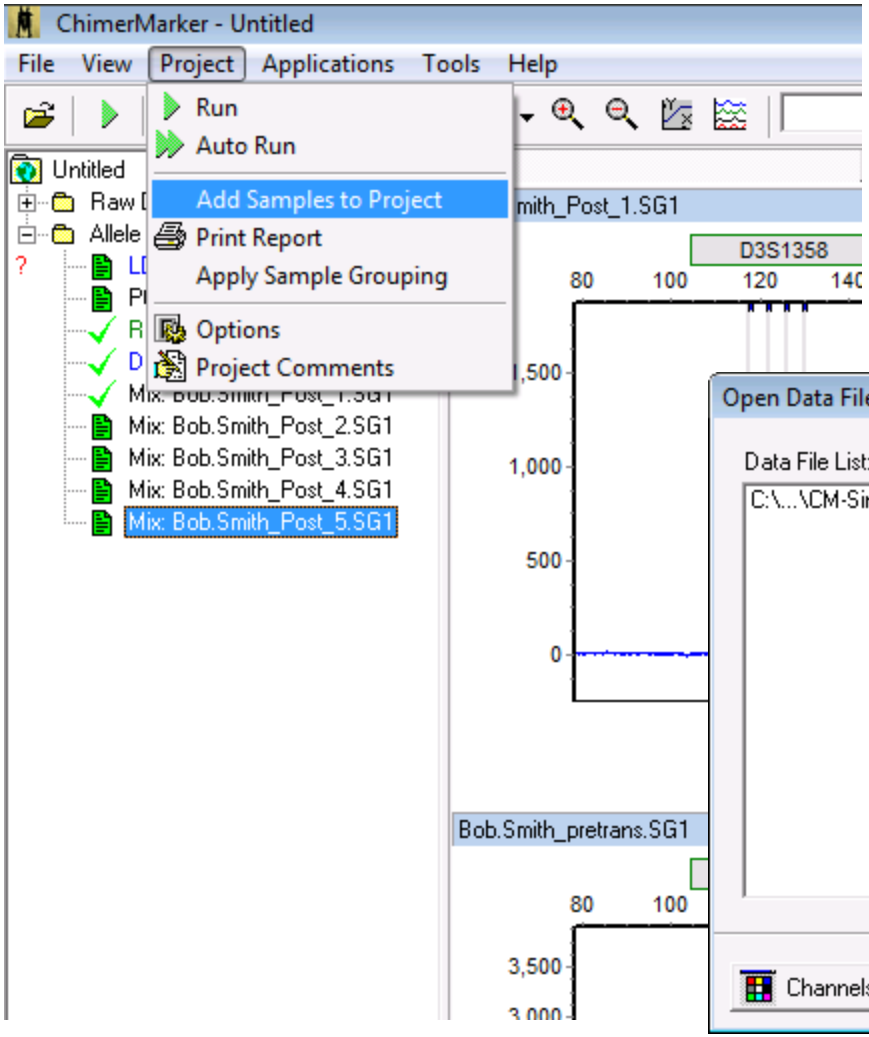


(2) How many recipients can I analyze at a time? – One. Because the Chimertyping panel is *project specific*, only one donor/recipient group may be analyzed at a time. This includes the pre-transplant donor and recipient files, and any number of post transplant (mixture) samples.

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

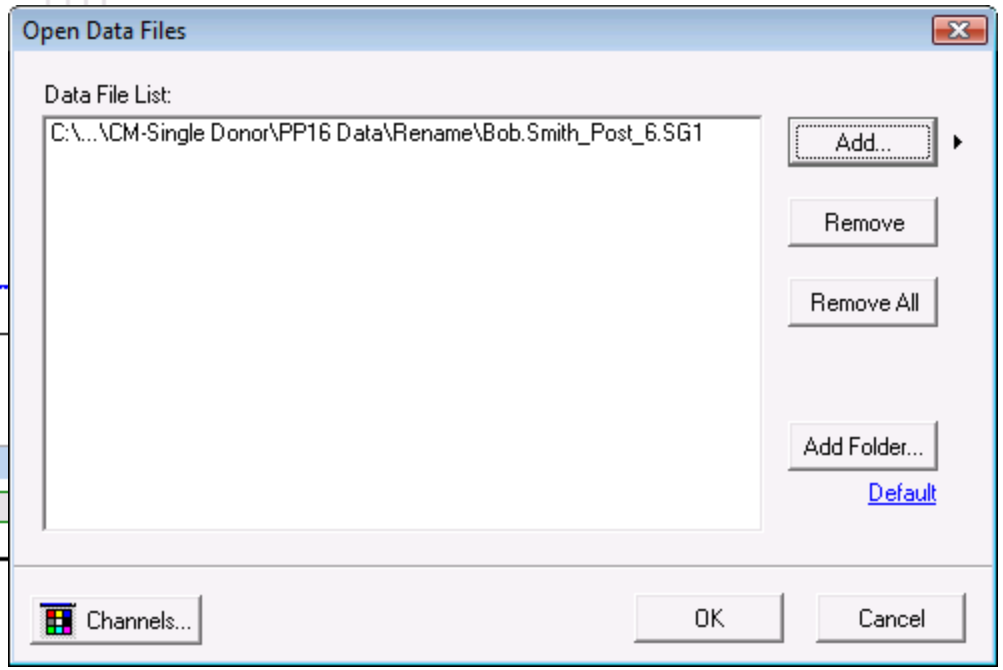


(3) How do I add samples to an existing project? – Simply open the project (file > open project) and click Project > Add Samples to Project.



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.

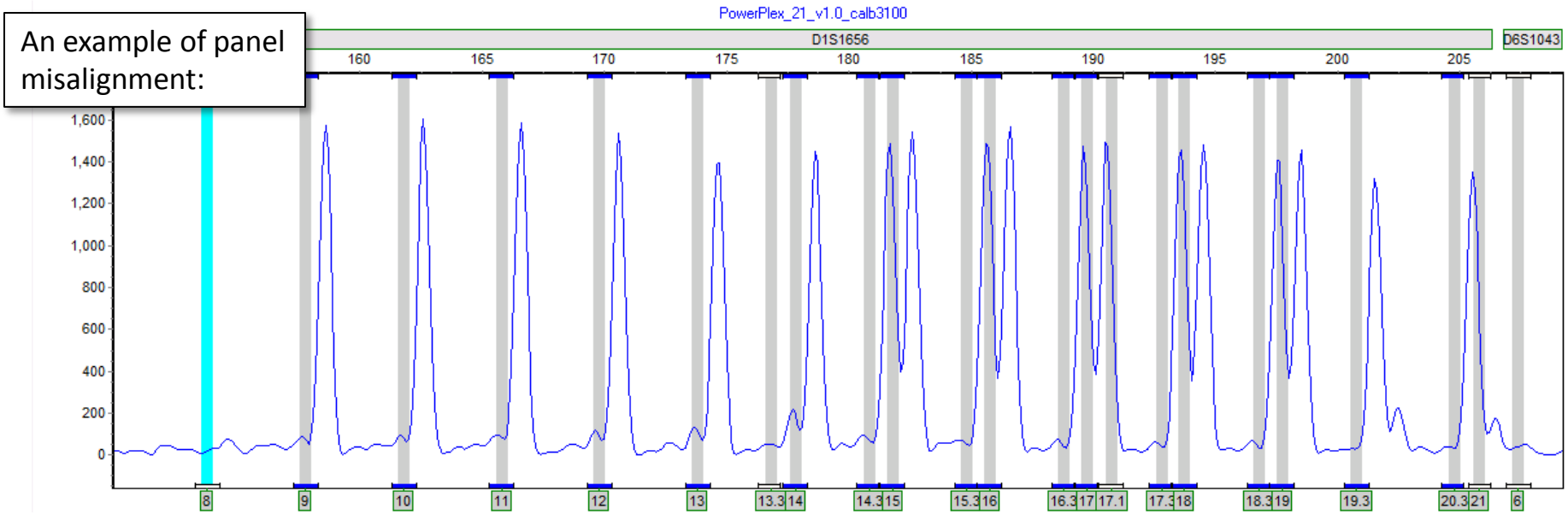


(4) How do I calibrate my panel? – Sometimes, particularly after running a new chemistry or maintaining your CE instrument, you may notice some amount of misalignment between your genotyping panel and your CE files.

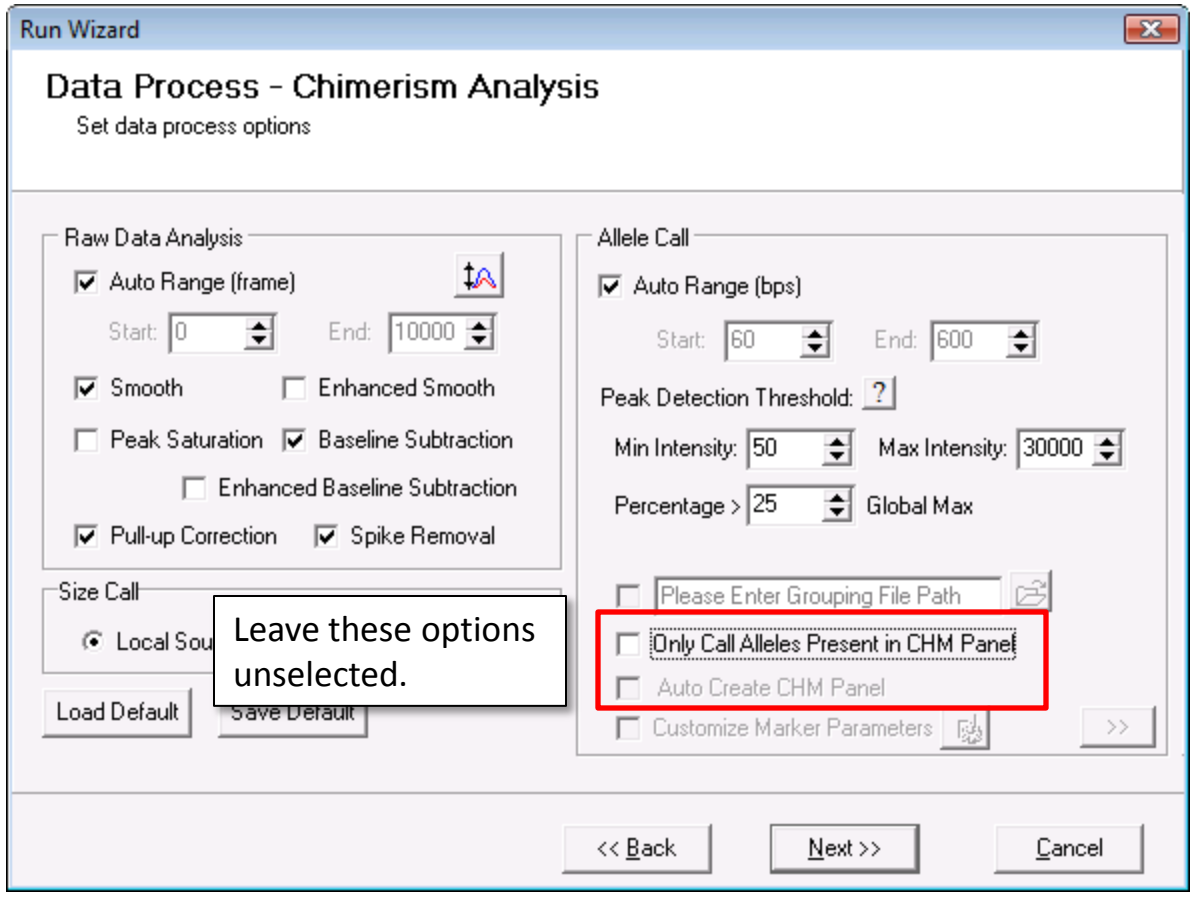
This can be an issue because when processing data, peaks may not “fall” into their correct bins, or they may be missed entirely.

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

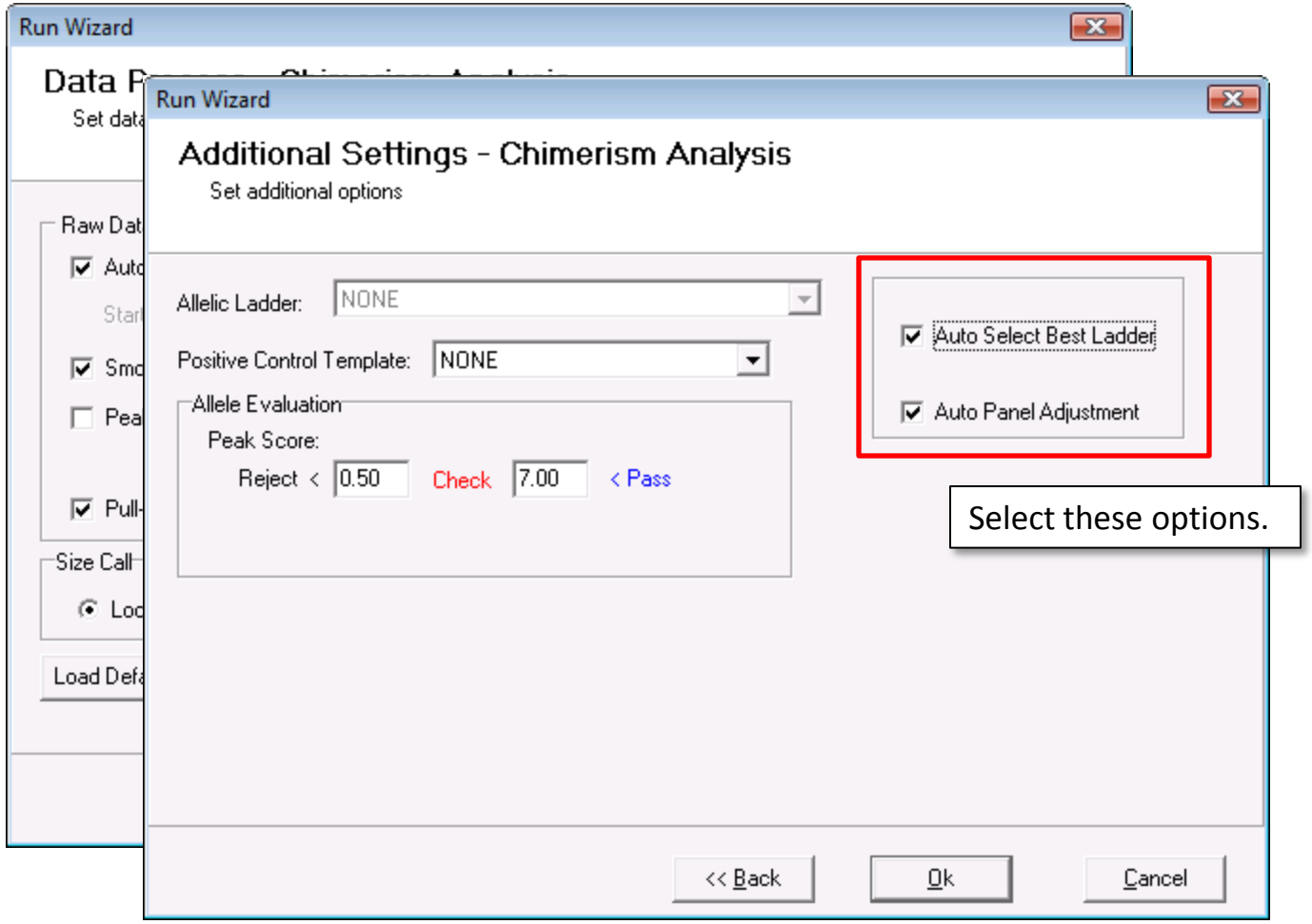
An example of panel misalignment:



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”.

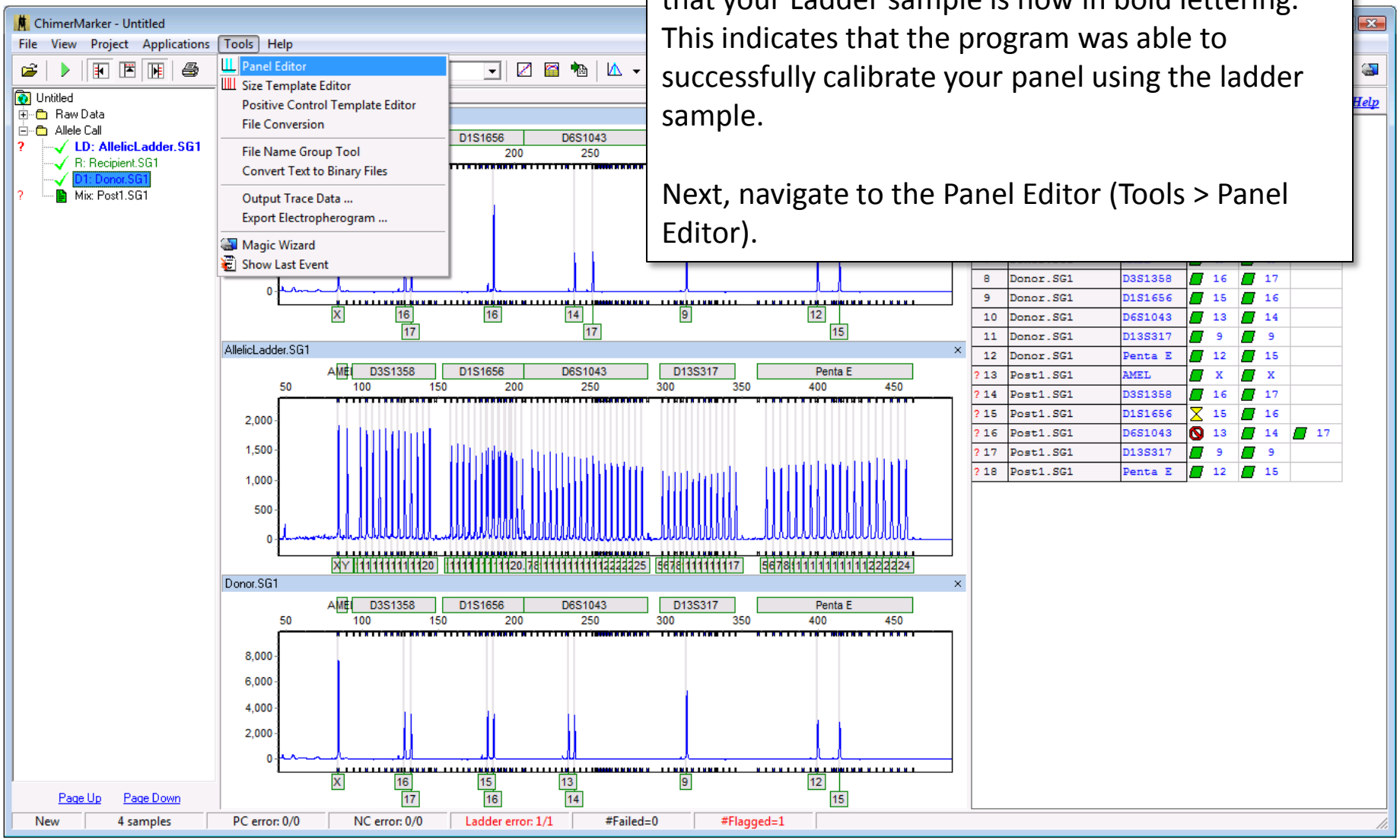


In the last page of the Run Wizard, select “Auto Select Best Ladder” and “Auto Panel Adjustment”.

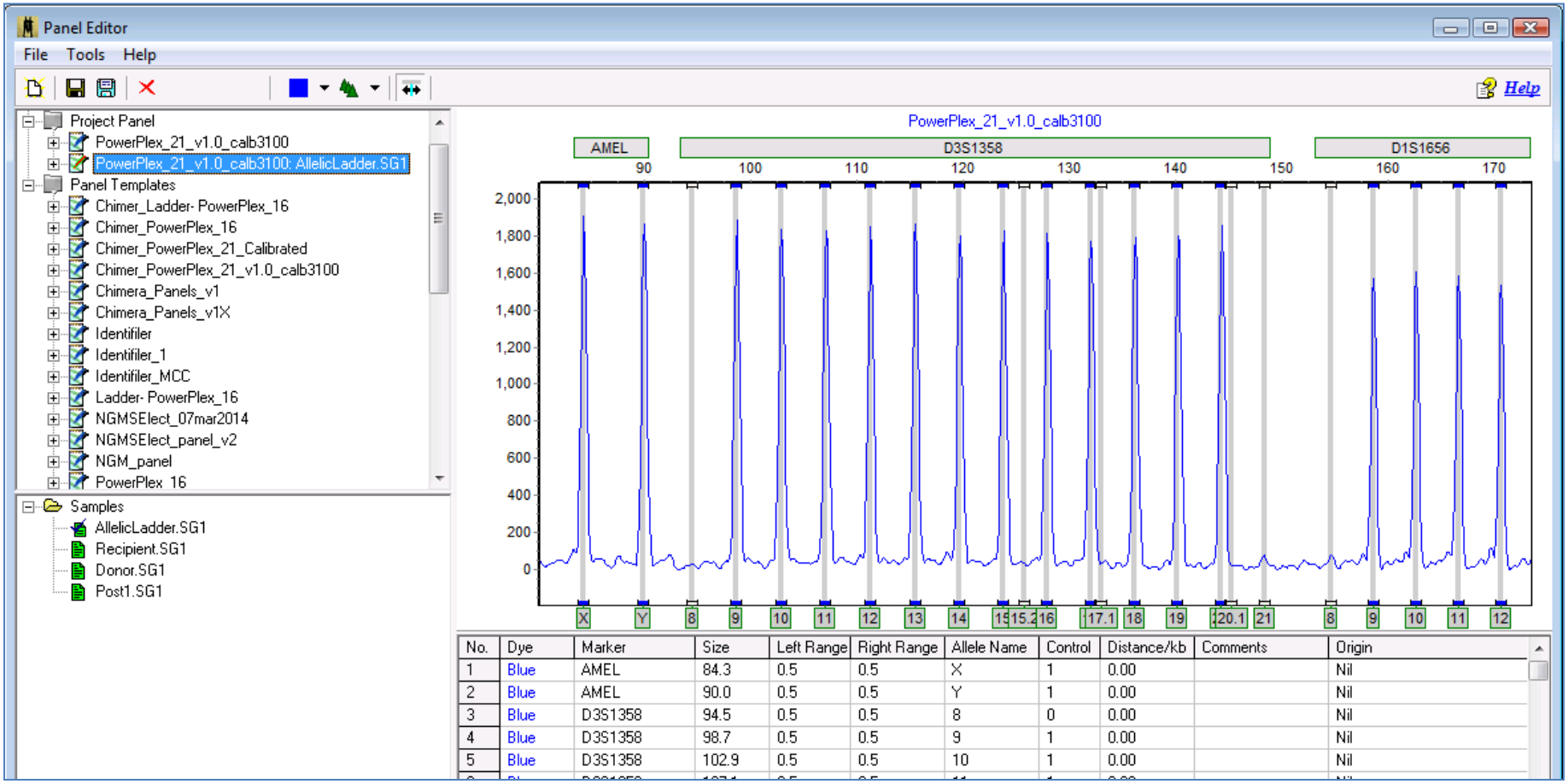


After Processing your samples, you should observe that your Ladder sample is now in bold lettering. This indicates that the program was able to successfully calibrate your panel using the ladder sample.

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



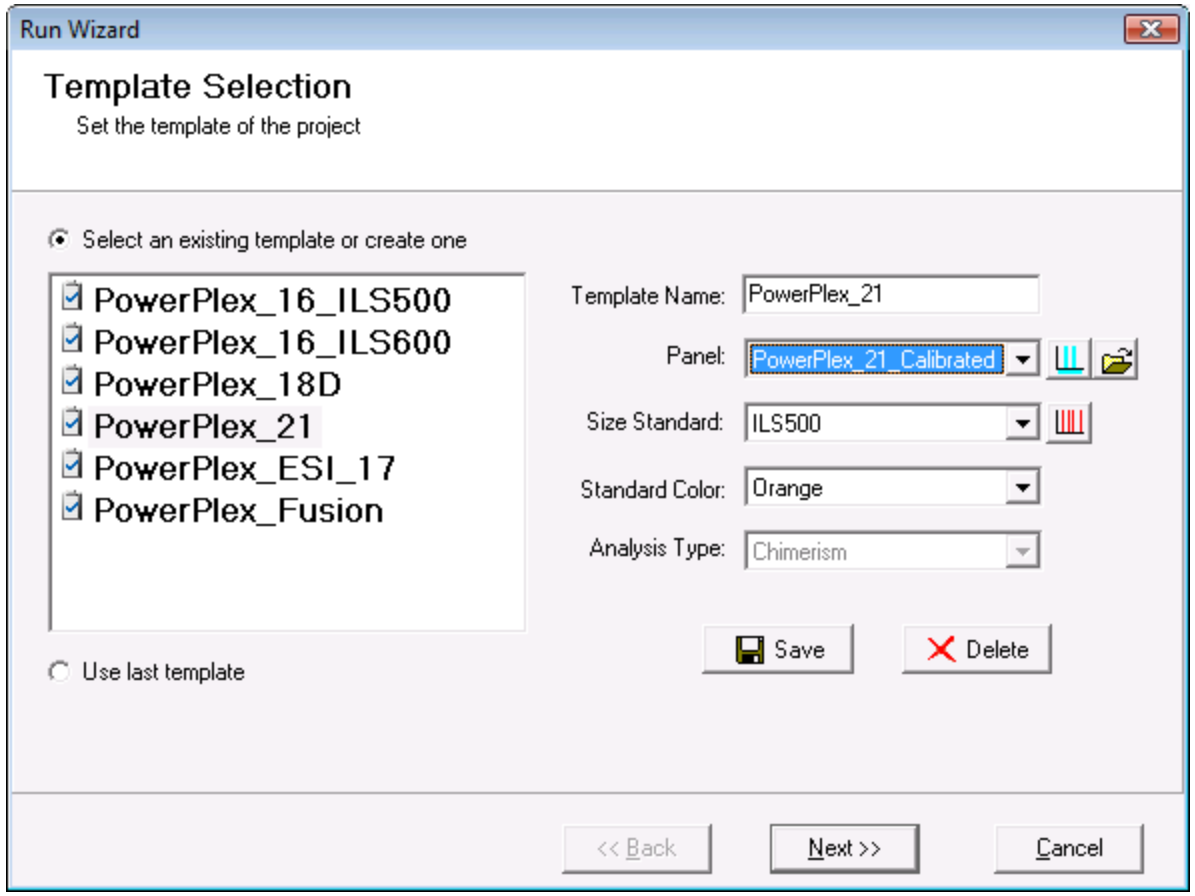
The panel editor contains all of the panel files saved in ChimerMarker. In the file tree to the left, you should see a section called **Project Panels** – these are the panels used to process your current project.



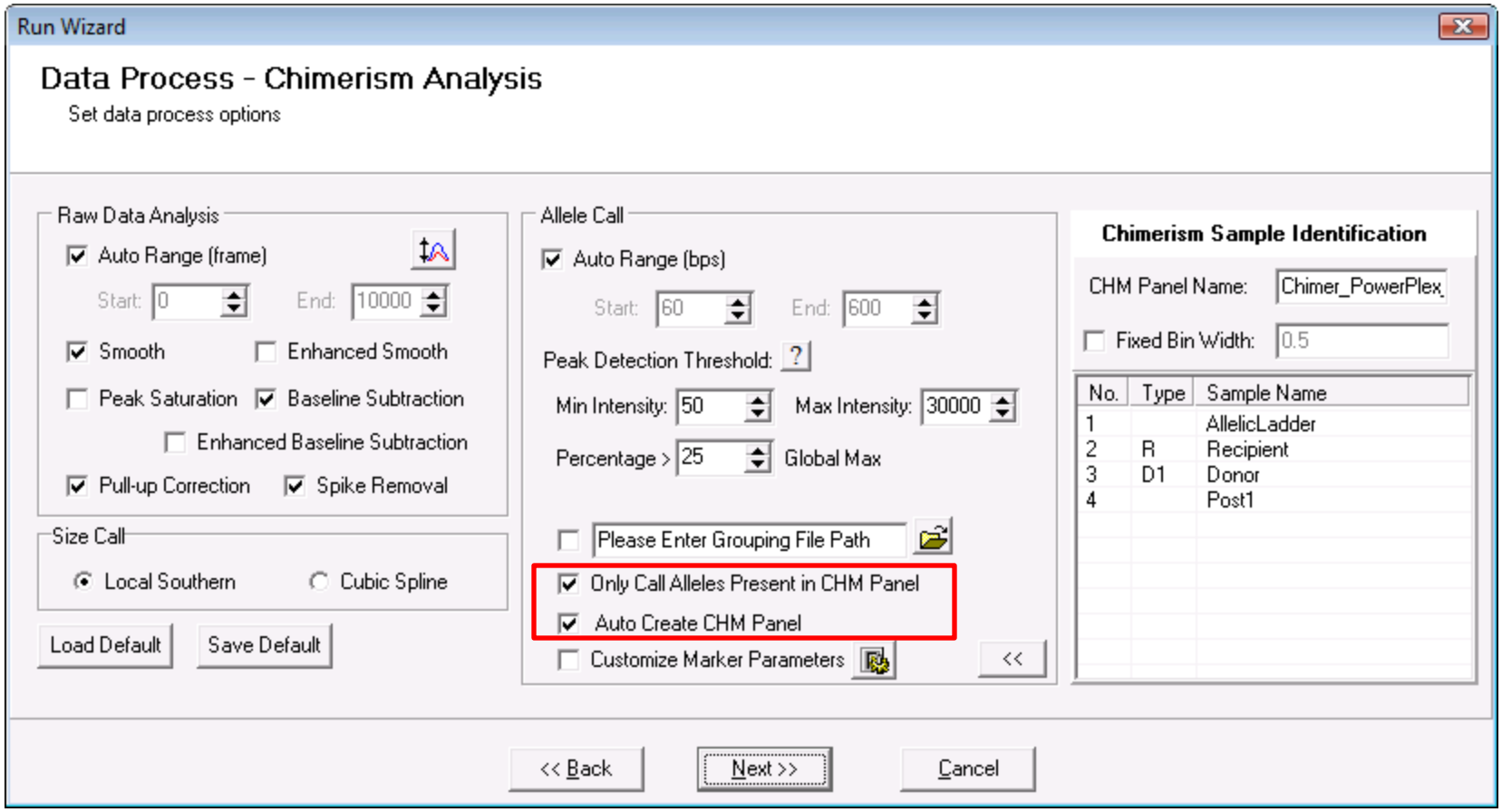
You should see two or more project panels – the original panel you used to genotype your samples, and another panel, appended with your ladder name.

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

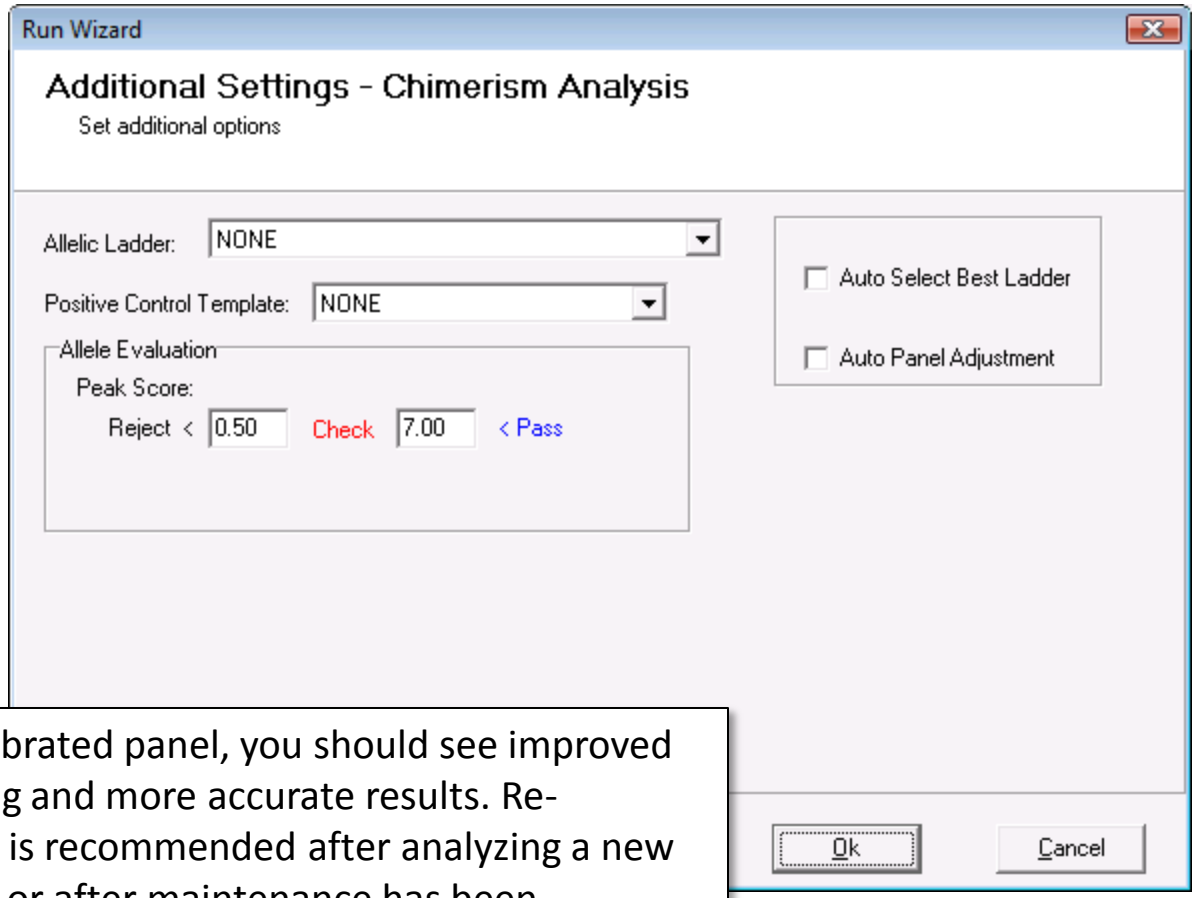
To reprocess your samples with this new calibrated ladder, simply reopen the Run Wizard (project > run) and be sure to select your new calibrated panel from the panel dropdown menu.



In the second page of the Run Wizard, select “Only Call Alleles Present in CHM Panel” and “Auto Create CHM Panel” to perform chimerism analysis.

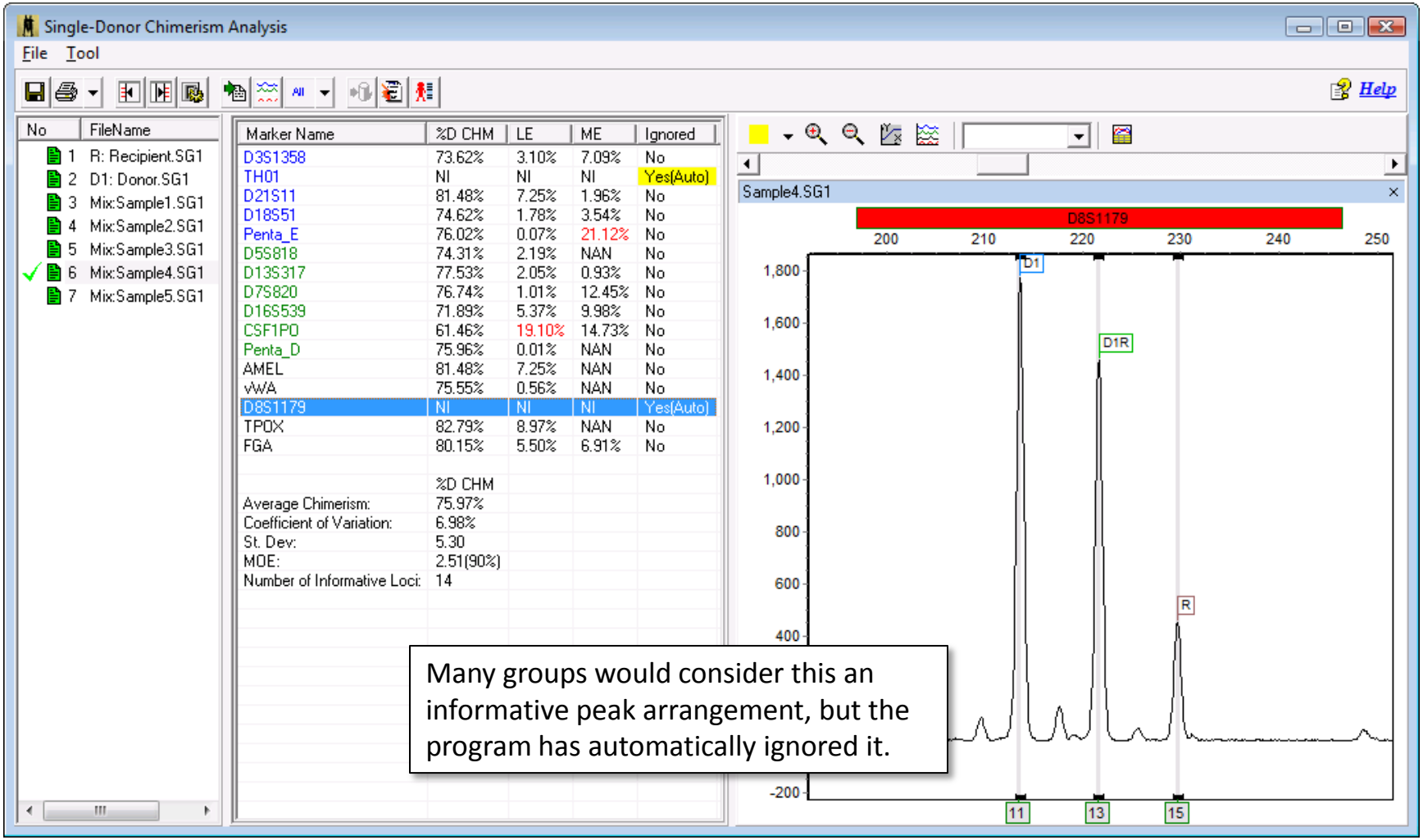


In the final page of the Run Wizard, do not select either “Auto Select Best Ladder” or “Auto Panel Adjustment”, as we have already done this.

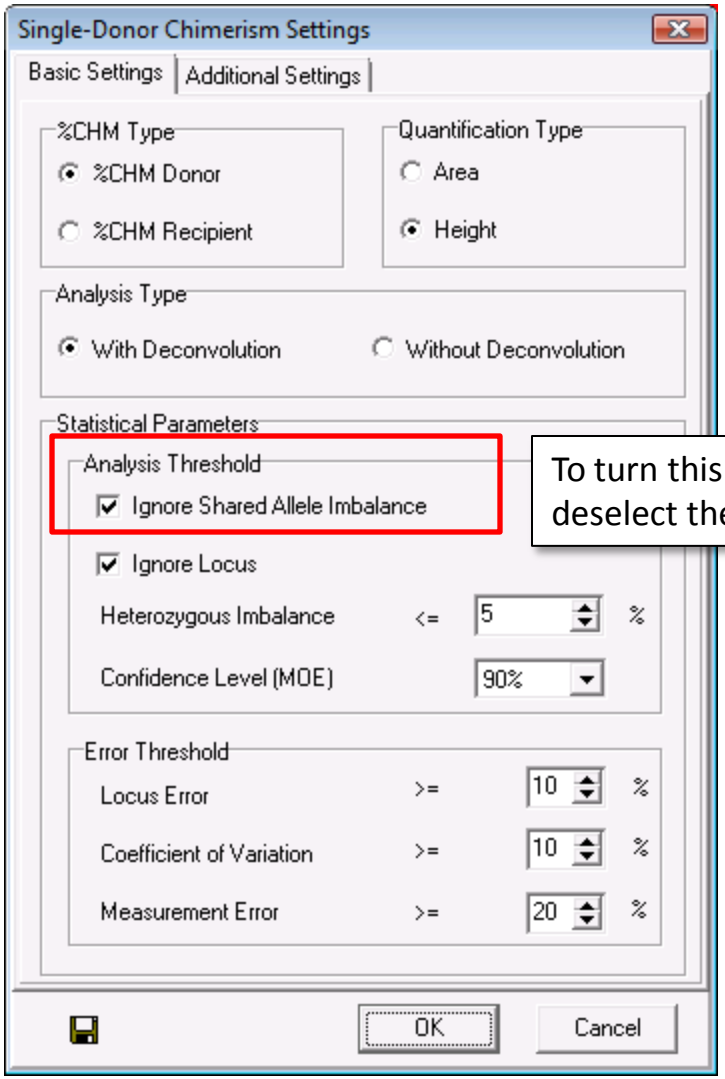


With a calibrated panel, you should see improved peak calling and more accurate results. Re-calibrating is recommended after analyzing a new chemistry, or after maintenance has been performed on your Genetic analyzer.

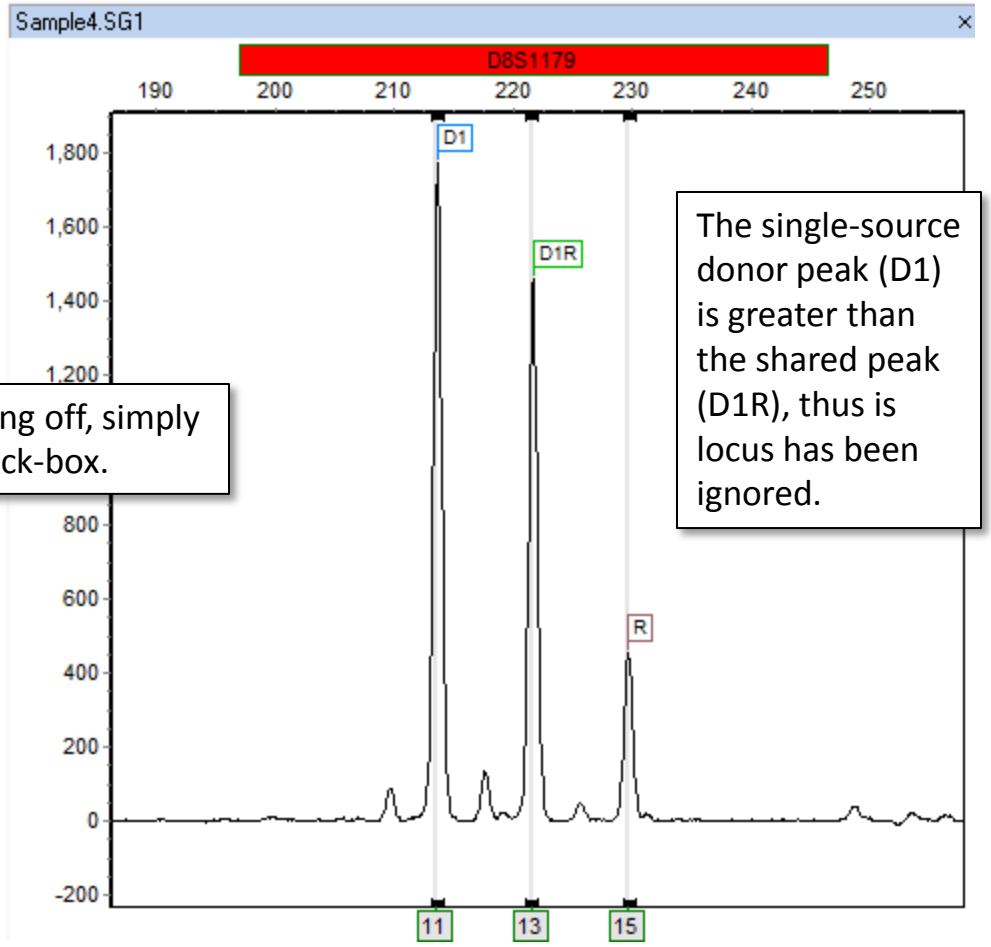
(5) Why are some informative loci being ignored? Sometimes, markers that appear informative may be ignored. This is usually related the settings that were chosen in the Chimerism Settings window. An example is shown below:



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.

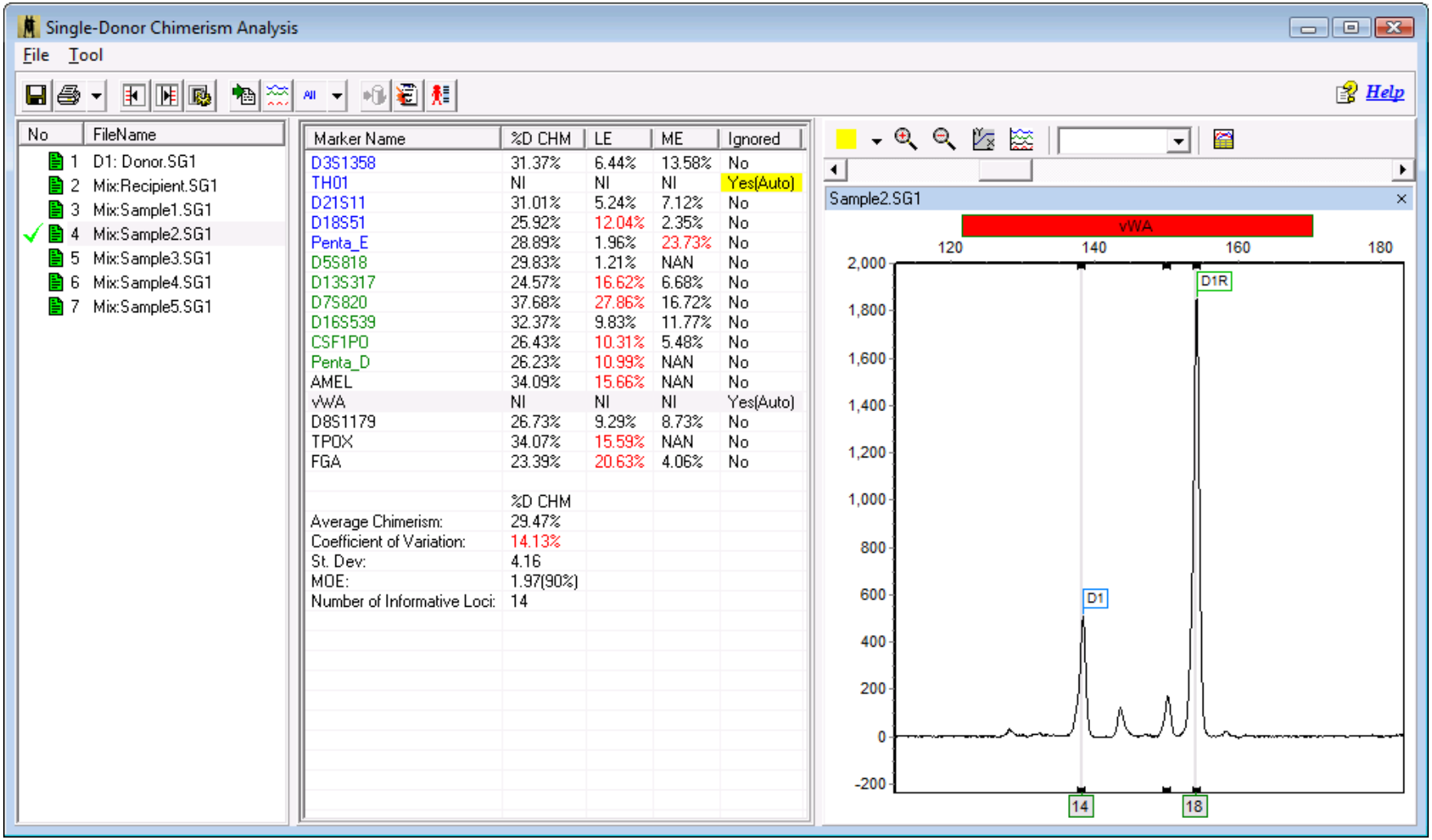


To turn this setting off, simply deselect the check-box.

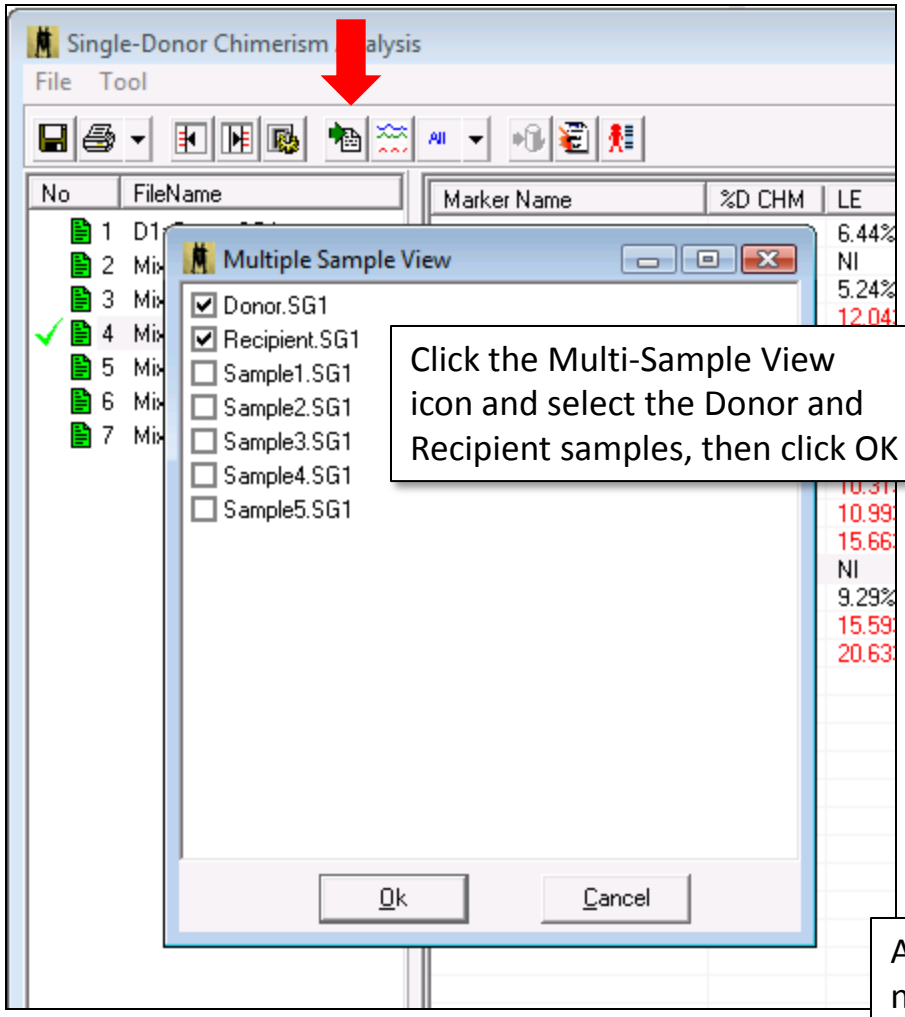


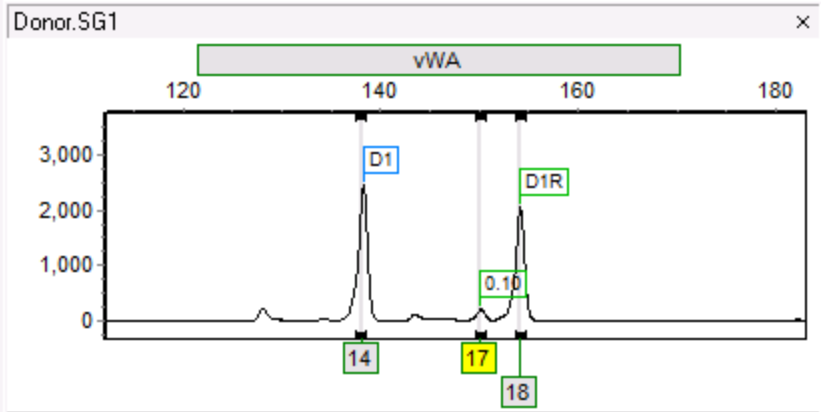
The single-source donor peak (D1) is greater than the shared peak (D1R), thus is locus has been ignored.

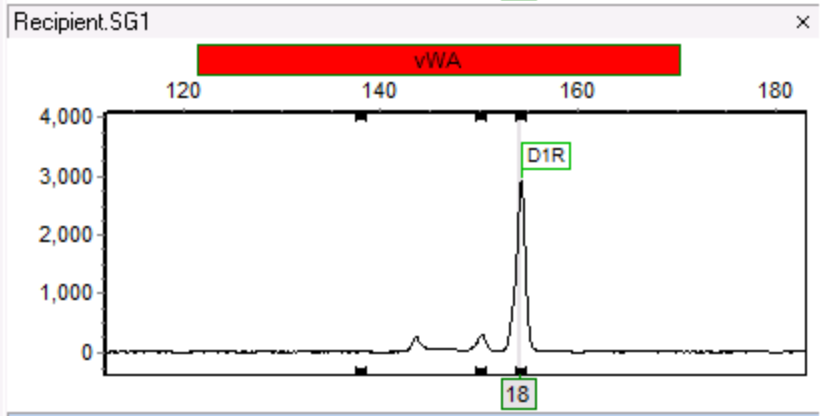
Here is another marker that is being ignored for no obvious reason:

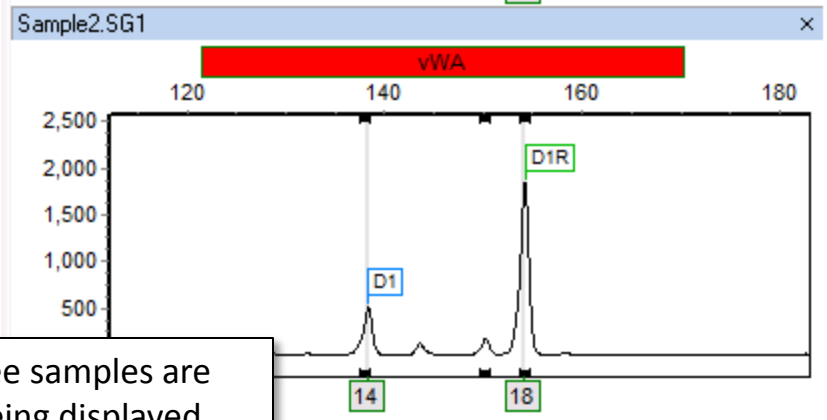


You can navigate back to the main analysis screen to look at this marker in more detail, or while in the chimerism analysis screen, you can use the "Multi Sample View" icon.









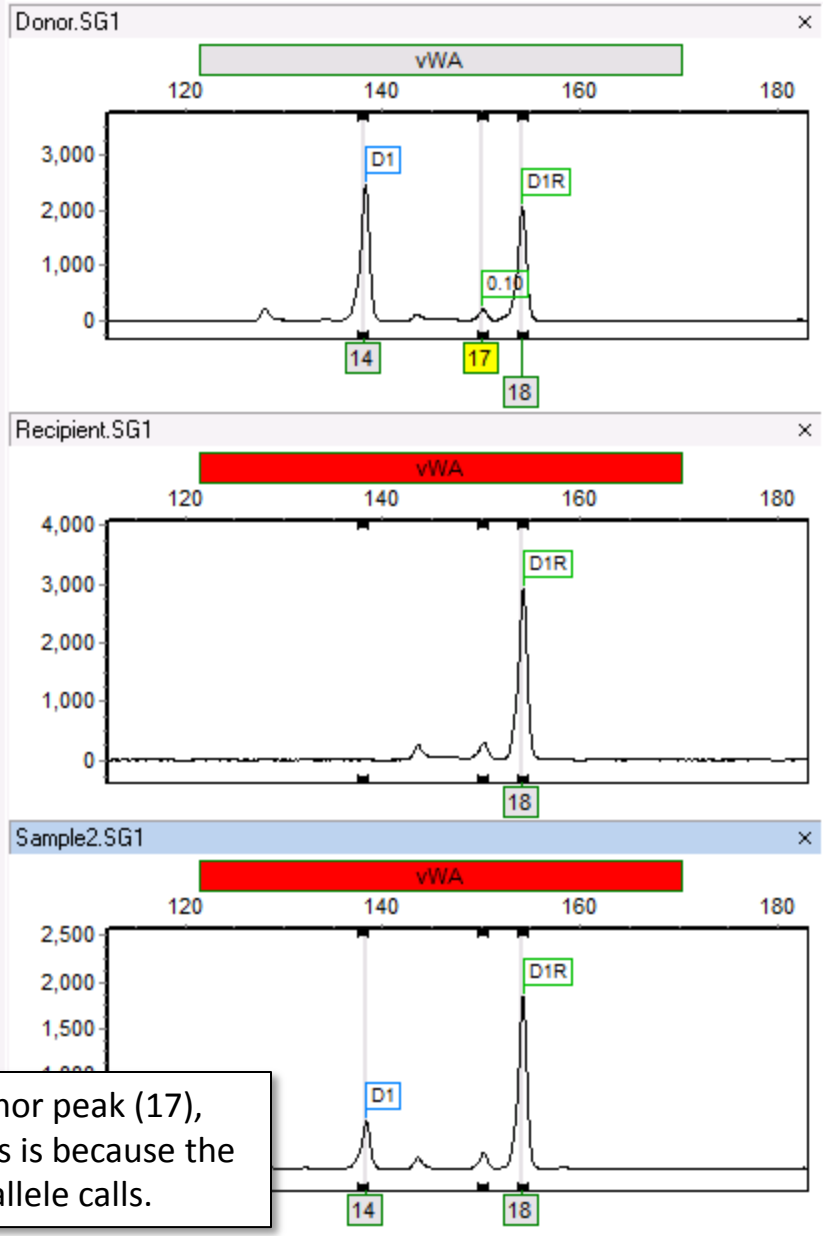
All three samples are now being displayed.

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).

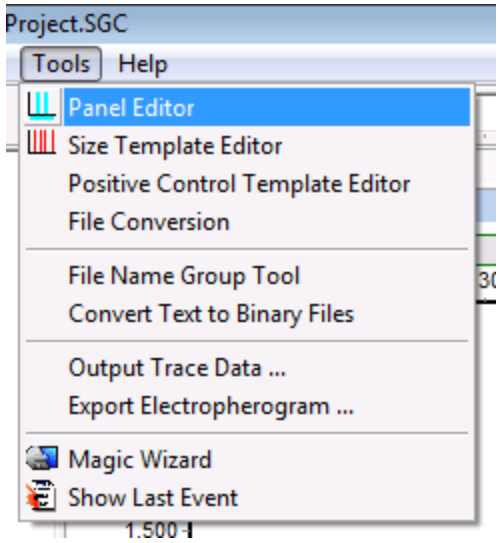
It may be tempting to simply delete this peak in the main analysis window. However this will not solve the problem.

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.

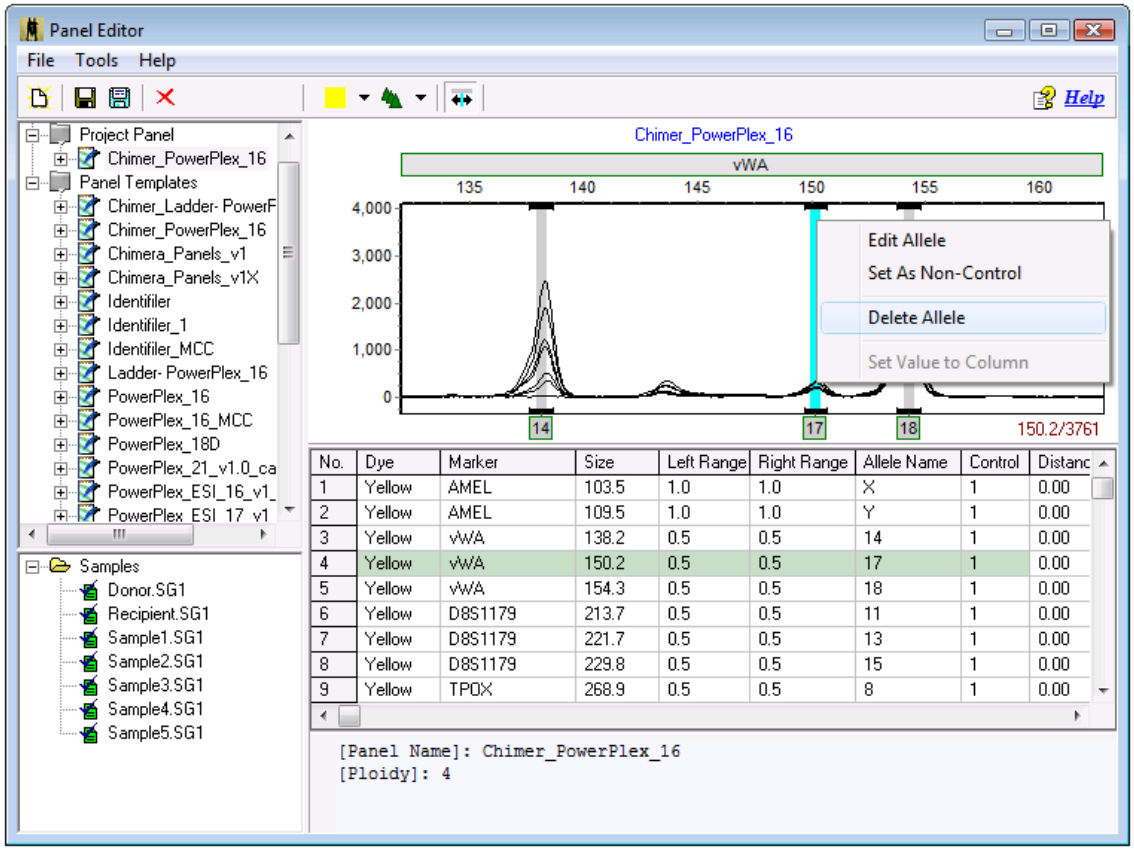


Note that the program has not only called a third donor peak (17), but it has also added a donor bin at this location. This is because the CHM panel is created based on donor and recipient allele calls.

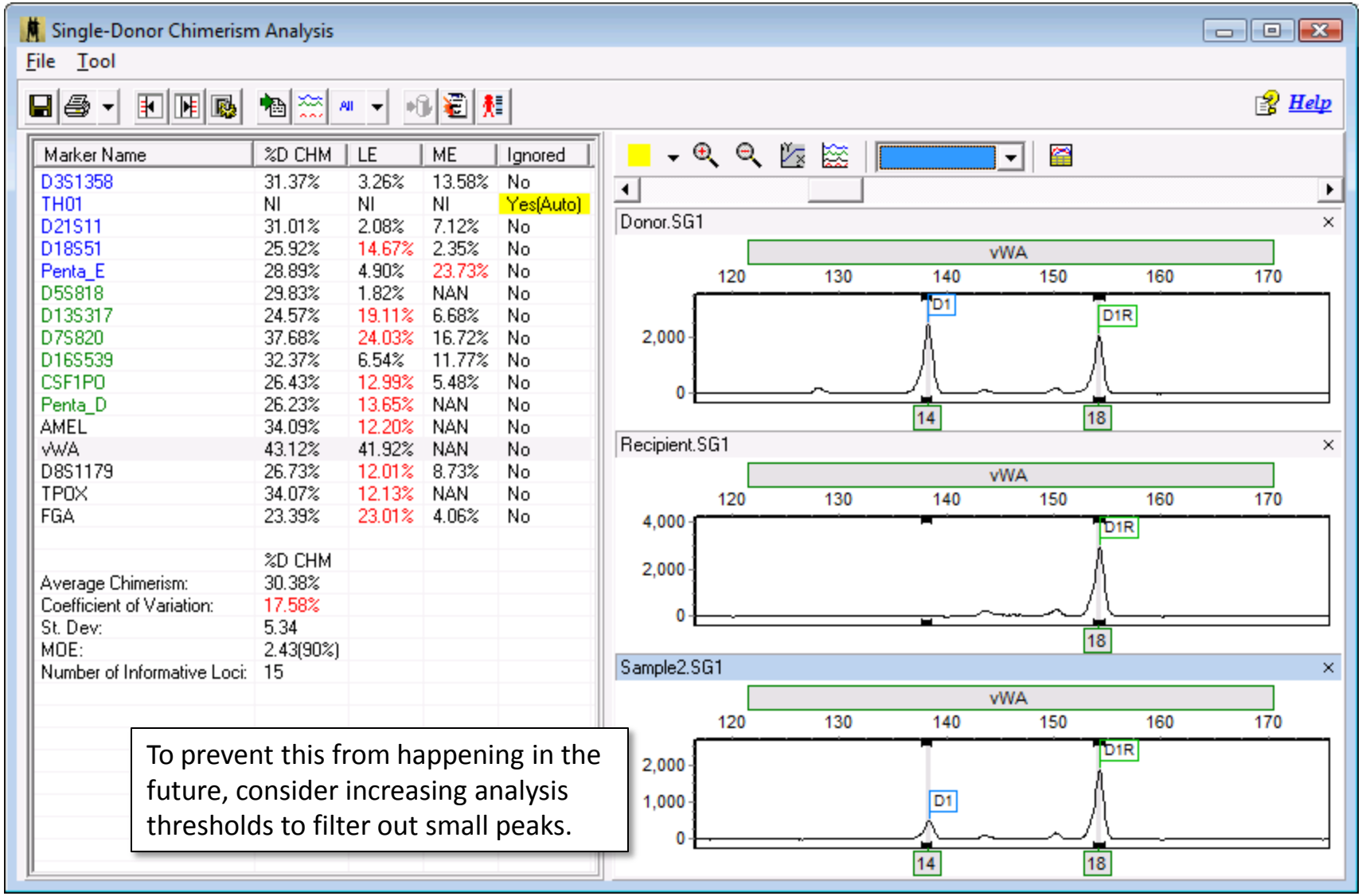
The Chimertyping panel can be viewed in the panel editor (Tools > Panel Editor). The Chimertyping panel for your current project will be listed under “Project Panel”.



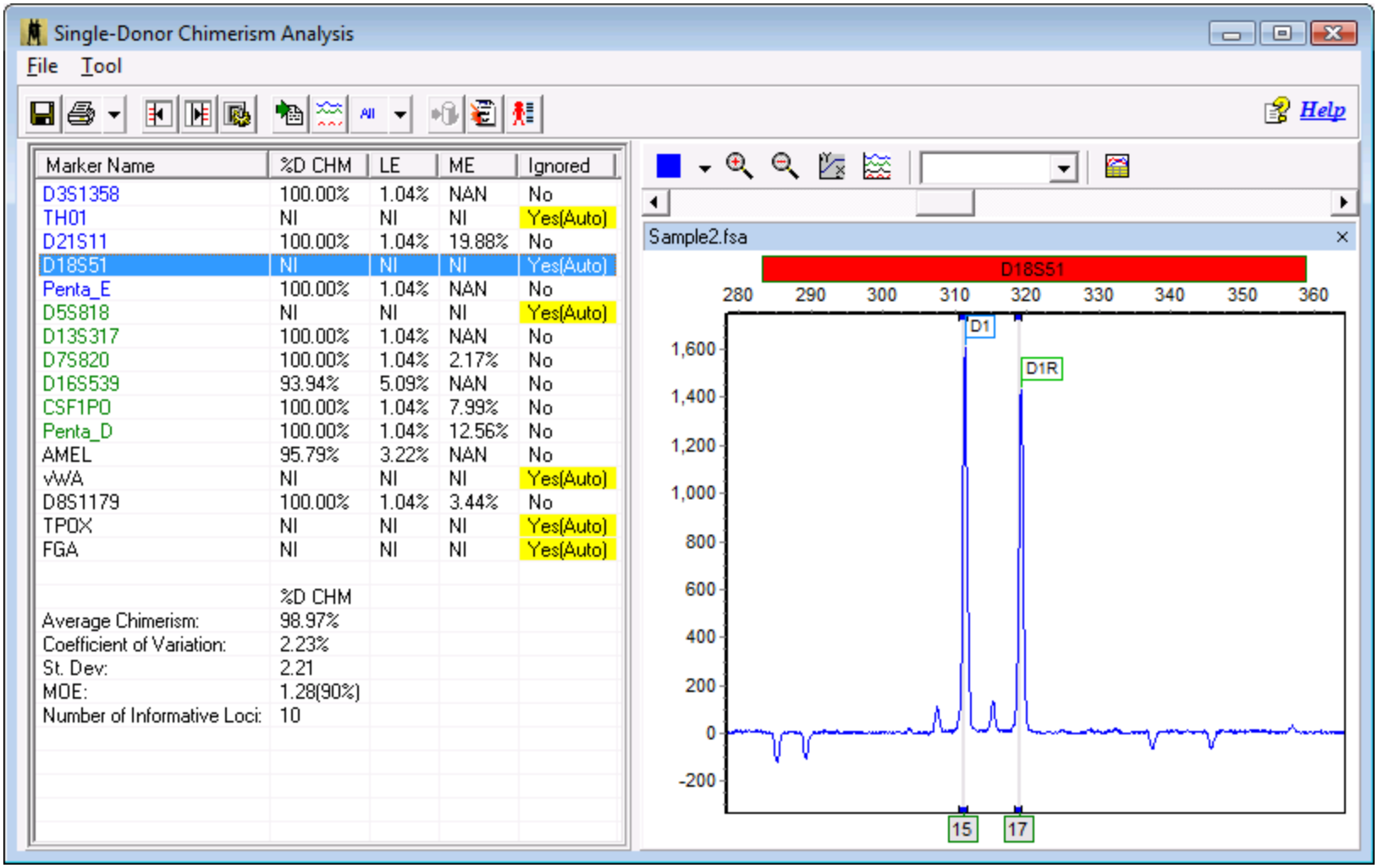
Simply right-click on the extra bin, and select Delete Allele. Save the changes by clicking the save icon



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.

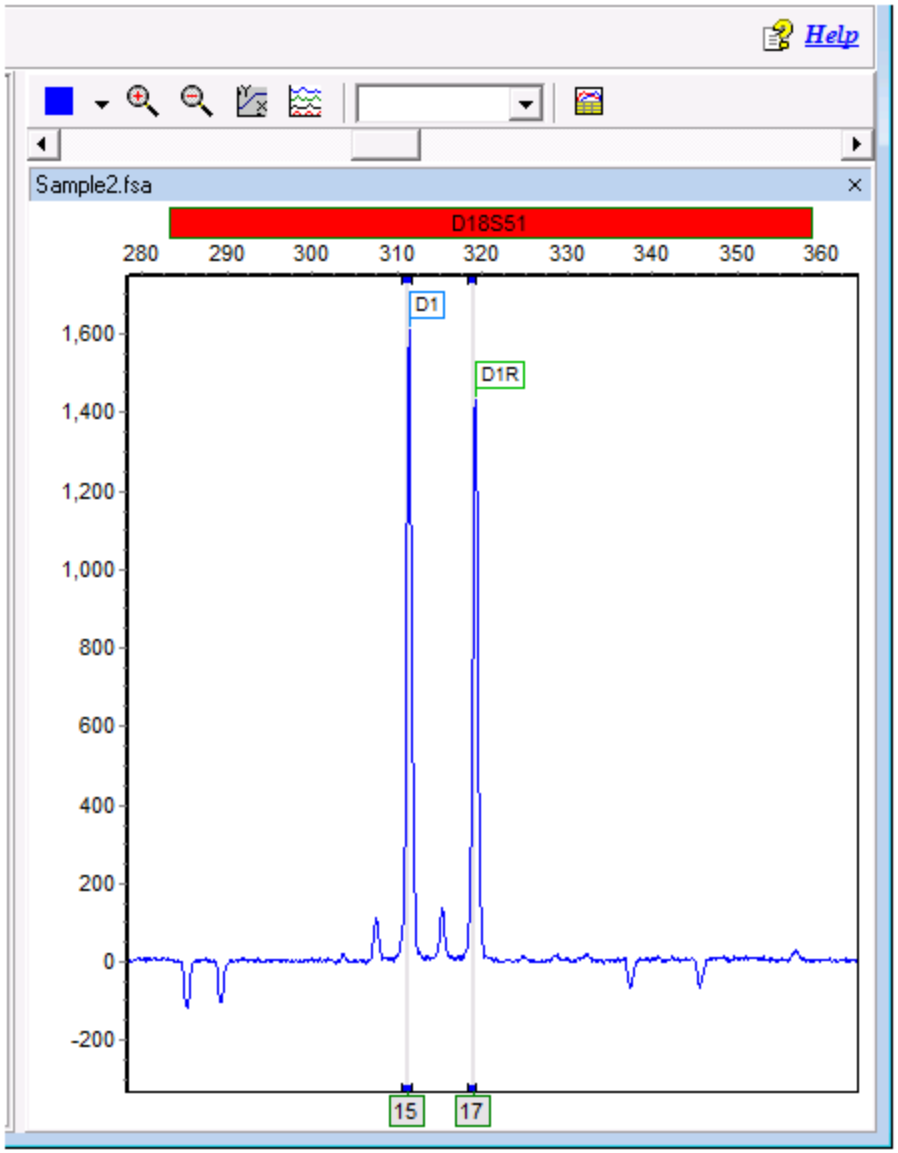


Our first impulse may be to blame the shared allele imbalance filter discussed on slide 17. However, even with this option deactivated, the marker will still be ignored.

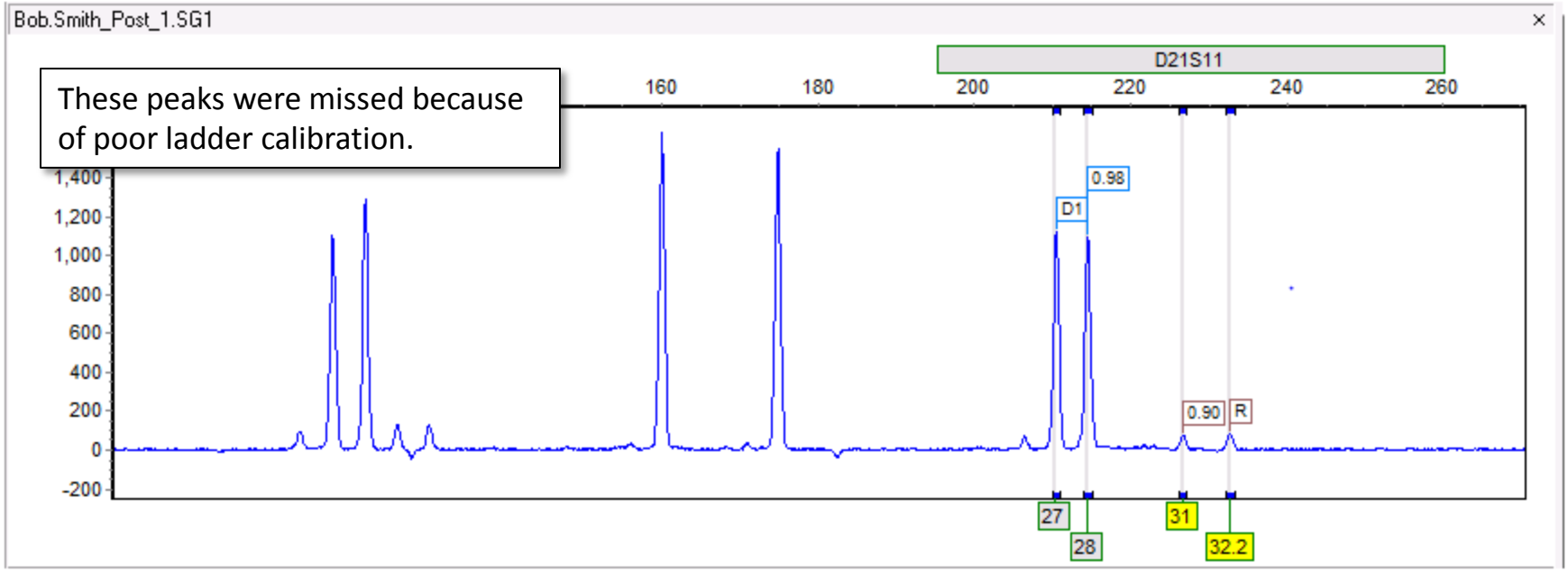
In this special case, only two alleles were called. Thus, when the program attempts to deconvolute the shared peak, it can only use the height of D1 as a reference.

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.



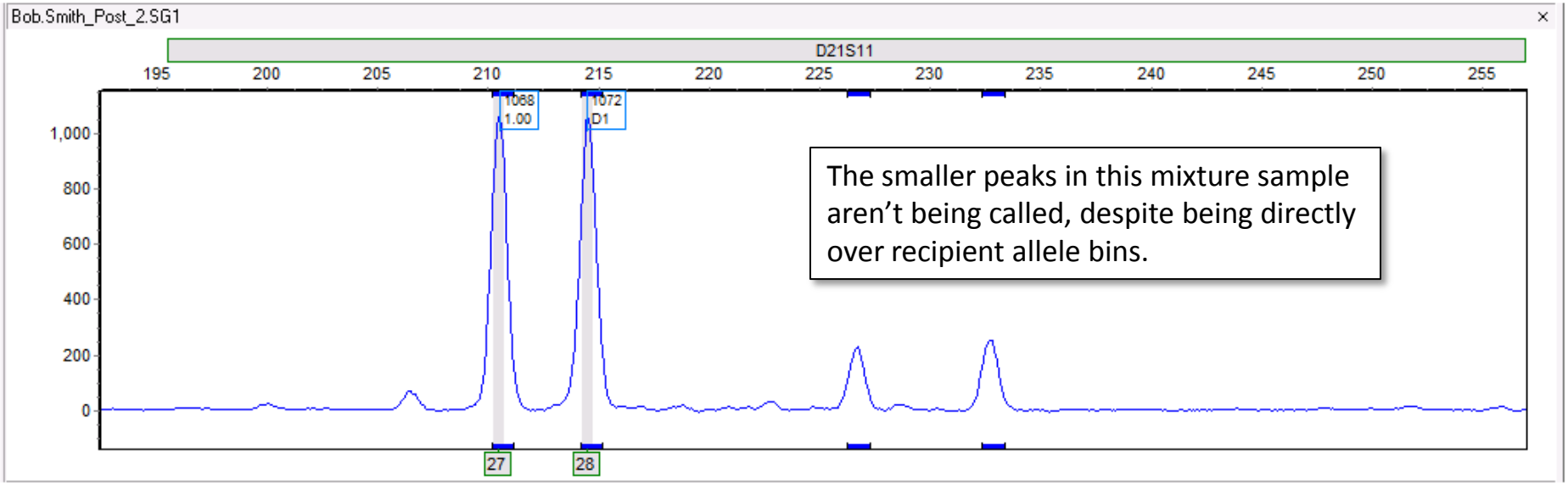
(6) Why aren't some peaks being called? - Occasionally you may notice that certain peaks, or even entire markers aren't being called by the program:



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.

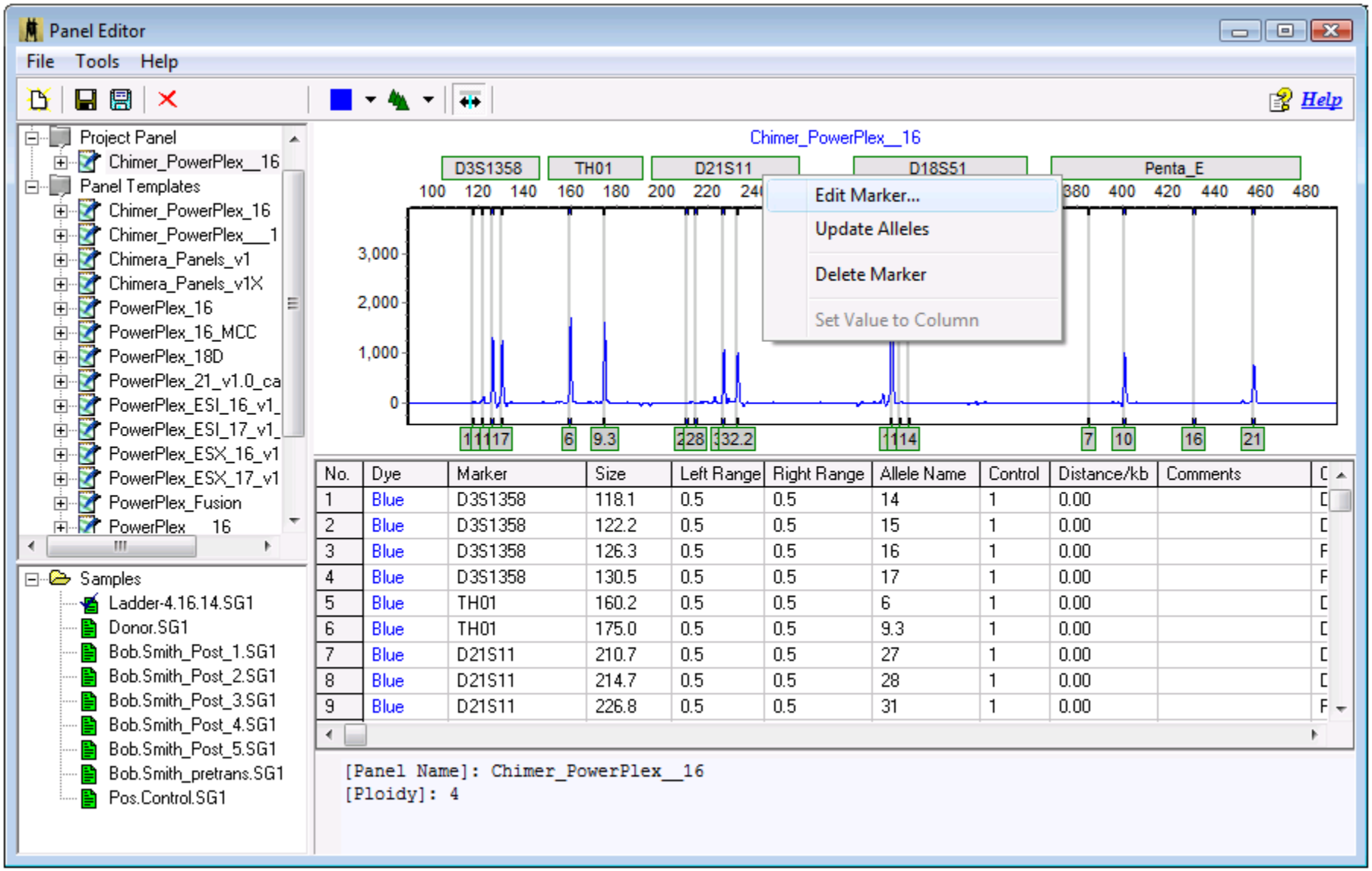
If you have confirmed that your panel *is* calibrated and peaks are still being missed, it could be that your analysis filters are set too high.

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



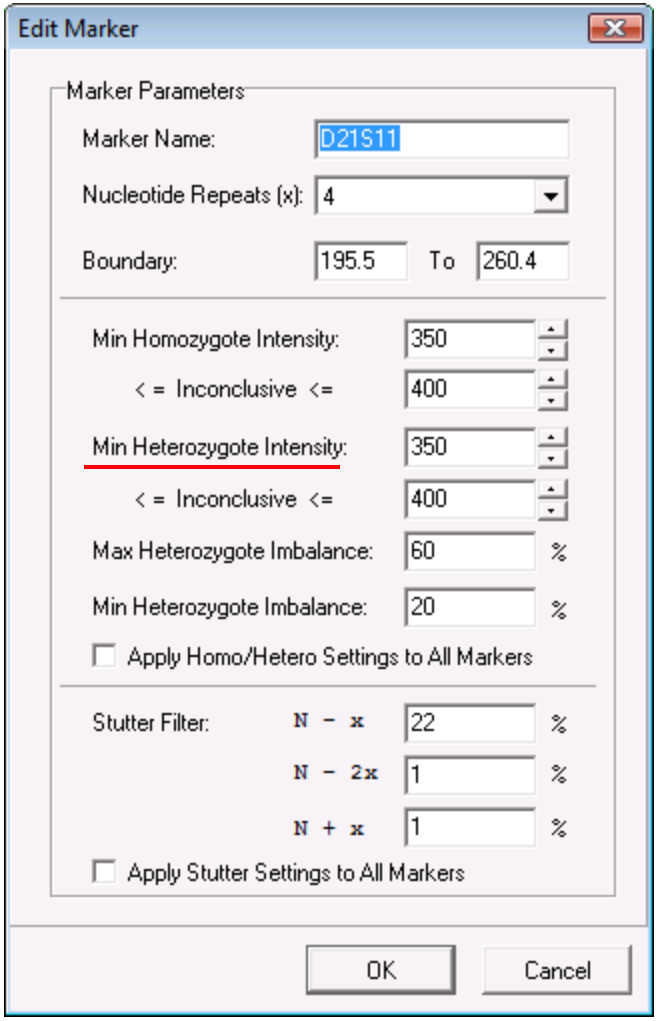
Thus, it is likely our analysis settings that are filtering them out. These peaks are within a marker – D21S11 – therefore we must check the marker specific settings for D21S11. These can be found in the panel editor (Tools > Panel Editor).

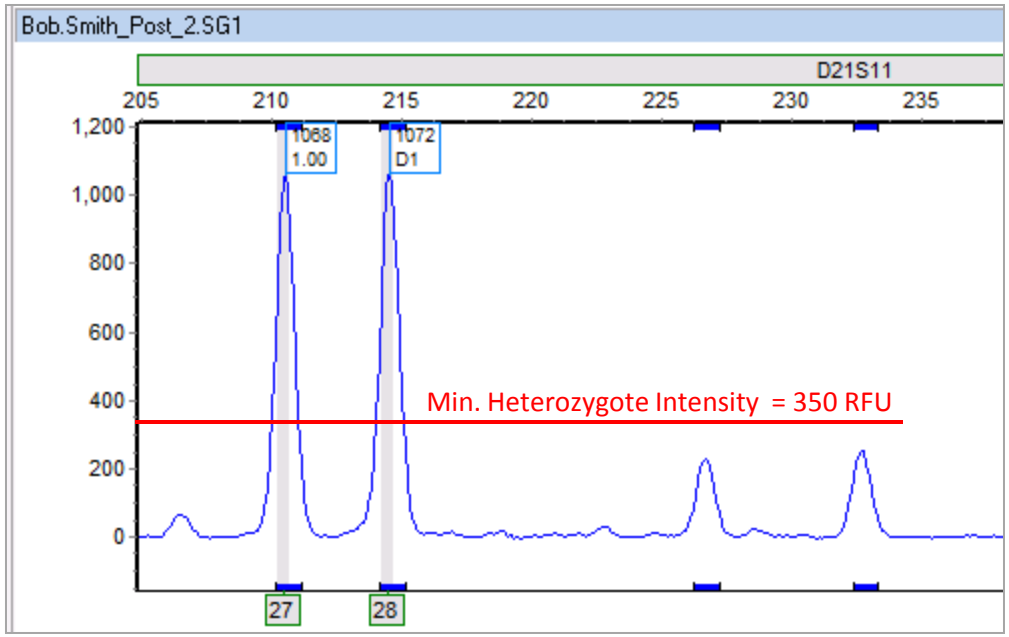
Click on your panel from the file tree to the left. Right click on a marker label (grey rectangle) and select **Edit Marker** to open the marker specific settings.



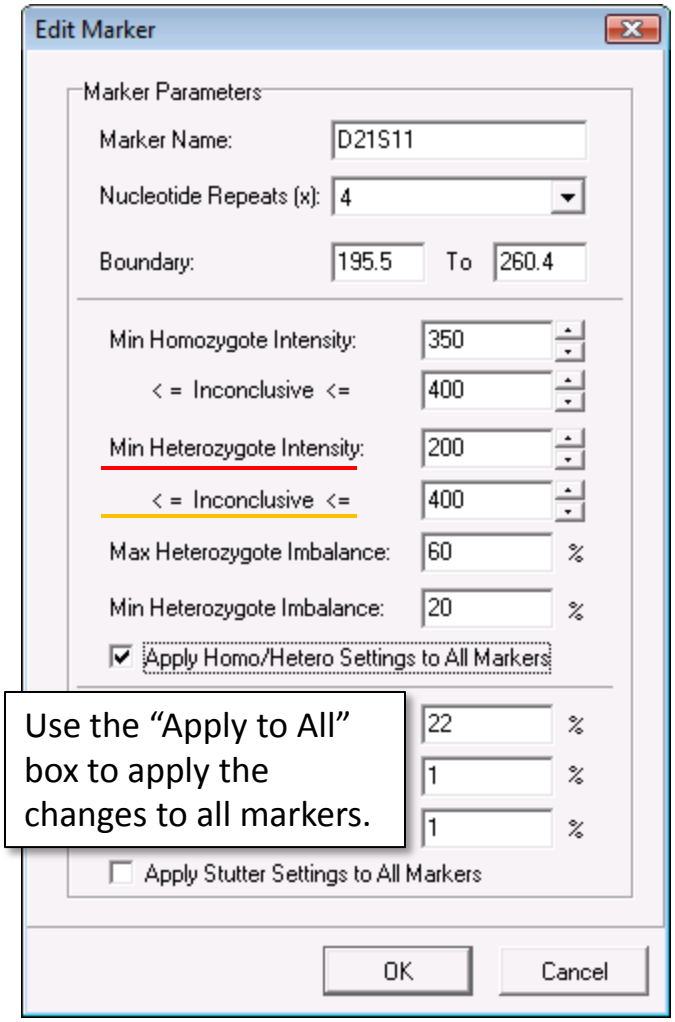
No.	Dye	Marker	Size	Left Range	Right Range	Allele Name	Control	Distance/kb	Comments
1	Blue	D3S1358	118.1	0.5	0.5	14	1	0.00	
2	Blue	D3S1358	122.2	0.5	0.5	15	1	0.00	
3	Blue	D3S1358	126.3	0.5	0.5	16	1	0.00	
4	Blue	D3S1358	130.5	0.5	0.5	17	1	0.00	
5	Blue	TH01	160.2	0.5	0.5	6	1	0.00	
6	Blue	TH01	175.0	0.5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

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.

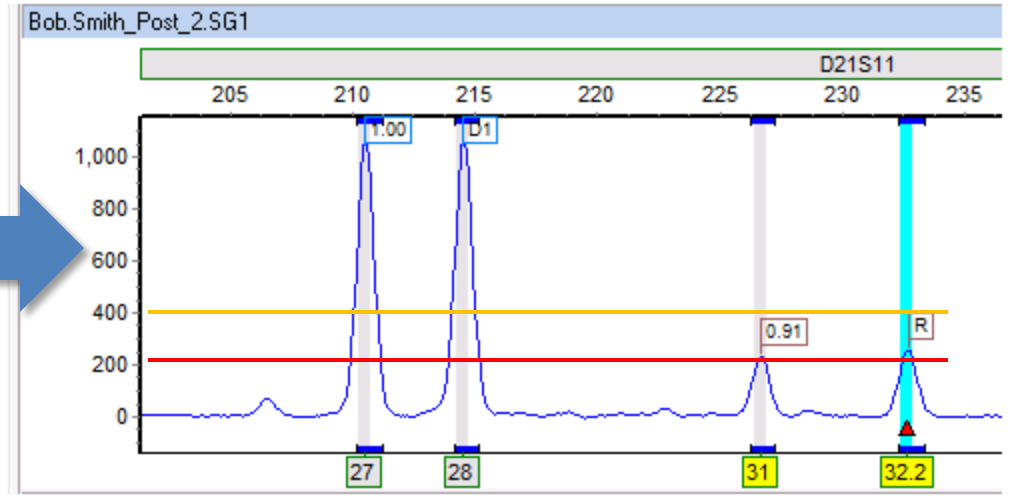




After lowering the threshold to 200 and saving the results, we can see that the peaks are now called, as excepted.



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

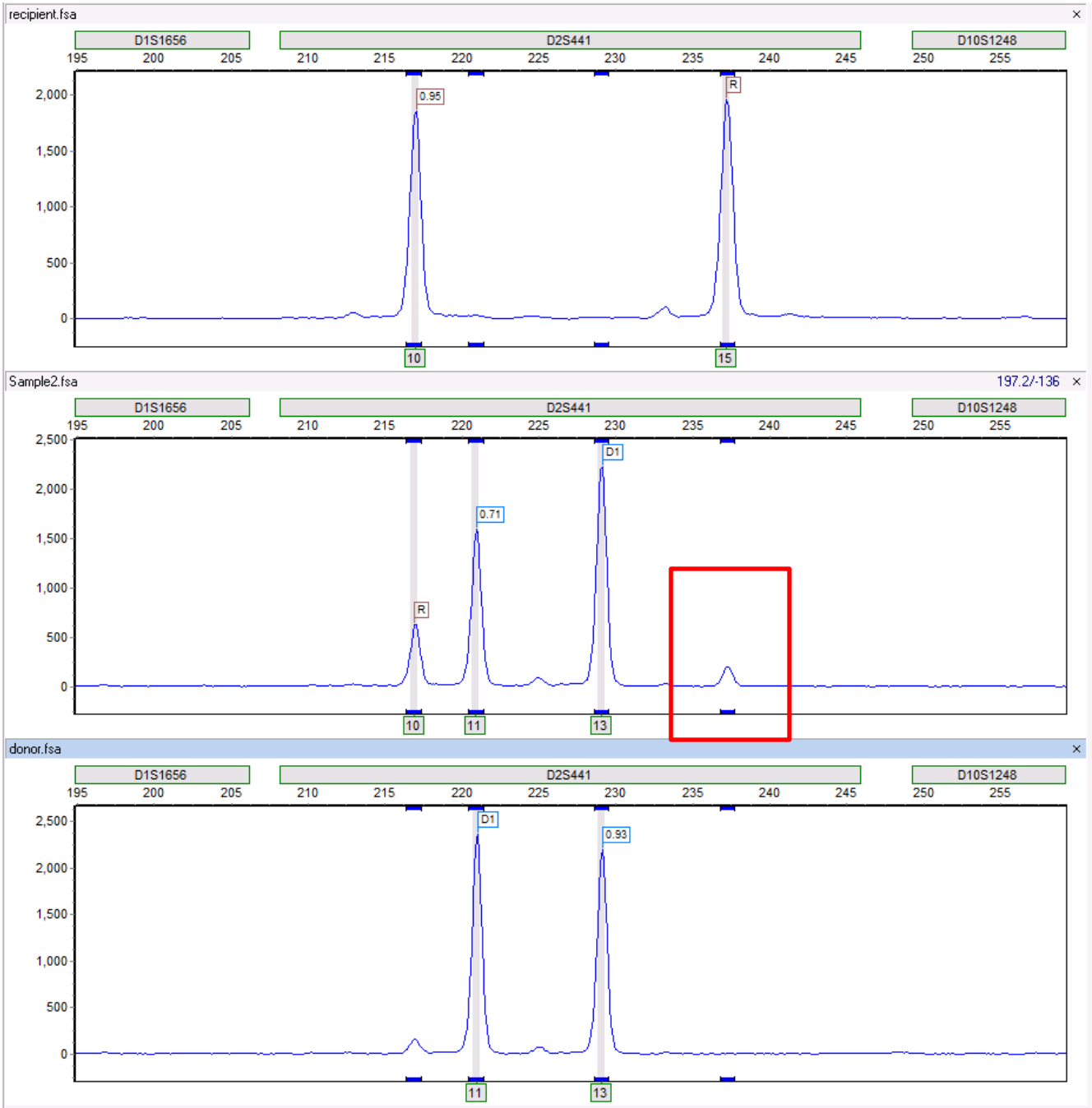


Note however that these peaks are flagged yellow. This is because despite being above the Min Heterozygote Intensity, the peaks are still below the upper range of 400 RFU, and are therefore still in the inconclusive range.

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

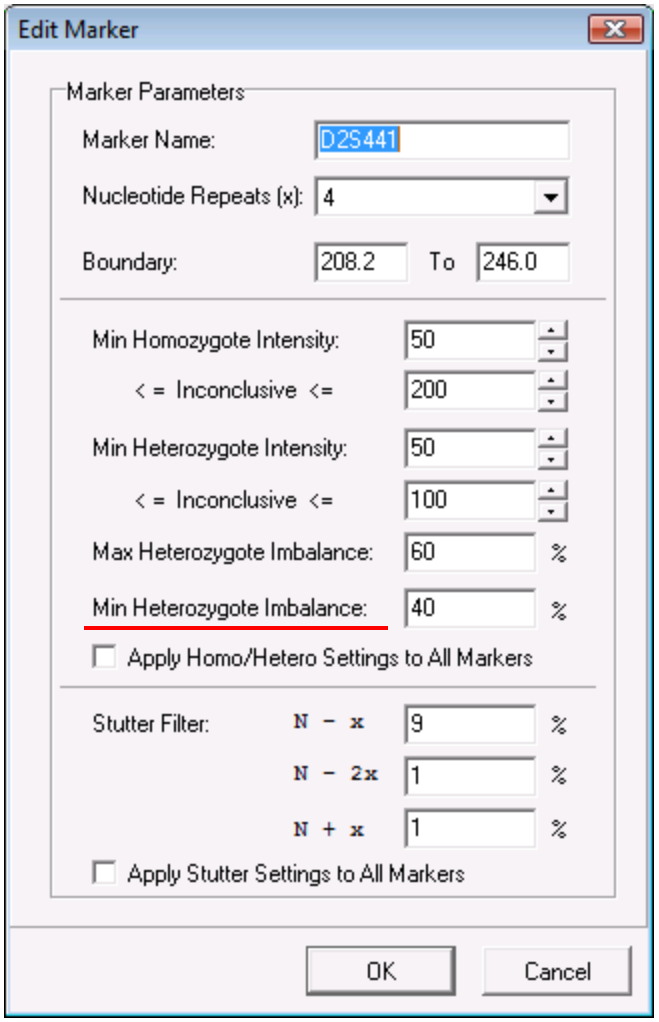
We can clearly see a recipient bin directly under the peak.

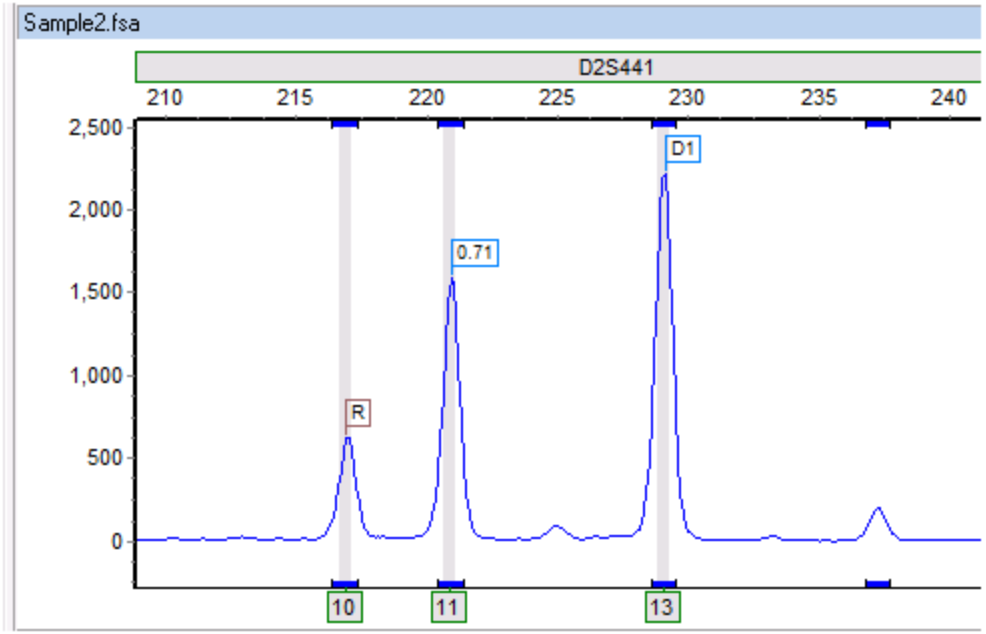
Again, we should check the marker-specific panel settings for this locus.



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.

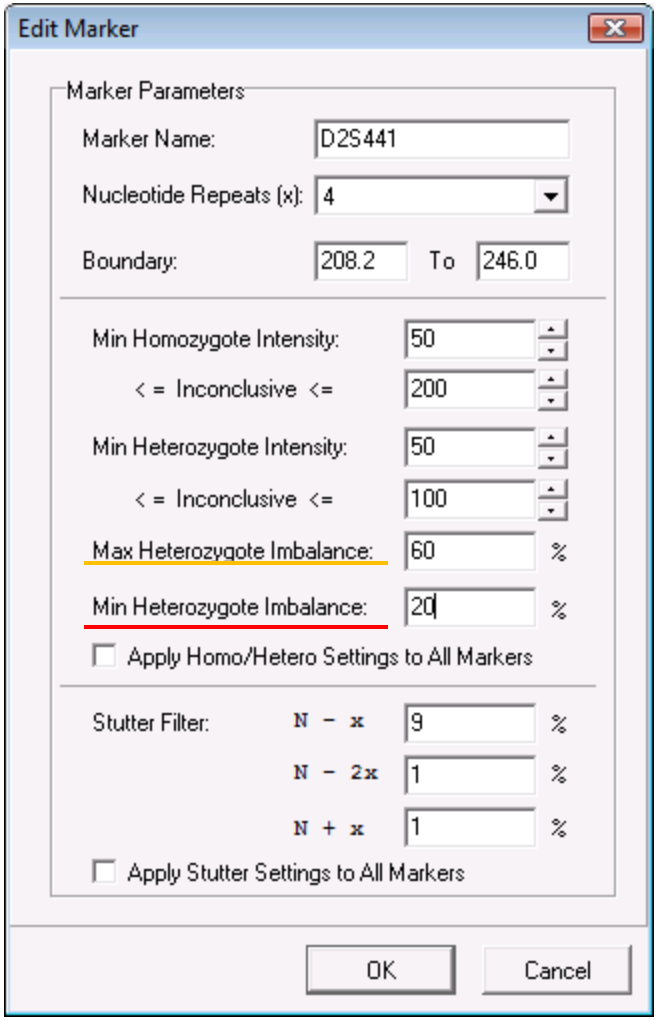
Here, it is the Min Heterozygote Imbalance filter, set currently at 40%, which is preventing this peak from being called.

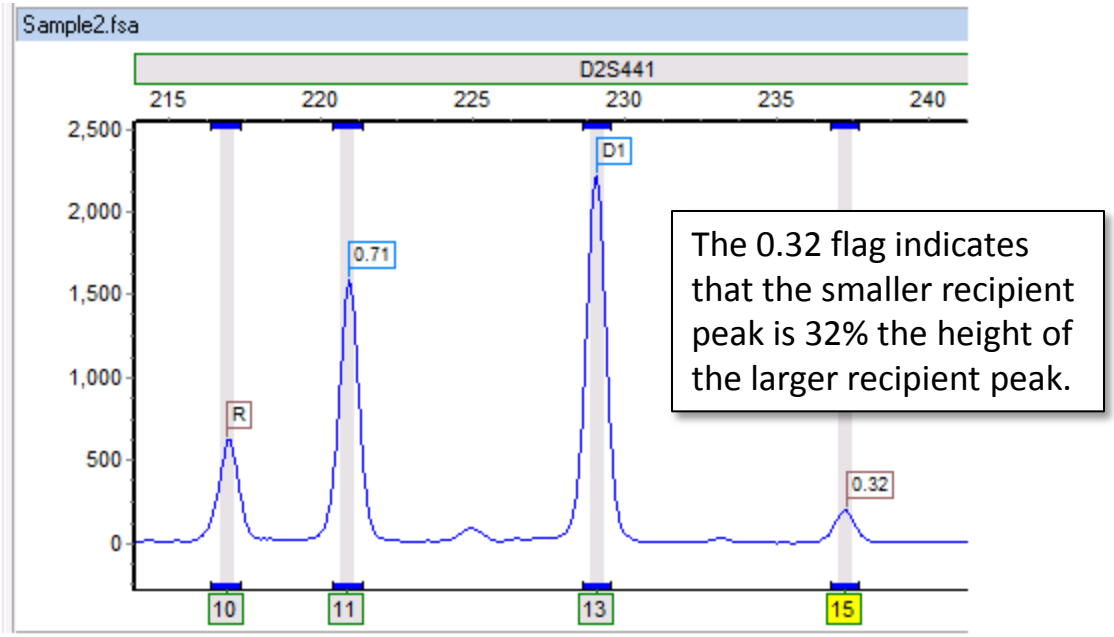




After lowering the Min Heterozygote Imbalance filter to 20%, saving the changes, and reprocessing the data, we can see that the peak is now called. Furthermore, we can see that the smaller peak is actually 32% the height of the other recipient peak, as indicated by the 0.32 flag.

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



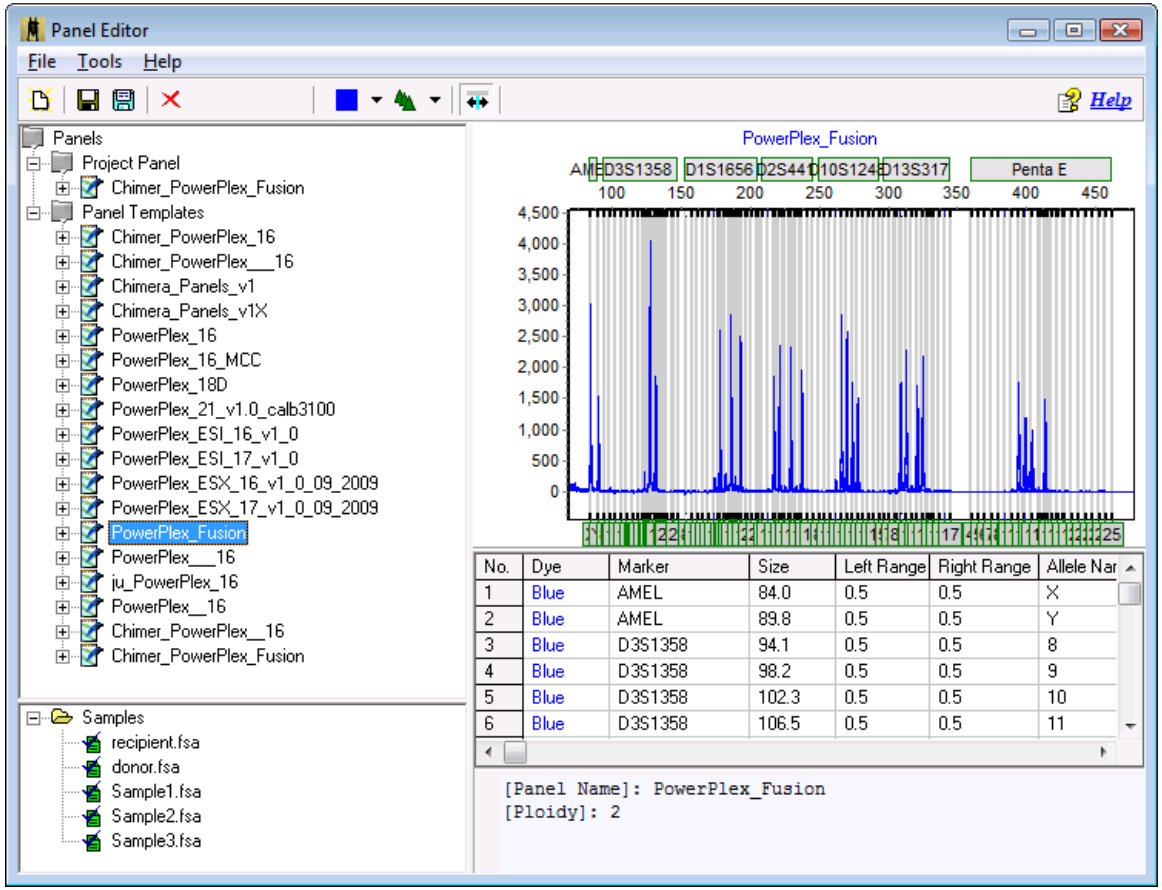


One final note about modifying panels: As mentioned in slides 2-4, The *Chimertyping panel* (prefix: Chimer) is a project specific panel derived from the original genotyping panel.

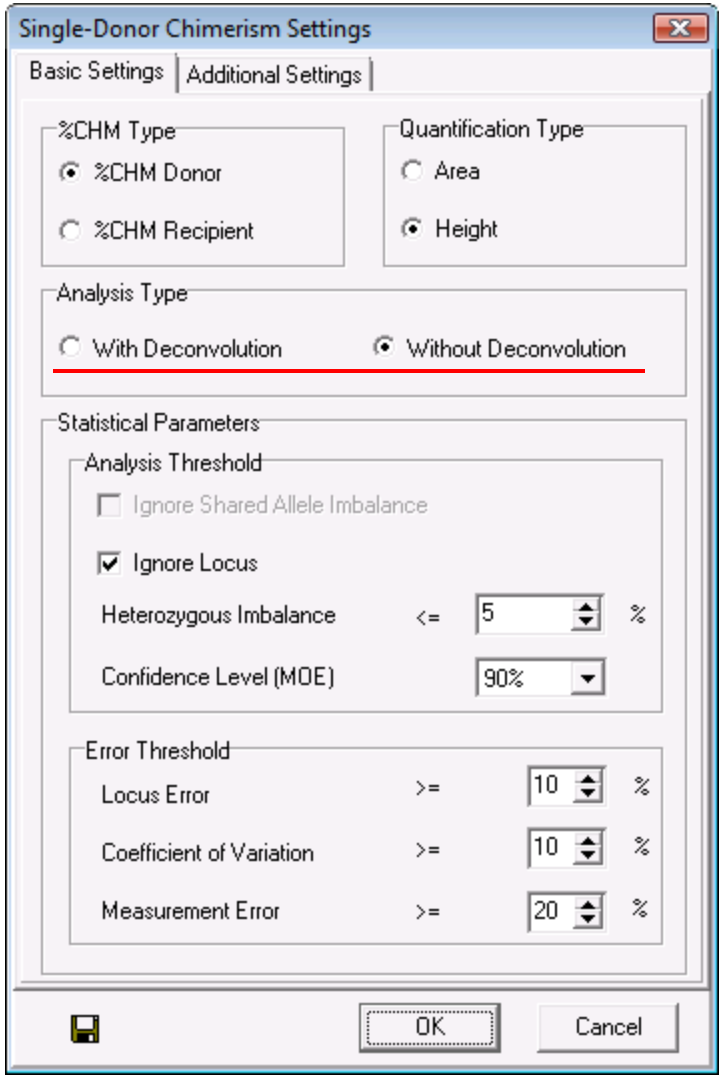
If you do choose to modify panel settings we recommend modifying the original genotyping panel, saving the changes, and then reprocessing your samples.

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.



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.