

Scoring Quail Alleles

A scoring guide for the available cross-utilized and developed Microsatellite markers.

Scoring Quail Alleles

This document has been set up to allow for easier scoring of the microsats that we have available for quail. You will see each Microsatellite listed below by the primer name (e.g. Primer 69, Primer 105) to facilitate ease of use of this guide. Locus name and citation are also given for each primer.

In putting this together, I have included some of the things that I find most confusing. Hopefully, this will make clear how to deal with these confusing situations. However, I cannot cover everything. If you come across something that is particularly confusing, please note it in your lab notebook and/or send me a jpeg of the “thing” of interest so that I may include it for future reference.

Finally, don't blame me if something is wrong in these pages. There are always problems with scoring, and you will notice that it seems subjective at times (which it can be).

Good luck, long-live the quail-bird. Peace.

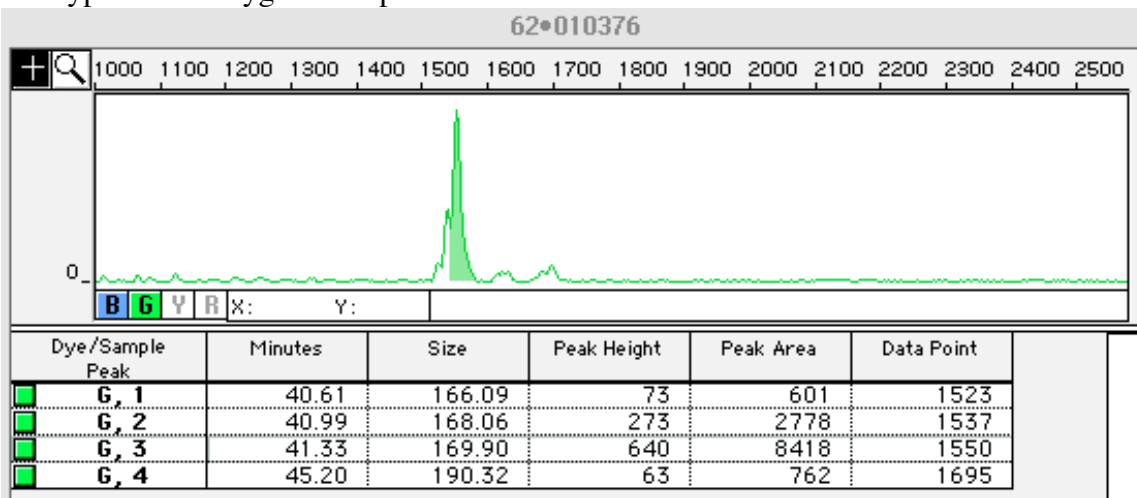
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Note: zooming and adjusting the vertical scale have two very different effects. These are in fact 2 different operations and both can be helpful (or a pain in the butt).

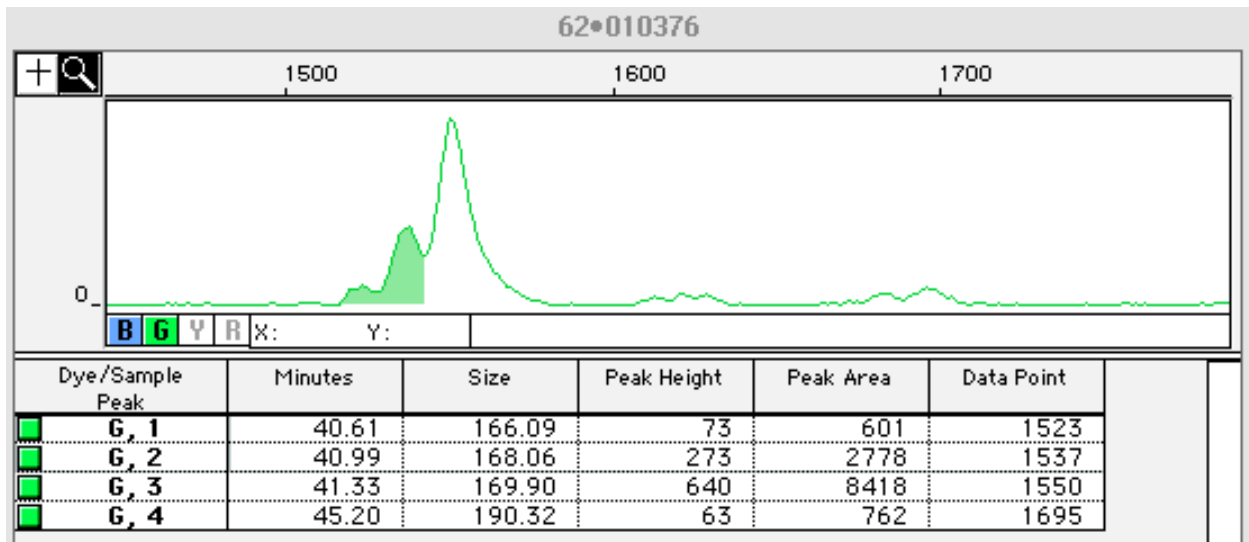
Primer 17; Locus LEI 70 Piertney and Dallas (1997)

Homozygotes

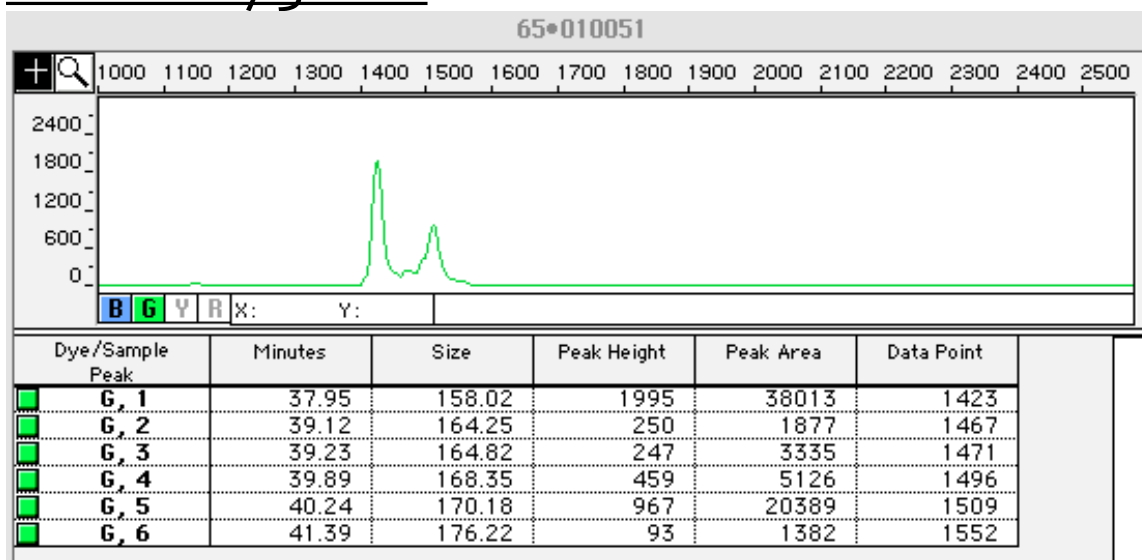
The typical homozygote will present itself as such:



Note in this example that the actual peak is highlighted (169.90/169.90 bp). Homozygotes tend to exhibit a bit more stutter than heterozygotes. Stutter, in this case, is indicated by the following highlighted stutter peaks. These peaks are the -4bp and -2bp stutter peaks (they are at 166.09 and 168.06 bps, respectively). These peaks help you identify the main peak as they “point” to it with increasing peaks heights. The following image is the same as the above except that it has been zoomed in upon:

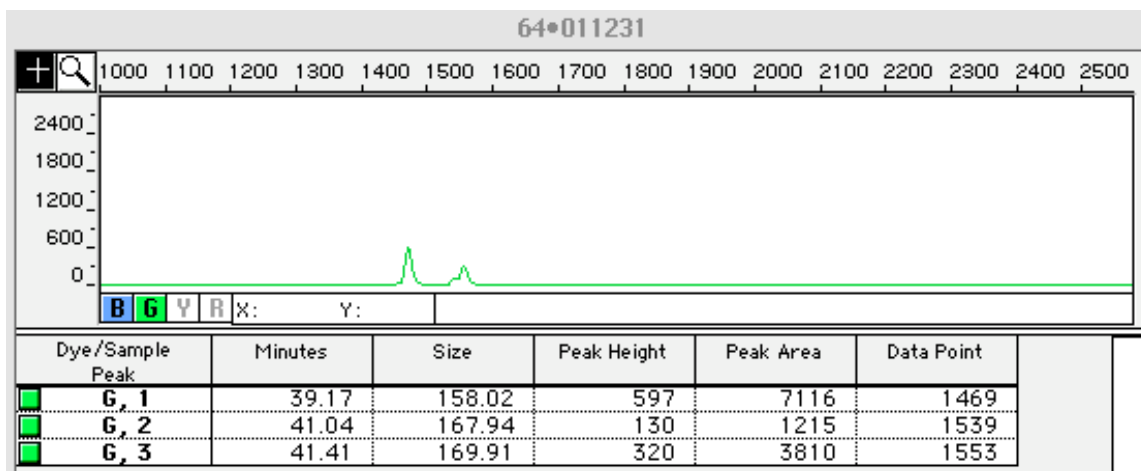


Heterozygotes

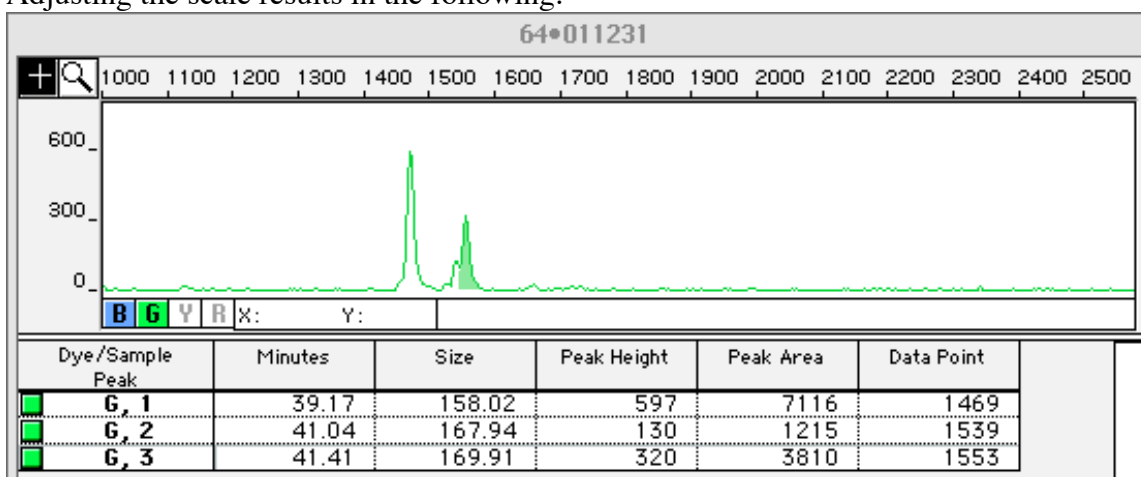


This image presents the typical looking heterozygote at P17. The peaks for this particular example fall at 158.02/170.18. When binning these alleles, they would fall out in the 158 and 170 bins, respectively (See Appendix A for binned allele sizes).

More commonly, you will see results such as the following:

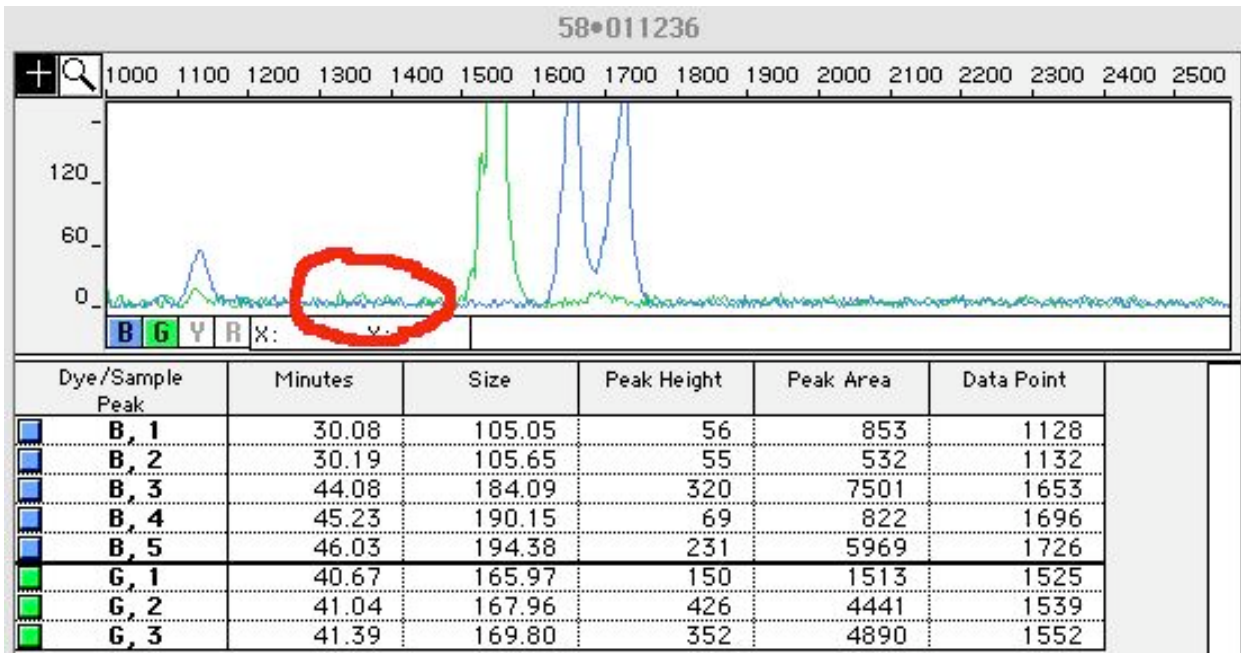


However, this is nothing to worry about too much as you can zoom in on the peaks to make them out a little better (->View->Vertical Scale-> the adjust the vertical scale to your needs). Adjusting the scale results in the following:

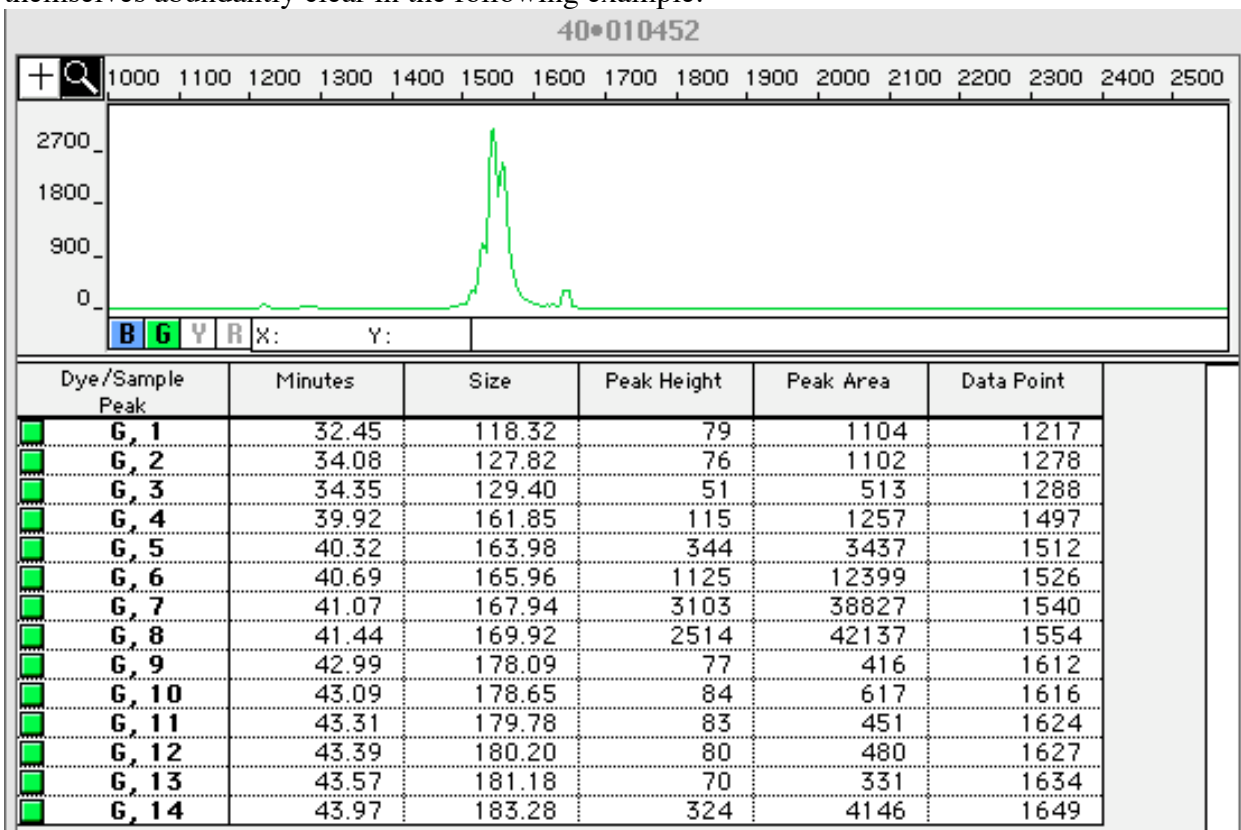


Now, you can more clearly see the peaks to score them. In this case the peaks are 158.02/169.91, again falling into the 158/170 bins.

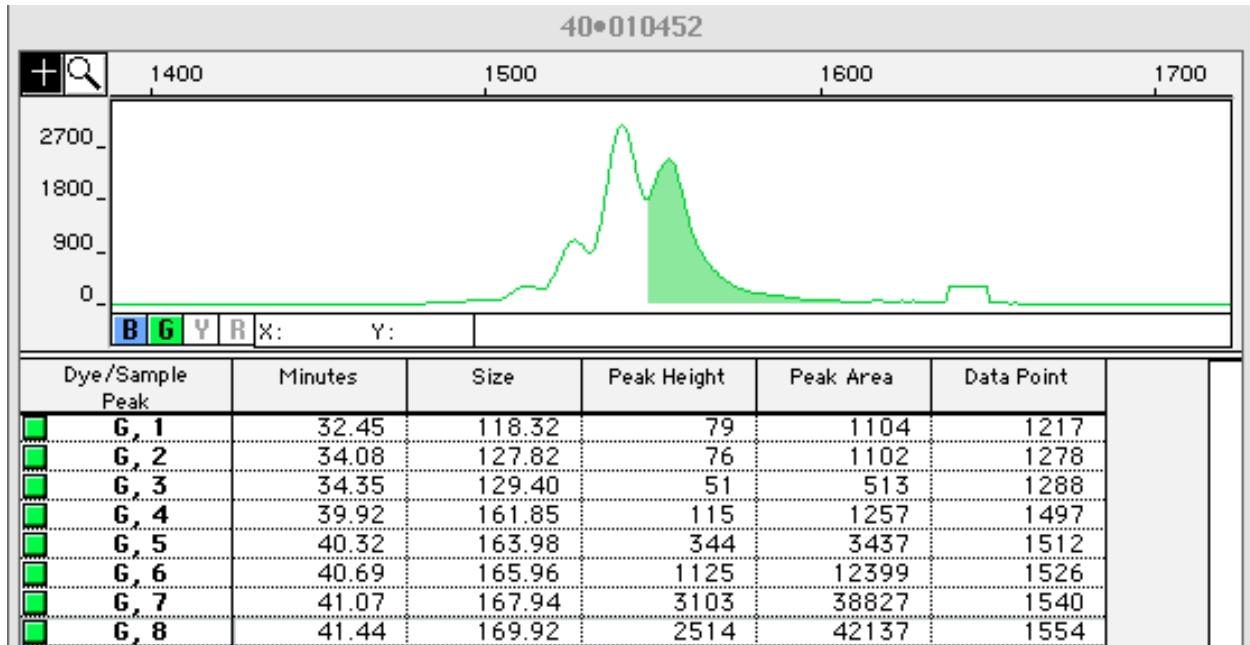
However, the scale can be adjusted to only a certain degree. If you set the vertical scale too low (below about 100), you will begin to notice noise from the particular gels that we use. If the peaks are within the “noisy” section of the baseline (e.g. inside the red circle surrounding the squiggly lines), it is better to go ahead and run the sample again.



Next, we have an example of a harder heterozygote. First, notice that the peaks do not make themselves abundantly clear in the following example:



Part of the problem lies in the fact that the peaks for each allele are very close to one another falling at 2 bps from each other (167.94/169.92). However, zooming in on the result allows us a little clearer view:

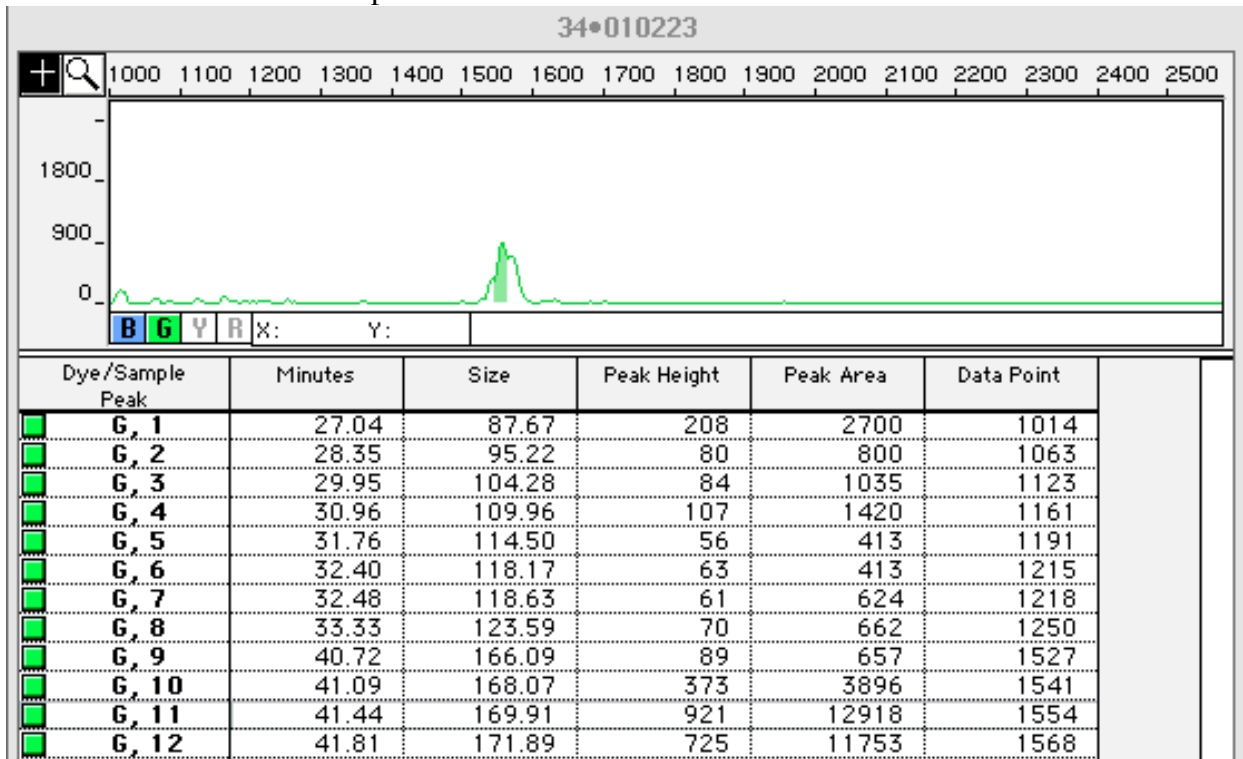


After zooming in, these peaks are much more clear. Notice that the trailing peak (the one on the right) is a bit smaller than the lead peak. This occurs because the leading peak typically has “its own” fluorescence and “absorbs” the -2bp stutter product for the trailing peak, therefore making it fluoresce more and appear “taller”. This is a fact of life. Also notice how visible the -4bp and -2bp stutter peaks are for the leading peak. So pretty.

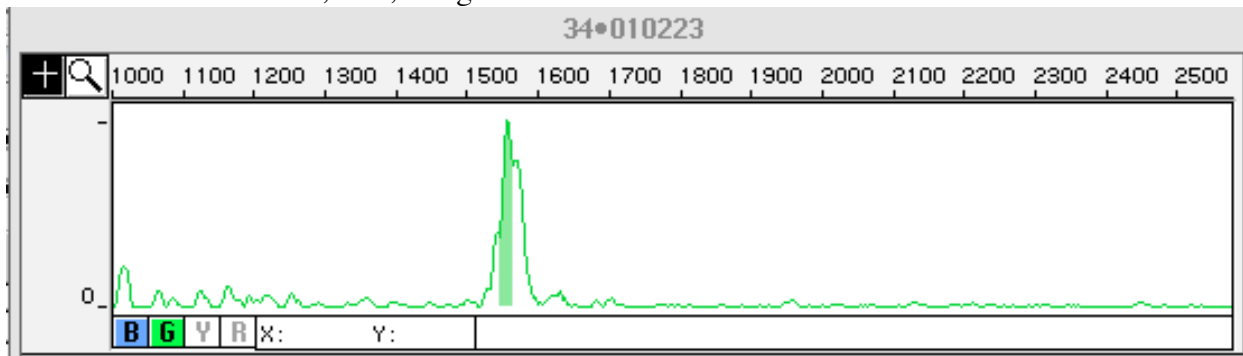
Usually, alleles that fall 2bp from each other will require you too zoom in to figure out which peak is which.

In most cases remember that: (1) trailing stutter is a relatively rare event, and (2) if the trailing peak exhibits decent amplitude (relative to the leading peak) and is of the size of a typical allele, then it probably really is one.

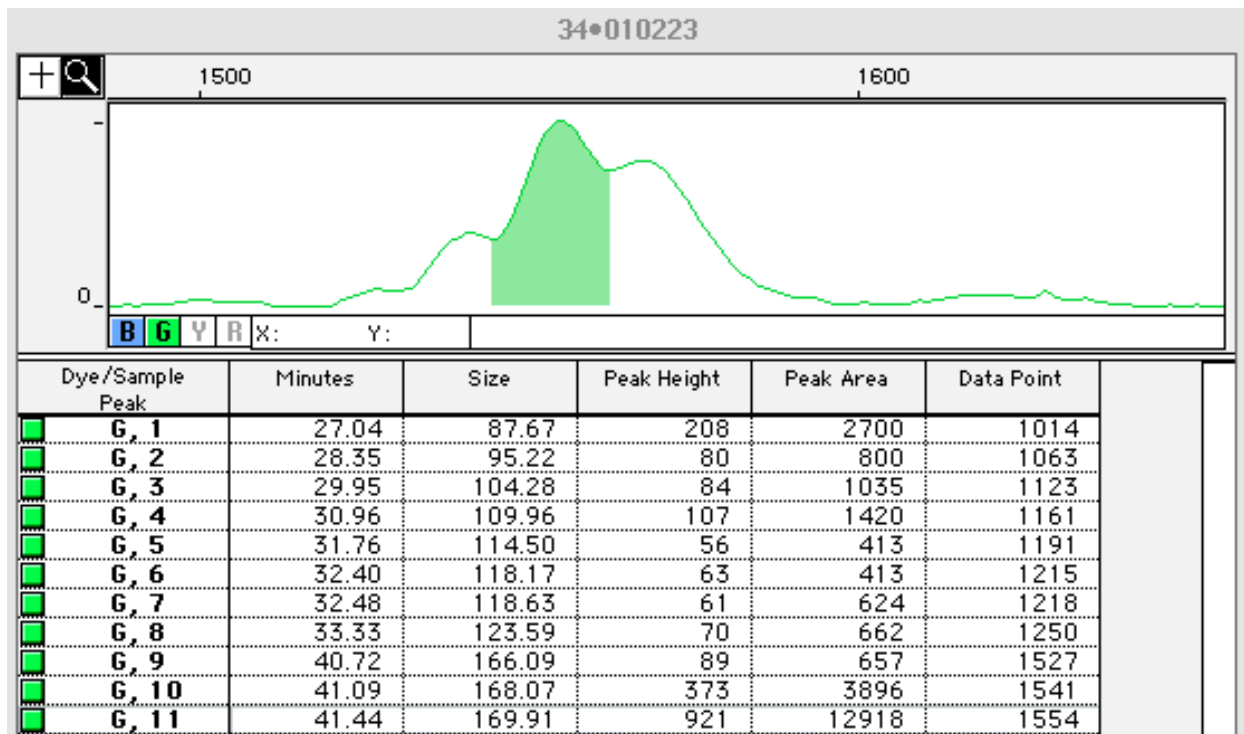
Here is an even harder example:



Basically, we cannot really tell what is going on with this peak. It looks pretty nasty even with the scale set at 3000. So, first, bring the scale down a bit:



Even after bringing the scale down to 1000, we still cannot tell much. So, the best option is to go ahead and zoom in on the peak to see what is going on and if we have to do the run for this sample again:



Now we are getting somewhere. I have just zoomed in over the peak to see what is going on. In this case, we have a heterozygote with peaks at 168.07 and 169.91. It is just not as pretty as what we have seen in the past few examples. Typically, when a peak exhibits this sort of triangular structure (even more apparent in examples for primer 105), the leading peak and the trailing peak both represent alleles. Again, see the “rules” given above. Sometimes the amplitude of the trailing peak is not very high making things even more difficult. However, keep an eye out for the triangle look. It is trying to tell you something.