

## Memo regarding use of Masking Stutter in Lab Retriever

September 1, 2016

This memo updates instructions for how to prepare a data file to accommodate stutter peaks that may potentially mask peaks from minor contributors. We had previously instructed that only stutter peaks that would potentially mask an allele in the profile of a proposed contributor should be added to the masking stutter profile. It has been clarified that this approach is incomplete as it only applies to the numerator. The complete approach requires that it also be applied to the denominator. In other words, all peaks in stutter positions above the empirical analytical threshold and below the stutter filter should be included both in the detected profile and in the masking stutter profile.

The key idea to consider is that a peak present in a stutter position could have been generated in either of two ways: First, it is possible that the peak is entirely due to stutter. It is also possible, however, that an allele from a minor contributor is present in addition to the stutter. Importantly, if a contributor carries an allele at one of these stutter positions, we do not wish to require drop-out of that allele to have occurred when computing the probability of the evidence. Thus, by placing the stutter peak in the “Assumed” column, drop-out will not be invoked. Additionally, if the contributor does not carry the allele, drop-in will not be invoked either, because stutter is a reasonable explanation as the sole cause of the peak. Importantly, this process needs to be applied for ALL possible potential contributors being evaluated by the program, both the proposed contributor (numerator) as well as all other potential contributors (denominator). Specifically, it is also necessary when summing over the probability of the evidence considering unknown contributors (denominator). Lab Retriever can accommodate uncertainty in any peak by designating it as potentially masking. Functionally, this is accomplished by entering it in the “Assumed” column in the program. The situation for potentially masking stutter is analogous to the situation for considering assumed donors. For an assumed donor, it is possible that the peaks originate entirely from assumed donors (i.e. there is no contribution from an additional minor contributor(s)) or that they contain contributions from both the assumed donor and a minor contributor. Peaks in stutter positions that could potentially mask a real contribution from a minor donor work in exactly the same way.

In general, we do not anticipate that this change in protocol will make a substantive difference in most profiles. But in very compromised profiles, the possibility exists that it could make a difference in the inference from the LR, so you might consider re-calculating those types of profiles.

For peaks in stutter positions, we suggest the following protocol for use in Lab Retriever: (see Appendix B)

1. Assess if the peak(s) of interest fall above the (EAT) and below the stutter filter typically employed.
  - a. If the peak is below the EAT then it should not be considered as potentially masking a minor contributor.
  - b. If the peak is above the stutter filter, and is called with stutter filters on, it should be typically be considered as containing at least some contribution from a real allele. If this allele does not appear in any assumed profile, then it will automatically appear in the “unattributed” column. You do not need to do anything else with this peak.
    - i. If concern exists that such a peak represents “high” stutter, then the sample should be re-amplified to attempt to resolve this issue.

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2. Assess if a minor component in the profile is present in a range such that some risk exists for masking of the minor profile by peaks in the stutter position of the major profile. In other words, determine if the profile from the minor contributor(s) is roughly less than or equal to the heights of peak in the stutter positions.

- a. If so, reanalyze the evidence profile with the stutter filter off, and export as a separate .csv file.
- b. Determine the alleles that have been called only when the stutter filter was off and create a separate “masking stutter” profile. It is often convenient to do this in the same .csv.
- c. DO NOT include the masking stutter peaks in the average RFU $\pi$ p calculation. The average RFUs should be determined from the evidence profile run with the stutter filter on.
- d. DO NOT include the masking stutter peaks in a determination of number of contributors. This should be determined from the evidence profile run with the stutter filter on.

3. In Lab Retriever:

- a. In the “detected” column, select the evidence profile run with the stutter filter off, in other words, including peaks  $> AT < St$ <sup>1</sup>.
- b. In the assumed column, select any assumed contributors.
- c. Additionally (by clicking on the green +) select the masking stutter profile you have prepared that includes all peaks  $> AT < St$ . These should match the extra peaks included in the evidence profile.
- d. Run the program as you normally would.

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<sup>1</sup> In this instance “St” refers to stutter, not to be confused with ST which is often used to refer to “stochastic threshold”

## **Memo regarding use of Masking Stutter in Lab Retriever**

### **Appendix A**

SWGAM 2010 Interpretation Guidelines excerpt:

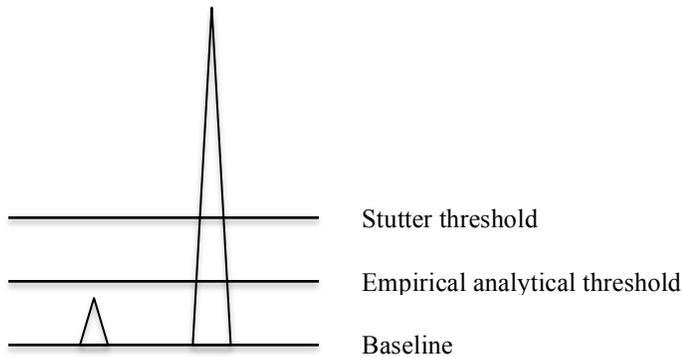
#### 3.5.8. Interpretation of Potential Stutter Peaks in a Mixed Sample

3.5.8.1. For mixtures in which minor contributors are determined to be present, a peak in stutter position (generally  $n-4$ ) may be determined to be 1) a stutter peak, 2) an allelic peak, or 3) indistinguishable as being either an allelic or stutter peak. This determination is based principally on the height of the peak in the stutter position and its relationship to the stutter percentage expectations established by the laboratory.

3.5.8.2. Generally, when the height of a peak in the stutter position exceeds the laboratory's stutter expectation for a given locus, that peak is consistent with being of allelic origin and should be designated as an allele.

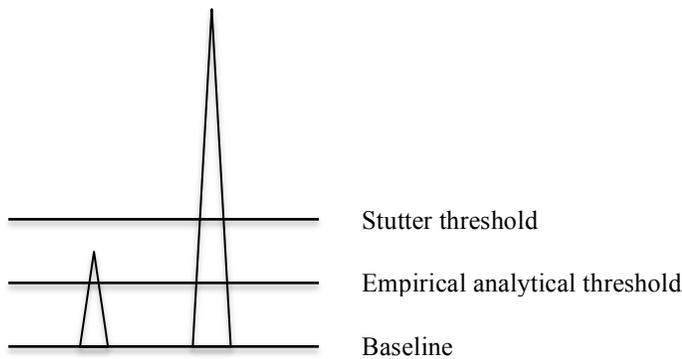
3.5.8.3. If a peak is at or below this expectation, it is generally designated as a stutter peak. However, it should also be considered as a possible allelic peak, particularly if the peak height of the potential stutter peak(s) is consistent with (or greater than) the heights observed for any allelic peaks that are conclusively attributed (i.e., peaks in non-stutter positions) to the minor contributor(s).

## Appendix B Masking Stutter



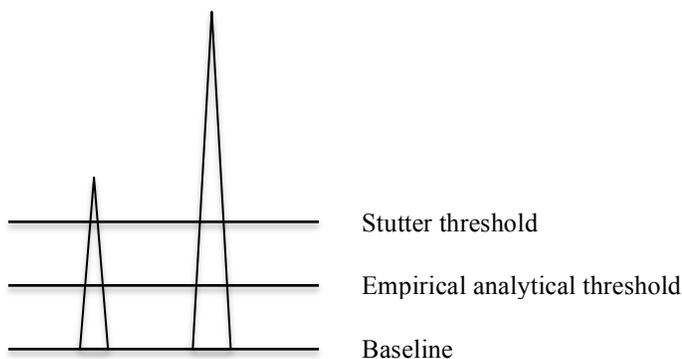
A.

The peak in the stutter position is above the EAT, but below the stutter threshold. It is not called with either stutter filter on or stutter filters off. It is not considered as having an allelic component. Nothing further need be done



B.

The peak in the stutter position is above the EAT; it is not called with the stutter filter on, but is called with the stutter filter off. It is considered as possibly having an allelic component. If a minor profile of interest is in the same RFU range as the stutter peak, it should be designated as an “uncertain” peak. It should be included both as an allele in the “detected profile,” and in a separate masking stutter file that would be placed in an “assumed” column



C.

The peak in the stutter position is above the EAT and above the stutter threshold; it is called with the stutter filter off. This would be considered an allelic peak and treated as any other allelic peak. Nothing special is done regarding masking unless it is otherwise present in the profile of an assumed contributor