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### **B97 Questioning the Unquestioned — Rethinking and Rejecting Traditional Mixture Concepts and Assumptions**

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After attending this presentation, attendees will appreciate that “contributor,” “number of contributors,” “allele present,” “stochastic threshold,” and other traditional mixture concepts are vague and not well-defined and, therefore, are not a solid foundation for mixture analysis.

This presentation will impact the forensic science community by highlighting pitfalls that are common to many or most mixture methods and which sometimes invalidate the results. Some remedies will be presented.

“Continuous” DNA mixture analysis is a big and welcome change from binary and other earlier methods, and with good reason it might be called a revolution or paradigm shift. A “mixture model” means a simplified description of DNA mixtures. A binary mixture model explicitly treats the mixture data as having just one dimension — for Short Tandem Repeat (STR) loci, it is the allelic repeat numbers. Phenomena such as dropout and stutter fit into the model awkwardly at best as ad hoc add-ons. A continuous mixture model acknowledges a full-fledged second dimension, namely the signal intensity or Relative Fluorescence Units (RFU) of each amplified allele. The extra (“continuous”) dimension is modeled as a template amount randomly modified by the combination of amplification stochastic variation and injection sampling variation. Two dimensions instead of one is in an obvious sense a complication, but it also leads to simplification because it lets analysts model both dropout and most drop-in, as well as variations in peak height and stutter ratios in a coherent fashion simply as aspects of the same random variation rather than as multiple black boxes.

Like most changes, the continuous approach has arrived gradually through various intermediate stages such as “semi-continuous” methods and ad hoc ways to cater to possible dropout. Consequently, there was no dramatic moment at which it became obvious to re-evaluate the original assumptions. Now is a good time to question some of the unquestioned principles and concepts about mixture analysis, and doing so unearths some surprises.

“Exclusion,” an ill-applied term, has been known as a charlatan for awhile. A suspect is non-excluded by one meaning of the word, then an exclusion probability is calculated using a different meaning. “Number of contributors” isn’t a definable number in any practical sense, yet traditionally some mixture protocols wrongly instruct the analyst to begin by determining it. Furthermore, it need not be the same number for prosecution and defense, contrary to typical mixture protocols and software. Realistic examples will illustrate and explain substitute concepts, such as “degree of contribution,” that make more sense.

Thresholds rarely have a natural role in the real world; “stochastic threshold” is a particularly unfortunate example as it was invented specifically for the analysis of *simple* stains and was borrowed even into binary mixture analysis only by mistake. In a continuous mixture model, it is natural to view all peak height ratios as more/less probable rather than yes/no possible. Hence, correcting the early mistake and banishing the “stochastic threshold” concept is a natural, though not inevitable, consequence of adopting a continuous mixture model.

In several ways, the continuous approach to mixture modeling tends to eliminate complication and encourages clear and explicit thinking about the analysis process; however, there are various ways to devise a continuous model and retaining some of the traditional misconceptions is possible. Critical analysis should begin with writing down the model. If that is done clearly, then assumptions can be better recognized and it will be easier to see misconceptions and know where to be wary.

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#### **Mixture, Pitfall, Continuous**