## B72 Developing a Dynamic Model of the DNA Laboratory Process to Characterize the Sources of Uncertainty in DNA Signal: Applications to Forensic DNA Education, Training, and Validation

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After attending this presentation, attendees will understand the ways in which the DNA laboratory process can be dynamically modeled using a systems-thinking approach. Attendees will also be made aware of how the model can be modified by the user in order to test the impacts of various systems and their errors.

This presentation will impact the forensic science community by demonstrating that a dynamic model can be utilized to train students and practitioners in the nuances associated with the forensic laboratory process. This presentation will demonstrate that modifying the Polymerase Chain Reaction (PCR) efficiency and the error in PCR can substantially impact the peak heights and peak height ratios. Further, it will be demonstrated that dilution of a concentrated stock solution during validation results in additional peak height variability and may need to be considered a process which introduces additional stochastic effects not typically introduced during evidence processing.

The model mimics the generation of a random 16-locus profile obtained by following these steps: extraction→quantification→dilution→amplification→electrophoresis. The input values that can be modified by the user are shown in the Table below.

| | Unit | Range | Description |
|---|---|---|---|
| Nominal Target based on qPCR | ng | 0-10,000 | The target value obtained in the DNA extract as per qPCR. |
| % RSD in qPCR | % | 0-100 | Relative standard deviation associated with qPCR results. |
| Buffer 1 switch | µL | 0-1,000 | Volume of buffer utilized when the DNA extract is diluted. |
| Buffer Pipette 1 Stdev | µL | 0-10 | Standard deviation of the pipette volume during the first dilution. |
| Buffer 2 switch | µL | 0-1,000 | Volume of buffer utilized when the DNA is serially diluted twice. |
| Buffer Pipette 2 Stdev | µL | 0-10 | Standard deviation of the pipette volume utilized during the second serial dilution. |
| Buffer 3 switch | µL | 0-1,000 | Volume of buffer utilized when the DNA extract is serially diluted three times. |
| Buffer Pipette 3 Stdev | µL | 0-10 | Standard deviation of the pipette volume utilized during the third serial dilution. |
| Number of PCR Cycles | | 28-29 | The number of PCR cycles. |
| Deviation in PCR Efficiency | | 0-0.2 | The PCR efficiency is different at each cycle and is normally distributed about 1. It is the standard deviation of the PCR efficiency. It is set such that the efficiency decreases from 1 as the number of amplicons increase, as per 1-e^(-4.5x10-10*[DNA]c-1), where c is the cycle number. |
| Stutter Ratio Information | | 0-1 | Mean and standard deviation of the stutter ratios for each locus. |
| Cumulative Population Statistics | | 0-1 | Cumulative allele frequencies for 15 STR loci. |
| CE Sensitivity | RFU | | The increase in average RFU signal with amplicon. It can be approximated by determining the slope of a the linear portion of a graph which plots the average peak signal ($\overline{RFURFU}$) for heterozygous loci versus 2Ctot[DNA]0, where Ctot is the total number of cycles (i.e., 28 or 29) and [DNA]0 is the template mass. |
| RSD of CE | | 0-1 | Relative standard deviation of the capillary electrophoresis set-up and injection. |

The dynamic model treats each copy of DNA as an independent molecule (i.e., no molecular interaction) without systematic preference for one allele over another. The number of copies varies as per the Poisson distribution for each aliquot. For example, if there is an average of 16 copies of DNA in an aliquot, the aliquot that is actually taken may contain 15 copies of allele 1 and 16 of allele 2. The PCR efficiency is different for each allele amplified and varies at each cycle. Thus, the PCR efficiency of cycle 27 may be 0.976 for one allele and 0.983 for another allele. During cycle 28 the PCR efficiency may be 0.979 and 0.980 for allele 1 and 2, respectively. Stutter ratios and CE sensitivity are treated similarly.

The output of the dynamic models gives the user information on: (1) the actual number of alleles/molecules added to the PCR; (2) the known genotypes; and, (3) the electropherogram.

Testing shows that decreasing the deviation in PCR efficiency decreases the differences in peak heights between alleles. Further, it has been determined that peak height differences increase when samples are serially diluted.

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**DNA Laboratory Validation, System Thinking, Dynamic Model DNA Laboratory**