BCI Validation Study: YFiler Plus Kit Validation

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Introduction

- Ohio Attorney General's Bureau of Criminal Investigation's (Ohio BCI) Forensic DNA/Biology Unit uses serological testing and DNA typing to provide forensic application in the court system.
- Federal Bureau of Investigation' Quality Assurance Standards (FBI-QAS) must be followed by DNA Units if they want to use the FBI's Combined DNA Index System (CODIS) which is essential in providing the most useful information and results to the DNA typing done in the laboratory.
- The FBI-QAS requires a laboratory to complete comprehensive validation studies on all equipment, materials, and methods used in the process of DNA analysis.
- YFiler Plus is a DNA amplification kit that only amplifies loci on the Y-Chromosome obtaining only male DNA profile(s).
- This validation study built off a study previously done with the Ohio BCI when they originally wanted to begin use of the amplification kit. It was found that additional validation work was needed it ensure that the kit was being used in an appropriate manner and was providing the most useful and quality results to the laboratory's clients.
- Components of this study included looking at male DNA sensitivity, the quality of a male DNA profile with increasing levels of female DNA present, and the qualities of male-male mixture DNA profiles.
- Additionally, the analytical threshold and stochastic threshold were reevaluated.

Methods

In Fall 2021, eight different single source male DNA samples were used to create the different scenarios of the validation study at the Ohio Attorney General's Center for the Future of Forensic Science at Bowling Green State University before being amplified at the BCI using the YFiler Plus amplification kit. The resulting DNA profiles were sent to the center at BGSU and edited using GeneMapper ID-X (v1.6). Data analysis proceeded for each study type as followed:

Analytical Threshold Study: Analysis of the negative controls and then the single source male samples in order to determine the new suggested analytical threshold.

Single Source Male Sensitivity Study: Analysis of the male DNA profiles, as DNA input concentrations decreased, under the new analytical threshold to determine the best DNA input concentration, the range of input concentrations that yield quality DNA profiles, and a suggested stochastic threshold.

Male with Increasing Excess Female Study: Analysis of male DNA profiles with large amounts of female DNA to determine when quality male profiles are still obtained.

2, 3, 4 Person Male-Male Mixtures Study: Analysis of the mixture samples is more complex and still occurring.

Results

Single Source Male DNA Profiles Peak Height by Dye

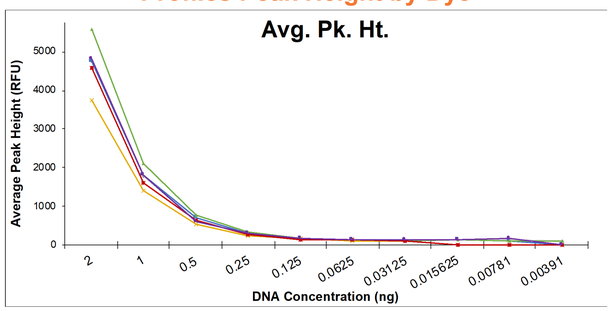

Figure 1. *Graph depicting each dye channel's average peak height (RFU) at each given DNA concentration (ng) for the single source male DNA profiles.*

Single Source Male DNA Profiles Overall Peak Height

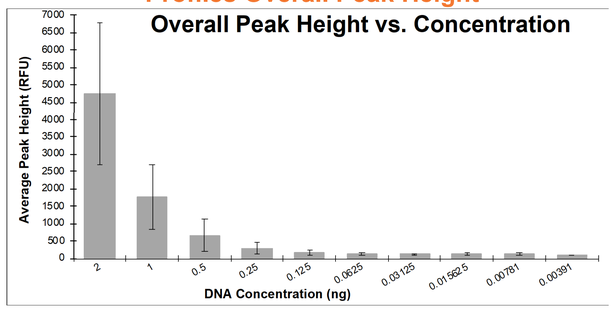

Figure 2. *Graph depicting the average peak height (RFU) of all the loci data at a given DNA input concentration (ng) in the single source male DNA profiles.*

Single Source Male with Excess Female Peak Heights

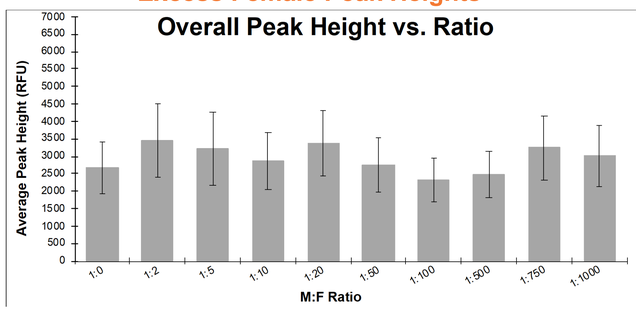

Figure 4. *Graph depicting the average peak height (RFU) for each given male to female ratio as the amount of female DNA increased.*

Discussion

Analytical Threshold Study:
- Negative controls suggested an analytical threshold of at least 45 RFUs
- Single source samples suggested a threshold between 75 RFUs and 140 RFUs
- Figure 1 indicates some difference between the dye channels.
- Ohio BCI decided to use an analytical threshold of 100 RFUs to continue.

Single Source Sensitivity Study:
- Figures 1 and 2 shows the breaking of linearity below 0.125 ng of DNA.
- The dynamic range was determined as 0.125 ng to 2 ng of input DNA.
- The ideal target concentration was found to be 0.5 ng to 1 ng.
- Figure 2 indicates that dropout occurred more in the yellow channel than the green channel. Figure 4 is an example of when this happened.
- Two loci, DYS385 and DYF387S1, can have duplicity in the number of intended peaks seen. The peak height of the tallest remaining peak when the other had dropped out was 365 RFU. This is used in determining the recommended stochastic threshold.

Male with Increasing Excess Female Study:
- Figure 3 depicts that there appears to be no significant difference at the different ratios as the female DNA increased with the constant 1 ng concentration of male DNA.

GeneMapper Example of Dropout

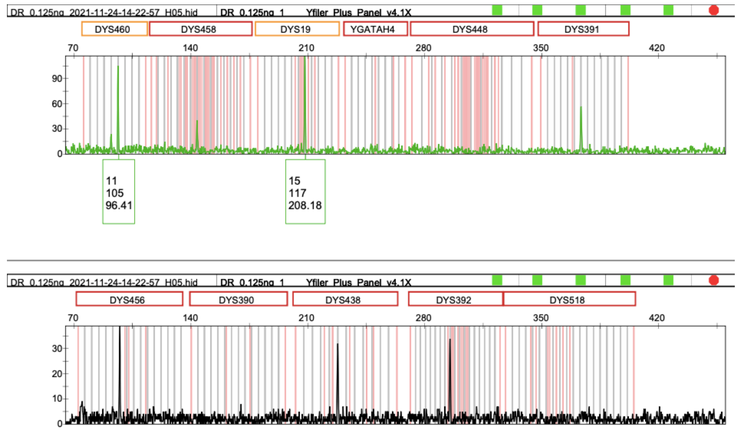

Figure 3. *GeneMapper screenshot depicting the green channel and yellow channel for a 0.125 ng sample for DR that shows the dropout differences between the two dye channels.*

Overall Conclusions

1. An analytical threshold of 100 RFU was established and utilized for all further DNA profile editing and will have continued use in the Ohio BCI laboratory system.
2. 400 RFU was suggested as the stochastic threshold for the laboratory system.
3. Good quality DNA profiles were obtained for single source males from 0.125 ng to 2 ng of DNA input concentrations.
4. The ideal target input DNA concentration should be 0.5 ng to 1 ng (0.75 ng) to ensure the most confidence in full male DNA profile.
5. Ratios of male to female DNA concentrations up to 1:1000 were shown to have a full male DNA profile if 1 ng of male is used.

References

1. Federal Bureau of Investigation. (2020). Quality assurance standards for forensic DNA testing laboratories. https://www.fbi.gov/file-repository/quality-assurance-standards-for-forensic-dna-testing-laboratories.pdf/view
2. Scientific Working Group on DNA Analysis Methods (SWGDAM). (2014). SWGDAM interpretation guidelines for Y-chromosome STR typing by forensic DNA laboratories. Retrieved from https://www.swgdam.org/publications
3. Jobling, M. A., Pandya, A., & Tyler-Smith, C. (1997). The Y chromosome in forensic analysis and paternity testing. International Journal of Legal Medicine, 110(3), 118–124. https://doi.org/10.1007/s004140050050
4. Roewer, L., Andersen, M. M., Ballantyne, J., Butler, J. M., Caliebe, A., Corach, D., D'Amato, M. E., Gusmão, L., Hou, Y., de Knijff, P., Parson, W., Prinz, M., Schneider, P. M., Taylor, D., Vennemann, M., & Willuweit, S. (2020). DNA commission of the International Society of Forensic Genetics (ISFG): Recommendations on the interpretation of Y-STR results in forensic analysis. Forensic Science International: Genetics, 48, 102308. https://doi.org/10.1016/j.fsigen.2020.102308