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## **VIRGINIA DEPARTMENT OF FORENSIC SCIENCE**

### **MINIMUM DNA TEMPLATE FOR AMPLIFICATION OF TRACE AND WEARER SAMPLES USING THE POWERPLEX® FUSION AND POWERQUANT® SYSTEMS VALIDATION SUMMARY Prepared in September 2024**

#### **PURPOSE**

This study is designed to determine the amount of input DNA measured using PowerQuant® for the amplification of trace and wearer samples with the PowerPlex® Fusion System below which one can expect that, in most instances, the resulting DNA profile would be of limited or no value.

Similar studies were previously conducted using samples amplified with the PowerPlex® 16 System as well as using samples quantitated with Plexor® HY (PLX) and amplified with PowerPlex® Fusion. During the PowerPlex® 16 validation study, trace and wearer profiles were considered separately from other body fluid samples in order to determine if there was a need to treat the two sample types differently with regard to a minimum DNA template amount or quantitation cutoff. That study demonstrated that the success rate of developing useable profiles either for direct comparison or searching the data bank from body fluid samples was significantly higher than that for trace and/or wearer samples. It was therefore determined that the implementation of a minimum quantitation amount for other body fluid samples would lose important investigative information. Therefore, only profiles from trace and wearer samples were examined for the PLX and PowerPlex® Fusion study, and, once again, only profiles from trace and wearer samples were examined for this current study.

The application of a minimum amount of input DNA template measured using PowerQuant® for the amplification of trace/wearer samples with the PowerPlex® Fusion System is designed alleviate the examiner and peer-reviewer time spent developing DNA profiles that are of no value for comparison or searching in the data bank. Although it is expected that implementation of this minimum input of DNA template may occasionally result in a sample that may have yielded an interpretable profile to be discontinued, this study is designed to determine a minimum value that will increase the overall success rate for these types of samples while limiting the loss of interpretable data.

#### **MATERIALS AND METHODS**

Data from 220 trace/wearer samples was collected from all four regional laboratories. Each of these samples was a routine evidential sample processed by a qualified examiner during normal workflow. All samples were extracted using the procedures as described in the Forensic Biology Section Procedures Manual (FB PM), Extraction of DNA. The samples were quantitated utilizing the PowerQuant® System as described in the FB PM, Quantitation of DNA.

All samples were amplified using the PowerPlex® Fusion System as described in the FB PM, PowerPlex® Fusion Amp and Storage. Samples and allelic ladders were separated on the 3500xl Genetic Analyzer (AB) as described in the FB PM, CE for PowerPlex® Fusion using the following settings at 12 or 24 second injection times: 1.2 kV injection, 1800 s electrophoresis time, 15 kV separation, 36 cm (length), 50  $\mu$ m i.d. capillary and POP-4 polymer (AB).

Analysis was completed by the GeneMapper® ID-X v1.4 software (AB). The stutter ratios and limit of detection (LOD) were defined based upon internal validation by the Department and were applied as described in the FB PM, GMID-X and FB PM, Interpretation of Fusion Data.

Samples classified as trace DNA included samples such as those described in 1.5.1 of the FB PM, Documentation and Evidence Handling Requirements. Samples classified as “wearer” DNA included samples taken from evidence such as clothing, gloves, caps, bandanas, sunglasses, and do-rags that were analyzed in an attempt to identify a possible wearer of such items.

The DNA profiles obtained were classified in one or more of the following categories:

- No profile detected/No result
- No value - limited
- No value – complex/full result
- Acceptable profile, suitable for direct comparison and/or data bank search

## RESULTS

Of the 220 sample profiles examined, 71 were of value for interpretation. The majority of the 71 samples contained greater than 0.0490 ng/μL of autosomal DNA. The remaining 193 profiles were deemed to be of no value either because no result was obtained, the results obtained were limited, the results obtained were too complex or a combination of these.

Quantitation data showed that 74 of the 220 samples examined contained 0.0100 ng/μL or less of autosomal DNA. No profiles developed from these samples with 0.0100 ng/μL or less of autosomal DNA were of value for interpretation.

Quantitation data showed that 106 of the 220 samples examined contained 0.0200 ng/μL or less of autosomal DNA. Profiles developed from 7 of these 106 samples with 0.0200 ng/μL or less of autosomal DNA were of value for interpretation. The remaining 99 of these 106 samples either did not amplify or were of no value due to the limited information developed.

Quantitation data showed that 116 of the 220 samples examined contained 0.0250 ng/μL or less of autosomal DNA. Profiles developed from 10 of these 116 samples with 0.0250 ng/μL or less of autosomal DNA were of value for interpretation. The remaining 106 of these 116 samples either did not amplify or were of no value due to the limited information developed.

Quantitation data showed that 131 of the 220 samples examined contained less than 0.0300 ng/μL of autosomal DNA. Profiles developed from 17 of these 131 samples with less than 0.0300 ng/μL of autosomal DNA were of value for interpretation. The remaining 114 of these 131 samples either did not amplify or were of no value due to the limited information developed.

Quantitation data showed that 143 of the 220 samples examined contained less than 0.0400 ng/μL of autosomal DNA. Profiles developed from 26 of these 143 samples with less than 0.0400 ng/μL of autosomal DNA were of value for interpretation. The remaining 117 of these 143 samples either did not amplify or were of no value due to the limited information developed.

Of the 220 sample profiles examined, 20 yielded profiles too complex to be interpreted per Department protocol due to the observation of four or more contributors. None of these 20 samples contained less than 0.0250 ng/μL of autosomal DNA. All but three of these 20 samples contained more than 0.0300 ng/μL of autosomal DNA per the quantitation data.

## DISCUSSION

Of the 26 out of 143 profiles that were interpretable with less than 0.0400 ng/μL of autosomal DNA, 8 were apparent single source profiles or mixtures from which a major contributor could be deconvoluted and compared. Eighteen of the 26 were 2- or 3-person mixture profiles deemed suitable for comparison purposes. The remaining profiles developed from the other 117 samples with less than 0.0400 ng/μL of autosomal DNA were of no value due to the limited information obtained. This data demonstrates that the success rate of developing interpretable profiles for trace/wearer samples with less than 0.0400 ng/μL of autosomal DNA is approximately 18.2%.

Of the 17 out of 131 profiles that were interpretable with less than 0.0300 ng/μL of autosomal DNA, none were apparent single source profiles. Two were mixtures from which a major contributor could be deconvoluted and compared. Fifteen of the 17 were 2- or 3-person mixtures deemed suitable for comparison purposes. The remaining profiles developed from the other 114 samples with less than 0.0300 ng/μL or less of autosomal DNA were of no value due to the limited information obtained. This data demonstrates that the success rate of developing interpretable profiles for trace/wearer samples with less than 0.0300 ng/μL of autosomal DNA is approximately 13.0%.

Of the 10 out of 116 profiles that were interpretable with 0.0250 ng/μL or less of autosomal DNA, none were apparent single source profiles. One was a mixture from which a major contributor could be deconvoluted and compared. Nine of the 10 were 2- or 3-person mixtures deemed suitable for comparison. The remaining profiles developed from the other 106 samples with 0.0250 ng/μL or less of autosomal DNA were of no value due to the limited information obtained. This data demonstrates that the success rate of developing interpretable profiles for trace/wearer samples with 0.025 ng/μL or less of autosomal DNA is approximately 8.6%.

Of the 7 out of 106 profiles that were interpretable with 0.0200 ng/μL or less of autosomal DNA, none were apparent single source profiles. One was a mixture from which a major contributor could be deconvoluted and compared. Five of the 6 were 2- or 3-person mixtures deemed suitable for comparison. The remaining profiles developed from the other 99 samples with 0.02 ng/μL or less of autosomal DNA were of no value due to the limited information obtained. This data demonstrates that the success rate of developing interpretable profiles for trace/wearer samples with 0.0200 ng/μL or less of autosomal DNA is approximately 6.6%.

Based upon this empirical data from all real casework samples evaluated, the overall success rate in obtaining an interpretable profile from trace/wearer samples without the implementation of a quantitation cutoff is approximately 32%.

By limiting the trace/wearer samples amplified with the PowerPlex® Fusion System to those containing more than 0.0400 ng/μL, the success rate is increased to approximately 58.4%. while

allowing for the discontinuation of a sample that may have yielded an interpretable profile approximately 18.2% of the time.

By limiting the trace/wearer samples amplified with the PowerPlex® Fusion System to those containing more than 0.0300 ng/μL, the success rate is increased to approximately 60.7%. while allowing for the discontinuation of a sample that may have yielded an interpretable profile approximately 13% of the time.

By limiting the trace/wearer samples amplified with the PowerPlex® Fusion System to those containing more than 0.0250 ng/μL, the success rate is increased to approximately 58.7%. while allowing for the discontinuation of a sample that may have yielded an interpretable profile approximately 8.6% of the time.

By limiting the trace/wearer samples amplified with the PowerPlex® Fusion System to those containing more than 0.0200 ng/μL, the success rate is increased to approximately 56.1%. while allowing for the discontinuation of a sample that may have yielded an interpretable profile approximately 6.6% of the time.

In comparing the gain in success rates and possible loss of interpretable samples at each of these potential cut off values, it is determined that limiting the trace/wearer samples amplified with the PowerPlex® Fusion System to those containing more than 0.0250 ng/μL as measured using PowerQuant® will substantially increase the success rate while limiting the loss of potentially interpretable profiles to an acceptable rate of approximately 8.6%. This has the potential to increase efficiency in casework analysis and alleviate time spent analyzing low level samples that are eventually determined to be of no value. Therefore, it is recommended that a quantitation cut off value of 0.0250 ng/μL for trace/wearer samples be implemented.