# Internal Validation of the AmpFℓSTR® Identifiler® Kit on the Applied Biosystems 3130 Instrument

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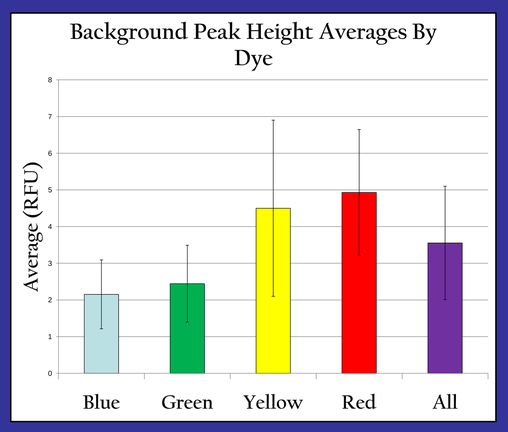
## ABSTRACT

An internal validation was completed on the Applied Biosystems 3130 instrument with the AmpFℓSTR® Identifiler® Kit [4]. The validation contained studies in: precision, reproducibility, concordance, sensitivity, background, stutter, peak height ratios, contamination, and overall observations throughout the study [8 & 13]. A qualifying test was conducted on the Identifiler® Kit at the end of the validation. The samples used were non-probative reference samples from the CODIS database with known profiles. The results showed that this kit is successful in producing reproducible and reliable data according to the Wyoming State Crime Laboratory (WSCL) protocols. Data analysis and a formal report resulted in a successful validation of the Identifiler® Kit.

Background Peak Height Averages By Dye




| | Blue | Green | Yellow | Red | All |
|---|---|---|---|---|---|
| # of Peaks | 1491 | 1579 | 1639 | 1670 | 6379 |
| Max | 12 | 10 | 29 | 23 | 29 |
| Average | 2.152247 | 2.442685 | 4.499085 | 4.929341 | 3.554162 |
| Std Dev | 0.939241 | 1.047528 | 2.402145 | 1.716878 | 1.545502 |

| LOD | LOQ |
|---|---|
| 8.190668 | 19.00918 |

**Graph 1 & Table 1 & 2**: Correspond to the Identifiler® background averages from 5 negative controls analyzed at a 1 RFU threshold. Limit of Detection and Limit of Quantitation were determined.

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## INTRODUCTION

The AmpFℓSTR® Identifiler® Kit is a single tube multiplex utilizing the thirteen core CODIS loci, D2, D19, and Amelogenin [4].
Laboratories using this chemistry allows analysts to:
- Amplify/genotype using only one kit for their samples
- Replace Profiler Plus® and COfiler®
- Reduce the time it takes to prepare samples
- Use less material (tubes, pipette tips, etc)
- Reduce costs

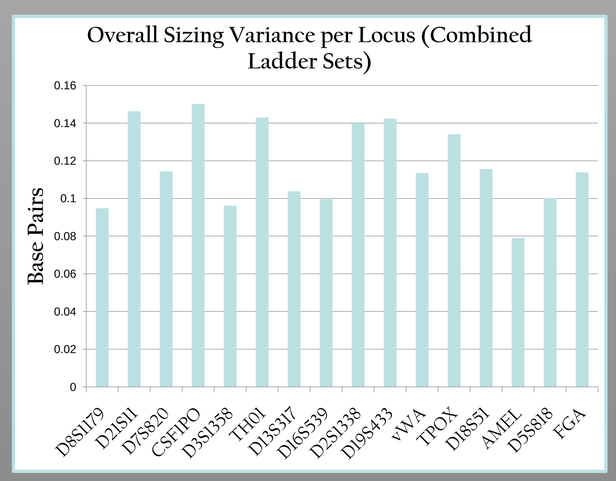
A half reaction (actually a 53.3% reaction) will be validated to use on single source reference samples (CODIS) which will significantly reduce the increased operational costs associated with the Identifiler® chemistry at the WSCL.

## MATERIALS AND METHODS

- 50 CODIS Samples: 25 dried blood samples on FTA® paper and 25 buccal cell samples on BODE DNA Collectors™
- Razor Blades
- Pipettes
- Pipette Tips
- Nanopure Water
- Microcentrifuge Tubes
- Qiagen EZ1® Robots
- Qiagen EZ1® Extraction Kit
- Quantitation Standards: concentrations (ng/ µL) of: 50.0, 16.7, 5.560, 1.850, 0.620, 0.210, 0.023, and 0.068

- Applied Biosystems Quantifiler™ Human DNA Quantification Kit
- AB 96-well Optical Plates
- AB Optical Covers
- AmpFℓSTR® Identifiler® Kit
- GeneScan™ 600 LIZ® Size Standard
- Multi-Capillary DS-33 (Dye Set G5)
- 10x Run Buffer
- POP-4™ Polymer
- Reservoir Septa
- AB 96 well Septa
- Applied Biosystems 3130 Instrument

1. 5x5 mm square cuttings of each sample were taken.
2. Samples were extracted using the Qiagen EZ1® robots.
3. Samples were quantitated with the Quantifiler™ Human DNA Quantification Kit then diluted appropriately.
4. The AmpFℓSTR® Identifiler® Kit was used to prepare the samples for amplification.
5. The samples were amplified on an AB 9700 Thermal Cycler.
6. A new capillary array and a new Multi-Capillary DS-33 (Dye Set G5) were installed on the AB 3130 Instrument; a spatial calibration and a spectral calibration were completed.
7. Allelic ladders were prepared and run to check for precision and reproducibility at the beginning and end of the validation.
8. The amplicons were prepared for genotyping on the Applied Biosystems 3130 Instrument using the AmpFℓSTR® Identifiler® Kit.
9. Genotyping was completed by running the samples on the AB 3130 Genetic Analyzer.




**Graph 2**: Precision and reproducibility results.

## RESULTS

**Precision Results:**
- Variance (3X St Dev) = 0.0789 to 0.1501 base pairs. See graph 2.
- Coefficient of Variation (CV %) = 0.0131 to 0.1622%.

**Reproducibility Results:** All alleles of each ladder consistently matched with the allele calls for the AmpFℓSTR® Identifiler® allelic ladder. See graph 2.
**Concordance Results:** Each DNA sample yielded a profile that matched the known profile.
**Sensitivity Results**:
- Concentration of 0.5 ng/µL displayed copious artifacts.
- Concentration of 0.2 ng/µL, minor homozygous off-scale data and less pull-up were exhibited.
- Concentrations of 0.1 ng/µL to 0.025 ng/µL demonstrated full profiles with no off-scale data and very few artifacts.
- Stochastic effect for the samples resulted in the highest heterozygous peak with dropout of one allele (analysis threshold of 60 RFU) was 145 RFU. Therefore, whenever a peak was greater than 145 RFU and an additional peak did not exist over 60 RFU, it was a true homozygous allele. See graph 3.

**Background Results:**
- Average peak height = 2.152 RFU to 4.929 RFU (blue and red, respectively). See graph 1 and table 1 &2.
- St Dev = 0.939 to 2.402 (blue and yellow, respectively).
- Minimum peak height = 1 RFU (each dye).
- Maximum peak height = 29 RFU (yellow).

| Stutter Comparison - Identifiler® | | |
|---|---|---|
| Marker | Applied Biosystems | WSCL ID Validation |
| CSF1PO | 9.2% | 6.2% |
| D13S317 | 8.0% | 7.1% |
| D16S539 | 10.4% | 7.8% |
| D18S51 | 17.0% | 14.8% |
| D19S433 | 13.3% | 13.1% |
| D21S11 | 9.4% | 8.6% |
| D2S1338 | 11.1% | 11.1% |
| D3S1358 | 10.7% | 10.0% |
| D5S818 | 6.8% | 6.0% |
| D7S820 | 8.2% | 7.4% |
| D8S1179 | 8.2% | 7.2% |
| FGA | 14.7% | 11.0% |
| TH01 | 5.1% | 3.7% |
| TPOX | 4.8% | 4.3% |
| vWA | 12.6% | 9.9% |

**Table 3**: Stutter study: the stutter comparison of Applied Biosystems stutter percentages and the WSCLs stutter percentages from the ID validation.

| Peak Height Ratio Comparison | | | | |
|---|---|---|---|---|
| | Applied Biosystems | | WSCL ID Validation | |
| Marker | Mean | Min | Mean | Min |
| AMEL | n/a | n/a | 89.8% | 58.9% |
| CSF1PO | 86.0% | 63.6% | 85.7% | 62.6% |
| D13S317 | 87.0% | 63.3% | 85.1% | 55.6% |
| D16S539 | 88.0% | 61.5% | 86.4% | 64.5% |
| D18S51 | 82.0% | 56.3% | 84.3% | 53.0% |
| D19S433 | 88.0% | 48.8% | 87.6% | 61.8% |
| D21S11 | 88.0% | 66.4% | 86.6% | 57.9% |
| D2S1338 | 84.0% | 42.8% | 85.2% | 50.7% |
| D3S1358 | 88.0% | 64.3% | 87.7% | 69.5% |
| D5S818 | 89.0% | 64.9% | 91.1% | 89.3% |
| D7S820 | 89.0% | 66.2% | 87.1% | 58.5% |
| D8S1179 | 90.0% | 57.5% | 89.1% | 79.2% |
| FGA | 85.0% | 60.9% | 83.5% | 54.8% |
| TH01 | 86.0% | 48.8% | 87.7% | 61.0% |
| TPOX | 87.0% | 55.9% | 87.4% | 68.6% |
| vWA | 86.0% | 62.8% | 84.2% | 57.7% |

**Table 4**: Peak Height Ratio study: the peak height means and minimums of Applied Biosystems and the means and minimums obtained during the ID Validation at the WSCL.

**Stutter Results:** When the samples were analyzed at 25 RFU the average n-4 stutter percentages ranged from 1.81% (TH01) to 8.16% (D18S51). The standard deviations ranged from 0.007 (TH01) to 0.020 (D18S51). See table 3.
**Peak Height Ratio Results:** The samples were analyzed at 60 RFU, the average peak height ratios ranged from 83.48% (FGA) to 91.10% (D5S818). See table 4.
**Contamination Results:** None of the DNA samples, positive controls, or negative controls showed any sign of contamination.
**Qualifying Test Results:** The qualifying test resulted in accurate data. All samples gave the correct profiles matching their respective known profiles. Negative controls and positive controls gave expected results.

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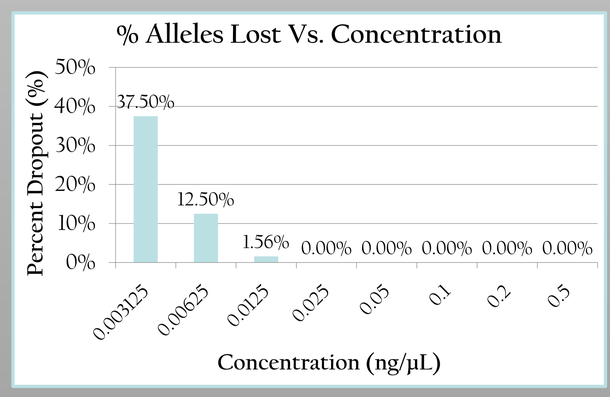
## DISCUSSION

**Concordance Discussion**: Samples 788 & 888 had discrepancies at the D5 locus. Sample 33 at the D7 locus was noted to have a micro-variant 11.3 allele while the known profile had a 12. Additionally, full profiles were obtained from samples 882, 888, and 893.
**Stutter Discussion:** The stochastic threshold was determined to be 145 RFU with this chemistry, but a conservative interpretative guideline of 200 RFU was established.
**Capillary Array Discussion:** A total of 220 injections were on the array without loss of resolution observed in the data. The manufacturer recommends 150 injections per array, though this should serve as evidence that an array may last for several more injection sets as long as the data appears acceptable.
**Evaporation Discussion**: Evaporation was noted due to a missing compression pad for use on the Thermal Cycler.
**GeneScan™ LIZ 600® Discussion:** WSCL determined that 0.40 µL of LIZ 600® size standard per sample yielded a better size standard peak height than the manufacturer's recommended concentration of 0.25 µL in a half reaction. [3]
**Spectral Calibration Discussion:** The manufacturer's spectral calibration protocol called for 10 µL of DS-33 standard with 190 µL of formamide. [3] This resulted in a "saturated" spectral calibration that failed, 5 µL (or less) should be used.

## CONCLUSIONS

The protocols used by the Wyoming State Crime Laboratory have been shown to be consistent, reliable, and reproducible with sufficient safeguards to prevent any cross-contamination. The data obtained from the qualifying test supports the conclusion that the protocols in use at the WSCL are accurate for genotyping reference samples. Additionally, the AmpFℓSTR® Identifiler® kit has been deemed reliable for use with reference samples. An appropriate target range of input DNA is between 0.025 ng/µL and 0.2 ng/µL. Also, a suitable DNA target amplification amount is 0.1 ng/µL. A stochastic limit of 200 RFU works for identifying true homozygous alleles.




**Graph 3**: Sensitivity Study: the number of alleles that were lost at each concentration.

## REFERENCES

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Note: Additional sources were used and are available.