Rapid DNA Maturity Assessment

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Integration of the extraction, amplification, separation, and detection processes for forensic DNA typing is a challenging goal. Several parallel efforts have been made to integrate the forensic workflow and utilize a simple swab in, answer out process within a single platform [1-3]. Of the multiple efforts, two platforms were tested as a part of a rapid DNA maturity assessment in 2014. The assessment was conducted with sets of blinded single-source reference samples to gauge the typing success of the current rapid DNA typing technology. Samples were provided to participants for testing on the individual rapid platforms, and data was returned to the National Institute of Standards and Technology (NIST) for review and analysis. Both automated and manual review of the data sets were conducted to assess the success of typing the CODIS core loci. Genotyping profiles from the multiple platforms, participants, and STR typing chemistry was combined into a single analysis to assess the current maturity of Rapid DNA technology. The presented results will focus on genotyping success rate, peak height ratios, and stutter artifacts across two platforms and multiple STR kit chemistries.

What is Rapid DNA Typing?

Rapid DNA, or Rapid DNA Analysis, describes the **fully automated** (hands free) process of developing a CODIS Core STR profile from a reference sample buccal swab in less than 2 hours. The "swab in – profile out" process consists of automated extraction, amplification, separation, detection and allele calling without human intervention.

https://www.fbi.gov/about-us/lab/biometric-analysis/codis/rapid-dna-addendum-to-qas-final-effective-12-1-2014

Rapid DNA Analysis vs. Modified Rapid DNA Analysis

Rapid DNA analysis describes the fully automated (hands-free) process of developing a CODIS Core STR profile from a known reference sample. The "swab in – profile out" process consists of automated extraction, amplification, separation, detection and allele calling **without human intervention**.

Modified Rapid DNA analysis describes the automated (hands-free) process of developing a CODIS Core STR profile from a known reference sample. This "swab in – profile out" process consists of automated extraction, amplification, separation, and detection without human intervention but **requires manual interpretation and technical review.**

https://www.fbi.gov/about-us/lab/biometric-analysis/codis/rapid-dna-addendum-to-qas-final-effective-12-1-2014

Integrated Rapid DNA Typing Devices Tested

As part of the Maturity Assessment

ANDE (NetBio)

- Electrophoresis takes place on chip
- Kit = one biochipset
  Stored at room temperature
  Shelf life ≈ 6 months
- RFID swabs tagged for sample tracking

PowerPlex 16 loci
≈86 min runtime
(5 samples)

RapidHIT 200 (IntegenX)

- Electrophoresis takes place on an 8 capillary array
- Kit = 4 separate components
  Stored between room temp and 4°C
  Shelf life ≈ 6 months at 4°C
- Cotton swabs

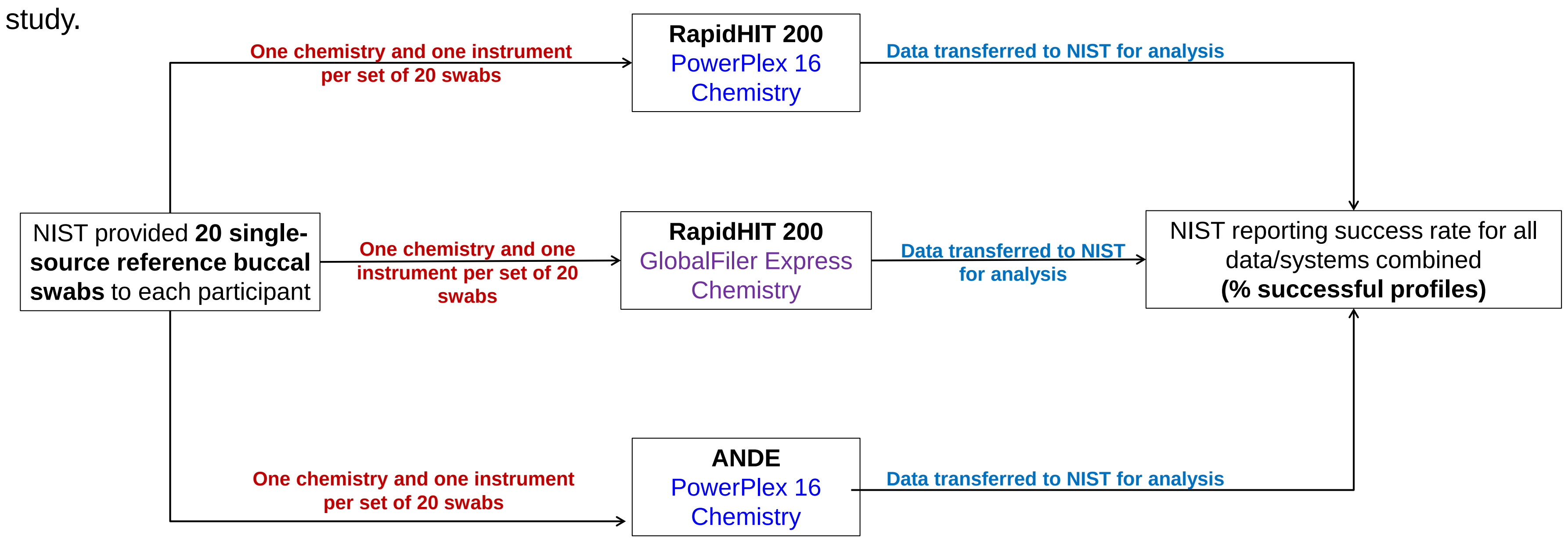
PowerPlex 16 loci
≈90 min runtime
(5 samples)

GlobalFiler Express loci
≈116 min runtime
(1-7 samples)

2014 NIST Rapid DNA Maturity Assessment

The purpose of the 2014 NIST Rapid DNA Maturity Assessment was to assess the current status of rapid DNA typing technology for the CODIS core loci in support of lab and future external (non-lab-based) Rapid DNA instrument implementation.

Only integrated (swab in – allele detection) instruments capable of genotyping the core CODIS 13 STR markers were eligible for this study.



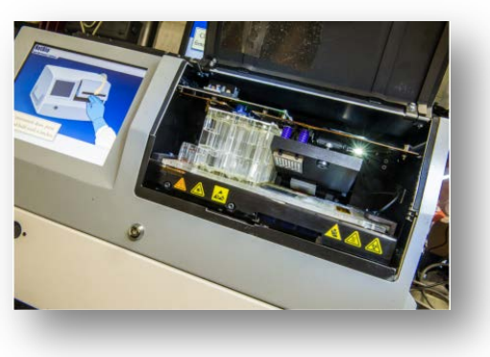
NIST provided **20 single-source reference buccal swabs** to each participant

One chemistry and one instrument per set of 20 swabs → **RapidHIT 200** PowerPlex 16 Chemistry → Data transferred to NIST for analysis

One chemistry and one instrument per set of 20 swabs → **RapidHIT 200** GlobalFiler Express Chemistry → Data transferred to NIST for analysis

One chemistry and one instrument per set of 20 swabs → **ANDE** PowerPlex 16 Chemistry → Data transferred to NIST for analysis

NIST reporting success rate for all data/systems combined **(% successful profiles)**

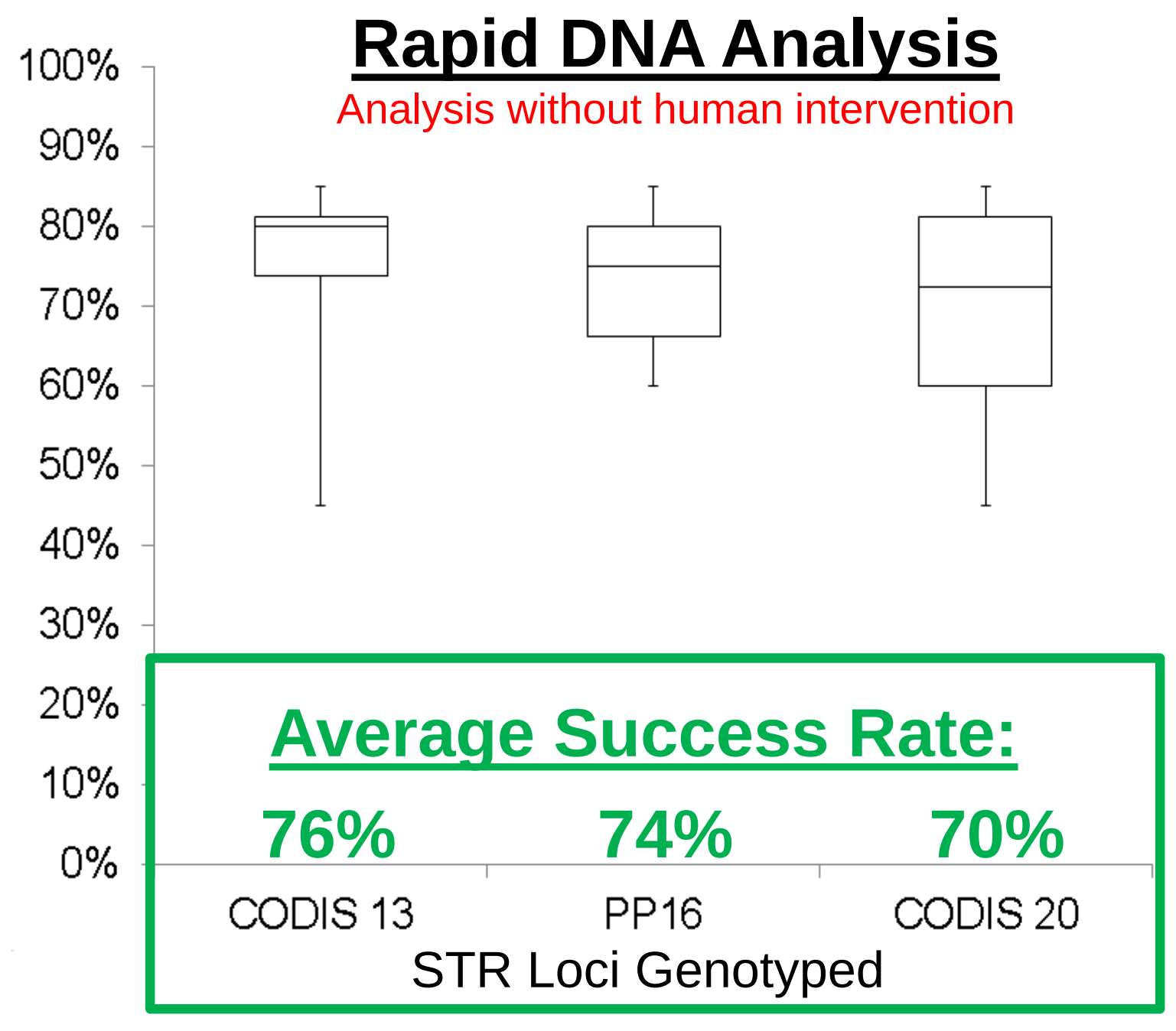
Participating Laboratories (7)	Instrument Platforms (2)	Independent Instruments (11)	Chemistry	Total Samples Tested (280)
U.S. Federal State Private	NetBio ANDE 	5	PowerPlex 16	100
	IntegenX RapidHIT 200 	6	PowerPlex 16	60
			GlobalFiler Express	120

Genotyping Success

Success was measured by **complete and concordant genotypes** produced by the integrated rapid DNA devices as compared to lab generated correct genotypes

Rapid DNA Analysis

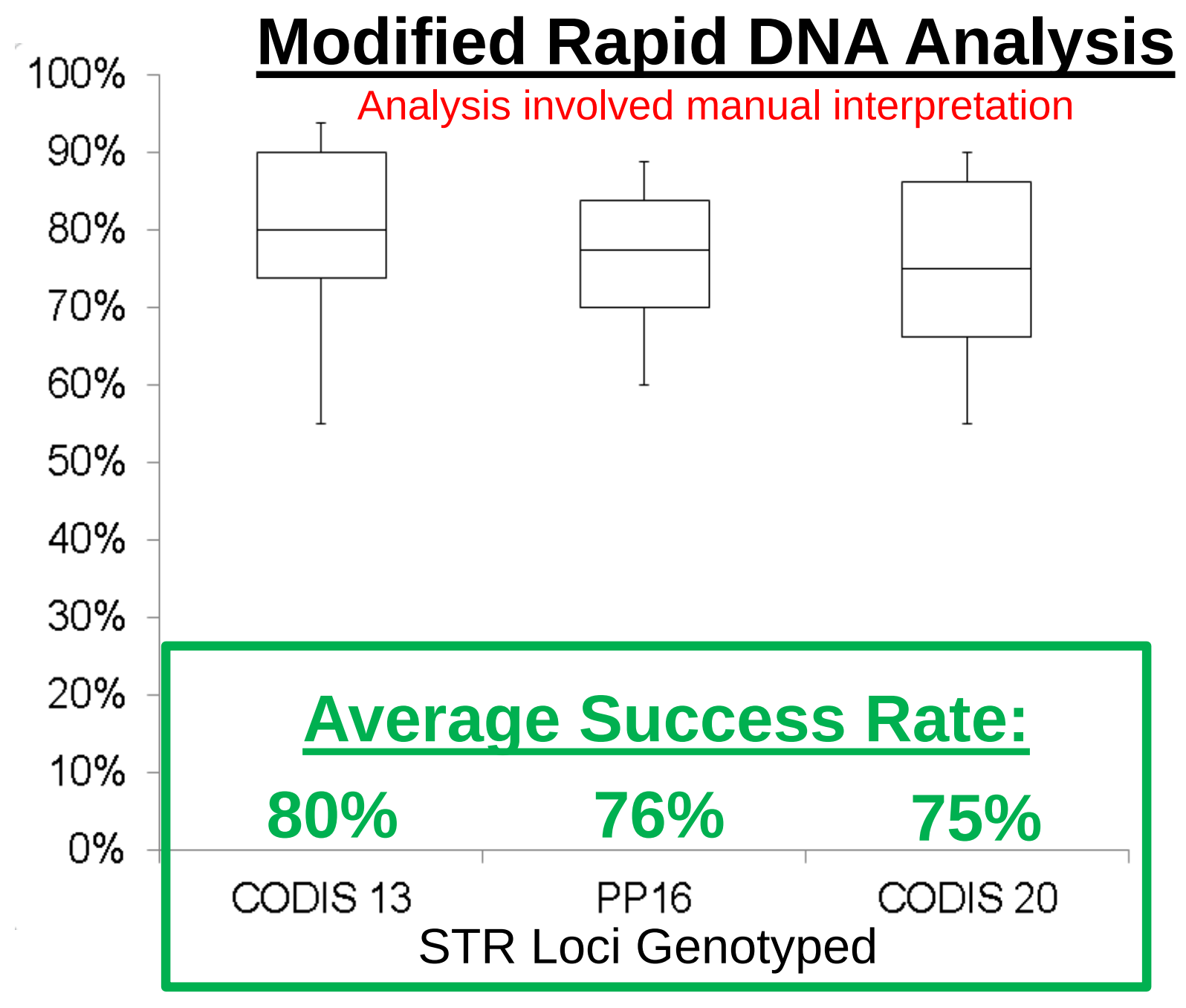
Analysis without human intervention



Average Success Rate:
76% 74% 70%

Modified Rapid DNA Analysis

Analysis involved manual interpretation



Average Success Rate:
80% 76% 75%

Success rates indicate the average success for each STR locus group genotyped (CODIS 13 loci, PowerPlex 16 loci, new CODIS 20 loci). The minimum and maximum success rates observed within individual participants within the Maturity Assessment is represented by the whiskers of the boxplot above.

Peak Height Ratios

Peak height ratios were calculated for all complete profiles for the PowerPlex 16 and GlobalFiler Express chemistries.

PowerPlex 16

Locus	Median
Penta_E	0.81
AMEL	0.83
Penta_D	0.84
D18S51	0.86
D3S1358	0.87
D8S1179	0.87
TPOX	0.87
D5S818	0.88
vWA	0.88
D21S11	0.88
D16S539	0.88
D13S317	0.89
CSF1PO	0.89
FGA	0.89
D7S820	0.89
TH01	0.93

n=118

The PowerPlex 16 data is a combination of the data generated from both ANDE and the RapidHIT 200.

GlobalFiler Express

Locus	Median
SE33	0.79
D2S1338	0.82
D5S818	0.85
D18S51	0.85
D12S391	0.86
D21S11	0.87
CSF1PO	0.87
vWA	0.88
D7S820	0.88
TPOX	0.89
D16S539	0.89
D1S1656	0.89
D22S1045	0.89
D8S1179	0.90
D13S317	0.90
AMEL	0.90
D3S1358	0.90
D19S433	0.90
D10S1248	0.91
TH01	0.91
FGA	0.92
D2S441	0.92

n=67

All peak height ratios above 79%

Stutter Percentages

Stutter percentages were calculated for all complete profiles for the PowerPlex 16 and GlobalFiler Express chemistries.

PowerPlex 16

Locus	Median
Penta_D	1.47
TH01	2.28
TPOX	2.82
Penta_E	4.12
D7S820	5.76
D13S317	6.48
D18S51	7.15
D8S1179	7.30
CSF1PO	7.52
D16S539	7.67
D5S818	8.36
FGA	8.78
vWA	9.30
D3S1358	10.23
D21S11	10.72

n=118

The PowerPlex 16 data is a combination of the data generated from both ANDE and the RapidHIT 200.

GlobalFiler Express

Loci	Median
TH01	1.27
TPOX	3.64
D7S820	4.75
D2S441	4.76
DYS391	5.73
D16S539	5.84
D13S317	5.90
CSF1PO	6.12
D8S1179	6.59
D18S51	6.67
D5S818	6.76
D22S1045	7.00
D19S433	7.10
FGA	7.20
D3S1358	8.31
D10S1248	8.36
D21S11	8.60
D2S1338	8.71
D1S1656	8.77
vWA	9.28
D12S391	9.46
SE33	15.56

n=67

Stutter within observed developmental validation range

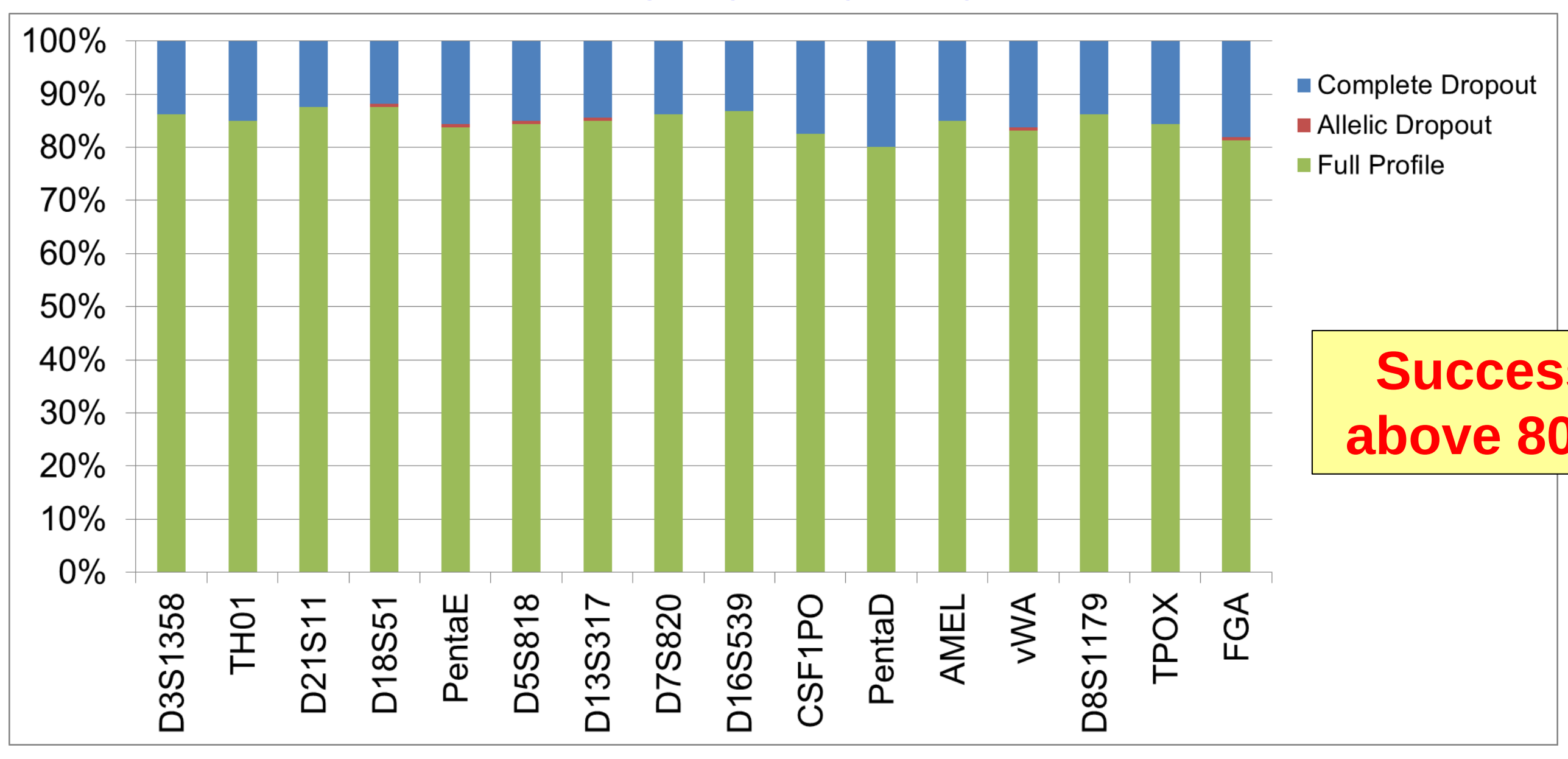
Additional Resources

1. https://www.fbi.gov/about-us/lab/biometric-analysis/codis/rapid-dna-analysis
2. http://www.swgdam.org/
3. https://www.fbi.gov/about-us/lab/biometric-analysis/codis/summary-of-rapid-dna-addenda-effective-12-1-14
4. https://www.fbi.gov/about-us/lab/biometric-analysis/codis/rapid-dna-addendum-to-qas-final-effective-12-1-2014
5. https://www.fbi.gov/about-us/lab/biometric-analysis/codis/audit-document-for-rapid-dna-qas-addendum-effective-12-1-2014

Success: Per Locus

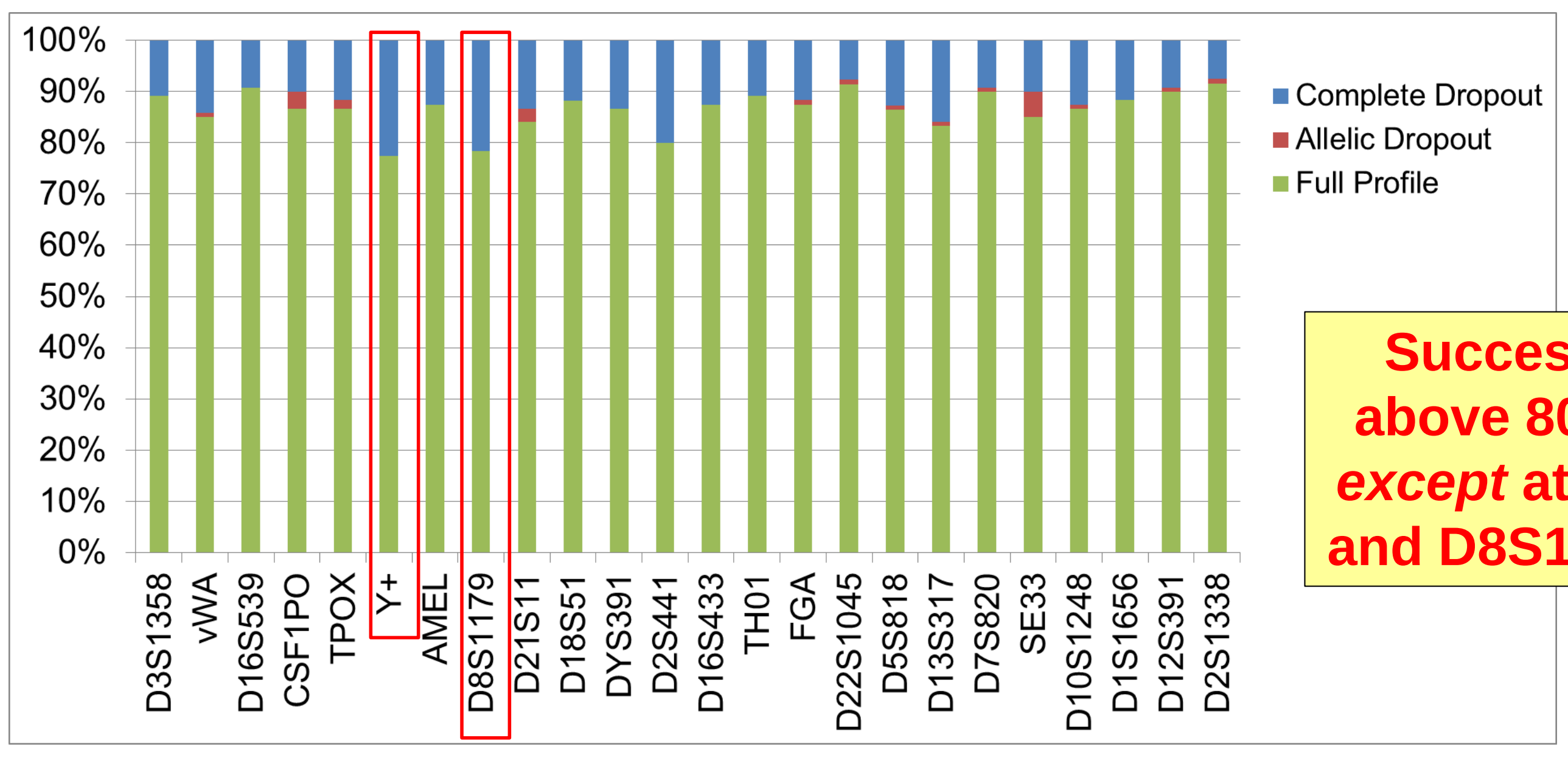
Percentage of successful genotypes generated per locus. The PowerPlex 16 data is a combination of the data generated from both ANDE and the RapidHIT 200.

PowerPlex 16



Success above 80%

GlobalFiler Express



Success above 80% *except* at Y+ and D8S1179

Conclusions

Two fully integrated platforms were included in the 2014 Rapid DNA Maturity Assessment. A total of 11 instruments were tested between 7 laboratories for a total of 280 samples examined. Data for the Maturity Assessment was generated from October-December 2014 and returned to NIST for data analysis. Updates to instrumentation and software may have taken place since this Maturity Assessment was completed. Observed success for the CODIS 13 Core Loci ranged from 76% for Rapid DNA Analysis to 80% with Modified Rapid DNA Analysis.

Results can be located: http://www.nist.gov/mml/bmd/genetics/dna_biometrics.cfm

References:
1. Tan E, Turingan RS, Hogan C, Vasantgadkar S, Palombo L, Schumm JW, et al. Fully integrated, fully automated generation of short tandem repeat profiles. Investig. Genet. 4 (2013) 2041-2223.
2. Jovanovich, S., Bogdan, G., Belcinski, R., Buscaino, J., Burgi, D., Butts, E. L. R., et al. Developmental validation of a fully integrated sample-to-profile rapid human identification system for processing single-source reference buccal samples. Forensic Sci. Int. Genet. 16 (2015) 181-194.
3. Hennessy LK, Mehendale N, Chear K, Jovanovich S, Williams S, Park C, et al. Developmental validation of the GlobalFiler express kit, a 24-marker STR assay, on the RapidHIT System. Forensic Sci. Int. Genet. 13 (2014) 247-258.

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