Internal Validation of Y-STRs for Casework at the Kentucky State Police Central Laboratory

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Abstract

Crime labs are interested in Y-STRs to amplify samples with low levels of male DNA. An internal validation of a Y-STR amplification kit was performed. The validation studies included: sensitivity, female/male mixtures, male/male mixtures, reproducibility of results, contamination, precision, adjusting stutter filters, and additional studies. In the validation studies performed, this kit proved to be sensitive and able to produce complete profiles from samples containing template DNA as low as 125 pg. Samples with as little as 50 pg had 88.2% of alleles called. At concentrations as strong as 100 ng, female DNA did not amplify or interfere. Male/male mixtures could be discerned at a ratio of 70:30 in samples with 1.2 ng, 0.6 ng, and 0.3 ng total DNA. For 90:10 mixtures, samples with 1.2 ng total DNA had 95.70% of alleles called and for samples with 0.3 ng total DNA, 77.42% of alleles were called. Based on the results, this internal validation shows that reproducible results from a Y-STR amplification kit will be beneficial for forensic casework purposes.

Introduction

Crimes such as sexual assaults may produce samples that involve a large amount of female DNA, from which a full female profile can be easily developed; however, while a small amount of male DNA may be detectable, a full profile is difficult to discern. An amplification kit that will specifically target male human DNA allows analysts to observe strong partial or even full profiles that otherwise would have not been picked up due to the strong concentration of female DNA (1). After completing an internal validation of a Y-STR amplification kit, the Kentucky State Police Central Laboratory (KSP) will soon be using this amplification kit as needed in casework.

Materials and Methods

- Differential Organic Extraction
- Real-time quantitative PCR using a dual human quantification kit on a 7500 Real-Time PCR Instrument (Applied Biosystems, Foster City, CA)
- Amplification with a commercial Y-STR PCR amplification kit on a GeneAmp 9700 thermal cycler (ABI)
- ABI Prism®310 Genetic Analyzer
- Data Analysis with GeneMapper®*ID* v 3.2

Figure 1. Commercial Y-STR kit

Figure 1 shows a commercial Y-STR PCR amplification kit which was validated for future use in casework.

Sensitivity

- Analyze samples with 1.0 ng to 0.05 ng template DNA using two male genotypes to determine optimal concentration for amplification

Specificity

- Amplify and run a known female sample with 0.5 ng template DNA to determine if amplification kit is male specific

Female/Male Mixtures

- Mixtures at 5:0.5 ng, 10:0.5 ng, 100:0.5 ng in female:male 25 µL reactions

Male/Male Mixtures

- A two part study that consisted of combinations that could be seen in casework-type samples
- Table 2 shows part one of the setup, with 1.2 ng total template DNA

Precision

- Used 10 ladders from 5 runs performed during the validation

Contamination

- Follow all KSP protocols

Reproducibility

- Amplify and run 60 reference samples and Y-STR kit male and female standards in triplicate

Accuracy

- Amplify and run NIST Standard Reference Material® (SRM) # 2395 samples

Table 1. Sensitivity

Table 1 sensitivity study results with samples run in triplicate at concentrations 1.0 ng to 0.05 ng per reaction at 5 and 10 second injections

	1.0 ng	0.75 ng	0.50 ng	0.25 0 ng	0.125 ng	0.070 ng	0.05 ng*
ANC 5s	0	0	0	0	1	16	13
ANC 10s	0	0	0	0	0	4	6
%AC 5s	100	100	100	100	98.0	68.6	61.8
%AC 10s	100	100	100	100	100	92.2	88.2
LNC 5s	0	0	0	0	1	13	11
LNC 10s	0	0	0	0	0	3	6
%LC 5s	100	100	100	100	93.8	18.8	31.3
%LC 10s	100	100	100	100	100	81.3	62.5

Table 1 defined: ANC = alleles not called, AC = alleles called, 5s = 5 seconds, 10s = 10 seconds, LNC = loci not called, LC = loci called
17 alleles/sample expected or 51 alleles/triplicate sample set, 17 loci/sample expected,
* 0.05ng only had two samples run at 5 seconds

Results

Sensitivity

- 88% of all samples had complete profiles with as low as 50 pg template DNA

Female/male mixtures

- Female DNA was not amplified at up to 200 times greater concentration than male DNA

Male/male mixtures:

- All alleles called except four in 90:10 mixtures
- The largest standard deviation was in 0.3 ng mixtures at 60:40

Table 2 Male/male mixture setup

Table 2 shows the setup for part one of the male/male mixture study.

Mixture	Major Contributor M49	Minor Contributor LC3
90:10	1.08 ng	0.12 ng
80:20	0.96 ng	0.24 ng
70:30	0.84 ng	0.36 ng
60:40	0.72 ng	0.48 ng
50:50	0.6 ng	0.6 ng

Table 2 includes male/male mixtures containing 1.2 ng total template DNA

Table 3. Male/Male Mixture Results

Table 3 shows the results from both parts one and two of the male/male mixture study. A sum of alleles called at both 5 and 10 seconds were recorded as well as the minor contributor and standard deviation.

	ANC 5s			ANC 10s			%AC 5s			%AC 10s		
	1.2ng	0.6ng	0.3ng	1.2ng	0.6ng	0.3ng	1.2ng	0.6ng	0.3ng	1.2ng	0.6ng	0.3ng
90:10	4	6	9	NA	3	7	95.70	80.65	70.97	NA	90.32	77.42
80:20	0	NA	5*	NA	0	2	100	NA	83.87	NA	100	93.55
70:30	0	NA	3	NA	0	1	100	NA	90.32	NA	100	96.77
60:40	0	NA	3	NA	0	2	100	NA	90.32	NA	100	93.55
50:50	0	NA	1	NA	0	0	100	NA	96.77	NA	100	100
	Av. % Minor Contributor 5s			Av. % Minor Contributor 10s			Standard Deviation % 5s			Standard Deviation % 10s		
	1.2ng	0.6ng	0.3ng	1.2ng	0.6ng	0.3ng	1.2ng	0.6ng	0.3ng	1.2ng	0.6ng	0.3ng
90:10	9.76	13.34	14.25	NA	10.61	13.44	3.71	6.08	2.55	NA	6.59	3.53
80:20	16.60	NA	20.38	NA	19.63	18.23	4.86	NA	7.34	NA	4.87	6.90
70:30	25.84	NA	30.33	NA	27.18	28.24	4.79	NA	8.85	NA	5.33	9.32
60:40	33.00	NA	40.06	NA	37.20	37.86	5.13	NA	8.66	NA	6.62	10.69
50:50	44.41	NA	42.79	NA	45.42	41.05	5.47	NA	9.58	NA	4.94	10.61

Table 3 defined: ANC = alleles not called, %AC = % alleles called, 5s = 5 seconds, 10s = 10 seconds, Av = average 17 alleles/sample expected or 51 alleles/triplicate sample set, 17 loci/sample expected
(*) one major allele not called DYS385a/b

Precision

- Sizing precision within a run had ≥ 99.9999% of all fragments fell within the ± 0.5 bp sizing range.
- Fragments > 300 bp had ≥ 82% falling within ± 0.5 bp

Contamination

- No contamination issues

Reproducibility

- All genotypes were reproducible

Accuracy

- Results matched those provided by NIST, therefore, the Y-STR kit proved to be accurate

Additional Studies

Stutter

- Comparison of ABI factory recommended stutter filters and Kentucky State Police
- Appropriate adjustments were made to meet

Artifact Identification

- All common artifacts were documented for casework purposes

Conclusions

The commercial Y-STR amplification kit validated is both sensitive and accurate. Female/male mixtures showed that this kit is sensitive to only human male DNA when female DNA concentrations are up to 200 times greater than that of male DNA. Male/male mixtures showed that not only were almost all alleles for all mixture samples called when at a concentration of 1.2 ng, but also major/minor contributors could be determined at 0.3 ng total DNA at loci without dropout. Stutter ranges were also determined and established. The KSP casework division will go online with this Y-STR amplification kit once validation protocols have been reviewed and approved.

Future Studies

- Increase concentration of female DNA in Female/Male mixtures to observe possible interference
- Increase PCR cycle number during amplification
- Analyze Male/Male mixtures with additional genotypes
- Perform post-amplification purification to remove primer peaks

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