

Validation of the Applied Biosystems AmpFISTR® Yfiler™ PCR Amplification Kit

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Abstract

The Applied Biosystems AmpF/STR® Yfiler™ PCR Amplification kit is a sensitive, precise human male specific kit. Validation studies showed no interference occurs due to the presence of female DNA, and identical profiles were produced between paternally linked males. Yfiler™ showed an optimal sensitivity range between 0.15-1.5 ng/μl and high precision. Successful amplification of DNA was seen in casework samples but inhibition can occur in the presence of rhodamine and NaCl. A comparison between Identifiler and Yfiler™ was performed during the non-probative study which demonstrated that while Identifiler is more sensitive to touch DNA, Yfiler™ yielded better profiles for sexual assault samples.

Introduction

- The Applied Biosystems AmpF/STR® Yfiler™ PCR Amplification Kit is used to produce male specific profiles by utilizing short tandem repeats at 17 loci in the SR Y region of the Y chromosome.

- Seven studies observing sensitivity, precision, inhibition, mixtures, family, nonhuman DNA, and non-probative casework with a comparison between Identifiler and Yfiler™, were performed to determine the Yfiler™ kit's optimal parameters for forensic casework samples.

Methods & Materials

- Organic extraction was performed on all samples using Pheol/Chloroform/Isoamyl Alcohol and a differential extraction was performed on mock sexual assault samples.

- Quantitation was performed using AB Quantifiler® Duo Quantitation kit on the AB 7500 Real-time PCR system.

- Amplification was performed using the AB AmpF/STR® Yfiler™ Amplification kit on a Genescan 9700 thermalcycler.

- Profile data was generated on the AB 3130xl Genetic Analyzer and data was analyzed using Genemapper® ID V3.

- All of the above methods were performed in accordance with the MDPD Forensic Services Bureau's standard operating procedures.

- With the exception of the non-probative, nonhuman, and family study, samples were collected from laboratory personnel via buccal swab or preexisting blood vial.

- Samples were analyzed using a 100 RFU minimum peak height threshold and a 50% major/minor determinant ratio.

Results

Sensitivity Study

Table 1.3: Profile Parameters at Various DNA Concentrations at Average Peak Heights

DNA Conc. (ng/μl)	Presence of Full Profile	Peak Morphology	(RFU's)	Artifacts Present
0.15	Yes	Normal	≤1200	≤2
0.30	Yes	Normal	≤2400	≤2
0.60	Yes	Normal	≤3800	≤2
1.25	Yes	Normal	≤6000	≥4
2.5	yes	Broader, some -A	≤8000	≥10
5	Yes	Broader, lots of -A	≤9000	≥12

Precision Study

Table 1.4: Standard Deviations Calculated from the Averages of Five Injections of Each Samples at Five Different Loci

Sample Name	DYS389 I	DYS448	DYS38 9II	DYS45 8	DYS393
007	0.076	0.046	0.081	0.043	0.091
BAVS 0.625	0.066	0.022	0.090	0.038	0.064
EGVS 0.625	0.078	0.083	0.091	0.041	0.077
JJVS0.625	0.005	0.055	0.075	0.052	0.017
VMVS 0.625	0.047	0.039	0.049	0.046	0.058

Inhibition Study

- Inhibitors used: wood oil, NaCl, rhodamine, leather, finger print powder, black denim

- Male blood samples were extracted and diluted to 1:10 and 1:100 ratios and incubated in the inhibitors for three days (total of 14 samples.)

- All samples yielded full profiles except the 1:100 rhodamine, the 1:100 NaCl which produced partial profiles, and the 1:10 and 1:100 finger print powder samples which produced no profiles.

- The lack of profiles from the finger print powder samples is due to the physical presence of the powder and not any chemical inhibition

Nonhuman Study

- Samples from: chicken, frog, mouse, pig, rabbit, trout, Old World monkey, and a chimpanzee were amplified with Yfiler™ and analyzed.

- No profiles were produced in any of the samples except for the chimpanzee.

- Allele calls in the chimpanzee profile were present in four loci but were uncharacteristic (>3 per loci) of expected human profile results.

Non-probative Study

- Samples ranging from touch to sexual assault samples from 6 closed cases were analyzed and compared with original profiles made using the Identifiler amplification kit.

Table 1.15: Profile Comparisons of Samples for Case 5

Sample Name	Sample Type	Yfiler™ Profile	Yfiler™ Result	ID: Profile	Identifiler™ (ID) Result:
13	Vaginal swab	F: Partial M: No Profile	Cannot Exclude 18	F: Full M: Full	Full Female Profile, No Male
14	Cervical swab	F: No Profile M: No Profile	No Result	F: Full M: Partial	F: Full Profile M: Inconclusive
15	Vaginal aspirate	F: No Profile M: None	No Result	F: Full M: None	F: Full Profile
18	Std male 1	Full Profile	Cannot be Excluded in 13F	Full	Female profile: no match

Mixture Study

Male:Female Mixtures:

- Male-Female mixture results using a constant concentration of male DNA, 1ng/μl, and increasing concentrations of female DNA at ratios of 1:0, 1:5, 1:10, 1:50, 1:100, and 1:200 showed no unusual allele calls and only a slight loss in peak height at ratios of 1:100 and 1:200.

- Male-Female mixture profiles for mixture ratios of 2.5:100, 1.25:100, 0.625:100, 0.31:100, and 0.16:100 showed no loss in peak height and unusual peaks were seen only at ratios of 2.5:100 and 0.625:100.

Table 1.9: Male-Male Mixture Profile Analysis at Various Ratios of Male DNA

Sample Name	MM1 1:0	MM2 19:1	MM3 9:1	MM4 4:1	MM5 2:1	MM6 1:1
Number of Distinguishable Loci	N/A	13	16	16	16	5
Sample Name	MM7 1:2	MM8 1:4	MM9 1:9	MM10 1:19	MM11 0:1	
Number of Distinguishable Loci	14	16	16	16	N/A	

Table 1.10: Male-Male-Male Mixture Profile Analysis at Various Ratios of Male DNA

Sample Name	MMMA (1:1:1)	MMMB (1:1:2)	MMC (1:2:1)	MMMD (2:1:1)
Number of Distinguishable Loci	0	6	5	6

Family Study

- Samples from: a father, paternal grandfather, maternal grandfather, and four male children of a family were amplified and analyzed.

- All members of the same paternal line, all except the maternal grandfather, possessed identical Yfiler™ profiles.

Conclusion

- The sensitivity study showed that the optimal DNA concentration range for producing profiles is between 0.2 and 2 ng/μl of DNA with the best profiles produced at a concentration of 1 ng/μl.

- The precision study demonstrated the high precision of the kit with the lowest standard deviation being 0.005 and the highest being 0.0912. This is well below the required 0.15 cut-off.

- The inhibition study suggested that NaCl and rhodamine can have notable effects as inhibitors if small DNA quantities are exposed to them.

- The family study showed that males of the same paternal lineage will have identical Yfiler™ profiles unless genetic mutation occurs.

- The nonhuman study demonstrated that the kit is human specific as none of the animal samples yielded profiles except the chimpanzee and the resulting profile was easily discernable as not being a valid profile.

- The female:male mixture study showed that the kit is male specific and not affected by the concentration of female DNA present in a sample.

- The male:male mixture study suggested that male profiles could be distinguished up to 1:2 ratios; the three male mixtures however could not be separated with any certainty.

- The non-probative study which compared Yfiler™ to Identifiler showed that while Identifiler was more sensitive to touch DNA, Yfiler™ yielded better results in sexual assault sample types.

- Further studies manipulating inhibition concentrations and further testing of Yfiler's sensitivity to touch DNA compared to Identifiler deserves further inquiry.

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