Integrated Use of the Quantifiler™ Y Human Male DNA Quantification Kit and the AmpFℓSTR® Yfiler™ PCR Amplification Kit in Sexual Assault Cases

Two kits from Applied Biosystems, the Quantifiler Y Human Male DNA Quantification Kit (PN 4343906) and the AmpFℓSTR Yfiler PCR Amplification Kit (PN 4359513), provide the most highly sensitive and successful quantification and amplification of male DNA available to date. Designed to be used in tandem, these kits form an integrated, sensitive and highly discriminating combination for analysis of sexual assault evidence, providing results that are accurate, reproducible and reliable. Among other benefits, the Quantifiler Y and Yfiler kits introduce a simple, more straightforward process that greatly reduces the need for repeat analysis and maximizes the amount of information that can be retrieved from mixed sexual assault samples and other difficult samples.

The Quantifiler Y kit first determines the quantity of male DNA present in a sample. If male DNA is present, the Yfiler kit, which provides a high level of discrimination, is used for short tandem repeat (STR) analysis (Figure 1). These kits are designed and optimized to integrate seamlessly with each other, and also with Applied Biosystems reagents and software. Developed and validated on Applied Biosystems thermal cyclers and capillary electrophoresis platforms, the kits include all components required for successful sexual assault casework.

Reliable, Reproducible, Real-time PCR

The Quantifiler Y kit was optimized for use with a simple protocol that is based on the 5' nuclease assay (real-time PCR) and TaqMan® probe-based technology. The kit uses PCR technology to specifically determine the amount of amplifiable human male DNA present in a sample. It also provides a more comparative indication of subsequent STR analysis than is possible with other quantification methods.

Quantifiler Y Human Male DNA Quantification Kit

The first step for successful analysis of sexual assault casework is quantifying the amount of amplifiable male DNA prior to PCR amplification. The Quantifiler Y kit is the first commercially available kit designed to provide this level of specificity. Additionally, a pre-formulated, internal PCR control (IPC) in each reaction allows the analyst to make informed decisions by quickly identifying samples that contain no male DNA or samples that contain a PCR inhibitor. The kit components have been optimized and developed to provide specific, robust quantification of male DNA within a range of 0.023 ng/µL – 50 ng/µL, or greater than 2,000-fold.

Analysis of Difficult Casework Samples

Detecting low levels of male DNA in a high background of female DNA samples, as is typically encountered in sexual assault cases, is difficult. The cause of this difficulty is that differential lysis of the male and female cellular fractions does not always result in complete separation of the fractions, resulting in difficulties in interpretation. Further, differential lysis is a laborious and time-consuming method of separation.

Differential extraction also is not applicable as a means of separating male and female cells when the male contributor has undergone a vasectomy or produces azoospermic semen. Additionally, when analyzing body fluids from more than one individual, e.g., two blood samples or a mixture of blood and saliva, differential lysis cannot separate the male and female

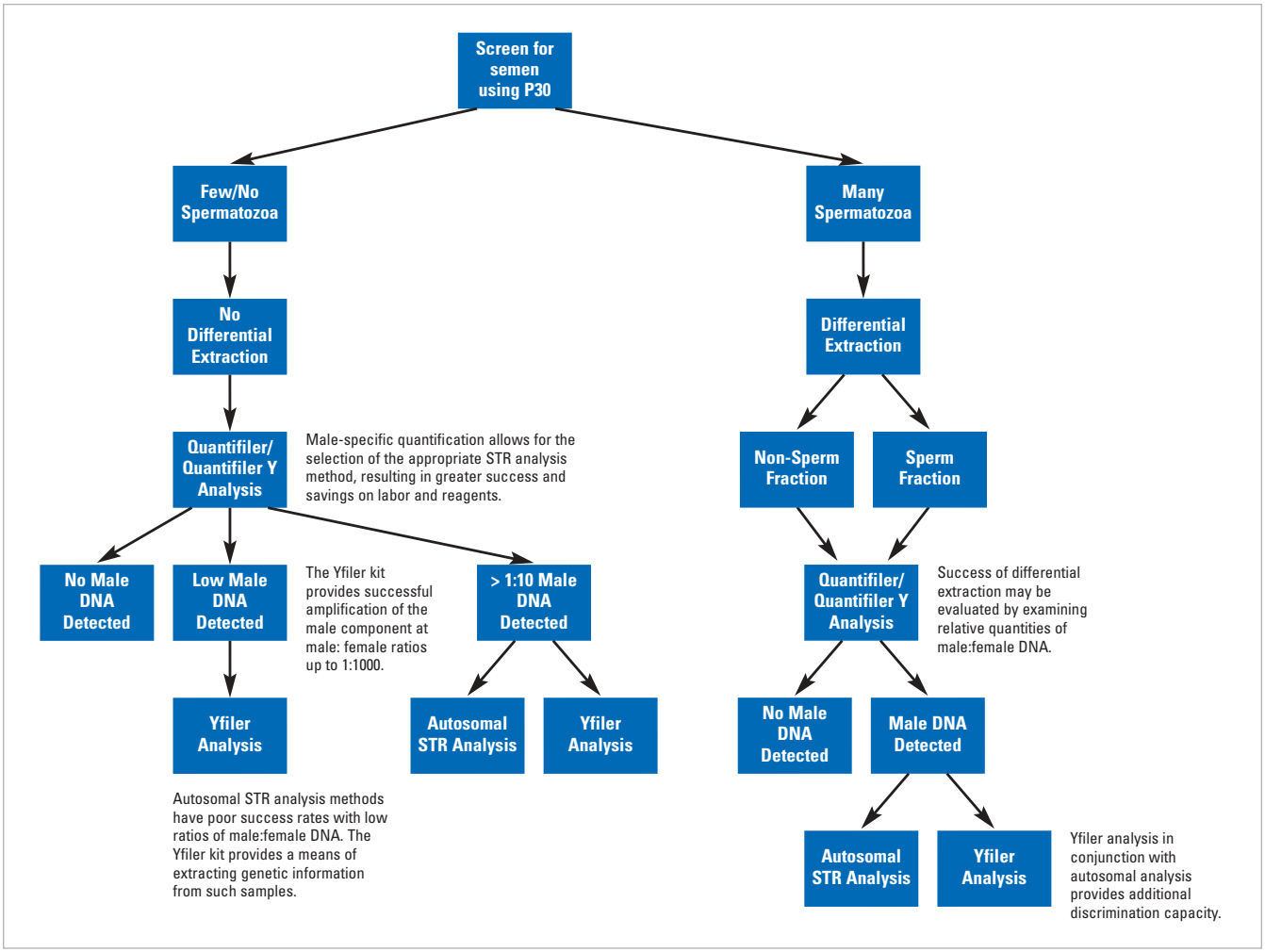

Figure 1. Workflow for the Quantifiler Y and Yfiler kits.

DNA fractions. In such cases, preferential amplification of the major component of the mixture (usually female DNA) can mask the genetic profile of the male component.

AmpFℓSTR Yfiler PCR Amplification Kit

The AmpFℓSTR Yfiler PCR Amplification Kit simplifies the interpretation of complex male:female DNA mixtures and helps resolve sexual assault cases. The Yfiler kit is especially successful with low levels of male DNA that are difficult to detect when found within a high background of female DNA. The kit chemistry is an STR assay that co-amplifies 17 Y chromosome STRs in a single PCR reaction. Included in the kit is an extensive allelic ladder that contains the most common 137 variants observed at each locus. The result is fast, accurate allele assignment.

Unmatched Performance and Sensitivity

The Yfiler kit can detect male DNA from a male:female ratio greater than 1:1000. This level of performance and sensitivity is especially important, not only for sexual assault cases, but also for laboratories required to analyze varied sample types that often comprise mixed male:female DNA. A full profile may be obtained using the Yfiler kit with as little as 62.5 pg of DNA.

The Yfiler kit uses five-dye fluorescence for automated DNA fragment analysis (Table 1). The capacity to amplify 17 Y-STR loci in a single reaction makes the Yfiler kit highly discriminating. Table 1 indicates the dye-labeled primers and the loci they detect. All loci are amplified simultaneously in a single tube and electrophoresed in a single capillary injection.

Dye	Detected Loci
6-FAM™	DYS456, DYS389I/II, DYS390
VIC®	DYS458, DYS19, DYS385 I/II
PET®	Y GATA H4, DYS437, DYS438, DYS448
NED™	DYS393, DYS391, DYS439, Y GATA C4 (DYS635), DYS392
LIZ®	This fifth dye is used for labeling the GeneScan™-500 Size Standard

Table 1. Fluorescent dyes in the Yfiler kit and detected loci

Features and Benefits of the Quantifiler Y and Yfiler Kits

Quantifiler Y Kit

- Provides precise quantification of male DNA
- When used in conjunction with the Quantifiler Human Quantification kit can be used to determine the ratio of male:female DNA allowing selection of the appropriate STR analysis method
- First kit exclusively for human male DNA quantification
- Valuable for sexual assault cases
- Contains IPC in each reaction for quick identification of inhibited samples or those lacking human DNA
- Quantifies samples with broad range of DNA concentrations from 0.023 ng/µL to > 50 ng/µL

Yfiler Kit

- Amplifies 17 Y-STR loci in a single PCR reaction, providing the greatest power of discrimination of any available Y-STR kit
- Detects low levels of male DNA in a high background (1:1000) of female DNA
- Greater sensitivity allows analysis of minimal amounts of DNA (< 100 pg)
- Uses five-dye detection chemistry for analysis of a greater number of loci within a smaller size range
- Improves the amplification success rate for challenging samples

Licensing and Validation

The Yfiler kit conveys specific rights for Paternity testing and, when used in conjunction with an Authorized Thermal Cycler, also conveys the PCR license rights for forensic and research use. The performance of the Yfiler kit is validated according to guidelines established by SWGDAM and DNA Advisory Board (DAB). The validation experiments and results are available in the Yfiler Kit User's Manual (PN4358101).

Conclusion

The tandem use of the Quantifiler Y kit and the Yfiler kit provides a complete solution for quantification and amplification of male DNA. The kits are expressly designed and validated for Applied Biosystems instruments, and integrate seamlessly with Applied Biosystems reagents and software. Additionally, the Quantifiler Y kit contains all components necessary for determining the quantity of male DNA in mixed samples or when the amount of male DNA is low, while the Yfiler kit provides the high level of discrimination that STR analysis demands.

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