

Utility of the [Auto]/[Y] Ratio When Using the Plexor® HY System

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The [Auto]/[Y] ratio can help predict the success of developing a male autosomal profile and direct the analyst to the appropriate STR amplification kit.

INTRODUCTION

Forensic scientists are often confronted with mixture samples, particularly sexual assault samples containing both male and female DNA. It is desirable to obtain the autosomal male profile for maximum statistical strength. However, female DNA present in the sample can mask male DNA alleles. The more female DNA that is present in the mixture in relation to male DNA, the fewer alleles from the male contributor that can be resolved. If there is too much female DNA in the mixture, autosomal STR analysis will reveal only a few or no alleles of the male profile (Figure 1). In this case, only Y-STR analysis is potentially informative. To avoid unnecessary effort and cost, it is desirable to have a measure that can direct the analyst to the appropriate STR amplification kit.

The Plexor® HY System^(h-n) quantifies human autosomal and Y DNA in one reaction. This article describes how the [Auto]/[Y] ratio, as calculated by the Plexor® Analysis Software, can help predict success or failure when developing a male autosomal profile.

CALCULATING THE [AUTO]/[Y] RATIO

The [Auto]/[Y] ratio is automatically calculated by the Plexor® Analysis Software, forensic release, which can be downloaded at: www.promega.com/plexorhy/. The [Auto] value signifies the concentration of total human autosomal DNA in a sample, and the [Y] value represents the concentration of human male DNA. The ratio is calculated as the quotient of autosomal and Y-chromosomal DNA concentrations.

INTERPRETING THE [AUTO]/[Y] RATIO

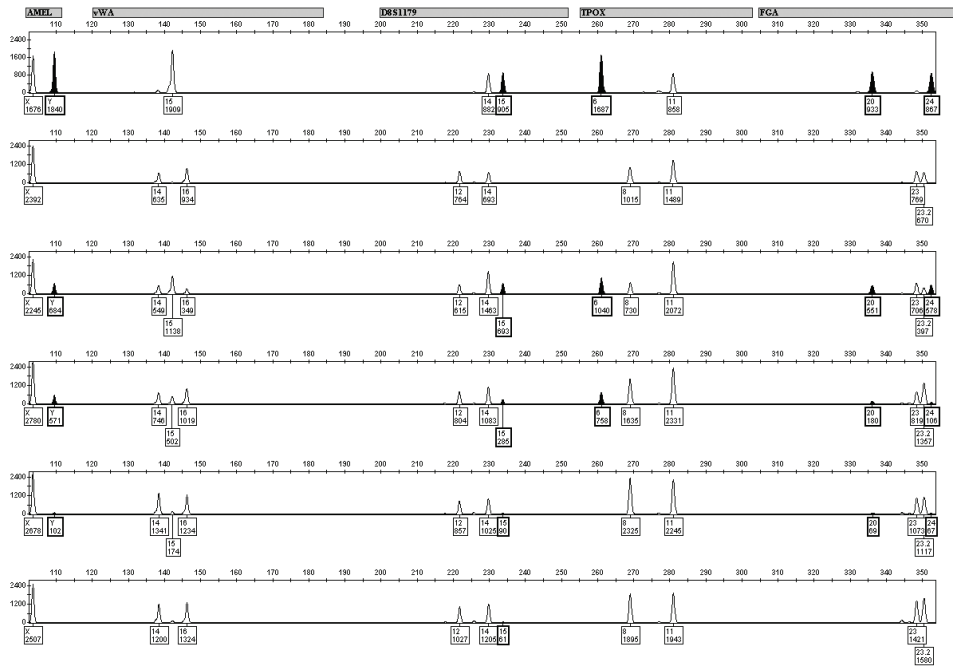
During the developmental validation of the Plexor® HY System Promega scientists performed experiments to relate the [Auto]/[Y] ratio to the percentage of male alleles observed in mixtures of male and female DNA (1). Figure 2 shows the results.

We found that for an [Auto]/[Y] ratio of:

- <10: PowerPlex® 16 amplification can be expected to yield a useful male STR profile.
- 10–80: The laboratory may choose to amplify both autosomal and Y-STRs or define a specific [Auto]/[Y] ratio above which one type of analysis is preferred, based on interpretation guidelines, laboratory preferences and internal validation results.
- >80: PowerPlex® Y amplification will yield greater information.

Thus, the [Auto]/[Y] ratio can help predict the success of an autosomal STR reaction. The ratio can be used to develop guidelines for STR analysis. Each laboratory should perform validation experiments to identify the appropriate STR analysis for [Auto]/[Y] ratios, as these decisions are affected by the choice of STR kits and laboratory interpretation guidelines.

TECH TIPS



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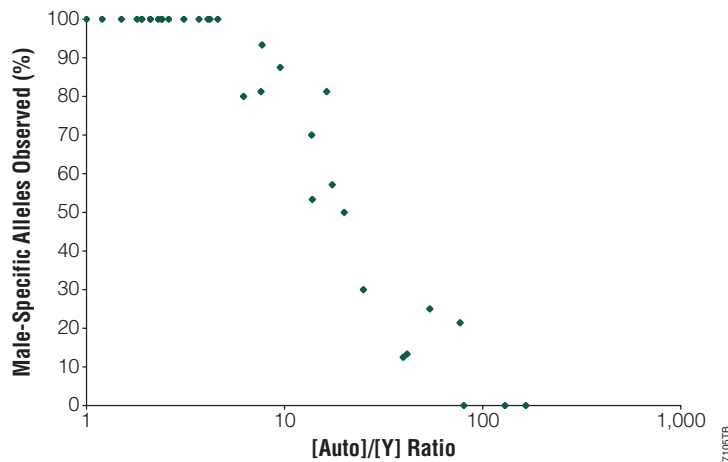
Figure 1. PowerPlex® 16 amplifications of a mixture series. Amplification of 0.5 ng of total DNA was performed to illustrate reduced amplification of male alleles as female DNA concentration increases. The TMR-labeled amplification products are shown for the male-only, female-only, 1:1 female:male mixture, 4:1 mixture, 16:1 mixture and 64:1 mixture amplifications (top to bottom panels). Highlighted peaks indicate male-specific alleles that are unshared and do not migrate in the stutter position of female alleles.

LEARN MORE ABOUT DNA QUANTIFICATION USING REAL-TIME PCR

To learn more about the Plexor® HY System for simultaneous quantification of human autosomal and male DNA, visit the Promega Web site at: www.promega.com/plexorhy/. There you will find a description of the system as well as links to publications and a free software download. If you are interested in a demonstration of the system, contact your local Forensic Regional Account Manager or Promega Branch Office or Distributor, or e-mail: genetic@promega.com

REFERENCE

1. Krenke, B.E. *et al.* (2009) Developmental validation of a real-time PCR assay for the simultaneous quantification of total human and male DNA. *Forensic Sci. Int. Genet.* 3, 14–21.



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Figure 2. Relationship of Plexor® HY [Auto]/[Y] ratio and ability to detect male alleles in autosomal STR analysis. Mixtures shown include duplicate analyses of approximately 1 ng/μl male DNA in a 1:1 to 64:1 female:male mixture. Percent of unshared male autosomal STR alleles is plotted versus the [Auto]/[Y] ratio observed for each sample (n = 34). Note that the X axis is plotted on a logarithmic scale.