TECH TIPS

The PowerPlex® Y System

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What is the PowerPlex® Y System?

The PowerPlex® Y System[(b)] allows amplification of 12 STR loci on the Y chromosome using 3-color detection. The system contains primers for the loci DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438 and DYS439. Amplified fragments are labeled with fluorescein (FL), carboxy-tetramethyl-rhodamine (TMR) or 6-carboxy-4´, 5´-dichloro-2´, 7´-dimethoxy-fluorescein (JOE). The 12 loci include the 9 European Minimal Haplotype loci and the core set of 11 SWGDAM-recommended loci. All 12 loci are amplified simultaneously in a single tube and analyzed in a single injection or gel lane. Fragment sizing can be performed using an internal size standard labeled with carboxy-X-rhodamine (Internal Lane Standard 600, Cat.# DG2611). An allelic ladder containing all 12 loci is provided to allow automated calling of alleles with the PowerTyper™ Y Macro (Cat.# DG3470). The PowerPlex® Y System is designed specifically for use with the ABI PRISM® 310 and 3100 Genetic Analyzers and ABI PRISM® 377 DNA Sequencer and is compatible with the ABI PRISM® 3700 DNA Analyzer and ABI PRISM® 373 DNA Sequencer.

Has a developmental validation paper been published for the PowerPlex® Y System?

Developmental validation has been completed, and the results will be published. Seventeen studies were performed by members of 7 laboratories. A variety of parameters were examined to test the sensitivity, robustness and reproducibility of the system. These included:

- Variation of cycle number
- Variation of reaction volume
- Concentration of AmpliTaq Gold® DNA polymerase
- Primer pair concentration
- Magnesium concentration
- Variation of annealing temperature
- Comparison of thermal cyclers
- Genotype consistency and reproducibility with standard specimens
- Sensitivity
- Male specificity
- Male/female mixture analysis
- Male/male mixture analysis
- Average stutter
- Sizing precision
- Nonhuman studies
- Nonprobative case studies
- Performance on different common instrument platforms
- Examination of reference standards and shared samples
- Population data

Does Promega have any resources for Y-STR population information?

Population studies have been completed and will be published. Promega has created the PowerPlex® Y Haplotype Database, an online tool that contains Y-STR haplotype data from a population study performed during initial validation of the PowerPlex® Y System. See “The PowerPlex® Y Haplotype Database” on page 15 of this issue. This database can be found at: **www.promega.com/ycalculator/**

What is the optimal amount of DNA template to add to PowerPlex® Y amplifications?

The PowerPlex® Y System is optimized to amplify 0.5–1ng DNA. Product specifications ensure amplification with 250pg of DNA, and Promega scientists and members of collaborating laboratories have obtained full profiles using less than 250pg of male DNA. When using more than the recommended amount of DNA, an imbalance of peak heights from locus to locus can be observed. In some cases, reducing the number of cycles in the thermal cycling program by 1 or 2 cycles can improve locus-to-locus balance, but we recommend re-amplifying a smaller amount of input DNA, if possible.

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Will female DNA interfere with the analysis of PowerPlex® Y data?

The PowerPlex® Y System specifically amplifies male DNA in the presence of female DNA. Each lot of the PowerPlex® Y System is tested for male specificity; an amplification with 100ng of female DNA must be devoid of amplification products. Promega scientists and members of collaborating laboratories have also amplified 0.1ng of male DNA in the presence of up to 100ng of female DNA (a 1,000-fold excess), and the male genotype is easily interpreted (1).

If I currently use other PowerPlex® systems, do I need to make a new matrix for the PowerPlex® Y System?

The PowerPlex® 16[b,c,d], PowerPlex® ES[b] and PowerPlex® Y Systems use the same dyes, so a new matrix is not required for instruments that are used with these systems. If the PowerPlex® systems have not been used with a particular instrument, a spectral calibration must be performed. The ABI PRISM® 310 Genetic Analyzer and the ABI PRISM® 377 DNA Sequencer use the Matrix FL-JOE-TMR-CXR (Cat.# DG2860), while the ABI PRISM® 3100 Genetic Analyzer requires the PowerPlex® Matrix Standards, 3100 (Cat.# DG3380). For a detailed discussion of matrix issues, please refer to reference 2. If you have questions or concerns regarding the matrix on your instrument or would like to discuss optimization of matrices, contact Promega Technical Services by phone at: 800-356-9526 in the U.S. or by email at: genetic@promega.com

Are there any special considerations for thermal cyclers used with the PowerPlex® Y System?

DNA samples can be amplified with the PowerPlex® Y System and the Model 480 or GeneAmp® PCR system 9600, 9700 or 2400 thermal cyclers. Specific protocol information can be found in the *PowerPlex® Y Technical Manual* #TMD018. Slight differences in balance between loci may be observed, depending on the thermal cycler and analysis instrumentation used.

What stutter bands might I see with the PowerPlex® Y System?

Stutter bands are a common amplification artifact associated with STR analysis. They are believed to result from slippage of the DNA polymerase as it crosses regions of DNA containing repeated elements (3,4). The pattern and intensity of stutter at a particular locus may differ, depending upon the primer sequence and annealing site, the labeled strand direction, and slight differences in reaction and amplification conditions. Promega has chosen primers that minimize the occurrence of stutter bands.

For samples with increased signal (>2,000RFU), stutter products are often observed one and occasionally two repeat units below the true allele peak. In addition to stutter peaks, several other stutter-like peaks, which differ in size from the true allele peak by a fraction of a repeat unit, can be observed at some of the PowerPlex® Y loci. The DYS392 locus and occasionally other loci with high signal may show a peak that is one repeat unit larger than the true allele. DYS19 and DYS389II can display low levels of products in the n–2 and n+2 positions (two bases below and above the true allele peak, respectively), with the DYS19 n–2 product being the most prominent. DYS437 and DYS385 also may show low-level peaks in the n–5 position, with DYS385 also displaying an n–9 product. The intensity of stutter and stutter-like peaks is directly related to signal intensity. Reducing the signal to <2,000RFU has been shown to minimize these artifacts. The initial stutter filters in the PowerTyper™ Y Macro are based on the observed stutter values obtained during initial development and are set at 2 standard deviations above the observed mean stutter value for each locus. Results may vary based on laboratory optimization. Internal laboratory validation should be performed. Additional stutter filters can be added to the PowerTyper™ Y Macro if desired. Contact Technical Services for assistance with PowerTyper™ Y Macro modifications.

REFERENCES

1. Krenke, B. *et al.* (2003) The PowerPlex® Y System. *Profiles in DNA* **6**(2), 6–9.
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3. Levinson, G. and Gutman, G.A. (1987) Slipped-strand mispairing: A major mechanism for DNA sequence evolution. *Mol. Biol. Evol.* **4**, 203–21.
4. Schlotterer, C. and Tautz, D. (1992) Slippage synthesis of simple sequence DNA. *Nucl. Acids Res.* **20**, 211–5.