TECH TIPS

Validation Questions and Answers

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Q: What variables should I consider when optimizing and validating an STR system?

A: Many factors contribute to signal intensity and sensitivity. One variable is the **instrument** used for analysis. During the PowerPlex® 16 System[a,c,d] validation study, 19 external laboratories compared ABI PRISM® 310 Genetic Analyzer sensitivities (1). Considerable differences were found between individual instruments. Differences in instrument sensitivity should be considered during validation so that laboratory protocols are written to allow analysts the flexibility to optimize their work for different instruments. Instrument sensitivity can change, particularly after service. A new matrix or spectral calibration must be created after service or major changes to an instrument.

The **amount of input DNA** can also affect signal intensity and balance between loci. Promega recommends 0.5–1ng of template, but laboratories have validated a wide range of template concentrations, depending on instrument sensitivity and other parameters. Initial experiments should include amplification of 0.5ng or 1ng of the positive control and of a known DNA to evaluate instrument sensitivity and matrix quality. Sensitivity studies typically cover a wide range of input DNA, ranging from 0.05–10ng.

Promega scientists have validated the PowerPlex® 16 System and PowerPlex® 16 BIO System[a,c,e] with several **thermal cyclers**, including the Perkin-Elmer GeneAmp® PCR System 9700, 9600 and 2400 and the Model 480 Thermal Cycler. Promega provides thermal cycling parameters for these instruments in the technical literature supplied with each kit. The type of **amplification tubes** used can also affect results. We recommend MicroAmp® 0.2ml thin-walled tubes for the GeneAmp® 9600, 9700 and 2400 thermal cyclers and 0.5ml GeneAmp® tubes for the Model 480.

The **amount of sample** loaded on the gel or capillary and the **injection parameters** can also affect results. We recommend loading 0.5µl of amplified sample on the ABI PRISM® 377 DNA Sequencer and 1µl of amplified sample on the ABI PRISM® 310 and 3100 Genetic Analyzers. Depending on instrument sensitivity, the amount of amplified sample can be increased or decreased. Intense samples may be diluted with 1X STR Buffer prior to loading. **Injection time** can also affect sample peak heights. We recommend a 3-second injection for the ABI PRISM® 310 and ABI PRISM® 3100, but individual laboratories have used injections of 2–10 seconds for the ABI PRISM® 310 and 5–30 seconds for the ABI PRISM® 3100, depending on instrument sensitivity.

Instruments are not static over time, and STR systems may vary slightly from one lot to the next. If protocols are created with flexibility in the injection times or the amount of amplified sample loaded, analysts can obtain maximum performance from their instrument and have the flexibility to re-analyze difficult samples without the need for re-amplification.

Q: Where can I obtain detailed information about the use of the PowerPlex® 16 System?

A: The technical manuals for Promega STR systems have detailed instructions and troubleshooting sections. They may be accessed online at:
www.promega.com/tbs/hmnid.htm

Promega has produced a "PowerPlex® 16 Training and Troubleshooting Guide" on CD-ROM. The CD provides detailed instructions for use of PowerPlex® 16 with the ABI PRISM® 310 Genetic Analyzer and the ABI PRISM® 377 DNA Sequencer. The CD also contains instructions on making a matrix for spectral calibration, amplification of samples, detection of amplified products and analysis of data using GeneScan® and Genotyper® software packages. An extensive troubleshooting section is included, making the CD an excellent resource for scientists new to the instrumentation or the PowerPlex® 16 System. Promega Technical Services can also provide information or answer questions about these systems and can be contacted by telephone in the U.S. at 1-800-356-9526 or by email at: genetic@promega.com

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Q: Is validation data available for Promega PowerPlex® 16 and PowerPlex® 16 BIO Systems?

A: The manufacturer's developmental validation, forensic casework validation and database validation (concordance) studies have been completed following TWGDAM (2) and DAB (3) guidelines and have been published (1,4,5). Reprints are available upon request from Promega.

Q: Are there recommendations for in-lab protocol validation?

A: In addition to TWGDAM guidelines and guidelines developed by individual laboratories, recommendations can also be found in the *Validation of STR Systems Manual*, a guide published by Promega and available at: **www.promega.com/techserv/apps/hmnid/referenceinformation/**

In addition to a summary of the manufacturer's validation, this manual contains recommended steps for internal validation in individual laboratories.

Q: During the validation process, I am observing bleedthrough peaks. What can I do to reduce or eliminate them?

A: When sample peak heights are below 2,000RFU, minimal bleedthrough should be apparent. The only exception to this is bleedthrough from the yellow channel into the red channel. Up to 15% bleedthrough of TMR signal into the CXR channel can be observed for the STR loci, and greater than 15% bleedthrough can be observed for the Amelogenin locus (approximately 104 and 110 bases in size). Bleedthrough into the internal lane standard (ILS) will not affect size calling, and the peak detection threshold may be increased for the red channel to reduce the number of bleedthrough peaks called.
If bleedthrough is unacceptable, a new matrix should be generated. The ABI PRISM® 310 Genetic Analyzer and 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, refer to *Profiles in DNA* (2003) **6**(1), 11–12 (www.promega.com/profiles/601/601_11.html). If you have questions or concerns about generating a matrix on your instrument or would like to discuss optimization further, you can contact Promega Technical Services by phone at 1-800-356-9526 or by email at: genetic@promega.com

Q: I am observing extra peaks in the red channel. Bleedthrough from yellow is less than 15%, but the peaks are called by the GeneScan® Software. What can I do to remove these peaks from my analysis?

A: If bleedthrough peaks are labeled in the CXR ILS, the analysis parameters can be adjusted. Increase the peak height threshold for the red channel to a point where the bleedthrough peaks are not identified by the GeneScan® Software and the ILS peaks are greater than the adjusted threshold. Changing the peak height threshold in the red channel will not affect the other channels. If the ILS peaks are low, additional ILS can be added to the samples. Likewise, if ILS peaks are high, the amount of ILS used should be reduced.

REFERENCES

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3. DNA Advisory Board (DAB) Guidelines: **www.cstl.nist.gov/biotech/strbase/dabqas.htm**
4. Budowle, B. *et al.* (2001) STR primer concordance study. *Forensic Sci. Int.* **124**, 47–54.
5. Budowle, B. and Sprecher, C. (2001) Concordance study on population database samples using the PowerPlex® 16 kit and AmpFlSTR® Profiler Plus™ kit and AmpFlSTR® COfiler™ kit. *J. Forensic Sci.* **46**, 637–41.