Ask Us: "Frequently Asked Questions"

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This article presents the answers to a number of "frequently asked questions" about STR* analysis using the *GenePrint*™ Fluorescent STR Systems.

Q: One locus seems to amplify better than another. Is this a result of primer imbalance?

A: Primers for the *GenePrint*™ PowerPlex™ Systems have been developed using the GeneAmp® PCR System 9600 Thermal Cycler for amplification and either the Hitachi FMBIO® II Fluorescent Scanner (for the PowerPlex™ 1.1 System) or the ABI PRISM® 310 Genetic Analyzer (for the PowerPlex™ 1.2 System) for detection of amplified products. Small differences in balance among loci or fluorophores are observed when using different thermal cyclers or detection instrumentation. The detection instrumentation itself can play a significant role in locus-to-locus balance. Our two ABI PRISM® 310 Genetic Analyzer instruments have shown significant differences in the relative efficiency of detection of various fluorescent dyes. When the two colors of the PowerPlex™ 1.2 System loci are balanced on one instrument, they are not balanced on the second instrument, even when using the same amplification product. In addition, the peak height display (i.e., relative fluorescent units (RFU)) of all alleles using the same amplified product is lower on one ABI PRISM® 310 Genetic Analyzer than on another.

However, the most dramatic differences in relative amplification product yields across loci come from one of the following situations. First, 1-2ng DNA template is recommended for use. Excessive amounts of DNA, especially 10ng or more, can lead to noticeable locus-to-locus imbalance in yields. The use of FTA™ punches for purification has also displayed lower yields of the larger loci using the PowerPlex™ 1.2 System. The amount of template available from an FTA™ punch is not known, but may be excessive for optimal performance. As described in the most recent technical manual for the PowerPlex™ 1.2 System, decreasing the number of cycles used in thermal cycling by 4 or 6 is recommended, to compensate for this property of FTA™ paper, and to provide good yield and balance. This effect is not observed with the PowerPlex™ 1.1 System for use with the Hitachi FMBIO® II Fluorescent Scanner.

Degraded sample template is a well-known cause of locus imbalance. As DNA is degraded to smaller pieces, the larger loci are the first to display diminished yield in multiplex amplification.

A third cause of locus imbalance, as well as an increase in stutter bands, is overloading of amplified sample material. Whenever the ABI PRISM® 310 Genetic Analyzer or ABI PRISM® 377 DNA Sequencer displays peak heights of more than 2000 RFU, there is a chance that large and small peaks may not be detected with the same efficiency. In this case, the representation of the smaller peaks is "relatively over-expressed" (i.e., a higher proportion of the total signal). When this occurs, dilute the sample in 1X amplification buffer before loading to lower the peak heights.

Suggested protocols for use of the *GenePrint*™ PowerPlex™ Systems or other *GenePrint*™ Multiplex Systems with a variety of thermal cyclers and detection instruments are available in the technical manuals, which are included without charge with each kit. Some protocols have also been reproduced later in this article.

Q: Do you have protocols for DNA amplification using the GenePrint™ *PowerPlex™ Systems and thermal cyclers other than the GeneAmp® PCR System 9600 Thermal Cycler?*

A: Thermal cyclers vary from one model to the next. Because of this, amplification protocols for STR analysis vary depending on the thermal cycler as well as on the *GenePrint*™ System used. The *GenePrint*™ PowerPlex™ Systems are optimized for use with the GeneAmp® PCR System 9600 Thermal Cycler. Other thermal cyclers, such as the Perkin-Elmer Model 480 or the GeneAmp® PCR System 9700 Thermal Cycler, can be used with the *GenePrint*™ PowerPlex™ 1.1 and 1.2 Systems. Small differences in the relative peak heights across loci may be observed with these thermal cyclers. Protocols for using the PowerPlex™ 1.1 and 1.2 Systems with the GeneAmp® 9700 Thermal Cycler are provided below. Protocols for use of the Perkin-Elmer Thermal Cycler Model 480 with the PowerPlex™ Systems are given in the technical manual provided with each product.

Amplification protocol for the *GenePrint*™ PowerPlex™ 1.1 System and the GeneAmp® PCR System 9700 Thermal Cycler

Ramps on this instrument are programmed as a percentage of the default ramp rather than by the number of seconds. We had excellent results with both AmpliTaq® and AmpliTaq Gold™ DNA polymerase.

Notes:

Use 0.2ml thin-walled MicroAmp® reaction tubes with this thermal cycler.

Ramp settings displayed below the hold settings are for the temperature ramp that follows the hold step.

When using AmpliTaq Gold™ DNA Polymerase, an additional incubation at 95°C for 11 minutes must be incorporated prior to initiation of the thermal cycling program.

Cycling profile:

96°C for 1 minute, then:

94°C for 30 seconds, ramp 100%
60°C hold for 30 seconds, ramp 29%
70°C hold for 45 seconds, ramp 23%
For 10 cycles, then:

90°C for 30 seconds, ramp 100%
60°C hold for 30 seconds, ramp 29%
70°C hold for 45 seconds, ramp 29%
For 20 cycles, then:

60°C for 30 minutes

Amplification protocol for the *GenePrint*™ PowerPlex™ 1.2 System and the GeneAmp® PCR System 9700 Thermal Cycler

Notes:

Use 0.2ml thin-walled MicroAmp® reaction tubes with this thermal cycler.

Ramp settings displayed in the cycling profile indicate the ramp to the temperature that follows.

When using AmpliTaq Gold™ DNA Polymerase, an additional incubation at 95°C for 11 minutes must be incorporated prior to initiation of the thermal cycling program.

Cycling profile:

96°C for 1 minute, then:

94°C for 30 seconds, ramp 100%
60°C for 30 seconds, ramp 29%
70°C for 45 seconds, ramp 23%
For 10 cycles, then:

90°C for 30 seconds, ramp 100%
60°C for 30 seconds, ramp 29%
70°C for 45 seconds, ramp 29%
For 22 cycles, then:

60°C for 30 minutes

Q: What is a matrix and why does it have to be generated?

A: Generation of a matrix file is crucial when using multi-dye systems on ABI detection instruments. This allows the machine to determine how much overlap will be seen with the different dyes. A matrix consists of at least 5 fluorescently labeled DNA fragments for each dye, run and analyzed in separate lanes. A matrix file should be generated every time a dye changes. When using the same dyes, a matrix need only be generated once per year.

Q: Are there any modifications to the cycling conditions of your STR systems?

A: A new amplification and cycling protocol has been developed that greatly improves the performance of the *GenePrint*™ Fluorescent STR Multiplex, F13A01, FESFPS, F13B, LPL (FFFL) with the 9600 thermal cycler. The cycling conditions when using AmpliTaq Gold™ and Gold ST*R 10X Buffer are given below; mineral oil is not needed.

Amplification protocol for the *GenePrint*™ Fluorescent STR Multiplex, F13A01, FESFPS, F13B, LPL (FFFL) with the GeneAmp® PCR System 9600 Thermal Cycler

Cycling profile:

95°C for 11 minutes, 1 cycle
96°C for 2 minutes, 1 cycle

ramp 50 seconds 94°C for 1 minute
ramp 34 seconds 60°C for 1 minute
ramp 25 seconds 70°C for 1.5 minutes
For 10 cycles, then:

ramp 45 seconds 94°C for 1 minute
ramp 30 seconds 60°C for 1 minute
ramp 25 seconds 70°C for 1.5 minutes
For 22 cycles, then:

60°C for 30 minutes

Q: Are the population statistics for the loci contained in the GenePrint™ *Systems published?*

A: The allelic frequencies for CSF1PO, TPOX, TH01, vWA, D16S539, D7S820, D13S317, D5S818, F13A01, FESFPS, F13B and LPL STR loci will be published in the November 1998 Journal of Forensic Sciences (JOFS), Vol. 43(6). Preprints are currently available from Promega and may be requested by e-mail at genetic@promega.com.

*See patent statement on page 2.