Ask Us

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Short tandem repeat (STR) polymorphisms are highly useful tools for human identification, paternity testing and genetic mapping (1). STRs contain tandemly repeated sequences that range from 3 to 7 base pairs in length (2-4). Alleles of STR loci are defined by the number of repeated sequences they contain and are easily amplified using the Polymerase Chain Reaction* (5-8). This allows analysis and interpretation of STR results in 1-2 days.

Q: How do STR systems work?

A: Genomic DNA is purified and the STR regions of interest are amplified. The amplification products, generally less than 400bp in size, are run on a 4% or 6% denaturing polyacrylamide gel. Allelic ladders containing fragments of the same size as several or all known alleles for each locus are run on the gel alongside the amplified samples. Comparison of sample and allelic ladders allows easy interpretation of amplified alleles. After electrophoresis, alleles are detected by silver staining, fluorescent detection, or the use of radioactivity. *GenePrint*™ STR Systems that utilize silver and fluorescent detection formats are available from Promega.

Q: Will these systems work with degraded DNA?

A: Degraded DNA is more amenable to STR analysis than VNTR analysis as the amplification products are short, generally under 400bp in size.

Q: How much DNA is needed for use with the GenePrint™ *STR Systems?*

A: The amount of template DNA is somewhat system-dependent; in general, the *GenePrint*™ STR Systems are designed for use with 1 to 25ng of template DNA.

Q: What are the common amplification artifacts seen with STR analysis?

A: Repeat slippage (9,10), also called "stutter bands", "n-4 bands" or "shadow bands", is a common phenomenon within STR analysis. This is due to the loss of one repeat during amplification and is seen as a band that is a full repeat smaller than the true allele. The frequency of the artifact varies with different loci as well as, to a lesser degree, primer design. We have selected loci and primers carefully to minimize the occurrence of this phenomenon. A second amplification artifact seen with STR analysis is due to terminal nucleotide addition (11,12). During amplification, *Taq* DNA Polymerase** adds a nucleotide, generally adenine, to the ends of the amplified DNA fragments. Terminal nucleotide addition artifacts appear because this process does not occur with 100% efficiency. The occurrence of the n-1 artifact (i.e., bands which have not received the terminal nucleotide) can be minimized by careful primer design as well as by the addition of a 60°C incubation for 30 minutes at the end of the amplification profile (13). The *GenePrint*™ STR Systems have been specifically engineered to minimize the occurrence of these artifacts.

Q: Will any Taq *DNA Polymerase function within the* GenePrint™ *STR Systems?*

A: Promega's STR systems have been developed for amplification using standard *Taq* DNA Polymerase. Specialized enzymes such as AmpliTaq Gold™ (Roche Molecular Systems) are not required for peak performance. If using AmpliTaq Gold™, use the GeneAmp® (Roche Molecular Systems) PCR buffer that is provided with the enzyme (instead of the 10X STR Buffer included in the *GenePrint*™ STR Systems) and add each dNTP to the reaction mix at a final concentration of 200µM. Use of AmpliTaq Gold™ also necessitates an additional incubation at 95°C for 11 minutes prior to the initiation of the thermal cycling profile. The STR Buffer (pH 9.0) currently provided with the *GenePrint*™ STR Systems does not function optimally with AmpliTaq Gold™ due to incompatibility with the pH of the modified enzyme.

Q: Are there any special considerations for the thermal cycler used with the GenePrint™ *STR Systems?*

A: All of the *GenePrint*™ STR Systems are optimized for the Perkin Elmer Model 480 Thermal Cycler with the exception of the *GenePrint*™ PowerPlex™ Fluorescent STR Systems which are optimized on the GeneAmp® PCR System 9600 Thermal Cycler. All of the *GenePrint*™ STR Systems are provided with protocols for use with both of these thermal cyclers. Customers have reported that the BIOTHERM (MJ Research) and the Perkin Elmer Model 2400 thermal cyclers also perform well.

Q: Are microvariant alleles seen within the GenePrint™ *STR Systems?*

A: Microvariants are alleles that differ from one another by lengths other than the repeat length (i.e., 4 bases). The occurrence of microvariants appears to be correlated with highly polymorphic STR regions and increased mutation rates (14,15). Because of this, we have chosen loci which are moderately high with respect to polymorphisms as well as those which have a minimal occurrence of microvariants. Microvariants are more common in many other STR systems which have been developed, adding complexity to allele assignment. There are two loci within the *GenePrint*™ STR Systems in which microvariants have been observed.

The TH01 locus contains the common 9.3 microvariant allele as well as the very rare 8.3 allele. The 3.2 allele has been observed within the F13A01 locus. Although microvariants can complicate interpretation, they are easily separated on a 4% denaturing polyacrylamide gel.

Q: What is the matching probability of the GenePrint™ *STR Systems?*

A: The matching probability increases with the number of STR loci that are amplified. The *GenePrint*™ PowerPlex™ STR System, in which eight loci are amplified in a single reaction, has a matching probability ranging from 1 in 118,000,000 for Caucasian-Americans to 1 in 261,000,000 for African-Americans. When using this system in combination with the *GenePrint*™ Fluorescent STR Multiplex-F13A01, F13B, FESFPS, LPL (FFFL Multiplex), the matching probability is 1 in 178,000,000,000 for Caucasian-Americans and 1 in 2,910,000,000,000 for African-Americans in just 2 reactions of 12 loci.

Q: What are the typical paternity indices for the GenePrint™ *STR Systems?*

A: The typical paternity index (PI) for the *GenePrint*™ PowerPlex™ STR System is 354 for Caucasian-Americans and 403 for African-Americans. When used in combination with the *GenePrint*™ Fluorescent STR Multiplex-F13A01, F13B, FESFPS, LPL (FFFL Multiplex) the PI value is 5,605 for Caucasian-Americans and 6,691 for African-Americans.

Q: What is included with the GenePrint™ *STR Systems?*

A: Each *GenePrint*™ System contains all of the materials required to amplify STR regions, with the exception of *Taq* DNA Polymerase. Each system includes sufficient STR Allelic Ladder to load every third lane. This means that each amplified sample can be run next to an Allelic Ladder to allow for easy identification of alleles. The *GenePrint*™ PowerPlex™ System also includes a fluorescent ladder which is labeled with carboxy-X-rhodamine. The Fluorescent Ladder (CXR), 60-400 Bases, contains 16 evenly spaced DNA fragments of 60, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, and 400 bases. This ladder can be used as an internal size marker to increase precision in analysis. The Fluorescent Ladder (CXR) and the *GenePrint*™ Multiplex Allelic Ladder can also be purchased separately.

Q: Which fluorescent dyes are used in the GenePrint™ *Fluorescent STR Systems?*

A: All of the *GenePrint*™ Fluorescent STR Systems which amplify 4 STR loci contain locus specific primers which are labeled with fluorescein. Within the *GenePrint*™ PowerPlex™ Fluorescent STR System, one primer for each of the loci D16S539, D7S820, D13S317 and D5S818 is labeled with fluorescein and one primer for each of the loci CSF1PO, TPOX, TH01 and vWA is labeled with carboxy-tetramethylrhodamine (TMR). The use of different fluorescent dyes allows simultaneous detection of overlapping loci within one reaction, in a single gel lane.

Q: Who do I contact with technical questions on the GenePrint™ *STR Systems or any other Promega product questions?*

A: Promega's Technical Services Department is committed to helping you with any questions you may have. Contact us either at 1(800) 356-9526 or by e-mail at: *genetics@promega.com*. Outside the U.S., please contact your local Promega Branch Office or distributor.

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