Ask Us: *GenePrint*® PowerPlex™ 2.1 System

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This article presents the answers to a number of questions about the new *GenePrint*® PowerPlex™ 2.1 System.

Q: What is included within the GenePrint® *PowerPlex™ 2.1 System?*

A: The *GenePrint*® PowerPlex™ 2.1 System contains Gold ST★R 10X Buffer, PowerPlex™ 2.1 Primer Pair Mix, K562 DNA, PowerPlex™ 2.1 Allelic Ladder Mix, Internal Lane Standard 600, Bromophenol Blue Loading Solution, Gel Tracking Dye and a Technical Manual.

Q: What is the Internal Lane Standard 600?

A: The Internal Lane Standard 600 (ILS 600 [Cat.# DG2611]) consists of carboxy-X-rhodamine (CXR)-labeled DNA fragments of known length. The fragment sizes are 60, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550 and 600 bases in length. The ILS 600 is designed to be used in each gel lane to increase precision in analyses. This practice reduces the number of allelic ladder lanes needed per gel and increases the number of lanes available for samples. As with the Fluorescent Ladder, CXR (Cat.# DG6221), fragments that are multiples of 100 bases are approximately twice the intensity as the others on a gel to help identify size range quickly when viewing results.

Q: How many loci can be amplified with the GenePrint® *PowerPlex™ 2.1 System?*

A: The *GenePrint*® PowerPlex™ 2.1 System allows simultaneous amplification of nine loci. These include Penta E, D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179 and vWA. As with the *GenePrint*® PowerPlex™ 1.1 System, one of the two locus-specific primers is labeled with either fluorescein or carboxy-tetramethyl-rhodamine (TMR). All nine of the loci are amplified simultaneously in a single tube and analyzed in a single gel lane.

Q: How were these loci chosen?

A: The loci within the *GenePrint*® PowerPlex™ 2.1 System include those loci that have been selected as standard loci throughout the world. In the United States, the Federal Bureau of Investigation requires that prior to inclusion in the Combined DNA Index System (CODIS) database, a DNA profile must include a genotype for each of the thirteen standard core loci. Loci were selected so that a combination of the *GenePrint*® PowerPlex™ 1.1 and 2.1 Systems gives the ability to amplify all thirteen loci in two reactions.

Outside the United States, the FGA, D21S11, TH01 and vWA loci have been established by INTERPOL as the European standard, and the European Network of Forensic Science Institutes (ENFSI) recommends that in addition to these four loci, the D3S1358, D8S1179 and D18S51 loci should also be used in laboratories throughout Europe.

In addition to providing the standard loci, the *GenePrint*® PowerPlex™ 2.1 System contains a newly introduced pentanucleotide repeat locus, Penta E. This pentanucleotide repeat locus is very polymorphic and has been shown to have a low occurrence of 'stutter' artifact (less than 1–2%). These two characteristics create an advantage in the interpretation of complicated mixtures.

Three loci (vWA, TH01 and TPOX) in *GenePrint*® PowerPlex™ 2.1 System are also found in the *GenePrint*® PowerPlex™ 1.1 System. This overlap of loci between the two multiplexes can be used as a form of quality control because it is highly unlikely that a mix-up of samples analyzed with both systems could go undetected.

Q: Are there microvariants found with this system?

A: The presence of microvariants can make it difficult to interpret data. The *GenePrint*® PowerPlex™ 2.1 System includes loci such as FGA and D21S11 that are highly polymorphic but also contain many mirovariant alleles. Most of the known microvariants have been included for each of the loci within the allelic ladder to provide easy interpretation. Penta E, also highly polymorphic, does not display frequent microvariants.

Q: What type of polyacrylamide gel should be used with the GenePrint® *PowerPlex™ 2.1 System?*

A: We recommend using a 43cm gel to ensure that sufficient separation of the alleles within each locus is obtained. The extra length of the gel assists in sufficient separation of the larger alleles in the allelic ladder. The largest allele within the allelic ladder is 474bp.

Due to the multiple microvariants of D21S11 and FGA included in the allelic ladder, we recommend using a 5% Long Ranger™ polyacrylamide gel, which gives better one-base resolution. Although a 4% or a 5% polyacrylamide gel can be used, we find that the band-finding software of the Hitachi FMBIO® II Fluorescence Imaging System performs better when a 5% Long Ranger™ polyacrylamide gel is used.

Either the 50% Long Ranger™ gel solution or the Long Ranger™ Singel™ Packs may be used. These can be purchased through the FMC Corporation.

Q: How much DNA should be used with the GenePrint® *PowerPlex™ 2.1 System?*

A: We recommend using 1–2ng of DNA in a 25µl reaction. We find that if more template DNA is used an imbalance between the loci will be seen, in which bands of the larger loci will generally be fainter than those of the smaller loci. This is seen particularly when using 5ng or greater template DNA. If the amount of template DNA cannot be reduced, the imbalance can be minimized if the number of cycles within the amplification program is decreased by two or four cycles. Locus imbalance can also be seen if 1–2ng of DNA is used in a reaction volume <25µl.

Q: Which Taq *DNA polymerase should be used?*

A: The *GenePrint*® PowerPlex™ 2.1 System has been optimized for use with AmpliTaq Gold™ DNA polymerase and the Gold ST★R 10X Buffer included in the kit.

Q: Are there any thermal cycler considerations?

A: The *GenePrint*® PowerPlex™ 2.1 System has been optimized for use with the GeneAmp® PCR System 9600 thermal cycler and AmpliTaq Gold™ DNA polymerase. Protocols for use with the GeneAmp® PCR System 9700 and 2400 thermal cyclers and the Perkin-Elmer Model 480 are given within the Technical Manual for the *GenePrint*® PowerPlex™ 2.1 System. Other thermal cyclers have not been tested.

Q: Can the GenePrint® *PowerPlex™ 2.1 System and the* GenePrint® *PowerPlex™ 1.1 System be run on the same gel?*

A: Yes, both systems can be run on the same gel; however, separate analyses need to be performed to account for the different allelic ladders for the two systems.

Q: Can the GenePrint® *Sex Identification System, Amelogenin (TMR) or (Fluorescein), be used with this system?*

A: No, neither of the *GenePrint*® Sex Identification Systems can be used with the *GenePrint*® PowerPlex™ 2.1 System. Because the 212 and 218 chromosomal repeats of the Amelogenin locus align with the D8S1179 locus for the TMR system and the D21S11 locus for the fluorescein system, the Amelogenin alleles would not be distinguishable. However, the Amelogenin TMR primers can be added to the *GenePrint*® PowerPlex™ 1.1 System.

Q: What are the typical Paternity Indices, Power of Exclusion and Matching Probability of the GenePrint® *PowerPlex™ 2.1 System?*

A: The typical Paternity Indices for the *GenePrint*® PowerPlex™ 2.1 System are 1 in 13,130 for African-Americans, 1 in 13,199 for Caucasian-Americans, 1 in 3,250 for Hispanic-Americans and 1 in 41,800 for Asian-Americans. The Power of Exclusion values for the *GenePrint*® PowerPlex™ 2.1 System are 0.9999219 for African-Americans, 0.9999242 for Caucasian-Americans, 0.9997134 for Hispanic-Americans and 0.9999759 for Asian-Americans. The Matching Probabilities for the *GenePrint*® PowerPlex™ 2.1 System are 1 in 3.0×10^{11} for African-Americans, 1in 8.46×10^{10} for Caucasian-Americans, 1 in 1.02×10^{11} for Hispanic-Americans and 1 in 1.52×10^{11} for Asian-Americans. Statistical analysis for each locus can be found in the GenePrint® *PowerPlex™ 2.1 System Technical Manual* #TMD011 (1).

REFERENCE

1. GenePrint® *PowerPlex™ 2.1 System Technical Manual* #TMD011, Promega Corporation.