IMPROVED PRIMER PAIRS FOR THE SE33 LOCUS IN THE POWERPLEX® ESI 17 PRO SYSTEM

Robert S. McLaren, Ph.D. [1], Jaynish Patel, Ph.D. [1], Carolyn R. Hill, M.S. [2], Margaret C. Kline, M.S. [2], John M. Butler, Ph.D. [2], Douglas R. Storts, Ph.D. [1],
[1]Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711, USA
[2]National Institutes of Standards and Technology, 100 Bureau Drive, Gaithersburg, MD 20899, USA

In September 2009 Promega launched the PowerPlex® ESX and ESI Systems to meet the needs of the next-generation European STR genotyping systems. These systems include five new loci (D1S1656, D2S441, D10S1248, D12S391, and D22S1045) recommended by the European Network of Forensic Science Institutes (ENFSI) and the European DNA Profiling Group (EDNAP) for expanding the European Standard Set (ESS) loci. Both systems are available with or without primers for amplifying the SE33 locus. The PowerPlex® ESX Systems are configured with the new ENFSI/EDNAP loci D10S1248, D22S1045 and D2S441 as miniSTRs (<125 bases) and D12S391 and D1S1656 as midiSTRs (125-185 bases). The PowerPlex® ESI Systems are configured with the existing ESS loci as small as feasible while providing the new ENFSI/EDNAP loci as larger amplicons.

Initial population studies performed in collaboration with the NIST Forensic/Human Identity Project Team, did not reveal any significant discordance at the SE33 locus between the PowerPlex® ESX and ESI Systems, or with shared loci in other commercial kits. Since this initial publication, there have been several reports in samples of West African descent of off-ladder alleles at the SE33 locus in amplifications performed with the PowerPlex® ESI 17 System, but not with the PowerPlex® ESX 17 System. These alleles migrated anywhere from 0.6 to 0.9 bases larger than the allele that was expected. The root cause of this migration shift is a set of up to three separate SNPs residing within the PowerPlex® ESI 17 SE33 amplicon, anyone of which is capable of causing this shift in migration. Each SNP on its own is capable of disrupting a stem-loop structure in the amplicon, which appears to be the basis of the altered migration. To alleviate this discordance we developed a new SE33 primer set for inclusion in the PowerPlex® ESI 17 Pro System. The new PowerPlex® ESI 17 Pro System incorporates changes to the SE33 primer pair based on the PowerPlex® ESX SE33 primer sequences. This change allows robust amplification of DNA samples containing the so-called “X.1/X.3 SNPs” and allows generation of amplicons which are not affected by the migration shift caused by these mutations. As the 3’ end of these new primer pairs are of the exact same sequence as those found in the PowerPlex® ESX 17 System, there is expected to be minimal if any effect on concordance at the SE33 locus given the high level of concordance between this primer set and the “Polymeropoulos primers”. Finally, the changes to this primer pair have been shown to eliminate non-specific amplification seen in some casework samples with the PowerPlex® ESI 17 System.