Improved Primer Pairs for the SE33 Locus in the PowerPlex® ESI 17 Pro System

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Abstract

The SE33 locus is one of the most polymorphic markers used in human identification. However, it also possesses a higher mutation rate than other STR loci which results in multiple microvariants both within the repeat and in the flanking regions. Such flanking region mutations can generate discordant allele calls between multiplexes using different primer pairs. Classically such discordance is due to a mutation within a primer binding site. However, a discordance observed in samples of primarily West African descent between the PowerPlex® ESI 17 and ESX 17 Systems does not fall in this category. The root cause is a set of up to three separate SNPs residing within the PowerPlex® ESI 17 SE33 amplicon which are capable of disrupting a stem-loop structure present in the wild-type sequence (1). These mutations retard the migration of the amplicon on capillary electrophoresis by 0.6 to 0.9 bases relative to the wild-type amplicon. As single-stranded DNA with a partial double-stranded region is known to migrate faster (2) these data are consistent with a stem-loop structure forming in the wild-type sequence post-electrokinetic injection while the mutants are unable to form this structure and hence migrate normally. Formation of a stem-loop (even one with a ΔG of only -5.77kcal/mol) is due to an inability to precisely control the temperature on the exposed cathode end of an array, thereby resulting in an incomplete denaturing environment (1). To alleviate this discordance we developed a new SE33 primer set for inclusion in the PowerPlex® ESI 17 Pro System. This change allows robust amplification of DNA samples containing these SNPs without 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. 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.

Introduction

Both the PowerPlex® ESX and ESI Systems released by Promega in September 2009 to meet the requirements for the new expanded European Standard Set (ESS) of loci are available with or without primers for amplifying the SE33 locus. Initial population studies did not reveal any significant discordance at the SE33 locus between the PowerPlex® ESX and ESI Systems, or with other commercial kits (3). 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 from 0.6 to 0.9 bases larger than expected. There are three separate SNPs residing within the PowerPlex® ESI 17 SE33 amplicon, which are each capable of causing this effect. All three of these SNPs are capable of disrupting a stem-loop structure (1). To alleviate this discordance we developed a new SE33 primer set for inclusion in the PowerPlex® ESI 17 Pro System which allows robust amplification of DNA samples containing these SNPs without the migration shift. These same changes to the SE33 primer pair are shown here to eliminate non-specific amplification seen in some casework samples with the PowerPlex® ESI 17 System.

Discordance Between PowerPlex® ESI 17 and ESX 17 SE33 Amplicons in DNA Samples with SNP Affecting Migration

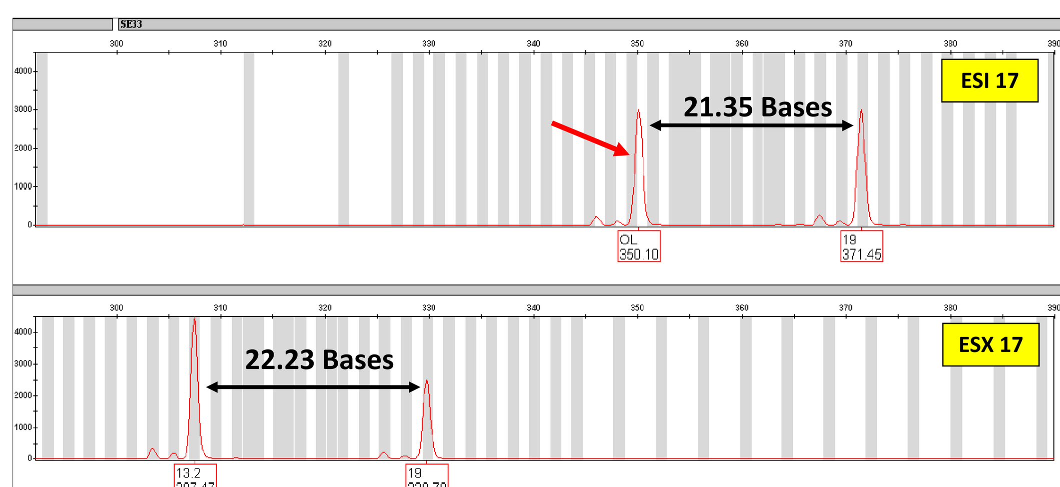

Figure 1. Amplification of GT37190 DNA with PowerPlex® ESI 17 and ESX 17 Systems. The SE33 locus range for both PowerPlex® ESI 17 and ESX 17 amplifications of DNA sample GT37190 are shown. The 13.2 allele that runs off-ladder in PowerPlex® ESI 17 is indicated by a red arrow.

SE33 Sequence Differences Identified at NIST

Total African American Samples Tested

258 Populations samples

190 Father/Son samples

46 New blood samples (not in ref #3)

12 samples with SNPs affecting migration out of 494 African American samples (2.43%)

9 from original study (not detected due to resolution issues on NIST 3130xl)

Structure of SE33 Locus and Location of Stem-Loop

Figure 2. SE33 Locus and Stem-Loop Structure with Location of SNPs. The SE33 locus is shown with the location of the "Polymeropoulos primers" (4) indicated in ***green italics***. The repeat structure is in bold with the underlined portion representing the region used for determining allele number (above sequence is a 25.2 allele). Inverted repeat responsible for stem-loop is indicated by **blue arrows** below sequence. Bases in **red** (in sequence and stem-loop) indicate location of SNPs (60, 61, and 68 bases downstream of repeat structure). A transition mutation at any one of these positions (G to A or C to T) results in a shift in migration on the capillary relative to the wild-type sequence of almost 0.6-0.9 bases.

PowerPlex® ESI 17 Pro SE33 Primer Set Unaffected by Stem-Loop SNPs

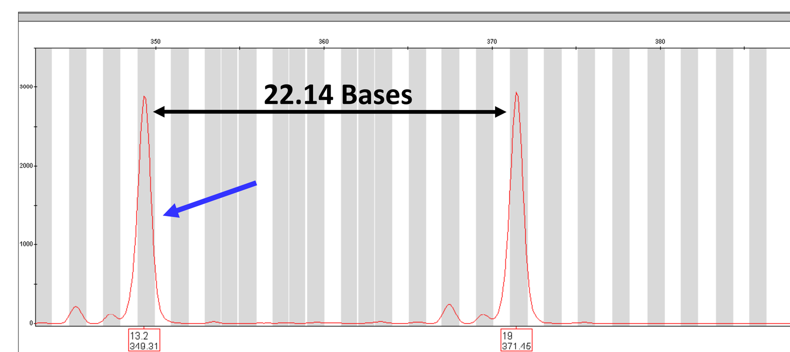

Figure 3. Amplification of GT37190 DNA with PowerPlex® ESI 17 Pro
The SE33 locus is shown for the DNA sample GT37190. The 13.2 allele that ran off-ladder with PowerPlex® ESI 17 (see Figure 1) now runs on ladder (blue arrow). Note size difference between alleles compared to amplification performed with PowerPlex® ESI 17 and ESX 17 in Figure 1.

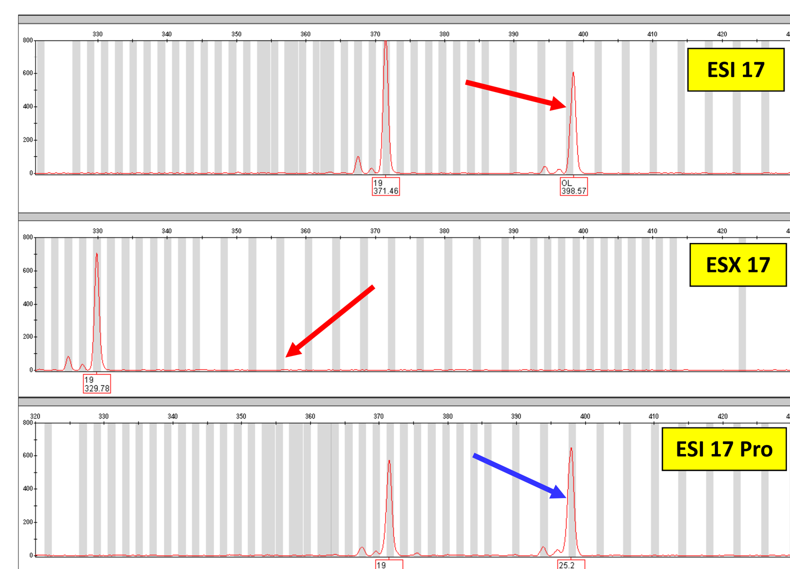

Figure 4. Amplification of PT83874 DNA with PowerPlex® ESI 17, ESX 17 and ESI 17 Pro
The SE33 locus is shown for the DNA sample PT83874. The 25.2 allele present in this sample migrates off-ladder with PowerPlex® ESI 17 and drops out with PowerPlex® ESX 17 (red arrows). This DNA sample contains a C to T SNP 60 bases downstream of the repeat structure. A SNP at this position has been shown to result in allelic drop-out with PowerPlex® ESX 17 (1). Amplification with PowerPlex® ESI 17 Pro generates an amplicon that runs on-ladder as the correct 25.2 allele call (blue arrow).

The PowerPlex® ESI 17 Pro System Eliminates Non-Specific Amplification

Non-specific amplification from non-human DNA samples was not detected during development of the PowerPlex® ESI 17 System (5). While these studies are useful in determining the likelihood of amplification from non-human DNA sources they are unfortunately limited by the fact that it is impossible to model every possible organism that might be encountered in a casework setting. Recently some labs encountered casework samples that showed apparent non-human amplification peaks, specifically in the red dye channel. These background peaks were eliminated when these samples were amplified with the PowerPlex® ESI 16 System, which lacks the SE33 primer pair. Amplification of such samples with the PowerPlex® ESI 17Pro System containing the new SE33 primer pair eliminates these amplification products.

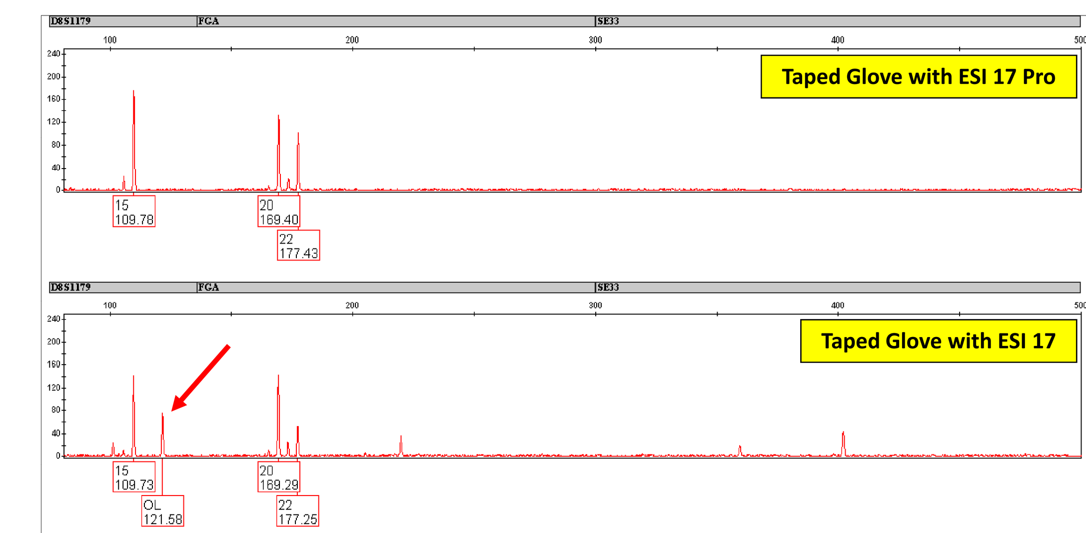

Figure 5. Amplification of Casework Sample with PowerPlex® ESI 17 and ESI 17 Pro Systems. Results are shown for a casework sample that was previously shown to exhibit an off-ladder peak at 121.58 bases in D8S1179 with PowerPlex® ESI 17 (indicated by arrow). Amplification with PowerPlex® ESI 17 Pro eliminates this peak along with other peaks under 50 RFU in FGA and SE33. Variation in peak heights is due to the low level nature of the available tested DNA sample.

Summary

- "Wild-type" SE33 amplicons contain a sequence capable of forming a stem-loop in single-stranded DNA. This structure appears to form in denatured DNA at the exposed cathode end of capillary arrays post-injection where it is difficult to achieve precise temperature control.
- Naturally occurring disruptive mutants of this stem-loop result in an altered migration on capillary electrophoresis instruments relative to "wild-type" sequences.
- SE33 amplicons generated with the PowerPlex® ESI 17 System include this stem-loop and are therefore affected by these mutants.
- The new SE33 primer pair in PowerPlex® ESI 17 Pro is not affected by the migration effect of the stem-loop or mutations that affect its formation.
- PowerPlex® ESI 17 Pro SE33 primer pair alleviates issues observed with amplification of non-human DNA in specific casework samples.

References

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