Why Use a Size Marker and Allelic Ladders in STR Analysis?

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GENE PRINT™ The advantages of including generic size markers in DNA typing have been recognized from the very beginning of VNTR analyses (1,2). Fragment lengths generated from DNA samples are calculated by direct comparison of their electrophoretic migration to that of a constant set of fragments of known size. However, size markers offer two additional benefits. Because the same markers are included in each gel in the same amounts, the reproducibility of the detection method is confirmed by observing the intensity of the marker fragments. In addition, the presence of the same multiple bands in several lanes in different regions of the gel provides information regarding possible lane-to-lane variation in the electrophoretic migration of sample material.

Size markers only give accurate readings of DNA length when the marker and unknown fragment have the same sequence and the same size.

It is not generally appreciated that the accuracy of determining base length using size markers is dependent upon the marker being used. It has been known for many years that DNA migration in gel electrophoresis is dependent not only upon the length of the DNA sample, but also upon its nucleotide sequence (3). Thus, size markers only guarantee accurate readings of DNA length when the marker and unknown fragment have the same sequence and the same size. If the sequences of marker and sample fragments are not the same, the migration of sample fragments relative to marker fragments has the potential to shift in response to changing environmental parameters. Examples of such parameters include the percentage acrylamide or cross-linker in the gel, the amount of salt in the buffer, the electrophoresis voltage, or the coupling of a fluorescent molecule to the sample for detection purposes.

For these reasons, we have long recommended the use of allelic ladders in the analysis of STR* systems (4-6). Because the components of the allelic ladder and the sample fragments have the **same length** and the **same sequence**, they will migrate the **same distance** during electrophoresis regardless of the types of environmental changes described above.

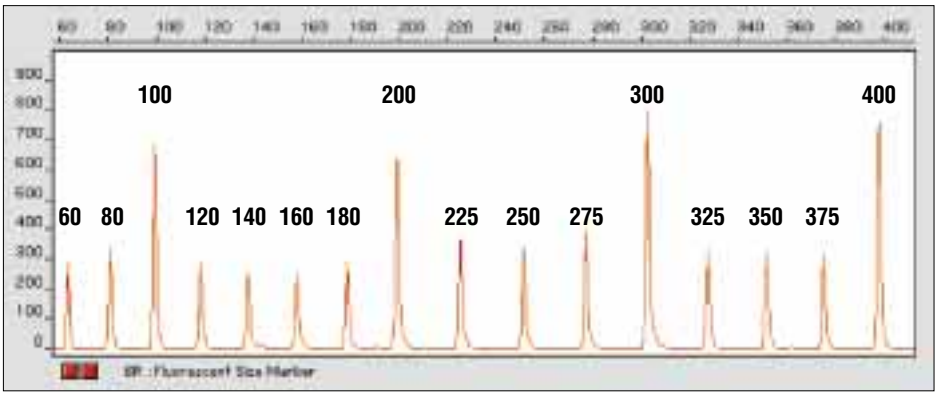

Figure 1. The Fluorescent Ladder (CXR), 60-400 Bases, detected using the ABI PRISM™ 377 DNA Sequencer. This image displays the 16 fragments (60, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, and 400 bases) contained within the fluorescent size marker. The carboxy-X-rhodamine-labeled fragments appear in red with their respective sizes, in bases, listed above each fragment.

In our early work with STRs, the discovery and characterization of microvariant alleles also illustrated the importance of including allelic ladders to provide precision and accuracy in STR analysis (4,7). Microvariant alleles are alleles that differ from one another by fewer bases than the true tetranucleotide repeat; examples include TH01 allele 9.3 and F13A01 allele 3.2 (4,7).

Since the development of STR allelic ladders (4) it has been recommended that allelic ladders be included in several lanes of each gel. The *GenePrint*™ Multiplex Systems developed for silver stain and fluorescent detection include enough allelic ladder to load in every third gel lane, ensuring that allelic ladders are placed next to all samples. The two-color, eight-locus, *GenePrint*™ PowerPlex™ Systems also include an additional size marker – the Fluorescent Ladder (CXR), 60-400 Bases (Figure 1).

The Fluorescent Ladder (CXR), 60-400 Bases, consists of 16 fragments which are evenly spaced across the range of 60 to 400 bases (60, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, and 400 bases) (Figure 1). These fragments have been labeled with the fluorescent dye, carboxy-X-rhodamine to allow placement of the marker in every lane. This provides a means for the detection of lane-to-lane variation in product migration in the gels, which can then be corrected using the detection instrument software. Instrument software also allows comparison of migration of the known sizes of allelic ladders with the unknown alleles in test samples. This provides the most accurate determination of alleles possible. By including the size marker in each lane, the number of lanes containing allelic ladder can be reduced to one or two, increasing the number of lanes available for samples, and generating high throughput (8).

Table 1. Lengths of Alleles of the TH01 Locus Determined By Sequence Analysis and By Different Fluorescence Detection Instrumentation.

Locus	Allele	Actual Size, Bases (Sequence Analysis)	Hitachi FMBIO® Fluorescent Scanner (4% Gel)	ABI PRISM™ 377 DNA Sequencer (5% Gel)
TH01	11	203	201.95	201.78
TH01	10	199	197.95	197.80
TH01	9	195	194.05	193.83
TH01	8	191	190.12	189.84
TH01	7	187	186.12	185.85
TH01	6	183	182.10	181.85
TH01	5	179	178.14	177.88

Figure 2 illustrates simultaneous electrophoresis and detection of the *GenePrint*™ PowerPlex™ 1.2 System (beta-test version 2.0) and the Fluorescent Ladder (CXR) with the ABI PRISM™ 377 DNA Sequencer. Figure 3 illustrates the *GenePrint*™ PowerPlex™ 1.1 System and the Fluorescent Ladder (CXR) detected using the Hitachi FMBIO® Fluorescent Scanner.

Table 1 illustrates the fact that sequence content and electrophoretic conditions may alter the relative migration of the size marker and STR alleles. The table displays the actual lengths of alleles for the TH01 locus in the ladder as determined by sequence analysis and comparison with GenBank® (U.S. Dept. of Health and Human Services) entries. It also shows the sizes calculated using the size marker and different gel electrophoresis conditions used with the two different fluorescence detection instruments. Note that the two instruments calculate the STR allele sizes differently due to the use of different electrophoresis conditions and 4% and 5% gels, respectively. This changes the relative migration of size marker and amplified STR fragments. Neither instrument calls exact integral lengths, and the assigned lengths differ from sequence-determined lengths. Using this method, different loci will vary from their sequenced lengths by different amounts. However, within an individual locus, the assigned lengths differ from each other by approximately four bases, as expected for true tetranucleotide variations.

The software included with both the ABI and Hitachi instruments calls the alleles by comparing the relative migration of the size marker fragments with those of the allelic ladders in the same lane. An acceptable measurement tolerance is assigned for each allele based on repeated runs of size marker with

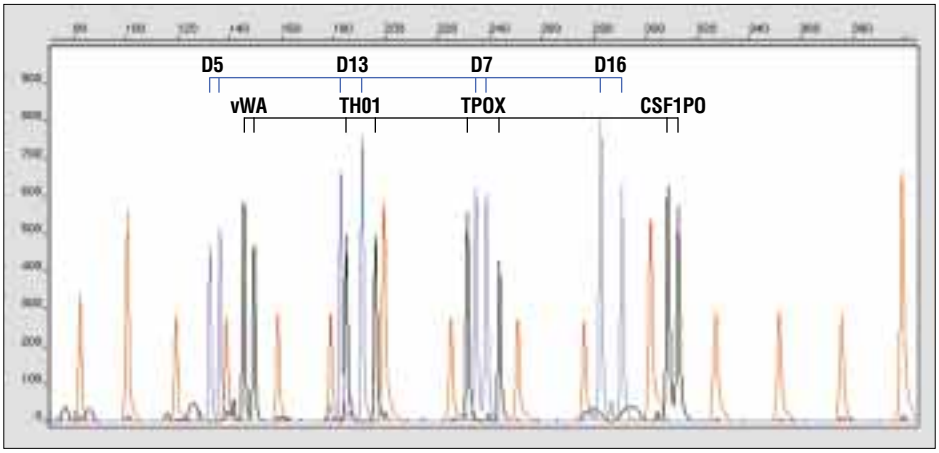

Figure 2. The *GenePrint*™ PowerPlex™ 1.2 System detected using the ABI PRISM™ 377 DNA Sequencer. The electropherogram displays the amplification products at eight STR loci and the Fluorescent Ladder (CXR), 60-400 Bases. The amplified products of the fluorescein-labeled loci, D16S539, D7S820, D13S317 and D5S818, are shown as purple peaks, while the TMR-labeled loci, CSF1PO, TPOX, TH01 and vWA are shown as black peaks. Fragments of the Fluorescent Ladder (CXR), 60-400 Bases, are displayed as red peaks. All materials were separated using a 5% polyacrylamide denaturing gel and detected with the ABI PRISM™ 377 DNA Sequencer.

allelic ladders. Any lane-specific influences which affect migration of the size marker fragments also alter the migration of allelic ladders correspondingly. Thus, despite occasional waviness in the gels, or differences in the way gels are run in different laboratories, allele calls can be made reliably using this approach.

Previously available size markers have been limited in their use by the uneven spacing of fragments contained within the marker set, and by different sequences contained within different fragments of the marker set. These limitations can cause imprecision in defining sizes of unknowns, especially in regions where there are few fragments, and may cause deviation from a linear sizing plot resulting from the varying sequences. Each of the 16 fragments of the Fluorescent Ladder (CXR), 60-400 Bases, contains the entire sequence of all smaller fragments in the set. That is to say, the small fragments are subsets of each larger fragment. This characteristic limits deviation from linearity in the measurements, while the even spacing provides increased precision. The Fluorescent Ladder (CXR) is also available as a stand-alone product for use with the *GenePrint*™ Fluorescent STR Multiplex Systems, CTTv, FFFL and GammaSTR™, or with any other compatible fluorescently labeled system.

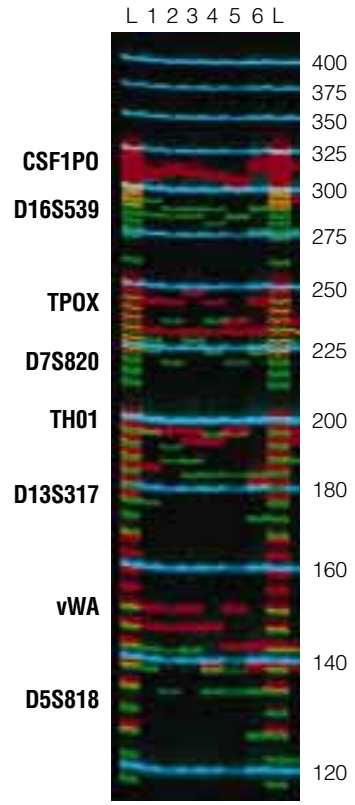

Figure 3. The *GenePrint*™ PowerPlex™ 1.1 System detected using the Hitachi FMBIO® II Fluorescent Scanner. Six DNA samples were amplified using the PowerPlex™ 1.1 System (lanes 1-6) and are shown with allelic ladders for the corresponding loci (lanes labeled L). Each lane contains the Fluorescent Ladder (CXR), 60-400 Bases (shown in blue). The 400, 375, 350, 325, 300, 275, 250, 225, 200, 180, 160, 140 and 120 base fragments are visualized in this image. The 100, 80 and 60 base fragments are not shown. The red fragments are TMR-labeled fragments amplified at the loci, CSF1PO, TPOX, TH01 and vWA. The green fragments are fluorescein-labeled amplified material from the D16S539, D7S820, D13S317 and D5S818 loci. All materials were separated using a 4% polyacrylamide denaturing gel.

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