

Multiplex STR Analysis by Capillary Electrophoresis

By D.B. Demers, C.M. Kelly and A.C. Sozer

Fairfax Identity Laboratories, Division of Genetics and IVF Institute, Fairfax, VA

e-mail ddemers@givf.com, ckelly@givf.com, asozer@givf.com

INTRODUCTION

While Southern-based RFLP testing is a powerful means of establishing biological relationships, the use of the polymerase chain reaction (PCR[†]) has gained popularity in paternity and forensic DNA testing. A PCR-based analysis is an excellent choice in cases where the amount of sample available is limiting or where the sample is degraded. The sample requirements and testing time for PCR-based techniques are greatly diminished compared to the requirements for RFLP. However, the average power of discrimination for any of the locations tested by PCR is also greatly reduced, prompting laboratories to test more loci in order to attain the power of discrimination offered by an RFLP analysis.

Amplified fragment length polymorphisms (AmpFLPs) can be divided into two categories: Long Tandem Repeats (LTRs) and Short Tandem Repeats (STRs). Both LTRs and STRs can be amplified using the polymerase chain reaction and detected by manual, semi-automated or automated procedures. One of the advantages of analyzing PCR-based variable number tandem repeat systems is that individual alleles can be resolved into more discrete fragments than with RFLP analysis. Discrete alleles enable phenotype determination by reference to allelic ladders in adjacent lanes of the gel or, in the case of some fluorescent-based detection systems, by reference to an internal lane standard.

Long tandem repeats generally have repeat units of 10-30 base pairs (bp). Some, such as D17S5 and D16S83, are considerably larger, having repeat lengths that are 70bp and 80bp, respectively. Allele sizes usually range from about 300bp to 1,000bp. Due to their size, LTRs are more susceptible to preferential amplification and allelic dropout, particularly with degraded DNA samples. However, they tend to be more polymorphic and hence are more informative than STRs.

STR MULTIPLEX SYSTEMS

Most short tandem repeat loci used in identity testing have repeats that are four base pairs in length, with allele sizes between 100bp and 300bp. Table 1 lists some of the commonly used STRs. The relatively small size of STR alleles reduces the effects of preferential amplification. Thus, more highly degraded samples can be analyzed than with LTRs. STRs tend to be less discriminating than LTRs, so a greater number of loci must be examined to obtain the same level of discrimination. STR multiplex systems allow the simultaneous amplification of multiple nonoverlapping loci in a single reaction, substantially increasing throughput. With multicolor fluorescent detection, even overlapping loci can be multiplexed. Commercial kits available for the analysis of STR loci generally provide all of the necessary reaction components and controls required for amplification.

The PCR products from LTR and STR analyses can be separated and detected using manual, semi-automated or automated methods (for a review, see reference 1). Semi-automated systems are gel-based and combine electrophoresis, detection and analysis into one unit. On a semi-automated system, gel assembly and sample loading are still manual processes; however, once samples are loaded onto the gel, electrophoresis, detection and analysis proceed automatically. Data collection occurs in "real time" as fluorescently labeled fragments migrate past the detector at a fixed point and can be viewed as they are collected. Most semi-automated systems also

The PowerPlex™ 1.2 System is a fluorescent-based system that is fully compatible with the ABI PRISM™ 310 Genetic Analyzer.

STR multiplex systems allow the simultaneous amplification of multiple, nonoverlapping loci in a single reaction, substantially increasing throughput.

[†]The PCR process is covered by patents issued and applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process.

Table 1. Commonly-Used STR Alleles.

Locus	Chromosomal Location	Locus Definition	K562 Allelic Pattern
CSF1PO	5q33.3-34	Proto-oncogene for the CSF-1 receptor	10,9
TH01	11p15.5	Tyrosine hydroxylase gene	9.3,9.3
TPOX	2p25.1-pter	Thyroid peroxidase gene	9,8
vWA	12p12-pter	von Willebrand antigen gene	16,16
LPL	8p22	Lipoprotein lipase gene	12,10
F13A01	6p24.3-25.1	Coagulation factor VIII subunit a gene	5,4
F13B	1q31-q32.1	Coagulation factor VIII subunit b gene	10,10
FESFPS	15q25-qter	c-fes/fps proto-oncogene	12,10
D16S539	16q24-qter	NA	12,11
D7S820	7q11.21-22	NA	11,9
D13S317	13q22-q31	NA	8,8
D5S818	5q23.3-32	NA	12,11

offer multicolor detection and, when coupled with small well combs that allow up to ninety-six samples on a single gel, throughput is considerable.

CAPILLARY ELECTROPHORESIS

At the time of this writing, the only completely automated system for LTR and STR analysis is capillary electrophoresis (CE). A CE system provides the automated electrophoresis, detection and analysis available on semi-automated systems as well as automated gel assembly and sample loading processes. As the name implies, capillary electrophoresis is carried out in a microcapillary tube rather than between glass plates. Once samples, gel polymer and buffer are loaded onto the instrument, the capillary is filled with gel polymer and the sample is loaded automatically. Up to ninety-six samples are automatically loaded and analyzed unattended.

The disadvantage of CE is that only single capillary systems are currently available – the ABI PRISM™ 310 Genetic Analyzer (Perkin-Elmer) and the P/ACE™ DNA System (Beckman Instruments) – so analysis proceeds one sample at a time and throughput is reduced compared to semi-automated systems. However, because CE is carried out at a much higher voltage, separation of fragments from a single sample is completed in twenty minutes. This helps to offset the reduced throughput. Also, since the CE is fully automated, the chance of human error in the post-amplification handling and analysis of samples can be reduced, a significant benefit when large numbers of samples are processed.

THE POWERPLEX™ 1.2 SYSTEM

The *GenePrint™* PowerPlex™ System allows for the simultaneous amplification

and two-color detection of eight polymorphic STR loci in a single reaction. The PowerPlex™ 1.2 Beta Test System contains all of the component loci of two *GenePrint™* quadriplex systems, the CTTv Multiplex (CSF1PO, TPOX, TH01 and vWA) and the GammaSTR™ Multiplex (D16S539, D7S820, D13S317 and D5S818). The PowerPlex™ 1.2 Beta Test System is compatible with use on the ABI PRISM™ 310 Genetic Analyzer. The CTTv component of the PowerPlex™ System is labeled with carboxy-tetramethylrhodamine (TMR) and appears as yellow on the ABI PRISM™ 310 instrument when the appropriate matrix is used. The GammaSTR™ loci are labeled with fluorescein and appear blue. The internal size marker supplied with the PowerPlex™ System, the Fluorescent Ladder (CXR), contains 16 fragments ranging from 60-400 bases in length. These fragments are labeled with carboxy-X-rhodamine (CXR), and appear red on the 310 instrument.

Although the loci within the CTTv and GammaSTR™ multiplexes overlap, they can be simultaneously separated and detected because they are labeled with different fluorescent dyes. Inclusion of the Fluorescent Ladder (CXR) in each run as an internal size standard enables the products of the PCR amplification to be sized with a high degree of precision.

RESULTS

The size range (window) for each allele of each locus in the PowerPlex™ System was determined by analysis of an allelic ladder (provided with the PowerPlex™ System) containing all of the common alleles except TH01 allele 9.3. (The final inventory product

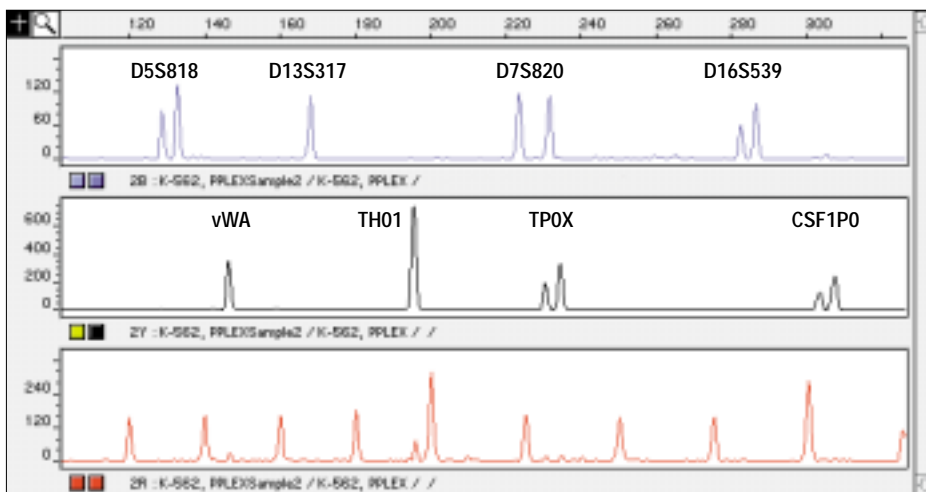


Figure 1. Electrophoretic profile of K562 DNA amplified using the *GenePrint™* PowerPlex™ 1.2 Beta Test System and detected using the ABI PRISM™ 310 Genetic Analyzer. DNA from the cell line K562 was amplified using the *GenePrint™* PowerPlex™ 1.2 Beta Test System version 2.0. Separation of PCR products was accomplished on the ABI PRISM™ 310 Capillary Electrophoresis System under standard conditions using the manufacturer's POP4 A Module for the 1ml polymer syringe. The conditions included a sample injection time of 5 seconds at 15.0 kV, with a run at the same voltage and a capillary temperature of 60°C. The K562 DNA contains imbalanced alleles at several loci. This result is due to the unusual chromosome content of this cell line and is not a function of the PowerPlex™ 1.2 System performance.

Table 2. Allele Size Windows for the GenePrint™ PowerPlex™ 1.2 Beta Test System Loci on the ABI PRISM™ 310 Genetic Analyzer.

Locus	Allele	Mean*	Minimum*	Maximum*	S.D.	Locus	Allele	Mean*	Minimum*	Maximum*	S.D.		
D5S818	7	112.18	112.01	112.28	0.06	vWA	11	126.27	125.85	126.38	0.10		
	8	116.34	116.15	116.41	0.05		13	134.30	133.77	134.42	0.10		
	9	120.54	120.35	120.91	0.09		14	138.32	137.65	138.46	0.12		
	10	124.72	124.63	124.83	0.05		15	142.31	141.69	142.40	0.12		
	11	128.80	128.68	128.88	0.05		16	146.37	145.80	146.54	0.11		
	12	132.95	132.76	133.04	0.05		17	150.35	149.80	150.47	0.10		
	13	137.03	136.93	137.10	0.05		18	154.35	153.92	154.47	0.08		
	14	141.14	141.06	141.19	0.05		19	158.37	158.05	158.45	0.08		
	15	145.28	145.15	145.47	0.07		20	162.34	162.02	162.49	0.08		
							21	166.22	165.93	166.33	0.07		
	D13S317	7	164.11	164.03	164.20		0.05	TH01	5	176.45	176.29	176.61	0.08
		8	168.11	168.04	168.20		0.05		6	180.42	180.24	180.49	0.07
		9	172.10	171.99	172.20		0.05		7	184.39	184.21	184.55	0.09
		10	176.02	175.70	176.16		0.07		8	188.34	188.19	188.55	0.09
		11	180.06	179.64	180.12		0.09		9	192.32	192.19	192.48	0.09
12		184.09	183.80	184.22	0.06	9.3	195.23		195.12	195.31	0.06		
13		188.08	187.73	188.21	0.08	10	196.30		196.11	196.44	0.08		
14		192.08	191.08	192.15	0.06	11	200.31		200.12	200.50	0.0		
15		196.07	195.89	196.19	0.06								
D7S820		6	211.24	211.16	211.34	0.05	TPOX		6	222.28	221.99	222.45	0.08
		7	215.26	215.14	215.37	0.06			7	226.26	225.90	226.41	0.07
		8	219.27	218.87	219.39	0.09			8	230.28	230.03	230.40	0.07
		9	223.28	222.99	223.38	0.08			9	234.30	234.18	234.43	0.06
		10	227.34	226.93	227.43	0.09			10	238.30	238.08	238.48	0.07
		11	231.39	231.07	231.53	0.08			11	242.33	242.16	242.48	0.07
	12	235.42	235.22	235.53	0.07	12		246.35	246.25	246.49	0.06		
	13	239.48	239.34	239.70	0.07	13		250.40	250.26	250.65	0.07		
	14	243.53	243.30	243.73	0.09								
	D16S539	5	258.08	257.91	258.31	0.08		CSF1PO	6	291.26	291.14	291.51	0.07
		8	270.22	270.13	270.47	0.07			7	295.18	295.06	295.43	0.07
		9	274.27	274.08	274.47	0.09			8	299.12	298.96	299.33	0.08
		10	278.27	278.01	278.50	0.09			9	303.11	302.96	303.33	0.08
		11	282.28	282.13	282.45	0.08			10	307.10	306.87	307.24	0.08
12		286.25	286.08	286.39	0.07	11	311.12		311.00	311.30	0.07		
						12	315.11		315.02	315.37	0.09		

*Values are in bases.

will contain this allele.) The allelic ladder was analyzed a total of 40 times. The window for the TH01 9.3 allele was determined by analysis of 6 different known 9.3 alleles run a total of 4 times each. Table 2 shows the minimum, maximum and average values in bases along with the standard deviation (S.D.) for each allele. The window for each allele is simply the size range bound by the minimum and maximum values of all observations.

Most allele windows are 0.5-0.6 bases in size or approximately +/-0.25 to 0.30 bases from the mean, including the TH01 allele 9.3.

Thus, single base resolution is readily achieved and TH01 alleles 9.3 and 10 can be separated. Figure 1 shows an electropherogram for K562 DNA. Studies on the resolution of alleles 9.3 and 10 are in progress.

The PowerPlex™ 1.2 Beta Test System is a robust, fluorescent-based system that is fully compatible with the ABI PRISM™ 310 Genetic Analyzer. This system provides simultaneous, rapid, reproducible and precise analysis of the STR loci CSF1PO, TPOX, TH01, vWA, D16S539, D7S820, D13S317 and D5S818.

REFERENCE

1. Sozer, A.C., Kelly, C.M. and Demers, D.B. (1997) *Molecular Analysis of Paternity. In: Current Protocols in Human Genetics* eds., Dracopoli et al. John Wiley and Sons, Inc., NY (in press).

GammaSTR, GenePrint and PowerPlex are trademarks of Promega Corporation

ABI PRISM is a trademark of the Perkin-Elmer Corporation. P/ACE is a trademark of Beckman Instruments, Inc.