DEVELOPMENT AND APPLICATIONS OF HIGH THROUGHPUT MULTIPLEX STR SYSTEMS

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INTRODUCTION

This chapter describes nine polymorphic tetrameric short tandem repeat (STR) loci (Edwards et al, 1991, 1992, Polymeropoulos et al., 1991) and the development of methods which are compatible with both silver and fluorescent detection, providing a pwerful set of markers for universal application. The creation of allelic ladders and multiplex sets involving these systems accelerates and simplifies analysis providing for rapid, efficient, and precise application to allele identification.

CHOICE OF STR SYSTEMS AND CONSTRUCTION OF ALLELIC LADDERS

Following an initial screening of over 35 STR loci, we selected 9 loci for additional applications development. These nine systems carry tetranucleotide repeats and display fewer artifacts than the rejected loci whether detected using silver stain analysis (Bassam et al, 1991) or fluorescence. Table 1 displays the chromosome location and the known allele size range for each of the 9 STR systems and the amelogenin locus (Sullivan et al, 1993). The amelogenin locus, which is not a true STR, generates a 212 bp fragment from the X chromosome and a 218 bp fragment from the Y chromosome, thus allowing its application to sex identification.

For each of the nine STR loci and the amelogenin locus, we constructed an allelic ladder, i.e. a mixture of many or all of the possible amplified alleles for the individual locus. Allelic ladders serve as size standards allowing rapid and precise comparison of amplified sample DNAs with well-characterized allelic ladder components (Puers et al, 1994). Using these size standards, there is no need for measurement of migration distance or calculation to determine the size of each allele. The components of the allelic ladder for each locus and the size ranges for these fragments are listed in Table 1.

MULTIPLEX STR SYSTEMS

To achieve high throughput with the STR systems, we have developed two triplex sets for use with silver stain (See Figure 1 for silver stain of the CTT triplex) or other post-electrophoresis staining technologies and two related quadriplex systems which

Table 1.

Locus	Chromosome Location	Size Range of Known Alleles (bases)	Size Range of Allelic Ladder (bases)	Allelic Ladder Component Names[1]
Amelogenin	X:Y	212, 218	212, 218	212, 218
CSF1PO	5q33.5-34	295 - 327	299 - 323	7 through 15
F13A01	6p24-25	281 -331	283 - 331	4 through 9, 11 through16
F13B	1q31-q32.1	169 - 189	169 - 185	6 through 10
FESFPS	15q25-qter	222 - 250	226 - 246	8 through 13
HPRTB	Xq26	259 - 303	259 - 303	6 through 17
LPL	8p22	105-133	105 - 133	7 through 14
TH01	11p15.5	179 - 203	179 - 203	5 through 11
TPOX	2p23-2pter	224 - 252	232 - 248	6 through13
vWF	12p12-pter	131 - 171	143 - 167	14 through 20

[1] Names of alleles represent the number of repeats within the alleles. The TH01 allele 9.3 (198 bases), F13A01 allele 10 (307 bases) and allele 3.2 (281 bases), F13B allele 11, FESFPS alleles 7 and 14, and vWF alleles 11, 13, and 21 are not currently included in these allelic ladders.

can be used in conjunction with fluorescence detection equipment such as the Hitachi FMBIO100, Molecular Dynamics FluorImager, or Applied Biosystems DNA Sequencer 373 or 377 instruments (Table 2). The combined use of the two quadriplexes provides matching probabilities ranging from 1 in 17.000.000 to 1 in 430.000.000 depending on the population being studied (Table 2). Either the CTT triplex or the CTTv quadriplex can be used in co-amplification with the sex identification locus, amelogenin, to generate a quadriplex or pentaplex, respectively, known as CTAT and CTATv.

Table 2.

	Matching Probability[1]		
	Caucasian-American	African-American	Hispanic-American
Multiplexes for Silver Detection			
CTT Triplex (CSF1PO,TPOX,TH01)	1 in 424	1 in 1639	1 in546
FFv Triplex (F13A01,FESFPS,vWF)	1 in 909	1 in 2785	1 in 1342
Both Triplexes (6 loci, above)	1 in 385000	1 in 4565000	1 in 733000
Multiplexes for Fluorescent Detection			
CTTv Quadriplex (CSF1PO,TPOX,TH01,vWF)	1 in 6623	1 in 25575	1 in 7194
FFFL Quadriplex (F13A01,FESFPS,F13B,LPL)	1 in 2632	1 in 16807	1 in 3279
Both Quadriplexes (8 loci, above)	1 in 17400000	1 in 430000000	1 in 23600000

[1] Matching probabilities have been determined as part of an unpublished collaborative study among S Creacy and RA Bever of Genetic Design (Greensboro, NC) and authors CJ Sprecher and JW Schumm.

The most efficient use of these systems is achieved by simultaneous detection of both quadriplexes in a single gel lane using the Hitachi FMBIO instrument. This can be performed with the CTTv quadriplex labeled with one fluorescent dye and the FFFL quadriplex with a second fluorescent label. This machine takes as little as 10

minutes to scan the gel following electrophoresis of the amplified products of both quadriplexes in the same gel lane using a standard gel rig. The resulting scans can be cleanly separated into individual black and white images for ease of interpretation as seen in Figure 2.

Figure 1

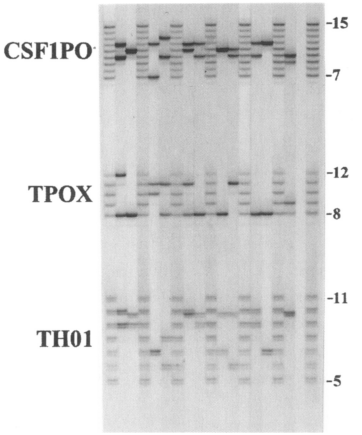

Figure 2

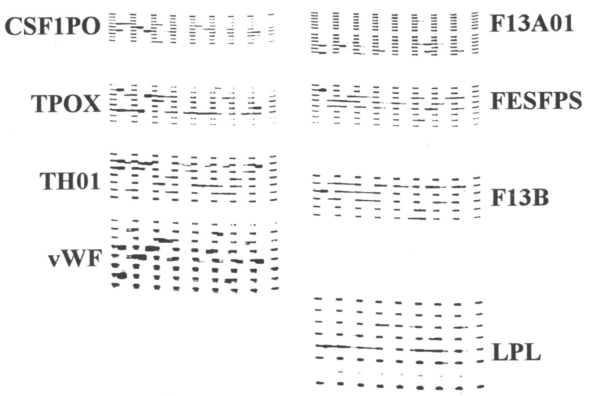

Figure 1 displays silver stain visualization of 11 samples (Lanes 1-11) amplified simultaneously at the three STR loci the CTT triplex. Allelic ladders for each locus have been included (Lanes L) to simplify interpretation of unknown samples. The numbers to the right of each locus indicate the number of tetranucleotide repeats present in each component of the allelic ladders.

Figure 2 shows simultaneous fluorescent detection of two STR quadriplex systems using the Hitachi FMBIO machine. The CTTv and FFFL quadriplexes were labeled with different fluorescent dyes and detected with 625nm and 505nm scans, respectively. Each STR locus is labeled. Allelic ladders for each locus have been included to simplify interpretation of unknown samples.

REFERENCES

Bassam BJ, Caetano-Anolles G, and Gresshoff PM (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal. Biochem. 196:80-83.

Edwards A, Civitello A, Hammond HA, and Caskey CT (1991) DNA typing and genetic mapping with trimeric and tetrameric tandem repeats, Am. J. Hum. Genet. 49:746-756

Edwards A, Hammond HA, Jin, L, Caskey CT, and Chakraborty R (1992) Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups, Genomics 12:241-253

Polymeropoulos, MH,Xiao H, Rath DS, and Merril CR (1991) Tetranucleotide repeat polymorphism at the human tyrosine hydroxylase gene (TH), Nucl. Acids Res. 19:3753

Puers, C, Lins AM, Sprecher CJ, Brinkmann B, and Schumm JW (1994) Analysis of polymorphic short tandem repeat loci using well-characterized allelic ladders. In: Proceedings from the Fourth International Symposium on Human Identification 1993. Promega Corporation, pp 161-172

Sullivan K, et al (1993) A rapid and quantitative DNA sex test: Fluorescence-based PCR analysis of X-Y homologous gene amelogenin, Biotechniques 15:636-641