SIMPLE AND RAPID TYPING FOR VNTR POLYMORPHISMS USING HIGH RESOLUTION ELECTROPHORESIS OF PCR PRODUCTS ON REHYDRATABLE POLYACRYLAMIDE GELS

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Introduction

The invention of the Polymerase Chain Reaction (PCR, Saiki et al 1988) has opened a wide new field of applications in molecular biology. Many new PCR-based techniques to type for polymorphic markers are now available and thus forensic haemogenetics is certainly moving to a new age. The highly polymorphic Variable Number of Tandem Repeat (VNTR) -loci offer a system of markers with high informativeness and have already been employed for paternity and identity testing for a few years. So far, most work has been done by analysis of enzyme-digested amplification products (RFLP) which is a cumbersome method with limited sensitivity. Therefore, typing of VNTR´s and STR´s (Short Tandem Repeats, a subset of VNTR´s with very short repeat sequences: 2-4bp) by Amplification Fragment Length Polymorphism (AMPFLP) is about to become the method of choice, since DNA-sequencing, of course the most precise way to type for a genetic polymorphism, will presumably not be available to most workers in the forensic field in the near future. We have undertaken studies to optimize conditions for high resolution electrophoresis on rehydratable polyacrylamide gels for the VNTR marker systems ApoB, COL2A1, D1S80, YNZ22 and for the STR loci SE33 and TC11 in order to obtain a simple protocol that leads to reproducible and reliable results and to further employ this protocol for local population and family studies and for the forensic casework in the future.

Material and methods

Samples

Genomic DNA was extracted from peripheral blood of healthy unrelated individuals by the salting out method described by Miller at al (1988). The DNA concentration was adjusted to 1 ng/µl.

PCR

The PCR protocols for the individual loci as well as the amplification conditions are listed in Tab 1.

Allelic Cocktails

were produced by coamplification of genomic DNA of 4-5 individuals.

Gel preparation

For typing of YNZ22 a commercially avaiable rehydratable polyacrylamide gel (CleanGel 48S, Pharmacia Biotechnology, FRG) was used. For typing of the other loci rehydratable gels were produced as described by Westermeier (1990).

Buffers

Rehydration and electrode buffers as originally described for the use of CleanGel (Pharmacia, FRG) were used:

Tris-Phosphate (Tris-P): 100mM Tris, 2mM Na_2EDTA, adjusted to pH 8.0 with phosphoric acid

Tris-Acetate (Tris-Ac): 112 mM Tris, adjusted to pH 6.4 with acetic acid

TBE: 0.27M Tris, 0.27M H_3BO_3, 7.5mM Na_2EDTA

Tris-Tricine (Tris-Tric): 0.2M Tris, 0.2M Tricine, 19mM SDS

Polyacrylamide gel electrophoresis

Horizontal electrophoresis was carried out at a temperature of 10°C on a Multiphor II electrophoresis equipment (Pharmacia Biosystems, FRG). The electrophoresis conditions are listed in Tab 1.

Silver staining

A simple and sensitive silver staining protocol (Bassam et al 1991) was used to visualize the amplification products.

Results and discussion

Sufficient resolution of the polymorphic alleles was achieved in all cases after modifications of the electrophoresis conditions, the rehydration and the sample buffers (Tab 1). Allelic cocktails that allow allele assignment were obtained for all systems. The number of alleles identified in a local population sample is listed in Tab 1. However, for D1S80 and SE33, allele assignment was difficult when comparing the results of the population studies (Schwartz et al 1993) to those of other workers. For SE33, a considerable high number of new high molecular weight alleles was observed which is presumably due to the enhanced electrophoretic separation conditions. The typing of D1S80 revealed microheterogenity of the V22-Allele and several new interalleles. From the results of our studies, we may conclude that PAGE with subsequent silver staining offers a reliable and convenient possibility to type for VNTR and STR-loci in forensic practice in the future. Since the combined Mean Exclusion Chance (Krueger at al 1968) of the 6 systems is 0.99975, it is possible to solve most forensic cases using only this technique with 6 systems. As a next step, sequencing of the individual alleles is necessesary to define a final nomenclature and cocktails composed of alleles with known sequences should be produced and shared by all workers in this field to further establish this procedure for forensic paternity and identity testing.

Acknowledgements

Thanks to B. Brinkmann and P. Wiegand for providing allelic cocktails for D1S80 and YNZ22 and for fruitful discussions.

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Tab.1 PCR and PAGE conditions for 6 microsatellite loci

Locus	APOB	COL2A1	D1S80	YNZ22	SE33	TC11
syn.	2p24-p23	12q13.1	pMCT118	D17S5, D17S30, 17p13.3	ACTBP2, Ac-psi-2	HUMTH01, 11p15.5
Lit.	Ludwig et al 1989	Wu et al 1990	Nakamura et al 1988 Budowle et al 1991	Horn et al 1989	Polymeropoulos et al 1992	Edwards et al 1992
Primer sequences	5´-ATG GAA ACG GAG AAA TTA TG-3´ 5´-CCT TCT CAC TTG GCA AAT AC-3´	5´-CCA GGT TAA GGT TGA CAG CT-3´ 5´-GTC ATG AAC TAG CTC TGG TG-3´	5´-GAA ACT GGC CTC CAA ACA CTG CCC GCC G-3´ 5´-GTC TTG TTG GAG ATG CAC GTG CCC CTT GC-3´	5´-AAA CTG CAG AGA GAA AGG TCG AAG AGT GAA GTG-3´ 5´-AAA GGA TCC CCC ACA TCC GCT CCC CAA GTT-3´	5´-AAT CTG GGC GAC AAG AGT GA-3´ 5´-ACA TCT CCC CTA CCG CTA TA-3´	5´-GTG GGC TGA AAA GCT CCC GAT TAT-3´ 5´-ATT CAA AGG GTA TCT GGG CTC TGG-3´
PCR-protocol	50 ng template DNA, 0.4 µM each primer, 200 µM each NTP, 5µl 10xPCR buffer[3], 2U DNA Polymerase[4], total volume 50 µl, overlaid with 50 µl oil					
Amplification conditions	94°C - 1 min 58°C - 1 min 72°C - 4 min 29 cycles	94°C - 1 min 60°C - 1 min 70°C - 1.5 min 25 cycles	94°C - 1 min 65°C - 1 min 72°C - 8 min 27 cycles	94°C - 1 min 63°C - 1 min 72°C - 5 min 29 cycles	93°C - 1 min 61°C - 1 min 72°C - 1.5 min 28 cycles	94°C - 1 min 64°C - 1 min 70°C - 2 min 30 cycles
polyacrylamide gels composition size(mm) rehydration buffer electrode buffer	 T5% C3% 230x110x0.45 Tris-P TBE	 T5% C3% 230x110x0.45 Tris-P TBE	 T6% C3% 230x110x0.45 Tris-P TBE	CleanGel[2] 110x250x0.43 Tris-Ac Tris-Tric	 T5% C3% 230x110x0.45 Tris-Ac Tris-Tric	 T6% C3% 230x110x0.45 Tris-Ac Tris-Tric
Electrophoresis[1] ramp final	 1200V,7mA, 30W 10 min 1200V,15mA, 30W	 1200V, 7mA, 30W, 10 min 1200V,15mA, 30W	 1200V, 7mA, 30W, 10 min 1200V, 15mA, 30W	 none 600V, 50mA, 30W	 1200V, 12mA, 30W, 10min 1200V, 25mA, 30W	 1200V, 12mA, 30W, 10min 1200V, 25mA, 30W
number of observed alleles	23	11	26	15	30	8

[1] Electrophoresis was stopped when the bromphenolblue-front reached the anode
[2] Pharmacia Biotechnology, consists of a T5% stacking gel and a T10% resolving gel
[3] Finnzymes Oy
[4] DynaZyme^{tm}