Introduction of Two New Electrophoresis Gel Systems for Screening and High Resolution Identification of STRs under Native Conditions

HP. Schickle, and R. Westermeier

ETC Elektrophorese-Technik, Bahnhofstr. 26,
D-72138 Kirchentellinsfurt, F.R.G.

INTRODUCTION

When polyacrylamide gels are employed for electrophoresis of DNA fragments, the mobilities are not only dependent on the fragment length, but also on the base sequence. Single strand conformation polymorphism SSCP and double strand conformation polymorphism DSCP (Barros et al. 1992) can be detected in these gels. However, when they are run under denaturing conditions, in the presence of high concentrations of urea or formamide and at elevated temperature (50 °C and higher), the mobilities are directly proportional to the sizes. This is the method for DNA sequencing, because under these conditions it is no problem to achieve a resolution of Δ 1 base.

Short tandem repeats (STR) analysis is performed in denaturing and in non-denaturing gels. In both cases, the assignment of alleles with well-defined (sequenced) allelic ladders of the respective STR locus, which are run in the same gel, proved to be the most reliable method (Puers et al. 1993; Möller et al. 1994). However, additionally to the regular length variation types, in some STR loci there are sequence variants existing, which can only be identified by sequencing the fragments or running them on non-denaturing polyacrylamide gels. For screening purposes and allel identification in the daily work, the latter is the method of choice.

Conventional native gels exhibit less resolution than denaturing gels. In the following contribution, two new electrophoresis gel systems are presented for screening and identification of STR alleles with adequate resolving power under native conditions.

MATERIAL AND METHODS

Samples: DNA was extracted according to Brinkmann et al. (1991); PCR[1] amplification was performed according to Möller et al. (1994a,1994b). 4 – 7 µL of each sample were directly after the PCR reaction applied to the electrophoresis gel.

[1] The PCR process is covered by U.S. patents 4,683,195 and 4,683,302 owned by Hoffman-La Roche Inc. Use of the PCR process requires a license.

Advances in Forensic Haemogenetics 6 (c) Springer-Verlag Berlin Heidelberg

Gels and electrophoresis: *Short gels:* CleanGel HyRes (25 × 11 cm, 430 µm thickness) for 48 samples of 7 µL, 9 cm separation distance; wicks: 5.0 × 25.3 cm special thick filter paper.
Long gels: CleanGels Long-Hyres (25 × 11 cm, 650 µm thickness) for 22 samples of 7 µL, 18 cm separation distance long direction, strips 1.8 × 11.7 cm special thick filter paper.

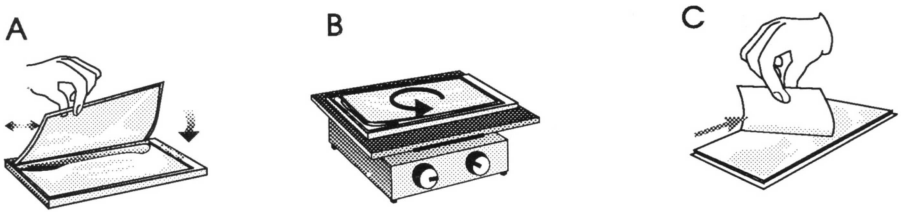

Fig. 1: Equilibration of the Cleangels HyRes in the gel buffer, and removing the excess buffer from the surface.

A GelPool is layed on a rotating platform; 50 mL equilibration solution is pipetted into the chamber. The gel-film – with the gel surface facing down – is layed into the rehydration buffer (fig. 1A) It is equilibrated for 1 hour 30 min (fig. 1B).After equilibration, excess buffer is removed with filter paper (fig. 1C).
The gels are laid on the center of the cooling plate of an horizontal electrophoresis apparatus. Special filter paper strips are soaked in the running buffer and applied over the gel edges. The gels are run at 15 °C; the short gel for 3 hours, the long gel for 3 hours 50 minutes. For *double loading*, the separation is interrupted after 1 hour 30 min, the wells are dried out with filter paper, and 48 more samples are loaded, electrophoresis is continued for another 1 hour 30 min.

Silver staining: The method according to Bassam et al. (1991) had to be modified for higher crosslinked gels: Fixing for 45 minutes with 15 % ethanol and 5 % acetic acid at 40 °C gives a lighter background; the times for all steps have to be doubled. The gels are preserved by impregnating them in 15 % glycerol and 15 % monoethylenglycol(v/v)for 30 minutes, drying at room temperature for 2 hours, then covering the surface with a polyester film, and storing the gels in a plastic bag.

RESULTS

CleanGel HyRes 48 S combined with the LongRun buffer system provide a resolution of down to less than Δ4 bp under native conditions (double strands) within a separation distance of 9 cm; the optimal separation is achieved between 50 and 200 bp. Up to 96 samples can be separated in one gel within a separation time of 3 hours by double loading (see fig. 2). With these gels, high sample throughput, good reproducibility, and high resolution for all these STR systems can be achieved. Thus, these gels are very useful for screening purposes. Figure 3 shows a complete gel with single loaded samples of the HumVWA and Hum THO1systems.
The "high-resolution gel" has the same size, however it is run along the long distance for 4 hours. In this gel a resolution of down to Δ 1 bp is obtained under non-denaturing conditions, even a complete separation down to the baseline between allel 9.3 and 10 of the HumTH01 allelic ladder (see fig. 4).
The gels shown also demonstrate the usefulness of running well-defined allelic ladders parallelly to the samples for allel identification.

Advances in Forensic Haemogenetics 6 (c) Springer-Verlag Berlin Heidelberg

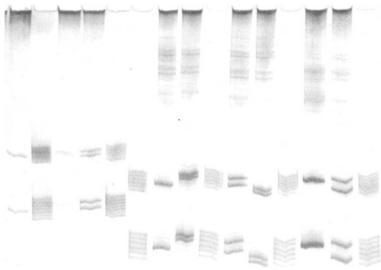

Fig. 2: Screening gel with double loaded samples.

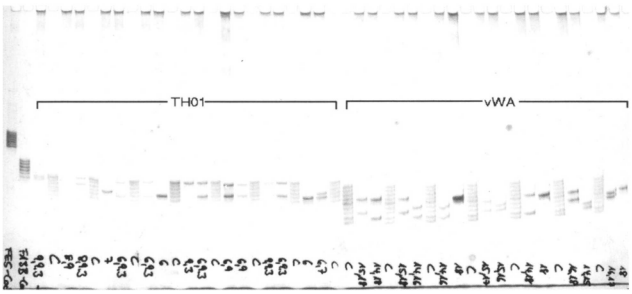

Fig. 3: Screening gel with separations of VWA and TH01 samples.

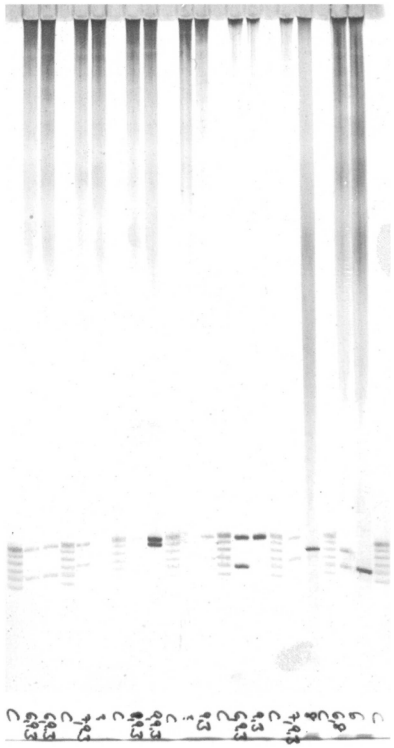

Fig. 4: High resolution gel with TH01 samples.

REFERENCES:

Barros F, Muños-Barús, Lareu MV, Rodriguez-Calvo MS, Carracedo A (1994) Double- and single-strand conformation polymorphism analysis of point mutations and and short tandem repeats. Electrophoresis 15:566-571

Bassam BJ, Caetano-Annollés G, Gresshoff PM (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal Biochem 80:81-84

Brinkmann B, Rand S, Wiegand P (1991)Population and and family data of RFLPs using selected single- and multi-locus systems. Int J Leg Med 104:81-86.

Möller A, Meyer E, Brinkmann B (1994a) Different types of structural variation in STRs: HumFES/FPS, HumVWA and HumD21S11. Int J Leg Med 106:319-323

Möller A, Wiegand P, Grüschow C, Seuchter SA, Baur MP, Brinkmann B (1994b) Population data and forensic efficiency values for the STR systems HumVWA, HumMBP and HumFABP. Int J Leg Med 106:183-189

Puers C, Hammond HA, Jin L, Caskey CT, Schumm JW (1993) Identification of repeat sequence heterogeneity at the polymorphic short tandem repeat locus HUMTH01[AATG]$_n$ and reassignment of allels in population analysis by using a locus-specific allelic ladder. Am J Hum Genet 53:953-958.

Advances in Forensic Haemogenetics 6 (c) Springer-Verlag Berlin Heidelberg