Analysis of the Short Tandem Repeat Polymorphism SE33: A New High Resolution Separation of SE33 Alleles by Means of Direct Blotting Electrophoresis

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Introduction

The analysis of the short tandem repeat (STR) polymorphism SE33 provides very useful information to both forensic casework and parentage testing. The advantage of this PCR based-system (1) is the high number of alleles showing an even distribution. The major problem of this system however is the separation of the alleles with a high resolution for an easy typing of alleles and interalleles. Sequencing gels are useful for such purposes but most laboratories no longer want to work with radioactivity or cannot afford an "automatic sequencer". For these reasons we chose the new technique of direct blotting electrophoresis (DBE) combined with a digoxygenin- detection protocol. This technique was developed by Beck et al. (2) and has the advantage of non-radioactive detection of products. Instead of a computer generated result, a hardcopy is produced.

Materials & Methods

DNA was extracted from whole blood according to standard procedures. PCR was carried out with the primers (1):

SE33/1: 5' - AAT CTG GGC GAC AAG AGT GA -3'
SE33/2: DIG- 5' - ACA TCT CCC CTA CCG CTA TA - 3'

The second primer was digoxygenin labeled.

Amplification conditions were:
50 mM KCl, 10 mM Tris pH 9.0, 0.1 % Triton X-100, 0.2 mM from each dNTP, 0.5 μM each primer, 1mM $MgCl_2$, 0.5 U Taq polymerase, 2.5 ng DNA.

The reaction volume was 12.5 μl. The cycler (Biometra TRIO- Thermoblock) was set to 80°C for 4-5 min for hot start conditions and the reaction started by adding primer SE33-2 and Taq polymerase to the heated reaction mix (DNA, SE33-1, $MgCl_2$). The reaction conditions were 94°C - 1 min, 60°C - 2 min, 70° - 1 min, for 30 cycles.

1μl of the amplification product was diluted 1:40 - 1:50 in H_2O dest. 1μl of this dilution was further diluted 1:5 in dye-mix (Formamid + 0.025% Bromphenolblue + 0.025% Xylenlcyanol). 1μl of the dye sample mix dilution was loaded onto a 0.19 mm thick, 30 cm long 4% denaturating polyacrylamide gel on the GATC 1500 DBE System (3) . The gel runs at constant 30W, about 1700 V and 17mA. After the xylencyanol dye reached the bottom of the gel, the nylon membrane fixed to a moving belt was started with a speed of 19 cm/h (Fig. 1).

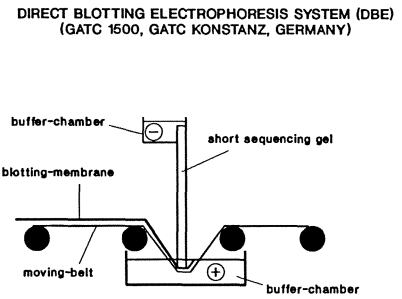

Figure 1: A schematic representation of the direct blotting electrophoresis system. DNA fragments are separated through a 30 cm long denturating 4% sequencing gel. Upon reaching the lower end of the gel the fragments are blotted onto a nylon membrane that is fixed to a moving belt and moved at a constant speed across the lower gel margin.

The total run time was 2h 45 min. After electrophoresis the membrane was dried overnight or for 1h at 80°C and exposed for 2 min to UV light in order to crosslink the DNA to the membrane. Visualization of PCR-products was carried out with the DIG Nucleic Acid Detection Kit (Boehringer Mannheim, Germany) according to the manufacturer's advice. DNA mixture experiments were carried out with DNA from two persons (X,Y). Initial concentration of the DNA in 1:1 mixtures was 2.5 ng each. Other mixtures tested were 0.5 ng Y (1:5), 0.25 ng Y (1:10), 0.125 ng Y (1:20), 0.05 ng Y (1:50), 0.025 ng Y (1:100) with constant 2.5 ng X. After PCR the samples were diluted 1:250. Two mixes (1:50 and 1:100) were also diluted directly 1:5 in dye mix. 1μl of these samples were loaded onto the gel. Sizes of the alleles were determined by comparing the products with a sequencing reaction ladder of M13mp9. The allele frequencies are the result of analyzing 217 unrelated persons from Northwest Germany.

Results and Discussion

Running under conditions described above the GATC 1500 DBE-system is able to resolve a 1 bp difference. It is therefore very easy to type interalleles of STR-systems. The usage of the DIG detection system enables us to reduce the sample volume and to increase the detection limits. It is possible to dilute a PCR product generated after 30 cycles from 2.5 ng of genomic DNA 1:250 and this could be visualized on the blot after 2 h color development (Fig. 2).

With the DBE system we were able to type more than 300 persons in a short time. Thus, the system is also ideal for population studies. Up to now, we observed 31 different alleles in a group of 217 unrelated German individuals (Fig. 3). Alleles ranged from 223 bp to 326 bp. Alleles showing a higher frequency than 9% could not be observed. The heterozygosity rate is about 96%. Length variation occur at a number of sites within the repeat region. Therefore the allele length could differ by 2 or 4 bp.

Mixtures of two DNA samples could be visualized down to a ratio of 1:20 in a standard 1:250 gel loading dilution. This is similar to other AMPFLP- and STR- systems. However, in contrast to other systems it is possible, by using a lower gel loading dilution of 1:5, to detect DNA in mixtures down to a ratio of 1:100. Higher dilutions could be visualized by increasing the time of color development from 2 h to 24 h. Thus, using the DIG detection system we achieved a very high sensitivity which is very important for case work. Due to increased sensitivity one has to be very careful with postamplification-handling.

A further advantage of the DBE system combined with the DIG detection is that the results can be kept as original hardcopies as opposed to automatically generated product sizes.

We think that DBE system is an easy, low cost and high resolution system which is ideal for separating STR systems like SE33. The gel is reusable and by using other labels (e.g. biotin, fluorescein) it is possible to separate multiplex PCR's with different staining for each label. The DBE system has multitasking capability so it is not restricted to sequencing or sequencing-like purposes. But with minor changes it is also usable for RFLP- and VNTR- analyses.

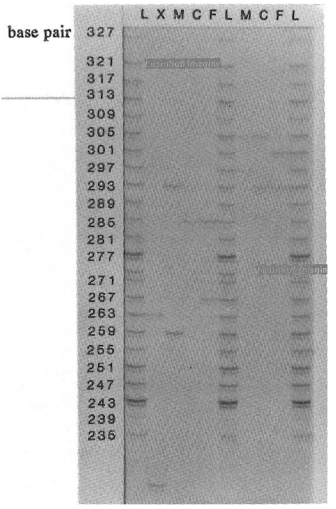

Figure 2

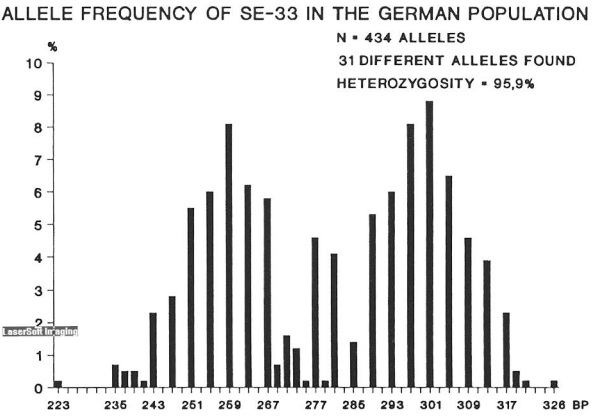

Figure 3

Figure 2: Blotting result of SE33: L = allelic ladder, X = unrelated person with the smallest allel (223 bp) found in our panel, M = mother, C = child, F = Father

Figure 3: Allele frequencies of a series of 217 unrelated German individuals. The allele length is plotted on the x-axis in base pairs (bp). Heterozygosity rate is 95.9%.

Literature

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3. Pohl T.M., Feger G. M. (1992). The DBE-DIG sequencing system. Boehringer Mannheim Colloquium 2/92

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