PRELIMINARY EXPERIENCES AND RESULTS WITH POWERPLEX 16™

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1 Abstract

The PowerPlex 16® Kit was evaluated with respect to amount of amplicon, peak height differences in heterozygotes, and balance between loci. The results were compared with those being observed after employing other commercial primers. Allele frequencies and mutation rates were compiled from German paternity cases.

2 Materials and Methods

DNA was extracted from EDTA blood samples either by the salting-out method described by Miller *et al*. [1] and quantified by spectrophotometry or prepared by the sodium hydroxide method [2]. PCR was performed with either 0.5 ng target DNA and 31 cycles (set 1, 82 samples) or 1 ng DNA and 30 cycles (set 2, 32 samples) in 12.5 µl reaction volume. The cycling was carried out as recommended in the manufacturer's manual. Depending on the minigel estimation of the amplicons, 0.5 to 4 µl amplicon was used for setting up the ABI 310 Genetic Analyzer. The peak height differences were calculated in percentage of the smaller allele. Paternity was proven by means of conventional blood group systems, DNA minisatellites and microsatellites at a PI of at least 10000.

3 Results

3.1 Peak height differences

The peak height differences of set 1 samples which were heterozygous at all 5 fluorescein labelled loci (D3S1358, TH01, D21S11, D18S51and Penta E) show a considerable sample to sample variation. Within an amplification, the different loci were well balanced as indicated by the low variation of the mean peak heights (Fig. 1). Especially, loci with larger allele sizes did not show weaker signals. As expected in most of the cases, the larger alleles of a heterozygous pair reveal lower peaks. This effect increased with the allele size itself (Fig. 2). In set 2, less sample to sample variation was observed (Fig. 3). The variation of the peak height differences was much lower than in set 1 (Fig. 4). A typical plot from set 1 and set 2 is shown in Fig. 5. The results for the Joe labelled loci D5S818, D13S317, D7S820, D16D539, CSF1PO, and Penta D and for the TMR labelled loci Amelogenin, vWA, D8S1179, TPOX, and FGA were similar and did show about the same mean peak heights.

3.2 Comparison of allele calls of results obtained with other primers

405 samples were analysed so far with PowerPlex 16®. 243 of this samples were analysed both with SGM Plus™ and Profiler 1™ (Applied Biosystems), 41 additional samples with SGM Plus™ and 20 samples with Profiler 1™ . Altogether 3971 results obtained with different kits could be compared. Different allele calling was observed in father and daughter at D8S117: Using PowerPlex 16®, the phenotype 10 / 15 was

obtained while the SGM Plus™ amplified allele *10 only. At locus TPOX, the allele *9 amplified weak in a father – son pair, whereas both alleles amplified normally using Profiler 1™. A weak PowerPlex 16® amplification of allele CSF1PO *8 (phenotype 8 / 10) shows normal peaks with Profiler 1™.

3.3 Population data and meioses

A total of 487 samples were analysed from 4 different local areas in Germany (Fig. 6 and Fig. 7). Although the number of each set is low, there is no hint for a considerable population substructure. The general exclusion chance was calculated as 0.657 for Penta D and 0.800 for Penta E, respectively. No deviation from Hardy-Weinberg-Equilibrium was observed ($P_{(Penta\ D)}$ =0.34, $P_{(Penta\ E)}$ =0.67). In 271 maternal and 253 paternal Penta D meioses no mutations were observed. In the Penta E system, 330 maternal and 325 paternal meioses followed the expected pathway.

3.4 Technical remarks

The bleeding-through of the TMR labelled peaks into the internal standard for TMR - peaks higher than 900 fluorescent units could only be avoided by using the 6 fold amount of the internal standard as recommended in the manual (Fig. 8).

4 Conclusion

The PowerPlex 16® amplifies a set of highly informative loci. Amplification of minute amounts of DNA leads to significant peak height differences in heterozygous loci, which can easily be avoided by increasing the target DNA and by reducing the number of PCR cycles. The new loci Penta E und Penta E did not show a mutation which was expected especially for Penta E, which is more polymorphic than loci FGA and vWA.

5 References

1 S.A. Miller, D.D. Dykes, H.F. Polesky, A simple salting out procedure for extracting DNA from human nucleated cells, Nucleic Acids Res. 16 (1988) 1215.

2 J. Dissing, L. Rudbeck, H. Marcher, in A. Carracedo, B. Brinkmann and W. Bär (eds.), Advances in Forensic Haemogenetics 6 (1995) 269-271

Fig. 1: Mean peak height in heterozygous samples in set 1. The error bars indicate the minimum and maximum peak height observed at each locus. (Y axis: relative fluorescent units)

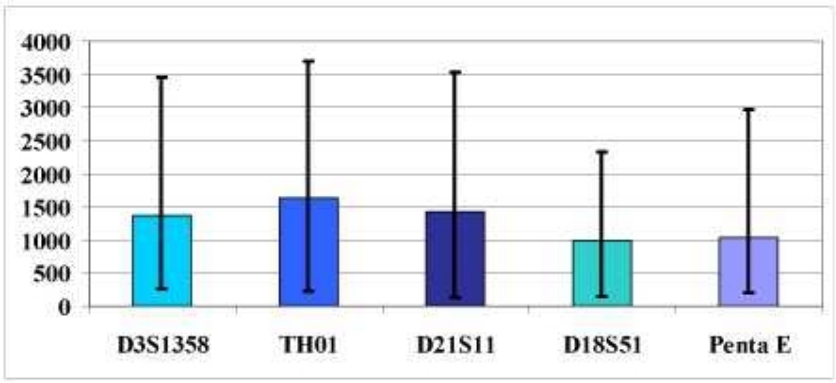

Fig. 2: Peak height differences (%) in set 1. Y axis: number of observations, X axis: bins of peak height differences

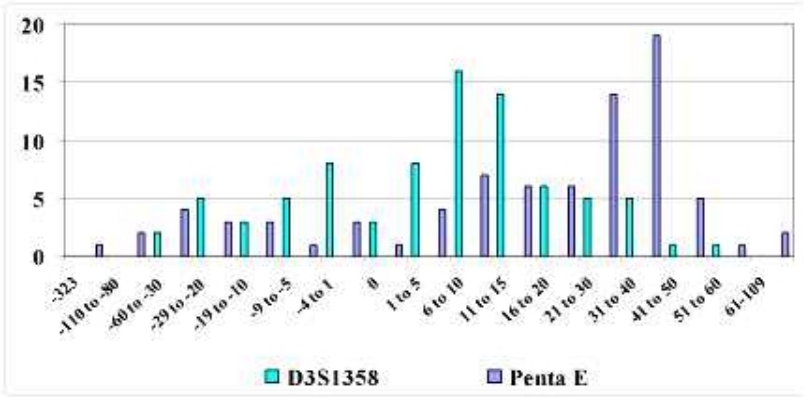

Fig. 3: Mean peak height in heterozygous samples in set 2. The error bars indicate the minimum and maximum peak height observed at each locus. (Y axis: relative fluorescent units)

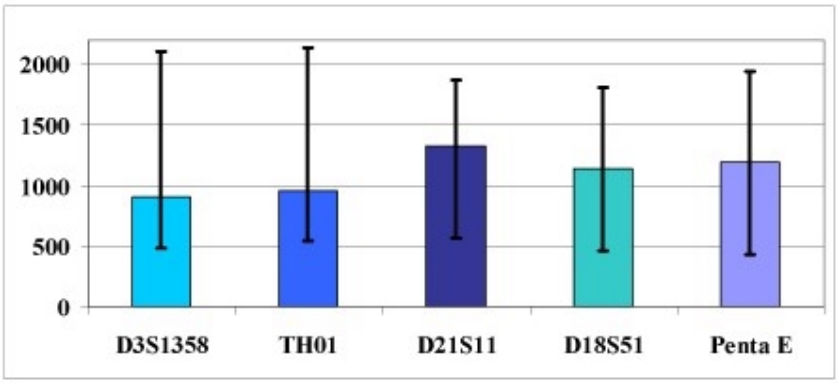

Fig. 4: Peak height differences (%) in set 2. Y axis: number of observations, X axis: bins of peak height differences

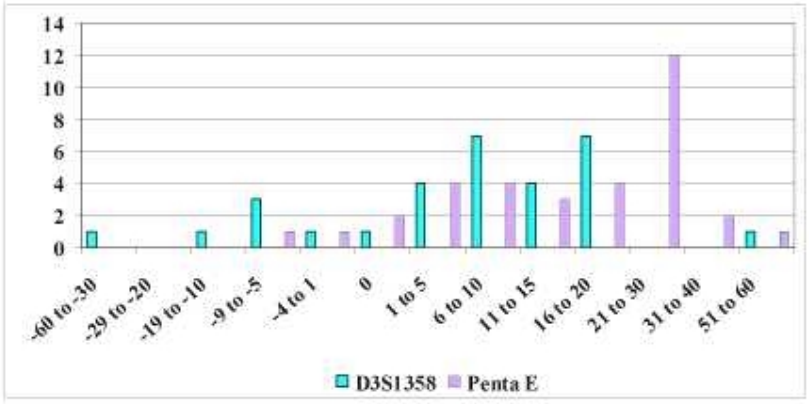

Fig. 5: Typical plots obtained from 1 ng and 0.5 ng target DNA

Fig. 6: Frequency distribution of Penta D alleles in 4 areas of Germany. [a] Institut f. Blutgruppenserologie und Genetik (Hamburg), [b] Institut f. humangenetische Analysen (Tuebingen), [c] Institut f. Rechtsmedizin (Mainz) and [d] Institut f. Blutgruppenforschung (Koeln)

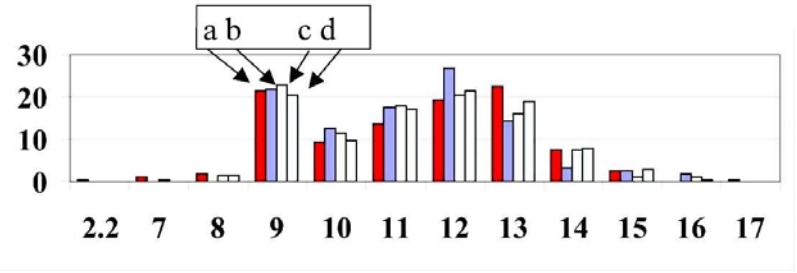

Fig. 7: Frequency distribution of Penta E alleles in 4 areas of Germany. [a] Institut f. Blutgruppenserologie und Genetik (Hamburg), [b] Institut f. humangenetische Analysen (Tuebingen), [c] Institut f. Rechtsmedizin (Mainz) and [d] Institut f. Blutgruppenforschung (Koeln)

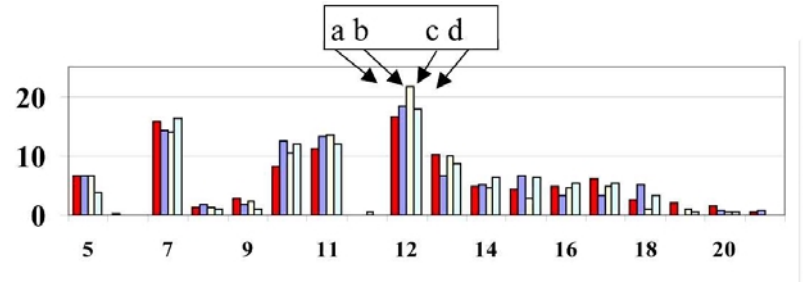

Fig. 8: Avoiding bleeding-through by increasing amount of internal standard