

DNA-PROFILING USING PROMEGA'S POWERPLEX® 16 KIT ON AN ABI 3100 SYSTEM

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Abstract

As a specialized lab for DNA analysis for different applications, Medigenomix offers DNA-profiling service for forensic Applications since 1998. Besides paternity testing and DNA trace analysis we have processed more than 20,000 samples for the German DNA database. Special experience has been gained with animal material from different species.

Our presentation will include an overview of the technical process using an ABI PRISM® 3100 multi capillary system. Details about quality management and logistics for high throughput will be shown as well as specific features of different kits, size standards and genotyper templates.

In our test the Power Plex® 16 kit in combination with the ILS 600 size standard showed clear peak patterns with high reliability and reproducibility for mouth swab samples. Despite high levels of multiplexing we recorded very few failures of markers. We have found this kit to be very satisfactory.

Genotyping for forensic questions: experiences•

- Participation on GEDNAP-comparison-tests since 1998
- Paternity-testing
- >20.000 DNA-profiles for the DNA-database of the Bundeskriminalamt
- DNA-trace-analysis on humans and animals

Genotyping for forensic questions: methods

- DNA-Extraction with affinity chromatography columns
(Macherey+Nagel, Qiagen)
- using 8-channel pipettes, 96-well plates
- QC each 348 samples: 24 allelic ladders, 8 to 9 known controls, 4 negative controls
- no determination of DNA-concentration of samples

Genotyping for forensic questions: ABI 3100 sequencer

Technical specs:

- 16 capillaries (36 or 50 cm)
- Polymer (POP 4 or POP 6)
- 4 (5) dyes
- PC (Dell), GeneScan data can be converted to MacIntosh data and analysed with GenoTyper 2.5
- Throughput: 384 samples (550 bp) per 10 h-day

Genotyping for forensic questions:

In the German DNA database eight systems are included:

D3S1358	D8S1179
D18S51	D21S11
FGA/FIBRA	TH 01
SE33/ACTBP2	vWA

Therefore we have to use kits shown on the next slide

Genotyping for forensic questions:

- Kits for Genotyping:
 - AmpF/STR Profiler Plus (Applied Biosystems) and SE33 in an additional reaction
 - AmpF/STR SGM Plus (Applied Biosystems) and SE33 in an additional reaction
 - Power Plex 16 (Promega) and SE33 in an additional reaction
 - SERAC MPX-2 (9 Systems incl. SE 33)
- Sizestandards:
 - ROX 500 (Applied Biosystems)
(retention time for fragments at 250 bp and 340 bp not constant)
 - HD 400 (Applied Biosystems)
(retention time for all fragments constant)
 - ILS 600 (Promega)
(retention time for all fragments constant)
 - SERAC LS
(retention time for all fragments constant)

Allelic Ladder:

On the next two slides allelic ladders are shown.
The calculated sizes showed the high reproducibility of size determination using ILS 600 on the ABI 3100 sequencer

Promega Powerplex® 16: Balance of peak intensity

In most cases of heterozygous genotypes the intensity of the two peaks is balanced.

Even if the sizes of the two alleles differs more than 80 bp, the peak intensity of the larger allele is 35 to 45 % of the intensity of the smaller allele.

On the next two slides examples were shown. In both cases large FGA/FIBRA alleles were present. Capillary Electrophoresis Artefacts. In the next slide an example of an unspecific peak is shown.

This peak is present in all four channels and could easily be identified as an artefact.

Capillary Electrophoresis Artefacts

In the next two slides also an example of an unspecific peak is shown. This peak is present only in the blue channel and therefore could not easily be identified as an artefact. Capillary Electrophoresis Artefacts
On the next slide the same PCR products as on the slide before were analyzed. The additional peak in the blue

channel is not detectable. This shows that the peak visible in the first analysis was an artefact.

Reproducibility of determined length of the fragments

In the next slides statistic data are shown:

We compared the determined length of the fragments of the allelic ladder, analyzed with ILS 600 on the Abi 3100 sequencer using four different arrays and several lots of polymer (POP4) over 6 months.

The results from all 16 capillaries were combined in the tables.

Summary

PowerPlex® 16 System is a powerful tool for analysis of buccal swabs.

- The kit allows analysis of 16 markersystems in one lane with very low failure rates.
- Microsatellite analysis using the ABI 3100 Sequencer and the size standard ILS 600 leads to very high reproducibility of the determined length of the fragments.
- Daily throughput is of 348 samples, 24 allelic ladders and 12 controls at a 10-hour working day.