

# Development of SNP microarray for supplementary paternity testing

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**Abstract.** We have developed a SNP microarray for paternity testing, which contains 10 SNPs and in addition X and Y chromosome-specific markers. SNPs were selected with an emphasis on their high allele frequency in the Finnish population. Coding regions of known genes were excluded. With this setup of SNPs, we have calculated the average exclusion power in paternity testing to be 86.5%. The array was validated by comparison with the results in 120 paternity trios obtained with microsatellite markers (Profiler™ and SGM+™ kits, Applied Biosystems). We conclude that the SNP microarray provides a feasible method to obtain additional genotype information especially in complex paternity cases. © 2003 Elsevier B.V. All rights reserved.

*Keywords:* SNP; Microarray; Paternity testing

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## 1. Introduction

Microsatellite (STR) marker sets are predominantly used in paternity testing. While genotyping of STR markers is everyday routine in laboratories, reliable and scalable SNP genotyping methods are intensely developed [1]. In complex paternity testing cases such as mutations or deficiency cases, when the DNA profiles of mother or alleged father are not available or father candidates are closely related additional genotype information would be helpful. For such instances, we have applied an alternative approach, a SNP microarray. Due to the mostly biallelic nature of SNPs, their information content is lower than in STR markers and thus larger number of markers are required to produce same information. On the other hand, SNPs have lower mutation rate, are abundant throughout the human genome and are more easily automated for high throughput genotyping.

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*Abbreviations:* STR, short tandem repeat; SNP, single nucleotide polymorphism; PCR, polymerase chain reaction.

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## 2. Material and methods

SNP genotyping was performed on microarray format with maximum of 80 samples per one microarray. Allele discrimination was performed with allele-specific oligonucleotides in a primer extension reaction (Fig. 1) described by Pastinen et al. [2].

Microarray slides were manufactured with OmniGrid microarrayer, which dispenses nanoliter volumes of allele-specific oligonucleotides on microscopic slides. Oligonucleotides attach to slide surface via aminated 5' termini by formation of a covalent bond. Each biallelic SNP requires two allele-specific oligonucleotides differing in the 3' nucleotides defining the alleles. The SNP loci were amplified in a single multiplex PCR reaction. Subsequently PCR products were in vitro transcribed by T7 RNA polymerase utilizing T7 promoter sequence introduced to the amplicons via PCR primers. After transcription, PCR products were enzymatically degraded to yield single-stranded RNA template to facilitate hybridization to allele-specific oligonucleotides on microarray surface. RNA samples were hybridized on a microarray slide in humid chamber at a constant temperature. After hybridization, allele-specific oligonucleotide/RNA hybrids were extended by MMLV-RT enzyme catalyzed reaction using fluorescently labeled deoxynucleotides. Oligonucleotides having complete hybridisation with sample RNA were extended in this reaction whereas incomplete hybridisation showed no or residual primer extension. Microarray slides were scanned with ScanArray4000 laser scanner instrument and fluorescent signals were quantitated by QuantArray software. Raw signals were converted to genotypes with the SNPSnapper software developed by Dr. Juha Saharinen in National Public Health Institute, Helsinki, Finland ([ajuha.saharinen@ktl.fi](mailto:ajuha.saharinen@ktl.fi)).

The 120 trios for the validation of the SNP array method were selected from the paternity cases analysed in our laboratory with microsatellite markers using Profiler™ kit (Applied Biosystems) and when needed SGM+™ kit (Applied Biosystems). The exclu-

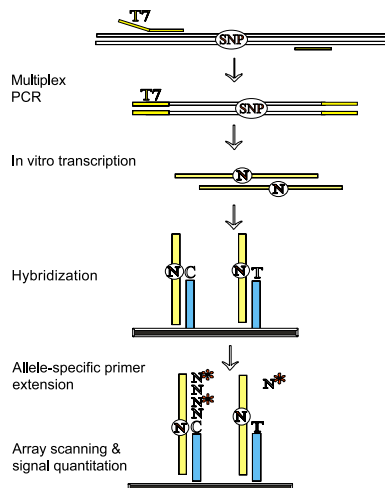


Fig. 1. Principle of the allele-specific primer extension SNP microarray.

Table 1  
The SNP loci used for the paternity array

Reference number	Minor allele frequency	Chromosomal location
rs2239393	0.44	22q11
rs324026	0.46	3q13
rs936461	0.42	11p15
rs904952	0.49	15q14
rs726283	0.40	6q25
rs6265	0.25	11p13
rs6279	0.38	11q23
rs6293	0.49	9q34
rs6311	0.43	13q14
rs1014531	0.42	13p14

sions were based at least on three loci and in the non-exclusion cases the power of evidence was at least 99.8%.

### 3. Results and discussion

The selected 10 SNP loci are shown in Table 1. SNPs were scattered in the genome and in addition X and Y chromosome-specific markers were included. These loci were not located within coding regions of genes. Based on the allele frequencies of the SNPs, the average exclusion power of these markers is 86.5% in the Finnish population. This provides reasonable supplementary genotype information which may be helpful in complex paternity cases such as mutation or in deficiency cases. The exclusion power can be increased by selecting more SNP loci for the array.

A set of 120 trios which were previously genotyped with the Profiler™ and SGM+™ kits were genotyped with allele-specific primer extension microarray. Blinded genotyping experiment produced genotype information to identify 33 out of 34 exclusion cases and no Mendelian errors were found among the 86 non-exclusion cases. SNP microarray produced reliable genotypes in all tested samples and no discrepancies were seen in duplicate genotypes. Validation experiments showed that allele-specific primer extension microarray provided a reliable and feasible method to produce additional information in complex paternity cases. Additional SNPs will be tested to increase the exclusion power of SNP microarray.

### Acknowledgements

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### References

- [1] A.-C. Syvänen, Assessing genetic variation: genotyping single nucleotide polymorphisms, *Nature Reviews Genetics* 2 (2001) 930–942.
- [2] T. Pastinen, et al., A system for specific, high-throughput genotyping by allele-specific primer extension on microarrays, *Genome Res.* 10 (2000) 1031–1042.