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Rapid applicable separation of DNA polymorphism on the human Y-chromosome by micro fabricated electrophoresis chip.

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Abstract

On a micro fabricated electrophoresis chip fast analysis of three genomic DNA polymorphisms on the human Y-chromosome has been realized. The total analysis time of these three polymorphisms, Y A*lu*, 47z/S*tu*l and 12f2, are all within 100 s, with RSD = 3.5% and relative error RE = 3.7%. Then a mixture of ten DNA markers on the human Y-chromosome have been separated with the smallest fragment size of 7bp.

Introduction

After the efficient rule of the CE in the Human Genome Project (HGP), Microchip electrophoresis will play a significant rule in Genomics and Proteomics, since it offers fast analysis and less sample consumption

Results and discussion Validation of microchip electrophoresis in DNA analysis has been carried out using an Agilent 2100 Bioanalyzer. the reproducibility and accuracy of fragment sizing of a 10bp DNA Ladder (Fig.1) has been show to be satisfactory with RSD = 1.0% and RE = 5.0% (n = 12). Based on such reliable results, fast analysis of three DNA polymorphisms on the human Y-chromosome has been realized with microchip electrophoresis. The YAP at locus DYS287 is a polymorphism resulting from the insertion of the A*lu* element on the long arm of the Y-chromosome so we have PCR product either YAP^- without Alu repeat (150 bp), or YAP+ with A*lu* repeat (455 bp). As shown in (Fig.2), YAP^- and YAP+ could be distinguish quite well within 90 s with a good reproducibility and accuracy of the size (table 1). The second polymorphism is the probe 47z which has two alleles, one of them can be digested with S*tu*l into three fragments, 370 bp,270 bp and 100 bp, while the other allele can not be digested, so that only one fragment of 370 bp could be obtained. From (Fig.3), We can see that all the digested products could be separated with high resolution within 90 s with good produciblity and accuracy (table 1). In order to examine the maximum resolution of the Agilent Bioanalyzer a mixture of ten DNA markers on the human Y-chromosome (Fig. 4), located on AZFa and AZFc and relative to examine the cause of spermatogenic failure, have been separated successfully with the smallest fragment size difference of 7 bp (Fig.5 and table 2). This affirms the superiority of the microchip for its rapid sizing with good reproducibility and high resolution.

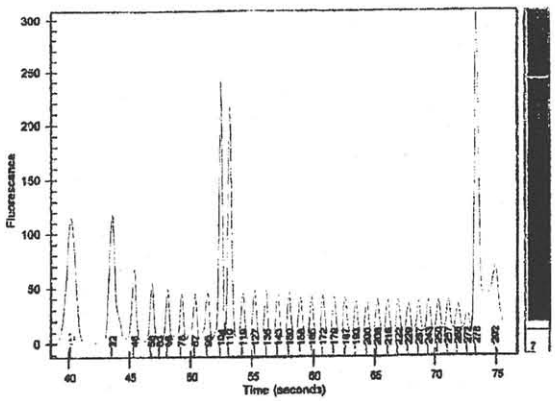

Figure 1. separation of 10 bp DNA Ladder.

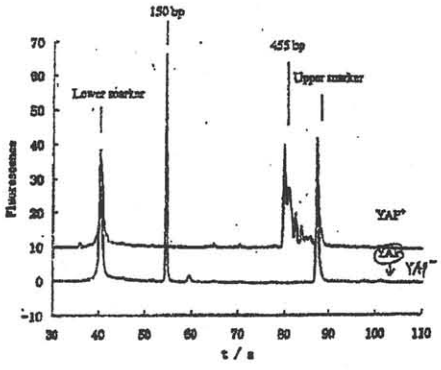

Figure 2. separation of YAP polymorphism.

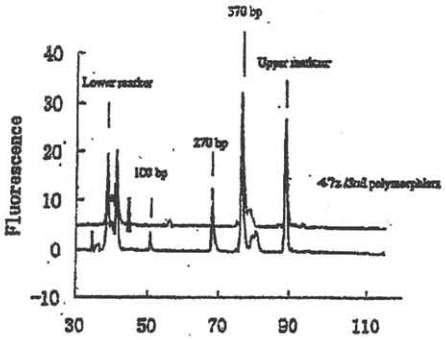

Figure 3. separation of 47z/*StuI* polymorphism.

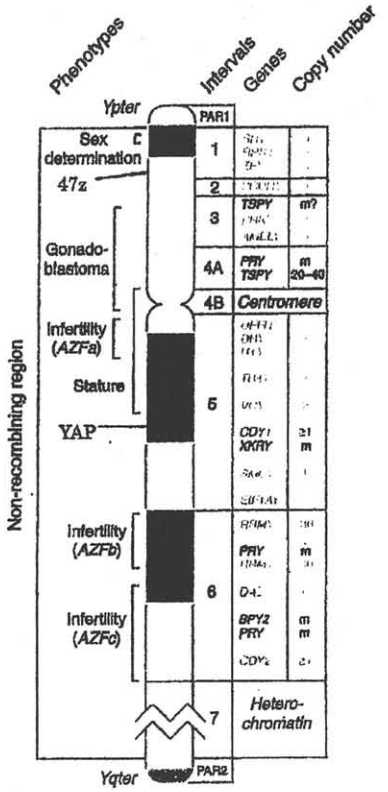

Figure 4. Human Y-chromosome.

Polymorphism		Ave. (bp)	RSD%	RE%
YAP	150 bp	149	0.68	-0.67
(n=39)	455 bp	449	1.0	-1.3
47z/StuI	100 bp	102	1.8	2.0
(n=42)	270 bp	280	1.2	3.7
	370 bp	372	0.97	0.54

Table 1. Validation of YAP, 47z polymorphism.

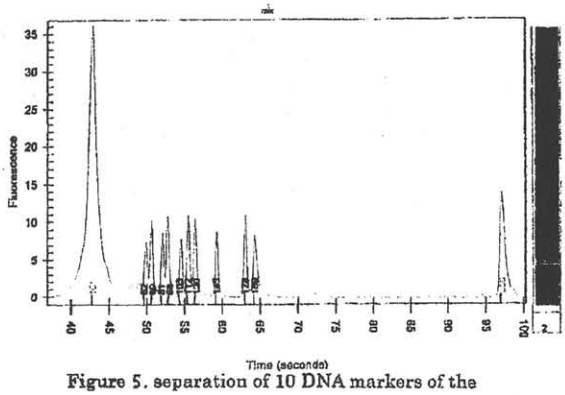

Figure 5. separation of 10 DNA markers of the human Y-chromosome

Y-chromosome marker	Avg. (bp)	RSD%	RE%
SY 619 (61 bp)	63	1.2	2.2
SY 592 (69 bp)	68	0.72	0.5
SY 595 (81 bp)	81	0.64	0.2
12f2 (88 bp)	87	0.69	1.2
SY 593 (102 bp)	103	0.44	0.9
12f2 inner marker (112 bp)	111	0.46	0.9
SY 202 (121 bp)	119	0.80	1.8
YAP (150 bp)	145	0.6	3.6
SY 90 (176 bp)	177	0.50	0.7
SY 200 (189 bp)	188	0.47	0.5

Table 2. Validation of ten DNA marker on the human Y-chromosome (n=30)