MULTIPLEX PCR ON MULTICHANNEL MICROCHIP ELECTROPHORESIS, AN ULTRAFAST TECHNOLOGY FOR GENETIC DIAGNOSIS

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Abstract

For the requirement of the Genetic Diseases Diagnosis a multiplex PCR for 6 markers located on the human Y-chromosome related to spermatogenic failure has been designed. These have been run on 12 channel microchip electrophoresis system, which can analysis the separation on these 12 channels simultaneously. By this method **72** markers (6*12= **72**) can be analyzed simultaneously, in about 100 second.

Key words: Multiplex PCR, Multichannel Microchip, Spermatogenetic Failure.

Introduction

The Human Genome Project (HGP) is moving quickly to the post genome era. The post genome era will stimulate the investigations for single nucleotide polymorphism (SNP), mutation analysis and proteome analysis. This will lead to gene therapy and DNA diagnosis of the human diseases and will result in a new class of drugs. High speed analysis for the mutations and the markers on the human Genes is a very important step for the high speed Diagnosis of the Genetic Diseases.

Experimental

- Agilent 2100 Bioanalyser microchip electrophoresis (Agilent technologies, Germany).
- Hitachi SV 1210 Microchip electrophoresis system with (LED) detector and with 12 channel each one has a detecting point (Hitachi Electronics Co.,

Table 1. Markers sizes.

The marker name	Marker size (bp)
SY610	61
SY202	121
SY90	176
SY276	216
SY624	256
SY57	288

Tokyo, Japan).

- The Genomic PCR products have been prepared in Prof. Nakahori Lab., School of Medicine, The University of Tokushima.

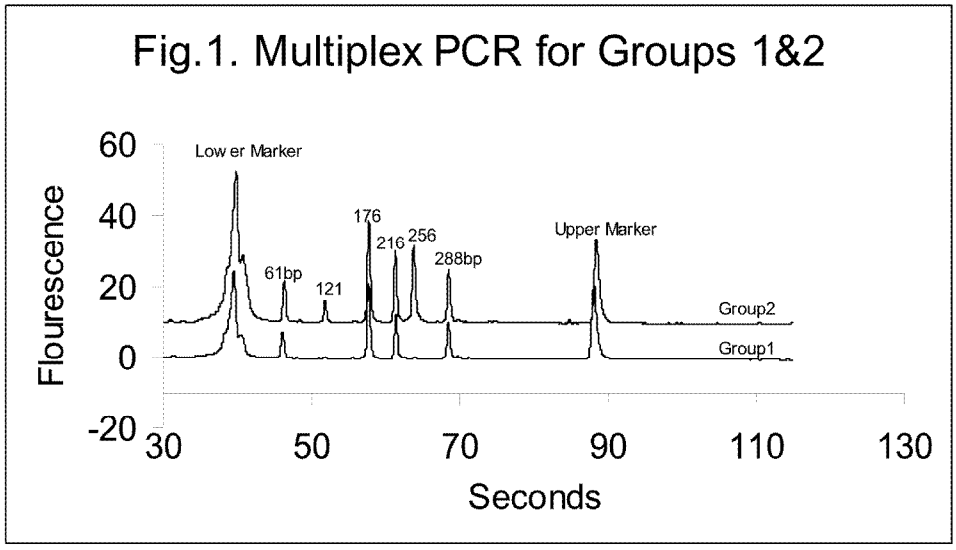
Results

In order to design a multiplex PCR for the six markers on the human Y-chromosome related to spermatogenic failure, we studied the PCR conditions for each one, the PCR condition for each was similar (we choose them to be like this) which is (94cfor 1 min., 58c for 1:30min., 72c for 2 min.) and all were cycled for 30 cycle.

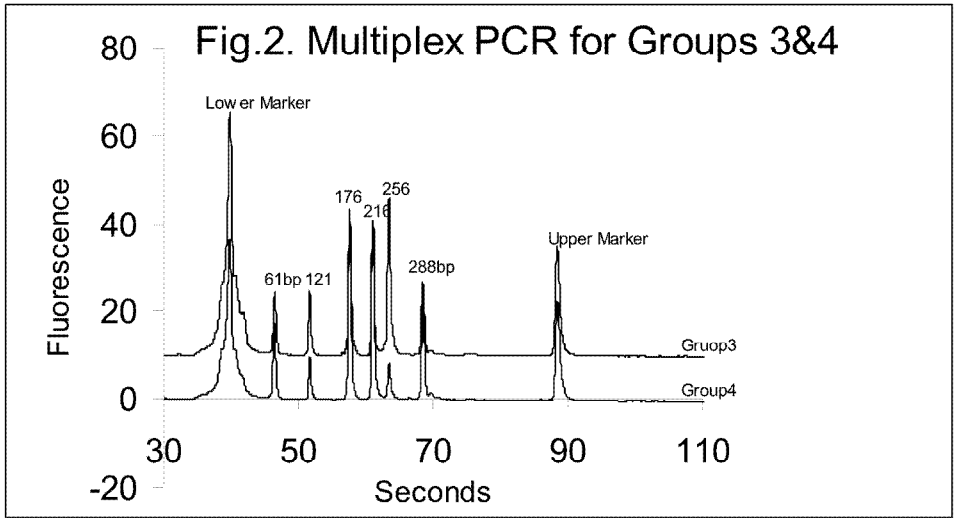
Table 2. The ratio of SY202, SY624 primers and enzyme

	Group 1	**Group 2 recommended**	Group 3	Group 4
DNA	2	2	2	2
10*buffer	2	2	2	2
dTNPs	2	2	2	2
SY202 F	**1**	**2**	**1**	**2**
SY202 R	**1**	**2**	**1**	**2**
SY624 F	**1**	**2**	**1**	**2**
SY624 R	**1**	**2**	**1**	**2**
$Mgcl_2$	2	2	2	2
Tag Gold	**0.1**	**0.1**	**0.2**	**0.2**

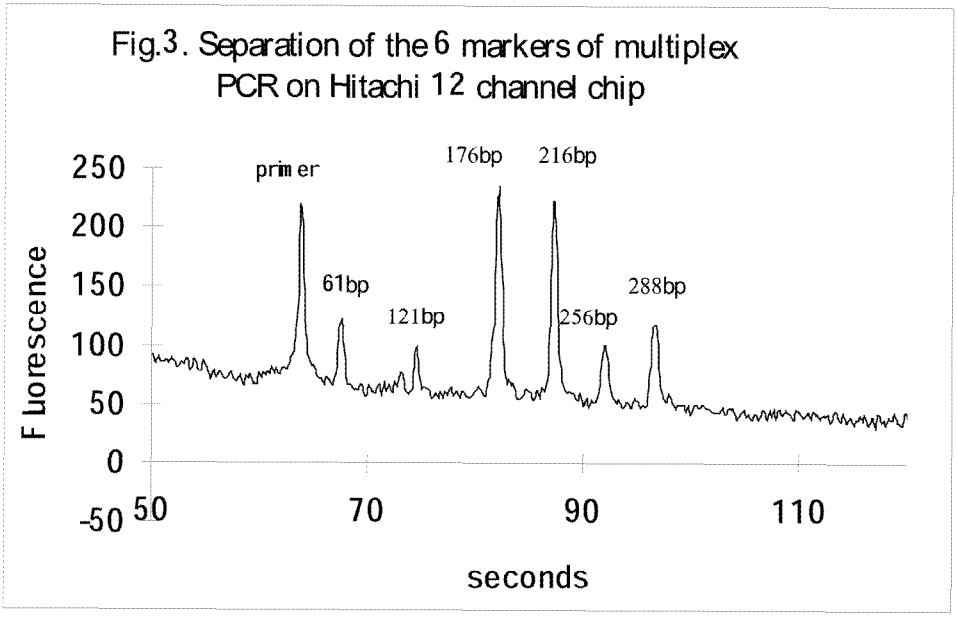
They were prepared in four groups (Table 2.). The first one (group 1) the fragments were in the same ratio, we found that we could magnify 4 markers of the six (Fig.1) while 2 markers (SY202, SY624) showed a very small peaks.
In order to over come this we designed 3 groups as shown in table 2 (groups 2, 3, 4), in group 2 we doubled the ratio of the primers for the 2 markers (SY202, SY624) and we could get the 6 primers clearly (fig.1);



in group 3 we doubled the ratio of the enzyme only and the result was also good (fig.2); while in group 4 we doubled both the ratio of the primers and the enzyme (SY202, SY624)the result was relatively better than groups 3 and 4 (fig.2).



So we found that doubling either the primers or the enzyme ratio is enough and no need for doubling them both, and since primers are less expensive than the enzyme so group 2 is the recommended one for this work.
Later on we run these 6 markers on Hitachi SV 12 10 microchip systems, this instrument has 12 channels which can detect simultaneously, so in 100 second we can detect 72 samples (6*12= 72).



This shows another horizon for Microchip Electrophoresis for the ultrafast analysis for genetic diseases.

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