

**CHROMOSOMAL ANEUPLOIDIES  
BY  
QUANTITATIVE FLUORESCENT PCR ANALYSIS  
(for Amniotic Fluid)**

**Extra Primer Set**



**Hong Kong DNA Chips Ltd**

**Notice**

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**1. Kit components**

The kit contains the following components sufficient to process approximately 25 amniotic fluid samples and should be stored at -20°C. Repeated freezing and thawing cycles will inactivate some components, therefore, keeping the components in small aliquots is highly recommended.

- PCR mastermix (1 x 530 l)
- Primer 21-4 (1 x 50 l)
- Primer 21-5 (1 x 50 l)
- Primer 18-3 (1 x 50 l)
- Primer 13-3 (1 x 50 l)

Note, the following additional materials are also required.

- DNA extraction kit for amniotic fluid (QIAamp DNA Blood Kit (cat No. 51104) or other method giving DNA of similar PCR quality)
- Gene Scan TAMRA 500 size standard (ABI cat. No. 401733)
- ABI Hi-Di™ formamide

**2. Procedure**

**2.1. DNA Extraction From Amniotic Fluid**

**Method**

1. Centrifuge 1.5 ml of amniotic fluid for 10 min at 4°C at 1000 g, then discard the supernatant.
2. Add 200 µl of ice-cold 1 X PBS to resuspend the pellet.
3. Follow instructions of Qiagen Blood Protocol with exception of final elution step: Add 100µl AE or distilled water to the QIAamp spin column, incubate for at least 5 min at room temperature. Centrifuge at 6000 g at 25°C for 1 min to collect DNA from the sample.

**2.2. Quantitative fluorescent PCR (QF-PCR)**

**Method**

1. For EACH PCR reaction, mix the following components in a total volume of 25 µl. One negative control (reaction without template) should be run with each set of experiments.

Components	Vol. for 1X
dd water	17.7 µl – n
PCR mastermix	5.3 µl
Primer	2 µl
Sample DNA	n
<b>Total volume</b>	<b>25 µl</b>

Note: For amniotic fluid extracted by the QIAamp DNA Blood Kit, 5 µl sample DNA is sufficient for PCR amplification. For other DNA extraction method, please use 50--100 ng sample DNA.

2. The PCR profile required to run the pre-natal screening reactions is optimized for PCR thermal cycler with temperature ramping rate of 3°C/s. Program these steps:

- 1 Cycle: 95°C, 11 min
- 28 Cycles: 94°C, 1 min  
59°C, 1 min  
72°C, 1 min
- 1 Cycle: 60°C, 45 min

3. Analysis of fluorescent PCR products.

- a. For each analysis, 4 µl of PCR product is mixed with 16 µl Hi-Di™ formamide and 1 µl of Gene Scan TAMRA 500 size standard.
- b. Heat the mixture at 95°C for 5 min.
- c. Cool the mixture immediately on ice for at least 30 s.
- d. Briefly centrifuge the mixture.
- e. Proceed to analysis using ABI PRISM Genetic Analyzer.

For detailed operation procedures on using the ABI PRISM Genetic Analyzer, please refer to the relevant User Manual provided by your supplier.

**3. Data collection, analysis and interpretation**

**Table 1: PCR product information**

Primer mix

Marker	Size of PCR Products (bp)	Color of Plot in ABI 310
21-4	163-187	Black
21-5	~200	Blue
18-3	~358	Blue
13-3	152-172	Blue

PCR products are resolved on an automated laser fluorescent sequencer e.g. ABI PRISM 310 Genetic Analyzer.

For each STR marker, a normal sample produces either a single peak (homozygous state) or two (diallelic) peaks with peak area ratio of about 1:1 (heterozygous state). Trisomy genotypes show characteristic three (triallelic) peaks of similar intensity or two (diallelic) peaks with a dosage ratio of 2:1 (measured as peak areas). Additional markers are used in case of ambiguous result or uninformative homozygous peaks. The combined heterozygosity produced by two or more STRs lowers the likelihood of uninformative results (i.e. homozygosity at the loci).

If you require more detailed analysis information please contact Hong Kong DNA Chips for technical assistance.

**4. Technical assistance**

Our technical staff are able to help troubleshoot any problems you may have in using this kit. Simply call +(852) 2111 2123 for assistance during our office hours:

Monday – Friday: 9:00 a.m. to 5:30 p.m.  
Saturday: 9:00 a.m. to 1:00 p.m.

A recorded message (in English, Cantonese or Putonghua) may be left at other times.

Alternatively, you are welcome to contact our technical staff by fax or email.

**Fax:** +(852) 2111 9762  
**Email:** technical@dnachip.com.hk

**5. Warranties and liabilities**

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**6. Limitations**

The pre-natal screening assay described here is intended for research purposes. The oligonucleotide primers have been tested against a limited number of examples of DNA extracted from known human chromosomal aneuploid and normal cells. False negatives may occur if the primers included in the product cannot amplify or detect nucleic acid from a particular aneuploidy. False positives may occur if the primers included in the product amplify or detect nucleic acid from a non-human source.

The manufacturer does not claim or intend that this product will be able to detect all known aneuploidies.