# QF-PCR as a Rapid Technique to be Introduced into Routine Prenatal Cytogenetic Diagnosis

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by

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# List of abbreviations

| | |
|---|---|
| AFP | Alfa-fetoprotein |
| AMXY | Amelogenin locus on X and Y chromosomes |
| bp | Base pairs |
| CVS | Chorionic villous sampling |
| DNA | deoxyribonucleic acid |
| DSCR | Down syndrome critical region |
| E3 | Unconjugated estriol |
| FISH | Fluorescent in situ hybridization |
| G banding | Giemsa banding |
| hCG | Human chorionic gonadotropin |
| IAM | infinite allele model |
| MAPH | Multiplex amplifiable probe hybridization |
| MBP | Mylein basic protein |
| MLPA | multiplex ligation-dependent probe amplification |
| MoM | Multiples of median |
| NOR-staining | Silver staining for nucleolar organizer regions |
| NT | Nuchal translucency |
| PAPP-A | Pregnancy associated plasma protein A |
| PCR | Polymerase chain reaction |
| Q- banding | Quinacrine banding |
| QF-PCR | quantitative fluorescent polymerase chain reaction assay |
| q-PCR | quantitative polymerase chain reaction |
| R- banding | Reverse banding |
| S1 | Markers Multiplex set 1 |
| S2 | Marekers Multiplex set 2 |
| SNP | Single-nucleotide polymorphism |
| STR | Short tandem repeats |
| T- banding | Telomere banding |
| Ta | Annealing temperature |
| β-hCG | Beta subunit-human chorionic gonadotropin |

# Introduction

Chromosomal aberrations are relatively uncommon, but because of their intractability they are among the most important causes of congenital malformation and mental handicap. They also contribute to many other problems including infertility, abnormal sexual development, and spontaneous abortion. This makes the diagnosis of these diseases before birth an aim of a lot of centers and physicians **(http://www.emro.who.int/Publications).**

The majority of chromosome abnormalities identified in prenatal samples are trisomy for chromosomes 13, 18 or 21 and sex chromosome aneuploidies. These are associated with the newborn phenotypes, Patau syndrome, Edwards syndrome and Down syndrome (trisomy 13, 18 and 21, respectively), and the less severe Turner (monosomy X) and Klinefelter (XXY) syndromes ***(Mann et al., 2004)***.

Prenatal diagnosis for women at increased risk for chromosome abnormalities is routinely undertaken for karyotype analysis. While standard cytogenetic studies detect structural and copy number changes of chromosomes, it requires prolonged cell culture resulting in average reporting times of about two to three weeks ***(Rooney and Czepulkowski 2001).***

Rapid methods, such as quantitative fluorescent polymerase chain reaction assay (QF-PCR) by using small tandem repeat (STR) markers, were developed for prenatal detection of sex and aneuploidies involving chromosomes 21, 18 and 13. This method can relieve parental anxiety, and improve the quality of pregnancy management ***(Mann et al., 2001).***

The use of QF-PCR analysis of short tandem repeats (STR) for the detection of aneuploidy was first reported by Mansfield (1993). It has

since been validated and successfully applied for the rapid diagnosis of prenatal aneuploidy by a number of European labs ***(Cirigliano et al., 2001; Levett et al., 2001 and Mann et al., 2004)***. The rapid result is issued to clinicians and is followed by full karyotype analysis in all cases where cultured cells are available. The technique has -been approved by the New York State Clinical Laboratory Evaluation Program ***(Brown et al., 2006)***. Another advantage of QF-PCR over other methods of prenatal diagnosis is that it is feasible on a few cells. Also, since the analysis can easily be automated, many samples can be processed at the same time ***(Cirigliano et al., 2001).*** Molecular cytogenetic testing by QF-PCR for pregnant women at increased risk of chromosome aneuploidy leads to rapid reassurance for those with normal results and earlier decisions on pregnancy management in the case of abnormality ***(Ogilvie et al., 2005).***

# Aim of the Work

In this study we aim to evaluate QF-PCR as a rapid technique to diagnose trisomy of chromosomes number 13, 18, 21 and sex chromosomes in comparison with the traditional karyotyping to be introduced into routine prenatal diagnosis.

# I. The cell division

The understanding of the cell division is essential to understand cytogenetics and cytogenetic abnormalities resulting from errors during division. Two types of cell division are known: mitosis and meiosis. Mitosis is the division of somatic cells, while meiosis is a special type of division that occurs only in gametic cells (Gersen and Keagle, 2005).

## A. Division of somatic cells

The average mammalian cell cycle lasts about 17-18 hours. It is the transition of a cell from one interphase through cell division and back to the interphase (Therman and Susman, 1993).

The cell cycle (Fig.1) is divided into four major stages variable in their length; the first three stages gap1 (G1), synthesis (S) and gap2 (G2) comprise the interphase. The fourth and final stage is mitosis (M) (Gersen and Keagle, 2005).

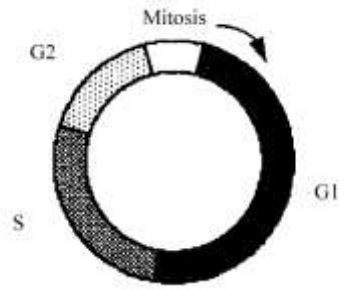


Fig (1): The cell cycle: G1, S, G2 and Mitosis (Gersen and Keagle, 2005).

1. **First stage (Gap 1 stage, G1):** Chromosomes exist as single chromatids. Cells are metabolically active during this stage; this is the stage when the protein synthesis takes place and cell prepares for DNA synthesis (Barch et al., 1997).
2. **Second stage (Synthesis stage, S):** It is when the DNA synthesis occurs. The DNA replicates itself and then forms chromosomes consisting of two identical chromatids (Therman and Susman, 1993).
3. **Third stage (Gap 2 stage, G2):** The cell prepares to undergo the cell division. The completion of G2 represents the end of the interphase (Therman and Susman, 1993).
4. **Final stage (Mitosis, M):** This stage lasts for only 1-2 hours in most mammalian cells. Mitosis is the process by which the cells reproduce themselves, creating two daughter cells that are genetically identical to one another and to the original parent cell (Gersen and Keagle, 2005).

It is further divided into stages (fig. 2):

***a) Prophase:*** This is when chromosomes begin to coil, become more condensed and begin to become visible as discrete structures (Nussbaum et al., 2004).

***b) Prometaphase:*** The nuclear membrane disappears and spindle fibers begin to appear. Chromosomes attach to the spindle fiber at their kinetochores (Nussbaum et al., 2004).

***c) Metaphase:*** The mitotic spindle is completed, their centrioles divide, move to opposite poles and chromosomes line up on the equatorial plate. It is the metaphase chromosomes that are traditionally studied in cytogenetics (Murray and Hunt, 1993).

***d) Anaphase:*** Centromeres divide longitudinally and the chromatids separate during this stage. Sister chromatids migrate to opposite poles (Miller and Therman, 2001).

***e)Telophase:*** The chromosomes uncoil and become indistinguishable again, the nuclear membrane is reconstructed (Miller and Therman, 2001).

***f) Cytokinesis:*** it is the process by which the cytoplasm cleaves.

***g) Interphase***: Following completion of cell division, the cell remains in this stage until the next cell division.

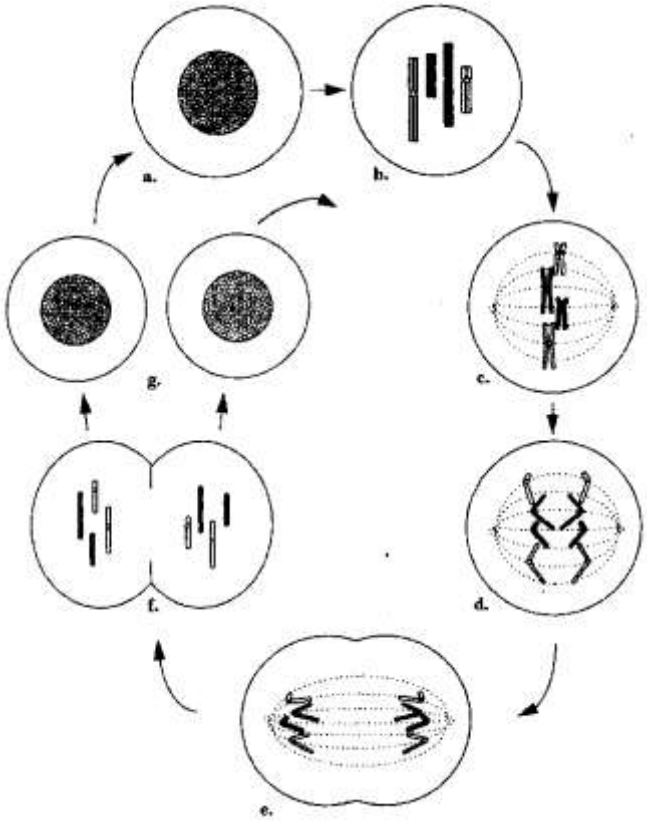

Fig. (2): Schematic representation of two pairs of chromosomes undergoing mitosis.
a: interphase, b: prophase, c: metaphase, d: anaphase, e: telophase, f: cytokinesis, g: interphase of the next cycle.
(Gersen and Keagle, 2005)

# B. Division of gametic cells

Meiosis is the type of cell division by which the diploid gametic cells (2n = 46 chromosomes) of the germline give rise to haploid gametes (n=23 chromosomes). It consists of one round of DNA synthesis followed by two rounds of chromosome segregation and cell division. It occurs in the primary spermatocytes or primary oocytes (Nussbaum et al., 2004).

It is further divided into meiosis I and II

## 1. Meiosis I :

Throughout this stage the chromosomes condense and become shorter and thicker (Nussbaum et al., 2004).

It is a complicated process with the following stages: (Fig. 3)

***a)*** ***Prophase I:*** (subdivided into leptotene, zygotene, pachytene, diplotene, diakinesis) all through the prophase stage the chromosomes shorten and thicken (Handel, 1998).

***b)*** ***Metaphase I:*** The bivalents line up on the equatorial plate with their centromere randomly oriented toward opposite poles (Handel, 1998).

***c)*** ***Anaphase I:*** *T*he centromeres separate and migrate to opposite poles (Handel, 1998).

**d)** ***Telophase I:*** The haploid sets of chromosomes reach opposite poles and the cytoplasm divides. The result is two cells with 23 chromosomes (Handel, 1998).

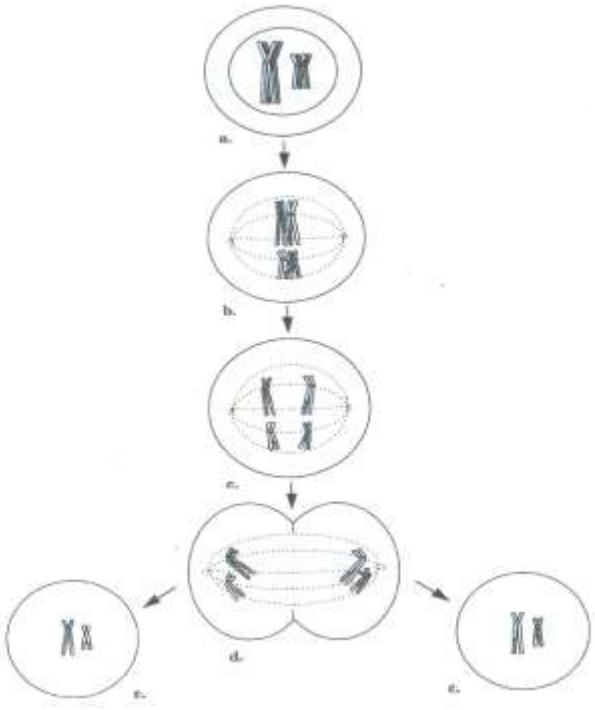

Fig. (3): schematic representation of chromosome pairs undergoing meiosis I.
a: prophase I, b: metaphase I, c: anaphase I, d: telophase I, e: products of meiosis I.
(Gersen and Keagle, 2005)

## 2. Meisosis II: (Fig.4)

The 23 chromosomes line up on the equatorial plate. The chromatids separate and move to opposite poles in anaphase II, and cytokinesis occurs in telophase II. The net result is four cells, each containing 23 chromosomes each consisting of a single chromatid. The effects of crossing-over and random assortment of homologs, each of the new cells differ genetically from one another and from the original cell (Gersen and Keagle, 2005).