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MQS-PCR: a simple and quick multiplex PCR molecular method for human chromosomal sex determination in DNA samples

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ABSTRACT. Quantitative PCR puts great demands on DNA quality and relies on a comparison of fragment amplification between two chromosomes using different primers. The use of a single primer pair capable of reliable relative comparative amplification would be a great advantage. Using this approach, we developed a rapid, high-throughput, semi-automated and cost-efficient multiplexed method for molecular determination of chromosomal sex called MQS-PCR – multiplex quantitative sexing PCR. DNA sequences located on different chromosomes and differing in length can be amplified and fluorescently labelled with a common fluorescent primer and conveniently separated and detected using capillary electrophoresis. In this method, the intensity of amplification of each of the fragments is compared to determine DNA dosage. MQS-PCR achieves 100% analytical sensitivity and specificity in detecting normal and abnormal sex chromosomal complements (45,X, 46,X,i(X)(q10), 46,X,i(X)(p10), 46,X,X(p-), 46,X,X(q-), 47,XXX, 47,XXY and 47,XYY). It is a reliable, low cost, and rapid detection method for the determination of chromosomal sex and sex chromosomal abnormalities in human samples.

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Key words: Chromosomal sex; Determination of sex; Sex chromosomal abnormalities; Multiplex fluorescent PCR; Karyotype

INTRODUCTION

Mutter and Pomponio (1991) reported on a simple molecular technique for sex identification that focused on differences between *ZFY* gene sequences on the Y chromosome and the homolog *ZFX* on the X chromosome. This allowed them to design a single primer pair that amplified both genes and produced different size X and Y amplicons. Taking advantage of these competitive internal genomic conditions, they were able to reliably diagnose individuals with a 47,XXY or a 47,XYY chromosome complement, since the relative amounts of *ZFX* and *ZFY* products were in 2:1 and 1:2 ratios, respectively, compared to 1:1 for normal controls. This very useful approach, based on homology of parts of the X and Y chromosomes, is not applicable to human autosomes. However, Mansfield (1993) reasoned that in heterozygous individuals, human polymorphic microsatellites could provide a simple and direct means of determining gene dosage, since one allele would serve as a competitive internal standard for the other. She used an automatic fluorescent DNA sequencer for computer-assisted laser densitometry and found that trisomic patients displayed, in informative microsatellite loci, three fragment peaks of equal intensity or two fragments at an average 2:1 dosage.

Soon afterwards, Pertl et al. (1994) used this approach as a rapid molecular method for prenatal detection of Down syndrome. This approach was called QF-PCR (quantitative fluorescent PCR) and became widespread since it is rapid and efficient. In 2012, a single service claimed to have performed more than 40,000 prenatal diagnostic tests solely using the QF-PCR methodology. This same approach could also be used to diagnose numerical and some structural sex chromosome abnormalities (Donaghue et al., 2003) although the more restrained polymorphism of STR, especially on the short arm of chromosome X, limited the diagnostic power.

An alternative to QF-PCR is the use of Homologous Gene Quantitative PCR (HGQ-PCR), a methodology developed by Lee et al. (1997). HCQ-PCR is based on the amplification of a sequence and simultaneously of another closely homologous sequence, sharing amplification primers, and thus with competitive comparative quantification similar to that of QF-PCR. It is more restricted in informativity because it is not compatible with three peak patterns in the case of trisomies. On the other hand, it does not depend on variability of the reference sequence since the DNA sequences only have to be paralogous and may even be monomorphic.

Indeed, we found that there is no need for extensive homology of the test and reference sequences; it is enough for them to share amplification primer pairs (Stofanko et al., 2013a; 2013b). Our procedure is based on finding genomic regions with high homology to segments of any critical chromosomal region. PCR amplification of both using the same primer pair establishes competitive kinetics and relative quantification of amplicons, as happens in microsatellite-based Quantitative Fluorescence PCR. It also makes it easy to build multiplex sets. The fact that it bypasses the need for polymorphic informative reference loci makes the construction of a single reaction enough for a diagnostic test of all samples.

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Here we report on a simple, quick, and highly effective multiplex diagnostic test for sexing of human DNA samples called MQS-PCR – multiplex quantitative sexing PCR. This can be used for the detection of all cases of human chromosomal sex abnormalities, including 45,X, 46,X,i(X)(q10), 46,X,i(X)(p10), 46,X,X(p-), 46,X,X(q-), 47,XXX, 47,XXY. It is a reliable, low cost, and rapid detection method for the determination of chromosomal sex and sex chromosomal abnormalities in human samples.

MATERIAL AND METHODS

The HGQ-PCR primer pairs used in the multiplex are shown in Table1 in their genomic locations, as follows: amplicon X-2 (Xq23/2p23.2), amplicon 1-X (1q32.1/Xp11.3) and amplicon Y-8 (Yq11.223/8p21.2). Degeneracies were introduced in some positions of primers (Table 1). A universal M13-40 extension (5‘-GTTTTCCCAGTCACGAC-3‘) was added to the 5’ end of the forward primer to allow for cost-efficient fluorescent labelling of amplicons (Schuelke, 2000) and a PIG-tail extension (5’-GTTTCTT-3’) was added to the 5’ end of the reverse primer to promote full adenylation of the 3’ end of the forward strand (Brownstein et al, 1996).

Table 1. Primers used in the MQS-PCR reaction.

Primer name	Chromosome location	Sequence 5' to 3'	Fragment sizes
X-2 F	Xq23/2p23.2	[a] AGACTGGGTGTGGTCTTTGG	88/92 pb
X-2 R		[b] CAGAGACTGCAAGGAAGTCAT	
1-X F	1q32.1/Xp11.3	[a] TCTGTGGCAATCCAAATTCA	255/259 pb
1-X R		[b] TGGAAGATGCAAATGAAATCC	
Y-8 F	Yq11.223/8p21.2	[a,c] CTTSRAAGGCRAGGAGACCC	171/181 pb
Y-8 R		[b,c] RSTGTAGTAGGACRGGAAGGA	
AMEL	Xp22.2/Yp11.2	CCCTGGGCTCTGTAAAGAATAGTG	106/112 pb
AMEL		GTTTTCCCAGTCACGAC	

[a] A Universal M13-40 extension (5‘-GTTTTCCCAGTCACGAC-3‘) was added to the 5’ end of the forward primer to allow for cost-efficient fluorescent labelling of amplicons (Schuelke, 2000).
[b] A PIG-tail extension (5’-GTTTCTT-3’) was added to the 5’ end of the reverse primer to promote full adenylation of the 3’ end of the forward strand (Brownstein et al, 1996).
[c] R = G or A, S = G or C, Y = T or C.

We also added amplification of the amelogenin gene to our multiplex reaction. Among known X-Y homologous genes, this gene is the most suitable for the sex test by PCR. Following a single PCR with one pair of primers, X- and Y-specific products with different sizes are simultaneously detected, because of differences in the lengths of corresponding introns, generating 106-bp and 112-bp PCR products from the X and Y homologues (Sullivan et al., 1993). Primers used in amplification were: 5'-CCCTGGGCTCTGTAAAGAATAGTG-3' (AmelA) and 5'-ATCAGAGCTTAAACTGGGAAGCTG-3' (AmelB). A universal M13-40 extension (5‘-GTTTTCCCAGTCACGAC-3‘) was added to the 5’ end of the forward primer. These primers flank a 6bp deletion within intron 1 of the X homologue, resulting in 106 bp and 112bp PCR products from the X and Y chromosomes, respectively.

RESULTS AND DISCUSSION

The multiplex reaction described here has sensitivity and specificity of 100% for sex diagnosis and is capable of diagnosing with 100% accuracy 45,X, 46,X,i(X)(q10), 46,X,i(X)(p10), 46,X,X(p-), 46,X,X(q-), 47,XXX, 47,XXY and 47,XYY.

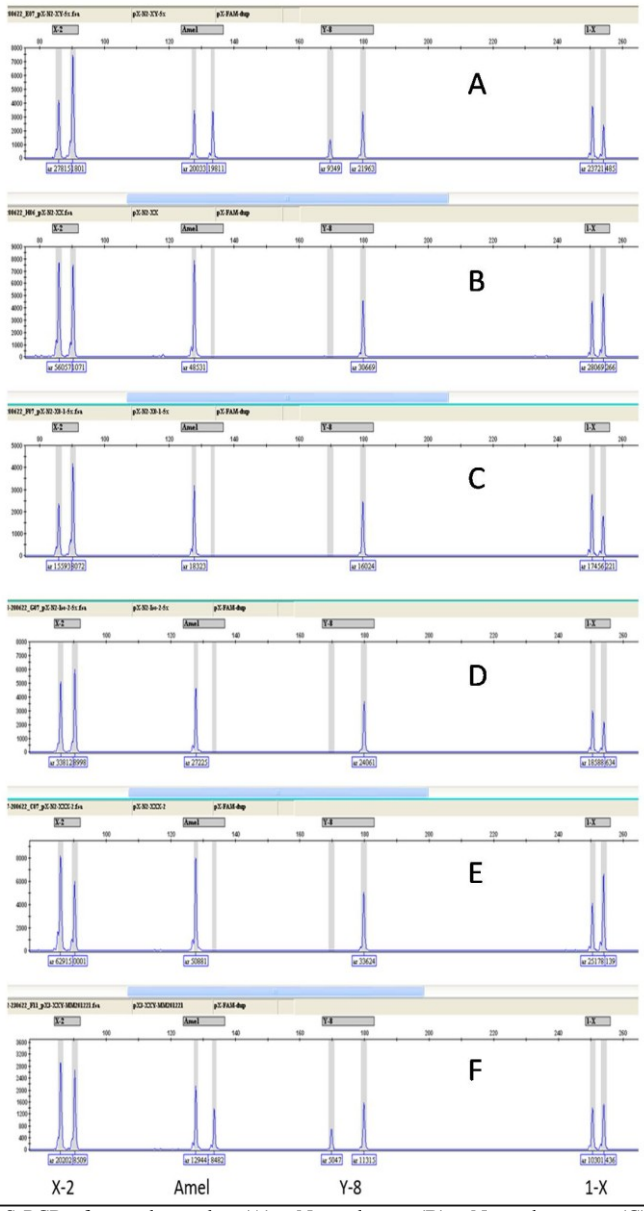

Figure 1. Figure 1. MQS-PCR of several samples: (A) = Normal man; (B) = Normal woman; (C) = Woman 45,X; (D) Woman with isochromosome Xq; (E) Woman 45,XXX; (F) Man 47,XXY. Please observe the relative height of the peak pairs: X-2, AMEL, Y-8 and 1-X.

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Santos et al. (1998) reported that the amelogenin incorrectly types some males as females because they lack the Y copy of the amelogenin gene because of a deletion polymorphism. This limitation does not apply to the method described here, since it makes use of not one, but two independent approaches for detecting a Y chromosome, identifying the chromosomal sex as male.

Also, we have reported previously the use of a single-tube multiplex PCR set of five polymorphic microsatellites for the diagnosis of monosomy X in fetal losses (Pereira et al., 2000). Since it depends on the polymorphism of the five loci tested, it does not provide incontrovertible evidence for or against the presence of a 45,X chromosomal sex when all five loci show a single peak, suggesting homozygosity. Indeed, application of Bayes' theorem allows us to calculate that given apparent homozygosity at all five loci, the probability of a first trimester spontaneously aborted female fetus having monosomy X is 98.4% (Pereira et al., 2000). Differently, the multiplex described here provides 100% sensitivity and specificity for the diagnosis of monosomy X.

In summary, the MQS-PCR (multiplex quantitative sexing PCR) approach described in this paper provides a reliable, low cost, and rapid detection method for the determination of chromosomal sex in human samples.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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