SensiFAST™ Probe

Employing Cell-free DNA from Maternal Plasma for Exclusion of X-linked Disorders

Advances in the analysis of cell free fetal DNA (cffDNA) found in maternal plasma have provided a non-invasive, lower-risk method for the analysis of fetal DNA. One of the applications of prenatal cell-free DNA (cfDNA) analysis is fetal sex determination, which is important in fetuses at risk of sex-linked genetic diseases. In this study the *SRY* gene located on the human Y-chromosome has been used to identify the gender of the fetus and the *CCR5* housekeeping gene used to confirm the presence of total DNA, using multiplex real-time PCR.

Prenatal analysis of single gene disorders and aneuploidy currently involves invasive procedures such as amniocentesis, chorionic villus sampling (CVS) and cordocentesis, with a combined risk of miscarriage of approximately 1%. Non-invasive prenatal analysis using cffDNA from maternal plasma can be used as an alternative to invasive procedures, reducing the risk of miscarriage. Furthermore, next generation sequencing has demonstrated that the entire fetal genome is represented within cfDNA and it has been shown to be pregnancy-specific as it is rapidly cleared from the circulation following delivery. Since cffDNA constitutes an average of 10% of maternal plasma cfDNA, the high maternal background makes it challenging to distinguish fetal from maternal alleles. At present, routine clinical applications using cfDNA are limited to the detection of alleles that are specific to the fetus and absent in the mother. These alleles include the ones used in the detection of the rhesus D gene (*RhD*) in rhesus negative mothers, and fetal sex determination, using sequences from the Y chromosome. For example, *DYS14* (a sequence located on the testis-specific Y encoded protein 1 (*TSPY*) gene, or the *SRY* (sex-determining region Y) gene can be used to detect a male fetus. The absence of these sequences is used to infer a rhesus negative or female fetus respectively.

Fetal sex determination is indicated for use in cases where parents are known to be carriers of an X-linked single gene disorder, such as Duchenne muscular dystrophy (DMD) or adrenoleucodystrophy, which primarily affect a male fetus. In these cases, if a male fetus is detected, women have the option to take an invasive test after 11 weeks gestation to confirm or exclude the condition in question. If a female fetus is detected, there is no need to take an invasive test, so removing any associated risk of miscarriage. Another key use of fetal sex determination is for pregnancies at risk of congenital adrenal hyperplasia, which can cause genital virilisation in female fetuses, these require steroid treatment during pregnancy if an invasive test confirms that the fetus is affected. If a male fetus is identified, women can avoid unnecessary steroid treatment and the invasive test.

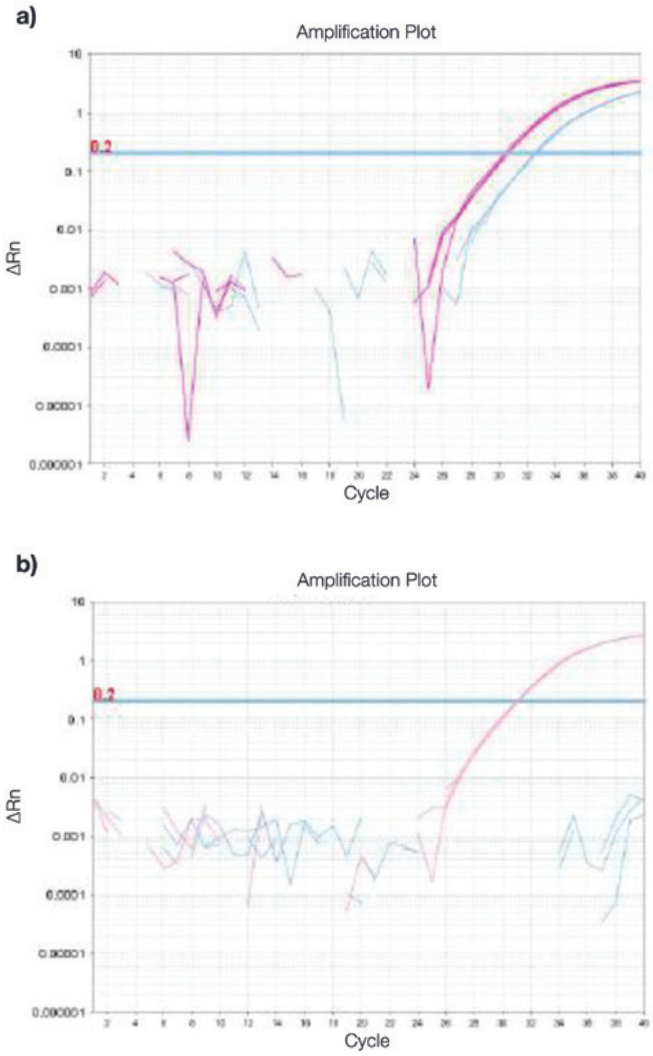

Fig. 1.
Quantitative real-time PCR for fetal sex determination. Two maternal cell-free DNA samples were tested using an assay for *SRY* (fetal DNA) and an assay for *CCR5* (total DNA). Six replicates were carried out for each assay. Positive amplification for both genes, indicating a male fetus is shown in (a), whilst only *CCR5* gives positive amplification for a female fetus (b).

Despite the relatively low abundance of fetal sequences within the maternal cfDNA, quantitative real-time PCR (qPCR) can be used to detect *SRY* or *DYS14* from as early as 7 weeks gestation, with a house-keeping gene such as *CCR5* (C-C chemokine receptor 5) used to confirm the presence of total DNA. It is important to note that the assay is looking for the presence of the Y marker to confirm a male fetus; absence is not definitive proof of a female fetus, since the possibility remains that fetal DNA levels are too low to detect. Therefore an efficient qPCR assay is essential. Here we have shown that the SensiFAST™ Probe LO-ROX Kit* can be used to reliably amplify male DNA sequences from maternal plasma DNA in a multiplex reaction (Fig. 1). The SensiFAST Kit therefore delivers fast, reproducible, highly specific and ultra-sensitive real-time PCR, making it ideal for screening in this application, other screening applications and for high-throughput assays.

REFERENCES

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