

Sex Determination of Oragene®•DNA samples using ReaX™ Mastermix PCR beads

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DNA samples collected using the Oragene•DNA self-collection kit were used in combination with ReaX Mastermix to successfully determine the sex of each DNA sample donor by employing a qPCR assay for the SRY gene.

Introduction

The number of studies requiring collection of genomic DNA from a large number of individuals is increasing rapidly. Oragene•DNA, a self-collection kit for the isolation, transport and storage of DNA from saliva, is ideal for such studies, as it provides an easy, non-invasive method for the collection of large amounts of high-quality DNA.

For sample collection, the donor is asked to abstain from eating or drinking half an hour before delivering 2 mL of saliva into the Oragene•DNA kit. This minimizes the presence of food particles in the sample. Upon closing the lid, the Oragene•DNA solution is released, and as it mixes with the saliva the DNA is stabilized at ambient temperature.

ReaX bead-based mastermixes are enhanced PCR formulations, which simplify PCR assays and provide robust, reproducible results. Each bead contains PCR buffer, Magnesium Chloride, dNTPs & Taq polymerase at final reaction concentrations.

ReaX Mastermix beads are fully optimised for end-point PCR and a range of real-time reporter chemistries. ReaX beads can be customized for any assay via co-encapsulation of specific probes & primers alongside standard PCR reagents.

A Taqman qPCR assay was developed to detect the presence of SRY gene. SRY, or Sex determining Region Y, encodes the testis determining factor which initiates male sex determination. Located on the short arm of the Y chromosome (Yp11.3), it is predominately found in males and can be utilised to distinguish between male- and female-donated DNA during qPCR. A primer / probe set specific to this locus was used to produce an amplicon of 80 base pairs. Hence in male samples, PCR results in amplification of the SRY gene with corresponding increase in 6-FAM fluorescence. Samples derived from female donors, which lack the template sequence, produce no increase in fluorescence and give a negative result.

This technical bulletin demonstrates that the combination of Oragene•DNA self-collection kits & ReaX SRY Mastermix beads, enables a simplified, streamlined workflow for the performance of molecular diagnostic assays. By simplifying DNA collection from the donor / patient and de-skilling & accelerating PCR set-up, reliable qPCR results can be obtained quickly without the need for doctors, phlebotomists or molecular biology specialists.

Materials and Methods

Primers and a 6-FAM hydrolysis probe were designed from a published description of the SRY gene to produce an 80bp target sequence in the qPCR assay. The primer and probe sequences are given in Figure 1 below.

Forward Primer	5' GCG ACC CAT GAA CGC ATT - 3'
Reverse Primer	5' AGT TTC GCA TTC TGG AGT TCT CT - 3'
Probe	6-FAM - TGG TCT CGC GAT CAG AGG CGC - BHQ - 1

Figure 1. SRY primer and probe sequences.

ReaX SRY custom beads were manufactured, containing the following PCR components within each bead.

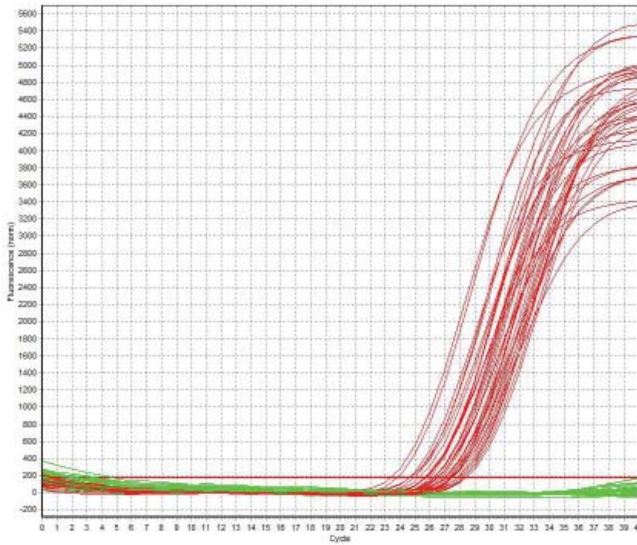
- 10pM of each primer
- 0.1pM of probe
- 800µM dNTPs (200µM of each)
- 5mM MgCl₂
- 1x ReaX PCR buffer
- 1 unit of HotStart Taq polymerase

Saliva samples were obtained from 100 donors. Collection and purification of DNA was carried out according to the protocol supplied with the Oragene•DNA kit. For each of 100 samples, a 25µL PCR reaction was set up, containing purified DNA (0.5µL diluted with 24 µl of ddH₂O). Each DNA dilution was then added to a single pre-wetted ReaX bead and each tube was sealed with a tube cap and then placed in the Mastercycler Realplex2® qPCR thermal cycle (www.eppendorf.com).

Thermocycle program:

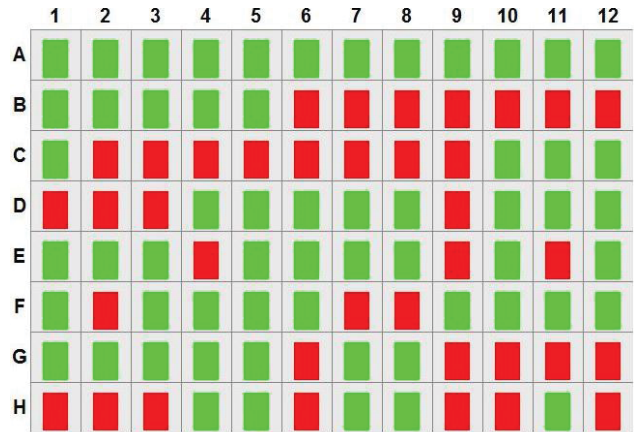
94°C – 300 secs

95°C – 15 secs } X39
58°C – 60 secs }



Threshold: 171 (Noiseband)
Baseline settings: automatic, Drift correction OFF

Figure 2: Oragene•DNA samples-ReaX amplification curves
Red – amplification of the SRY gene occurs indicating that the DNA sample is derived from a male individual.
Green – no amplification curve is produced therefore no SRY gene is present in the DNA sample indicating that the DNA sample is derived from a female individual.






 Inconclusive
 Positive
 Negative

Figure 3: Oragene•DNA-ReaX beads qPCR results represented diagrammatically to easily identify positive/negative results and therefore the sex of each sample DNA

Results

The presence or absence of an amplification curve for each Oragene•DNA sample was recorded. Results are shown below in Figures 2 and 3. Note that the concentration of DNA in each sample was not standardized prior to PCR.

Conclusions

The results obtained were 100% concordant for all of the Oragene•DNA samples tested with the SRY ReaX bead qPCR assay.

References

1. Clepet C, Schafer AJ, Sinclair AH, Palmer MS, Lovell-Badge R, Goodfellow PN: The human SRY transcript. Hum Mol Genet 2:2007-2012 (1993)