

Sex determination of DNA from Oragene®/saliva samples[†] using ReaX™ Mastermix PCR beads

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DNA samples collected using the Oragene® 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, 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 kit. This minimizes the presence of food particles in the sample. Upon closing the lid, the Oragene 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 and 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 and primers alongside standard PCR reagents.

A Taqman qPCR assay was developed to detect the presence of SRY gene¹. 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 DNA from Oragene/saliva samples and 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 and 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 80 bp 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.

- 10 pM of each primer
- 0.1 pM of probe

[†] Saliva samples were collected with Oragene®•DNA or Oragene®•DISCOVER.





- 800 μM dNTPs (200 μM of each)
- 5 mM 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 prepIT•L2P protocol² for use with Oragene/saliva sample. For each of 100 samples, a 25 μL PCR reaction was set up, containing purified DNA (0.5 μL diluted with 24 μL of ddH2O). Each DNA dilution was then added to a single pre-welled 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

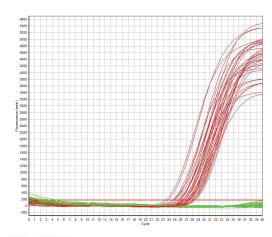
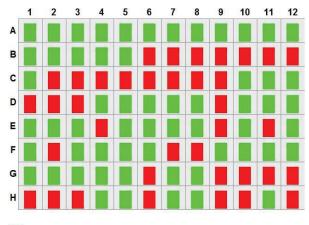


Figure 2: DNA from Oragene/saliva samples - ReaX amplification curves.

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: ReaX beads qPCR results represented diagrammatically to easily identify positive/negative results and therefore the sex of each sample of DNA from Oragene/saliva samples.

Results

The presence or absence of an amplification curve for each Oragene/saliva sample was recorded. Results are shown 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/saliva samples tested with the SRY ReaX bead qPCR assay.

References

- Clepet C., Schafer A.J., Sinclair A.H., Palmer M.S., Lovell-Badge R., and Goodfellow P.N. (1993). The human SRY transcript. Hum Mol Genet 2:2007-2012.
- ² Laboratory protocol for manual purification of DNA from 0.5 mL of sample. DNA Genotek. PD-PR-006.

Oragene®•DNA is not available for sale in the United States.

 $Oragene \hbox{$^\circ$-DISCOVER} is for research use only, not for use in diagnostic procedures.$

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