

## Sex determination of DNA from Oragene®/saliva samples<sup>†</sup> using ReaX™ Mastermix PCR beads

R. Groves, D. Palmer, R. Calder

Q Chip Ltd. Cardiff, UK

2008-09-28

*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 gene<sup>1</sup>. 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

<sup>†</sup> Saliva samples were collected with Oragene®•DNA or Oragene®•DISCOVER.

