



## Oragene®/saliva samples<sup>†</sup> and DNA sequencing with ABI PRISM®

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*DNA from Oragene®/saliva samples is reliably sequenced using the ABI PRISM® 377 DNA sequencer.*

### Introduction

Blood leukocytes are the traditional source of DNA for molecular testing, but collection is invasive and specimens require infectious precautions. Buccal swabs are a less invasive method of collecting DNA for sequencing applications such as HLA typing and prelingual deafness screening<sup>1, 2</sup> but they tend to have an appreciable failure rate<sup>3</sup>. Oragene is a non-invasive DNA self-collection kit from saliva that provides significantly higher DNA yields than buccal swabs<sup>4</sup>. The purpose of this study was to investigate the suitability of DNA from Oragene/saliva samples for DNA sequencing.

### Materials and methods

#### DNA collection

Saliva samples were collected from 5 donors using the Oragene kit. Collection and purification of DNA was carried out according to the prepIT™•L2P purification protocol<sup>5</sup>.

#### Primer design

PCR primers for the human Thymidylate Synthetase (TYMS) gene were designed based on the publicly available DNA sequence (GenBank accession no. AP001178). The primers generate a 560 bp fragment. Table 1 above shows the primer sequences.

Primer name	Sequence (5' - 3')
TS 560-forward	ATGCTTAGTAGGCAATTCTG
TS 560-reverse	TTTGGTTGTCAGCAGAGG

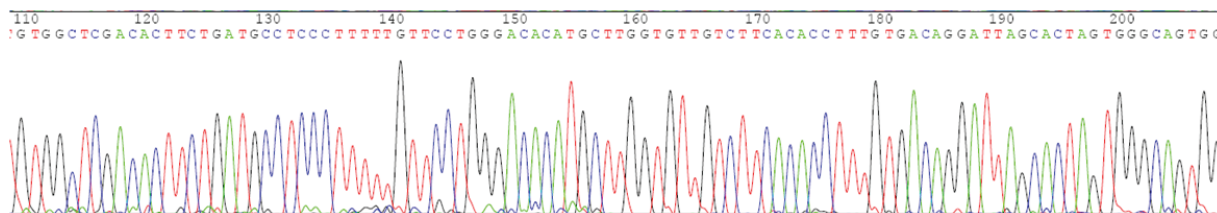
**Table 1:** PCR primers for the Thymidylate Synthetase gene.

### DNA sequencing

Purified DNA from each of the 5 Oragene/saliva samples was used as the template for PCR with the TS 560 primers. The PCR products were sequenced in both directions by Cortec DNA Service Laboratories (Kingston, ON) using an ABI PRISM 377 DNA sequencer (Applied Biosystems) and the DYEnamic™ ET dye terminator kit (Amersham Biosciences). Prior to sequencing, the PCR products were purified using the microCLEAN™ DNA clean-up reagent (Microzone).

### Results

DNA sequencing results were aligned using CLUSTAL W (Version 1.83), a multiple sequence alignment program. All 5 samples correctly aligned to the original human Thymidylate Synthetase gene sequence. Figure 1 shows a representative ABI PRISM sequencing read-out.



**Figure 1:** ABI PRISM sequencing read-out.

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

