

## Oragene™ and DNA sequencing with ABI PRISM®

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*DNA from Oragene 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 (ref. 1, 2) but they tend to have an appreciable failure rate (ref. 3). Oragene is a non-invasive DNA self-collection kit from saliva that provides significantly higher DNA yields than buccal swabs (ref. 4). The purpose of this study was to investigate the suitability of DNA from Oragene for DNA sequencing.

### Materials and Methods

#### DNA collection

Saliva samples were collected from 5 donors. Collection and purification of DNA was carried out according to protocols supplied with the Oragene kit.

#### Primer design

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

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

**Table 1.** PCR primers for the Thymidylate Synthase gene.

### DNA Sequencing

Oragene-purified DNA from each of the 5 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

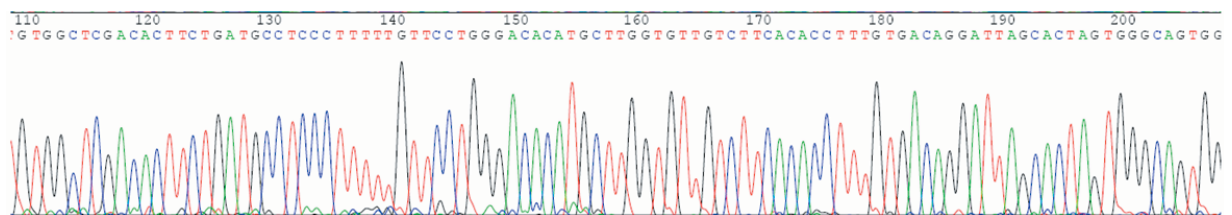
DNA sequencing results were aligned using CLUSTAL W (Version 1.83), a multiple sequence alignment program. All 5 samples correctly aligned to the original human Thymidylate Synthase gene sequence. Figure 1 shows a representative ABI PRISM sequencing read-out.

### Discussion and Conclusions

Oragene is a non-invasive and reliable method of collecting DNA from saliva. DNA from Oragene works well for DNA sequencing with the ABI PRISM 377 DNA Sequencer.

### References

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