

Oragene®•DNA and DNA sequencing with ABI PRISM®

J. Chartier
DNA Genotek Inc., Ottawa, ON, Canada

DNA from Oragene•DNA 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•DNA 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•DNA 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•DNA 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 | TTTGGTTGTCAGCAGAGG |

Table 1. PCR primers for the Thymidylate Synthase gene.

DNA Sequencing

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

References

- Liu, C., Chu, C., Lee, Y., Shi, Y., and M. Lin. (2004). Identification of two novel HLA-DRB1 alleles, DRB1*0903 and DRB1*1145. *Tissue Antigens*. 64: 99-101.
- Sugata, A. et al. (2002). High-throughput screening for GJB2 mutations—its clinical application to genetic testing in prelingual deafness screening for GJB2 mutations. *Auris, Nasus, Larynx*. 29: 231-239.
- Elit, L., Jack, E., Kwan, E., Baigal, G., and S. Narod. (2001). A unique BRCA1 mutation identified in Mongolia. *International Journal of Gynecological Cancer*. 11: 241-243.
- DNA Genotek (2004). DNA Yield with Oragene•DNA, DNA Genotek PD-WP-001

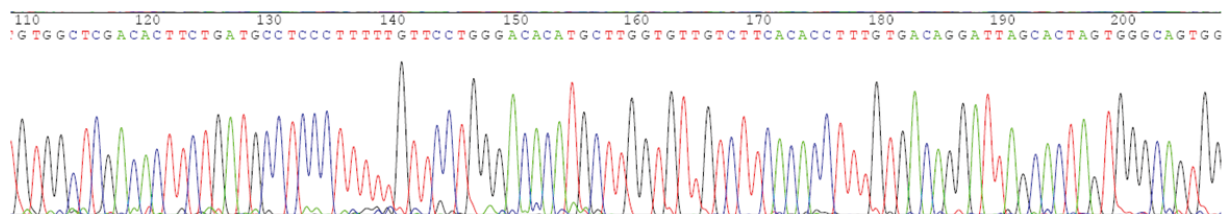


Figure 1. ABI PRISM sequencing read-out.