DNA GENOTEK

Oragene®/saliva samples⁺ 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^{1, 2} but they tend to have an appreciable failure rate³. Oragene is a non-invasive DNA self-collection kit from saliva that provides significantly higher DNA yields than buccal swabs⁴. 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 protocol⁵.

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.

Sequence (5' - 3')
ATGCTTAGTAGGCAATTCTG
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.

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





Discussion and conclusions

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

References

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- 2 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.
- 3 Elit, L., Jack, E., Kwan, E., Baigal, G. and Narod S. (2001). A unique BRCA1 mutation identified in Mongolia. *International Journal of Gynecological Cancer*. 11: 241-243.
- 4 Birnboim, H.C. (2004). DNA yield with an Oragene self-collection kit. DNA Genotek. PD-WP-001.
- 5 Laboratory protocol for manual purification of DNA from 0.5 mL of sample. DNA Genotek. PD-PR-006.

Oragene®•DISCOVER is for research use only, not for use in diagnostic procedures.

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

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