



DNA from Oragene®/saliva samples[†] is ideal for long-range PCR

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For long-range PCR, DNA from Oragene®/saliva samples is equal in performance to DNA isolated from blood.

Introduction

Long-range PCR has a number of applications in human genetics including the detection of insertions and deletions¹ and SNP genotyping^{2, 3}. To be successful, long-range PCR requires DNA of high quality and high molecular weight such as that obtained with the Oragene self-collection kit, which has a molecular weight greater than 23 kilobases⁴. This study compares the long-range PCR performance of DNA from Oragene/saliva samples purified with prepIT™•L2P versus DNA purified from blood.

Materials and methods

DNA purification

Saliva was collected using the Oragene self-collection kit and DNA was purified using the prepIT™•L2P purification protocol⁵. Additional DNA was isolated from blood and purified using the MagneSil® Blood Genomic, Max Yield System (Promega) on the KingFisher 96 automated robotic system (Thermo Electron).

Long-range PCR

A 7.3 kb fragment of the human GBA gene was amplified using the Expand Long PCR kit with Buffer System 2 (Roche Diagnostics). Primers specific to the human GBA gene were synthesized by Integrated DNA Technologies Inc. (Table 1). All PCR reagents were used at the final concentrations recommended by the manufacturer (Table 2).

Primer	Sequence
GBA Forward	5'- TTC TCC ATG CAA ATC TGT GT-3'
GBA Reverse	5'- GAA CCA GAT CCT ATC TGT GC-3'

Table 1: Primer sequences.

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

Roche expand long PCR kit	Final concentration
Buffer	1×
MgCl ₂	2.25 mM
dNTPs	500 μM
Forward primer	20 pmol
Reverse primer	20 pmol
Enzyme mix	1 U
Template DNA	200 ng

Table 2: Final PCR reagent concentrations.

Thermal cycling was performed using a DNA Engine Tetrad machine (MJ Research) and the PCR program in Table 3. PCR products were loaded on 0.8% agarose gels and run for 1 hour and 20 minutes at 80 V. After electrophoresis, gels were stained with ethidium bromide and photographed.

Cycle	Temperature	Time
1×	93°C	2 minutes
10×	93°C	10 seconds
10×	60°C	30 seconds
10×	68°C	4 minutes 40 seconds
20×	93°C	10 seconds
20×	60°C	30 seconds
20×	68°C	4 minutes 40 seconds + 20 seconds per cycle
1×	68°C	7 minutes
1×	4°C	Hold

Table 3: Thermal cycling parameters.

Results

Figure 1 shows the results of long-range PCR using DNA from Oragene/saliva samples and DNA from blood. Lanes 1 to 3 show the results from Oragene and Lane 5 from blood. Both DNA types produced the expected 7.3 kb amplicon from the human GBA gene.

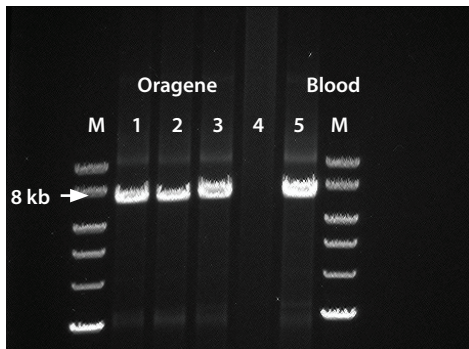


Figure 1: Agarose gel of long-range PCR reactions. Lanes 1 to 3 used DNA from Oragene/saliva samples and Lane 5 used DNA from blood. Lane 4 is a negative control.

Discussion and conclusions

The results show that Oragene-purified DNA gives equal performance to DNA isolated from blood. The Oragene kit minimizes DNA shearing and generates high-molecular-weight DNA that is ideal for long-range PCR applications.

References

- ¹ Dabora, S., Nieto, A., Franz, D., Jozwiak, S., Van Den Ouweland, A. and Kwiatkowski, D. (2000). Characterisation of six large deletions in TSC2 identified using long-range PCR suggests diverse mechanisms including Alu mediated recombination. *Journal of Medical Genetics*. 37(11), 877-883.
- ² Nagano, M., Nakamura, T., Ozawa, S., Maekawa, K., Saito, Y. and Sawada, J. (2003). Allele-specific long-range PCR/sequencing method for allelic assignment of multiple single nucleotide polymorphisms. *Journal of Biochemical & Biophysical Methods*. 55(1), 1-9.
- ³ Plaschke, J., Ruschoff, J. and Schackert, H. (2003). Genomic rearrangements of hMSH6 contribute to the genetic predisposition in suspected hereditary non-polyposis colorectal cancer syndrome. *Journal of Medical Genetics*. 40(8), 597-600.
- ⁴ Birnboim, H.C. (2004). Long-term stability of DNA from saliva samples stored in the Oragene self-collection kit. DNA Genotek. PD-WP-005.
- ⁵ Laboratory protocol for manual purification of DNA from 0.5 mL of sample. DNA Genotek. PD-PR-006.

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

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

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All DNA Genotek protocols, white papers and application notes, are available in the support section of our website at www.dnagenotek.com.