

Oragene®•DNA is ideal for long-range PCR

M. Keddache¹ and P. Lem²

¹DNA Sequencing Services, Cincinnati Children's Hospital Research Center, Cincinnati, OH, USA

²DNA Genotek Inc., Ottawa, ON, Canada

For long-range PCR, DNA from Oragene•DNA 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 deletions (ref. 1) and SNP genotyping (ref. 2, 3). To be successful, long-range PCR requires high-quality and high molecular weight, such as Oragene•DNA purified DNA which has a molecular weight greater than 23 kilobases (ref. 4). This study compares the long-range-PCR performance of DNA from saliva purified with Oragene•DNA versus DNA purified from blood.

Materials and Methods

DNA purification

Saliva was collected using the Oragene•DNA Self-Collection Kit and DNA was purified according to the supplied protocol. 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.

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 min
10×	93°C	10 sec
10×	60°C	30 sec
10×	68°C	4 min 40 sec
20×	93°C	10 sec
20×	60°C	30 sec
20×	68°C	4 min 40 sec + 20 sec per cycle
1×	68°C	7 min
1×	4°C	Hold

Table 3. Thermal cycling parameters.

Results

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

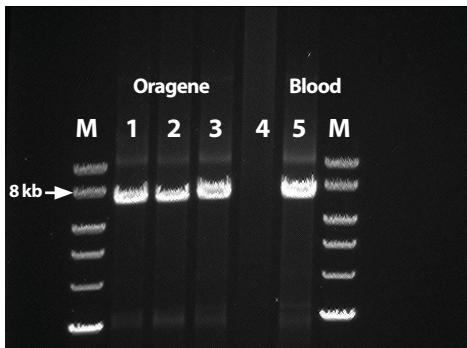


Figure 1. Agarose gel of long-PCR reactions. Lanes 1 to 3 used DNA from Oragene and Lane 5 used DNA from blood. Lane 4 is a negative control.

Discussion and Conclusions

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

References

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