DNA genotek

Comparison of DNA purified with prepIT™•L2P and the QIAamp™ mini kit

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Saliva samples† collected with the Oragene® self-collection kit may be purified using the prepIT™•L2P purification protocol or QIAamp™ DNA mini kit (Qiagen). Compared to the prepIT•L2P protocol, samples purified with QIAamp gave equivalent A_{260}/A_{280} ratios, but the yield was lower and the DNA was of lower molecular weight.

Introduction

The Oragene self-collection kit allows saliva samples to be collected and stored at ambient temperature. The DNA purification process begins as soon as the saliva mixes with the Oragene solution. Purification is completed in the lab with the prepIT•L2P purification protocol, which uses an alcohol precipitation procedure to obtain pure DNA[1]. QIAamp kits use a silica-gel membrane and a bind-wash-elute procedure. Nucleic acids are adsorbed to the silica membrane in the presence of high concentrations of chaotropic salts. After a wash step, DNA is eluted under low-salt conditions[2].

The purpose of this technical bulletin is to compare DNA yield, purity, and molecular weight of saliva samples purified using the prepIT•L2P protocol and the QIAamp DNA mini kit.

Materials and methods

Saliva collection

Two milliliters of saliva were collected from 8 donors using the Oragene kit. In the collection kit, the saliva was automatically mixed with Oragene preserving solution. The Oragene/saliva samples were stored at ambient temperature before processing.

prepIT•L2P purification

A 200 µL aliquot from each of the Oragene/saliva samples was purified according to the prepIT•L2P purification protocol. In brief, 8 µL (1/25th vol.) of prepIT•L2P was added to each aliquot. The mixture was centrifuged and the supernatant was transferred to a fresh tube. An equal volume of 95% ethanol was added to the clear supernatant. The precipitated DNA was centrifuged and collected as a pellet. The ethanol supernatant was discarded and the DNA pellet was rehydrated in TE buffer.

QIAamp purification

A 200 µL aliquot from each of the Oragene/saliva samples was purified according to the QIAamp purification protocol[3]. In brief, proteinase K and 200 µL of Buffer AL were added and the sample was incubated at 56°C. After incubation, 200 µL of 95% ethanol was added. The sample was transferred to a QIAamp spin column and centrifuged. The filtrate was discarded. The column was washed once with Buffer AW1 and then with Buffer AW2. The DNA was eluted in 200 µL of Buffer AE.

prepIT•L2P followed by QIAamp purification

prepIT•L2P purified DNA in TE buffer was added to a QIAamp spin column and centrifuged.

DNA analysis

DNA samples purified with the prepIT•L2P, QIAamp, or prepIT•L2P + QIAamp protocols were quantified by absorbance. The A_{260}/A_{280} ratios were determined. To determine molecular weight, the DNA was run on a 0.8% agarose gel and compared to a Lamda-Hind III digest ladder.

Results

The average A_{260}/A_{280} ratios and DNA yields from the Oragene/saliva samples are summarized in Table 1. The A_{260}/A_{280} ratios were similar for the three purification protocols. In contrast, DNA yields were highest with the prepIT•L2P protocol and lowest with QIAamp. Some loss of DNA was observed when the prepIT•L2P purified DNA was passed through a QIAamp column.

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

	Average DNA yield (µg)	A_{260}/A_{280}
prepIT•L2P	11.2	1.73
QIAamp	4.1	1.75
prepIT•L2P + QIAamp	8.7	1.80

***Table 1**: Average DNA yield and A_{260}/A_{280} ratios.*

Figure 1 shows the molecular weights of DNA extracted using the three purification protocols. DNA extracted using the prepIT•L2P protocol consistently had a higher molecular weight compared to DNA that had passed through a QIAamp spin column.

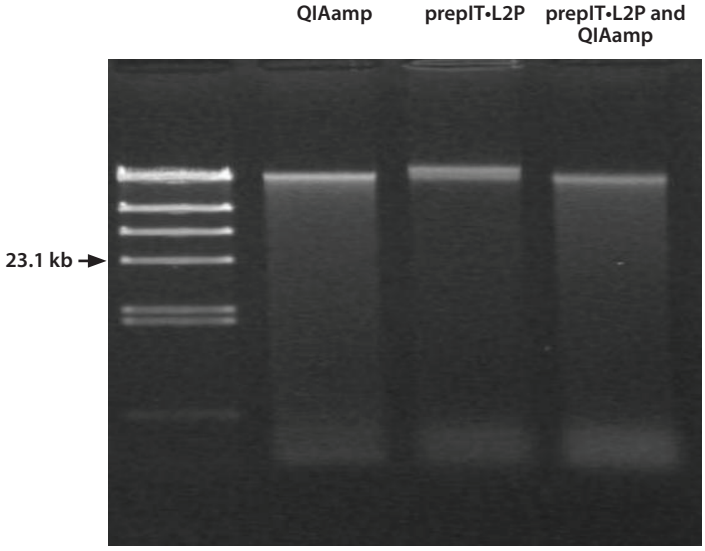

***Figure 1**: Agarose gel electrophoresis of purified DNA. A Lambda-Hind III digest was used as the marker in Lane 1.*

Discussion and conclusions

The A_{260}/A_{280} ratios of prepIT•L2P and QIAamp-purified DNA were similar. This suggests that both kits are effective at removing contaminants from the saliva sample. However, DNA yields were lowest with the QIAamp protocol. This may be because high-molecular-weight DNA elutes slowly from the silica-gel membrane. Less DNA loss was observed when prepIT•L2P purified DNA was passed through a QIAamp column. In this case, the DNA was more solubilized, and less may have been trapped by the column. This trapping effect may also explain the lower molecular weight of the QIAamp-purified DNA as seen by agarose gel electrophoresis. In summary, the prepIT purification protocol gave the highest yield and the highest molecular weight of DNA.

References

1 Laboratory protocol for manual purification of DNA from 0.5 mL of sample. DNA Genotek. PD-PR-006.
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