DNA genotek

Automated extraction of gDNA from Oragene® saliva samples using the QIAGEN® QIAsymphony® SP

Saliva collected using the Oragene® self-collection kit from DNA Genotek is a proven source of high quantity and high quality genomic DNA. The kit facilitates research by enabling unsupervised, non-invasive collection of samples from a large number of people. In order to assist in the processing of such large numbers of samples an Oragene-specific protocol has been developed for the QIAsymphony® SP. The QIAsymphony SP saves time and increases efficiency by processing up to 96 Oragene saliva samples in a single run.

Introduction

In large-scale population studies it is common to collect samples from thousands of donors. Extraction of the DNA from these samples using manual methods is time-consuming and labour-intensive. The QIAsymphony SP is an innovative system that enables the preparation of genomic DNA from a wide variety of biological samples using paramagnetic silica particle-based technology.

The purpose of this study is to evaluate the performance of an Oragene-specific QIAsymphony SP protocols for the extraction of gDNA from Oragene saliva samples.

Materials and methods

Sample collection

Oragene self-collection kits were used to collect saliva from 50 donors according to the standard instructions included in the kits. Three 2 mL saliva samples collected from 23 of the donors were heated at 50°C for two hours, pooled and then split into three replicate samples. A single 2 mL sample was collected from each of the remaining 27 donors which, together with the 23 triplicate sets, resulted in a set of 96 samples in total. All samples were incubated at 50°C for two hours in a laboratory oven prior to DNA extraction on the QIAsymphony SP.

DNA extraction

DNA was extracted from a 1 mL aliquot of each Oragene saliva sample on the QIAsymphony SP using the QIAsymphony DSP DNA Midi Kit (QIAGEN® catalogue number: 937255 or 937236) and protocol Oragene_ID372_V2.xml. This protocol was specifically developed for use with Oragene saliva samples and includes a heated elution (37°C) into 60 µL of elution buffer, among other customized parameters. The setup of the QIAsymphony SP was conducted according to the procedures contained in the QIAsymphony Handbook.

DNA analysis

The DNA yield and concentration for each eluted sample was determined using Quant-iT™ PicoGreen® reagent (Life Technologies). Samples were diluted 1/50 prior to quantification. To evaluate DNA purity the absorbance (260, 280 and 320 nm) of each sample was measured using a microplate reader. The 260 nm and 280 nm readings were corrected by subtracting the 320 nm reading from each before calculating the A_{260}/A_{280} ratio. DNA integrity was evaluated by running approximately 100 ng of DNA from each eluted sample on 0.8% agarose gel (90 V, 50 minutes) and staining with ethidium bromide. A Lambda HindIII digest ladder was used to determine the approximate size of the eluted DNA.

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

ORAgene®•DNA (outside the USA) ORAgene®•DISCOVER (in the USA)

Results

DNA yield and concentration

From 96 Oragene saliva samples extracted on the QIAsymphony SP, the median DNA yield was 5.3 ug and the average yield was 6.2 ug (Figure 1, right axis).

The median concentration of the extracted DNA was 88.1 ng/µL and the mean was 104 ng/µL (Figure 1, left axis) with a 95% confidence interval of 91.9 ng/µL to 116.1 ng/µL. The concentration range was 14.8 ng/µL to 278.9 µL. Less than 5% of samples fell below 30 ng/µL.

The average A_{260}/A_{280} ratio of the extracted DNA samples was 1.8.

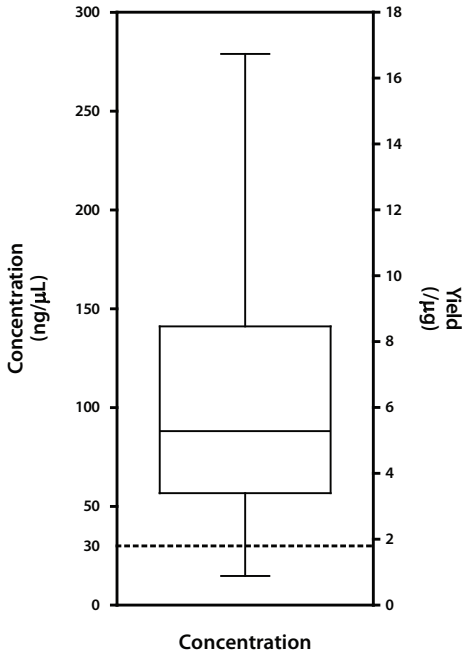

***Figure 1**: DNA concentration and yield obtained from extracting a 1 mL aliquot of Oragene/saliva on the QIAsymphony SP, eluting in a 60 µL volume of elution buffer. The box plot, from top to bottom, represents the maximum, upper quartile, median, lower quartile and minimum values.*

Molecular weight of extracted DNA

As assessed by agarose gel, the DNA extracted from each sample had a molecular weight equal to or greater than 23 kb (Figure 2).

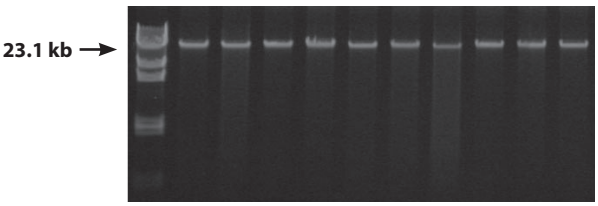

***Figure 2**: Agarose gel of DNA extracted by the QIAsymphony SP from 10 randomly selected Oragene saliva samples. Gel image depicts samples representative of the results obtained from the 96 samples tested. The first lane contains a Lambda* HindIII *ladder.*

Reproducibility

Saliva samples were collected in triplicate from 23 of the 50 donors and the DNA extracted as described above. Comparison of the DNA concentrations of replicate eluates indicates a high degree of reproducibility. All samples exhibited a %CV <20 across three replicates with the mean %CV being 8.0 across replicate groups.

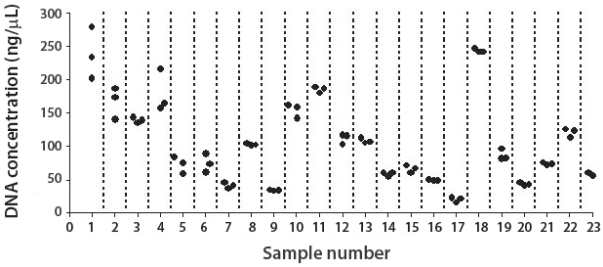

***Figure 3**: Reproducibility of extraction was tested using replicate aliquots of Oragene/saliva collected from 23 donors. Each dot on the graph represents an individual aliquot. There are three replicates per sample tested.*

Discussion and conclusions

Using the QIAsymphony SP with an Oragene-specific protocol we were able to obtain high quality DNA from Oragene saliva samples. DNA concentrations suitable for a variety of downstream applications including PCR and microarray analysis were achieved from >95% of samples. Agarose gel electrophoresis revealed that the DNA is intact and reproducibility testing indicates that the QIAsymphony SP can replicate results when multiple aliquots of a sample are extracted.

The QIAsymphony SP, when used with the QIAsymphony DSP DNA Midi Kit and an Oragene-specific protocol, was able to extract DNA from 96 Oragene saliva samples in approximately 110 minutes with no user interaction beyond initial instrument setup.

Available protocols

The protocol evaluated in this study (Oragene_ID372_V3.xml) was designed for extracting DNA from a 1000 µL volume of Oragene saliva using the QIAsymphony SP. An alternate protocol (Oragene_ID381_V2.xml) for extracting DNA from a 350 µL aliquot of Oragene saliva is also available. Results obtained using the alternate protocol may differ from those presented here. Both protocols can be obtained directly from QIAGEN by contacting your QIAGEN Sales Specialist or the QIAGEN Applications Lab.

Sample input volume	Sample elution volume	Protocol name
1000 µL	60 µL	Oragene_ID372_V3.xml
350 µL	50 µL	Oragene_ID381_V2.xml†

† An alternate protocol for extracting DNA from a lower sample input volume is available. This protocol may yield different results than those presented here.
The presented QIAsymphony application is for Research Use Only. Not for use in diagnostic procedures. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.
Oragene®•DNA is not available for sale in the United States.
Oragene®•DISCOVER is for research use only, not for use in diagnostic procedures.

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

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