Automated DNA purification from Oragene® saliva samples using the MagMAX™ Saliva gDNA Isolation Kit on the KingFisher™ Flex

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Introduction

Saliva is an easily accessible, non-invasive, and reliable source of genomic DNA (gDNA) suitable for downstream genetic analysis[1,2,3]. The Oragene® saliva self-collection kit (DNA Genotek, Canada) enables easy collection of high quality human gDNA and provides long-term stability at ambient temperatures[4]. Current growth in saliva-based genetics research is also driving the need for a rapid, high-throughput gDNA isolation method suitable for this sample type. The purpose of this study was to assess the compatibility of Oragene saliva samples with the MagMAX™ Saliva gDNA Isolation Kit, and to demonstrate the high-throughput automated workflow on the KingFisher™ Flex Purification System.

Materials and methods

Sample collection

Saliva samples were collected from 48 healthy adult donors according to the instructions in the Oragene (OG-500) self-collection kit. Samples were incubated overnight at 50°C prior to DNA isolation.†

DNA purification

DNA was isolated from 500 µL of Oragene/saliva using MagMAX™ Saliva gDNA Isolation Kit (Thermo Fisher Scientific Catalog Numbers: A39059, A39060)[5]. All isolations were automated on the KingFisher™ Flex Purification System in 96 deep-well plate format, with 100 µL final elution volume. Donors 1-24 were processed in triplicate, and single isolations were performed for Donors 25-48, resulting in a total of 96 samples.

DNA analysis

Total DNA concentration was measured with the Qubit dsDNA High Sensitivity Assay. This assay uses a fluorescent dye that specifically intercalates into double stranded DNA (dsDNA), allowing quantification of total DNA (human and microbial) concentration in each sample. The total DNA yield was calculated by multiplying the concentration (ng/µL) by the elution volume (100 µL).

Human-specific DNA yield was quantified by qPCR using the human RNase P TaqMan™ Assay. A standard curve was generated with the Human DNA standard curve dilution series (at 0.6, 1.2, 3, 6, 12 ng/µL) from the TaqMan™ DNA Template Reagents Kit. The concentration of human DNA was calculated, and multiplied by elution volume (100 µL) to determine total human DNA yield.

DNA purity was determined using the double stranded DNA Nucleic Acid application (DNA-50) on the NanoDrop One Spectrophotometer. Absorbance was measured at 260 nm and 280 nm, and the ratio of A_{260}/A_{280} was calculated.

Genomic DNA size and intactness were measured on the Agilent 4200 TapeStation. Briefly, 1 µL of purified DNA sample was mixed with 10 µL Genomic DNA Sample Buffer in a 96-well plate and analyzed using the Genomic DNA ScreenTape Assay on the Agilent 4200 TapeStation instrument. Samples were assigned a genomic DNA peak size (in bp) and DNA Integrity Number (DIN) by the Agilent software.

† Minimum incubation time at 50°C is 1 hour in a water bath or 2 hours in a dry incubator. Overnight incubation was performed for workflow convenience.

Results

DNA isolation workflow

All 96 samples were processed in a single KingFisher™ Flex run. The total workflow was 63 minutes, including hands on steps (loading reagents, transferring saliva) and automated steps (instrument run time) (Table 1).

Step	Time to complete (500 µL saliva input)
Loading reagents to plates	6 minutes
Transferring saliva to plates	35 minutes
KingFisher run	22 minutes
TOTAL	63 minutes

Table 1

DNA yield and purity

Total DNA yield was measured by Qubit (Figure 1A). Across all 48 donors, median DNA yield was 2.1 µg, and average yield was 4.3 µg. Ninety-eight percent of donors yielded greater than 10 ng/µL DNA in 100 µL final elution volume, corresponding to total yields greater than 1 µg total DNA for 47 out of 48 donors.

Additional quantification was performed to determine total DNA yield of human origin using the TaqMan™ RNase P assay (Figure 1B). In this assay, the median human DNA yield was 3.0 µg, with an average human DNA yield of 4.4 µg. In total, 96% of samples yielded greater than 1 µg of human DNA. This extrapolates to a total DNA yield of at least 8 µg from the entire 2 mL saliva sample.

The A_{260}/A_{280} ratio was used to determine DNA purity. Across all 48 donors, the average A_{260}/A_{280} was 1.93, with 98% of donors having a ratio ≥1.8, indicating high sample purity (Figure 1C).

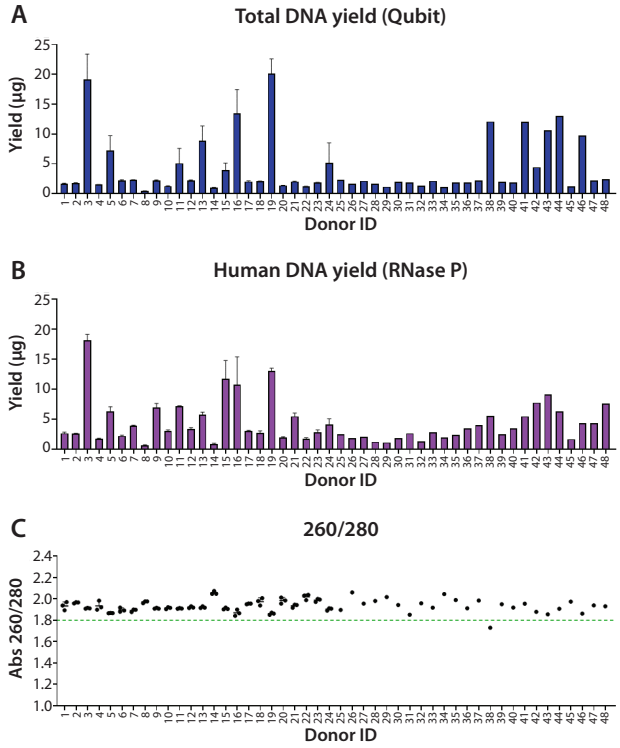

Figure 1*: Quantification of total DNA yield by Qubit assay (A) and TaqMan™ RNase P assay (B). Triplicate isolations were performed and quantified separately for Donors 1-24 (error bars = replicate SD), single isolations were performed for Donors 25-48. C) The 260/280 absorbance ratio plotted for all isolated samples. Each dot represents an individual isolation. Triplicate isolations were performed for Donors 1-24 (error bars = replicate SD), and single isolations for Donors 25-48. Green dotted line indicates $A_{260}/A_{280} = 1.8$.*

Genomic DNA size

The isolated DNA was visualized using the Agilent 4200 TapeStation. A single, high molecular weight genomic DNA band (>50,000 bp) was present in samples from all 48 donors. The Agilent software was used to determine the DNA Integrity Number (DIN) for each sample, with an average DIN = 9.5 ± 0.4. (Figure 2), indicating highly intact genomic DNA with little to no degradation.

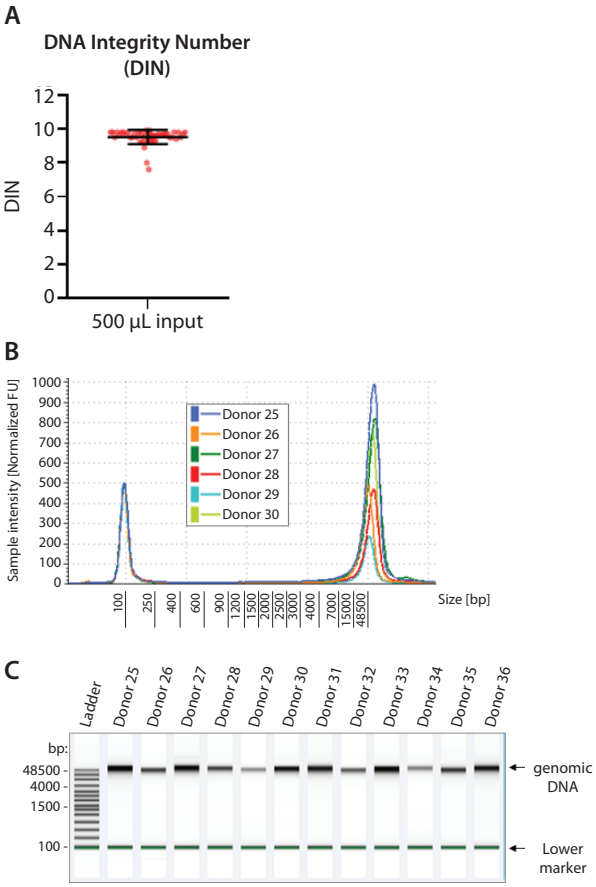

Figure 2: *DNA size and integrity was analyzed on the Agilent 4200 TapeStation. A) Scatter plot of DNA Integrity Number (DIN) assigned to each sample. Error bars = SD. B) Electropherograms of purified DNA from six representative donors. A single high molecular weight peak is present (>50,000 bp), corresponding to intact genomic DNA (100 bp peak = assay marker). C) TapeStation gel images for representative samples, Donors 25-36. Saliva genomic DNA bands and the 100 bp lower marker assay band are indicated.*

Conclusions

The MagMAX™ Saliva gDNA Isolation Kit is compatible with the Oragene self-collection device, and allows rapid purification of high quality, intact genomic DNA. In this study, a total of 96 samples (500 µL input) were processed on the KingFisher™ Flex in only 63 minutes, demonstrating utility for high throughput applications. The kit also offers automated protocols for scaled saliva inputs (200 µL – 2 mL), allowing flexibility in sample processing and elution volumes to achieve the desired DNA concentrations and yields.

In this study, DNA yield was measured by Qubit and RNase P assays to demonstrate differences between quantification methods. The Qubit assay relies on a fluorescent dye that intercalates specifically into double stranded regions of DNA, and is unable to differentiate between human or microbial DNA. It may also under-quantify samples containing single stranded regions of DNA. Therefore, we recommend Qubit for rapid estimation of total DNA concentration that is sufficient for many downstream applications. For studies requiring accurate quantification of human-specific DNA, the TaqMan™ RNase P Assay is recommended.

In conclusion, this study demonstrates a robust, high throughput method for purification of human genomic DNA from saliva preserved with the Oragene self-collection device using the MagMAX™ Saliva gDNA Isolation Kit and KingFisher™ Flex Processing System.

References

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