# Automated extraction of gDNA from Oragene®saliva samples† using the Tecan Freedom® EVO liquid handling platform and the Promega MagaZorb® DNA mini-prep kit

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2009-07-10

*The Oragene® self-collection kit facilitates the non-supervised, non-invasive collection of gDNA from a large number of individuals. In order to assist in the processing of such large numbers of samples we have validated the purification of Oragene/saliva samples using the Tecan Freedom® EVO liquid handling robot in conjunction with the Promega MagaZorb® DNA mini-prep kit. Using the hands off procedure described in this technical note, 96 500 µL aliquot samples of Oragene/saliva can be processed in 2 hours with median yields of 7.2 µg with median concentration of 143 ng/µL.*

## Introduction

Large-scale population studies may involve the collection of thousands of donor samples. Manual purification of DNA from these samples is both time and labour intensive. The Promega MagaZorb DNA mini-prep kit is a paramagnetic particle-based system for the purification of genomic DNA from a variety of biological samples. This system is ideally suited for use with high-throughput liquid handling platforms such as the Freedom EVO series from Tecan. The purpose of this study was to evaluate the performance of the MagaZorb DNA mini-prep kit used in conjunction with a Tecan Freedom EVO liquid handling robot for the purification of DNA from Oragene/saliva samples.

## Materials and methods

### *Sample collection*

Oragene kits containing 2 mL of DNA-preserving solution were used to collect 2 mL of saliva from 96 donors according to the kit instructions. The samples were heated at 50°C overnight in an incubator prior to purification on the Tecan Freedom EVO.

### *Instrument configuration*

DNA was extracted from 500 µL of the Oragene/saliva sample using the Freedom EVO 150 liquid handling platform (Tecan) outfitted with the following:

- an 8-channel liquid handling arm with disposable pipette tips,
- a robotic manipulator arm with centric grippers,
- labware carriers for 96 x 16 mm Oragene sample tubes,
- three 96-well plates (Axygen Inc., Cat P-DW-20-C),
- a deep-well plate magnet was affixed to position 3 of the 96-well plate carrier,
- a Te-Shake™ orbital mixer with heating block and 96-deep well plate adapter was used for mixing samples, and
- the MagaZorb DNA mini-prep kit (Promega) was used for purification.

### *DNA purification*

DNA was purified from 96 Oragene/saliva samples. The purification was performed in triplicate. A 500 µL aliquot of MagaZorb binding buffer was transferred to a round bottom, 2 mL 96-deep well plate. A 500 µL aliquot of Oragene/saliva sample was added to the binding buffer in the plate. In additional experiments to detect any possible cross-contamination between samples, 'blank' samples (water) and Oragene/saliva samples were alternated in the sample racks so that a checkerboard pattern of saliva and blank samples was obtained in the purification plate. The binding buffer and sample were thoroughly mixed using the Te-Shake (1.5 mm orbit, 1100 RPM, 30 seconds). The MagaZorb reagent was thoroughly mixed by pipetting prior to adding 30 µL to the mixed binding buffer/sample. The plate was then

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

incubated at room temperature for 20 minutes with mixing on the Te-Shake every 2 minutes. The plate was transferred to the magnet and incubated for 5 minutes to allow complete sedimentation of the magnetic particles. The supernatant was removed and discarded. The plate was transferred off the magnet and 900 µL of MagaZorb Wash Buffer was added to each well. The plate was mixed on the Te-Shake before being transferred back to the magnet and incubated for 5 minutes. The wash buffer supernatant was removed and discarded and the wash procedure was repeated. After removal of the second wash supernatant, the plate was transferred off the magnet and 50 µL of MagaZorb elution buffer was added. DNA was eluted from the magnetic particles by incubating the plate on the Te-Shake at 60°C for 5 minutes with brief shaking every 2 minutes (1.5 mm orbit, 1100 RPM, 15 seconds). The plate was transferred to the magnet and incubated for 5 minutes. The clear eluate was transferred to a 96-well storage plate.

### *DNA analysis*

DNA yield and concentration were determined by fluorescence using SYBR® Green I dye[1]. Samples were diluted 1/40 prior to quantification. This method of quantifying DNA is more accurate than quantification by absorbance. Approximately 100 ng of purified DNA was run on 0.8% agarose gel (90 V, 50 minutes) and visualized by ethidium bromide staining. A Lambda-HindIII digest ladder was used to determine the molecular weight of the DNA. The DNA was diluted 5-fold with water, the absorbance (260, 280 and 320 nm) was measured using an Infinite® M200 Microplate Reader (Tecan). The 320 nm reading, which measures residual turbid material, was subtracted from both the 260 nm and 280 nm readings. The corrected $A_{260}/A_{280}$ ratio was then calculated[2].

To evaluate the performance of the purified DNA in PCR, primers were used to amplify regions of the human thymidylate synthetase gene and the human amelogenin gene. 'Blank' samples were also analyzed by PCR to detect cross-contamination. Amplification reactions were conducted in a quantitative thermal cycler. The thymidylate synthetase PCR was monitored in real-time; the amelogenin PCR[3] was analyzed by running the product on 1% agarose gel and visualizing the PCR product by ethidium bromide staining.

As a further test for sample cross-contamination, 2 µL of the eluate from the Oragene/saliva samples and 20 µL of the adjacent 'blank' samples were run on a 0.8% agarose gel. The DNA was visualized by ethidium bromide staining. An empty lane was left between each sample on the gel to guard against cross-well contamination during loading of the gel.

## Results

### *DNA yield, concentration and purity*

From 96 Oragene/saliva samples purified in triplicate, the median DNA yield for a 500 µL aliquot of starting sample was 7.2 µg and the average yield was 8.4 µg.

The median DNA concentration of the purified DNA was 143 ng/µL and the mean was 169 ng/µL with a 95% confidence interval of 146 ng/µL to 191 ng/µL. The concentration range of the purified DNA was 16.9 to 582 ng/µL. In each run, only 5% of the samples fell below 30 ng/µL.

The average $A_{260}/A_{280}$ ratio of the purified DNA samples was 1.6.

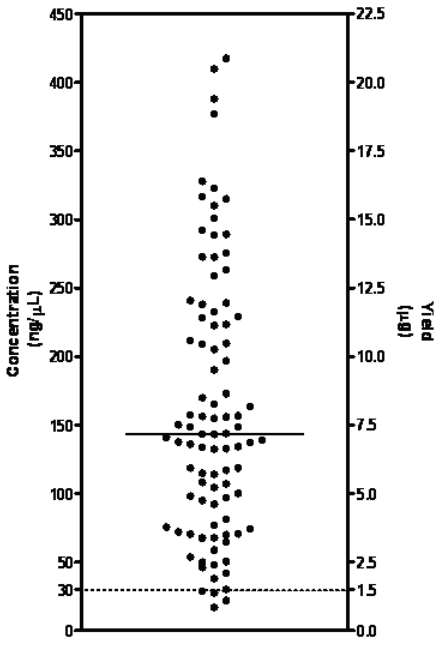


***Figure 1:*** *Scatter plot of DNA concentration and yield obtained from purifying a 500 µL aliquot of Oragene/saliva sample on the Tecan Freedom EVO using the Promega MagaZorb DNA mini-prep kit.*

### *Molecular weight of extracted DNA*

The integrity of the DNA extracted from Oragene/saliva was assessed by running the DNA on an agarose gel. The DNA in each sample had a molecular weight > 23 kb (Figure 2).

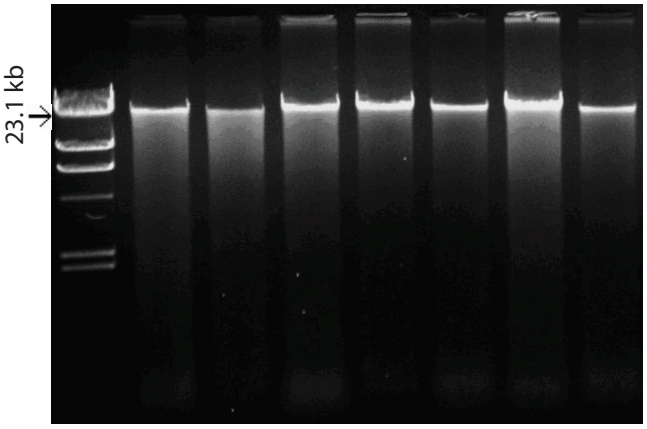


***Figure 2:*** *Agarose gel electrophoresis of DNA purified from seven randomly selected Oragene/saliva samples using the Tecan Freedom EVO and the Promega MagaZorb DNA mini-prep kit. A Lambda-HindIII digest was used as the marker with the top band representing 23.1 kb.*

### *Performance in PCR*

The suitability of the purified DNA for use in PCR was assessed by amplifying fragments of two human genes, thymidylate synthetase and amelogenin. The data demonstrates that the DNA performs well in real-time PCR (Figure 3, blue curves) and in conventional PCR as evidenced by the single (female samples) and double (male samples) bands on the agarose gel (Figure 4). The real-time PCR data confirms that 'blank' samples contain no detectable DNA (Figure 3, red curves).

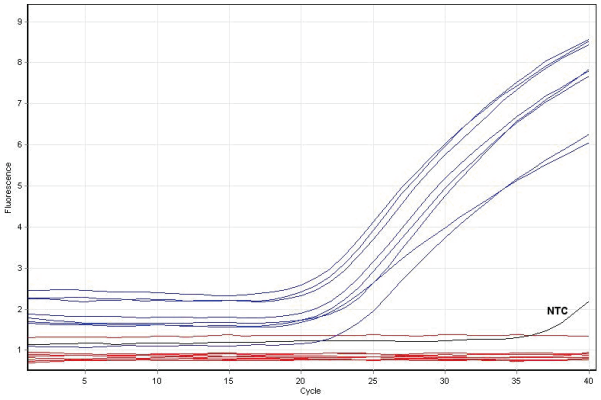


***Figure 3:*** *Real-time PCR data for eight randomly- elected Oragene/saliva samples (blue) and eight 'blank' samples (red). Primers selective for a fragment of the human thymidylate synthetase gene were used in the amplification. A no template control was included (black).*

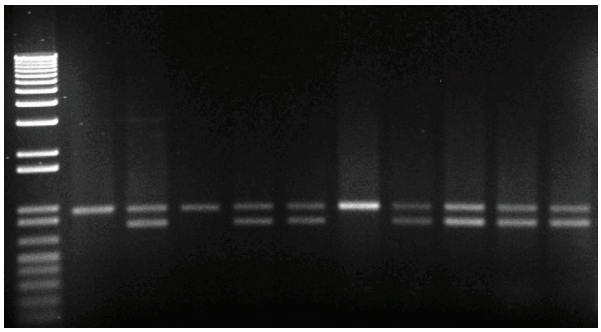

***Figure 4:*** *Agarose gel of PCR product. MagaZorb-purified DNA was used as template and primers selective for a fragment of the human amelogenin gene were used in the amplification.*

## Assessment of cross-contamination

Cross-contamination of samples is a concern in any high-throughput DNA purification system. To verify that cross-contamination was not occurring during purification of DNA using the MagaZorb kit on the Freedom EVO platform, 'blank' samples were tested by real-time PCR of the thymidylate synthetase gene as described previously. No detectable signal was observed for the 'blank' samples indicating the blanks were not contaminated by DNA from neighbouring samples during the purification procedure (Figure 3, red curves). Additionally, there was no DNA visible in the blank samples run on the agarose gel (Figure 5).

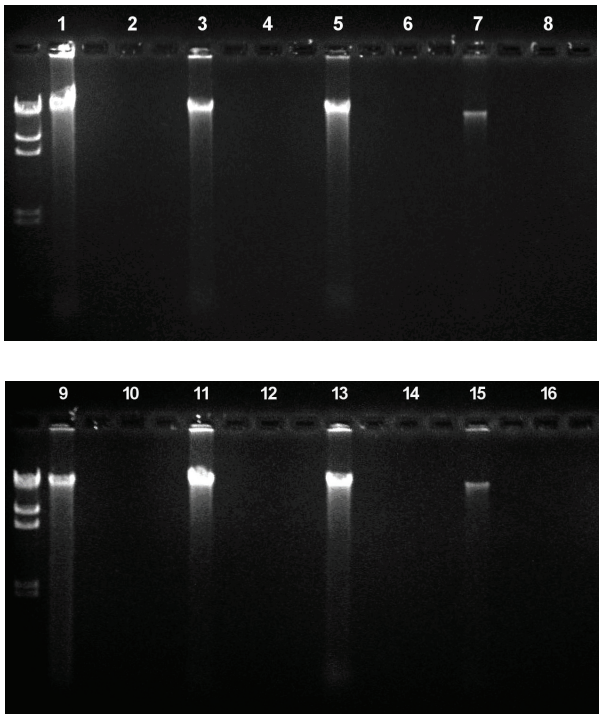


***Figure 5:*** *Agarose gel of 2 µL of MagaZorb-purified DNA (odd-numbered lanes) or 20 µL of eluate from 'blank' samples (even-numbered lanes) demonstrated that there is no cross-contamination between samples during purification on the Freedom EVO.*

## Discussion and conclusions

High yields of high quality DNA were purified from Oragene/saliva samples using the MagaZorb DNA mini-prep kit and Freedom EVO. DNA concentrations suitable for a wide variety of assay platforms including microarray analysis and array CGH were readily achieved. Analysis by agarose gel electrophoresis showed that the integrity of the DNA was excellent. The extracted DNA also performed well in both conventional and real-time PCR.

In summary, the MagaZorb DNA mini-prep kit, when used on the Freedom EVO liquid handling platform, is capable of purifying DNA from 96 Oragene/saliva samples in approximately 2 hours 15 minutes with no detectable cross-contamination of samples. For even higher throughput, the Freedom EVO can be equipped with additional tube and plate carriers to purify up to 196 samples in a single run.

### References


1 DNA quantification using the Fluorescence/DNase (F/D) assay. Replaced by DNA quantification using SYBR Green I dye and a micro-plate reader. DNA Genotek. PD-PR-075.

2 From turbidity to clarity: Simple methods to improve the $A_{260}/A_{280}$ ratio of Oragene-purified DNA samples. DNA Genotek. MK-AN-017.

3 Ong, K.K., et. al. (2004). Maternal-fetal interactions and birth order influence insulin variable number of tandem repeats allele class associations with head size at birth and childhood weight gain. *Diabetes*. 53,1128-1133.