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High-throughput Isolation of Genomic DNA From Buccal Swab on the Eppendorf ep*Motion*® 5075 VAC

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Abstract

The method adapted to the automated liquid handling system ep*Motion*® 5075v or VAC, offers a complete system for automated purification of high quality genomic DNA from 96 samples. The procedure is very easy to perform, user-friendly and yields DNA suitable for various downstream applications. From buccal swabs, approximately 20 ng/µL DNA with an A260/A280 ratio of 1.8 – 2 could be isolated. The ep*Motion* 5075 can easily process one 96-well microplate in 90–105 minutes.

Introduction

The automation and optimization of the DNA extraction from buccal swabs is considered a key point in the applied research and genetic testing. The high quality of the end product highly depends on the experimental conditions, while many extraction protocols have critical steps that may impair the end product purity.

Here we describe a simple, fast and automated method for genomic DNA isolation from buccal swab sample. Buccal swabs are one of the least invasive ways of collecting genomic DNA from humans, and also have the added benefit of minimizing the exposure to blood-borne pathogens. Cells collected on buccal swabs do not require special storage conditions. Due to the high purity, the isolated genomic DNA is ready to use for a broad panel of downstream applications such as conventional and quantitative PCR, restriction enzyme digestion, southern blotting or any kind of enzymatic reaction. This method has been validated for use with several types of swabs, and will most likely be compatible with others.

Here we are introducing a fully automated system to extract high quality genomic DNA from buccal swabs in a 96 well format. The entire procedure has been optimized for use on the Eppendorf ep*Motion* 5075 VAC workstation, a fully automated liquid handling system, but can easily be transferred to the newer ep*Motion* 5075v version as well.

Material and Methods

Required Labware

> ep*Motion* 5075 VAC or 5075v
> Dispensing Tool TM 1000-8
> Dispensing Tool TM 50-8
> Thermorack for 24 tubes
> Thermoblock DWP 2000
> Eppendorf Biophotometer® plus.
> UVette® Cuvettes.
> Eppendorf BioPhotometer Data transfer Software.
> Easygen-gDNA extraction buccal swabs kit, Bioeasy SL
> Ethanol 100 %
> Proteinase K, Sigma-Aldrich®
> Buccal swabs, Invasive sterile EUROTUBO® Collection swab, Deltalab®

Required Consumables

> Deepwell plate 96/2,000 µL, Eppendorf
> Microplate 96/F
> Filterplate, Macherey-Nagel
> epT.I.P.S.® Motion 50 µL, filtertips
> epT.I.P.S. Motion 1000 µL, filtertips

Sample Material

Buccal smear samples are collected according to the manufacturer's instructions using the swabs. Rinse mouth with water and wait for 10 minutes. Collect the sample of buccal cells with the swab, rub the swab on the inside of the cheek and gums with a firm pressure about 20 times on each side of the face and each side of the broom.

Swabs can either be used immediately for extraction or need to be dried for 30 minutes at room temperature to allow shipping/storage for 1 week at 22–37 °C. If the latter is the case after drying, the swabs need to be put into the respective container. Long term storage of dried swabs can be for 6 months at -20 °C.

Method

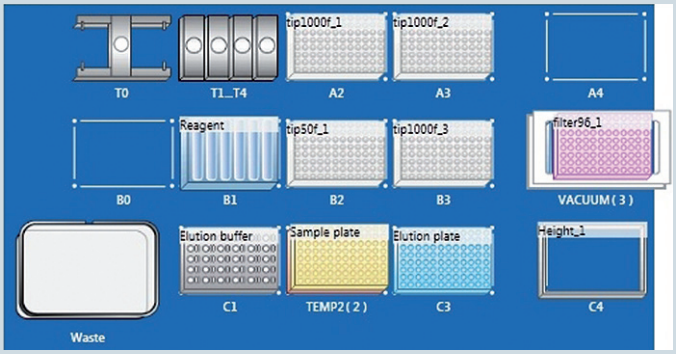

Figure 1: Screenshot of the worktable allocation

Worktable layout

Worktable Position	Labware
A2	1000 µL filtertips
A3	1000 µL filtertips
A4	1000 µL filtertips
B1	ReservoirRack with reservoirs
B2	50 µL filtertips
B4	> Waste tub > VacFrame2 > Filter plate
C1	Sample plate
C3	Elution plate
C4	VacFrame holder

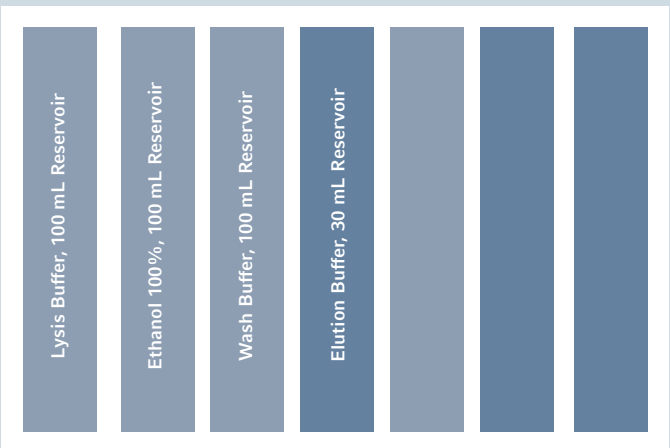

Figure 2: ReservoirRack Layout

Procedure

Collect samples by scraping a buccal swab firmly against the inside of a donor's cheek, approximately 20 times. The donor should not drink, eat or smoke 30 minutes prior to sampling.

Place the swab (cotton) into a tube containing Lysis buffer then rotate the swab 15 times (spin between fingers) to release cells from the swab into the buffer.

Place the swab (cotton) into a tube containing Lysis buffer, then snap off the handle region of the swab so that it fits entirely inside the tube allowing the cap to close. Incubate at 58 °C for 10 minutes.

Remove the swab (cotton) from the tube, pressing it against the side of the tube to ensure most of the liquid remains in the tube.

The following steps are done by the ep*Motion* 5075 automated liquid handling system: 300 µL Lysis buffer is dispensed into each well of a sample plate (2000 µL Deep-well plate). 5 µL of Proteinase K solution is added into each well. The lysate is mixed by pipetting up and down 10 times and incubated at 58 °C for 15 minutes on the thermo module C2 on the worktable of ep*Motion*.

200 µL of binding buffer is added and mixed thoroughly by pipetting 30 times (white precipitates will be formed). 200 µL of 100 % ethanol is added to the sample and mixed by pipetting 15 times.

Incubation of the sample plate at 58 °C for 5 minutes, again on thermo module C2 (precipitates will dissolve during this step).

The lysate is transferred to the binding filter plate on the vacuum station. Incubation for 5 minutes at room temperature. Vacuum is applied until all lysates have passed through the columns (900 mbar for 10 minutes).

500 µL of washing solution is dispensed by the ep*Motion* into each well of the binding filter plate. A vacuum step is performed until all the buffer has passed through the filter with the vacuum parameters 900 mbar and 10 min.

Again, 500 µL of washing solution is dispensed into each well of the binding filter plate. The vacuum step is programmed until all the buffer has passed through the filter (900 mbar for 10 minutes).

After the final washing step, the filter plate should be nearly dry. The gripper removes the filter plate from the vacuum system. If necessary, remove any residual washing buffer on the filter plate, via tapping the outlet onto a clean paper until no drops come out. Insert the filter plate into the vacuum system again. Continue with the next vacuum step (900 mbar for 10 minutes) to dry the membrane completely. This step is necessary to eliminate traces of ethanol.

During the next step, the binding filter plate and the waste container are removed via gripper from the vacuum system. The microplate is inserted and the filter plate is placed on top. 60 µL of elution buffer is added directly to the bottom of each well and preheated at 70 °C in the thermorack on the thermo module. Incubation for 3 minutes at room temperature follows.

The vacuum is applied for elution (600 mbar and 2 minutes). 60 µL of pre-heated elution buffer is pipetted directly into the bottom of each well. Incubation: 3 minutes at room temperature. The vacuum for DNA elution is applied (600 mbar and 2 minutes).

Results and Discussion

When performing high-throughput nucleic acid purification, it is essential to achieve consistent, reproducible results with respect to DNA quality and yield. Here, we examined DNA isolated from eight donors of buccal sample, using buccal swabs to obtain biological samples and high throughput isolation of genomic DNA from buccal swab on the Eppendorf ep*Motion* 5075 VAC. Spectrophotometer analysis demonstrated high quality DNA in all samples.

Inhibition of downstream reactions of contaminating substances, such as lipids and proteins, is a major obstacle when isolating DNA from buccal swabs. We included steps like proteinase K digestion and special composition wash buffer to remove typical PCR-inhibitors and used the extracted DNA for several enzymatic reactions.
DNA yield and purity were examined using the UVette with the Eppendorf BioPhotometer plus, revealing average yields of 2419.74 ng in 120 µL, an average concentration of 20.16 ng/µL per sample, with A260/280 ratios ranging from 1.8 – 2.0 (Table 1).

Conclusion

The introduction of an automated method with silica membrane binding offers the possibility to optimize and automate the extraction and purification of gDNA. This method was developed with maximum simplicity and accuracy, in order to optimize this routine procedure and save time compared to the manual method approach.

The results demonstrate that the method using high-throughput isolation of genomic DNA from buccal swab on the Eppendorf ep*Motion* 5075 VAC is ideally suited for the automation of DNA extraction from buccal swabs and FTA® cards. Furthermore, the non-contact dispensing of the ep*Motion* workstation makes it ideal for DNA applications; the ep*Motion* is capable of pipetting in 96-well format with no detectable cross contamination between wells.

The time taken for the new extraction process is approximately one hour and 30–45 minutes for 96 samples. This time is highly favorable when compared with the manual method which typically takes five to six hours.

Table 1: Final yields using 1:10 diluted DNA solutions in Nuclease free water (10 mm light path) – UVette with Eppendorf BioPhotometer plus.

Donors	A230	A260	A280	Ratio A260/A230	Ratio A260/A280	Concentration (ng/µl)	Yield (ng)
Donor 1	0.056	0.110	0.056	1.96	1.96	22.00	2640
Donor 2	0.055	0.108	0.055	1.96	1.96	21.60	2592
Donor 3	0.042	0.078	0.040	1.86	1.95	15.60	1872
Donor 4	0.050	0.099	0.051	1.98	1.94	19.80	2376
Donor 5	0.068	0.120	0.065	1.76	1.85	24.00	2880
Donor 6	0.051	0.097	0.049	1.90	1.98	19.40	2328
Donor 7	0.055	0.099	0.050	1.80	1.98	19.80	2376
Donor 8	0.052	0.101	0.056	1.94	1.80	20.20	2424
Means	0.050	0.100	0.050	1.90	1.93	20.16	2419.74
Standard desviation	0.01	0.01	0.01	0.08	0.07	2.44	292.82
CV	0.14	0.12	0.14	0.04	0.03	0.12	0.12

Ordering information

Description	Order no. international
ep*Motion*® 5075v	5075 000.303
Reservoir Rack	5075 754.002
Dispensing Tool TM 1000-8	5280 000.258
Dispensing Tool TM 50-8	5280 000.215
epT.I.P.S® Motion 1000 µL with filter	0030 014.499
epT.I.P.S® Motion 50 µL with filter	0030 014.413
Reservoir 30 mL	0030 126.505
Reservoir 100 mL	0030 126.513
Thermorack 24 tubes	5075 771.004
Thermoblock DWP 2000	5075 751.054
Microplate 96/F	0030 601.106
Deepwell Plate 96/2,000 µL	0030 501.306
Macherey-Nagel	
Filterplate	740665

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