

Applications

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Reproducible, convenient walk away purification of genomic DNA from whole blood with the Invisorb® Blood Mini HTS 96 Kit/ep on the epMotion® 5075 VAC from Eppendorf

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Abstract

For large genotyping projects like HLA genotyping assays for tissue compatibility, screening projects, or the diagnosis of hereditary diseases like cystic fibrosis, the DNA purification from hundreds or thousands of samples often represents a bottleneck in sample analysis. The use of an automated, easy to handle method is therefore highly desirable to increase productivity. The automated method for the purification of genomic DNA consisting of the Invitek Invisorb® Blood Mini HTS 96 Kit/ep and the Eppendorf automated pipetting system epMotion® 5075 VAC realizes these requirements and is described in this application note. Various human blood samples were used as starting material and the extracted genomic DNA was subjected to PCR analysis. The quality of individual samples was assessed in a cross contamination assay.

Introduction

With the wide application of DNA in SNP analysis, genotyping experiments and other molecular diagnosis, the request for fully automated high throughput DNA isolations has increased in the past. We have addressed this demand with the integration of the well-established technology of the Invisorb Blood Mini HTS 96 Kit into the automated pipetting system epMotion 5075 VAC from Eppendorf. This flexible and accurate system allows the simultaneous isolation of 96 DNA samples from up to 200 µl human or mammalian blood (whole blood treated with EDTA or citrate*). The Invisorb Blood Mini HTS 96 Kit/ep provides high quality DNA for downstream applications such as PCR, short random amplification of polymorphic DNA (SNP), and sequence repeat/short tandem repeat analysis (SSR / STR) and human leukocyte antigen typing (HLA). The purification procedure requires neither phenol / chloroform extraction nor prior separation of leukocytes or alcohol precipitation, allowing safe handling of potentially infectious samples. The procedure is designed to avoid sample-to-sample cross-contamination.

Due to the high purity, the isolated genomic DNA is ready to use for a broad panel of downstream applications (see below) or can be stored at -20°C for subsequent use.

Downstream Applications

- PCR
- Restriction Enzyme Digestion
- SNP Analysis
- HLA typing
- Cloning

For reproducible and high yields an appropriate sample storage is essential**.

The purified DNA can be used for in vitro diagnostic analysis. The epMotion 5075 VAC is equipped with an 8-channel pipetting tool, a plate gripper, an electronically controlled vacuum processing chamber and a heating platform and therefore enables a fully automated procedure with very short hands-on time for the user.



*) These stabilization agents were tested, others may work, heparin for example causes problems in downstream reactions

**) The reproducibility and yield of DNA from blood samples depends on many factors which include the age, health and medication of the donor as well as the storage conditions. So the yield of typical human blood can differ between 4 –12 µg per 200 µl of sample. Wrong storage (e.g. long time storage at room temperature) can dramatically reduce the yield and quality of the DNA by degradation.

Materials and Methods

Equipment

- epMotion 5075 VAC with Gripper
- epMotion 8-channel dispensing tool TM 1000-8
- epMotion Reservoir 400 ml
- Vac frame 1 and Vac frame Holder

- Invisorb® Blood Mini HTS 96 Kit /ep
- epT.I.P.S Motion 1000 µl

Sample Material

- EDTA Blood from humans

Real-Time PCR Analysis

Real time PCR analysis for cross contamination assay and reproducibility were done on a Rotor-Gene™ 3000 (Corbett Life Science) with a GAPDH PCR setup as described in the following table. (Tab. 1 and Tab. 2).

Table 1: PCR setup

Component	Volume /µl	Final concentration
Template DNA	5	2.8 ng/µl
Water	13.85	
10 X Reaction Buffer	2.5	1x
50 mM Magnesiumchlorid Solution	1	2 mM
50 X dNTP Master Mix (12,5 mM)	1	0.15 mM
Forward Primer	0.075	0.15 µM
Reverse Primer	0.075	0.15 µM
SYBR Green I dilution of 1:1000	1	1:25000
InviTaq DNA Polymerase	0.5	2.5 U

Table 2: PCR program

Cycle	Cycle Point
Hold @ 95 °C, 5 min 0 secs	
Cycling (40 repeats)	Step 1 @ 95 °C, hold 30 secs
	Step 2 @ 53 °C, hold 30 secs
	Step 3 @ 72 °C, hold 30 secs, acquiring to Cycling A(FAM/Sybr)
Melt (66-99 °C) , hold 45 secs on the 1st step, hold 5 secs on next steps, Melt A(FAM/Sybr)	

Automated Sample Processing with the epMotion 5075VAC

Before starting the method the worktable was prepared according to Fig. 1. Detailed information on the required labware for each position on the worktable can be found in Tab. 3. The needed amount of the buffers were added to each tray of the reagent reservoir holder. The automatic procedure starts with transferring 200 µl of each blood

sample to the appropriate wells of the provided u-bottom plate. If less than 200 µl blood is used, water may be added to the sample until 200 µl sample volume is reached. Changing the amount of starting volume in the epMotion software is also possible. The processing time is approximately 2 hours for 96 samples.

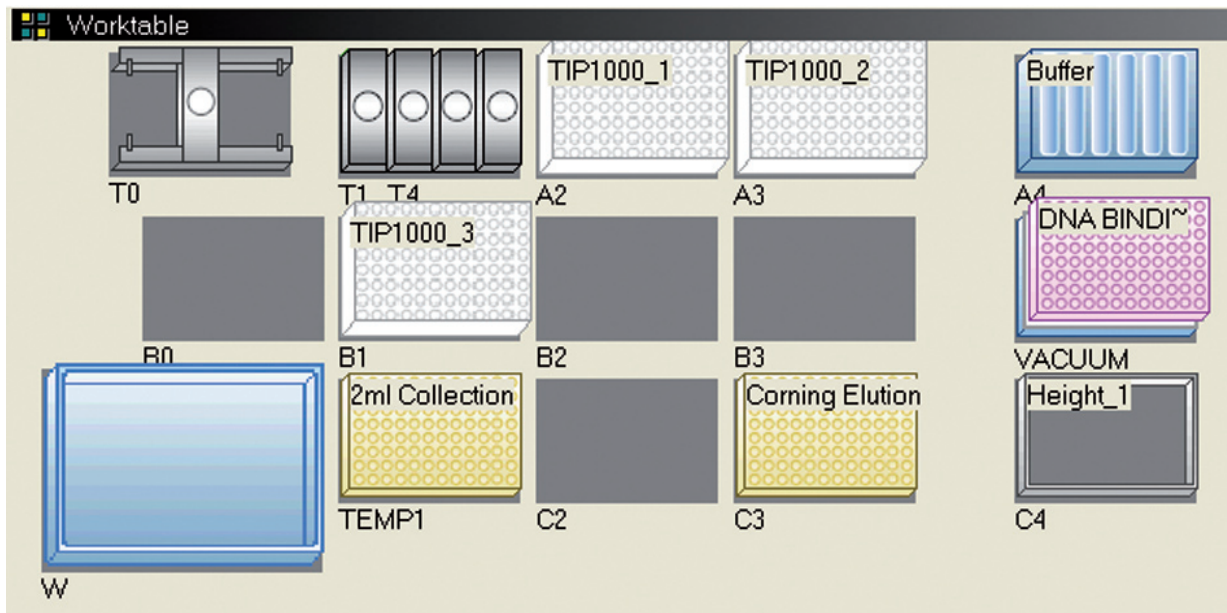


Figure 1: Screenshot from the epMotion Editor showing the setup of the epMotion 5075 VAC work-table for genomic DNA extraction with Invisorb Blood Mini HTS 96 protocol

Table 3: epMotion 5075 VAC worktable details for the Blood mini 96 protocol

Position	Lab ware	Comment
A2	epT.I.P.S Motion Filter 1000 µl	96 tips for 96 samples
A3	epT.I.P.S Motion Filter 1000 µl	96 tips for 96 samples
A4	Reagent Reservoirs	
	Lysis Buffer HL	100 ml reservoir
	Binding Buffer HL	100 ml reservoir
	Wash Buffer I	100 ml reservoir
	Wash Buffer II	100 ml reservoir
	Elution Buffer	30 ml reservoir
	Proteinase K	30 ml reservoir
B1	epT.I.P.S Motion Filter 1000 µl	96 tips for 96 samples
VACUUM	DNA Binding Plate D	Binds DNA onto membrane
	Vacuum Frame 1	Collar for vacuum chamber
	Reservoir 400 ml	Collects flow through
C1	2.0 ml Collection Plate on TEMP1	Heating for lysis
C3	Elution Plate	
C4	Vacuum Holder	Holder for Vacuum Frame 1
T0	TG-T	Gripper for epMotion
T1	TM 1000-8	8-channel pipetting tool

DNA isolation from human blood

The automatic procedure started with transferring 200 µl of each blood sample to the appropriate wells of the provided u-bottom plate. Then the samples were automatically mixed with Lysis Buffer HL and Proteinase K and the mixtures were incubated for 10 min at 56 °C. After lysis, Binding Buffer HL was added, mixed by pipetting up and down and the whole mixtures were transferred to the DNA Binding Plate D.

The mixtures were incubated at RT for 2 min. During this step, the DNA will bind to the membrane. The following washing steps remove all contaminants and afterwards the ethanol will be dried away. Finally the DNA will be eluted in an EDTA free Elution Buffer.

Results

Reproducibility

As illustrated in Fig. 2 genomic DNA from various blood samples can be isolated with the Invisorb Blood Mini HTS 96 Kit /ep and the automated epMotion 5075 VAC method. The procedure consistently delivered high molecular weight DNA as indicated by clear bands without detectable RNA contamination. The DNA was suitable for PCR amplification which is demonstrated by the successful amplification of the GAPDH - sequence in all of the samples (Fig. 3).

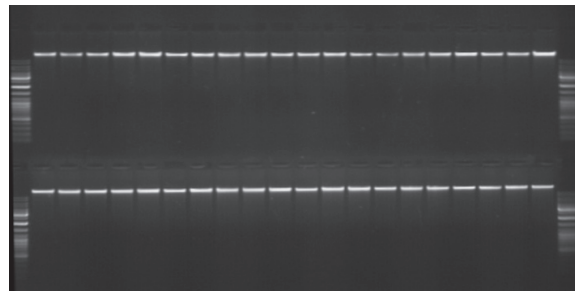


Figure 2: 10 µl of the eluted DNA from 96 isolated DNA samples were analyzed on 1 % TAE agarose gel stained with Ethidiumbromide

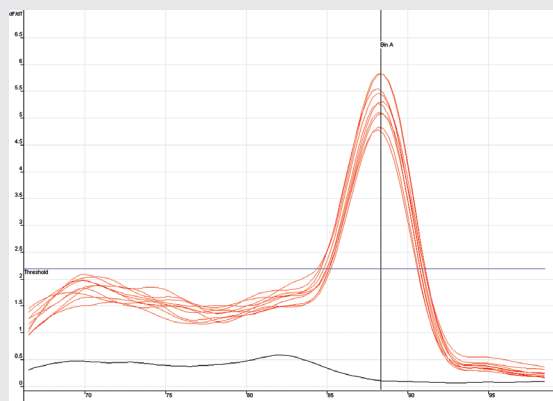
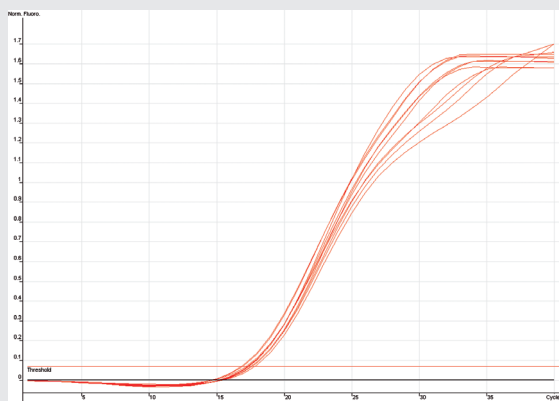


Figure 3: Amplification of GAPDH out of 10 randomly taken samples (left side) and corresponding melting curve for the resulted PCR products (right side).

Quality of extracted DNA

The yields may vary from sample to sample depending on factors such as the health of the donor, patient medication, sample type, leukocytes content, sample source, transport, storage, and age. In the presented run, an average yield of 2.3 µg with an averaged ratio A260/A280 of 1.7 was obtained (Fig. 4).

The yields are in average between 2-6 µg, depending on the blood. The DNA amounts for the recommended amount of blood (numbers of leukocytes) are significantly below the maximum binding capacity of each well.

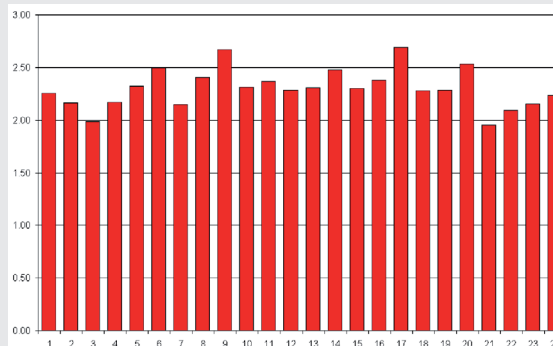
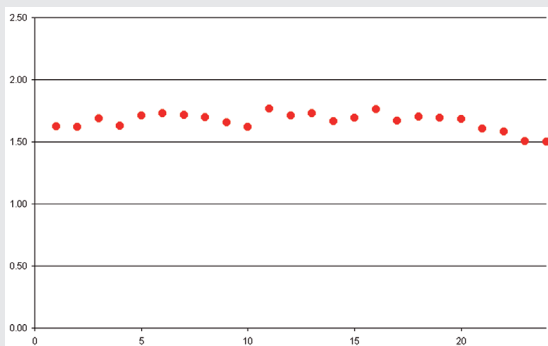


Figure 4: Typical ratio A260/A280 and yields of 24 of the 96 samples of the run presented above.

Cross Contamination Assay

A cross contamination assay was performed to assess if the automated process ensures precise and reliable pipetting within each well without affecting adjacent samples. Following the DNA extraction with the Invisorb Blood Mini HTS 96 Kit /ep, every second well was filled with water instead of the blood sample to create a chessboard pattern, as illustrated in Fig. 5 After extraction the eluted samples and negative controls were subjected to agarose gel electrophoresis analysis and real-time PCR as a very sensitive method for detecting any contaminating DNA in the controls.

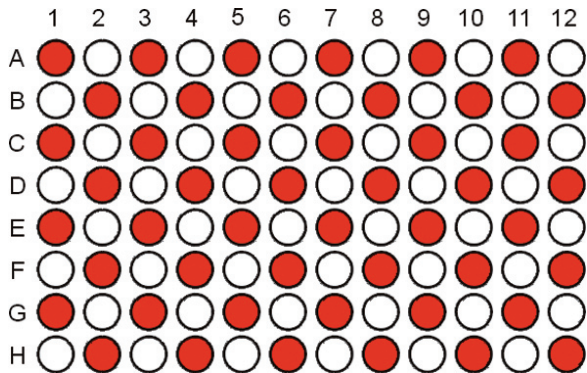


Figure 5: Chessboard pattern as used for cross contamination assay. Arranged are blood samples (red) and water as no sample controls (white).

The resulting genomic DNA is shown in Fig. 6. No visible genomic DNA can be detected in the no sample control wells with water. The results of the PCR amplification of these samples are shown in Fig. 7. In the no sample controls no amplification of the GAPDH sequence could be detected. In Tab. 2 the high reproducibility with mean Ct values and standard deviations is shown again.

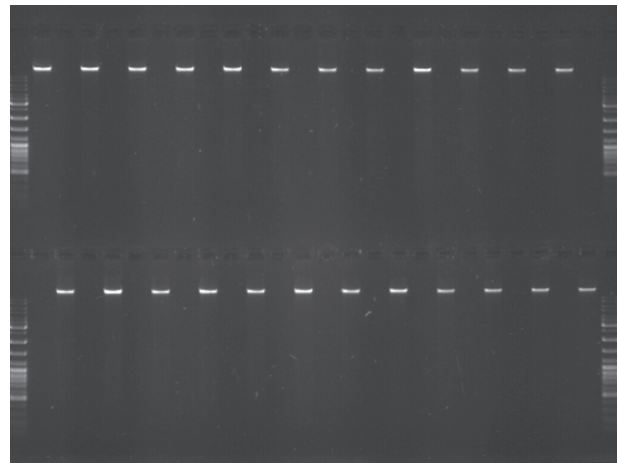


Figure 6: 10 µl of the eluted DNA from 24 samples and 24 no template controls were analyzed on 1% TAE agarose gel stained with Ethidium-bromide.

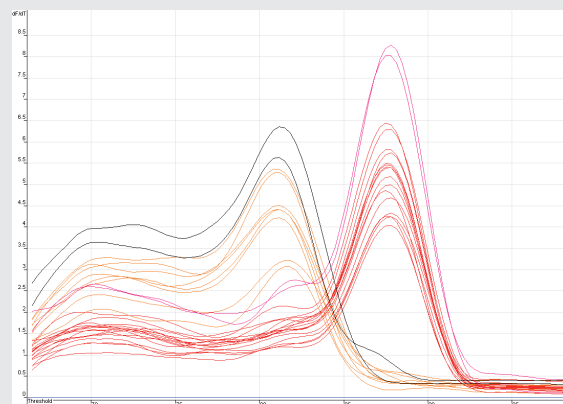
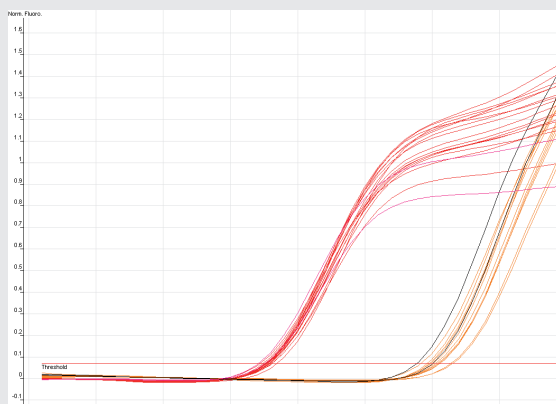






Figure 7: GAPDH was amplified in real-time PCR with the 48 samples of the chessboard pattern (24 blood samples, red lanes, and 24 no sample controls, orange lane). On the left the increase of amplified product is shown and on the right the melting curve of the resulting product. Only the red samples show a PCR product, were the yellow show no product but amplification of primer dimers. Black lines are PCR negative controls (NTC) which show also amplification of primer dimers.

Table 4: Overview of the resulting Ct values from the real-time PCR above. The results show a good reproducibility for the samples which indicate the same amount of extracted DNA in all blood samples. NTC (no template control); PTC (positive template control from in-house human DNA pool with unknown concentration).

Colour	Name	Rep. Ct	Rep. Ct Std. Dev.
	blood sample	20.06	0.28
	no sample controls	28.07	2.32
	NTC	28.56	0.97
	PTC	18.89	0.14

Conclusion

In combination with the epMotion 5075 VAC workstation the Invisorb Blood Mini HTS 96 Kit /ep provides automated and economical purification of genomic DNA from 96 blood samples in less than two hours with minimal hands-on time. This rapid and convenient method can be adapted to various blood samples by adjusting the individual liquid handling parameters within the automated method protocol. The process can flexibly adapted to different sample types, e.g. with animal blood samples, the lysis time is increased.

All of these modifications can easily be done with the intuitive software structure of the epMotion control panel or more quickly and convenient with the new epMotion PC software epBlue. Apart from time-saving and productivity-enhancing aspects the automated sample preparation process on the epMotion 5075 VAC with the Invisorb Blood Mini HTS 96 Kit /ep allows reliable DNA extraction of high quality for sensitive downstream applications. In particular the performed cross contamination assay did not show any PCR amplification from control wells.

References

- [1] Eppendorf, Instrument Manual for the epMotion® 5075 VAC
- [2] Invitex, Invisorb® Blood Mini HTS 96 Kit /ep Handbook for processing the Invisorb® Blood Mini HTS 96 Kit /ep on the Eppendorf epMotion® 5075 VAC workstation.

Eppendorf Ordering information

Description	Order no. International	Order no. North America
epMotion® 5075 VAC 230 V	5075 000.016	n/a
epMotion® 5075 VAC 120 V	n/a	960020014
Dispensing tool TM 1000-8	5280 000.258	960001061
Gripper	5282 000.018	960002270
Holder for gripper	5075 759.004	960002211
Reservoir Rack	0030 128.648	960002148
Reservoirs 100 ml (10 x 5 reservoirs in bags/case, PCR clean)	0030 126.513	960051017
Reservoirs 30 ml (10 x 5 reservoirs in bags/case, PCR clean)	0030 126.505	960051009
epTIPS Motion 1000 µl Filter	0030 003.993	960050100

Invitex Ordering information

Description	Order no. International
Invisorb® Blood Mini HTS 96 Kit /ep	
2 x 96 preparations	71313202
4 x 96 preparations	71313203
24 x 96 preparations	71313204

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