

Application Note

CyBio CyBi®-Genomics Workstation/Invitex Invisorb® Blood Mini HTS 96-Kit/V Automated genomic DNA extraction from whole Blood with CyBi®-Genomics workstation using Invisorb® Blood Mini HTS 96-Kit

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The CyBi®-Genomics workstation has been used to automate Invitex's Invisorb® Blood Mini HTS 96-Kit/V technology for purification of genomic DNA from whole blood. The use of a 96-well pipetting head, a stationary plate gripper and electronically controlled vacuum processing enables an extremely rapid (50 minutes) fully automated procedure and also maintains the purification technology's highly reliable performance. This results in very good yield (0,24 µg DNA from 50 µl blood) and excellent DNA quality (PCR tested) without cross-contamination.

Introduction: This purification method is based on DNA binding onto the Invisorb® filter surface without the need for hazardous chaotropic buffer components. The automated purification protocol consists of cell lysis, lysate binding onto the filter surface, two washing steps with ethanol containing buffers, an optimized drying process for ethanol removal and two elution steps.

Methods: The extraction protocol was set up on a CyBio system comprising: 96-well automated pipettor with a volume range of 1 – 250 µl, 10 position deck, an electronically controlled vacuum pump (ControlElute software), the patented SafeElute sealing system, a stationary plate gripper, a universal vacuum manifold and an active tip wash station. The purification procedure was established according to Invitex's standard protocol for Invisorb® Blood Mini HTS 96-Kit/V. The whole protocol was carried out with 50 µl mammalian blood per well with heparin as anticoagulant. The quality and size of the DNA obtained was analysed by gel electrophoresis and ethidium bromide staining. Extraction yield was calculated by DNA quantification with SYBR Green I. For a quality check, PCR was set up for amplification of *amelogenin* (977 bp) with 35 PCR cycles. To test for cross-contamination, blood samples and water were loaded into the collection plate in chessboard pattern, purified with CyBio CyBi®-Genomics Workstation and analysed by PCR amplification with 50 cycles instead of 35.

Automated extraction procedure:

1. Add 220 µl Lysis Buffer including Proteinase K to the collection plate containing 50 µl blood, mix thoroughly by pipetting up and down and incubate for 15 min. Wash tips in parallel.
2. Add 110 µl Binding Buffer A, mix thoroughly by repeated pipetting and transfer sample to a Binding Plate C positioned on the vacuum manifold
3. Tightly seal the Binding Plate C to vacuum manifold using SafeElute and apply vacuum at a pressure difference of 500 mbar to ambient for 2 min. Wash tips in parallel.
4. Wash sample in the Binding Plate C by adding 400 µl Wash Buffer I and by repeating the vacuum step (step 3) but for 1 min only. Wash tips in parallel.
5. Wash sample in the Binding Plate C by adding 800 µl Wash Buffer II and by repeating the vacuum step. Proceed with step 6 without interruption in vacuum process.
6. Remove ethanol by applying the automated drying process (vacuum at a pressure difference of 500 mbar for at least 10 min). Wash tips in parallel.
7. Reassemble vacuum manifold by the stationary plate gripper with the elution plate inside.
8. Elute purified sample into the elution plate by adding 60 µl Elution Buffer D, incubate 3 min and apply vacuum at a pressure difference of 500 mbar to ambient for 2 min. Wash tips in parallel.
9. Add another 60 µl Elution Buffer D and repeat the vacuum step (step 6) without incubation.

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Results: Purified genomic DNA showed high quality, as analyzed by gel electrophoresis (figure 1) and could be successfully used for downstream applications like PCR amplification (figure 2). DNA extraction yield was calculated at 0,24 µg from 50 µl blood (n = 32, cv = 0,039). Volume left in filter plate after elution of DNA was about 20 µl per well ('dead volume'). Automated sample processing took only 50 minutes. No cross-contamination between neighbouring wells occurred, as analyzed by PCR with 50 cycles (figure 3). A summary of these results is listed in table 1.

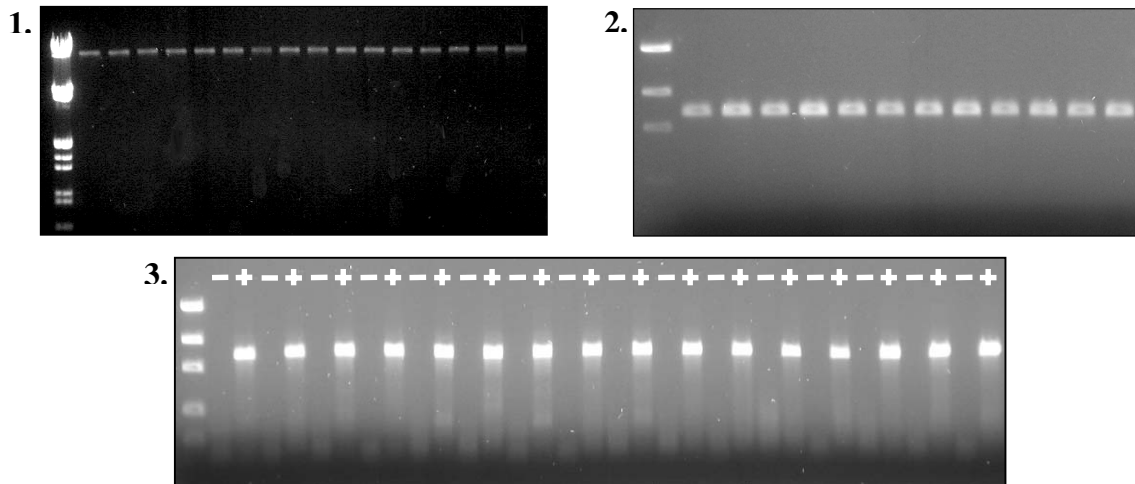


Figure 1: Analysis of extracted DNA by agarose gel electrophoresis (10 µl sample, marker: Lambda DNA/*EcoRI/Hind III*).
 Figure 2: Analysis of extracted DNA for their usage in the downstream application PCR: The 977 bp long *amelogenin* was amplified by PCR with 35 cycles and analyzed by gel electrophoresis (10 µl sample, marker: 4 µl Low DNA Mass Ladder, Invitrogen).
 Figure 3: Cross-contamination test: Blood samples (+) and water (-) were loaded into the collection plate in a chessboard pattern, purified, subjected to PCR with 50 cycles instead of 35 and analyzed by gel electrophoresis (10 µl sample, marker: 4 µl Low DNA Mass Ladder, Invitrogen).

Table 1: Technical specifications obtained by automated genomic DNA extraction

Specification	CyBi® Genomics workstation
Purity	Successful in downstream applications like PCR
Extraction yield	0,24 µg from 50 µl blood (n = 32, cv = 0,039)
Dead volume	20 µl
Time	50 min
Process safety	No PCR detectable cross-contamination (50 cycles)

Discussion: By applying the special features of CyBio's robotic system to the DNA extraction method it was possible to establish a very safe and fast process with excellent specifications (table 1). By processing 96 wells at the same time and using parallel processing software for simultaneous execution of vacuum steps and tip washing, a very fast protocol was established. Using CyBio EluteControl software for automated filter drying, resulted in a further decrease in the required preparation time. Careful adjustment of pipetting speed was implemented to ensure safe handling of the very viscous blood samples. Very thorough mixing of samples with Lysis Buffer (step 1) and with Binding Buffer (step 2) proved to be important and was done efficiently by repeatedly pipetting the samples. To prevent cross-contamination during the extraction process both the pipetting and tip washing procedures were optimised. The results clearly demonstrate that the CyBi®-Genomics workstation can be used for fast and reliable automation of genomic DNA extraction from whole blood with Invitex's Invisorb® Blood Mini HTS 96-Kit/V technology.