Challenge

Automated purification of genomic DNA with high quantity and quality thanks to a simple and robust method.

Solution

SmartExtraction significantly simplifies the entire automated workflow of DNA extraction, setting new standards with regard to efficiency, yield, and quality of the DNA:

- Simple method, fast routines
- Extraction of high-molecular-weight DNA
- High yield while achieving good purity

Automated Extraction of High-Molecular-Weight DNA – From High-Volume Blood Samples

Introduction

Due to the steadily growing requirements placed on the samples to be processed, in academic environments as well as in the industry, automation solutions are taking on an ever increasing importance. Purification of genomic DNA (gDNA) is the starting point for a large number of subsequent processes where the quantity and purity of the isolated DNA is important. Conventional extraction methods use expensive reagents and are based on handling of small sample quantities as well as on the resulting purification procedures, which are repeated multiple times. In addition, this increases the risk of losing valuable sample material.

SmartExtraction is Analytik Jena's innovative technology for extracting high-molecular-weight DNA. This technology forms the basis of a fundamentally new method for the automated isolation and purification of nucleic acids. SmartExtraction combines the patented extraction chemistry (DC Technology) with an intelligent surface, called "Smart Modified Surface". SmartExtraction significantly simplifies the entire automated workflow of DNA extraction and setting new standards with regard to efficiency, yield, and quality of the DNA. It is possible to obtain particularly large quantities of DNA. In addition, it is feasible to isolate high-molecular-weight DNA (200 kb → 500 kb) using an automated process. The "Smart Modified Surface" also facilitates mapping of the entire extraction process inside a pipette tip via simple pipetting steps.

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The SmartExtraction technology does not require phenol/chloroform, ion exchangers, filter columns, or filter plates, nor is a suspension of magnetic or paramagnetic particles needed to bind the DNA. In addition, pre-filled and sealed reagent plastics make handling easier and can, in analogy to the functionalized pipette tips, be used directly on the CyBio FeliX pipetting system. For automated extraction of nucleic acids applying smartExtraction the CyBio FeliX can be used for 96 samples in parallel. Next to smart Blood DNA Midi prep additional protocols for different starting materials are available. This technology is particularly suited to meet the growing demand for automated handling of liquids.

CyBio FeliX is a flexible, fully automated pipetting system with 1 to 384 channels in the volume range from 1 to 1000 µL. In addition to the highly precise, parallel transfer in the 96- and 384-well format, pipetting can be also performed by single-channel, column, or row. The appropriate accessory is automatically determined and changed within a pipetting routine. CyBio FeliX combines highest flexibility with minimum space requirements, which is particularly reflected by its unique design with 12 positions on 2 levels. Owing to the modular concept of the CyBio FeliX, application specific configurations can be added at any time. On the basis of pre-configured and optimized pipetting routines, the focus always lies on the application.

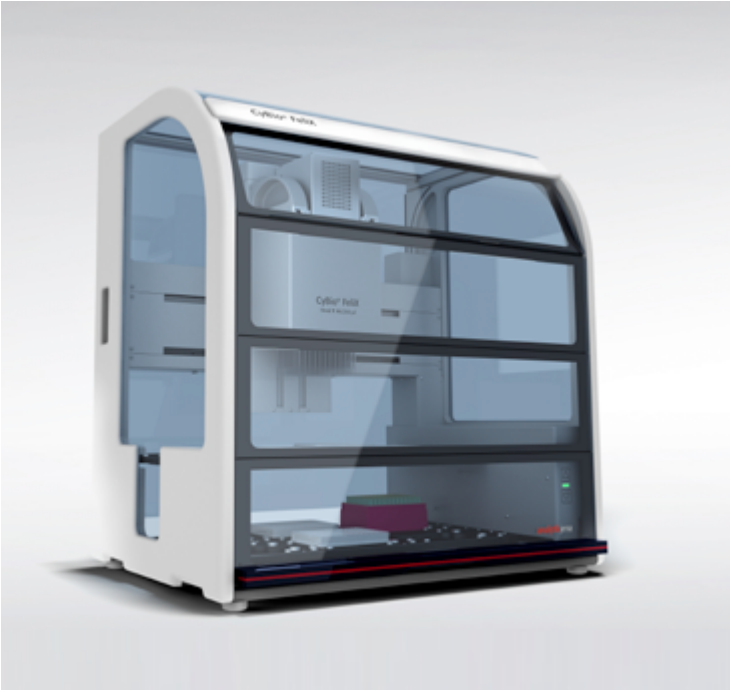

Figure 1: CyBio FeliX basic unit

Material

- CyBio FeliX basic unit (OL5015-24-100, Analytik Jena AG)
- CyBio FeliX head R 96/1000 µL (OL3316-14-950, Analytik Jena AG)
- 96-channel magazine; head R 96/1000 µL (OL3810-13-024, Analytik Jena AG)
- smart Blood DNA Midi prep (a96) (845-ASP-1296096, Analytik Jena AG)
- centrifuge (e.g. Eppendorf 5424R)
- thermal mixer (e.g. BioShake iQ, 848-1808-0506, Analytik Jena AG)
- gel electrophoresis system (e.g. Compact M electrophoresis chamber for agarose gels, 846-025-200, Analytik Jena AG)
- 1 x PBS buffer (137 mM NaCl; 2,7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4)
- ddH_2O (S15-012, GE Healthcare)
- 1 x TBE buffer, pH 8.0
- LE agarose (Biozym, 840004)
- Roti®-Load DNA with glycerol (Roth, X904.1)
- spectrophotometer (ScanDrop 250, Analytik Jena AG)
- whole blood (stabilized with EDTA, stored for 2 months at -80° C)

Methods

Lysis of erythrocytes

3 mL of whole blood from each donor were used. Lysis of erythrocytes and pelleting of nucleated blood cells was performed in accordance with the information in the manual (Kit: smart Blood DNA Midi prep(a96)). After pelleting, the nucleated blood cells were completely resuspended in 120 µL 1 x PBS.

Enzymatic lysis (proteolysis) and extraction

Proteolytic lysis of the cells was performed externally. To this end, 200 µL of a lysis buffer (Lysis Solution CBV) and 30 µL Proteinase K were added to the resuspended cells followed by incubation for 30 min on the BioShake iQ for 800 rpm at 55° C. Then the lysate was transferred into the reagent plate and placed onto device position 10 of the CyBio FeliX. Automatic processing and elution of the DNA samples was performed according to the information of the smart Blood DNA Midi prep(a96) protocol. The reagent plates of the kit required for automated DNA extraction are pre-filled with all reagents.

Verification of the DNA extraction

The extracted DNA was verified by using agarose gel electrophoresis. This involved applying 10 µL of each eluate and 7 µL of the DNA ladder to a 0.8 % TBE gel (contains ethidium bromide, 5 µL of ethidium bromide for 100 mL of agarose gel). Electrophoretic separation took place in a horizontal gel electrophoresis system (Analytik Jena AG); by constantly applying of 127 mA for optimal running conditions. The resulting DNA bands were visualized using UV light. The yield and the quality of the DNA were determined by using a spectrophotometer (ScanDrop 250, Analytik Jena AG). To this end, 4 µL of each eluate were filled into an appropriate CHIPCUVETTE and measured by a path length of 1.0 mm.

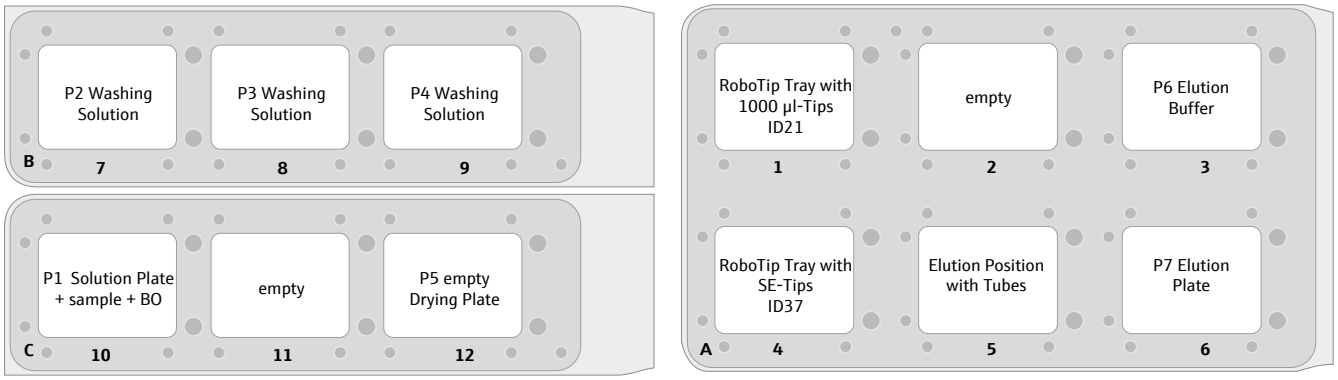

Figure 2: CyBio FeliX deck layout for automated extraction using smart Blood DNA Midi prep (a96).

Results and discussion

Automated extraction of genomic DNA (gDNA) from 10 different whole blood samples (3 mL) was performed by means of the combination of smart Blood DNA Midi prep (a96) with CyBio FeliX. Agarose gel electrophoresis (Fig. 3) and spectrophotometric measurements (Table 1) were used to determine the quality and the yield of the isolated DNA. Table 1 shows the purity (A_{260}:A_{280} and A_{260}:A_{230}) and concentration of the extracted DNA. The purity and concentration measurements were performed by using Analytik Jena's ScanDrop 250. With respect to the A_{260}:A_{280} and A_{260}:A_{230} evaluation, the extracted DNA indicates excellent quality.
The extraction method facilitates a high yield of high-molecular-weight DNA. In addition, the samples were distributed randomly on the plate in order to demonstrate the comparability and reproducibility of sample preparation within the reaction plate.

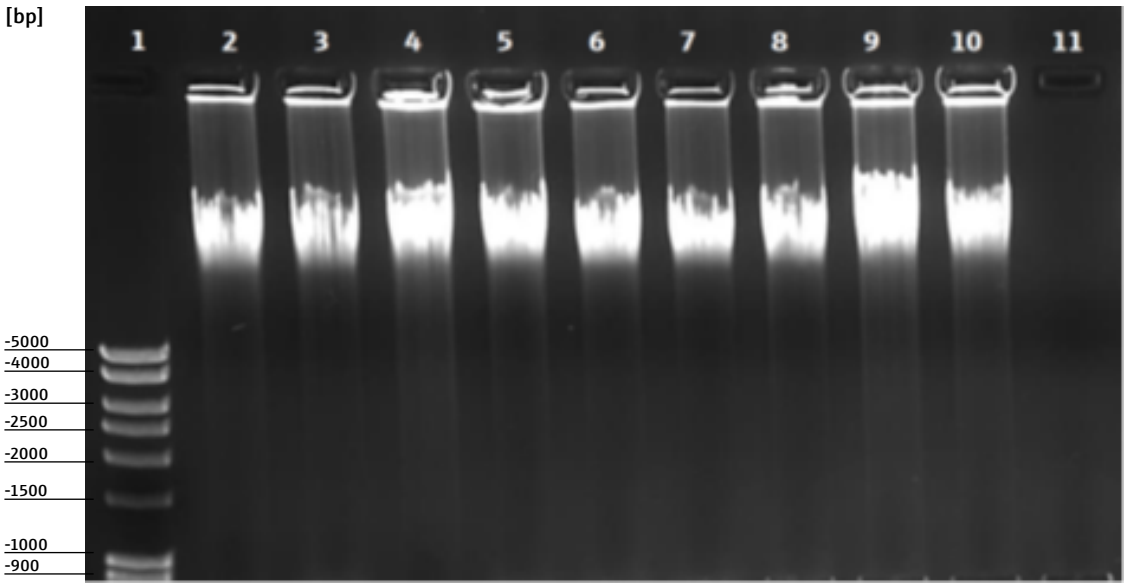

Figure 3: Results of horizontal agarose gel electrophoresis, gDNA from whole blood samples (pelleted nucleated cells), extracted by means of an automated process using smart Blood DNA Midi prep (a96) in combination with the CyBio FeliX pipetting system. Visualized is a used quantity of 10 µL of eluate on a 0.8 % agarose gel with added ethidium bromide. Column 1: DNA ladder; Columns 2 – 10: DNA extracted from nucleus containing cells from 3 mL whole blood; Column 11 : negative control (CO)

Lane	Whole blood [mL]	A_{260}:A_{280}	A_{260}:A_{230}	Conc. [ng/µL]	Yield [µg]
1	DNA ladder	–	–	–	–
2	3 mL	1.8	2.4	188	94
3	3 mL	1.8	2.3	175	87.5
4	3 mL	1.8	2.6	216	108
5	3 mL	1.8	2.3	229	114.5
6	3 mL	1.8	2.2	213	106.5
7	3 mL	1.8	2.7	194	97
8	3 mL	1.8	2.3	216	108
9	3 mL	1.8	2.8	178	89
10	CO	–	–	0.0	0.0

Table 1: Results of the gDNA extraction from whole blood samples, spectrophotometrically determined using a ScanDrop 250

Conclusion

SmartExtraction, a novel and innovative technology for isolating nucleic acids, can be elegantly and efficiently automated by using the flexible CyBio FeliX pipetting system for isolation of up to 96 samples in parallel. High-quality, reproducible pipetting results can be achieved, allowing the isolation of high yield of high-molecular-weight DNA. On the basis of a modular system, the degree of automation can be freely configured by the customer. The flexible automation solution contributes to improved reproducibility of the results and increases the efficiency of lab processes. Automatic extraction of DNA for downstream processes leads to simple, parallel preparation of samples with minimum effort and maximum consistency. In addition, the CyBio FeliX can also be used for other downstream routine liquid handling tasks.

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