DNA EXTRACTION: NO BEADS REQUIRED

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Nucleic Acid Isolation through the Ages

Today, we know that DNA is the carrier of genetic information, that it is composed of fixed ratios of adenine, thymine, cytosine, and guanine bases, and that it structurally forms a double helix. What is now common knowledge began to emerge in 1869 with a discovery by Swiss doctor Johann Friedrich Miescher[1]. Working at the University of Tübingen, Miescher identified a novel substance when extracting leukocyte nuclei for study. He called the substance "nuclein," and went on to develop the first-ever protocol for DNA extraction. First, wash the cells with warm alcohol to lyse them and remove most of the cytoplasm. Next, digest the proteins and remaining cytoplasm with pepsin, then wash with water to remove residual lipids. Finally, wash the resulting extract with alcohol, alkaline solutions, and acidic solutions to yield a precipitate containing purified DNA[1].

Almost one hundred years passed between Miescher's groundbreaking achievement and the next revolutionary step in DNA isolation. In 1967, Kathleen Kirby and Ezra Cook developed a three-step protocol for nucleic acid isolation that eventually became the phenol/chloroform phase separation-based method popularly used in laboratories around the world[2]. When tissues and cells are placed in a phenol/chloroform solution and centrifuged, three distinct phases form: the aqueous phase containing nucleic acids, the organic phase containing lipids, and an interphase between the two containing proteins. From here, the aqueous phase is removed and alcohol is added to precipitate the nucleic acids, which are then pelleted using centrifugation.

While the phenol/chloroform three-phase separation method provides good yields, it requires considerable user expertise. Not only are phenol and chloroform hazardous chemicals, but the lengthy protocols involve many liquid transfer steps, increasing the possibility of contamination. These drawbacks led researchers to develop solid-phase DNA extraction[3]. Here, DNA is bound to a solid mineral surface such as a silica matrix or glass. Chaotropic salts typically facilitate this binding by disrupting hydrogen bonds, rendering DNA hydrophobic[4]. Ethanol then washes away unwanted non-bound materials, and a low-salt buffer detaches the DNA from the solid phase[5].Similarly, DNA will bind reversibly to magnetic beads under the correct salt conditions.

A half-century on from the work of Kirby and Cook, solid-phase DNA extraction method development is now moving towards more universal and "hands-off" solutions. With custom engineered modified nucleic acid-binding surfaces replacing traditionally used solid-phase materials and apparatuses, it is now possible to extract DNA in mere minutes without using harmful chemicals, ion exchangers, particle suspensions, spin-filter columns, or centrifugation—all within a single storage vessel. As the 21st century marches on, DNA extraction technology and methodology continues to promote the technique's accessibility, accuracy, and ease of use.

References:

1. R. Dahm, "Discovering DNA: Friedrich Miescher and the early years of nucleic acid research," *Hum Genet,* 122(6):565-81, 2008.
2. K.S. Kirby, E.A. Cook, "Isolation of deoxyribonucleic acid from mammalian tissues," *Biochem J,* 104(1): 254-57, 1967.
3. S.C. Tan, B.C. Yiap, "DNA, RNA, and protein extraction: the past and the present," *J Biomed Biotechnol,* 2009:574398, 2009.
4. B. Vogelstein, D. Gillespie, "Preparative and analytical purification of DNA from agarose," *Proc Natl Acad Sci U S A,* 76(2):615-619, 1979.
5. R. Boom et al., "Rapid and simple method for purification of nucleic acids," *J Clin Microbiol,* 28(3):495-503, 1990.

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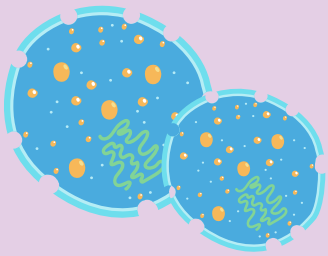
DNA EXTRACTION
in a Single Tube

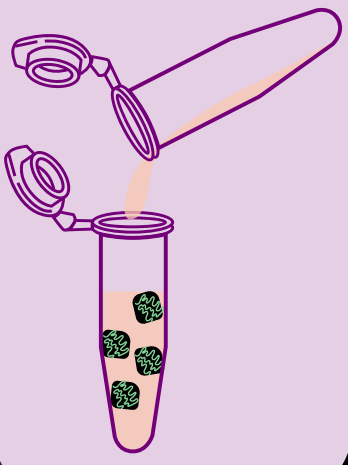
Lysis

- Chemicals, enzymes, and osmotic pressure lyse cells with minimal damage to internal components.
- DNase and RNase inhibitors prevent post-lysis nucleic acid degradation.



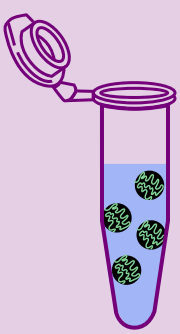
Binding

- Custom engineered SmartExtraction "modified surface" materials are incubated with lysate.
- DNA binds to SmartExtraction surfaces upon contact. Samples are agitated for three minutes to promote maximum binding.



Wash

- Bound DNA is subjected to a one minute wash using a specialized solution.
- DNA is then allowed to dry in the tube.



Elute

- Treatment of SmartExtraction complexes reverses binding.
- DNA dissolves into the elution buffer, creating an eluate that can be isolated.

Fully Prepped Direct Extraction of DNA

To extract nucleic acids, one must separate the heterogeneous intracellular milieu into distinct fractions so that the DNA- or RNA-containing fraction can be isolated and the nucleic acid subsequently purified. Throughout history, this process has been relatively lengthy and laborious, requiring considerable amounts of centrifugation, reconstitution, precipitation, and liquid transfer. Common methods in use today require hazardous chemicals or specialized reagents such as silica matrices and magnetic beads.

While these cumbersome extraction protocols arose from the work of Johann Friedrich Miescher 150 years ago, protocols widely used today are dramatically different from his original approach for isolating "nuclein." This spirit of change is alive and well, as scientists look for simpler, faster, and more precise ways to extract nucleic acids. To that end, it is now possible to extract nucleic acids using a single tube-based system, without the need for centrifuges, beads, or harsh chemical reagents.

Single-tube nucleic acid SmartExtraction relies on a custom engineered "modified surface" that binds nucleic acids and can be manipulated into desired shapes and modalities. For this approach, the nucleic acid-containing sample lysate simply incubates with SmartExtraction particles possessing modified nucleic acid-binding surfaces—a much more straightforward process than fractionating either a phenol/chloroform-treated lysate or setting up and centrifuging a spin column. In single-tube extraction, the lysate-particle mixture agitates for a mere three minutes to facilitate nucleic acid binding with the modified surfaces.

Following extraction, washing the bound nucleic acids using a single-tube extraction protocol simply entails immersing the smart modified material particles in wash buffer and agitating for one minute. After washing, simply remove the buffer to dry the particle-bound nucleic acids. Finally, elute the nucleic acids by immersing the particles in a buffer that reverses the nucleic acid-smart modified material bond, allowing the nucleic acids to solubilize. Steps for pelleting, precipitating, or centrifuging the samples are not necessary during this process.

Single-tube nucleic acid SmartExtraction is not only fast and simple, it is remarkably effective. By limiting the number of times the user has to manipulate the sample during the extraction process, single-tube extraction minimizes contamination risk, decreases the amount of stress that the nucleic acids are subjected to, and limits potential yield decreases caused by incomplete sample transfers. Single-tube SmartExtraction can generate three-fold greater yields with equal or superior quality to spin filter extraction when isolating genomic DNA from rodent tails. Similarly, single-tube extraction has achieved double the yield of magnetic beads when extracting DNA from whole blood samples. Moreover, the gentle purification procedure minimizes DNA fragmentation, benefitting multiple downstream applications such as next generation sequencing.

Old nucleic acid extraction methods might be tried and true, but they are no longer the only option. Single-tube SmartExtraction, made possible by a custom engineered nucleic acid-binding "smart modified surface," offers a simple, fast, and effective solution—one that is amenable to automation for even higher throughputs.

Challenge

Simple, effective, automated extraction of high quality DNA.

Solution

SmartExtraction Technology applied on the automated nucleic acid extraction system InnuPure® C16/C16 *touch* for high quality and quantity DNA extraction from multiple sample types with minimum hands-on time.

Nucleic Acid Extraction from Multiple Sample Types

Introduction

Modern molecular biology technologies are based on the manipulation and analysis of nucleic acids. Nucleic acids are part of each individual cell or viral particle. A prerequisite of molecular biology applications is the availability of nucleic acids in a certain content, a certain purity and integrity. Therefore, the sample material must be disintegrated by physical or chemical processes followed by the separation of the nucleic acids from other components of the sample material. Besides manual extraction of nucleic acids, automated extraction is of growing importance. This development is driven by growing sample numbers as well as the requirement for stringent documentation in highly regulated environments like hospitals or food safety laboratories. A drawback of automated nucleic acid extraction is that extracted DNA can be cross-contaminated with magnetic particles.

The presented method combines automated extraction with InnuPure® C16/C16 *touch* and SmartExtraction Technology. InnuPure® C16/C16 touch enables parallel extraction of up to 16 samples. Commonly used automated nucleic acid extraction technologies rely on binding of the nucleic acid to silica-coated magnetic particles. Automated SmartExtraction-Technology omits magnetic particles by binding the DNA to the Smart Modified Surface of macro beads, which are located within a disposable and filtered pipette tip called Smart Modified Tip. Using a Smart Modified Tip makes the classical steps of nucleic acid purification — binding, washing, elution — as easy as simple up and down pipetting.

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This application note describes the extraction of DNA from bacteria, yeast and blood cells using the smart DNA prep (a) and smart Blood DNA Midi prep (a) kits on InnuPure® C16.

SmartExtraction Technology based kits allow the use of large sample volumes (e.g. 10^9 bacterial cells, 3 ml blood) resulting in high DNA yields. Besides impressive yields, quality of extracted DNA is excellent.

Materials and Methods

DNA from multiple bacterial species (as indicated in the sections below), Saccharomyces cerevisiae and EDTA-stabilized human blood was extracted. Bacterial and S. cerevisiae cells were cultured with standard methods. Blood samples were delivered from a local blood bank and prepared according to commonly used procedures. The smart DNA prep (a) kit was used for the extraction of DNA from bacteria and S. cerevisiae while smart Blood DNA Midi prep (a) kit was used for human blood samples. Extraction from bacteria was improved by the use of innuPREP Bacteria Lysis Booster, which supports lysis of hard to lyse bacteria cells by application of an enzyme mix. All samples were prepared using the standard procedures described in the kit manuals and protocols predefined for InnuPure® C16.

Samples and Reagents

- Cultured Escherichia coli
- Cultured Klebsiella oxytoca
- Cultured Staphylococcus aureus
- Cultured Bacillus cereus
- Cultured Saccharomyces cerevisiae
- Human blood, EDTA stabilized
- smart DNA prep (a)
- innuPREP Bacteria Lysis Booster
- smart Blood DNA Midi prep (a)

Instrumentation

- InnuPure® C16
- Standard thermal shaker
- Standard spectrophotometer
- Standard equipment for agarose gel electrophoresis and gel documentation

Results and Discussion

DNA was extracted from four different bacteria species comprising gram- (*E. coli, K. oxytoca*) and gram+ bacteria (*S. aureus, B. cereus*). DNA was extracted using SmartExtraction Technology, innuPREP Bacteria Lysis Booster and InnuPure® C16 as well as manual extraction from a third-party supplier using anion exchange chromatography.

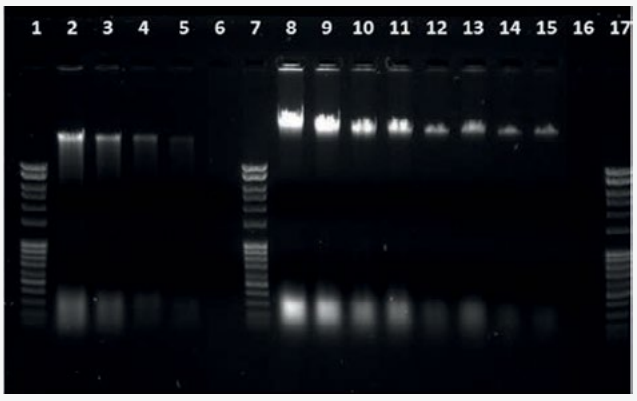


Fig. 1: Gel electrophoresis of DNA extracted from E. coli. Lanes 1, 7 and 17 DNA ladder; lanes 2–6 competitor product based on anion-exchange technology; lanes 8–16 smart Bacteria DNA prep (a); lanes 6 and 16 negative control.

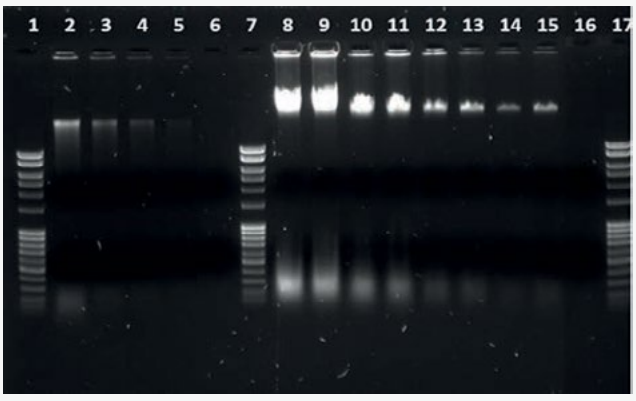


Fig. 2: Gel electrophoresis of DNA extracted from K. oxytoca. Lanes 1, 7 and 17 DNA ladder; lanes 2–6 competitor product based on anion-exchange technology; lane 8–16 smart Bacteria DNA prep (a); lanes 6 and 16 negative control.

Table 1: Purity and yield of DNA extracted from *E. coli*.

lane	cell count [CFU]	A_{260}/A_{280}	A_{260}/A_{230}	c [ng/µl]	m [µg]
1	DNA ladder	-	-	-	-
2	2.2×10^9	2.0	2.0	127.0	38.1
3	1.1×10^9	2.0	2.0	64.0	19.2
4	0.55×10^9	2.0	1.8	25.0	7.5
5	0.275×10^9	2.1	1.7	12.5	3.8
6	neg	0.0	0.3	0.5	0,2
7	DNA ladder	-	-	-	-
8	2.2×10^9	1.9	1.8	288.0	86.4
9	2.2×10^9	1.9	1.7	228.0	68.4
10	1.1×10^9	1.9	1.8	127.0	38.1
11	1.1×10^9	1.9	1.8	125.0	37.5
12	0.55×10^9	1.9	1.4	37.0	11.1
13	0.55×10^9	1.9	1.7	63.0	18.9
14	0.275×10^9	1.9	1.7	19.0	5.7
15	0.275×10^9	1.8	1.9	17.0	5.1
16	neg	0.0	0.0	0.0	0.0
17	DNA ladder	-	-	-	-

Table 2: Purity and yield of DNA extracted from *K. oxytoca*.

lane	cell count [CFU]	A_{260}/A_{280}	A_{260}/A_{230}	c [ng/µl]	m [µg]
1	DNA ladder	-	-	-	-
2	1.2×10^9	1.9	1.2	46.5	14.0
3	0.6×10^9	1.9	1.0	14.5	4.4
4	0.3×10^9	1.8	0.8	7.0	2.1
5	0.15×10^9	1.5	0.6	4.5	1.4
6	neg	0.0	0.3	0.5	0.2
7	DNA ladder	-	-	-	-
8	1.2×10^9	1.9	1.8	230.0	69.0
9	1.2×10^9	1.9	1.8	216.0	64.0
10	0.6×10^9	1.9	1.7	100.0	30.0
11	0.6×10^9	1.9	1.7	79.5	23.9
12	0.3×10^9	1.8	1.7	34.0	10.2
13	0.3×10^9	1.8	1.8	25.5	7.7
14	0.15×10^9	1.6	1.6	9.5	2.9
15	0.15×10^9	1.6	1.6	16.0	4.8
16	neg	0.0	0.0	0.0	0.0
17	DNA ladder	-	-	-	-

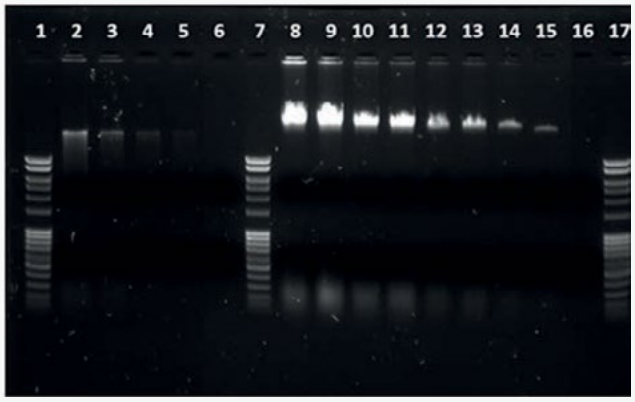


Fig. 3: Gel electrophoresis of DNA extracted from *S. aureus*. Lanes 1, 7 and 17 DNA ladder; lanes 2–6: competitor product based on isolation using anion-exchange technology; lanes 8–16: smart Bacteria DNA prep (a); lane 6 and 16 negative control.

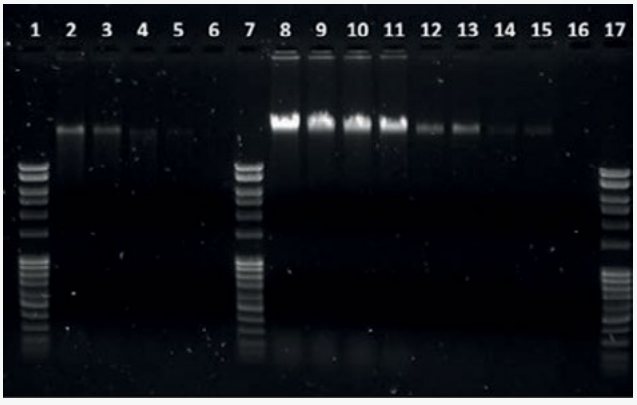


Fig. 4: Gel electrophoresis of extracted DNA from B. cereus. Lanes 1, 7 and 17 DNA ladder; lanes 2–6 competitor product based on isolation using anion-exchange technology; lanes 8–16 smart Bacteria DNA prep (a); lane 6 and 16: negative control.

Table 3: Purity and yield of DNA extracted from *S. aureus*.

lane	cell count [CFU]	A_{260}/A_{280}	A_{260}/A_{280}	c [ng/µl]	m [µg]
1	DNA ladder	-	-	-	-
2	2.25×10^9	1.7	1.0	31.0	9.3
3	1.13×10^9	1.6	0.8	14.5	4.4
4	5.65×10^8	1.6	0.7	5.5	1.7
5	2.83×10^8	1.2	0.6	3.5	1.1
6	neg	0.5	0.3	0.5	0.2
7	DNA ladder	-	-	-	-
8	2.25×10^9	1.8	1.6	84.0	25.2
9	2.25×10^9	1.9	2.1	107.0	32.1
10	1.13×10^9	1.9	2.2	50.5	15.2
11	1.13×10^9	1.9	2.4	53.0	15.9
12	5.65×10^8	1.8	2.3	23.0	6.9
13	5.65×10^8	1.8	2.1	22.5	6.8
14	2.83×10^8	1.7	1.9	9.5	2.9
15	2.83×10^8	1.3	1.0	4.0	1.2
16	neg	0.0	0.0	0.0	0.0
17	DNA ladder	-	-	-	-

Table 4: Purity and yield of DNA extracted from *B. cereus*.

lane	cell count [CFU]	A_{260}/A_{280}	A_{260}/A_{280}	c [ng/µl]	m [µg]
1	DNA ladder	-	-	-	-
2	5.20×10^7	1.8	1.2	14.0	4.2
3	3.47×10^7	1.6	1.0	8.0	2.4
4	1.74×10^7	1.6	1.0	2.5	0.8
5	0.87×10^7	1.0	0.6	1.5	0.5
6	neg	0.0	0.0	0.0	0.0
7	DNA ladder	-	-	-	-
8	5.20×10^7	1.9	1.9	53.0	15.9
9	5.20×10^7	1.8	2.1	36.0	10.8
10	3.47×10^7	1.8	2.1	36.0	10.8
11	3.47×10^7	1.9	1.9	32.0	9.6
12	1.74×10^7	1.9	1.5	7.5	2.3
13	1.74×10^7	1.9	1.7	7.5	2.3
14	0.87×10^7	1.7	1.0	2.5	0.8
15	0.87×10^7	3.0	0.6	1.5	0.5
16	neg	0.0	0.0	0.0	0.0
17	DNA ladder	-	-	-	-

DNA was extracted from *S. cerevisiae* using SmartExtraction Technology and InnuPure® C16 as well as manual extraction from a third-party supplier using anion exchange chromatography.

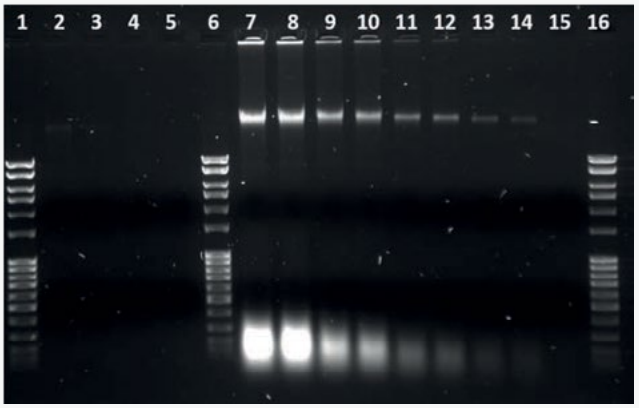


Fig. 5: Gel electrophoresis of extracted DNA from *S. cerevisiae*. Lanes 1, 6 and 16 DNA ladder; lanes 2–5 competitor product based on anion-exchange technology; lanes 7–15: smart Yeast DNA prep (a); lane 15 negative control.

DNA from increasing sample volumes (1–3 ml) was extracted from whole blood (PBMC's) using smart Blood DNA Midi prep (a) and InnuPure® C16.

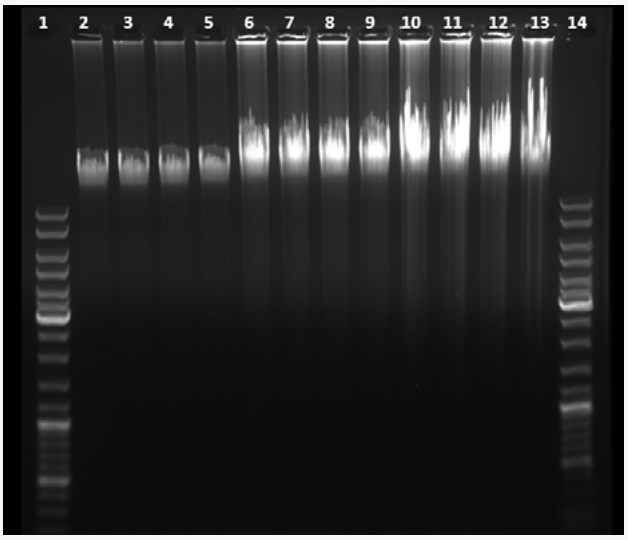


Fig. 6: Gel electrophoresis of extracted DNA from Blood. Lanes 1 and 14 DNA ladder; lanes 2–5 DNA extracted from 1 ml whole blood, lanes 6–9 DNA extracted from 2 ml whole blood; lanes 10–13 DNA extracted from 3 ml whole blood.

Table 5: Purity and yield of DNA extracted from *S. cerevisiae*.

lane	cell count [CFU]	A_{260}/A_{280}	A_{260}/A_{280}	c [ng/µl]	m [µg]
1	DNA ladder	-	-	-	-
2	1.76×10^8	1.6	0.7	3.8	0.8
3	8.8×10^7	1.6	0.7	3.7	0.7
4	4.4×10^7	1.8	0.7	3.4	0.7
5	2.2×10^7	2.2	0.7	4.1	0.8
6	DNA ladder	-	-	-	-
7	1.76×10^8	1.9	1.6	610.8	122.2
8	1.76×10^8	1.9	1.7	613.3	122.7
9	8.8×10^7	1.9	1.7	282.0	56.4
10	8.8×10^7	1.9	1.8	224.3	44.9
11	4.4×10^7	1.9	1.8	103.2	20.6
12	4.4×10^7	2.0	1.9	77.2	15.4
13	2.2×10^7	2.0	2.0	33.7	6.7
14	2.2×10^7	2.0	1.8	23.0	4.6
15	neg	2.9	1.0	2.8	0.6
16	DNA ladder	-	-	-	-

Table 6: Purity and yield of DNA extracted from blood.

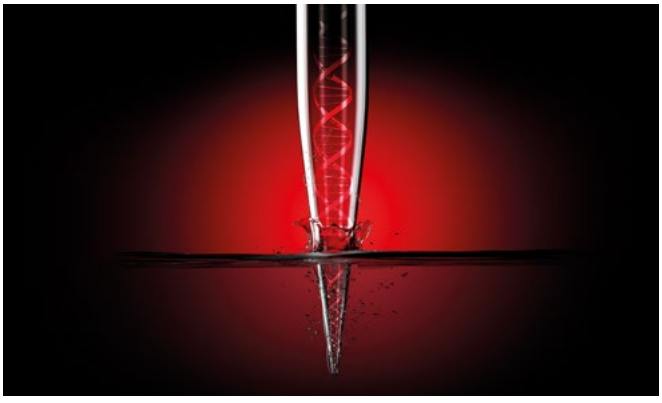
lane	cell count [CFU]	A_{260}/A_{280}	A_{260}/A_{280}	c [ng/µl]	m [µg]
1	DNA ladder	-	-	-	-
2	[1]	1.9	2.2	79	23.7
3	1	1.9	2.2	71	21.3
4	1	1.9	2.2	68	20.4
5	1	1.9	2.2	63	18.9
6	2	1.9	2.3	150	45.0
7	2	1.9	2.3	156	46.8
8	2	1.9	2.2	143	42.9
9	2	1.9	2.3	147	44.1
10	3	1.8	2.3	216	68.8
11	3	1.9	2.3	234	70.2
12	3	1.9	2.3	228	68.4
13	3	1.9	2.3	240	72.0
14	DNA ladder	-	-	-	-

Conclusion

SmartExtraction Technology together with the patented Dual-Chemistry-Technology® applied on the automated nucleic acid extraction system InnuPure® C16 enable the extraction of high amounts of nucleic acids from both, gram- as well as gram+ bacteria and yeast (Tables 1-5). Direct comparison with the long-established and well-accepted technology of anion exchange chromatography shows that SmartExtraction is comparable with respect to purity according to A_{260}/A_{280}. However, except from *E.coli* (Table 1) purity according to A_{260}/A_{230} is much better with SmartExtraction. Using same amounts of bacteria or yeast, SmartExtraction results in higher yields as compared to anion exchange chromatography with all extracted species.

Using blood, it is possible to scale to volumes which are high for automated extraction (Figure 6). This means that doubling the sample volume (e.g. 1 ml to 2 ml) results in doubling of the yield (e.g. 21,1 mg in average to 44,7 mg in average, see Table 6).

Taken together, these results show that SmartExtraction allows purification of DNA with high yields and quality. In direct comparison with anion exchange chromatography SmartExtraction is superior with respect to yield and quality of the extracted DNA. Due to its high binding capacity, sample amounts are scalable over a broad range.



Automating DNA Extraction

In the past, the prospect of automating nucleic acid extraction was a relative afterthought. Not only were traditional DNA and RNA extraction protocols too complex for easy automation, but the main advantages afforded by automation—higher throughputs, higher quality, and superior yields—were not seen as necessary, especially for smaller research-focused laboratories. However, technological advances have fueled the ever-increasing depth and breadth of our understanding of genetics and genomics. These advances have led researchers to develop new applications such as next generation sequencing, genetic engineering, and high-throughput screening. Today, automating DNA extraction offers great advantages in terms of allowing scientists to meet the sample quality, yield, and throughput requirements of these new applications.

Technology has not only created a need for automated DNA extraction, it has also created the means for it by making single-tube nucleic acid extraction possible. Automating DNA extraction is much simpler without the need for centrifugation or fractionation. A single-tube SmartExtraction protocol where the DNA-binding particles containing smart modified surfaces are placed inside the pipette tip can be carried out entirely through the aspiration and dispensation of various liquids. These protocols are something that can be performed on a multitude of the automated liquid handling stations that have become available over the last decade.

Automating DNA extraction saves time and effort for the researcher, freeing them from mundane tasks like determining and confirming mouse genotypes. It also allows for throughput rates that cannot be achieved manually. This makes automated DNA extraction ideal for environments where data is needed as quickly as possible, such as a hospital or diagnostic laboratory performing genetic screening on patient samples or a drug manufacturing laboratory investigating the genetic-level effects of pharmaceutical compound candidates. Finally, automation helps increase data accuracy by eliminating the prospect of user error and reducing contamination risk—accomplishing the latter by removing contact with human skin and limiting the workspace to a confined, controllable area. All of these things serve to promote experimental reproducibility.

Automated single-tube DNA SmartExtraction protocols are also flexible and adaptable to a researcher's situation and requirements. It can be run on 16 or 96 well formats and can be adapted for problematic situations, such as viscous samples with high DNA concentrations or high molecular weight DNA. Here, since pipetting is problematic, samples are processed in deep wells containing modified surface particles. The deep well plate can still be handled by automated pipetting platforms, with shaking replacing repeated aspiration/dispensation as the means of sample agitation. This process is capable of extracting high-quality DNA from samples containing over 10,000,000 cells, and is very useful for microbiologists and other individuals studying the microbiome who have to deal with bacterial samples containing billions of cells.

Traditionally, DNA extraction involved either a considerable number of chemicals and reagents, or specialized solid-phase filters and repeated centrifugation. Not only were these protocols time- and labor-consuming, but they were also prone to contamination of the nucleic acid fraction, DNA damage, and sub-optimal yields. Single-tube SmartExtraction using custom engineered DNA-binding smart modified surfaces offers a solution to the issues presented by traditional DNA extraction workflows. Featuring streamlined workflows, higher yields of superior quality, and automation capability, SmartExtraction represents a leap forward for DNA extraction during a time when the need has never been greater!

Task

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.

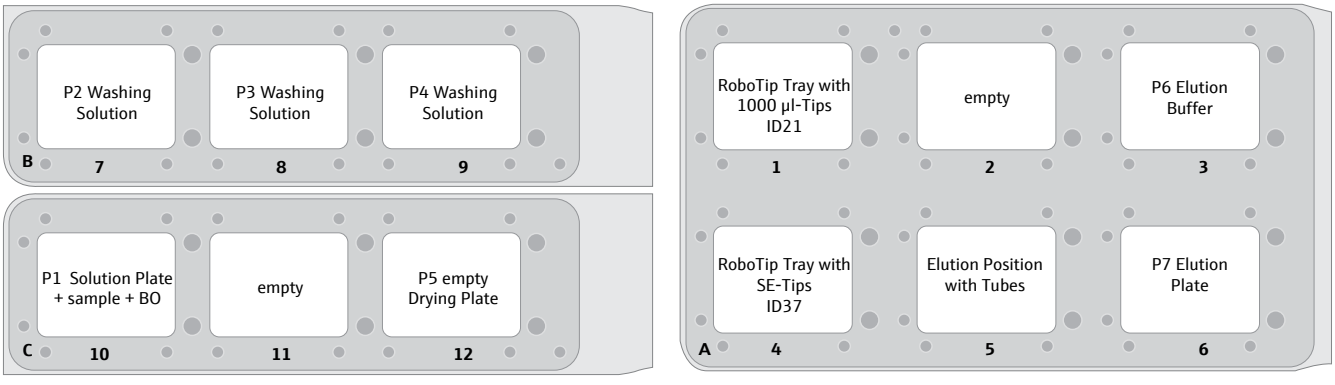


Figure 2: CyBio® FeliX deck layout for automated extraction using smart Blood DNA Midi prep (a96).

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 AG'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.

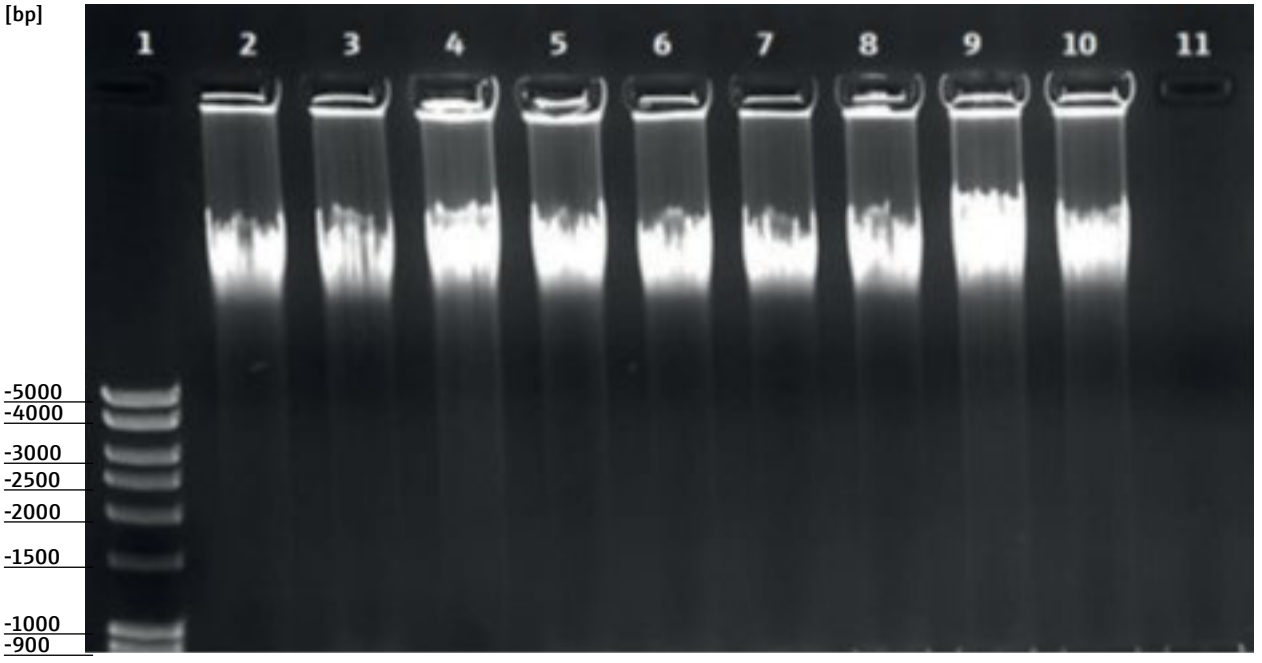


Figure 3: Results of horizontal agarose gel electrophoresis, gDNA from whole blood samples (pelleted nucleated cells), extracted by means of an automated process using smart Blood DNA Midi prep (a96) in combination with the CyBio® FeliX pipetting system. Visualized is a used quantity of 10 µl of eluate 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

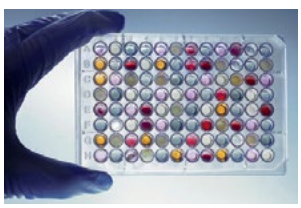
Table 1: Results of the gDNA extraction from whole blood samples, spectrophotometrically determined using a ScanDrop® 250

Summary

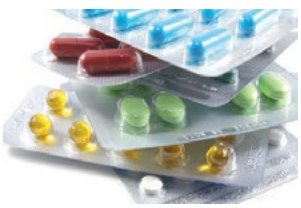
SmartExtraction, a novel and innovative technology for isolating nucleic acids, can be elegantly and efficiently automated by using the flexible CyBio® FeliX pipetting system for isolation of up to 96 samples in parallel. High-quality, reproducible pipetting results can be achieved, allowing the isolation 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.

Versatility for Your Applications

The CyBio® FeliX can be equipped according to various application needs. With endless possibility for future upgrades and additions.



Analysis
Sample preparation
Extraction
ELISA
Kits



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Clean-up
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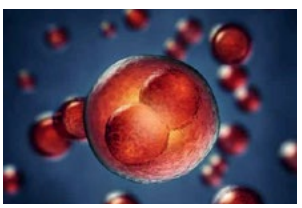

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