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.

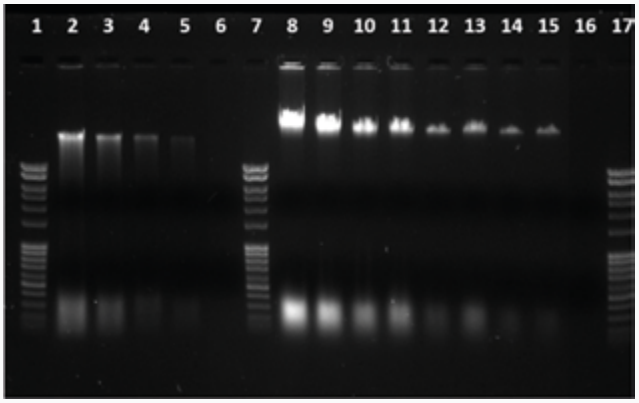

Fig. 1: Gel electrophoresis of DNA extracted from E. coli. Lanes 1, 7 and 17 DNA ladder; lanes 2–6 competitor product based on anion-exchange technology; lanes 8–16 smart Bacteria DNA prep (a); lanes 6 and 16 negative control.

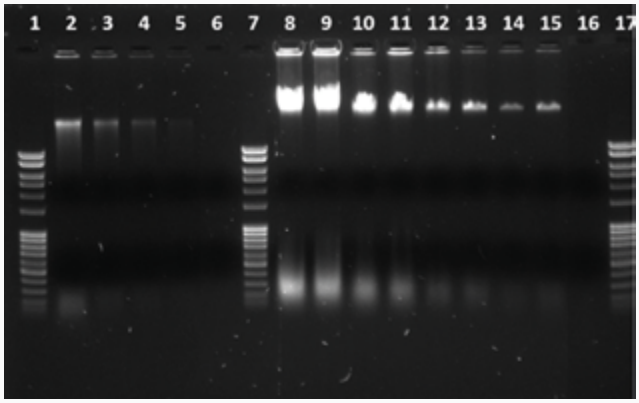

Fig. 2: Gel electrophoresis of DNA extracted from K. oxytoca. Lanes 1, 7 and 17 DNA ladder; lanes 2–6 competitor product based on anion-exchange technology; lane 8–16 smart Bacteria DNA prep (a); lanes 6 and 16 negative control.

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	-	-	-	-

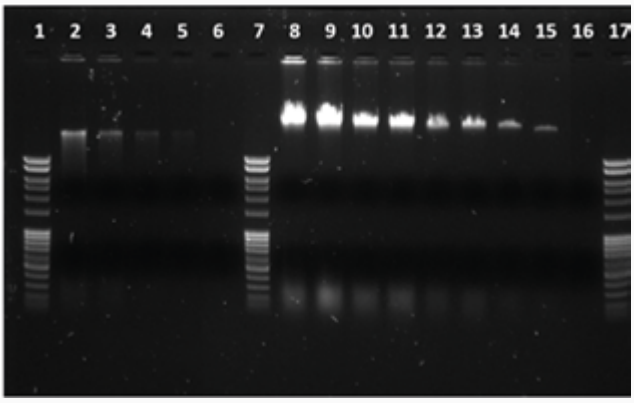

Fig. 3: Gel electrophoresis of DNA extracted from *S. aureus*. Lanes 1, 7 and 17 DNA ladder; lanes 2–6: competitor product based on isolation using anion-exchange technology; lanes 8–16: smart Bacteria DNA prep (a); lane 6 and 16 negative control.

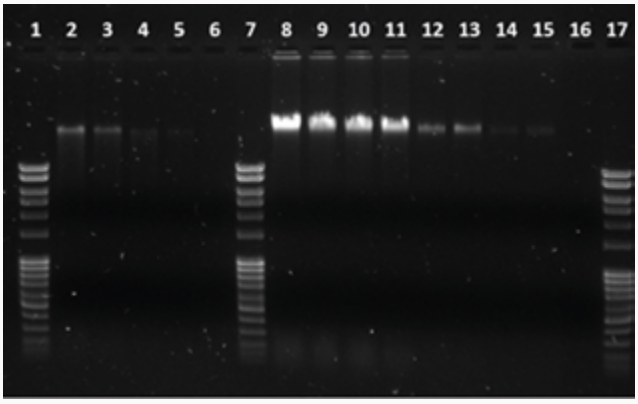

Fig. 4: Gel electrophoresis of extracted DNA from B. cereus. Lanes 1, 7 and 17 DNA ladder; lanes 2–6 competitor product based on isolation using anion-exchange technology; lanes 8–16 smart Bacteria DNA prep (a); lane 6 and 16: negative control.

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.

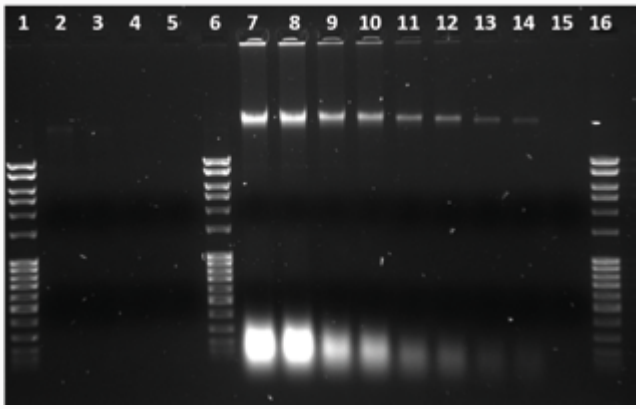

Fig. 5: Gel electrophoresis of extracted DNA from *S. cerevisiae*. Lanes 1, 6 and 16 DNA ladder; lanes 2–5 competitor product based on anion-exchange technology; lanes 7–15: smart Yeast DNA prep (a); lane 15 negative control.

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.

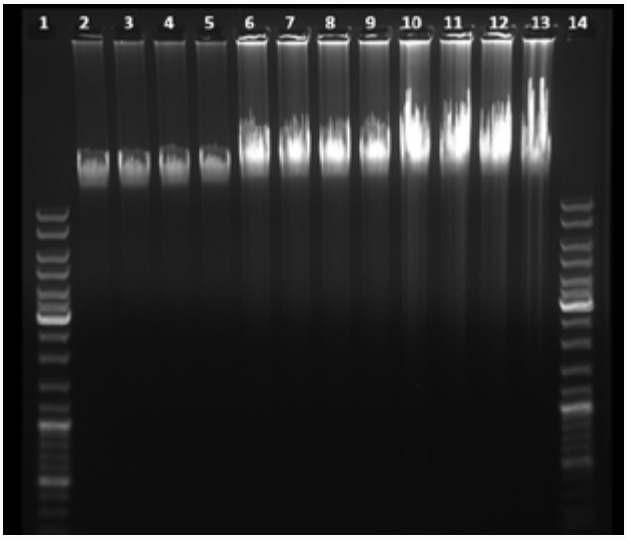

Fig. 6: Gel electrophoresis of extracted DNA from Blood. Lanes 1 and 14 DNA ladder; lanes 2–5 DNA extracted from 1 mL whole blood, lanes 6–9 DNA extracted from 2 mL whole blood; lanes 10–13 DNA extracted from 3 mL whole blood.

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.

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Version: 1.2 I Author: ChGr
en · 12/2020