Protocol	#5.1
Title	**BOMB plasmid DNA extraction**
Keywords	HT DNA miniprep, Silica beads, plasmid extraction
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Summary

Plasmid extraction from cultured *E. coli* cells is probably one of the most common laboratory practices. In the late 1960s the first protocols for isolation of plasmid DNA were published [1–3], of which the alkaline lysis of bacterial cells in a slightly modified form became today's primarily used method [4–6]. Based on silica-coated magnetic beads (BOMB protocol #2.1) we have developed a high-throughput miniprep protocol that provides highly pure plasmid DNA and that can be used to purify 96 samples in parallel.

Chemicals

Name	Provider	PN	MW [g/mol]	Safety codes	
Acetic acid (CH_3COOH)	Roth Chemicals	6755.2	60.05	 Danger	H: 314-226-290 P: 210-280-303+361+353-305+351+338-310
Dipotassium phosphate (K_2HPO_4)	Roth Chemicals	P030.2	141.96	n.a.	n.a.
Ethanol (C_2H_6O, 99.9%)	Honeywell / Riedel-de Haën	34963	46.07	 Danger	H: 225-319 P: 210-280-305+351+338-308+313
Glycerol ($C_3H_8O_3$, 86%)	Roth Chemicals	7533.2	92.09	n.a.	n.a.
Guanidinium chloride / Gu-HCl ($CH_5N_3 \cdot HCl$)	Roth Chemicals	0037.1	95.53	 Warning	H302-315-319 P280-302+352-305+351+338
Ethylenediaminetetraacetic acid dihydrate / EDTA ($C_{10}H_{16}N_2O_8 \cdot 2\ H_2O$)	Roth Chemicals	8043.1	372.24	 Warning	H: 332-373 P: 260-314
Hydrochlorid acid fuming ($HCl_{(aq)}$, 37%)	Roth Chemicals	4625.2	36.46	 Danger	H: 290-314-335 P: 280-303+361+353-304+340-305+351+338-310
Monopotassium phosphate (KH_2PO_4)	Roth Chemicals	3904.1	136.09	n.a.	n.a.
Potassium acetate (CH_3CO_2K)	Riedel-de Haën	32309	98.15	n.a.	n.a.

Name	Provider	PN	MW [g/mol]	Safety codes	
RNase A	Serva	34390.02	n.a.	 Danger	H: 334 P: 261-284-304+340-342+311
Sodium dodecyl sulfate / SDS ($C_{12}H_{25}NaO_4S$)	Roth Chemicals	CN30.3	288.38	 Danger	H 302-315-318-412 P: 280-301+312-302+352-305+351+338-332+313
Sodium hydroxide (NaOH)	Roth Chemicals	6771.2	40.00	 Danger	H: 290-314 P: 280-301+330+331-305+351+338-310
Tris(hydroxymethyl)-aminomethane / Tris ($C_4H_{11}NO_3$)	Roth Chemicals	AE15.3	121.14	 Warning	H: 315-319-335 P: 280-302+352-305+351+338-312
Yeast extract	BD Biosciences	212750	n.a.	n.a.	n.a.

Please consult appropriate MSDS information before working with these chemicals! Use lab coat, gloves and eye protection at all times! The chemicals are available from other providers as well. No preference is given to the indicated vendors.

Buffers and solutions

TB media [7] (2 ml per sample, 200 ml per 96) – autoclaved, can be stored at RT for at least 6 months

1.2% tryptone
2.4% yeast extract
72 mM dipotassium phosphate (autoclave separately as 10x stock)
17 mM monopotassium phosphate (autoclave separately as 10x stock)
0.4% glycerol

P1 buffer (250 µl per sample, 25 ml per 96) – can be stored at 4 °C for up to 12 months

50 mM Tris-HCl pH 8.0
10 mM EDTA
100 µg/ml RNase A

P2 buffer (250 µl per sample, 25 ml per 96) – can be stored at RT for up to 12 months

200 mM sodium hydroxide
1% SDS

N3 buffer (500 µl per sample, 50 ml per 96) – can be stored at RT for up to 12 months, prepare fresh if change of colour is observed

4.2 M Gu-HCl
0.9 M potassium acetate
(adjust pH to 4.8 with acetic acid)

PE (1 ml per sample, 100 ml per 96) – can be stored at RT for at least 12 months

10 mM Tris-HCl pH 7.5
80% ethanol

Elution buffer (40 µl per sample, 4 ml per 96) – can be stored at RT for at least 12 months

10 mM Tris-HCl pH 8.5

Consumables and equipment

Temperature controlled incubation shaker (e.g. Infors HT Multitron Pro)

Plate centrifuge with swing-out rotor (e.g. Eppendorf, Centrifuge 5804R)

Microtiter plate orbital shaker (e.g. IKA MS 3 basic)

Heat block (e.g. Roth, Rotilabo®-block thermostat H250 – PN: Y264.1) or **incubator** (e.g. Thermo Scientific, Heratherm™ Advanced Protocol Microbiological Incubator – PN: 51028066)

Magnetic stand for 96-well plate (e.g. BOMB microplate magnetic rack)

Multichannel pipettes

30 – 300 µl (e.g. Eppendorf, **Eppendorf Research® plus,** 8-channel – PN: 3125000052)

50 – 1200 µl (e.g. VWR, Multi-channel pipette, 8-channel – PN: 613-5422)

96-well deep well plates

2.2 ml (e.g. Sarstedt, MegaBlock® 96 Well – PN: 82.1972.002)

1.2 ml (e.g. Sarstedt, MegaBlock® 96 Well – PN: 82.1971.002)

Multistep pipette with 500 µl tips (e.g. Eppendorf, Multipette® M4 – PN: 4982000012)

Reservoirs (e.g. Roth, Rotilabo®-liquid reservoirs – PN: E830.2)

Seals (e.g. Bio-Rad, Microseal® 'B' Adhesive Seals – PN: MSB1001)

BOMB Plasmid preparation

Step	Bacterial culture growth	Time	☑
1	Seed bacteria in TB with the appropriate antibiotics in a deep well plate (**2.2 ml 96-well**) and grow at 250 rpm at 37 °C for 15 to 24 h		☐
	Transfer 50-100 µl of the bacteria culture to a 96-well plate, seal and store at 4 °C (positive clones can be used to inoculate cultures after sequencing/confirmation)		
Step	**Plasmid DNA extraction**		
2	Centrifuge in a swing-out rotor (20 min, 2000 g, 4 °C) and discard the supernatant	20 min	☐
	At this point, the pellets can be frozen at -20 °C and processed later		
3	Add 250 µl **P1 buffer** and shake at 1300 rpm until the pellets are completely resuspended	5 min	☐
	At this point, the cell suspension can be frozen at -20 °C and processed late		
4	Add 250 µl **P2 buffer**, shortly mix at 500 rpm and keep at room temperature until the lysis is complete (~5 min)	5 min	☐
5	Add 500 µl **N3 buffer** and shake at 750 rpm for 5 min *Ensure a complete neutralization of the sample*	10 min	☐
6	Centrifuge in a swing-out rotor (20 min, 2000 g, RT)	20 min	☐
7	Transfer 500 µl of the supernatant to a fresh deep well plate (**1.2 ml 96-well**) *Pipette carefully to avoid transferring the cell debris/precipitate*	10 min	☐
8	Add 500 µl **ethanol** to bind the DNA to the silica beads and shake at 750 rpm for 5 min	10 min	☐
9	Add 20 to 50 µl (optimal 50 µl) stock solution of **silica-coated magnetic beads (BOMB protocol #2.1)**	5 min	☐
10	Shake at 500 rpm for 15 min	15 min	☐
11	Settle the magnetic beads on a magnetic stand and discard the supernatant *Ensure that the beads are completely pelleted*	5 min	☐
Opt	*Repeat steps **7-11** once with another 300 µl of supernatant for a maximal yield* *Avoid transferring cell debris!*	45 min	
12	Remove the plate from the magnetic stand and add 500 µl **PE** and mix well (by pipetting, or seal and vortex and/or inverting)	5 min	☐☐
13	Settle the magnetic beads on a magnetic stand and discard the supernatant	5 min	☐☐
14	**Repeat** the washing steps **12-13** once	10 min	☐
15	Remove the supernatant completely and dry the beads at 65 °C for ~20 min *The beads are dry as soon as they turn brownish. Do not elute earlier!*	30 min	☐
16	Add at least 40 µl of **elution buffer** or **ddH_2O** to elute the DNA from the beads, mix well for 15 min (shake at 1300 rpm) and spin down briefly.	35 min	☐
17	Settle the magnetic beads on a magnetic stand and transfer the supernatant to a 96-well collection plate (or separate tubes/strips)	10 min	☐
End	Measure concentration and purity	**~3.5 h** (1.5 h hands-on)	
	Store @ -20 °C or 4 °C		

Troubleshooting

Problem	Solution
Beads sticking to the wells	• Sonicate shortly in a sonic bath and/or centrifuge briefly
Pipetted unequal amounts of beads or water	• The silica-coated magnetic beads can settle quite quickly. Ensure they are fully suspended before pipetting
Bad DNA quality (260/280 < 1.6)	Step 7 is particularly critical. Transferring a small or medium amount of cell debris may reduce the sample quality significantly, although the debris disappears during the following wash steps. • Aspirate slowly • Stay at the rim of the well (debris tends to be in the centre) • Do not insert the pipette too far into the well In case any debris was picked up by the pipette, centrifuge the respective samples at higher g for another 5 min (e.g. in microcentrifuge tubes in a microcentrifuge). This will allow the debris to form a more compact pellet and thus simplify the transfer of the liquid
Low DNA concentration	Low DNA concentration can have multiple reasons **Buffers:** • Make sure the pH of N3 was properly adjusted • Ethanol tends to evaporate over time, leading to a reduced concentration in PE, which might reduce the binding of the DNA to the beads during washing **Amount of beads:** Make sure the stock solution used during the process was not diluted (e.g. as for BOMB protocol #8.1) **Remaining ethanol:** before elution you have to ensure that the beads are completely dry. Their colour will turn from shiny black to brittle brown. If this was not the case, dry the beads again and elute a second time.
RNA contamination	Add fresh RNase A to P1.

Exemplary Results

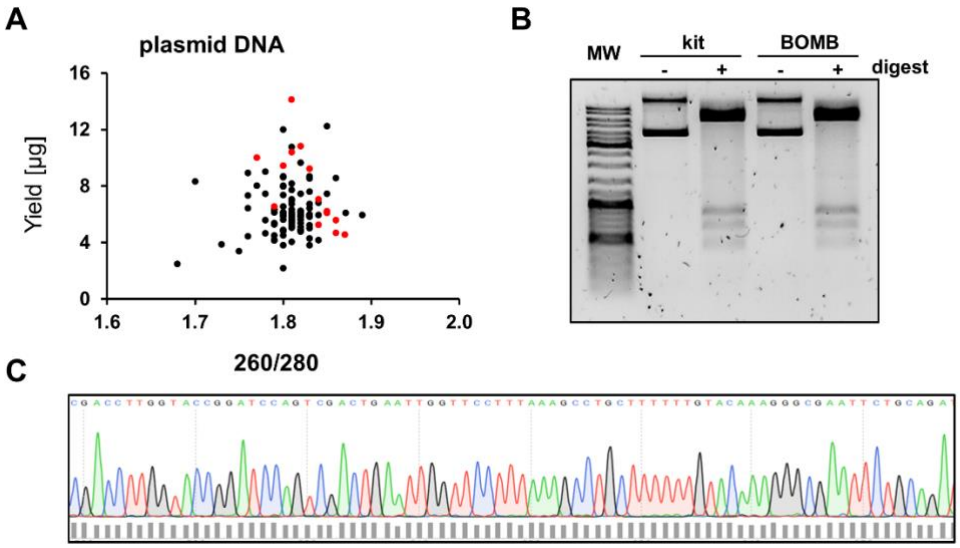

Fig 1: Quality control of BOMB plasmid extraction. *(A) Total plasmid DNA yield [µg] extracted from E. coli, plotted against the A260 nm/A280 nm ratio for each sample. DNA concentration and purity were measured with UV-Spectroscopy (NanoDrop). Black dots represent samples extracted using the BOMB plasmid extraction protocol #5.1, red dots represent samples processed using a commercial kit. (B) Comparison of commercially purified plasmid DNA and BOMB extracted DNA with and without restriction enzyme digestion. MW: Gene Ruler DNA Ladder (Thermo). (C) Exemplary sequencing trace of BOMB extracted plasmids with Sanger sequencing. A sequencing read length of at least 800-1000 nt is typically observed.*

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