Protocol	#5.2
Title	**BOMB plasmid DNA extraction using MNP-pulldown**
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	21270	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 (160 µl per sample, 16 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 (200 µl per sample, 20 ml per 96) – can be stored at RT for up to 12 months

200 mM sodium hydroxide
1% SDS

N3a buffer (100 µl per sample, 10 ml per 96) – can be stored at RT for up to 12 months, prepare fresh if change of colour is observed

2.3 M potassium acetate
(adjust pH to 4.8 with acetic acid)

N3b 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

90% ethanol

(heat to 65 °C to dissolve)

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 160 µl **P1 buffer** and 40 µl stock solution of **silica-coated magnetic beads (BOMB protocol #2.1)** and shake at 1300 rpm or vortex shortly 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 200 µl **P2 buffer**, shortly mix at 750 rpm and keep at room temperature until the lysis is complete (~5 min)	5 min	☐
5 	Add 100 µl **N3a buffer** and shake at 750 rpm for 5 min *Ensure a complete neutralization of the sample*	10 min	☐
6 	Settle the magnetic beads on a magnetic stand and transfer the supernatant to a fresh deep well plate (**1.2 ml 96-well**) *Ensure that the beads are completely pelleted*	5 min	☐
7	Add 500 µl **N3b buffer**	5 min	☐
8	Add 20 to 50 µl (optimal 50 µl) stock solution of **silica-coated magnetic beads (BOMB protocol #2.1)**	5 min	☐
9	Shake at 750 rpm for 15 min	15 min	☐
10 	Settle the magnetic beads on a magnetic stand and discard the supernatant *Ensure that the beads are completely pelleted*	5 min	☐
11	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	☐☐
12	Settle the magnetic beads on a magnetic stand and discard the supernatant	5 min	☐☐
13	**Repeat** the washing steps **11-12** once	10 min	☐
14 	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	☐
15	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	☐
16	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	**~3.0 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
Low DNA concentration	Low DNA concentration can have multiple reasons **Buffers:** • Make sure the pH of N3a was properly adjusted • Ethanol tends to evaporate over time, leading to a reduced concentration in N3a and 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

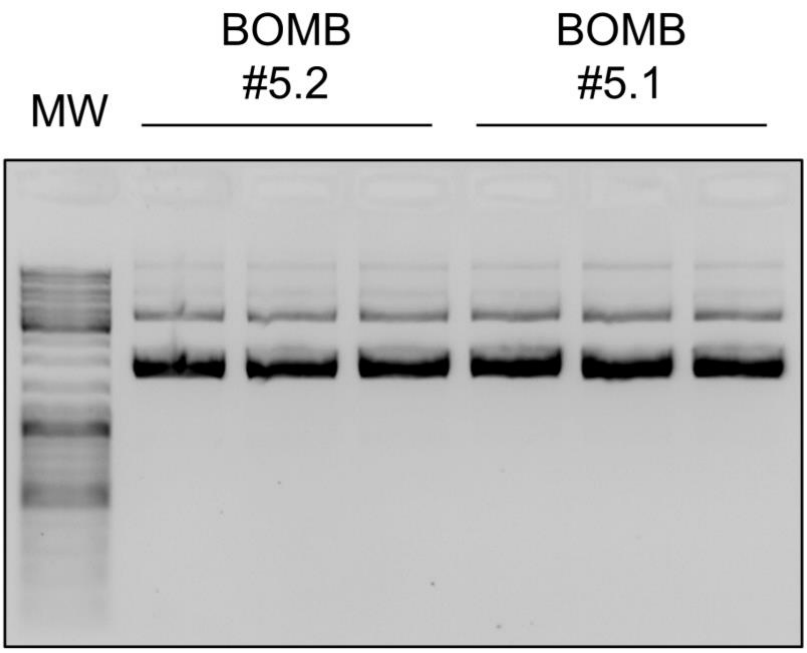

Fig 1: Quality control of BOMB plasmid extraction using MNP-pulldown. *Comparison of exemplary plasmid isolations performed with BOMB protocols #5.1 or 5.2, respectively. MW: Gene Ruler DNA Ladder (Thermo).*

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