Community Contribution

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Protocol	#5.4
Title	**BOMB plasmid MIDI prep using Sera-Mag carboxylated beads**
Keywords	midiprep, Sera-Mag, plasmid extraction
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Chemicals

Name	Provider	PN	MW [g/mol]	Safety codes	
Acetic acid (CH_3COOH)	Merck	1.09951	60.05	 Danger	H: 290-314 P: 280-301+330+331-305+351+338-308+310
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
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
Polyethylene glycol 8000	AppliChem	A2204	~8000	n.a.	n.a.
Potassium acetate (CH_3CO_2K)	Riedel-de Haën	32309	98.15	n.a.	n.a.
RNase A	Serva	34390.02	n.a.	 Danger	H: 334 P: 261-284-304+340-342+311
Sodium Chloride (NaCl)	Roth Chemicals	3957.2	58.44	n.a.	n.a.
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

Name	Provider	PN	MW [g/mol]	Safety codes	
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
Tween 20	Roth Chemicals	9127.1	1228	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

1 M Tris (50 ml)
6.057 g – TRIS
40 ml of MilliQ water
Adjust the pH to 8.0 with concentrated HCl
Bring the final volume to 50 mL
Filter sterilize.

0.5 M EDTA (50 ml)
9.31g – EDTA
40 ml of MilliQ water
Adjust the pH to 8.0 NaOH. You will need around one gram of NaOH. When you get close to pH 8, it is better to use NaOH solution, to avoid overshooting the pH.
Bring the final volume to 50 mL
Filter sterilize.

1 M NaOH (50 ml)
2g -NaOH
Dissolve in 40 ml of MilliQ water.
Bring the final volume to 50 ml

P1 buffer – can be stored at 4 °C for up to 12 months

Reagent	Concentrations	For 50 ml
Tris-HCl pH 8.0	50 mM	2.5 ml of 1 M stock
EDTA	10 mM	1 ml of 0.5 M stock
RNAseA	200 µg/ml	100µl from a 100mg/ml stock

adjust the volume with MilliQ water to 50 ml

P2 buffer – can be stored at RT for up to 12 months

Reagent	Concentrations	For 50 ml
NaOH	200 mM	10 ml of 1 M stock
SDS	1%	0.5 g

adjust the volume with MilliQ water to 50 ml

P3 buffer – can be stored at RT for up to 12 months

Reagent	Concentrations	For 50 ml
Potassium acetate	2.3 M	11.3 g

Add water till you have around 30 ml solution. Adjust the pH of the solution to **4.8** using **acetic acid.** Afterwards adjust the volume with MilliQ water to 50 ml

PB buffer – can be stored at 4 °C for up to 12 months

Reagent	Concentrations	For 50 ml
NaCl	2.5 M	25 ml of 5 M stock
Tris-HCl pH 8.0	10 mM	0.5 ml of 1 M stock
EDTA	1 mM	0.1 ml of 0.5 M stock
PEG 8000	20%(w/v)	10g
Tween 20	0.05%	0.25 ml of 10% stock
Sera-Mag carboxylated beads	1.14% (v/v)	570 µl of stock (wash first, see below)

adjust the volume with MilliQ water to 50 ml

PEG buffer – can be stored at 4 °C for up to 12 months

Reagent	Concentrations	For 50 ml
NaCl	2.5 M	25 ml of 5 M stock
Tris-HCl pH 8.0	10 mM	0.5 ml of 1 M stock
EDTA	1 mM	0.1 ml of 0.5 M stock
PEG 8000	20%(w/v)	10g
Tween 20	0.05%	0.25 ml of 10% stock

adjust the volume with MilliQ water to 50 ml

PE buffer – can be stored at RT for up to 12 months

Reagent	Concentrations	For 50 ml
Tris-HCl pH 7.5	10 mM	0.5 ml of 1 M stock
Ethanol	80 %	41.7 ml of 96% EtOH
MilliQ water	N/A	7.8 ml

EB buffer – can be stored at RT for up to 12 months

Reagent	Concentrations	For 50 ml
Tris-HCl pH 8.5	10 mM	0.5 ml of 1 M stock

adjust the volume with MilliQ water to 50 ml

Consumables and equipment

Centrifuge with swing-out rotor (e.g. Rotixa 500 RS Centrifuge)

Vortex (e.g. SciQuip Vortex Mixer)

Tube rotator /shaker (e.g. Green BioResearch, Centrifuge Tube Rotator Shaker – PN: 82422201)

Magnetic stand for Eppendorf tubes (e.g. BOMB microtube racks)

Beads Washing procedure

Step	Washing	Time	☑
1	Resuspend the **stock of Sera-Mag carboxylated beads** by vortexing. Ensure that the beads are resuspended completely before proceeding forward	1 min	☐
2	Take out **570 µl** of beads as quickly as possible (to avoid the beads settling) and transfer them to a new microcentrifuge tube.	1 min	☐
3	Settle the magnetic beads on a magnetic stand and discard the supernatant.	5 min	☐
4	Add 1 ml of MilliQ water. Remove the tube from the magnet, resuspend the beads and centrifuge briefly. Put back the tube on the magnet and pellet the beads	2 min	☐
5	Remove the supernatant and add 1 ml of MilliQ. Remove the tube from the magnetic stand and resuspend the beads. After resuspension, the beads are ready to be used in preparing the **PB buffer.**	5 min	☐

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BOMB plasmid MIDI prep using Sera-Mag carboxylated beads

Step	Clean-up	Time	☑
1	Pellet the bacteria from a 100 ml culture in a centrifuge at 6000 x g for 5 minutes. Discard the supernatant. *If you have a **low-copy** number vector, you can use up to 500 ml of culture. However, in that case you will need to multiply the volumes in the steps **2, 3, 4** by **3**, as well as adjust the volume of the **PEG buffer** in step 8 according to the notes.*		☐
2	Resuspend the pellet in **2 mL** of **P1 buffer**. Make sure the pellet is completely resuspended.		☐
3	Add **3 ml** of **P2 buffer**, mix by flipping the tube a few times and incubate at room temperature for 5 minutes.	5 min	☐
4	Add **2.5 ml** of **P3 buffer,** mix by flipping the tube a few times. *Ensure a complete neutralization of the sample*	1 min	☐
5	Centrifuge for 10 minutes at >14.000 x g	10 min	☐
6	Transfer **7 ml** of the supernatant to a new tube. *Pipette carefully to avoid transferring the cell debris/precipitate. Transferring cell debris can cause protein contamination.*	1 min	☐
7	Resuspend the beads in the **PB buffer** by vortexing. Make sure that all the beads are resuspended before proceeding forward. *Ensure the beads are fully resuspended otherwise you might lose your DNA*.	1 min	☐
8	Add **3 ml** of **PB buffer** and **4 ml** of **PEG buffer,** mix by flipping the tube a few times. Incubate on a shaker for 30 min at RT. *Make sure that **always** the ratio of the recovered supernatant in step 6 and the sum of PB and PEG buffer volume is 1:1. Otherwise the DNA might not bind the beads! If you need to adjust the volumes, always adjust the **PEG volume** not the **PB**.*	30 min	☐
9	Centrifuge the tube briefly (2-3 seconds). Settle the magnetic beads on a magnetic stand and discard the supernatant. *Ensure that the beads are completely pelleted*	5 min	☐
10	Remove the tube from the magnetic stand and add 5 ml of **PE buffer.** Mix well (by pipetting, or vortex and spin down).	2 min	☐
11	Settle the magnetic beads on a magnetic stand and discard the supernatant.	2 min	☐
12	Remove the tube from the magnetic stand and add 1 ml of **PE buffer.** Mix well (by pipetting, or vortex and spin down).	2 min	☐
13	At this stage, transfer the mixture in a new microcentrifuge tube for easier work.	1 min	☐
14	Settle the magnetic beads on a magnetic stand and discard the supernatant.	2 min	☐
15	While the tube is on the magnet, add 1 ml of **PE buffer.** Wait for 30 seconds and then remove the supernatant.		
16	Remove the tube from the magnetic stand and add 500 µl of **PE buffer.** Mix well (by pipetting, or vortex and spin down).	2 min	☐
17	Remove the tube from the magnet and centrifuge it briefly to pellet all the liquid.	1 min	☐
18	Put back the tube on the magnet and using a 10 µl pipette, remove all the remaining liquid with care to not take up any beads.	1 min	☐

19	Put the tube on a heat block and dry the beads for approximately 10 minutes at 37°C. *The beads are dry when you see the beads pellet starting to crack**. Do not over-dry.*** *For midiprep, it might be that even in 10 minutes, the beads don't dry so leave them more if needed.*	5 min	☐
20	Add 300 µl of **EB buffer** to elute the DNA from the beads. Pipette well to resuspend and make sure that all the beads from the side of the tube are resuspended. Incubate for 15 min at 40°C. *If you have bigger plasmid (>6kbp) it is recommended to incubate the elution at 50°C.* *Do not elute above 50°C.*	15 min	☐
21	Settle the magnetic beads on a magnetic stand and transfer the supernatant to a new tube. *Make sure the beads are completely settled before transferring the supernatant. Avoid transferring any beads. If possible, leave some supernatant in the tube to minimize carryover.*	5 min	☐

Exemplary results

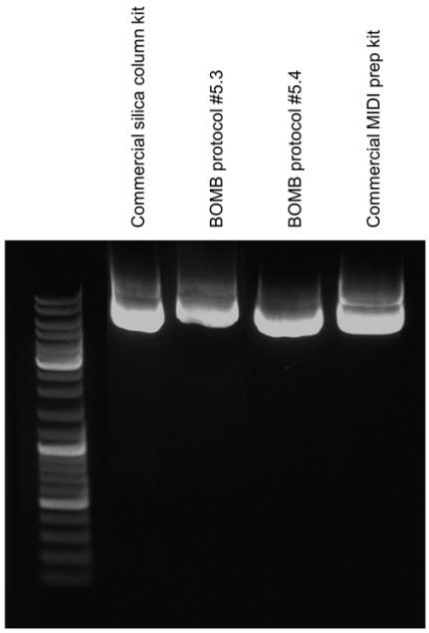

Fig 1: Comparison of commercial kits and BOMB protocols #5.3 and #5.4.MW: Gene Ruler DNA Ladder (Thermo).