Protocol	#4.2
Title	**BOMB clean-up and size exclusion using carboxyl beads**
Keywords	Carboxyl-beads, nucleic acid clean-up, size exclusion
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Summary

This clean-up and size exclusion protocol is designed to purify DNA from enzymatic reactions. The procedure relies on high concentrations of NaCl and PEG-8000 to facilitate DNA binding to carboxyl-coated magnetic beads [1,2] (BOMB protocol #3.1), followed by two wash steps with 80% ethanol to remove proteins, dNTPs, primers, salts and other contaminants. Furthermore, reducing the concentration of the binding buffer increases the size cut-off of the DNA that can stay bound to the beads, enabling size selection of the desired fragment sizes (works from <100 to 1000 bp).

Chemicals

Name	Provider	PN	MW [g/mol]	Safety codes	
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
Sodium Chloride (NaCl)	Roth Chemicals	3957.2	58.44	n.a.	n.a.
Polyethylene glycol 8000	AppliChem	A2204	~8000	n.a.	n.a.
Tween 20	Roth Chemicals	9127.1	1228	n.a.	n.a.
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
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
Ethylenediamine tetraacetic acid disodium salt dihydrate (EDTA ($C_{10}H_{14}N_2Na_2O_8 \cdot 2\ H_2O$)	Roth Chemicals	8043.1	372.24	 Danger	H: 332-373 P: 260-314

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.

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Buffers and solutions

Binding buffer (100 µl per 50 µl sample) – can be stored at 4 °C for at least 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)	10 g
Tween 20	0.05%	0.25 ml of 10% stock
Carboxyl-coated paramagnetic beads	2% (v/v)	1 ml of stock (wash first)

adjust the volume with water to 50 ml

80% ethanol (2x200 µl per sample, 40 ml per 96) – can be stored at RT for at least 12 months if closed properly

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

5 mM Tris-HCl pH 8.5

Consumables and equipment

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

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

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 PCR plate (e.g. Sarstedt – PN: 72.1979.102)

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

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BOMB Clean-up and size exclusion using carboxyl-coated magnetic beads

Step	Clean-up	Time	☑
1	Combine your DNA sample with 2.5 volumes of **binding buffer** (for size exclusion see Modification 1) in a 96-well PCR plate	5 min	☐
2	Seal and shake at RT at 1300 rpm for 5 min	5 min	☐
3 ⚠	Settle the magnetic beads on a magnetic stand and discard the supernatant *Ensure that the beads are completely pelleted*	5 min	☐
4	Remove the plate from the magnetic stand and add 200 µl **80% ethanol** and mix well (by pipetting, or seal, vortex and spin down)	5 min	☐☐
5	Settle the magnetic beads on a magnetic stand and discard the supernatant	5 min	☐☐
6	**Repeat** steps **4-5** once for a total of **2 washes**	10 min	☐
7 ⚠	Remove the supernatant completely and incubate at RT for approximately 5 min *Make sure to remove all remaining ethanol*	5 min	☐ ☐
8	Add 15-20 µl of **elution buffer** or **ddH_2O** to elute the DNA from the beads, mix well for 5 min (shake at 1300 rpm) and spin down briefly	5 min	☐
9	Settle the magnetic beads on a magnetic stand and transfer the supernatant to a collection plate/tube	10 min	☐
End	Measure concentration	**~1 h** (45 min hands-on)	
	Store @ -20 °C or 4 °C		☐

Modifications

1. **Size exclusion**
   To achieve accurate size exclusion lower **binding buffer** to sample ratios need to be used. Therefore, benchmark every batch of silica-coated magnetic beads and Binding buffer using the ratios as recommended in Fig 1 of the exemplary results section of this protocol (p. 4).

2. **Microcentrifuge tubes**
   The protocol above was designed to be performed in a 96-well format. It is also possible to use **1.5 ml microcentrifuge reaction tubes**. This allows an easier and faster handling for smaller sample numbers (up to 24). The clean-up of up to 24 samples using microcentrifuge tubes takes about 30 min total.

3. **Separate binding buffer and beads**
   The magnetic beads and the binding buffer (without magnetic beads) can be added separately to the sample. Therefore one can control the amount of beads that is added to each sample.

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Troubleshooting

Problem	Solution
Low DNA recovery	• **DNA/beads-ratio:** use the volume ratios outlined in the exemplary results section of this protocol • **Buffer composition:** use freshly prepared ethanol solution, as ethanol evaporates over time. Too low ethanol concentration might cause the DNA to detach from the beads during washing • **Buffer volume:** During elution the beads need to be covered completely with liquid, make sure to use enough water or **elution buffer** • **Incubation:** elute the DNA from the beads for a longer time (~5 min) • **Remaining ethanol:** please ensure that no ethanol is present in the sample before elution
Bad DNA quality (260/280 < 1.6)	• **Carried-over magnetic beads**: Increase time of magnetic pelleting and pipette carefully to avoid aspirating beads. Re-pellet the beads and move the eluate to a new container

Exemplary results

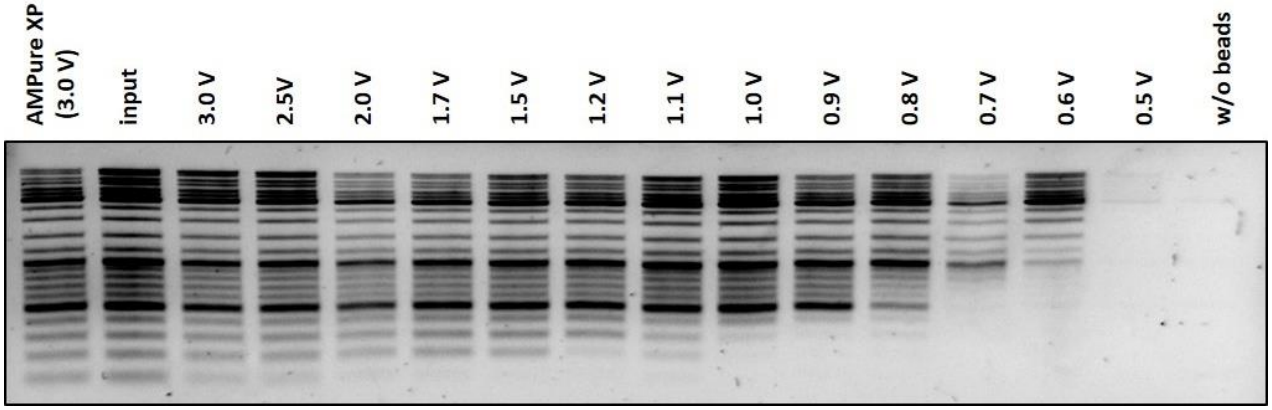

Fig 1: Size selection using the BOMB protocol. *Size exclusion of GeneRuler DNA Ladder mix (Thermo) using carboxyl-coated magnetic beads. Different volumes ratios of binding buffer compared to sample volume were used to achieve size exclusion.*

References

1. Deangelis MM, Wang DG, Hawkins TL. Solid-phase reversible immobilization for the isolation of PCR products. Nucleic Acids Res. 1995;23: 4742–4743. doi:10.1093/nar/23.22.4742

2. Jolivet P, Foley JW. Solutions for purifying nucleic acids by solidphase reversible immobilization (SPRI). Ludmer Center, Neuroinfromatics & Mental Health; 2015. pp. 1–6.

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