Protocol	#4.1
Title	**BOMB clean-up and size exclusion using silica beads**
Keywords	Silica 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 utilises the effects of chaotropic salts on the binding affinity of the negatively charged DNA backbone [1] to silica-coated magnetic beads (BOMB protocol #2.1), allowing the size specific clean-up of the desired molecules, depending of the amount of added binding buffer (Fig 1A). Furthermore, we were able to optimise the clean-up and buffer conditions to a degree where we were able to recover up to 95% of the purified DNA (Fig 1B).

Chemicals

Name	Provider	PN	MW [g/mol]	Safety codes	
2,2-Bis(hydroxymethyl)-2,2',2''-nitrilotriethanol / Bis-Tris ($C_8H_{19}NO_5$)	Sigma-Aldrich	B9754-100G	209.24	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
Guanidinium chloride / Gu-HCl ($CH_5N_3 \cdot HCl$)	Roth Chemicals	0037.1	95.53	 Warning	H: 302+332-315-319 P: 261-280-301+312-330-304+340+312-305+351+338-337+313
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
Phenolsulfonephthalein / Phenol Red ($C_{19}H_{13}NaO_5S$)	Honeywell / Riedel-de Haën	10418120	376.35	 Warning	H: 315-319-335 P: 302+352-362-305+351+338-280
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

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

Binding buffer (100 µl per 50 µl sample) – can be stored at RT for at least 12 months, prepare fresh if colour changes

Reagent	Concentrations	For 50 ml
Gu-HCl	3 M	14.33 g
Bis-Tris	10 mM	104.6 mg
Ethanol	90%	45 ml
Phenol Red (optional)	40 µM	50 µl of 40 mM stock in ddH_2O

adjust pH with HCl until the solution turns yellow (pH < 6.5) and adjust the volume with water to 50 ml

80% ethanol (200 µl per sample, 20 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)

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

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

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

Step	Clean-up	Time	☑
1	Combine your DNA sample with 5 µl stock solution of **silica-coated magnetic beads (BOMB protocol #2.1)** and 2 volumes of **binding buffer** (for size exclusion see Modification 1) in a 96-well PCR plate and mix until you receive a homogenous suspension	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 settled*	5 min	☐
4	Remove the plate from the magnetic stand and add 100 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 (or higher) for approximately 5-10 min to dry the beads *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 1A of the exemplary results section of this protocol (p. 4).

2. **Microcentrifuge tubes**
   The protocol above was designed for a 96-well microplate 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). For the washing step, up to 500 µl 80% ethanol can be used. The clean-up of up to 24 samples using microcentrifuge tubes takes about 30 min total.

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

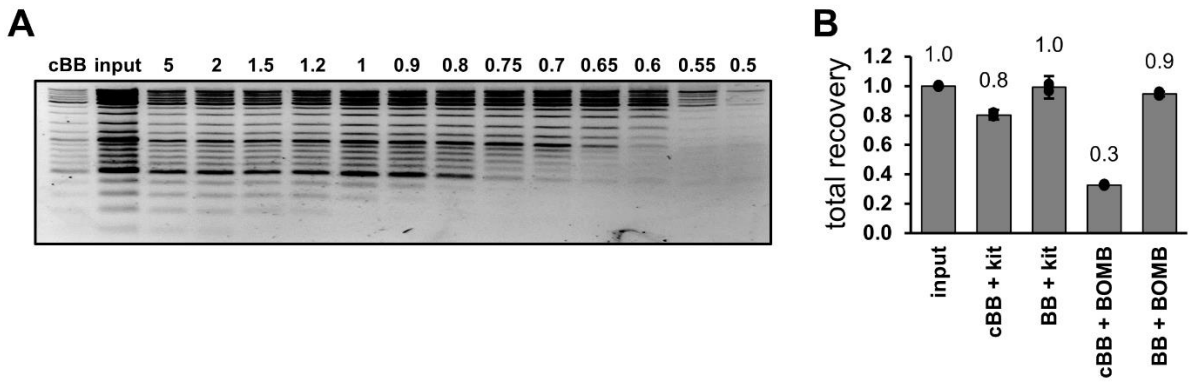

Figure 1: Clean-up and size exclusion of DNA. *(A) Size exclusion of GeneRuler DNA Ladder mix (Thermo) using silica-coated magnetic beads (see protocol above). Different volumes of binding buffer (BB) compared to sample volume were used to achieve size exclusion; as a comparison 2 volumes of commercial BB were used. (B) Total recovery of ~6 µg plasmid DNA (pUC19, input) using either a commercial kit utilising silica-packed columns (kit) or the #4.1 clean-up protocol with silica-coated beads (BOMB). For binding either commercial binding buffer (cBB) or the binding buffer (BB) described in the BOMB protocol above was used. Error bars show standard deviation, n=3.*

References

1. Vogelstein B, Gillespie D. Preparative and analytical purification of DNA from agarose. Proc Natl Acad Sci. 1979;76: 615–619. doi:10.1073/pnas.76.2.615