Protocol	#4.4
Title	**BOMB clean-up and nucleotide removal using silica beads**
Keywords	Silica beads, nucleic acid clean-up, nucleotide removal, size exclusion
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Online	https://bomb.bio/protocols/
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Summary

This clean-up and nucleotide removal protocol is designed to purify DNA from single nucleotides as well as other components of 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. If high salt concentrations are used (in this protocol ~2.7 M), it is possible to remove single nucleotides and simultaneously purify small DNA fragments. Using this protocol single stranded oligo nucleotide as small as 25 nts can be purified with a recovery rate of 30%. Larger oligos yield up to 80% for 70 nts (Fig 1).

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

Name	Provider	PN	MW [g/mol]	Safety codes	
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

Dissolve the Gu-HCl and Bis-Tris by adding Ethanol (99.9%) to a final volume of 45 ml and add an additional 4 ml ddH_2O. Dissolve by shaking, vortexing and incubation at e.g. 65 °C for a few minutes. 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 nucleotide removal 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 10 volumes of **binding buffer** 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. **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	• **Salt concentration:** higher salt concentrations (than 2.7 M) can potentially lead to better yields. However, as Gu-HCl does not dissolve easily in water in higher concentrations than 3 M, adding more than 10 volumes of BB in order to approximate 3 M might help to increase the recovery rate. • **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

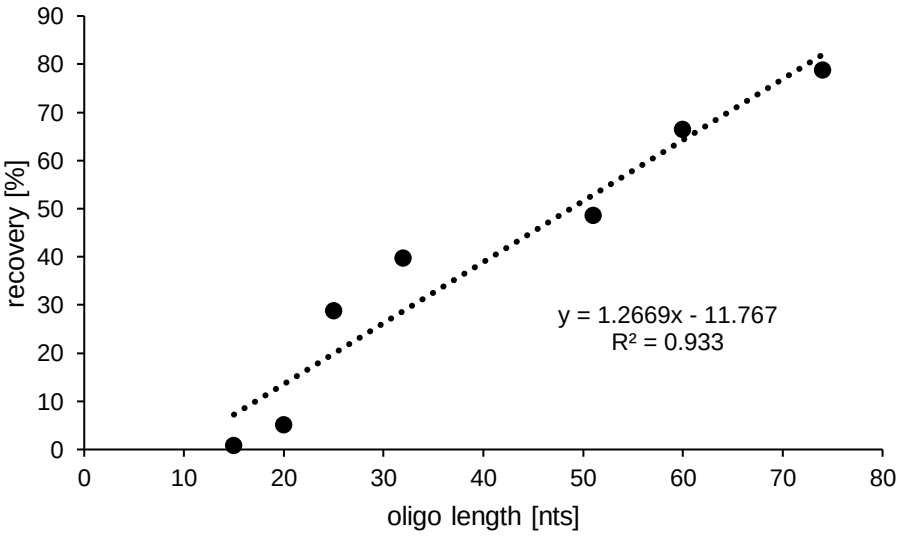

Figure 1: Clean-up of single stranded oligo nucleotides. *The recovery rate depends on the length of the respective DNA fragment. Fragments ≤ 20 nts resulted in ~10 % recovery, while larger fragments (74 nts) were purified with a yield of ~80% of the input.*

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