Protocol	#4.3
Title	**BOMB Gel extraction**
Keywords	Gel-extraction, silica-beads, carboxyl beads
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Summary

The BOMB gel extraction protocol is designed to purify DNA of specific sizes from agarose gels [1]. The protocol assumes users know how to run an agarose electrophoresis gel and safely cut out the band they want. Following this, the gel slice is melted at 50 °C in 1.5 volumes of GITC buffer [2] (also used for BOMB protocols #6.1 and #6.3). DNA is then captured on either silica- or coated beads using isopropanol to trigger precipitation. Standard isopropanol and ethanol washes are used to further purify the DNA ahead of resuspension. In our test samples, the extraction efficiency ranged between 20 and 50% of the input (dependent on the fragment size).

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: 210-240-305+351+338-403+233
Guanidine Isothiocyanate (GITC, $C_2H_6N_4S$)	Roth Chemicals	2628.4	118.16	 Warning	H: 302+312+332-412-EUH032 P: 273-280-302+352-304+340-312
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
N-Lauroylsarcosine sodium salt (Sarkozyl, $C_{15}H_{29}NO_3Na$)	Sigma (Merck)	L9150-50G	293.38	 Danger	H: 315-318-330 P: 260-280-284-305+351+338-310
Antifoam 204	Sigma (Merck)	A8311-50ML	n.a.	n.a.	n.a.
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
Isopropanol (C_3H_8O)	Acros Organic	184130025	60.01	 Danger	H: 225-319-336 P: 210-233-240-305+351+338-403+235

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

GITC buffer (stable for at least several weeks at RT)

Reagent	Concentrations	For 50 ml
GITC	4 M	23.64 g
Tris HCl pH 7.6-8.0	50 mM	2.5 ml of 1 M stock
Sarkosyl	2%	1 g
EDTA	20 mM	2 ml of 0.5 M stock
Antifoam (optional)	0.1 %	50 µl

adjust pH with HCl to 7.6-8.0 and adjust the volume with water to 50 ml

Isopropanol (~0.5 ml – 1 ml per sample)

80% ethanol (1 ml per sample)

Elution buffer (stable, can be stored for at least 1 year at RT; 30 µl per sample)

5 mM Tris-HCl, pH 8.5

Equipment and setup

Heat block without shaker function (e.g. Roth, Rotilabo®- block thermostat H250 – PN: Y264.1) or with shaker function (e.g. Sigma (Merck), Eppendorf® Thermomixer Compact – PN: T1317)

Magnetic stand for 1.5 ml microcentrifuge tubes (see BOMB protocols #A.1 or use a commercial solution)

Pipettes

20 – 200 µl (e.g. Gilson Pipetman P200)

200 – 1000 µl (e.g. Gilson Pipetman P1000)

1.5 ml microcentrifuge tubes (numerous vendors available)

BOMB Gel extraction

Step	Melt gel slice	Time	☑
1	Collect a gel slice containing the desired DNA fragment or amplicon into a 1.5 ml microcentrifuge tube		☐
2	Weigh tube containing gel slice (and subtract mass of empty tube)		☐
3 ⚠	Add 1.5 volumes of **GITC buffer** to 1 volume of gel slice (e.g. 150 µl of GITC buffer for 100 mg gel slice) *The GITC buffer contains guanidine isothiocyanate, a chemical which can cause burns and sensitivity and produces hydrogen cyanide when mixed with acid*	1 min	☐
4	Incubate gel slice in GITC buffer at 50 to 55 °C for 10 to 20 min or until fully dissolved (mixing by inversion or by using a heat block with shaker function during incubation helps accelerate process)	10 min	☐
Step	**Task - DNA capture and purification**		
5	Add 5 to 10 µl stock solution of **silica-coated magnetic beads (BOMB protocol #2.1)** or **carboxyl-coated magnetic beads** (**BOMB protocol #3.1**) and 2.5 volumes of **isopropanol** to the sample	1 min	☐
6	Shake at RT at 1300 rpm for 5 min or mix by pipetting and incubate for 5 min.	5 min	☐
7 ⚠	Settle the magnetic beads on a magnetic stand and discard the supernatant *Ensure that the beads are completely pelleted*	1 min	☐
8	Remove the plate from the magnetic stand and add 500 µl **isopropanol** and mix well (by pipetting, or vortex and spin down)	1 min	☐
9 ⚠	Settle the magnetic beads on a magnetic stand and discard the supernatant *Ensure that the beads are completely pelleted*	1 min	☐☐
10	Remove the plate from the magnetic stand and add 500 µl **80% ethanol** and mix well (by pipetting, or vortex and spin down)	1 min	☐☐
11	**Repeat** steps **9-10** once for a total of 2 washes	2 min	☐
12 ⚠	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	☐
13	Add 20-30 µl of **elution buffer** or **ddH_2O** to elute the DNA from the beads, mix well for 5 min (e.g. shake at 1300 rpm) and spin down briefly	5 min	☐
14	Settle the magnetic beads on a magnetic stand and transfer the supernatant to a collection plate/tube	2 min	☐
End	Measure concentration and proceed	**~35 min** (20 min hands-on)	
	Store @ -20 °C or 4 °C		

Troubleshooting

Problem	Solution
Beads sticking to the tube	• Sonicate shortly in a sonic bath and/or centrifuge briefly
Beads stay at the bottom of the tube when mixing	• Close lid and invert tube a few times
Low DNA concentration	Low DNA concentration can have multiple reasons • **Gel slice incompletely solubilised:** After addition of GITC buffer to gel slice, mix by inverting tube 5 times every 2-3 min or shake at 1300 rpm during 50 to 55 °C incubation and incubate for another 10 min (DNA will remain in any undissolved agarose) • **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

Exemplary Results

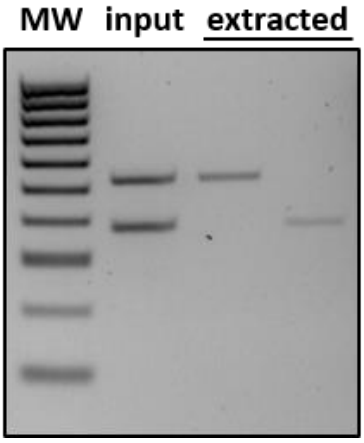

Fig 1: Gel extraction using BOMB. *The second lane contains the product of a CDH1 PCR using female chicken gDNA as a template, according to Fridolfsson and Ellegren [3]. The two rightmost lanes contain the gel extracted 519 bp and 373 bp bands from lane two using the BOMB protocol with carboxyl-coated magnetic beads. The volumes loaded are proportional (i.e. the right hand 2 lanes represent the efficiency of capture from the left hand lane). Depending on the fragment size between ~20% (373 bp) and ~50% (519 bp) of the input could be recovered (as judged by image densitometry of the extracted bands). MW: Hyperladder IV (Bioline).*

References

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