Isolation of DNA from GF/F or Polycarbonate Filters: Caron Lab (USC)

Roll the filter loosely and place into 15 ml Falcon Tube.

Add 2 ml 2x lysis buffer (see recipe below), swirl tube to coat filter, freeze in dry-shipper for storage (Liq. N_2), transfer to -80C freezer ASAP.

Thaw tube in 70C water bath and add about 200 µl of 0.5 mm zirconia/silica beads (www.biospec.com, Cat.# 11079105z) to the sample in the Falcon Tube.

Bead-beat (vortex) for ~30 seconds and then heat @ 70 ºC for 5 minutes. (Repeat 3x total)

Transfer the filter and lysate to the barrel of a 10 ml syringe (make sure you have a microfuge tube under the syringe to catch drips). The syringe will allow you to squeeze as much lysate out of the filter as possible. Dispense the lysate into a new sterile 14 ml Falcon tube.

Make the extract at least 0.7M with NaCl, add 10% CTAB (cetyltrimethyl ammonium bromide) to make a 1% CTAB solution and incubate at 60-70 ºC for 10 minutes. (See chart below for volumes to use with variable lysate volumes)

DNA Extraction: reagent volumes	Starting	Desired		
	M	M		
NaCl	2.5	0.7		
	%	%		
CTAB	0.1	0.01		
Lysate Volume (ml)	1000	1500	2000	2500
CTAB volume (ml)	161	242	323	403
NaCl (0.7M) volume (ml)	452	677	903	1129
Total Volume (ml)	1613	2419	3226	4032
% CTAB (final)	0.998	1.000	1.001	1.000

Transfer 800-1000 µl aliquots of the lysate into as many 2 ml sterile centrifuge tubes as needed. Add an equal volume of (25:24:1) Phenol:chloroform:isoamyl alcohol (Sigma Cat. # P2069). Vortex to mix, then spin @ 14,000 RPM for 3 minutes in desktop centrifuge under the hood.

Carefully remove supernatant to a new sterile epitube. Repeat P:C:I extraction, transfer supernatant to a new epitube.

Extract supernatant with an equal volume of (24:1) Chloroform:Isoamyl alcohol. Vortex, then spin at 14,000 RPM for 3 minutes. Transfer supernatant to a new tube and repeat this CI (24:1) extraction step. Transfer to a new tube.

Precipitate supernatant with 1X volume of ice-cold 95% Ethanol and 0.1X volume of 10.5M Ammonium Acetate.

Mix well, then chill overnight at -20ºC before spinning at 14,000RPM (4ºC) for 30 minutes on Eppendorf.

The next day…Decant liquid, rinse pellet with 500 µl ice-cold 70% Ethanol, spin at 14,000 RPM (4ºC) for 15 minutes, decant supernatant and air-dry the pellet.

Resuspend the pellet in 20-100 µl sterile TE (10 mM Tris, 1 mM EDTA, pH=7.5). May need to heat at 40ºC for ~30 minutes to help dissolve pellet.

Recombine the DNA suspensions into a single epitube. Determine the concentration by fluorescence (Pico-Green).

Freeze at -20ºC (ideally, archive half of sample at -80ºC)

Note: We have achieved similar DNA recoveries from GF/F and 0.8 µm polycarbonate filters when an equivalent volume of cultured cells were filtered down. The GF/F filters are generally much faster to filter than the polycarbonate filters. However, if you only have a small number of cells and you're concerned about recovery, use the polycarbonate filters since the entire filter can be dissolved in the P:C:I extraction step.

Recipe for 2X Lysis buffer: 100 mM Tris (pH = 8), 40 mM EDTA, 100 mM NaCl, 1% SDS.

To prepare 200 ml of 2X lysis buffer:

Stock	Vol. used (ml)	Final Conc (in 200 ml)
0.25M EDTA, pH 8	32	40 mM
1M Tris, pH 8	20	100 mM
2.5M NaCl	8	100 mM
10% SDS (w/v)	20	1%

Adjust final volume to 200 ml with sterile milli-Q water to achieve final concentrations listed above, Autoclave mixture.

Note: Tris can be made from "Tris-Base" and pH adjusted with NaOH or HCl