Barton Lab

Low-Biomass Carbonate Rock Extraction Protocol

(*JMM **66**:21-31)*

1) Use dremel tool to cut approximately 1 g of rock from frozen sample. Place rock in ethanol sterilized steel pestle and mortar (when cooled under hood). Cover with sterile aluminum foil, bring out of hood and onto bench. Crush to a powder, return to hood.

2) Place ~0.5 g of crushed rock into eppendorf. Add 500 μl 2X Buffer AE, 10 μl poly dI-dC, and 30 μl lysozyme. Mix by gentle inversion, then incubate at 37°C for 30 minutes. (Bubbling will occur from EGTA binding Ca^{2+} ions, releasing CO_2 – watch for lid popping off when you open it!).

3) Add 10 μl 20% SDS and 60 μl proteinase K. Mix by gentle inversion, incubate at 50°C for 30 minutes.

4) Add 200 μl 20% SDS, mix gently, and 500 μl SEVAGE [phenol:$CHCl_3$:IAA (25:24:1)]. Bead-beat on low setting for 2 min, increase to homogenize for 30 s.

5) Spin at ~ 13,000 x *g* for 5 min. Pipette 700 μl of supernatant into fresh tube.

6) Extract again with SEVAGE. Remove ~650 μl supernatant, add 2 μl poly dI-dC and precipitate with 1/10th volume 3M NaAc and 2 volumes 100% ethanol.

7) Pellet by centrifugation at ~ 13,000 x *g* at 4°C for 20 minutes. Wash pellet with 70% ethanol, spin briefly to remove excess ethanol. Air dry in laminar flow-hood (~15 min).

8) Resuspend pellet in 30 μl Fluka water. Place suspension in slide-a-lyzer MINI dialysis unit. Dialyze against 100 ml filter sterilized 20 mM EGTA in glass beaker for >4 hours at 4°C (usually overnight). [**NB:** Use sterile aluminum foil to make float]

9) Take supernatant from dialysis tube and precipitate with 3M NaAc/2 volumes ethanol. Pellet and air dry again. Resuspend in 20 μl Fluka water.

Note: The addition of the poly dI-dC generates a template 'smear' when the PCR products are separated on an agarose gel. This DNA will help in the gel purification of the PCR product before cloning, but will not affect cloning unless the 'smear' is really significant. A bright 'smear' will generally indicate that all carbonate binding sites were blocked in the initial extraction and excess poly dI-dC is being carried over. To reduce this affect, do not add additional poly dI-dC at step #6.

Reagent[a]	Preparation	Storage
2X Buffer AE	200 mM NaCl 200 mM Tris (pH 8.0) 50 mM EDTA 300 mM EGTA Make ~20 ml with DNAase/RNAse free Fluka water (Add EGTA as a powder, pH to 8.0 with 1M NaOH drop-by-drop. EGTA will go into solution when pH reaches 8.0 – use pH paper to confirm pH with small volume) Filter sterilize (0.2 μm)	-20°C
Lysozyme (10 mg/ml)	Make in 1.5 ml tubes with Fluka water	-20°C
Poly dI-dC (0.5 mg/ml)	Resuspend in Fluka water. Decontaminate by pipetting 50 μl aliquots onto Petri dish and exposing to 5000 μJ on Stratalinker. Store aliquots individually.	-20°C
Proteinase K (20 mg/ml)	Make in 1.5 ml tubes with Fluka water.	-20°C
20% SDS	Make in 1.5 ml tubes with Fluka water.	-20°C
Ethanol	Keep dedicated 'clean' bottle – open in laminar hood only.	-20°C

Chemical Reagent	Source
EGTA	Sigma-Aldrich cat# E-4378
Fluka water	Sigma-Aldrich cat# 95284
Poly dI-dC	Sigma-Aldrich cat# P-4929
Slide-A-Lyzer MINI	Pierce Biotech. Cat# 69550

[a] All reagents (except ETOH) are filter sterilized and stored as eppendorf aliquots.