PEG precipitation minipreps

(This protocol gives high yield, sequencing quality DNA.)

BUFFERS

TS
25% sucrose
50 mM Tris pH 8.0

BL
62.5 mM EDTA
50 mM Tris pH 8.0
0.1% Triton X-100

BP
20% PEG (polyethylene glycol)
20 mM Tris pH 8.0
1M NaCl

frozen stock of lysozyme 20 mg/ml
stock of 0.5M EDTA pH 8.0
TE pH 8.0
RNase A (DNAse-free, 10 mg/mL frozen stock)
EtOH (100%, 70%)
10 M NH_4Ac

PROTOCOL

Spin down 1-3 mL culture in eppendorf tube.
Resuspend pellet in 400 μL TS (by vortexing).
Add 600 μL of [500 μL BL + 50 μL lysozyme (10/mL stock) + 50 μL 0.5 M EDTA].
Mix by inverting -> ice 10 min -> 70 degC 15 min -> ice 5 min
Spin 14K rpm 15 min.
Take SN to new tube, add 550 μL BP.
-> Room temp at least one hour (ok to leave overnight).
Spin 10 min, 14K rpm.
Remove SN completely.
Resuspend pellet in 100 μL TE pH8.0. Store at 4 degrees C.

Use 4 μL for restriction enzyme digests, including RNAse in digest reaction (0.1 μL of 10 mg/mL stock per 20 μL reaction).

For correct clone(s):

Add 2 μL RNAse (10 mg/mL) -> 37 degC 30 min.
Add 1/10 vol. of 10M NH_4Ac + 2 vols. 100% EtOH -> -80 degC at least 30 min.
Spin 10 min, 4 degC, 14K rpm.
Wash pellet with 250-500 μL 70% EtOH, re-spin (4 degC).
Resuspend pellet in 50 μL TE or H_2O.
Sequence if needed. (From 3 mL culture I typically get ~ 100 ng/μL F.C.)