Using Exonuclease V (RecBCD) to eliminate residual genomic DNA when purifying low copy plasmids with the Monarch® Plasmid Miniprep Kit

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Introduction

The use of low and/or single-copy plasmids to clone large pieces of DNA (up to 200 kb) or to drive expression of slow folding or toxic proteins in *E.coli* is a commonly used strategy. Purification of low-copy plasmids or bacterial artificial chromosomes (BACs) presents some challenges that are not evident when working with higher copy number plasmids such as pUC19. The ratio between bacterial gDNA and plasmid DNA is higher, thereby reducing yield of the desired plasmid produced by typical plasmid miniprep protocols. Additionally, elevated levels of host gDNA are often co-purified, reducing the accuracy of quantitation by UV absorbance or dsDNA specific dyes. Neither method can distinguish the contribution from gDNA to the overall nucleic acid content. Co-purification of host gDNA also affects the appearance of the sample when resolving by gel electrophoresis and adds unwanted contaminating DNA for any amplification-based application.

Exonuclease V (RecBCD, NEB #M0345) is an exonuclease that degrades both linear ss- and dsDNA, while keeping the circular DNA intact. Treatment of miniprep DNA samples of low copy plasmids with this exonuclease degrades the contaminating gDNA, restoring purity and ease of use in downstream applications.

Protocol

1. Transform an *endA-* strain (e.g. NEB 10-beta, NEB #C3019) with the BAC plasmid DNA and plate outgrowth onto a media plate with appropriate antibiotic. Incubate overnight at 30°C. BACs with Cam^R require reduced stringency selection. Chloramphenicol levels should be maintained between 10-15 µg/ml on the selective plate.
   Note: strains with an F' plasmid are not compatible with BACs or miniF plasmids.
2. Pick a colony, inoculate 10 ml LB + antibiotic, and incubate overnight at 30°C (200-250 RPM).
3. Check OD_{600} nm (usually it will be around 4 O.D./ml of cells).
4. Harvest 3 ml of the overnight culture and purify the plasmid DNA using the Monarch Plasmid Miniprep kit (NEB #T1010) following the recommended protocol.
5. In the final elution step, elute the DNA with 30 µl of Monarch DNA Elution Buffer (pre-heated to 50°C).
6. To the eluted DNA, add 4 µl of NEBuffer 4 (10X), 4 µl of 10 mM ATP, and 2 µl of Exonuclease V (RecBCD). Mix reaction and incubate at 37°C for 1 hr.
7. Heat-inactivate the Exonuclease V by incubating at 70°C for 30 min. The plasmid DNA is now ready for restriction enzyme digestion, PCR or transformation.
   Note: Typically 30-60 ng of single-copy plasmid can be purified from 3 ml of an overnight E.coli culture with (O.D. 600 nm = 4 O.D/ml)

Materials

Endonuclease V (RecBCD) (NEB #M0345)

NEB 10-beta Competent *E coli* (High Efficiency)(NEB #C3019)

Antibiotic, typically Chloramphenicol

LB Media

Monarch Plasmid Miniprep Kit (NEB #T1010)

Results:

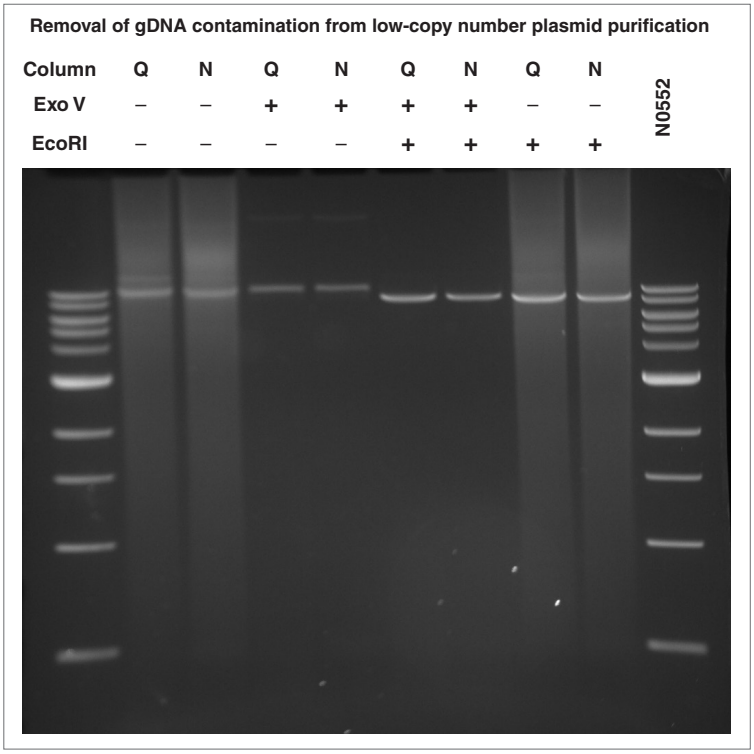
Three milliliters of an overnight culture of NEB-10 beta competent *E. coli* cells transformed with pBAC were processed using the Monarch Plasmid DNA Kit and an equivalent Miniprep kit from another vendor. After isolating the DNA, samples were treated with Exonuclease V (RecBCD) and then digested with EcoRI. Samples were run on an agarose gel to assess the quality of the isolated DNA, and whether or not the Exonuclease V-treated DNA still was able to be digested to completion. The Exonuclease V-treated samples showed no gDNA contamination (#3-6) while the untreated samples exhibited a significant amount of gDNA as seen by the smear observed in those samples (#1,2,7,8).

These results indicate that Endonuclease V can be used to efficiently degrade contaminating gDNA from plasmid purification steps, including those of low copy number.

FIGURE 1:

pBAC samples exhibit no bacterial gDNA contamination after treatment with Exonuclease V (RecBCD).

Miniprep plasmid DNA samples isolated with the Monarch Plasmid Miniprep kit (N) and a similar kit from a competitor (Q) were either treated (+) or not treated (-) with Exonuclease V, and then digested with EcoRI. The samples treated with Exonuclease V showed no contaminating gDNA and they were correctly cut with EcoRI.



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