plasmidPrep Mini Spin Kit

FAST, EFFICIENT PLASMID DNA PURIFICATION

The plasmidPrep Mini Spin Kit rapidly purifies high-quality plasmid DNA from small culture volumes (1 to 3 mL) of transformed *E. coli*. The system is fast and efficient, and routinely generates highly pure plasmid DNA yields of up to 15 µg. The yield and quality of the purified plasmid DNA ensures exceptional performance in downstream applications such as restriction enzyme analysis, ligation, cloning, DNA sequencing, PCR and other molecular biology applications.

plasmidPrep Mini Spin Kit delivers:

- **Fast results:** Isolates plasmid DNA in less than 10 min.
- **High purity:** Yields plasmid DNA samples that are free of RNA and genomic DNA contamination, that lack any residual nuclease activity, and exist in a predominantly supercoiled configuration.
- **High quality:** Excellent performance in several down- stream applications including, restriction enzyme analysis, ligation, cloning, DNA sequencing, and PCR.
- **Simpler purification:** A streamlined process with minimal changes in pipetting volume from one part of the protocol to the next, fewer centrifugation steps, color-coded kit components, and no organic solvents.

Method overview

The protocol consists of three phases:

1. A modified alkaline lysis method is used to disrupt the bacteria (1, 2, 3).
2. A centrifugation step to pellet bacterial cellular debris.
3. Loading of the cleared supernatant onto a purification column that contains a novel silica membrane.

The novel membrane facilitates the removal of denatured contaminants using a single wash and drying step prior to plasmid DNA elution. The plasmidPrep Mini Spin Kit employs chaotropic salts to denature potential contaminants and promote the selective binding of plasmid DNA to the silica membrane (4, 5).

Extraction time

Plasmid DNA extraction can be completed using plasmidPrep Mini Spin Kit in as little as 10 minutes using the included protocol.

Amount of supercoiled plasmid DNA

The amount of supercoiled plasmid DNA present in a sample can be used to assess the extent of physical damage suffered in the course of the mini extraction procedure. The data indicates that 60 to 70% of the plasmid DNA isolated from all 4 cultures were in a supercoiled configuration (Fig 1).

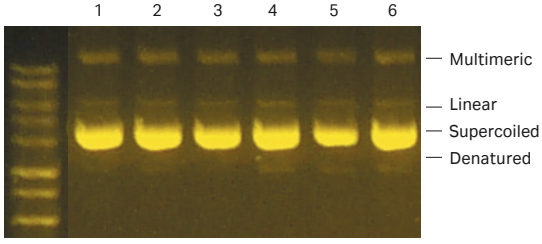


Fig 1. A representative image of undigested plasmid DNA (400 ng) extracted from culture 1. Plasmid mini preparations were performed using the plasmidPrep Mini Spin Kit.

Nuclease activity in plasmid DNA samples derived from the *EndA*$^{+}$ strain HB101

Residual nuclease activity was investigated in plasmid DNA samples purified from *E. coli* HB101 with the plasmidPrep Mini Spin Kit. The presence of a partially degraded plasmid DNA smear suggests that residual nuclease activity was present in samples that were not subjected to the nuclease removal wash. Equivalent plasmid DNA samples that had undergone the additional wash did not show any plasmid DNA degradation, indicating the removal of nuclease activity (Fig 2).

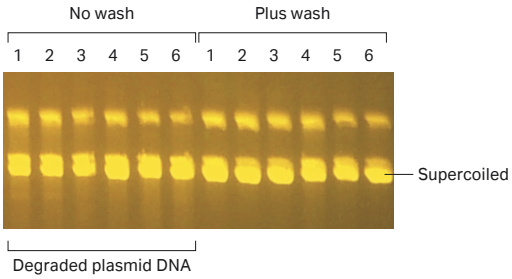
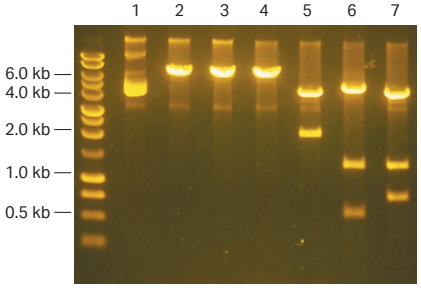


Fig 2. Plasmid DNA samples after incubation for potential nuclease activity. A representative image is shown of plasmid DNA samples extracted using the plasmidPrep Mini Spin Kit from *E. coli* strain HB101 (*EndA*$^+$) containing pCORON1002/EGFP-C1 plasmid.

Compatibility with downstream applications

The plasmid DNA samples isolated with plasmidPrep Mini Spin Kit was digested to completion in all the restriction digests performed in this study, including digests involving low concentrations of the restriction enzyme HindIII (1 unit at 37°C for 1 h) (Fig 3). HindIII activity is diminished in the presence of elevated salt concentrations; therefore, HindIII digestion can be utilized to indicate the presence of prohibitively high salt contamination in the purified plasmid DNA. Plasmid samples were digested to completion with HindIII, suggesting that plasmidPrep Mini Spin Kit produced high quality plasmid with negligible salt content (Fig 4).



1. Undigested plasmid DNA
2. NheI (6313 bp)
3. SacI (6313 bp)
4. BamHI (6313 bp)
5. HindIII (4273 and 2040 bp)
6. PsiI (4497, 1252, and 564 bp)
7. HindIII/NheI (4273, 1278, and 762 bp)

Fig 3. Restriction enzyme digestion of plasmid DNA samples (400 ng, 5 units, 37°C for 1 h). A representative image is shown of single plasmid DNA samples extracted from culture 1.

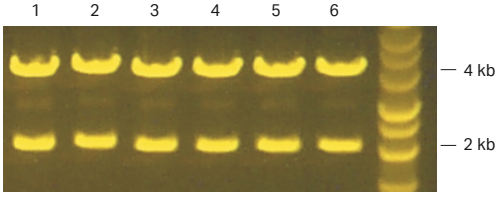


Fig 4. HindIII restriction digests (1 unit, 37°C for 1 h) of plasmid DNA samples. A representative image is shown of plasmid DNA samples extracted from culture 1.

Endpoint PCR

Plasmid purified using the plasmidPrep Mini Spin kit was used to generate a1187-bp product by PCR (Fig 5). The number of PCR cycles did not affect the amplification result, thus indicating similar amplification efficiencies for all plasmid DNA templates. The enzymes were chosen to highlight the efficacy of plasmidPrep derived plasmid DNA as reliable templates for the amplification of PCR products using either nonproofreading (*Taq* DNA polymerase) or proofreading polymerases (*PfuTurbo*™ and Vent™ DNA polymerases).

Ligation and cloning

Plasmid DNA isolated using the plasmidPrep Mini Spin Kit was shown to be a suitable template for T4 DNA ligase-mediated cloning experiments (Fig 6). The ligation, cloning, and transformation efficiencies were found to be as expected (> 300 ampicillin-resistant colonies. Negative control reactions [absence of ligase] produced < 50 colonies).

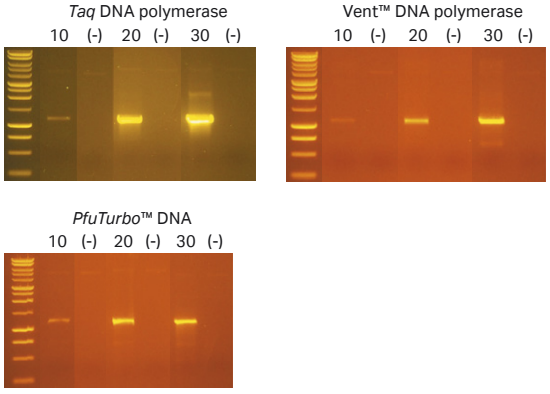


Fig 5. Endpoint PCR analysis was performed on several individual plasmid purifications. The numbers 10, 20, and 30 donate the number of PCR cycles performed; (-) represents no template controls.

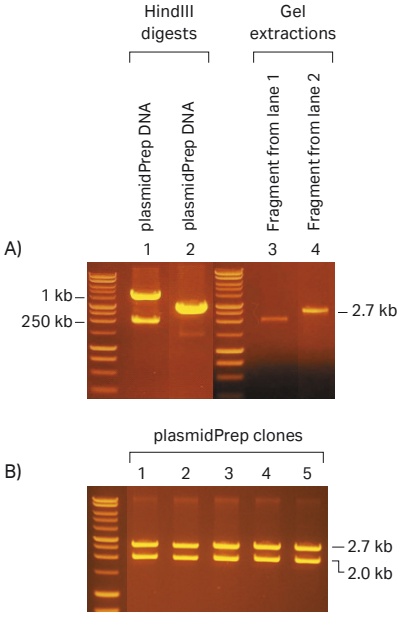


Fig 6. Ligation and cloning results. (A) Lane 1 (pCORON1002/EGFP-C1); lane 2 (pUC18) are HindIII digests of plasmid isolated with the plasmidPrep Mini Spin Kit. The pCORON1002/EGFP-C1 digest products are ~ 2 and ~ 4 kb in size, that of pUC18 is ~ 2.7 kb. Lanes 3 and 4 are the gel-extracted fragments. (B) Five randomly selected recombinant plasmid DNAs were digested with HindIII. The correct religation pattern is ~ 2.0 and 2.7 kb.

Summary: efficiency without compromise

The plasmidPrep Mini Spin Kit is a versatile plasmid DNA purification system enabling fast processing without compromising plasmid DNA yield, purity, or quality.

References

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2. Ish-Horowicz, D. and Burke, J.F., Rapid and efficient cosmid cloning. *Nucl. Acids Res*. 9, 2989 (1981).
3. Sambrook, J. *et al.*, Cold Spring Harbor laboratory, 2nd eds., (1989).
4. Vogelstein, B. and Gillespie, D., Preparative and analytical purification of DNA from agarose. *Proc. Natl. Acad. Sci.* USA 76, 615 (1979).
5. Marko, M.A., *et al.*, A procedure for the large-scale isolation of highly purified plasmid DNA using alkaline extraction and binding to glass powder. *Anal. Biochem.* 121, 382 (1982).
6. Schoenfeld *et al.,* DNA purification: effects of bacterial strains carrying the endA1 genotype on DNA quality isolated with Wizard Plasmid Purification. *Promega Notes,* 12-19, 53 (1995).

Ordering information

Product	Quantity	Product code
plasmidPrep Mini Spin Kit	50 preps	28904269
plasmidPrep Mini Spin Kit	250 preps	28904270

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