Protocol

NANO FLEX

GeneJET Plasmid Miniprep Kit, K0502

Introduction

For 50 preps.

The GeneJET Plasmid Miniprep Kit is designed for rapid and cost-effective small-scale preparation of high quality plasmid DNA from recombinant E. coli cultures. The kit utilizes an exclusive silica-based membrane technology in the form of a convenient spin column. Each GeneJET spin column can recover up to 20 µg of plasmid DNA. The kit can be successfully used for efficient purification of any size plasmids and cosmids. The actual plasmid yield and optimal culture volume depend on the plasmid copy number and medium used for cultivation.

Pelleted bacterial cells are resuspended and subjected to SDS/alkaline lysis (1) to liberate the plasmid DNA. The resulting lysate is neutralized to create appropriate conditions for binding of plasmid DNA on the silica membrane in the spin column (2). Cell debris and SDS precipitate are pelleted by centrifugation, and the supernatant containing the plasmid DNA is loaded onto the spin column membrane. The adsorbed DNA is washed to remove contaminants, and is then eluted with a small volume of the Elution Buffer (10 mM Tris-HCl, pH 8.5). The purified plasmid DNA is ready for immediate use in all molecular biology procedures such as conventional digestion with restriction enzymes, fast digestion with FastDigest® restriction enzymes, PCR, transformation and automated sequencing.

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For Plasmid DNA purification using vacuum manifolds, see link above.

Materials

- Buffer preparation
  - 20 mL wash Solution (concentrated)
  - 35 mL ethanol 96%
- GeneJET Plasmid Miniprep Kit, K0502

Procedure

Buffer preparation - Important Notes

1. Add the provided RNase A solution to the Resuspension Solution and mix. After addition of RNase A, the Resuspension Solution can be used for 6 months when stored at 4°C.

2. Add ethanol (96-100%) to the Wash Solution prior to first use.
3. Check the Lysis Solution and the Neutralization Solution for salt precipitation before each use. Redissolve any precipitate by warming the solution at 37°C, then cool back down to 25°C before use. Do not shake the Lysis Solution too vigorously.
4. Both the Lysis Solution and the Neutralization Solution contain irritants. Wear gloves when handling these solutions.
5. When using EndA+ strains, use Wash Solution I (#R1611) which is available to purchase separately.

Growth of Bacterial Cultures

6. Pick a single colony from a freshly streaked selective plate to inoculate 1-5 mL of LB medium supplemented with the appropriate selection antibiotic. Incubate for 12-16 hours at 37°C while shaking at 200-250 rpm. Use a tube or flask with a volume of at least 4 times the culture volume.
7. Harvest the bacterial culture by centrifugation at 8000 rpm (6800 × g) in a microcentrifuge for 2 min at room temperature. Decant the supernatant and remove all remaining medium.

   Do not overload the column:

   - For high-copy-number plasmids, do not process more than 5 mL of bacterial culture. If more than 5 mL of such a culture are processed, the GeneJET spin column capacity (20 µg of dsDNA) will be exceeded and no increase in plasmid yield will be obtained.
   - For low-copy-number plasmids, it may be necessary to process larger volumes of bacterial culture (up to 10 mL) to recover a sufficient quantity of DNA.

Plasmid DNA purification using centrifuges

Note:

- Read IMPORTANT NOTES before starting.
- All purification steps should be carried out at room temperature.
- All centrifugations should be carried out in a table-top microcentrifuge at >12000 × g (10 000-14 000 rpm, depending on the rotor type).

8. Use 1-5 mL of E. coli culture in LB media for purification of high-copy plasmids.
9. For low-copy plasmids use up to 10 mL of culture.
10. Resuspend the pelleted cells in 250 µL of the Resuspension Solution. Transfer the cell suspension to a microcentrifuge tube. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain. Note. Ensure RNase A has been added to the Resuspension Solution.
11. Add 250 µL of the Lysis Solution and mix thoroughly by inverting the tube 4-6 times until the solution becomes viscous and slightly clear. Note. Do not vortex to avoid shearing of chromosomal DNA. Do not incubate for more than 5 min to avoid denaturation of supercoiled plasmid DNA.

12. Add 350 µL of the Neutralization Solution and mix immediately and thoroughly by inverting the tube 4-6 times. Note. It is important to mix thoroughly and gently after the addition of the Neutralization Solution to avoid localized precipitation of bacterial cell debris. The neutralized bacterial lysate should become cloudy.
13. Centrifuge for 5 min to pellet cell debris and chromosomal DNA.
14. Transfer the supernatant to the supplied GeneJET spin column by decanting or pipetting. Avoid disturbing or transferring the white precipitate. Note. Close the bag with GeneJET Spin Columns tightly after each use!
15. Centrifuge for 1 min. Discard the flow-through and place the column back into the same collection tube. Note. Do not add bleach to the flow-through
16. Optional: use this preliminary washing step only if EndA+ strains which have high level of nuclease activity are used. Wash the GeneJET spin column by adding 500 µL of Wash Solution I (#R1611, diluted with isopropanol) and centrifuge for 30-60 sec. Discard the flow-through. Note. This step is essential to remove trace nuclease activity.
17. Add 500 µL of the Wash Solution (diluted with ethanol prior to first use as described in Buffer preparation - Important Notes) to the GeneJET spin column. Centrifuge for 30-60 seconds and discard the flow-through. Place the column back into the same collection tube.
18. Repeat the wash procedure (step 16) using 500 µL of the Wash Solution.
19. Discard the flow-through and centrifuge for an additional 1 min to remove residual Wash Solution. This step is essential to avoid residual ethanol in plasmid preps.
20. Transfer the GeneJET spin column into a fresh 1.5 mL microcentrifuge tube (not included). Add 50 µL of the Elution Buffer to the center of GeneJET spin column membrane to elute the plasmid DNA. Take care not to contact the membrane with the pipette tip. Incubate for 2 min at room temperature and centrifuge for 2 min. Note. An additional elution step (optional) with Elution Buffer or water will recover residual DNA from the membrane and increase the overall yield by 10-20%. For elution of plasmids or cosmids >20 kb, prewarm Elution Buffer to 70°C before applying to silica membrane.
21. Discard the column and store the purified plasmid DNA at -20°C.