BPGene® Filter-Based Plasmid DNA Extraction Kit

For small-scale (mini) preparations of purified plasmid DNA

Cat. No. BPGene PEF011400 25 Reactions

Store the kit at +4 °C to +8 °C.

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شرکت برنا پویش ژن (سهامی خاص)
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1. General Information

The BPGene® **Filter-Based Plasmid DNA Extraction Kit** is designed for rapid and efficient purification of high and low copy plasmid DNA from bacterial lysates of *E. coli* strains. Special buffers provided in the kit are optimized to enhance binding DNA onto a specially-treated glass filter membrane for efficient recovery of highly pure plasmid DNA. The supplied RNase helps to get highly pure DNA which is suitable for downstream applications including PCR and cloning.

1.1. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

1.2. Contents

No.	Vial /Bottle	Label	Content
1	1	**BPGene Suspension Buffer/RNase A**	6.5 ml
2	1	**BPGene Lysis Buffer**	6.5 ml
3	1	**BPGene Binding Buffer**	9 ml
4	1	**BPGene Wash Buffer I**	8.12 ml (add 4.88 ml absolute ethanol before using)
5	1	**BPGene Wash Buffer II**	3.6 ml (add 14.4 ml absolute ethanol before using)
6	1	**BPGene Elution Buffer**	3 ml
7	1	**Spin column**	25x
8	1	**Collection tubes**	25x

1.3. Additional Reagents and Equipment Required

- Absolute ethanol
- Microcentrifuge capable of 13,000 rpm centrifugal force
- Microtubes, 1.5 ml, sterile
- Adjustable-volume micropipettes

1.4. Before you begin

1. Add 4.88 ml absolute ethanol to **BPGene Wash Buffer I** before using it for the first time.

2. Add 14.4 ml absolute ethanol to **BPGene Wash Buffer II** before using it for the first time.

3. All solutions must be clear and should not be used when precipitates formed. Allow the solutions to warm at room temperature before use to become clear.
4. 5.0 ml bacterium culture (OD_{600}, 1.5-2.5) which grown for 12 to 16 hours in desired broth medium (e.g., LB) containing a selective antibiotic need to give the best results.

2. Protocol

- ✓ **You must place the total buffers at room temperature for 15 min before starting the procedure except for BPGene Suspension Buffer/RNase A which must store at +4 °C to +8 °C till use.**
- ✓ **Then, you must place the BPGene Binding Buffer on ice before starting the procedure.**
- ✓ **Close bottles immediately after use.**

1- After preparation of the starting material, transfer the culture medium containing bacteria into a 1.5 ml sterile microtube and centrifuge at 8000 rpm for one minute and then discard the supernatant.

2- Pellet the bacterial cells from 5.0 ml of bacteria culture. Discard the supernatant.

3- Add 250 µl **BPGene Suspension Buffer/RNase** to the centrifuge tube containing the bacterial pellet. Resuspend the bacterial pellet using mild pipetting by a micropipette.

4- Add 250 µl **BPGene Lysis Buffer**. Mix gently by inverting the tube 3 to 5 times. To avoid shearing genomic DNA, do not vortex. Incubate for 5 minutes at temperature between +15 °C and +25 °C (Do not incubate for more than 5 minutes).

5- Add 350 µl chilled **BPGene Binding Buffer**. Mix gently by inverting the tube 3 to 5 times. Incubate on ice for 5 minutes. The solution should become cloudy and a flocculants precipitate should form.

6- Centrifuge for 10 minutes at approx. 13,000 rpm (full speed).

7- After centrifugation, insert one **Spin Column** into one **Collection Tube**. Transfer entire supernatant from Step 6 into Spin Column. Centrifuge for 1 minute at full speed. After centrifugation, Remove the Spin Column from the Collection Tube, discard the flowthrough liquid, and re-insert the Spin Column in the same Collection Tube.

8- Add 500 µl **BPGene Wash Buffer I** to the upper reservoir of the Spin Column. Centrifuge for 1 minute at full speed and discard the flowthrough.

9- Add 700 µl **BPGene Wash Buffer II** to the upper reservoir of the Spin Column. Centrifuge for 1 minute at full speed and discard the flowthrough.

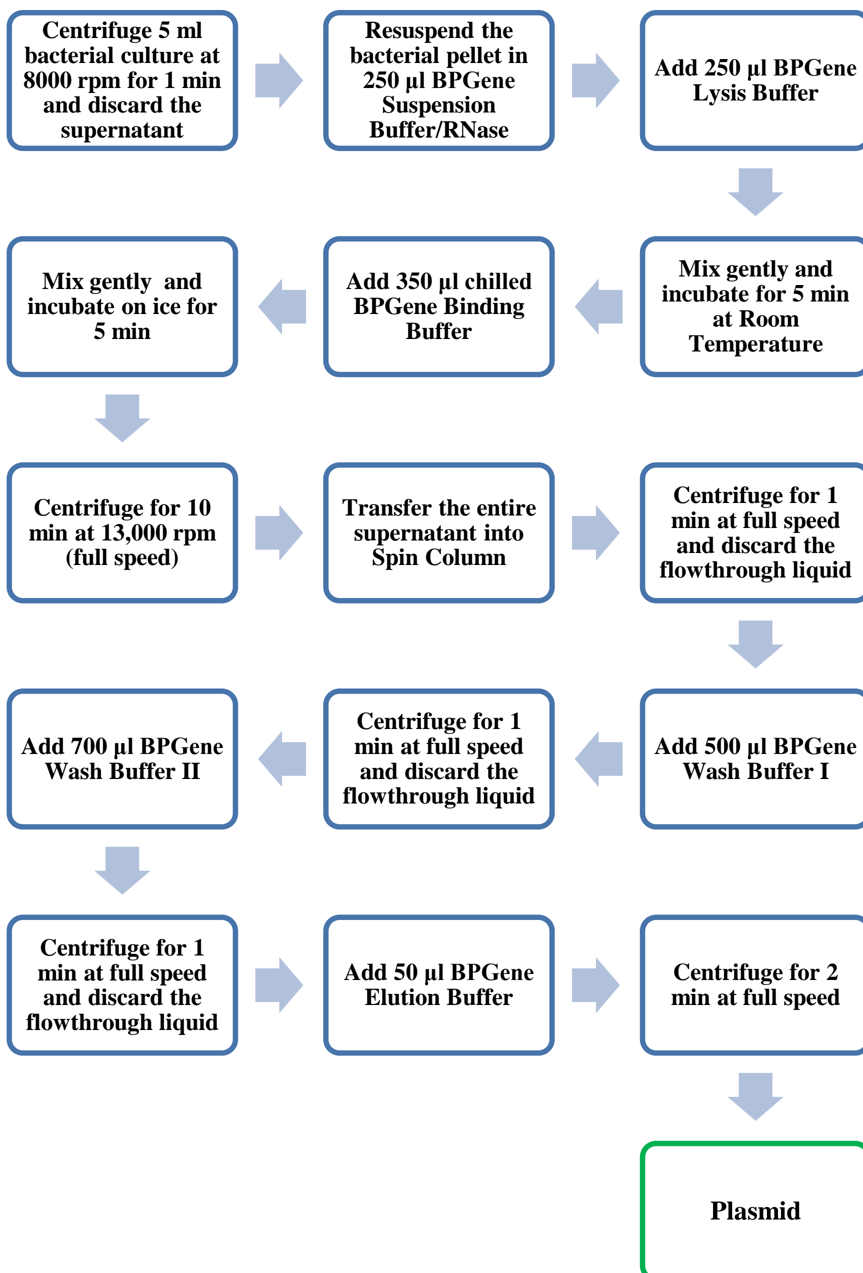
10- After discarding the flowthrough liquid, centrifuge the entire Spin Column assembly for additional 1 minute. Discard the Collection Tube.

11- Insert the Spin Column into a clean, sterile 1.5 ml microtube.

12- Add 50 µl **BPGene Elution Buffer** to the center part of the Spin Column. Centrifuge the tube assembly for 2 minute at full speed.

13- The microtube now contains the eluted plasmid DNA.

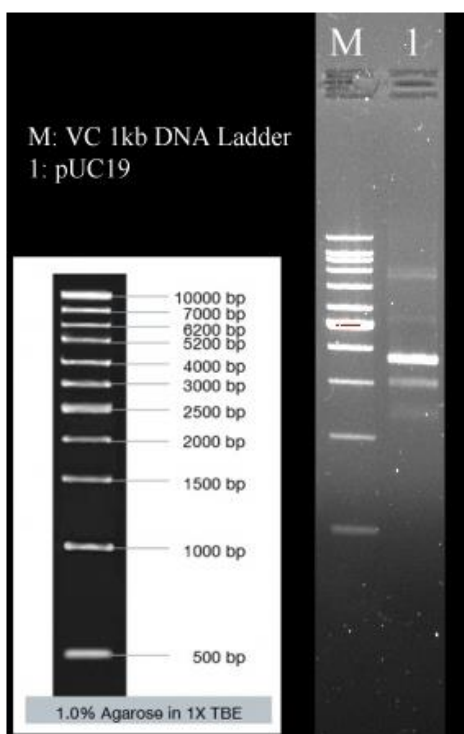
3. Experimental Overview



4. Results

4.1. Expected Yield

Yield is variable and depends both on the particular bacterium strain used and the cell density of the bacterial culture.



Plasmid DNA (pUC19) extracted from *Escherichia coli* strain XL1 blue using the **BPGene®** **Filter-Based Plasmid DNA Extraction Kit.**

A standard procedure of measuring DNA quality is determination of the absorption ratio of 260 nm/280 nm. For a pure DNA preparation, this ratio is between 1.7 and 2.0.

5. Troubleshooting

Observation	Possible cause	Recommendation
Low plasmid yield	Too few cells in starting material	Grow bacterium to an absorbance (OD_{600}) of 1.5-2.5 before harvest.
	Incomplete cell lysis	Be sure the bacterium pellet is completely resuspended in **BPGene Suspension Buffer.**
		Make sure the lysate is clear and viscous after the lysis step (incubation with **BPGene Lysis Buffer**).
		Make sure a cloudy white precipitate forms when **BPGene Binding Buffer** is added to the lysate. The precipitate should pellet completely during centrifugation.

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