BPGene® Non-Filter Plasmid DNA Extraction Kit

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

Cat. No. BPGene PEN011400 25 Reactions

Store the kit at +15 °C to +25 °C.

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1. General Information

The **BPGene®** **Non-Filter 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.

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 Solution I**	3 ml
2	1	**BPGene Solution II**	3 ml
3	1	**BPGene Solution III**	3 ml
4	1	**BPGene Solution IV**	4 ml

1.3. Additional Equipment and Reagents Required

- Microcentrifuge capable of 13,000 rpm centrifugal force
- Microtubes, 1.5 ml, sterile
- Adjustable-volume micropipettes
- Absolute ethanol
- 70% ethanol

1.4. Before you begin

- 5.0 ml bacterium cultures (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 BPGene Solution I and the BPGene Solution IV 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 10,000 rpm for 5 minute and then discard the supernatant.

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

3- Add 100 µl chilled **BPGene Solution I** to the centrifuge tube containing the bacterial pellet. Resuspend the bacterial pellet mildly using pipetting by a micropipete.

4- Add 100 µl **BPGene Solution II** and 100 µl **BPGene Solution III**. Mix gently by inverting the tube for 10 times. Incubate for 5 minutes at temperature between +15 °C to +25 °C (Do not incubate for more than 5 minutes).

5- Add 150 µl chilled **BPGene Solution IV**. Mix gently by inverting the tube for 10 times.

6- Centrifuge for 5 minutes at 13,000 rpm.

7- After centrifugation, transfer the supernatant of Step 6 into 1.5 ml sterile microtube and centrifuge for 5 minute at 13,000 rpm.

8- After centrifugation, transfer supernatant of Step 7 into 1.5 ml sterile microtube and add two-volume chilled absolute ethanol.

9- Centrifuge for 5 minute at 13,000 rpm and then discard the supernatant.

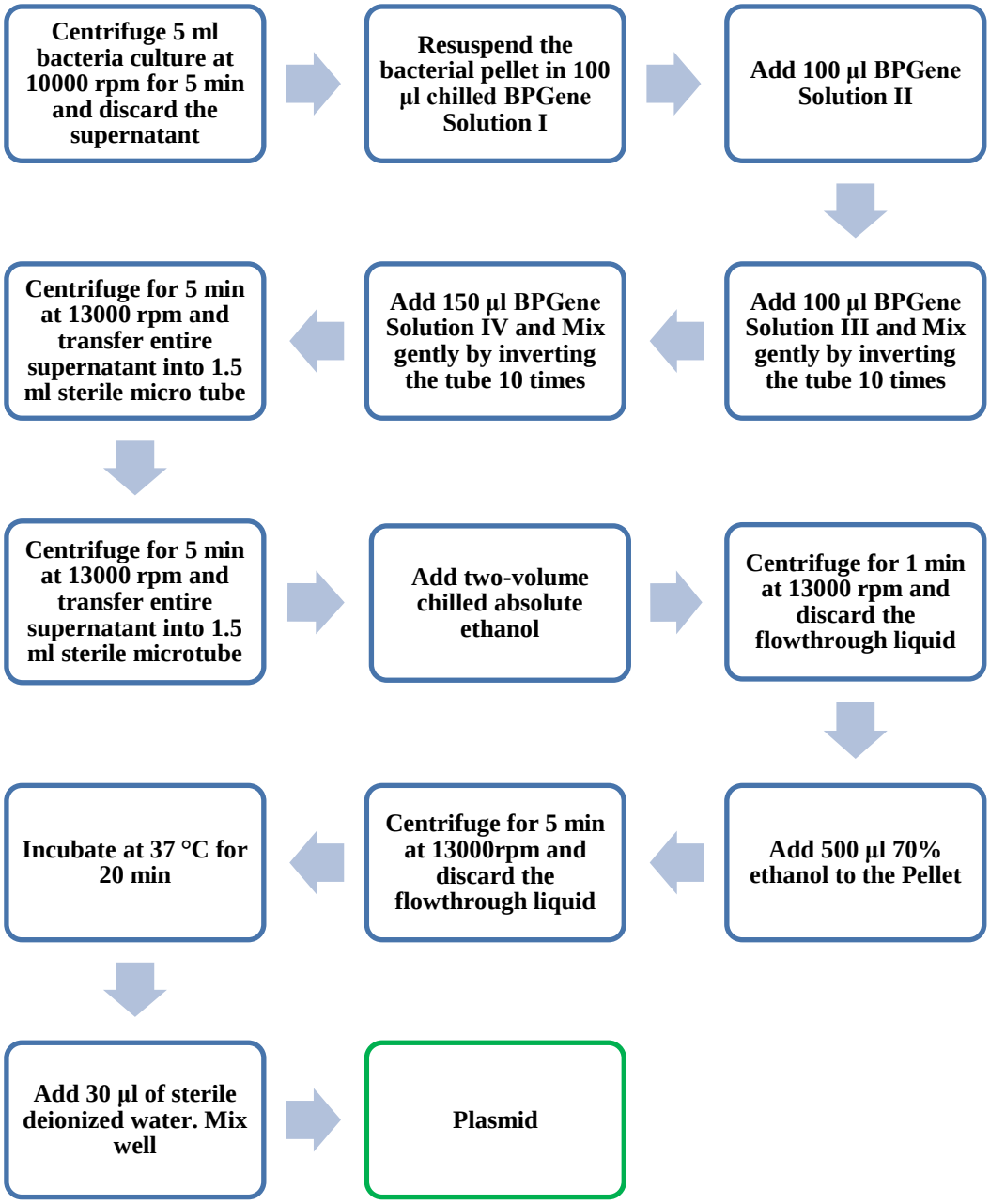
10- Add 500 µl of 70% ethanol to the pellet. Centrifuge for 5 minute at 13,000 rpm and discard the supernatant.

11- Place microtube containing pellet at 37 °C for 20 min.

12- Add 30 µl of sterile deionized water to the microtube. Mix well.

13- The microtube now contains the plasmid DNA.

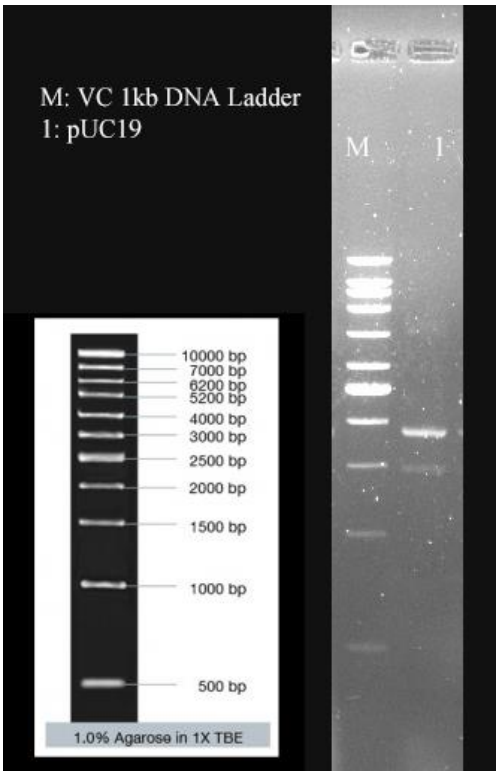
3. Experimental Overview



4. Results

4.1. Expected Yield

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



Plasmid DNA (pUC19) extracted from *Escherichia coli* strain XL1 blue using the **BPGene®** **Non-Filter 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 Solution I**
		Make sure the lysate is clear and viscous after the lysis step (after using **BPGene Solution I** and **BPGene Solution II**).
		Make sure a cloudy white precipitate forms when **BPGene Solution IV** is added to the lysate. The precipitate should pellet completely during centrifugation.