Plasmid DNA extraction V.3

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ABSTRACT

This protocol is used to extract plasmid DNA from E. coli.

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GUIDELINES

This protocol is based on E.Z.N.A.® Plasmid DNA Mini Kit I Protocol - Spin Protocol.

MATERIALS TEXT

- 100% ethanol
- Isopropanol
- Microcentrifuge capable of at least 13,000 x g
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Culture tubes
- Optional: sterile deionized water
- Optional: water bath or incubator capable of 70°C
- Optional: 3M NaOH solution

SAFETY WARNINGS

Please wear gloves before start.

BEFORE STARTING

Prepare DNA Wash Buffer, HBC Buffer, and Solution I .
1. Add the vial of RNase A to the bottle of Solution I and store at 2-8˚C;
2. Dilute DNA Wash Buffer with 100% ethanol 120ml and store at room temperature;
3.Dilute HBC Buffer with isopropanol and store at room temperature;
4.Check Solution II and Solution III for precipitation before use. Redissolve any precipitation by warming to 37˚C.

1 Solate a single colony from a freshly streaked selective plate, and inoculate a culture of 1- 5 mL LB medium containing the appropriate selective antibiotic. Incubate for ~12-16 hours at 37°C with vigorous shaking (~ 300 rpm). Use a 10-20 mL culture tube or a flask with a volume of at least 4 times the volume of the culture. It is strongly recommended that an endA negative strain of E. coli be used for routine plasmid isolation. Examples of such strains include DH5a® and JM109®.

2 Centrifuge at 10,000 x g for 1 minute at room temperature. 1m

10000 x g, Room temperature , 00:01:00

3 Decant or aspirate and discard the culture media.

4 Add 250 µL Solution I/RNase A. Vortex or pipet up and down to mix thoroughly.
Complete resuspension of cell pellet is vital for obtaining good yields.

RNase A must be added to Solution I before use.

5 Transfer suspension into a new 1.5 mL microcentrifuge tube.

6 Add 250 µL Solution II. Invert and gently rotate the tube several times to obtain a clear lysate. A 2-3 minute incubation may be necessary.

Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. Do not allow the lysis reaction to proceed more than 5 minutes. Store Solution II tightly capped when not in use to avoid acidification from CO2 in the air.

7 Add 350 µL Solution III. Immediately invert several times until a flocculent white precipitate forms.

It is vital that the solution is mixed thoroughly and immediately after the addition of Solution III to avoid localized precipitation.

8 Centrifuge at maximum speed (≥13,000 x g) for 10 minutes. A compact white pellet 10m

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will form. Promptly proceed to the next step.

15000 x g, Room temperature , 00:10:00

9 Insert a HiBind® DNA Mini Column into a 2 mL Collection Tube.

10 Transfer the cleared supernatant from Step 8 by CAREFULLY aspirating it into the HiBind® DNA Mini Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind® DNA Mini Column.

11 Centrifuge at maximum speed for 1 minute. 1m

15000 x g, Room temperature , 00:01:00

12 Discard the filtrate and reuse the collection tube.

13 Add 500 µL HBC Buffer

HBC Buffer must be diluted with isopropanol before use. Please see Page 6 for instructions.

14 Centrifuge at maximum speed for 1 minute. 1m

15000 x g, Room temperature , 00:01:00

15 Discard the filtrate and reuse collection tube.

16 Add 700 µL DNA Wash Buffer.

17 Centrifuge at maximum speed for 1 minute. 1m

15000 x g, Room temperature , 00:01:00

18 Discard the filtrate and reuse the collection tube.

19 Repeat step 16~18 once.

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20 Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at maximum speed to dry the column matrix.

21 Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

22 Add 30-100 µL Elution Buffer or sterile deionized water directly to the center of the column membrane.

The efficiency of eluting DNA from the HiBind® DNA Mini Column is dependent on pH. If using sterile deionized water, make sure that the pH is around 8.5.

23 Let sit at room temperature for 1 minute.

24 Centrifuge at maximum speed for 1 minute. 1m

15000 x g, Room temperature , 00:01:00

This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

25 Suck out the solution from the tube and re-add it to the center of the column membrane to give a second centrifuge. 1m

15000 x g, Room temperature , 00:01:00

26 Test the concentration and purity of DNA using NanoDrop.

27 Store DNA at -20°C.

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