2024 NEFU_China

Plasmid DNA extraction

ABSTRACT

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

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 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,W25113 and BWΔCD.

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

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

6.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.

7.Centrifuge at maximum speed (≥13,000 x g) for 10 minutes. A compact white pellet will form. Promptly proceed to the next step.

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

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

9.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.

10.Centrifuge at maximum speed for 1 minute.
15000 x g, Room temperature, 00:01:00
11.Discard the filtrate and reuse the collection tube.
12.Add 500 µL HBC Buffer HBC Buffer must be diluted with isopropanol before use.
13.Centrifuge at maximum speed for 1 minute.
15000 x g, Room temperature, 00:01:00
14.Discard the filtrate and reuse collection tube.
15.Add 700 µL DNA Wash Buffer.
16.Centrifuge at maximum speed for 1 minute.
15000 x g, Room temperature, 00:01:00
17.Discard the filtrate and reuse the collection tube.
18.Repeat step 16~18 once.
19.Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at maximum speed to dry the column matrix.
20.Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.
21.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.
22.Let sit at room temperature for 1 minute.
23.Centrifuge at maximum speed for 1 minute.
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.
24.Suck out the solution from the tube andre-add it to the center of the column membrane to give a second centrifuge.
15000 x g, Room temperature, 00:01:00
25.Test the concentration and purity of DNA using NanoDrop.
26.Store DNA at -20°C.