Isolation and Purification of Plasmid DNA from E. coli

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Abstract

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Guidelines

MATERIALS:

1) *E. coli*, strain ____, containing plasmid
2) LB media

    1.0% Bacto-tryptone

    0.5% Bacto-yeast extract

    1.0% NaCl

    Add the appropriate antibiotic to the growth media for the plasmid to be purified.
3) Chloramphenicol, 34 mg/mL, made up in 100% EtOH, filter sterilized.
4) Spectinomycin, 10 mg/mL, made up in d-H_2O, filter sterilized.
5) Lysozyme solution

    25 mM Tris-HCl, pH 8.0

    10 mM EDTA

    50 mM Glucose

    Add lysozyme to a concentration of 2 mg/mL just before use.
6) Alkaline SDS solution

    0.2 M NaOH

    1.0% SDS

Make up fresh from stock solutions for each use.

7) High salt solution, per 100 mL

60.0 mL of 5 M KOAc

11.5 mL of glacial acetic acid

28.5 mL of d-H_2O

8) 50 mM Tris-HCl, pH 8.0, 100 mM NaOAc

9) 10 mM Tris-HCl, pH 8.0, 1 mM EDTA (1X TE buffer)

10) Ethidium bromide, 10 mg/mL in d-H_2O

11) CsCl, solid

12) 100% EtOH

13) Isopropanol, CsCl/TE buffer saturated

14) 3 M NaOAc

15) Beckman Ti50 rotor quick seal tubes

16) Syringes, with 18g needles

17) Phenol, buffer-saturated

References

- C. Birnboim. A rapid Alkaline Extraction Method for the Isolation of Plasmid DNA.

  Methods In Enzymology **100**: 243-255.

- Maniatis, E.F. Fritsch, and J. Sambrook. Large-scale Isolation of Plasmid DNA.

  Molecular Cloning: A Laboratory Manual. Pages 86- 94.

Protocol

Step 1.
Inoculate 1000 mL of L broth containing the appropriate antibiotic with 10 mL of an overnight culture of the *E. coli* strain containing the plasmid to be purified.

Step 2.
Incubate at 37°C overnight.

DURATION
18:00:00

Step 3.
If amplification of the plasmid DNA is necessary (because of low copy number of the plasmid under normal conditions), after 4-8 hours of growth, add chloramphenicol (34 mg/mL) to a final concentration of 170 ug/mL or spectinomycin (10 mg/mL) to a final concentration of 50 ug/mL (if the bacteria carries chloramphenicol resistance).

Step 4.
Incubate at 37°C overnight.

DURATION
18:00:00

Step 5.
Centrifuge the bacterial cells in the Sorvall GSA rotor at 5,000 rpm, 5 min, 4°C.

DURATION
00:05:00

Step 6.
Use 3-250 mL GSA bottles per liter of bacteria.

Step 7.
Discard the supernatants.

Step 8.
Resuspend the cells with 60 mL (total, 20 mL/bottle) of lysozyme solution.

Step 9.
Incubate on ice for 30 min.

DURATION
00:30:00

Step 10.
Add 120 mL (total, 40 mL/bottle) of the alkaline SDS solution.

Step 11.
Gently mix (by inversion) until the solution clears.

Step 12.
Incubate on ice for 5 min.

DURATION
00:05:00

Step 13.
Add 90 mL (total, 30 mL/bottle) of the high salt solution.

Step 14.
Mix well and incubate on ice for 30-60 min.

DURATION
01:00:00

Step 15.
Centrifuge the bottles in the Sorvall GSA rotor at 10,000 rpm, 10 min, 4°C.

DURATION
00:10:00

Step 16.
Decant the supernatants to clean bottles.

Step 17.
Precipitate the DNA with 2X volumes (approximately 170-180 mL) of 100% EtOH.

Step 18.
Hold at -80°C for 60 min.

DURATION
01:00:00

Step 19.
Centrifuge the bottles in the Sorvall GSA rotor at 10,000 rpm, 10 min, 4°C.

DURATION
00:10:00

Step 20.
Discard the supernatants.

Step 21.
Resuspend the pellets with 10 mL each of 50 mM Tris-HCl, pH 8.0, 100 mM NaOAc.

Step 22.
Transfer the material to 30 mL corex tubes.

Step 23.
Add an equal volume of buffer-saturated phenol to each tube.

Step 24.
Mix well and centrifuge in the Sorvall SS34 rotor at 10,000 rpm, 10 min, room temperature.

DURATION
00:10:00

Step 25.
Remove the upper aqueous layers to clean 30 mL corex tubes.

Step 26.
Precipitate the DNAs with 2X volumes of 100% EtOH.

Step 27.
Hold at -80°C for 45 min.

DURATION
00:45:00

Step 28.
Centrifuge the tubes in the Sorvall HB-4 rotor at 10,000 rpm, 10 min, 4°C.

DURATION
00:10:00

Step 29.
Discard the supernatants.

Step 30.
Dry the pellets briefly (10-15 min) in the vacuum desiccator to remove the EtOH.

DURATION
00:15:00

Step 31.
Resuspend each plasmid DNA with a total of 10 mL of 1X TE buffer.

Step 32.
Weigh out exactly 6.8 gm of CsCl into each of 2-30 mL corex tubes for each plasmid DNA.

Step 33.

Divide each plasmid DNA solution between the 2 tubes of CsCl, measuring the volume.

Step 34.
Add 1X TE buffer to the tubes of CsCl to a final volume of 6.4 mL added to each tube.

Step 35.
Mix well, until all the CsCl is in solution.

Step 36.
Add 512 µL of ethidium bromide (10 mg/mL) to each tube.

Step 37.
Mix well.

Step 38.
Centrifuge the tubes in the Sorvall SS34 rotor at 10,000 rpm, 15 min, 4°C.

DURATION
00:15:00

Step 39.
Transfer the supernatants to Beckman polyallomar Ti50 rotor quick-seal tubes using a syringe and needle (18g).

Step 40.
Fill the tubes to capacity with a CsCl/ethidium bromide solution (6.8 gm CsCl, 6.4 mL 1X TE buffer, 512 µL 10 mg/ml ethidium bromide).

Step 41.
Seal the tubes with the Beckman tube sealer.

Step 42.
Centrifuge the gradients in the Beckamn Ti50 rotor at 35,000 rpm, 72 hours, 25°C.

DURATION
12:00:00

NOTES
Irina Agarkova 19 Apr 2016
Alternatively, centrifuge in the Beckman Ti70.1 rotor at 45,000 rpm, 24-40 hours, 25°C.

Step 43.
Visualize the DNA using a UV light source.

Step 44.
Remove the plasmid DNA from the gradients by puncturing the side of the tube with an 18g needle and syringe.

Step 45.
Place a small amount of Vaseline on the side of the tube and puncture through the Vaseline.

Step 46.
Smear the Vaseline over the puncture hole when the syringe is removed to seal the hole.

Step 47.
Circular plasmid DNA is the lower band in the gradient. Chromosomal DNA and nicked circular DNA is in the upper band in the gradient.

Step 48.
Place the DNA solution into 30 mL corex tubes.

Step 49.
Extract the ethidium bromide from the DNA solution by adding an equal volume of CsCl/TE buffer

saturated isopropanol.

Step 50.
Mix gently and centrifuge in the Sorvall SS34 rotor at 3,000 rpm, 1 min, 4°C.

DURATION
00:01:00

Step 51.
Pipet off the upper isopropanol layer.

Step 52.
Repeat the isopropanol extraction 1X.

Step 53.
Add 1.0 mL of 3 M NaOAc to each tube of DNA and adjust the volume in each tube to 10.0 mL with 1X TE buffer.

Step 54.
Precipitate the DNAs with 2X volumes of 100% EtOH.

Step 55.
Hold at -20°C overnight.

DURATION
18:00:00

Step 56.
Centrifuge the tubes in the Sorvall HB-4 rotor at 10,000 rpm, 10 min, 4°C.

DURATION
00:10:00

Step 57.
Discard the supernatants.

Step 58.
Dry the pellets briefly (10-15 min) in the vacuum desiccator to remove the EtOH.

DURATION
00:15:00

Step 59.
Resuspend the plasmid DNAs with 1X TE buffer.

Step 60.
Determine the DNA concentration.

Step 61.
Store the DNAs at 4°C.