Jun 14, 2016

Isolation and Purification of Plasmid DNA from E. coli

DOI
https://dx.doi.org/10.17504/protocols.io.ertbd6n

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DOI: https://dx.doi.org/10.17504/protocols.io.ertbd6n

Protocol Citation: David Dunigan and Irina Agarkova 2016. Isolation and Purification of Plasmid DNA from E. coli. **protocols.io** https://dx.doi.org/10.17504/protocols.io.ertbd6n

Protocol status: Working

Created: March 25, 2016

Last Modified: February 15, 2018

Protocol Integer ID: 2579

Keywords: purification of plasmid dna, plasmid dna, plasmid, dna, purification

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.

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.

2 Incubate at 37°C overnight.
18:00:00

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).

4 Incubate at 37°C overnight.
18:00:00

5 Centrifuge the bacterial cells in the Sorvall GSA rotor at 5,000 rpm, 5 min, 4°C.
00:05:00

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

7 Discard the supernatants.

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

9 Incubate on ice for 30 min.
00:30:00

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

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

12 Incubate on ice for 5 min.
00:05:00

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

14 Mix well and incubate on ice for 30-60 min.
01:00:00

15 Centrifuge the bottles in the Sorvall GSA rotor at 10,000 rpm, 10 min, 4°C.
00:10:00

16 Decant the supernatants to clean bottles.

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

18 Hold at -80°C for 60 min.
01:00:00

19 Centrifuge the bottles in the Sorvall GSA rotor at 10,000 rpm, 10 min, 4°C.
00:10:00

20 Discard the supernatants.

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

22 Transfer the material to 30 mL corex tubes.

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

24 Mix well and centrifuge in the Sorvall SS34 rotor at 10,000 rpm, 10 min, room temperature.
00:10:00

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

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

27 Hold at -80°C for 45 min.
00:45:00

28 Centrifuge the tubes in the Sorvall HB-4 rotor at 10,000 rpm, 10 min, 4°C.
00:10:00

29 Discard the supernatants.

30 Dry the pellets briefly (10-15 min) in the vacuum desiccator to remove the EtOH.
00:15:00

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

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

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

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

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

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

37 Mix well.

38 Centrifuge the tubes in the Sorvall SS34 rotor at 10,000 rpm, 15 min, 4°C.
00:15:00

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

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).

41 Seal the tubes with the Beckman tube sealer.

42 Centrifuge the gradients in the Beckamn Ti50 rotor at 35,000 rpm, 72 hours, 25°C.
72:00:00

Note

Alternatively, centrifuge in the Beckman Ti70.1 rotor at 45,000 rpm, 24-40 hours, 25°C.

43 Visualize the DNA using a UV light source.

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

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

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

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.

48 Place the DNA solution into 30 mL corex tubes.

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

50 Mix gently and centrifuge in the Sorvall SS34 rotor at 3,000 rpm, 1 min, 4°C.
00:01:00

51 Pipet off the upper isopropanol layer.

52 Repeat the isopropanol extraction 1X.

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.

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

55 Hold at -20°C overnight.
18:00:00

56 Centrifuge the tubes in the Sorvall HB-4 rotor at 10,000 rpm, 10 min, 4°C.
00:10:00

57 Discard the supernatants.

58 Dry the pellets briefly (10-15 min) in the vacuum desiccator to remove the EtOH.
00:15:00

59 Resuspend the plasmid DNAs with 1X TE buffer.

60 Determine the DNA concentration.

61 Store the DNAs at 4°C.