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Recovering Plasmid DNA from Bacterial Culture

Forked from Recovering Plasmid DNA from Bacterial Culture

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NCI Genomics

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol is for recovering plasmid DNA from Bacterial Culture. To see the full abstract and other resources, visit **https://www.addgene.org/protocols/purify-plasmid-dna/**.

Guidelines

Tips and FAQ

- Plasmid purification kits provide the fastest way to obtain a high concentration of clean plasmid DNA. To improve the purity of plasmid DNA purified without a kit it is advisable to perform a phenol/chloroform extraction of the supernatant after step 6 and before step 7. This will help to remove proteins and other contaminants from the plasmid DNA.

- It is also advisable to add RNAse to the supernatant after step 6 to eliminate RNA contamination. This is included in the resuspension buffer of most kits.

Materials

Equipment

- Desktop microcentrifuge
- Desktop vortexer
- Vacuum (optional)

Reagents

- Overnight culture of bacteria transformed with your plasmid
- Resuspension buffer
- Denaturing solution
- Renaturing solution
- 2 mg/mL RNase A
- TE or water-saturated phenol-chloroform
- Chloroform
- 100% ethanol or isopropanol
- 90% ethanol
- 70% ethanol
- TE buffer
- 3 M Na-acetate (pH 4.8)

Troubleshooting

Generalized DNA Purification

1 Grow an **overnight culture of bacteria.**

Note

Pro-Tip
Refer to appropriate DNA prep protocol for volume of bacteria to grow (low copy plasmids require larger cultures).

2 Centrifuge the culture to pellet the bacteria before proceeding with DNA preparation.

Note

Pro-Tip
If your entire overnight culture cannot fit into a single centrifuge tube, aliquot it into several tubes/bottles.

3 Remove the supernatant and resuspend the bacteria in buffer.

Note

Note, this step gets all of the bacteria back into suspension, but within a smaller volume of buffer that is compatible with the next solution.

4 Add a denaturing solution to the resuspended bacteria.

Note

Note, this step causes the bacteria to lyse, releasing their contents, including plasmid DNA, into solution.

5 Add a renaturing solution to the denatured bacteria.

Note

Note, this step brings the pH back down causing the proteins and genomic DNA to precipitate, while leaving the smaller plasmids free in solution.

6 Pellet the proteins and genomic DNA by centrifugation, and remove the plasmid-containing supernatant.

7 Add either ethanol or isopropanol to precipitate the plasmid DNA.

8 Either spin to pellet the DNA or apply the solution to a column that will bind the now precipitated DNA.

9 Wash the pellet or column with 70% ethanol to remove excess salt.

10 Resuspend the DNA pellet, or elute the DNA off of the column using water or a neutral buffer such as TE. You will now have plasmid DNA that has been purified away from the bacterial proteins and genomic DNA. Depending on the method used, the DNA concentration and purity will vary. For more information on determining DNA concentration and purity click **here**.

Protocol: Kit-free Alkaline Lysis Plasmid Miniprep

11 **Solution I - Resuspension Buffer**
25 mM Tris-HCl (pH 8)
50 mM glucose
10 mM EDTA

Note

Store Solution I at 4 °C.

12 **Solution II - Denaturing Solution**
0.2 N NaOH
1.0% SDS

Note

Store Solution II at Room temperature.

13 **Solution III - Renaturing Solution (Potassium Acetate)**

120 mL 5M Potassium acetate

23 mL glacial acetic acid

57 mL of dH_2O

Note

Store Solution III at 4 °C.

14 Grow 2 mL overnight cultures from single colonies of bacteria containing your plasmid of interest.

15 Add 1.5 mL of the stock culture to a 1.75 mL microfuge tube.

16 Centrifuge in microfuge tube at 10000 x g for 00:00:30.

17 Pour off the supernatant, being careful not to disturb the bacterial pellet.

18 Resuspend the pellet in 100 µL of cold Solution I.

19 Vortex the solution for 00:02:00 or until all bacteria are fully resuspended.

20 Add 200 µL of Solution II and invert the tube carefully 5 times to mix the contents. The contents will become clear and thicker as the proteins and DNA are denatured.

Note

Pro-Tip
Do not vortex at this stage or the genomic DNA will become sheared and will therefore contaminate your purified plasmid DNA.

21 Incubate solution on ice for 00:05:00 .

22 Add 150 µL of cold Solution III to each tube.

23 Mix by inverting several times. A white precipitate will be formed which contains the bacterial proteins and genomic DNA.

24 Incubate tube on ice for 00:05:00 .

25 Centrifuge the tube for 00:05:00 at 12000 x g .

Note

Notes:
- Pellet contains proteins, cell fragments, salt and other extra particles from solutions.
- Supernatant contains the plasmid DNA separated from bacterial chromosomes.

26 Collect the supernatant into a new tube by pipetting or carefully pouring.

27 (Optional) Add 5 µL of 2 mg/ml RNase A to the supernatant in the new tube and incubate at 37 °C for 00:05:00 .

Note

Note, Ribonuclease A (RNase A) is a pancreatic ribonuclease that digests single-stranded RNA.

28 (Optional) Perform phenol-chloroform extraction - see protocol below.

Note

Note, phenol-chloroform extraction removes remaining contaminant proteins and RNase A from the DNA sample. When phenol is mixed with the aqueous solution containing DNA, proteins will move into the phenol phase and will be separated from the aqueous DNA.

29 Add either 700 µL of cold 100% ethanol or 350 µL room temperature isopropanol to the solution to precipitate the plasmid DNA; see detailed protocol section below.

Note

Pro-Tip
If precipiating with ethanol, it is often thought that an incubation of 00:20:00 to overnight at -20 °C or -80 °C will improve precipitation.

30 Pour out the supernatant.

31 (Optional) Wash the pellet with 70% ethanol.

Note

Note, this step removes excess salt from the pellet which can cause problems with some common reactions.

32 Air dry the pellet (can be done by inverting the tube at an angle over kimwipe) for 00:20:00 - 00:30:00 .

33 Resuspend pellet with 25 µL - 50 µL of TE.

Protocol: Phenol-Chloroform Extraction of DNA Samples

34 Add an equal volume of TE-saturated phenol-chloroform to the aqueous DNA sample.

Note

Pro-Tip
Water-saturated phenol-chloroform can be used if TE-saturated is not available.

35 Vortex microfuge tube for 00:00:30 - 00:01:00 .

36 Centrifuge the tube for 00:05:00 at Room temperature on the highest speed setting.

Note

Note, you should see clearly separated layers:

Top Phase - Aqueous DNA phase
Middle phase - A white layer may appear, consisting of precipitated protein particles
Bottom phase - Organic phase (protein)

37 Pipet the aqueous DNA layer and place it in a new microfuge tube.

38 Add equal volume of chloroform to the recovered aqueous DNA layer.

39 Repeat steps 2-4.

Safety information

Phenol-chloroform is a hazardous waste - DO NOT pour down sink.

Protocol: Ethanol Precipitation

40 To your DNA solution, add 2-2.5 volumes 95% or 100% ethanol and 1/10 volume of 3 M Na-acetate (pH 4.8).

41 Invert the microfuge tube to mix.

42 (Optional) Place the tube either at -20 °C overnight OR -80 °C for 00:30:00 OR on dry ice for 00:05:00 .

Note

Note, this freezing may help the DNA to precipitate.

43 Centrifuge solution at high speed (at least 12000 rpm) for 00:15:00 - 00:30:00 at 4 °C .

Note

Notes:
- Pellet contains the precipitated DNA.
- Supernatant contains residues, salts, and water.

44 Pour out the supernatant in the sink.

45 Open and invert the tubes on a paper towel to drain them out.

46 Wash pellet by adding 500 µL cold 70% ethanol.

Note

Note, this helps to remove excess salt from the DNA pellet.

47 Centrifuge solution at high speed (at least 12000 rpm) for 00:05:00 at Room temperature .

48 Pour out the supernatant in the sink.

Note

Pro-Tip
Be careful, the pellet is harder to see and less well attached to the tube after the 70% ethanol wash. You can also pipet the supernatant out of the tube if you are worried about losing the pellet.

49 Dry with vacuum or by inverting over paper towel for 00:05:00 - 00:20:00 .

50 Resuspend dry DNA with TE (10 mM Tris-HCl pH 8, 0.1 mM EDTA).

Note

Pro-Tip
DNA resuspension can take time, it is a good idea to let it sit for several hours to overnight at room temperature before quantifying and using.

51 Store DNA at 4 °C .