Maxiprep - Alkaline Lysis

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ATTENTION: This is a low priced protocol. Use it preferably!

1. Pick colony and grow in 3 ml LB over night at 37°C
2. Inoculate 500 ml LB with antibiotics and grow over night at 37°C
3. Spin down **5 min at 4000 G at 4°C**
4. Resuspend pellet in **100 ml cold STE** buffer
5. Spin down **5 min** at 4000 G at 4°C
6. Resuspend pellet in **20 ml ALS-I** buffer
7. Add **400 µl Lysozym**
   Mix well by swirling the tube
8. Incubate for **5 min** at room temperature
9. Add **40 ml ALS-II**
   Mix well by swirling the tube
10. Incubate for **10 min** at room temperature
11. Add **30 ml ice cold ALS-III** buffer
12. Incubate for **10 min on ice** and mix again by swirling
13. Spin down for **15 min** at **5000 G**
    ...
14. Filter the supernatant through 4 layers of cheesecloth/miracloth
15. Add **55 ml Isopropanol**
16. Precipitate for at least **10 min** at room temperature
17. Spin down for **20 min at 6000 G**
18. Decant the supernatant
19. **Transfer the pellet** with 5-10 ml Ethanol (70 %) into a 15 ml tube
20. Spin down for **20 min at 4000 G**
21. **Decant the supernatant** and let dry at room temperature
22. **Dissolve** the pellet in **2.5 ml TE**
23. Add **40 µl RNAse A** solution
24. Incubate for at least **10 min** at room temperature
    ...
25. Add **1.5 ml phenol, 1.5 ml chloroform and 60 µl isoamylalcohol**
    Mix well by inverting the tube
26. Spin down for **5 min at 4000 G**

27. Transfer the **upper phase** into a new 15 ml tube
28. Add **3 ml chloroform and 120 µl isoamylalcohol**
    Mix well by inverting the tube
29. Spin down for **5 min** at 4000 G
30. Transfer the **upper phase** into a new 15 ml tube
31. Add **3 ml chloroform and 120 µl isoamylalcohol**
    Mix well by inverting the tube
32. Spin down for **5 min** at 4000 G
33. Transfer the **upper phase** into a new 15 ml tube
    ...
34. Add **300 µl NaAcetate** (3 M, pH 5.2) and **1.8 ml isopropanol**
    Mix well by inverting the tube several times
35. Precipitate for at least **10 min** at room temperature
36. Spin down for **20 min** at 4000 G
37. Remove the supernatant and add **15 ml of 70% ethanol**
    Mix well by inverting the tube several times
38. Spin down for **10 min** at 4000 G
39. **Remove the supernatant** and add **15 ml of 70% ethanol**
    Mix well by inverting the tube several times
40. Spin down for **10 min** at 4000 G
41. **Remove the supernatant** and spin down for **5 min** at 4000 G
    Pipett of the reminding liquid
42. Dry at room temperature for 5 min
43. Add **500-1000 µl water**

Buffers (for 10 Maxipreps):

ALS-I:	**ALS-II:**
2.3 g Glucose	4 g NaOH
6.3 ml TrisHCl (Stock: 1 M; pH 8.0)	50 ml SDS (Stock: 10 %)
5 ml EDTA (Stock: 0.5 M; pH 8.0)	
add water to 250 ml, store at 4 °C	add water to 500 ml, store at room temperature

ALS-III:

73.8 g potassium acetate

28.8 ml glacial acetic acid

should have pH 4.8

add water to 250 ml,
store at room temperature

STE:

5.8 g NaCl

10 ml TrisHCl (Stock: 1 M; pH 8.0)

2 ml EDTA (Stock: 0.5 M; pH 8.0)

add water to 1 liter, store at 4 °C

Stock Solutions:

1 M TrisHCl (pH 8.0)

0.5 M EDTA (pH 8.0)

10 mg / ml RNAse A

10 % (w/v) SDS

50 mg / ml Lysozym

Buffer-Concentration:

ALS-I:

50 mM Glucose

25 mM TrisHCl (pH 8.0)

10 mM EDTA (pH 8.0)

ALS-II:

0.2 M NaOH

1 % SDS

ALS-III:

3 M potassium acetate

11.5 % v/v glacial acetic acid

should have pH 4.8

STE:

0.1 M NaCl

10 mM TrisHCl

1 mM EDTA

Materials needed:

Miracloth (# 475855, 1R) by Calbiochem

Commented Protocol:

1. Pick colony and grow in 3 ml LB over night at 37°C

Most of the times you have a rest of the miniprep in the fridge what can be used now.

2. Inoculate 500 ml LB with antibiotics and grow over night at 37°C

Use 100 µl to inoculate a maxiprep, normally I just use some and dont care about amounts so much.

3. Spin down 5 min at 4000 G at 4°C

Most protocols state here 15 min. I prefer to loose some bacteria and to work on faster. If you see that with your bacteria you can not spin down 95 % in 5 min, then extend the time.

If 500 ml dont fit in one bucket, you can just spin down, decant the supernatant, load more solution, spin down, decant and so on. The bacteria don't mind (and will be lysed anyway).

4. Resuspend pellet in 100 ml cold STE buffer

To wash the medium of the bacteria.

5. Spin down 5 min at 4000 G at 4°C

6. Resuspend pellet in 20 ml ALS-I buffer

Do not leave any pieces of the pellet undissolved, or the lyses will be incomplete.

7. Add 400 µl Lysozym

Mix well by swirling the tube

Add the Lysozyme and immediately mix (because lysis starts fast turning the liquid into gel). Now the enzymatic degradation of the cell wall happens.
Most protocols recommend to add the Lysozym in the ALS-I buffer before resuspension. I found that very unhandy. The bacteria lyse before you have the complete pellet resuspended and everything gets messy. If you add the Lysozyme afterwards you can first resuspend very relaxed all your pellets and then you add it into all buckets and you get a homogenious lysis.

8. Incubate for 5 min at room temperature

9. Add 40 ml ALS-II

Mix well by swirling the tube

10. Incubate for 10 min at room temperature

Now the alkali lysis happens. Do not extend the time, longer denaturation time can result in useless DNA

11. Add 30 ml ice cold ALS-III buffer

To neutralize the NaOH.

12. Incubate for 10 min on ice and mix again by swirling

Now the SDS and some proteins flocks out. The solution can be stored much longer at this step if you want.

13. Spin down for 15 min at 5000 G

...

To remove the SDS and some proteins that flocked out.

14. Filter the supernatant through 4 layers of cheesecloth/miracloth

You can also spin again until you separated the liquid from the solid - but cheesecloth/miracloth is really handy for that. Maybe gauze as it is used in hospitals works also well, take 4 - 6 layers.

15. Add 55 ml Isopropanol

To precipitate the DNA.

16. Precipitate for at least 10 min at room temperature

The solution can be stored much longer at this step if you want.

17. Spin down for 20 min at 6000 G

18. Decant the supernatant

The pellet may be spread over the whole outside wall. So watch your bucket carefully.

19. Transfer the pellet with 5-10 ml Ethanol (70 %) into a 15 ml tube

Just flush the pellet of the wall by pipetting ethanol at it. I pipet the DNA as a suspension into the 15 ml tube. I repeat it several times until the bucket is clean and the 15 ml tube full.

20. Spin down for 20 min at 4000 G

21. Decant the supernatant and let dry at room temperature

Just remove all liquid on top, it does not have to be dry.

22. Dissolve the pellet in 2.5 ml TE

I normally break first the pellet in smaller pieces with a yellow tip, than it dissolves much faster when I add the TE. It can be stored in this solution over night at -20 °C

23. Add 40 µl RNAse A solution

Mix well by pipetting up and down with a 5 ml pipet.

24. Incubate for at least 10 min at room temperature

...

25. Add 1.5 ml phenol, 1.5 ml chloroform and 60 µl isoamylalcohol

Mix well by inverting the tube

From here on work in a **fume hood**!!!! If you pipet up and down in the chloroform BEFORE you transfer it into you sample you can prevent leaking from the tip (the gas phase in the pipet gets saturated with chloroform).

Also a premix can be used. I prefer to add it like that because I only have 4-6 samples at one and this are not very homogenious as a mixture. This step removes remaining proteins.

26. Spin down for 5 min at 4000 G

27. Transfer the upper phase into a new 15 ml tube

Thats the water phase...

28. Add 3 ml chloroform and 120 µl isoamylalcohol

Mix well by inverting the tube

To remove rests of phenol.

29. Spin down for 5 min at 4000 G

30. Transfer the upper phase into a new 15 ml tube

31. Add 3 ml chloroform and 120 µl isoamylalcohol

Mix well by inverting the tube

32. Spin down for 5 min at 4000 G

33. Transfer the upper phase into a new 15 ml tube

...

Can be stored for a long time at -20°C

34. Add 300 µl NaAcetate (3 M, pH 5.2) and 1.8 ml isopropanol

Mix well by inverting the tube several times

To precipitate the DNA again.

35. Precipitate for at least 10 min at room temperature

Time can be extended.

36. Spin down for 20 min at 4000 G

This is enough time and force. Most of the DNA is spin down after 5 min.

37. Remove the supernatant and add 15 ml of 70% ethanol

Mix well by inverting the tube several times

Be careful!!! Try to keep an eye on it during the removal of the supernatant to now loose it. It does not sick very well to the eppi, that is why I always pipet of the supernatant, just to be sure.

38. Spin down for 10 min at 4000 G

39. Remove the supernatant and add 15 ml of 70% ethanol

Mix well by inverting the tube several times

Sometimes I only wash once.

40. Spin down for 10 min at 4000 G

41. Remove the supernatant and spin down for 5 min at 4000 G

Pipett of the reminding liquid

Its the fastest way to remove the last drops of liquid.

42. Dry at room temperature for 5 min

Not too much, otherwise it won't dissolve any more.

43. Add 500-1000 µl water

If it gets too jelly, add more water. You can expect concentrations up to 2 µg / µl.

Known Issues:

- If you don't add RNAse A you can not digest the DNA and load it on gel. The big amounts of RNA will outshine your bands. This happened to me many times....

References and Comments:

This is in my hands the best protocol for prepping DNA in large amounts. You can get 1-10 mg out of 500 ml bacteria culture.

I did it as described before many times and never had any problems.

How to cite this page in publications:

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