# Miniprep - Alkaline Lysis

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Version: 1.0 - Print Version (.PDF)

**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. Spin down 1.5 ml for **2 min** at 8000 rpm (6000 G) store the rest at 4°C
3. Resuspend pellet in **150 µl ALS-I** buffer
4. Add **300 µl ALS-II** buffer
5. Mix well by inverting the tube several times
6. Incubate for **10 min** at room temperature
7. Add **225 µl ALS-III** buffer
8. Spin down for **10 min** at 13000 rpm (18000 G)
9. Optional: (only needed for Agrobacterium)
   For E.coli go directly to step 16
10. Transfer supernatant to a tube containing **350 µl phenol and 350 µl chloroform**
11. Mix well by shaking
12. Spin down for **5 min** at 13000 rpm (18000 G)
13. Transfer supernatant to a tube containing **700 µl chloroform**
14. Mix well by shaking
15. Spin down for **5 min** at 13000 rpm (18000 G)
16. Transfer **600 µl supernatant** to a tube containing **360 µl isopropanol**
17. Mix well by inverting the tube several times
18. Spin down for **10 min** at 13000 rpm (18000 G)
19. Remove the supernatant and add **700 µl of 70% ethanol**
20. Mix well by inverting the tube several times
21. Spin down for **5 min** at 13000 rpm (18000 G)
22. Remove the supernatant and dry at room temperature for 1 min
23. Add **50 µl TER**

# Buffers:

**ALS-I:**

0.9 g Glucose

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

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

add water to 100 ml, store at 4 °C

**ALS-II:**

0.8 g NaOH

10 ml SDS (Stock: 10 %)

add water to 100 ml,
store at room temperature

**ALS-III:**

29.5 g potassium acetate

11.5 ml glacial acetic acid

should have pH 4.8

add water to 100 ml,
store at room temperature

**TER:**

10 µl TrisHCl (Stock: 1 M; pH 8.0)

2 µl EDTA (Stock: 0.5 M; pH 8.0)

2 µl RNAse A

add water to 1 ml, store at 4 °C

**Stock Solutions:**

1 M TrisHCl (pH 8.0)

0.5 M EDTA (pH 8.0)

10 % (w/v) SDS

10 mg / ml RNAse A

# 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

**TER:**

10 mM TrisHCl (pH 8.0)

1 mM EDTA (pH 8.0)

0.02 mg / ml RNAse A

# Commented Protocol:

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

3 ml LB are fine if you prep 1.5 ml, use 4 ml if you want to prep 3 ml. Then you have still enough to inoculate a maxiprep, 500 µl for a glycerol stock or similar things...

## 2. Spin down 1.5 ml for 2 min at 8000 rpm (6000 G) store the rest at 4°C

Most other protocols recommend longer and higher centrifugation steps, but this step gives you a pellet that is easy to resuspend and would not fall off during the following steps. Do not pipet off the supernatant - open the eppi, discard all liquid inside and beat the eppi hard and several times upside-down on a piece of paper towel! The pellet will stand it for sure and the liquid is efficient and fast removed.

## 3. Resuspend pellet in 150 µl ALS-I buffer

You have to resuspend each pellet with a fresh tip. Do not leave any pieces of the pellet undissolved, or the lyses will be incomplete.

## 4. Add 300 µl ALS-II buffer

Just open all eppis, use one tip to pipet into all eppis.

## 5. Mix well by inverting the tube several times

Shake the whole eppi-rack strongly.

## 6. Incubate for 10 min at room temperature

Protocols state here 5-10 min. Do not extend the time, longer denaturation time can result in useless DNA. With some strains the solution clears after some time.

## 7. Add 225 µl ALS-III buffer

Just open all eppis, use one tip and pipet into all eppis, close them and shake the whole eppi-rack strongly. The solution can be stored much longer at this step if you want.

## 8. Spin down for 10 min at 13000 rpm (18000 G)

Probably 5 min are enough.

## 9. Optional: (only needed for Agrobacterium)

For E.coli go directly to step 16.

This is not needed for E.coli strains, but I guess it improves DNA quality for other strains as well.

## 10. Transfer supernatant to a tube containing 350 µl phenol and 350 µl chloroform

Don't do that in the lab - do it inside a fume hood!!!! If you pipet up and down in the chloroform BEFORE you transfer a aliquot to you sample you can prevent leaking from the tip (the gas phase in the pipet gets saturated with chloroform).

## 11. Mix well by shaking

Shake the whole eppi-rack strongly.

## 12. Spin down for 5 min at 13000 rpm (18000 G)

## 13. Transfer supernatant to a tube containing 700 µl chloroform

Don't do that in the lab - do it inside a fume hood!!!!

## 14. Mix well by shaking

Shake the whole eppi-rack strongly.

## 15. Spin down for 5 min at 13000 rpm (18000 G)

## 16. Transfer 600 µl supernatant to a tube containing 360 µl isopropanol

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

## 17. Mix well by inverting the tube several times

Shake the whole eppi-rack strongly.

## 18. Spin down for 10 min at 13000 rpm (18000 G)

You can see a pellet!!!

## 19. Remove the supernatant and add 700 µl of 70% ethanol

**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.

## 20. Mix well by inverting the tube several times

Shake the whole eppi-rack strongly.

## 21. Spin down for 5 min at 13000 rpm (18000 G)

**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.

## 22. Remove the supernatant and dry at room temperature for 1 min

If you extend this to 15 min it would not be a problem as well. Do not use a speedvac for a long time, because if the DNA is overdried it is difficult to dissolve it later.

### 23. Add 50 µl TER

Allow some time to dissolve and pipet up and down a few times when you take out the DNA for further use. Do not use water here!!! If you don't add RNAse A at this step then you can not digest the DNA and load it on gel. The big amounts of RNA on the bottom will take up all Ethidium bromide and will outshine your bands.

## Known Issues:

- If you don't add RNAse A at the last step then 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 from Agrobacterium strains. I dislike this protocol to use it for E. coli strains, because you have to write a lot of tubes and it takes some time (don't worry, it works also perfect for E. coli). For E. coli strains I prefer the **Rapid Boiling Method**, because all happens there in the eppi you start with and it is far less pipetting from one eppi to the other.

I did it as described before many times and never had any problems (also the students).

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Untergasser, Andreas. “Miniprep - Alkaline Lysis” *Untergasser's Lab*. Summer 2006. (include here the date when you accessed these page).
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