# Miniprep - Alkaline Lysis for BACs

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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. Incubate on ice for **5 min**
5. Add **300 µl ALS-II** buffer
6. Mix carefully by inverting the tube 8 times
7. Incubate on ice for **5 min**
8. Add **225 µl ALS-III** buffer
9. Mix carefully by inverting the tube 8 times
10. Spin down immediately for **6 min** at 13000 rpm (18000 G)
11. Transfer **600 µl supernatant** to a tube containing **360 µl isopropanol**
12. Mix well by inverting the tube several times
13. Spin down for **10 min** at 13000 rpm (18000 G)
14. Remove the supernatant and add **700 µl of 70% ethanol**
15. Mix well by inverting the tube several times
16. Spin down for **5 min** at 13000 rpm (18000 G)
17. Remove the supernatant and dry at room temperature for 1 min
18. Add **50 µl TER** and incubate at **60 °C** for 5 min

# 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, prepare fresh

**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 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. Incubate on ice for 5 min

## 5. Add 300 µl ALS-II buffer

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

## 6. Mix carefully by inverting the tube 8 times

Invert the tubes carefully or the BACs will break and you get fractioned DNA.

## 7. Incubate on ice for 5 min

Do not extend the time, longer denaturation time can result in useless DNA. With some strains the solution clears after some time.

## 8. Add 225 µl ALS-III buffer

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

## 9. Mix carefully by inverting the tube 8 times

Close the eppis and invert carefully the whole eppi-rack.

## 10. Spin down immediately for 6 min at 13000 rpm (18000 G)

This is the biggest difference to the normal alkaline lysis which incubates samples some time on ice at this step.

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

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

Invert carefully the whole eppi-rack.

### 13. Spin down for 10 min at 13000 rpm (18000 G)
You can see a pellet!!!

### 14. 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.

### 15. Mix well by inverting the tube several times
Invert carefully the whole eppi-rack.

### 16. 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 sticks even less to the eppi, that is why I always pipet of the supernatant.

### 17. 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.

### 18. Add 50 µl TER and incubate at 60 °C for 5 min
Allow some time to dissolve but do NOT pipet up and down (to prevent shearing of the DNA). Do not use water here!!! If you dont add RNAse A at this step then you can not digest the DNA and load it on gel. The big amounts of RNA 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 BACs. For regular use 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.

## How to cite this page in publications:
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