# Miniprep - Quick & Dirty Alkaline Lysis

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Version: 1.0 - Print Version (.PDF)

**ATTENTION:** This is a low priced protocol. Use it preferably!
People like it because it's fast and you can pour over the liquids (no need to pipett). Be aware that the DNA is not very clean. It works for digests, but more tricky things may get difficult.

1. Pick colony and grow in 3 ml LB over night at 37°C
2. Spin down 1.5 ml for **20 sec** at max speed; store the rest at 4°C
3. **Pour off supernatant** leaving about 50 - 100 µl
4. Resuspend cells by vortexing
5. Add **300 µl TENS** (0.1M NaOH/0.5% SDS in TE)
6. Mix by inverting about 5 times
7. Add **150 µl of 3M NaAc** pH5.2
8. Mix by inverting about 5 times
9. Spin down for **5 min** at max speed
10. Transfer the supernatant to new eppi
    Here you can pour over, no need to pipett.
11. Add **900 µl of 96% ethanol**
12. Mix by vortexing for a few secs
13. Spin down for **5 min** at max speed
14. Pour off and **discard supernatant**
15. Add **500 µl 70% ethanol**
16. Mix by vortexing for a few secs
17. **Pour off all supernatant**
18. Spin for 30 seconds
19. Remove all remaining supernatant by pipetting it off
20. Leave the tubes open for at least 10 minutes to evaporate all ethanol
21. Dissolve the dried pellet in **30 µl water containing 20 µg/ml RNase**
22. Vortex until a milky suspension is formed
23. Spin down the insoluble white stuff for 2 min at max speed
24. Use the clear supernatant in subsequent digestions

# 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 20 sec at max speed; store the rest at 4°C

Here we spin very short so the pellet is not too compact. Therefore resuspeding by vortexing will still work.

## 3. Pour off supernatant leaving about 50 - 100 µl

We reuse the medium to resuspend the cells. This does not have to be acurate.

## 4. Resuspend cells by vortexing

The cells should resuspend within 15 - 30 sec. If it takes longer, reduce the time for centrifugation.

## 5. Add 300 µl TENS (0.1M NaOH/0.5% SDS in TE)

This will start the lysis of the bacteria.

## 6. Mix by inverting about 5 times

## 7. Add 150 µl of 3M NaAc pH5.2

## 8. Mix by inverting about 5 times

## 9. Spin down for 5 min at max speed

It will pellet the debris.

## 10. Transfer the supernatant to new eppi

Here you can pour over, no need to pipett.

## 11. Add 900 µl of 96% ethanol

This will precipitate the DNA.

## 12. Mix by vortexing for a few secs

## 13. Spin down for 5 min at max speed

It will pellet the DNA.

## 14. Pour off and discard supernatant

Keep an eye on the pellet to avoid losing it.

**15. Add 500 µl 70% ethanol**

**16. Mix by vortexing for a few secs**
Don't vortex too much, the pellet should still be intact at the end.

**17. Pour off all supernatant**

**18. Spin for 30 seconds**

**19. Remove all remaining supernatant by pipetting it off**
This is still the fastest way to remove it completely.

**20. Leave the tubes open for at least 10 minutes to evaporate all ethanol**
Ethanol can interfere with enzymatic reactions.

**21. Dissolve the dried pellet in 30 µl water containing 20 µg/ml RNase**
Don't forget the RNAse or you will have huge amounts of RNA on your gel.

**22. Vortex until a milky suspension is formed**

**23. Spin down the insoluble white stuff for 2 min at max speed**

**24. Use the clear supernatant in subsequent digestions**
This DNA solution is sufficient for digestions.

# Known Issues:

- The DNA is not very clean and can cause complications in enzymatic reactions.

# References and Comments:

I got this protocol from Jan Verver. He likes it because it's fast and simple. I never did it, because I prefer the rapid boiling method which results in cleaner DNA.

# How to cite this page in publications:

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