

DNA Miniprep using CTAB

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Version: 1.0 - [Print Version \(.PDF\)](#)

ATTENTION: This is a low priced protocol. Use it preferably! It is a great protocol to extract DNA from plants.

1. Add **1% beta-mercaptoethanol** to the CTAB-buffer
2. Preheat the CTAB-buffer to 65 °C
3. Prepare **600 µl CTAB-buffer** in an eppi
4. Grind tissue into a fine powder
5. Transfer tissue-powder to the eppi and mix very well
6. Incubate at **65 °C for 30 min**
7. Add **600 µl chloroform** and mix very well
8. Spin down at max speed for **2 min**
9. Transfer **upper phase** into a new eppi
10. Add **600 µl chloroform** and mix well
11. Spin down at max speed for **2 min**
12. Transfer **upper phase** into a new eppi
13. Add **450 µl of isopropanol** and mix well
14. Optional: Store for **30 min at -20 °C**
15. Spin down at max speed for **15 min**
16. Keep the **pellet**, discard supernatant
17. Add **600 µl 70% ethanol** to wash and mix well
18. Spin down at max speed for **5 min**
19. Keep the **pellet**, discard supernatant
20. Dissolve pellet in **90 µl water** for 15 min at 65 °C
21. Spin down at max speed for **5 min**
22. Transfer the **liquid** into a new eppi
This contains RNA and DNA. If you prefer to use RNase to get rid of RNA:
Add 1 µl RNase solution and incubate 30 min at 37 °C.
If you do not want to purify further go directly to step 34.
Most protocols end here!

23. Add **30 µl 8 M LiCl** and mix well
24. Precipitate the RNA for at least **30 min at -20 °C**
25. Spin down at max speed for **30 min at 4 °C**
26. Keep the **supernatant**, discard pellet
27. Add **80 µl isopropanol** and mix well
28. Spin down at max speed for **20 min**
29. Keep the **pellet**, discard supernatant
30. Add **100 µl 70% ethanol** to wash and mix well
31. Spin down at max speed for **5 min**
32. Keep the **pellet**, discard supernatant
33. Dissolve pellet in **100 µl water** for 15 min at 65 °C
34. Store DNA at -20 °C for long term storage

Buffers and Solutions

CTAB Buffer

| | | 100 ml | 500 ml |
|--|--------|---------------|---------------|
| CTAB (Cetyl Trimethyl Ammonium Bromide) | 2% | 2 g | 10 g |
| NaCl | 1.4 M | 8.1 g | 40.6 g |
| 0.5 M EDTA, pH 8.0 | 20 mM | 4 ml | 20 ml |
| 1 M TRIS, pH 8.0 | 100 mM | 10 ml | 50 ml |
| Polyvinylpyrrolidone (PVP40) (M.W. 40,000) | 2% | 2 g | 10 g |
| water | | add to 100 ml | add to 500 ml |

The buffer takes some time to dissolve. Some people like to autoclave it before use (me), others prefer to make it fresh.

Before use add 1 % beta-mercaptoethanol!

Commented Protocol:

1. Add 1% beta-mercaptoethanol to the CTAB-buffer

The buffer is not stable, use it within one day.

2. Preheat the CTAB-buffer to 65 °C

The buffer is not stable, use it within one day.

3. Prepare 600 µl CTAB-buffer in an eppi

Directly go to the next step after each sample.

4. Grind tissue into a fine powder

This is best done in a mortar for big amounts with liquid nitrogen or in a cap-shaker with glass beads for smaller amounts. If a cap shaker is used, 600 µl CTAB-buffer can be added directly following shaking.

5. Transfer tissue-powder to the eppi and mix very well

Do not take too much or too few. Use not more than 5 mg of dried tissue for extraction. 75 mg fresh poplar leaves works also well (just to give some guidance values). If you take too much, the lysis will not work, if you take too few, the DNA will not precipitate efficient enough.

6. Incubate at 65 °C for 30 min

Shake from time to time. Some protocols only incubate for 10 min. Probably even shorter times are sufficient. If you are in a hurry try first if shortening time decreases yield. I extended already up to 1.5 hours without decreasing the yield (due to many samples).

7. Add 600 µl chloroform and mix very well

After this step I wait till I processed all samples.

8. Spin down at max speed for 2 min

Handle the eppis carefully to not disturb the interphase.

9. Transfer upper phase into a new eppi

It is no problem if you take a little chloroform. But do not take anything of the interphase.

10. Add 600 µl chloroform and mix well

11. Spin down at max speed for 2 min

This time no white interface should form.

12. Transfer upper phase into a new eppi

Try to take none of the chloroform at this step.

13. Add 450 µl of isopropanol and mix well

This precipitates all nucleic acids. Some protocols use 600 µl.

14. Optional: Store for 30 min at -20 °C

This may increase yield, but also more RNA may precipitate.

15. Spin down at max speed for 15 min

Probably 10 min are sufficient.

16. Keep the pellet, discard supernatant

The pellet should be white and easily visible.

17. Add 600 µl 70% ethanol to wash and mix well

This washes off the salts.

18. Spin down at max speed for 5 min

Probably even 1 min would be sufficient.

19. Keep the pellet, discard supernatant

Do not dry the pellet at this step.

20. Dissolve pellet in 90 µl water for 15 min at 65 °C

21. Spin down at max speed for 5 min

Probably even 1 min would be sufficient.

22. Transfer the liquid into a new eppi

This contains RNA and DNA. If you prefer to use RNase to get rid of RNA:

Add 1 µl RNase solution and incubate 30 min at 37 °C.

If you do not want to purify further go directly to step 34.

Most protocols end here!

23. Add 30 µl 8 M LiCl and mix well

This will precipitate the RNA only.

24. Precipitate the RNA for at least 30 min at -20 °C

25. Spin down at max speed for 30 min at 4 °C

26. Keep the supernatant, discard pellet

The RNA pellet might be transparent and hardly visible.

27. Add 80 µl isopropanol and mix well

To precipitate the DNA.

28. Spin down at max speed for 20 min

29. Keep the pellet, discard supernatant

30. Add 100 µl 70% ethanol to wash and mix well

This washes off the salts.

31. Spin down at max speed for 5 min

Probably even 1 min would be sufficient.

32. Keep the pellet, discard supernatant

Do not dry the pellet at this step.

33. Dissolve pellet in 100 µl water for 15 min at 65 °C

Most of the time pipetting up and down is sufficient.

34. Store DNA at -20 °C for long term storage

Known Issues:

- This protocol is very common and has been modified so many times. Many other protocols only incubate for 10 min at 65 °C and make only one chloroform extraction. It can probably be further adapted, this protocol aims to succeed and provide high yields.
- Works well on Tobacco, Tomato and Arabidopsis.
- If you don't get DNA with this protocol, search for protocols specific for your plant and be prepared for difficulties.

References and Comments:

This is a basic protocol used in the plant field to extract DNA from all kinds of tissues. It is popular because it works really well. I did it several times and the DNA was always great.

How to cite this page in publications:

This document can be cited like this:

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<http://www.untergasser.de/lab/protocols/miniprep_dna_ctab_v1_0.htm>.

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