DNA-Miniprep. - Rapid boiling

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

1. Pick colony and grow in 3 ml LB + antibiotic over night at 37°C
2. Prepare the STET++ and switch on the heat block to 95 °C
3. Spin down 1.5 ml for **2 min at 8000 rpm** (6000 G) store the rest at 4 °C
4. Resuspend pellet in **300 µl STET++** buffer
5. Incubate at room temperature for **10 min**
6. Incubate at **95 °C** (in heat block) for **2 min (not longer!)**
7. Spin down for **15 min** at 13000 rpm (18000 G)
8. **Discard pellet(!)** with a toothpick a toothpick
9. Add **500 µl Isoprop/NH4Ac** and mix
10. **Incubate for 20 min** at room temperature
11. Spin down for **15 min** at 13000 rpm (18000 G)
12. Discard supernatant and wash with **500 µl 70% Ethanol**
13. Spin down for **10 min** at 13000 rpm (18000 G)
14. Discard supernatant
15. Spin down for **2 min** at 13000 rpm (18000 G)
16. Pipett remaining supernatant of
17. Let pellet dry for 1 min
18. Resuspend pellet in **30 µl demi water**
19. **Digest 5 µl** of DNA solution in a total volume of 30 µl **(MasterMix !)**
20. Load **10 µl** of the digest on agarose gel

Buffers:

STET:	**Isoprop/NH4Ac:**	**LB:**
8 % Sucrose (w/v)	75 ml Isopropanol 100%	10 g Tryptone
0.1 % Triton X-100	19.3 g Ammoniumacetat (NH4Ac)	5 g Yeast Extract
50 mM EDTA pH 8.0		5 g NaCl
50 mM TrisCl pH 8.0		
	add water to **100 ml** (takes some time to dissolve)	add water to **1 litre**

Stock solutions:

Lysozyme 50 mg / ml
RNAse A 10 mg / ml

Prepare STET++:

	1x	10x	15x	20x	30x	60x	90x
STET	300 µl	3 ml	4.5 ml	6 ml	9 ml	18 ml	27 ml
Lysozym	5 µl	50 µl	75 µl	100 µl	150 µl	300 µl	450 µl
RNAse A	1 µl	10 µl	15 µl	20 µl	30 µl	60 µl	90 µl

Freeze unused STET++ at -20°C until further use

Commented Protocol:

1. Pick colony and grow in 3 ml LB + antibiotic 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. Prepare the STET++ and switch on the heat block to 95 °C

The STET++ should always be prepared fresh - and I always do. But the frozen rest works fine as well, so probably you can also prepare STET++ and freeze in aliquots.

3. 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.

4. Resuspend pellet in 300 µl STET++ buffer

That is the most annoying step in the protocol. 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.

5. Incubate at room temperature for 10 min

That is the minimum time. I incubated for longer times up to one hour without any problems (for example because of many samples).

6. Incubate at 95 °C (in heat block) for 2 min (not longer!)

That is really important. All protocols warn to not extend this step - so I never did.

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

Some do spin shorter, but than you will not get a nice pellet easy to remove.

8. Discard pellet(!) with a toothpick a toothpick

Works perfect, because the pellet sticks to it. But the first 1000 minipreps I picked the pellet with a yellow tip, which works with some training.

9. Add 500 µl Isoprop/NH4Ac and mix

Just open all eppis, use one tip and pipet into all eppis, close them and shake the whole eppi-rack strongly.

10. Incubate for 20 min at room temperature

This is also not time critical - The longer the better. But do not freeze it, otherwise NH4Ac may precipitate.

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

Probably 10 min are sufficient. **You will not see a pellet!!!** Do not panic, everything is fine, the pellet is fine and transparent, it sticks to the wall and I never lost one - but it's invisible.

12. Discard supernatant and wash with 500 µl 70% Ethanol

Like before, do not pipet off the supernatant - open the eppi, discard all liquid inside by turning it over 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.

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

Probably 5 min are sufficient.

14. Discard supernatant

Like before, do not pipet off the supernatant - open the eppi, discard all liquid inside by turning it over 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.

15. Spin down for 2 min at 13000 rpm (18000 G)

Enough to spin down the remaining rest.

16. Pipett remaining supernatant of

That is the most efficient way to remove the rest. You can also dry or speedvac, but it will take a long time and over dried DNA does not dissolve well.

17. Let pellet dry for 1 min

If you are finished with pipetting, the DNA is dry enough. If you extend this to 15 min it would not be a problem as well.

18. Resuspend pellet in 30 µl demi water

The DNA solution will be around 0.2 µg/µl. Allow some time to dissolve and pipet up and down a few times when you take out the DNA for the digest.

19. Digest 5 µl of DNA solution in a total volume of 30 µl (MasterMix !)

Remember, this is a digest for testing the DNA, not for further cloning! So you need low amounts of restriction enzyme. I always use 0.3 µl enzyme in this assay, which is more than enough (if you use 0.1 µl all DNA will be digested after 5-10 min). Calculate how much samples you have, add 3 and make a MasterMix for that amount! I always incubate one hour - probably half an hour will do the job, too. Do not use expensive Enzymes. Sty I is a good choice. It will cut several times and the pattern of the bands will tell you if the clone is correct. **For more information on restriction digests, check the special protocols.**

20. Load 10 µl of the digest on agarose gel

Remember: 0.7% for bands from 12.000 to 5.000 bp, 0.8% for 8.000 to 2.000 bp, 1.0% for 6.000 to 1.200 bp and 1.5% for 2.500 to 200 bp.

References and Comments:

I got this protocol from Heinz Schaller in the ZMBH, Heidelberg. I did it many thousand times and never had any problems. Many people say that the DNA can not be digested by some restriction enzymes, but I never had any problems. I even used it for sequencing without problems, but then it's probably better to do a phenol/chloroform extraction first.

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