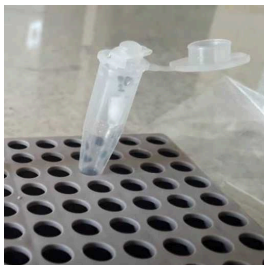
Oct 18, 2023

extract dropped plasmid DNA from filter paper



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Protocol status: Working
We use this protocol and it's working

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Disclaimer

this protocol is unofficial and not published as far as i know in any formal resource.

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I want to thank my supervisor Prof. A. AL Daoud for teaching me this protocol, and Prof. J. Molloy for the plasmid which are photographed in the images.

Abstract

this is a short yet effective protocol to extract plasmid DNA on filter paper, this protocol could benefit plasmid production labs and companies and also receiving researchers or universities from underdeveloped countries.

Guidelines

be fast and prepare your materials ahead in one place.

Materials

materials needed are:
- syringe needle.
-forceps
- TE buffer (elution buffer) or DH2O
- surgical scalpel (sterile)
- 0.2 or 0.5 centrifuge tube
- 1 or 1.5 or 2 ul centrifuge tube
- alcohol to sterile the bench and tools.
- sterile adhesive
-marker to label tubes
-gloves
-lighter to sterile forceps

excuse the messy table, i was in a hurry (you don't need mortar grinder)

Troubleshooting

Safety warnings

the centrifuge should be steady to handle two tubes or the cap will break and make a mess.

Before start

STERILE EVERYTHING!! to avoid contamination, and wear gloves.

extraction of DNA from filter paper

1m

1 locate the dropped plasmid on filter paper, it is usually circled with a marker or a pencil, it is preferable to take a photo and look at the texture if it looks "powdery" then you have a high concentration, if it looks flat then you have a low concentration and need additional time in incubation in step 4.
all steps should be sterile to the max.

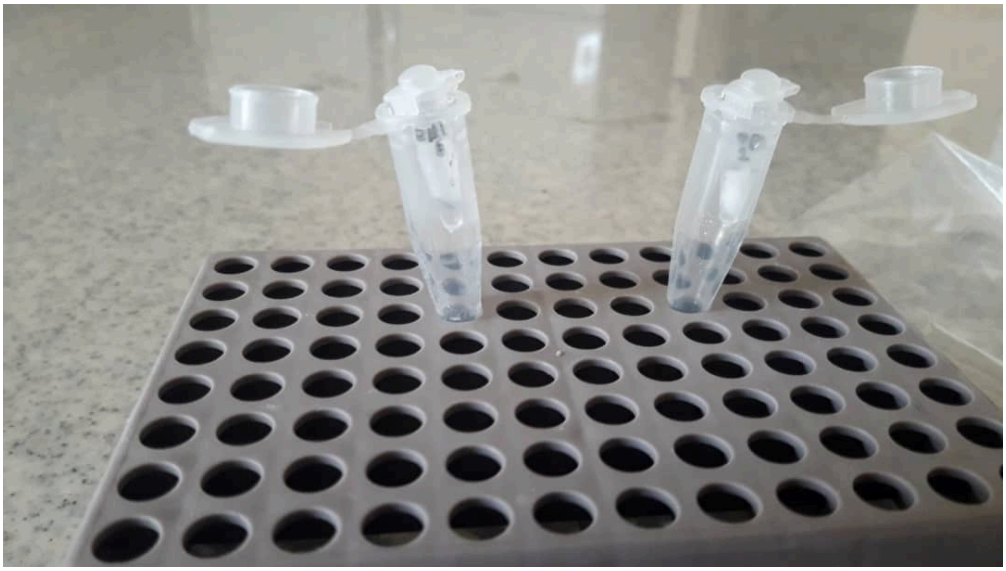
2 Cut the desired piece of paper with a sterile surgical scalpel, try to avoid ink and not to tremble the paper, and put your elbows on a table to be more steady. 2m

filter paper containing plasmid is cut out by surgical scalpel without the ink.

3 pierce a 0.2 ml centrifuge tube with a sterile syringe from the bottom, then Place the piece of cutted paper containing the plasmid with sterile forceps the tube (you can use 0.5 tube also if 0.2 not available). 1m

4 Add 30 µL of elution buffer to the paper to absorb it (you can use TE or DH2O), you can modify the volume according to the miniprep information, it could be from 20-50 µL . 2m

5 The 0.2 ml tube containing the paper is then placed in a 1.5 ml Eppendorf tube or you can place it in a 1ml tube if possible, it is necessary that the tube doesn't sink and the tip of the 0.2 tube hold steady on the edge of the 1.5 tube 2m



0.2 ul bottom pierced tube located inside 1.5 ul tube with the edge attached to prevent it from falling inside the bigger tube.

6 The soaked paper is incubated for 00:30:00 01:30:00 at room temperature 25 °C (if the concentration is low and you want the maximum yield you can leave it 4h or overnight) 2h

7 Centrifuge the tubes at 16,000 r/min for 2 minutes, but be careful that the small tube doesn't sink inside the bigger one, you can but a small sterile adhesive tape. 5m

8 the hole in the 0.2 tube allows the liquid where the plasmid is dissolved in to pass to the bigger tube, so you will get **25 ul** filtrate from 30 ul original liquid, no you can take the filtrate and place it on ice for 00:10:00 10 minutes. 10m

9 test your dissolved plasmid in nanodrop or make a quick electrophoresis to check the quality of dna.

10 store your dissolved plasmid in -4 °C or -20 °C C for downstream application.

in the end it should look something like this but less (this is 100ul from DNA extraction, i only want to share the concept)

Protocol references

http://wang.ucsd.edu/protocol/2.%20molecular%20cloning/2.3%20Amplification/Shipping_and_Receiving_Plasmids_on_Filter_Paper.pdf