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Miniprep (Ethanol Lysis)

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ABSTRACT

For the purification of Plasmid DNA from E. coli or similar Gram-Negative E. Coli

GUIDELINES

This process is optimised for the purification of Plasmid DNA from bacteria. If you wish to purify genomic DNA, or plasmids from another source - this protocol may not work as expected.

Ideally plasmid DNA will be produced in a strain of nuclease-deficient bacteria such as DH5α

MATERIALS

NAME	CATALOG #	VENDOR
Centrifuge		
Micropipettes and tips		
Eppendorf tubes 1.5 mL uncolored	022363204	Eppendorf Centrifuge
Ethanol		
PB Buffer (DIY)		
P1 Buffer (DIY)		
P2 Buffer (DIY)		
N3 Buffer (DIY)		
TE Buffer (DIY)		
Elution Buffer (DIY)		
Silica Spin Columns Collection Tubes		
Water Bath or Heat Block at 50-60°C		
LB Media with the appropriate antibiotic (e.g. LB-Amp)		

SAFETY WARNINGS

Miniprep buffers contain some extremely nasty chemical compounds. Use appropriate PPE (Gloves, Lab coat and googles) when performing miniprep. Be especially careful with:

- P2 (Lysis Buffer)
- N3 Buffer
- PB Buffer

Day 1 15m

1 Inoculate a 10-15ml tube of LB-Antibiotic media with your plasmid-containing bacteria. Place in a 37°C incubator or water bath (ideally shaking) and grow overnight.

Day 2 1h 30m

2 Pellet cells in 15mL Falcon tube by centrifuging at max speed for 5 minutes. Pour off supernatant into liquid waste.

3 Resuspend pelleted bacterial cells in 250µl Buffer P1 and transfer to a micro-centrifuge tube.

4 Add 250µL buffer P2. Mix gently by inverting the tube.
- Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear.
- Do not allow the lysis reaction to proceed for more than 5 min. If LyseBlue or another indicator has been added to P2, mix until a homogenous blue.

5 Add 350µl Buffer N3 and mix immediately and thoroughly by inverting the tube 4–6 times.
- To avoid localized precipitation, mix the solution thoroughly, immediately after addition of Buffer N3. Large culture volumes (e.g. ≥5 ml) may require inverting up to 10 times. The solution should become cloudy.
- If LyseBlue reagent has been used, the suspension should be mixed until all trace of blue has gone and the suspension is colorless. A homogeneous colorless suspension indicates that the SDS has been effectively precipitated.

6 Centrifuge for 10 min at max speed in a centrifuge. A compact yellow-white pellet will form.
- If the solid matter fails to form a pellet, try centrifuging for longer or add more P2 next time.
- It is possible to proceed by carefully decanting the liquid fraction with a pipette, but the excess matter will clog the filter and reduce the amount of plasmid purified

7 The plasmid DNA is now in the supernatant. Seperate supernatant from pellet using a micropipette and add to a microcentrifuge tube, mix in a 1:1 ratio with ethanol. (Roughly 750µL of Ethanol). Mix tube by inverting 10 times.

8 Apply the ethanol-supernatant from previous step to the silica spin column by decanting or pipetting 750µL into the top of the column. Centrifuge for 30–60s. Place flow through back into the spin column Centrifuge again for 30–60 s. The plasmid DNA is now captured in the silica, pour flow-through in the collection tube into the liquid waste.

9 Repeat above step for remaining 750µL of ethanol-supernatant mixture.
- All plasmid DNA should now be captured in the silica column, collection tube should be empty for next step.

10 Wash the silica spin column by adding 500µL of Buffer PB and centrifuging for 30–60 s. Discard the flow-through to liquid waste.

11 Wash silica spin column by adding 0.75 ml Buffer PE and centrifuging for 30–60s.

12 Discard the flow-through, and centrifuge at full speed for an additional 1 min to remove residual PE buffer.
- Important: Residual wash buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions

13 Remove the silica spin column from the collection tube and place onto a kimwipe in a 50°C oven. Alternatively place the spin column into a fresh collection/eppendorf tube and into a heat block at 50-60°C. Leave for 10-15 minutes to evaporate residual ethanol.

14 Place the silica spin column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 30-100 µl TE/Water/EB to the center of each silica spin column, let stand for 1 min, and centrifuge for 1 min.
- Using less elution liquid here will result in a higher concentration of DNA, albeit with less volume.
- Optional: Pipette flow-through back into the centre of the silica column and centrifuge again to capture more of the plasmid DNA and increase final concentration

15 Quantify yield by nanodrop or by running on an agarose gel