Protocol: Miniprep Plasmid DNA Extraction

This is a generic protocol for plasmid DNA extraction and purification. Many plasmid miniprep kits are sold that operate on similar principles. You should follow the specific instructions for the kit that you buy.

Material	Amount		Material	Amount
Resuspension Buffer	250 µl		Miniprep Spin Column	1
Cell Lysis Buffer	250 µl		Ethanol Wash Solution	1 mL
Neutralization Buffer	550 µl		Elution Buffer or Water	50 µl

The night before:

1) Start a 5 mL overnight culture of the bacteria carrying your plasmid.
Include a selective antibiotic.

2) Centrifuge 2 mL of culture for 2 minutes at 6000 rpm.
Keep the cell pellet and discard the supernatant.

3) Resuspend the cell pellet in 200 µl Resuspension Buffer.

4) Add 250 µl Cell Lysis Buffer.
Mix gently and wait 5 minutes for lysis to proceed.

5) Add 350 µl Neutralization Buffer.
Mix gently.

6) Centrifuge the lysed cells for 5 minutes at maximum speed.
Keep the supernatant and discard the cell debris.

7) Add the supernatant to a Miniprep Spin Column and spin for 1 minute.
Keep the column and discard the flow-through.

8) Add 500 µl Ethanol Wash Solution to the column and spin for 1 minute.
Keep the column and discard the flow-through.

8) Again add 500 µl Ethanol Wash Solution to the column and spin for 1 minute.
Keep the column and discard the flow-through.

9) Spin the column for 2 minutes at maximum speed to dry it.
Ensure that no ethanol remains on the column

10) Transfer the column to a clean Eppendorf tube.

11) Add 30-100 µl of water directly to the column membrane.
Wait a few minutes to allow the DNA to elute.

12) Spin the column at max speed for 1 minute to collect the plasmid DNA.
Keep the flow-through and discard the column.