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

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Plasmid Extraction (Plasmid Isolation)

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ABSTRACT

2023 NUS-Singapore iGEM team followed this protocol to isolate the plasmid from the cells.

PROTOCOL MATERIALS

- Buffer PE Qiagen Catalog #19065 — In 2 steps
- Buffer P1 Qiagen Catalog #19051 — Step 5
- RNase A Qiagen Catalog #19101 — Step 5
- Buffer N3 Qiagen Catalog #19064 — Step 9
- PB buffer Qiagen Catalog #19066 — Step 15
- Buffer P2 Qiagen Catalog #19052 — Step 7

SAFETY WARNINGS

- Proper lab PPE must be worn at all times.

BEFORE START INSTRUCTIONS

Glycerol stock (900 µL of cell stock in 300 µL of 100% glycerol solution) may be prepared and keep it in the -80 °C fridge before the plasmid extraction procedure.

PROTOCOL integer ID:
88484

Keywords: Plasmid Extraction, Plasmid, Buffer P1, Buffer P2, Buffer N3, Buffer PB, Buffer PE, Plasmid Isolation, Isolation

Cell Culture

1 Incubate the cells containing the plasmid of interest in a Falcon tube with 5 mL of LB media and 5 µL of the appropriate antibiotics Overnight at 37 °C before starting the plasmid extraction.

Plasmid Extraction

5m

2 Take out the Falcon tube with the cultured cells from the incubator.

3 Centrifuge the Falcon tube at 5000 rpm, 4°C, 00:05:00 . 5m

4 Discard the supernatant in the Falcon tube and keep the cell pellet.

5 Add 250 µL of Buffer P1 Qiagen Catalog #19051 (with RNase A Qiagen Catalog #19101 added, kept in the 4 °C fridge) into the Falcon tube and resuspend the cell pellet.

6 Transfer the whole solution into a Eppendorf tube.

7 Add 250 µL of Buffer P2 Qiagen Catalog #19052 into the Eppendorf tube to lyse the cells.

8 Shake the Eppendorf tube for mixing.

9 Add 350µL of Buffer N3 Qiagen Catalog #19064 into the Eppendorf tube.

10 Shake the Eppendorf tube for mixing, a cloudy solution shall be observed.

11 Centrifuge the Eppendorf tube at 13 rpm, 00:10:00. 10m

12 Transfer the supernatant into a Mini Prep tube and discard the cell debris.

13 Centrifuge it at 13 rpm, 00:01:00. 1m

14 Discard the flow-through and place the Mini Prep tube back into the same tube.

15 Add 500 µL of PB buffer Qiagen Catalog #19066 into the Mini Prep tube.

16 Centrifuge it at 13 rpm, 00:01:00.

17 Discard the flow-through and place the Mini Prep tube back into the same tube.

18 Add 700 µL of Buffer PE Qiagen Catalog #19065 into the Mini Prep tube.

19 Centrifuge it at 13 rpm, 00:01:00.

20 Discard the flow-through and place the Mini Prep tube back into the same tube.

21 Centrifuge it at 13 rpm, 00:01:00 to remove the Buffer PE Qiagen Catalog #19065 residual.

22 Transfer the Mini Prep column into the newly labelled Eppendorf tube.

23 Add 50 µL of DI water into the Eppendorf tube.

24 Centrifuge it at 13 rpm, 00:01:00, ensuring that the direction of the Eppendorf tube's cap is the same as the direction of spinning to avoid breaking.

25 Discard the Mini Prep column, the solution left in the Eppendorf tube is the plasmid.

26 Use NanoDrop

Equipment	
NanoDrop™ One/OneC Microvolume UV-Vis Spectrophotometer	NAME
UV-Vis Spectrophotometer	TYPE
Thermo Scientific	BRAND
ND-ONE-W	SKU

to measure the concentration (under "dsDNA" mode) and the purity of the plasmid.

27 Store the isolated plasmid at room temperature.