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Plasmid Extraction (Plasmid Isolation)

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Protocol status: Working

We use this protocol and it's working

Created: September 27, 2023



Last Modified: September 28, 2023

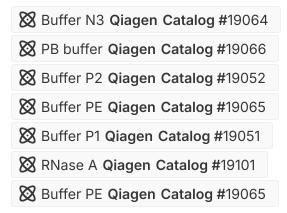
Protocol Integer ID: 88484

Keywords: Plasmid Extraction, Plasmid, Buffer P1, Buffer P2, Buffer N3, Buffer PB, Buffer PE, Plasmid Isolation, Isolation, plasmid isolation, plasmid extraction, plasmid from the cell, plasmid, singapore igem team, isolation, extraction

Abstract

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

Protocol materials



Troubleshooting

Safety warnings



Proper lab PPE must be worn at all times.

Before start

Glycerol stock ($4900 \, \mu L$ of cell stock in $4300 \, \mu L$ of 100% glycerol solution) may be prepared and keep it in the $8-80 \, ^{\circ} C$ fridge before the plasmid extraction procedure.



Cell Culture

Incubate the cells containing the plasmid of interest in a Falcon tube with $\[\] 5 \]$ of LB media and $\[\] 5 \]$ of the appropriate antibiotics $\[\bigcirc \]$ 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.
- Centrifuge the Falcon tube at \$\ 5000 \text{ rpm, 4°C, 00:05:00} \tag{.}

5m

- 4 Discard the supernatant in the Falcon tube and keep the cell pellet.
- 6 Transfer the whole solution into a Eppendorf tube.
- 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 3 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 \triangle 700 μ L of \bigotimes Buffer PE Qiagen Catalog #19065 into the Mini Prep tube.
- 19 Centrifuge it at 3 rpm, 00:01:00
- 20 Discard the flow-through and place the Mini Prep tube back into the same tube.
- 21 Centrifuge it at 3 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 \perp 50 μ L of DI water into the Eppendorf tube.



- 24 Centrifuge it at 3 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.