

VERSION 1

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Protocol status: In development We are still developing and optimizing this protocol

Created: Sep 28, 2023

ONA Isolation V.1

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ABSTRACT

2023 NUS-Singapore iGEM team followed this protocol to isolate the DNA fragments from the agarose gel after the gel electrophoresis.

PROTOCOL MATERIALS

⊠ Buffer QG **Qiagen Catalog** #19063

Step 5

In 3 steps

SAFETY WARNINGS



- Proper lab PPE must be worn at all times.
- When using the LED transilluminator, wear eyewear that is designed to block blue light to protect the eyes.

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88509

Keywords: DNA Isolation, DNA, Gel, Gel Electrophoresis, Buffer QG, Buffer PE

- 1 Prepare and label an Eppondoft tube.
- 2 Place the agarose gel onto the LED Transilluminator (blue light) to observe the DNA band(s).

Safety information

- Wear protective eyewear to protect the eyes from blue light.
- Turn off the LED transilluminator immediately when it is not in use.
- 3 Cut out the target DNA band from the agarose gel.
- 4 Put the gel piece into the Eppendorf tube.
- 5 Add \underline{A} 450 μL of \bigotimes Buffer QG Qiagen Catalog #19063 into the Eppendorf tube.
- 6 Heat the Eppendorf tube at 55 °C for 00:20:00 in the Thermo-Shaker.

20m

- 7 Add \angle 150 μ L of 100% isopropanol (IPA) into the Eppendorf tube and shake the tube to mix the solution well.
- Transfer the whole solution into a QIAquick Spin Column (purple tube with a maximum volume of \pm 750 μ L).
- 9 Centrifuge it for (3) 13 rpm, 00:01:00

- 10 Discard the flow-through and place the QIAquick column back into the same tube.
- 11 Add Δ 700 μL of 🔀 Buffer PE Qiagen Catalog #19065 into the QIAquick column.
- 12 Centrifuge it for (13 rpm, 00:01:00
- 13 Discard the flow-through and place the QIAquick column back into the same tube.
- Add △ 700 µL of ☒ Buffer PE Qiagen Catalog #19065 again into the QIAquick column.

- 15 Centrifuge it for (13 rpm, 00:01:00
- 16 Discard the flow-through and place the QIAquick column back into the same tube.
- Centrifuge the emptied QIAquick column at 13 rpm, 00:01:00 to remove residual Buffer PE Qiagen Catalog #19065.

1m

- 18 Transfer the QIAquick column into the newly labelled Eppendorf tube.
- 19 Add \underline{A} 30 μL of DI water into the QIAquick column.
- Centrifuge the tube 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.

1 100

- 21 Discard the QIAquick column, the solution left in the Eppendorf tube contains the DNA fragment of interest.
- Use the Nanodrop to measure and record the purity and concentration of the DNA fragment.

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

Keep the isolated DNA fragment in the Room temperature