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

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PCR Clean-up

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ABSTRACT

2023 NUS-Singapore iGEM team followed this protocol to isolate the known DNA fragments from the PCR product without running the gel electrophoresis.

PROTOCOL MATERIALS

PB buffer Qiagen Catalog #19066 — Step 1

Buffer PE Qiagen Catalog #19065 — In 3 steps

SAFETY WARNINGS

Proper lab PPE must be worn at all times.

PROTOCOL integer ID:
88521

Keywords: PCR Clean-up,
PCR, DNA, DNA Isolation

1 Add 5 times the sample's volume of PB buffer Qiagen Catalog #19066 into the PCR tube with the PCR product.

2 Transfer the whole solution into a QIAquick Spin Column (purple tube) and ensure that the sample is dripped onto the white membrane in the column.

3 Centrifuge the tube at 13 rpm, 00:01:00. 1m

4 Discard the flow-through and place the QIAquick column back into the same tube.

5 Add 700 µL of Buffer PE Qiagen Catalog #19065 into the QIAquick column.

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

7 Discard the flow-through and place the QIAquick column back into the same tube.

8 Add 700 µL of Buffer PE Qiagen Catalog #19065 again into the QIAquick column.

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

10 Discard the flow-through and place the QIAquick column back into the same tube.

11 Centrifuge the emptied QIAquick column at 13 rpm, 00:01:00 to remove residual Buffer PE **Qiagen Catalog #19065**. 1m

12 Transfer the QIAquick column into the newly labelled Eppendorf tube.

13 Add 30 µL of DI water into the QIAquick column.

14 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. 1m

15 Discard the QIAquick column, the solution left in the Eppendorf tube contains the DNA fragments.

16 Use the Nanodrop to measure and record the purity and concentration of the DNA fragments.

Equipment

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