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Version 2

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Amplicon clean-up using SPRI beads for RAPID nanopore kit RBK004 V.2

Forked from Amplicon clean-up using SPRI beads

Muhammad Faisal[1], Olin Silander[1], Nikki Freed[1]

[1]Massey University

1 *Works for me* This protocol may be deleted by the owner

Coronavirus Method Development Community

Nikki Freed

MATERIALS

NAME	CATALOG #	VENDOR
Agencourt AMPure XP beads		

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Agencourt AMPure XP	A63880	Beckman Coulter
QuantiFluor(R) ONE dsDNA System, 100rxn	E4871	Promega

MATERIALS TEXT

Freshly prepared 80% ethanol
10 mM Tris-HCl pH 8.0 with 50 mM NaCl

EQUIPMENT

NAME	CATALOG #	VENDOR
Quantus	E6150	

Ampure XP bead clean up

1 Vortex SPRI beads thoroughly to ensure they are well resuspended, the solution should be a homogenous brown colour.

Agencourt AMPure XP
by Beckman Coulter
Catalog #: A63880

2 Add an equal volume (1:1) of SPRI beads to the sample tube and mix gently by either flicking or pipetting. For example add **50 µl** room temperature SPRI beads to a **50 µl** reaction.

3 Pulse centrifuge to collect all liquid at the bottom of the tube.

4 Incubate for **00:05:00** at room temperature.

5 Place on magnetic rack and incubate for **00:02:00** or until the beads have pelleted and the supernatant is completely clear.

6 Carefully remove and discard the supernatant, being careful not to touch the bead pellet.

7 Add **200 µl** of freshly prepared room-temperature **80 % volume** ethanol to the pellet.

8 Keeping the magnetic rack on the benchtop, rotate the bead-containing tube by 180°. Wait for the beads to migrate towards the magnet and re-form a pellet. Remove the ethanol using a pipette and discard.

9 and repeat ethanol wash.

10 Pulse centrifuge to collect all liquid at the bottom of the tube and carefully remove as much residual ethanol as possible using a P10 pipette.

11 With the tube lid open incubate for **00:01:00** or until the pellet loses it's shine (if the pellet dries completely it will crack and become difficult to resuspend).

12 Remove the tube from the magnetic rack. Resuspend pellet in **10 µl** 10 mM Tris-HCl pH 8.0 with 50 mM NaCl, mix gently by flicking and incubate for **00:02:00** .

13 Place on magnet and transfer sample to a clean 1.5mL Eppendorf tube ensuring no beads are transferred into this tube.

14 Quantify **1 µl** product using the Quantus Fluorometer using the ONE dsDNA assay.

QuantiFluor(R) ONE dsDNA System, 100rxn
by Promega
Catalog #: E4871

Quantus
Fluorometer

Promega E6150