May 22, 2020 Version 3

Amplicon clean-up using SPRI beads for RAPID nanopore kit RBK004 V.3

Version 1 is forked from Amplicon clean-up using SPRI beads

DOI
https://dx.doi.org/10.17504/protocols.io.bgsrjwd6

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Coronavirus Method De...

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Protocol Citation: Nikki Freed, Olin Silander 2020. Amplicon clean-up using SPRI beads for RAPID nanopore kit RBK004. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bgsrjwd6

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

Created: May 22, 2020

Last Modified: July 02, 2020

Protocol Integer ID: 37425

Keywords: spri beads for rapid nanopore kit rbk004, rapid nanopore kit rbk004, using spri bead, amplicon clean, amplicon, bead

Materials

MATERIALS

Agencourt AMPure XP beads

STEP MATERIALS

Agencourt AMPure XP **Beckman Coulter Catalog #**A63880

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

Protocol materials

Agencourt AMPure XP beads

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

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Ampure XP bead clean up

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 [M] 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 go to step #7 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 at room temperature 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.