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Amplicon clean-up using SPRI beads

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

Created: September 25, 2019

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Protocol Integer ID: 28087

Materials

STEP MATERIALS

Elution Buffer (EB) **Qiagen Catalog #**19086

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

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

Protocol materials

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

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

Agencourt AMPure XP **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 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 room-temperature [M] 70 % volume ethanol to the pellet.

8 Carefully remove and discard ethanol, being careful not to touch the bead pellet.

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 Resuspend pellet in 30 µL Elution Buffer (EB), mix gently by either flicking or pipetting and incubate for 00:02:00 .

Elution Buffer (EB) **Qiagen Catalog #**19086

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 **Promega Catalog #**E4871

Equipment

Quantus	NAME
Fluorometer	TYPE
Promega	BRAND
E6150	SKU
https://www.promega.co.uk/products/microplate-readers-fluorometers-luminometers/fluorometers/quantus-fluorometer	LINK