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Modified protocol for AMPure XP clean-up

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We use this protocol and it's working

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Disclaimer

The duration times presented are estimations only.

Abstract

This modified AMPure XP bead clean-up protocol is intended for the purification and/or concentration of high-molecular-weight (HMW) DNA for use as input in Oxford Nanopore or Pacific Biosciences long-read sequencing. The bead-to-sample ratio (v:v) determines the size-selection effect.

Guidelines

Important note from Nanopore protocols:
When the AMPure beads-to-sample ratio (v:v) is lower than 0.4:1, DNA fragments of any size will be lost during the clean-up.

Materials

- AMPure XP Beckman Coulter (COD. A63880)
- Hula mixer or rotator mixer (do not use thermomixer instead of a Hula mixer / rotator mixer)
- Thermoblock / Thermomixer (350 rpm)
- Magnetic separation rack
- Freshly prepared 80 % ethanol
- Vortexer
- Minicentrifuge
- Pipettes and pipette tips
- 1.5 ml DNA LoBind tubes

Troubleshooting

Problem

The concentration of this first eluate is too low compared to the expected one.

Solution

Check the presence of DNA in the saved supernatant with Qubit. If the supernatant contains a high DNA amount, repeat the purification procedure adding fresh beads to the supernatant. Then, in step 9, resuspend the bead pellet using as a buffer the first eluate (i.e., the one containing the “less than expected” DNA amount) to increase the final concentration. If the supernatant does NOT contain DNA, perform a second elution from the saved beads, and in step 9 resuspend the pellet using the first eluate as buffer.

Safety warnings

The operator must wear a lab coat, powder-free nitrile gloves and safety specs to perform the laboratory procedures in this protocol.

Waste needs to be collected in a suitable container and disposed of in accordance with local regulations.

Liquid waste needs to be collected in a suitable container and disposed of in accordance with local regulations.

See best practices, thawing and handling instructions, and safety instructions for all the kit reagents, as well as instructions and safety data sheets for other reagents used in this protocol **before starting**.

Before start

- Allow the samples to reach room temperature, then gently mix by pipetting.
- Allow the beads to warm to room temperature for at least 30 minutes prior to use. Vortex beads to resuspend.
- This protocol assumes you're working with your sample in 1.5 ml DNA LoBind tubes.

AMPure XP clean-up

1h 40m

1 Resuspend the AMPure XP Beads by vortexing. 2m

2 Add the resuspended AMPure XP Beads to the sample and mix by pipetting. The AMPure XP beads-to-sample ratio (v:v) is adjusted based on the specific aim. 3m

For purification and/or concentration of a genomic DNA sample:

- **0.4X** (0.4 volume of beads added per 1 volume of sample): for **concentration and size selection** with removal of fragments < 1.5- 2 kb
- **From 1X to 1.8X** (1 to 1.8 volumes of beads added per 1 volume of sample): for **contaminant removal and volume concentration**, without size selection

Note

To pipette the precise volume of needed AMPure XP beads, adjust the volume of the sample with the original buffer in order to have a volume of beads that can be easily set and pipetted.

3 Incubate on a Hula mixer or rotator mixer for 00:30:00 at RT. 30m

Note

In case a small amount of the total volume remains at the bottom of the tube during the rotation, flick to resuspend.

4 Prepare 2 ml of fresh 80% ethanol in Nuclease-free water per sample. 2m

5 Spin down the sample and pellet on a magnet for 00:05:00. Keep the tube on the magnetic rack until the eluate is clear and colourless, and pipette off the supernatant. 6m

Note

Save the supernatant as a precaution. If, at the end of the protocol, the DNA is not in the eluate, it can be recovered from this supernatant.

6 Keep the tube on the magnetic rack and wash the beads with at least 700 µl (ethanol must cover the beads) of freshly prepared 80% ethanol without disturbing the pellet. Remove the ethanol using a pipette and discard. 1m

7 Repeat the previous step. 1m

8 Spin down and place the tube back on the magnetic rack. Pipette off any residual ethanol. Allow to dry for ~30-60 seconds 00:01:00 but do not dry the pellet to the point of cracking. 2m

9 Remove the tube from the magnetic rack and resuspend the pellet in XX µl TE or TE with low EDTA concentration. Resuspend by flicking or tapping, not by pipetting. 2m

Note

Set the elution volume, XX, according to the expected yield and the downstream application. An elution in 50 µl from 180 µl of beads was also successfully performed.

10 Incubate for 00:30:00 at 37°C at 350 rpm on a thermomixer, flick if beads precipitate. Extend the incubation time up to 40–45 min 00:45:00 in case of problematic beads pelleting or viscous/bubbling samples. 45m

Note

Incubation at 37°C facilitates elution of long fragments of HMW-DNA.

11 Pellet the beads on a magnetic rack for 00:05:00 . 5m

12 Remove and retain XX µl of eluate into a clean 1.5 ml Eppendorf tube. 1m

Note

Save both the beads and the supernatant until the end of the Qubit check of this first eluate, especially in case of sample with problematic beads pelleting or viscous/bubbling samples.

Protocol references

Ligation sequencing DNA V14 (SQK-LSK114) (GDE_9161_v114_revAC_24Sep2025) https://nanoporetech.com/document/genomic-dna-by-ligation-sqk-lsk114#issues-during-dna-rna-extraction-and-library-preparation