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Protocol status: Working

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DOH Workshop Part 2: Promega Pronex protocol

In 2 collections

Vesa Qarkaxhija[1], Bryan Wee[1], Natalie Ring[1]

[1]The Roslin Institute, University of Edinburgh

Bryan Wee
The University of Edinburgh

ABSTRACT

This protocol is to purify extracted dsDNA, removing contaminants (e.g., buffers, proteins, salts, etc.) and low molecular weight DNA (e.g., dsDNA adapters, ssDNA oligonucleotides and nucleotides).

ATTACHMENTS

pyxtbzpxx.pdf

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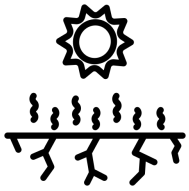
PROTOCOL integer ID: 96353

GUIDELINES

Important points throughout protocol:

Be gentle when pipetting, too much fast pipetting/ extended vortexing can shear the DNA which will result in poorer sequencing outcomes.



Do not let the beads dry after removal of supernatant. This can be avoided by keeping Eppendorf tubes closed if beads are not submerged.
Note: When bead pellet is moist, it appears shiny. As they start to dry, the shine reduces, and cracks start to form.

When working with beads ensure they are thoroughly mixed before using. This can be achieved by vortexing for at least 10 seconds before use and vortexing between use to prevent beads settling.

Troubleshooting:

1. My DNA yield is low, do I have to redo the extraction?

There are a few things you can do to increase yield before having to redo the extraction.

Try these solutions:

- Try re-eluting the DNA from the remaining Pronex beads from step 16 of the **Pronex protoco**l. Repeat steps 14 –16.

- Repeat **Pronex protocol**.
- Try re-eluting the DNA from the remaining MagAttract Suspension G beads from step 32 of the **DNA extraction protocol**. Repeat steps 30, and re-elute by placing the tube onto the thermomixer and incubate at 21°C for **6 min** at 1400 rpm.

MATERIALS

Materials

- Extracted genomic DNA

Equipment

- P1000 pipette (Micropipette with 100 µL – 1000 µL range)
- P200 pipette (Micropipette with 20 µL – 200 µL range)
- P20 pipette (Micropipette with 2 µL – 20 µL range)
- P10 pipette (Micropipette with 0.5 µL – 10 µL range)
- DNA fluorometer (Promega Quantus or Themofisher Qubit)
- Vortex mixer

Consumables

- P1000 filter pipette tips (with 100 µL – 1000 µL range)
- P200 filter pipette tips (with 20 µL – 200 µL range)
- P20 filter pipette tips (with 2 µL – 20 µL range)
- P10 filter pipette tip (with 0.5 µL – 10 µL range)
- 1.5 ml Eppendorf DNA LoBind tubes
- Qubit dsDNA HS Assay Kit OR Promega QuantiFluor® ONE Dye
- Qubit™ Assay Tubes
- Absolute ethanol (>96%)
- ProNex® Size-Selective Purification System

1. Pronex beads
2. Wash buffer (Ethanol must be added)

BEFORE START INSTRUCTIONS

Ensure that the Pronex Wash Buffer is prepared according to instructions on them (i.e.adding appropriate amount of Ethanol).

Promega Pronex protocol

25m 10s

1 Resuspend the Pronex beads by vortexing for 00:00:10 or longer. 10s

Note

Shake well before use

2 Into your extracted DNA tube pipette 80 µL of Pronex beads and mix into the sample by slowly pipetting 10 times.

Note

If sticky clumps of bead-bound DNA form, be careful not to take any beads either in the pipette tip or on the outside of the pipette tip.

3 Leave at Room temperature for 00:10:00 . 10m

4 Place the sample on a magnetic rack for 00:02:00 (or longer if necessary) until the solution becomes clear and the beads form a pellet. 2m

5 While leaving tubes on the magnet, carefully remove and discard supernatant without disturbing the beads using a P1000.

6 While the tube is on the magnetic rack, add 200 µL of Pronex wash buffer without flushing directly onto the pellet. If 200 µL is not enough to submerge the pellet, use more wash buffer.

Note

Wash Step 1

7 Allow to incubate for 00:00:30 - 00:01:00 . 1m

8 While leaving tubes on the magnet, carefully remove and discard wash buffer without disturbing the beads using a P1000.

9 Keeping the tube on the magnetic stand, carefully add 200 µL of Pronex Wash Buffer without flushing directly onto the pellet. If 200 µL is not enough to submerge the pellet, use more wash buffer.

Note

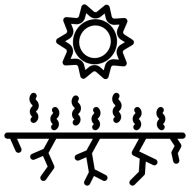
Wash Step 2

10 Allow to incubate for 00:00:30 - 00:01:00 . 1m

11 While leaving tubes on the magnet, carefully remove and discard wash buffer without disturbing the beads using a P1000.

12 Allow the sample to air dry with lids open for 00:02:00 - 00:05:00 watching it until the pellet is no longer shiny. 5m

Note



Be careful not to over-dry

13 Remove the sample from the magnetic stand.

14 Remove the tube from the magnetic rack and add 32 µL of nuclease free water. Resuspend the beads by slowly pipetting or stirring with the pipette tip.

Note

Be as gentle as possible while ensuring that pellet is resuspended.

Pipette gently, be careful not to shear DNA

15 Leave for 00:05:00 at Room temperature to elute the DNA. 5m

16 Pellet beads on magnet for 00:01:00 until solution becomes clear and slowly pipette DNA eluate into a new LoBind tube. 1m

Note

Save the tube with Pronex beads in case of incomplete DNA elution, so you can repeat.

Pipette gently, be careful not to shear DNA

17 Quantify 1 µL of DNA elute using the Qubit™ dsDNA HS Assay Kit or QuantiFluor® ONE dsDNA System.