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Title of resource
GN_13: PCR product purification using MinElute spin columns
Authored by
When using this protocol, the following should be referenced: Harrup, L.E (2018). The Pirbright Institute DNA Barcoding Protocols Version 3.
DOI
10.13140/RG.2.2.14879.41129
Description
This method details the protocol and guidance notes for the purification of PCR products prior to sequencing and other downstream applications. Protocol from the Gnatwork Bangladesh workshop, September 3-6th 2018.
Intended use
Scientific research use and training purposes.
Restrictions on use
Content is not to be redistributed in the public domain (e.g. presentation, lecture, online or in publications).
Resource history
Updated version of: Harrup, L.E. (2014). The Pirbright Institute *Culicoides* DNA Barcoding Protocols, Version 2, available at www.ibvnet.com

GN_13: PCR product purification using MinElute spin columns

Citation: Harrup, L.E (2018). The Pirbright Institute DNA Barcoding Protocols Version 3

A. Introduction

This method details the protocol and guidance notes for the for the purification of PCR products prior to sequencing and other downstream applications.

B. Materials

Equipment

- Pipettes (2-20 µl, 20-200 µl, 100-1000 µl)
- Vortex
- Centrifuge with rotor capable of holding 1.5 ml centrifuge tubes and spin columns at up to 20,000 x g
- Fridge (+4°C)
- Freezer (-20°C)
- Disposable pipette tips containing hydrophobic filters (2-20 µl, 20-200 µl, 100-1000 µl)
- 1.5 ml flip-top microcentrifuge tubes (e.g. Eppendorf Safe Lock Tubes: 0030 123.328)
- Label printer
- Microcentrifuge tube labels
- Solvent resistant cryopen
- Nitrile gloves

Reagents

- Analytical grade 100% ethanol
- DNAZap™ Solutions (ThermoFisher Scientific: AM9890) (or equivalent)
- 3 M pH 5.0 sodium acetate (NaOAc) (e.g. Sigma-Aldrich: S7899-100ML)
- Qiagen MinElute® PCR Purification Kit (Qiagen 50 columns: 28004; 250 columns: 28006), includes:
    - MinElute® spin columns
    - 2ml collection tubes
    - pH Indicator
    - Buffer PB
    - Buffer PE
    - Buffer EB (10 mM Tris HCL pH 8.0)

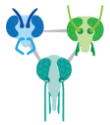
C. Method

C.1 Put on nitrile gloves.

C.2 Use DNAZap™ (or equivalent) to clean the working area and any equipment e.g. pipettes as required, to reduce/remove any potential residual DNA contamination.

C.3 Discard used gloves and put on a new pair of nitrile gloves and wear throughout the remainder of the procedure.

C.4 Add 100% ethanol to Qiagen MinElute Buffer PE (see bottle label for volume), tick the box on the Buffer PE Label to indicate ethanol has been added. Label the bottle with your initials and the date. Shake bottle to mix, store at room temperature.



C.5 Add 1:250 volume pH indicator to Qiagen Buffer PB (120 µl pH indicator to 30 ml Buffer PB). The yellow colour of Buffer PB with pH indicator I indicates a pH ≤ 7.5. Label the bottle with your initials and the date. Store at room temperature.

C.6 Pre-cool the centrifuge to 21°C.

C.7 Aliquot five volumes of Buffer PB to one volume of PCR product (i.e.100 µl per 20 µl PCR product). Use a new tip per well to prevent cross-contamination. Seal tubes and vortex briefly.

C.8 PCR product/Buffer PB mix should be pale yellow indicating a pH ≤ 7.5 if the solution is orange or violet add 10 µl 3 M sodium acetate (NaOAc) pH 5.0 then reseal the tubes and vortex briefly (solution should turn pale yellow). If CoralLoad Concentrate has been used in the PCR mastermix add 10 µl 3 M NaOAc pH 5.0 to all tubes.

C.9 Arrange MinElute® collection tubes in a microcentrifuge tube rack and place one appropriately labelled MinElute® PCR purification column per collection tube.

C.10 Transfer the PCR product/Buffer PB mix to the corresponding MinElute® PCR purification column and seal the cap on the spin column. Use a new tip per tube to prevent cross-contamination.

C.11 Centrifuge all MinElute® PCR purification columns in their collection tubes at 18,500 x g (~13,000rpm) at 21°C for 1 minute.

C.12 Discard flow through in each collection tube, returning each column to its original collection tube to prevent cross-contamination.

C.13 Aliquot 750 µl of Buffer PE to each MinElute® PCR purification column. Use a new tip per column to prevent cross-contamination. Seal the cap on the column.

C.14 Centrifuge all MinElute® PCR purification columns with their collection tubes at 18,500 x g (~13,000 rpm) at 21°C for 1 minute.

C.15 Discard flow through in each collection tube and return each column to its original collection tube to prevent cross contamination.

C.16 To dry the column, centrifuge all MinElute® PCR purification columns with their collection tubes at 18,500 x g (~13,000 rpm) at 21°C for 1 minute.

C.17 Place double-labelled 1.5 ml microcentrifuge tubes in a microcentrifuge tube rack.

C.18 Transfer each MinElute® PCR purification column to the corresponding appropriately labelled microcentrifuge tube, discard Qiagen collection tubes.

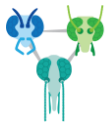
C.19 Add 10 µl Buffer EB (10 mM Tris-HCL, pH 8.5) to the centre of each MinElute® PCR purification column membrane. Use a new tip per column to prevent cross-contamination.

C.20 Allow MinElute® PCR purification columns to stand for 1 minute at room temperature (~21°C).

C.21 Centrifuge all MinElute® PCR purification columns with their microcentrifuge tubes at 18,500 x g (~13,000 rpm) at 21°C for 1 minute.

C.22 Discard MinElute® PCR purification columns and seal all microcentrifuge tubes containing purified PCR product.

C.23 Store purified PCR product at 4°C if to be used within 24 hours, otherwise store at -20°C.



C.24 Use DNAZap™ (or equivalent) to clean all surfaces used to reduce/remove any potential DNA contamination.

C.25 Remove and discard gloves.

D. Results

D.1 Results and sample details should be recorded in the appropriate laboratory notebook.

D.2 PCR product concentration may be checked using a NanoDrop spectrophotometer or using a Qubit® Fluorometer with either the Qubit® High Sensitivity dsDNA Assay kit or the Qubit® Broad Range dsDNA Assay Kit (choice of kit is dependent upon expected PCR product yield, see manufacturers guidance for further information).

E. Tips and troubleshooting

- PCR products purified using the method described in this protocol are ready to be either sent to a commercial sequencing facility for Sanger sequencing or for preparation for Sanger sequencing in-house using an appropriate kit and protocol e.g. the BigDye™ Terminator v3.1 Cycle Sequencing Kit (ThermoFisher Scientific).
- See the Qiagen MinElute® PCR Purification Kit handbook for further information on this purification kit.

F. References

Qiagen MinElute® PCR Purification Kit handbook