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PCR normalization and size selection with magnetic beads

In 1 collection

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ABSTRACT

This protocol describes how to clean up and normalize PCR products or DNA extracts and perform a size selection with carboxylated-magnetic beads and a PEG-NaCl buffer. It works by diluting the beads so that the binding capacity is lower than the PCR yield which leads to a normalization of all PCR products to the binding capacity.

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PROTOCOL CITATION

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COLLECTIONS

Invertebrate bulk sample metabarcoding protocol collection

KEYWORDS

pcr cleanup, normalization, magnetic beads, library prep

CREATED

Oct 10, 2022

LAST MODIFIED

Oct 11, 2022

PROTOCOL INTEGER ID

71079

PARENT PROTOCOLS

Part of collection

Invertebrate bulk sample metabarcoding protocol collection

GUIDELINES

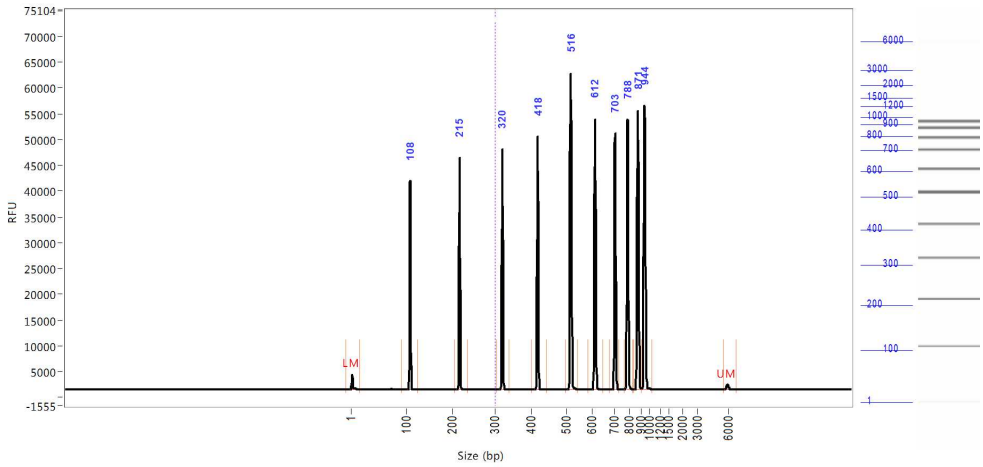
Follow general lab etiquette. Wear gloves to prevent contaminating the samples. Clean the workspace before starting with 80% EtOH.

Ratio Guide:
To get an estimate the performance of different ratios the protocol was tested using a DNA Ladder

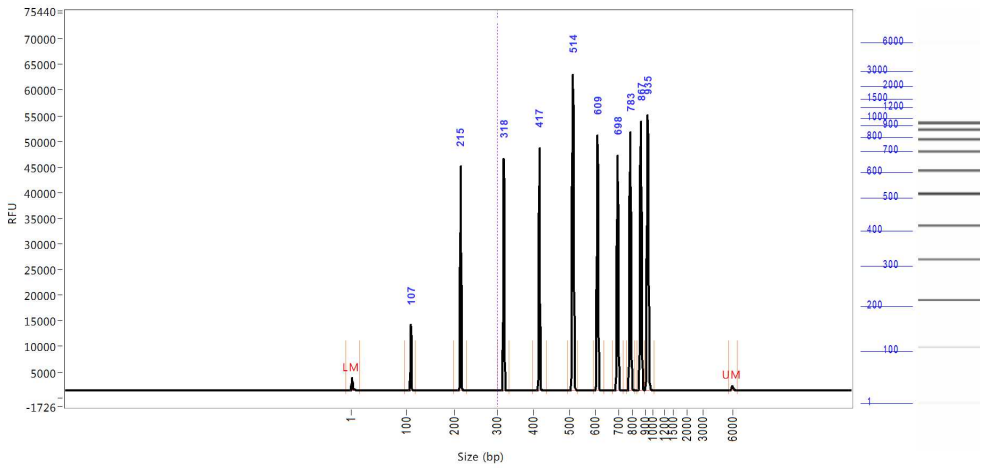
GeneRuler 100 bp DNA Ladder ready-to-use **Thermo Fisher Scientific Catalog #SM0243**

. The eluate was then measured using a Fragment Analyzer with the High Sensitivity Kit.

Input DNA:



Ratio 1.8:



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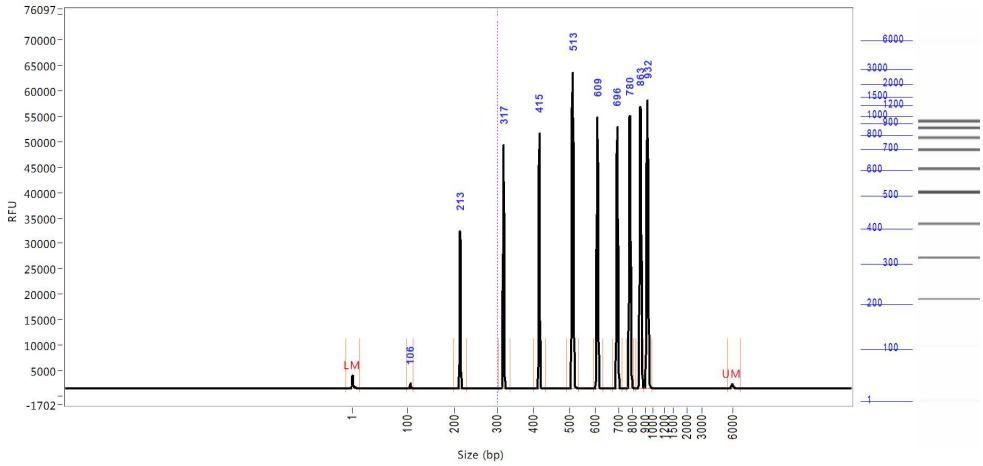
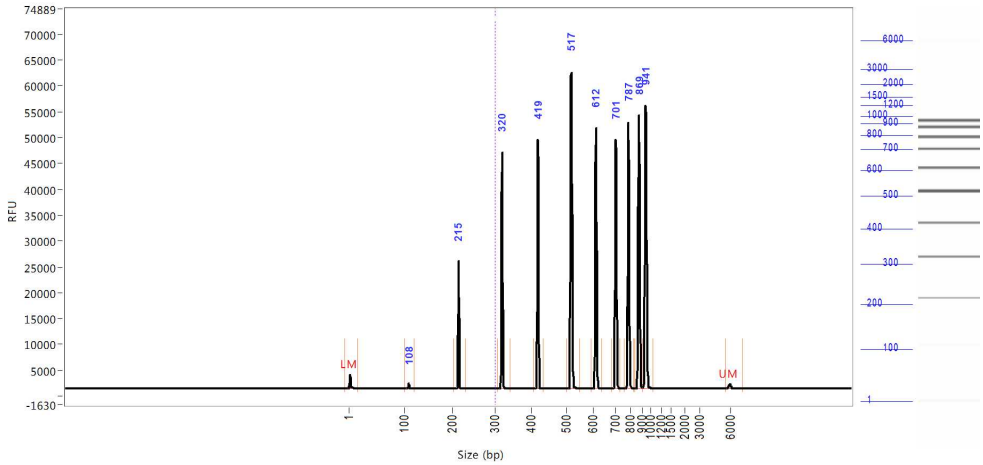
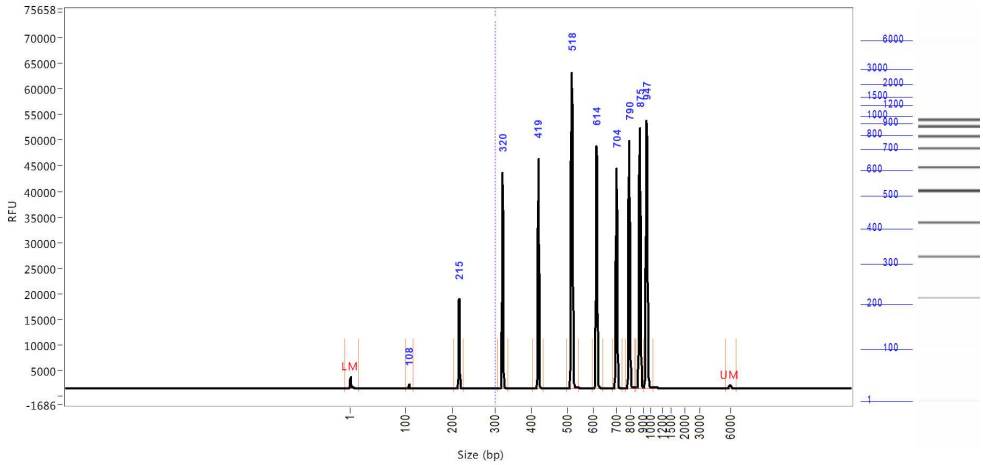
Ratio 1:



Ratio 0.9:



Ratio 0.85:



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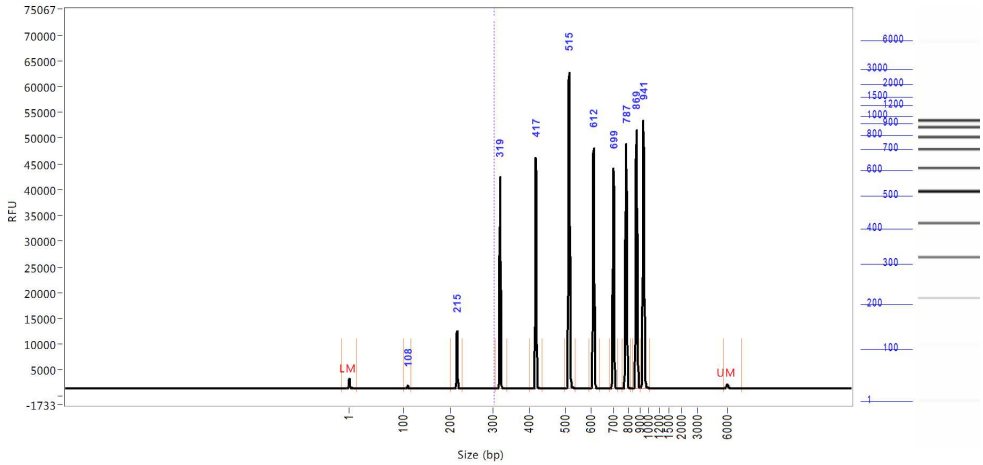
Ratio 0.8:



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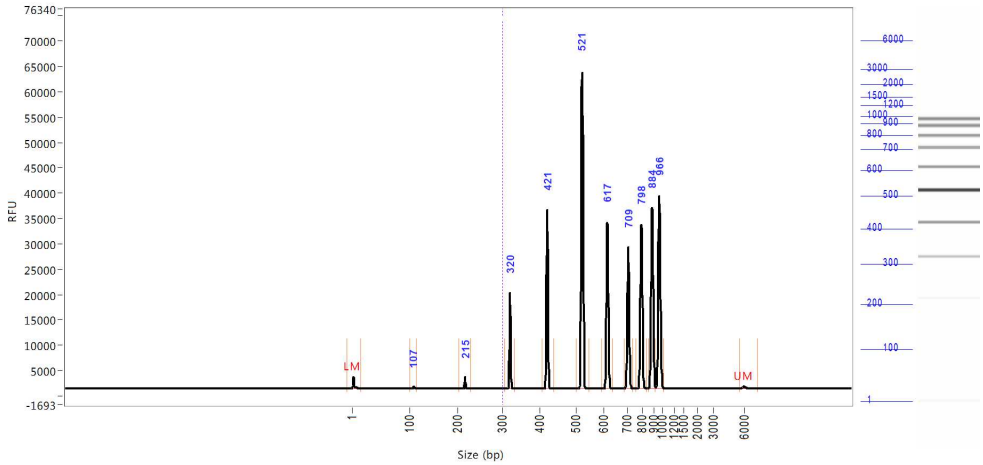

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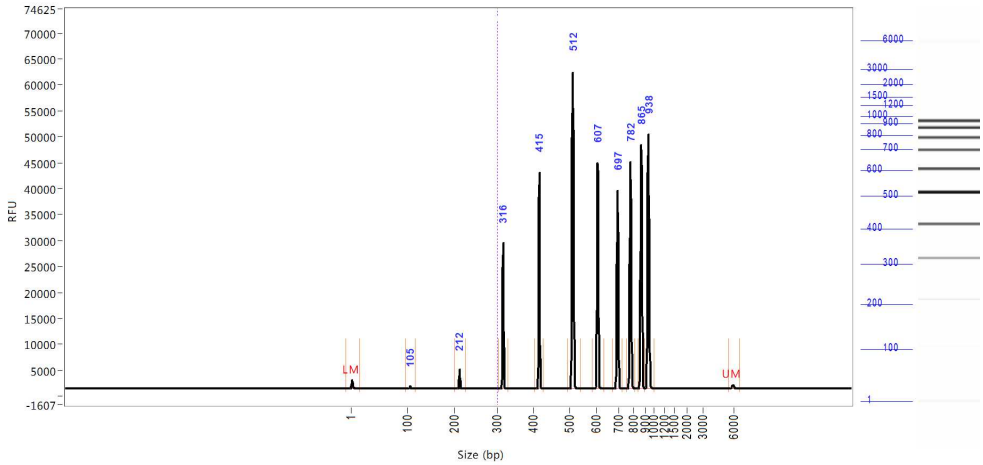

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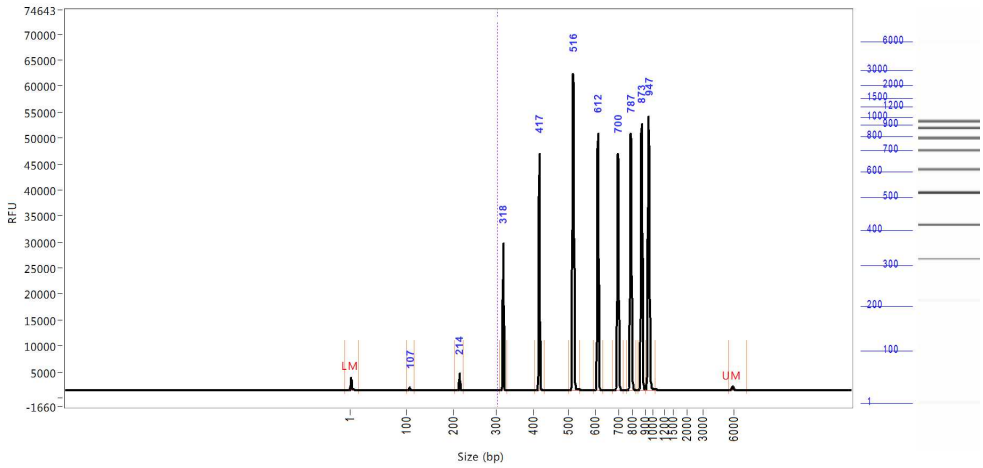

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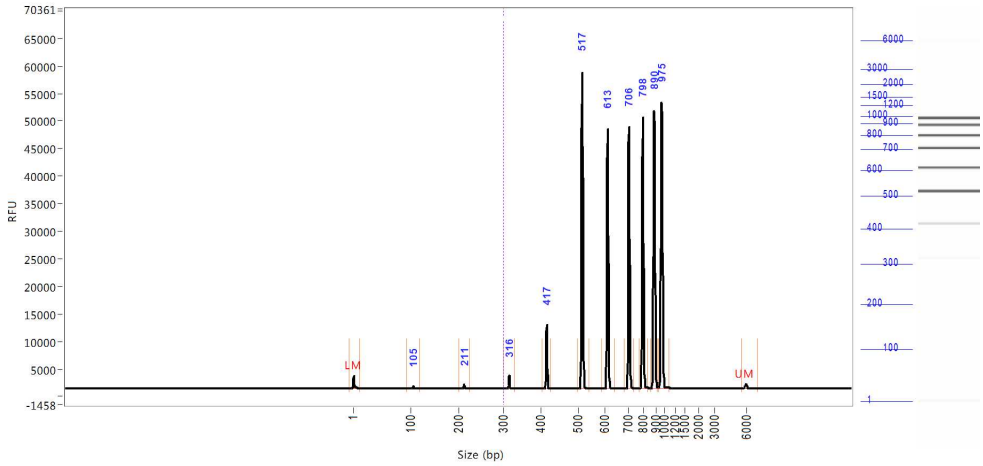

Ratio 0.55:

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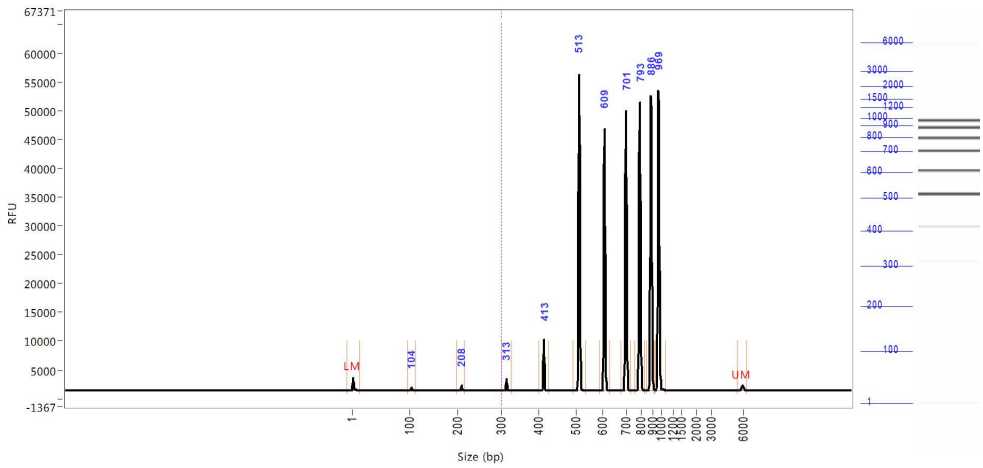

MATERIALS TEXT

Materials required:

Below all materials needed for the protocol are listed. Vendors and part numbers are listed but interchangeable depending on the supply situation.

Chemicals:

Ethanol absolute Ethanol absolute 99.8% **Fisher Scientific Catalog #11994041**

Hydrochloric acid fuming 37% Hydrochloric acid fuming 37% **Sigma Aldrich Catalog #1003171011**

Tris ultrapure Tris ultrapure 99.9% 99.9% **Diagonal Catalog #A1086.1000**

EDTA disodium salt EDTA disodium salt **Sigma Aldrich Catalog #E5134-50G**

Tween 20 Tween 20 **Carl Roth Catalog #9127.1**

Sera-Mag SpeedBeads

Sera-Mag SpeedBeads carboxylate modified particles **Sigma Aldrich Catalog #GE45152105050350**

PCR-grade water

Invitrogen UltraPure DNase/RNase-Free Distilled Water **Fisher Scientific Catalog #11538646**

Labware:

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125 mL Nalgene Wide-Mouth Bottle Therm

Thermo Scientific Nalgene Wide-Mouth LDPE Bottle with Closure **Fisher Scientific Catalog #10044180**

Neodyme

Large magnet magnet **Magnethandel Catalog #3935**

MM-Seperator M96 **Carl**

96-well plate magnet **Roth Catalog #2141.1**

Hard-Shell PCR Plate Hard-Shell 96-well

Hard-Shell 96-well PCR plate **BioRad Sciences Catalog #HSP9601**

Clear Polystyrene 96-Well Microplate

Corning Clear Polystyrene 96-Well EIA/RIA Microplate **Fisher Scientific Catalog #10380982**

Stock solutions:

1 L Tris stock solution [M]**1 Molarity (M)** pH**8.5**

- Add **121.14 g Tris ultrapure 99.9%** to a beaker
- Adjust volume to **800 mL** with ddH2O
- Adjust pH to pH**8.5** with HCl
- Adjust volume to **1 L** with ddH2O
- Sterilize by filtering and store at **Room temperature**

1 L Tris stock solution [M]**1 Molarity (M)** pH**8**

- Add **121.14 g Tris ultrapure 99.9%** to a beaker
- Adjust volume to **800 mL** with ddH2O
- Adjust pH to pH**8** with HCl
- Adjust volume to **1 L** with ddH2O
- Sterilize by filtering and store at **Room temperature**

1 L Tris stock solution [M]**1 Molarity (M)** pH**7.5**

- Add **121.14 g Tris ultrapure 99.9%** to a beaker
- Adjust volume to **800 mL** with ddH2O
- Adjust pH to pH**7.5** with HCl
- Adjust volume to **1 L** with ddH2O
- Sterilize by filtering and store at **Room temperature**

1 L EDTA stock solution [M]**0.5 Molarity (M)** pH**8**

- Add **186.12 g** EDTA disodium salt to a beaker

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- Adjust volume to 1 L with ddH2O
- Adjust pH to pH 8 with sodium hydroxide
- Sterilize by filtering and store at Room temperature

1 L wash buffer stock solution (50 millimolar (mM) Tris) pH 7.5

- Add 50 mL Tris stock solution pH 7.5 to a beaker
- Adjust volume to 1 L with ddH2O
- Sterilize by filtering and store at Room temperature

1 L PEG-NaCl buffer (2.5 Molarity (M) NaCl, 20 Mass / % volume PEG 8000, 10 millimolar (mM) Tris, 1 millimolar (mM) EDTA, 0.05 % (v/v) Tween 20) pH 8

- Add 200 g NaCl to a beaker
- Add 146.2 g PEG 8000
- Add 10 mL Tris stock solution pH 8
- Add 2 mL EDTA stock solution pH 8
- Add 250 µL of Tween 20
- Adjust volume to 1 L with ddH2O
- Dissolve the PEG and NaCl by stirring and heating to 80 °C the solution will become milky at this point.
- Let the solution cool down to Room temperature
- Sterilize by filtering and store at 4 °C

Working solutions:

1 L TE minimum buffer (10 millimolar (mM) Tris, 1 millimolar (mM) EDTA) pH 8

- Add 10 mL Tris stock solution pH 8 to a beaker
- Add 200 µL EDTA stock solution pH 8
- Adjust volume to 1 L with ddH2O
- Sterilize by filtering and store at Room temperature

1 L wash buffer (10 millimolar (mM) Tris, 80 % (v/v) Ethanol) pH 7.5

- Add 200 mL wash buffer stock solution
- Adjust volume to 1 L with Ethanol absolute
- Sterilize by filtering and store at Room temperature

1 L elution buffer (10 millimolar (mM) Tris) pH 8.5

- Add 10 mL Tris stock solution pH 8.5 to a beaker

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- Adjust volume to **1 L** with ddH2O
- Sterilize by filtering and store at **Room temperature**

100 mL cleanup solution pH **8**

- Add **2 mL Sera-Mag SpeedBeads carboxylate modified** to a clean **125 mL** Nalgene bottle
- Add **25 mL TE minimum buffer**
- Shake the bottle to wash the beads
- Place the bottle on a large magnet for **00:05:00** to pellet the beads
- Discard the supernatant
- Add **25 mL TE minimum buffer**
- Shake the bottle to wash the beads
- Place the bottle on a large magnet for **00:05:00** to pellet the beads
- Discard the supernatant
- Add **100 mL PEG-NaCl buffer**
- Shake well to resuspend the beads
- Store at **4 °C**

100 mL normalization solution pH **8**

- Add **95 mL PEG-NaCl buffer** to a clean **125 mL** Nalgene bottle
- Add **5 mL cleanup solution**
- Shake well to resuspend the beads
- Store at **4 °C**

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SAFETY WARNINGS

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Reagents are potentially damaging to the environment. Dispose waste responsibly.

BEFORE STARTING

Make sure all buffers are prepared before starting.
For easier pipetting let the normalization solution adjust to Room temperature.

The protocol described here is designed for the use of **250 µL U-bottom assay plates** , but can also be done in tubes, PCR plates, strips, or any sufficient reaction vessel. The recommended shaking speeds are adjusted to the plates mentioned in the materials.

1 Shake the **normalization solution** until the beads are homogeneously resuspended

The protocol described here uses a **normalization solution** to **sample** ratio of 0.7:1. This is sufficient for the removal of primer and primer dimers below a size of 200 bp. For the removal of shorter or larger fragments, the ratio has to be adjusted accordingly. For more information on ratios refer to the material provided in the tab "Guidelines".

The protocol described here is designed for **9 µL PCR product** . If the PCR assay is larger, less water has to be added in step two. It's recommended to keep the amount of normalization solution as is to achieve an output concentration of about **2 ng/µL** .

2 Add **31 µL PCR-grade water** and **28 µL of normalization solution** to a 250 µL U-bottom assay plate

It's recommended to increase the volume of the sample with PCR-grade water for easier liquid handling but also to lower relative pipetting error (e.g. if the pipette is off by **2 µL** the effect on the ratio is larger if working with a **10 µL** assay than when working with a **80 µL** assay.

The amount of beads is calculated as follows:
(sample volume + water volume) * ratio = cleanup solution volume

In this example:
(9 µL PCR product +30 µL PCR-grade water) * 0.7 = 28 µL cleanup solution

For higher sample numbers PCR-grade water and cleanup solution can be prepared as a master mix.

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3 Add **9 µL of PCR product**

4 To bind the DNA to the beads shake at **900 rpm, Room temperature , 00:05:00**

If the protocol is not done in plates mixing can also be accomplished by pipetting or vortexing.

5 Place the plate on a magnet to pellet the beads for **00:02:00** 2m

The bead pellet might be barely visible at this point.

Depending on the magnet and volume used separation times may vary and have to be adjusted accordingly.

6 Discard the supernatant by pipetting

7 With the plate still on the magnet, add **100 µL of wash buffer** to each sample

8 Incubate for at least **00:00:30** 30s

9 Discard the supernatant by pipetting

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10 **go to step #7 and repeat once for a total of 2 washes**

11 With the plate still on the magnet, incubate the plate for **00:05:00** at **Room temperature** to dry off residuals of wash buffer 5m

12 Add **50 µL** of elution buffer to each sample

13 **900 rpm, Room temperature , 00:05:00 to elute the DNA from the beads**

14 Place the plate on a magnet to pellet the beads for **00:02:00** 2m

> The bead pellet might be barely visible at this point.

15 Transfer **40 µL** of the DNA to a new PCR plate. Store at **-20 °C**

> Leaving **10 µL** of elution buffer is recommended to avoid carry-over of beads. If all of the DNA is needed for subsequent analysis try to pipette slowly without disturbing the pellet.

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