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PCR normalization and size selection with magnetic beads V.3

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We use this protocol and it's working

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Keywords: pcr cleanup, normalization, magnetic beads, library prep, normalization of all pcr product, pcr normalization, size selection with magnetic bead, pcr product, magnetic bead, pcr yield, dna extract, lower than the pcr yield, dna, bead, normalization, nacl buffer, size selection

Abstract

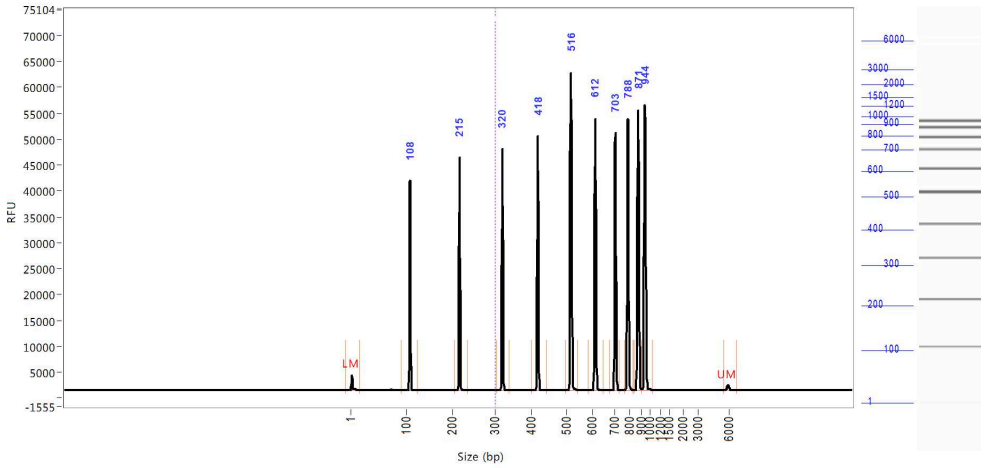
This protocol describes how to clean up and normalize PCR products or DNA extracts and perform a size selection with carboxylated-magnetic beads and a PEG-NaCl buffer. It works by diluting the beads so that the binding capacity is lower than the PCR yield which leads to a normalization of all PCR products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

Guidelines

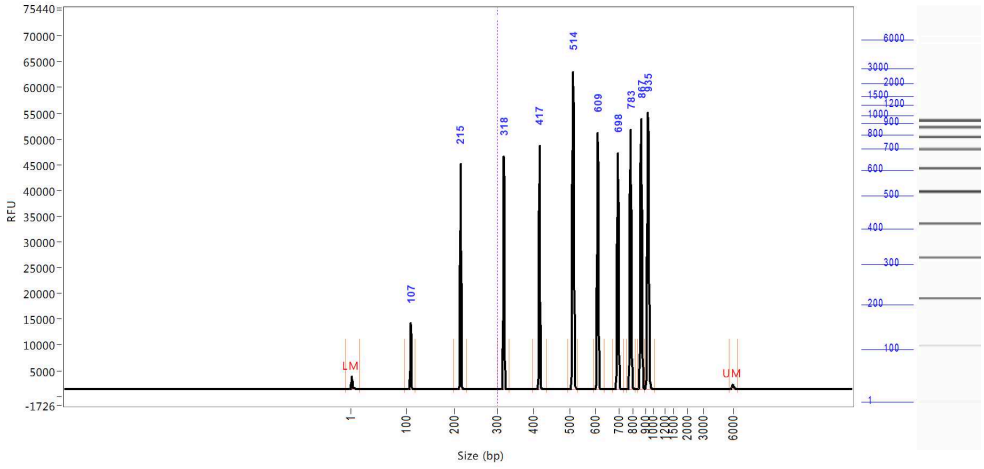
Follow general lab etiquette. Wear gloves to prevent contaminating the samples. Clean the workspace before starting with 80% EtOH.

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.

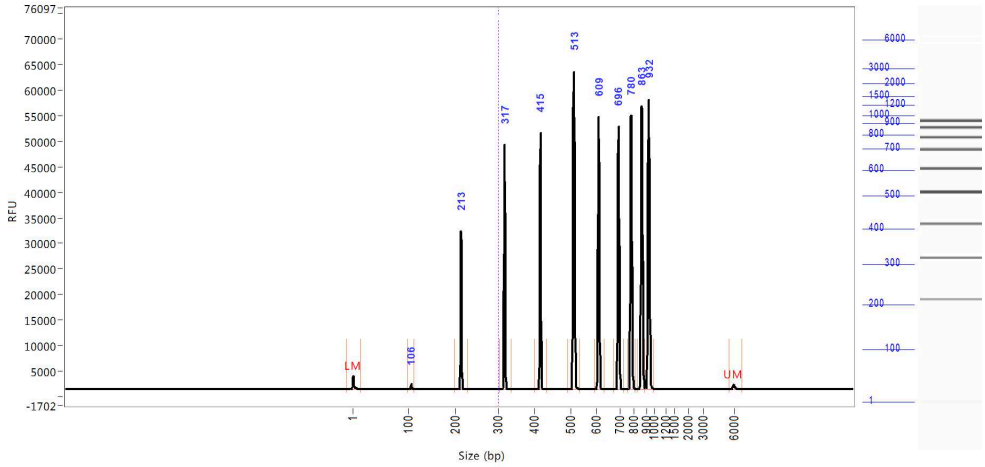
Input DNA:



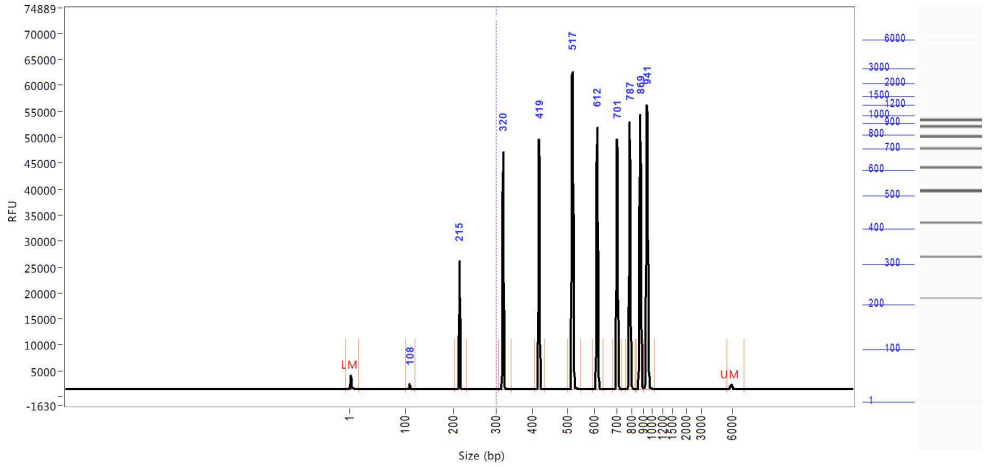
Ratio 1.8:



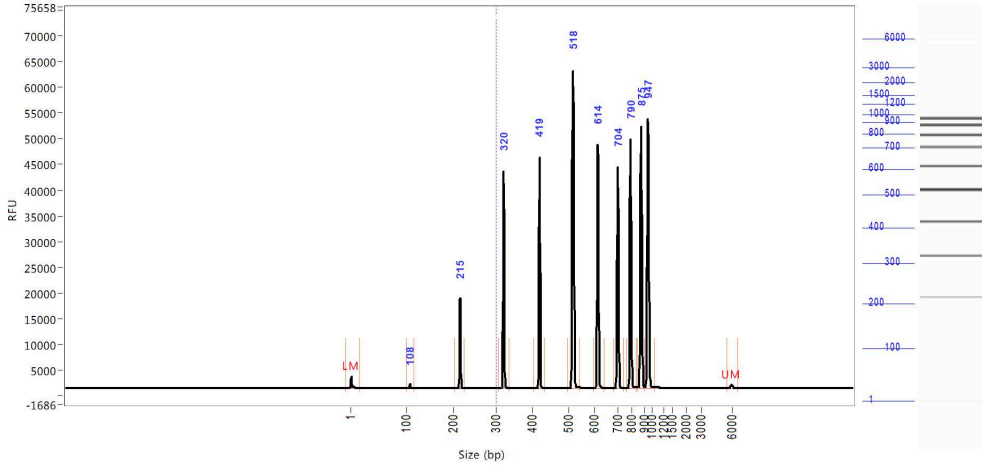
Ratio 1:



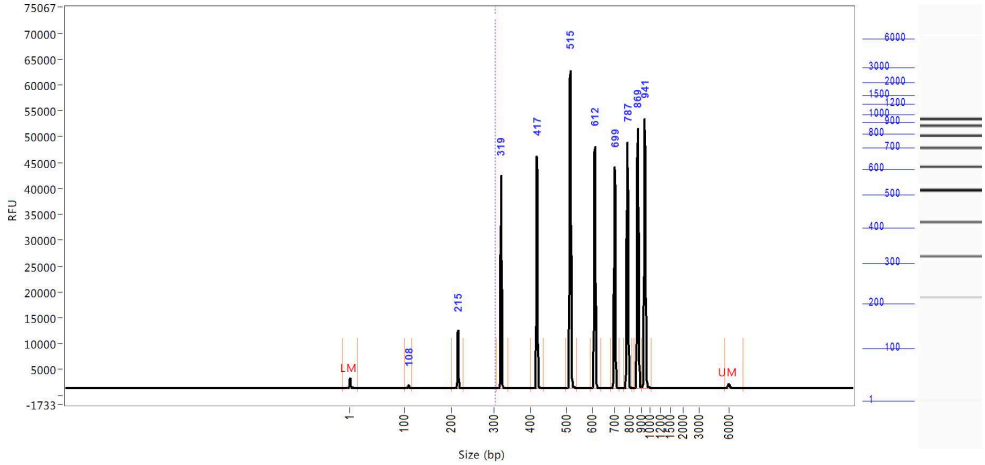
Ratio 0.9:



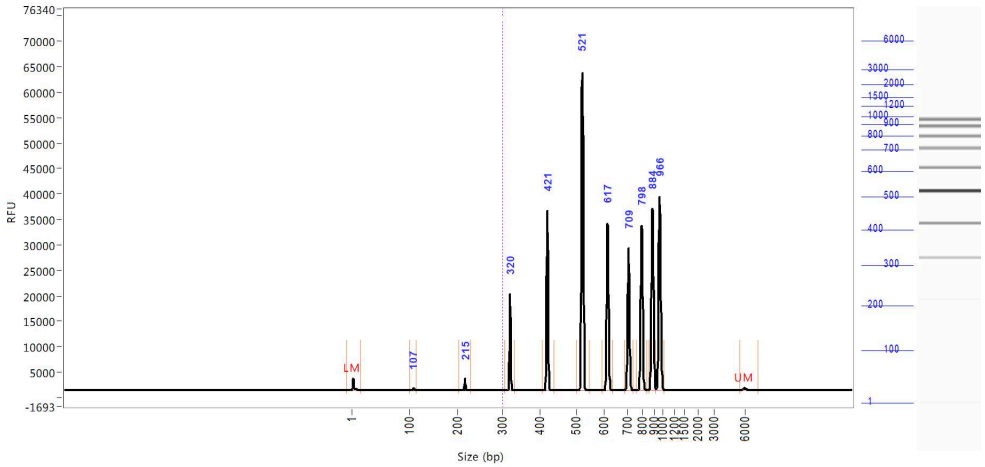
Ratio 0.85:



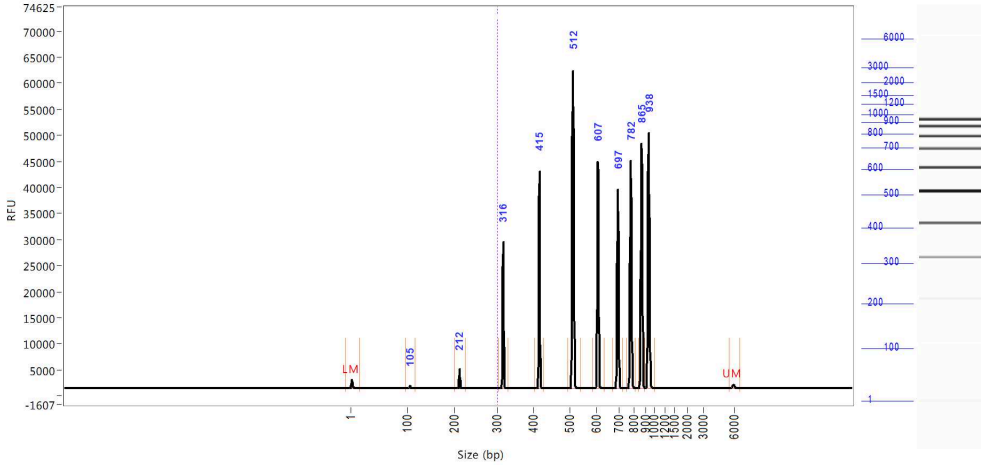
Ratio 0.8:



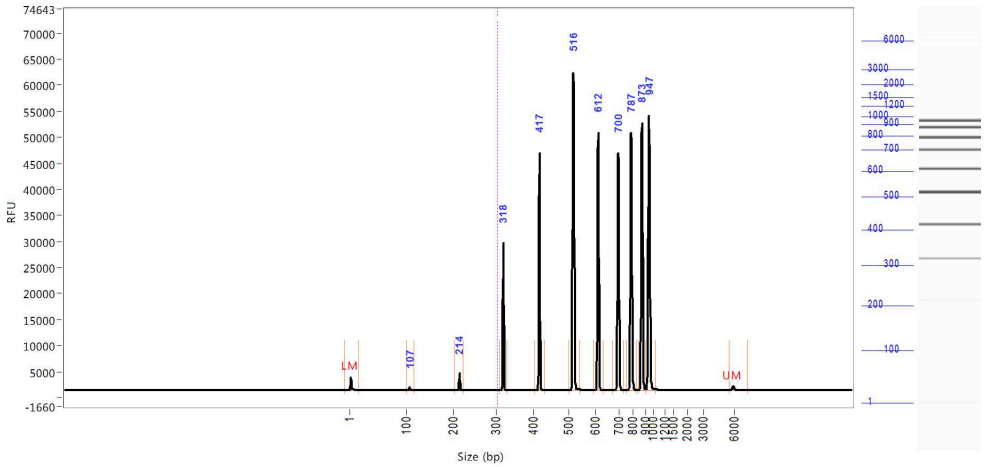
Ratio 0.75:



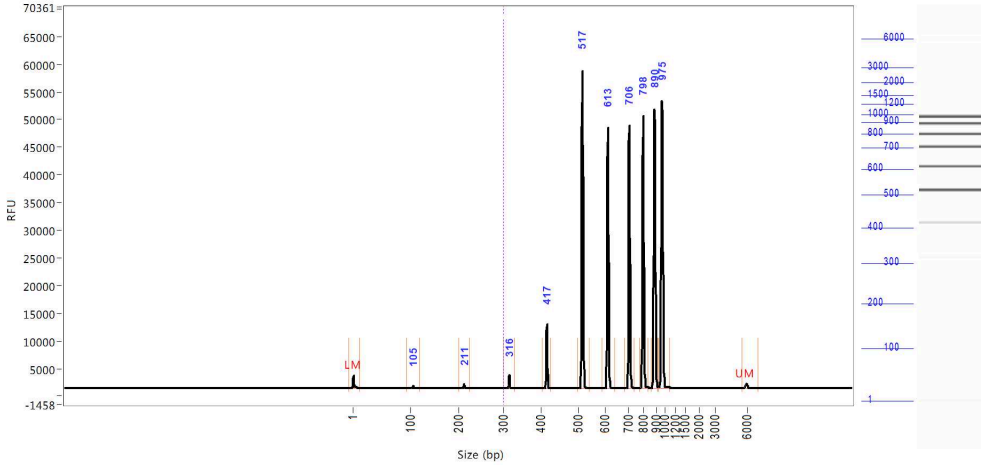
Ratio 0.7:



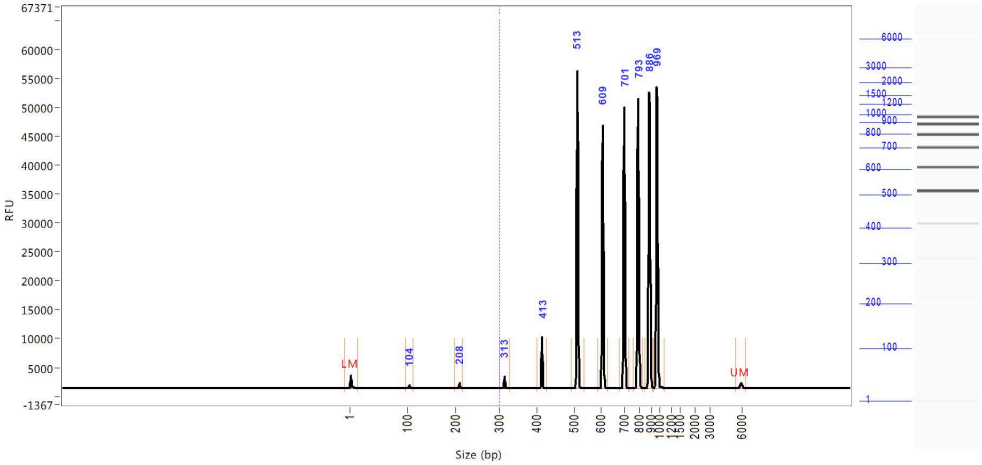
Ratio 0.65:



Ratio 0.6:



Ratio 0.55:


RFU
Size (bp)
LM
UM
104
208
313
413
513
609
701
793
809

Materials

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% **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**1003171011
Tris ultrapure 99.9% Tris ultrapure 99.9% **Diagonal Catalog #**A1086.1000
EDTA disodium salt EDTA disodium salt **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**E5134-50G
Tween 20 Tween 20 **Carl Roth Catalog #**9127.1
Sera-Mag SpeedBeads
Sera-Mag SpeedBeads carboxylate modified particles **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**GE45152105050350
PCR-grade water
Invitrogen UltraPure DNase/RNase-Free Distilled Water **Fisher Scientific Catalog #**11538646

Labware:

125 mL Nalgene Wide-Mouth Bottle Therm
Thermo Scientific Nalgene Wide-Mouth LDPE Bottle with Closure **Fisher Scientific Catalog #**10044180
Large magnet Neodyme magnet **Magnethandel Catalog #**3935
96-well plate magnet MM-Seperator M96 **Carl Roth Catalog #**2141.1
Hard-Shell PCR Plate Hard-Shell 96-well
Hard-Shell 96-well PCR plate **Bio-Rad Laboratories 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 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) 8

- Add 121.14 g Tris ultrapure 99.9% to a beaker
- Adjust volume to 800 mL with ddH2O
- Adjust pH to 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) 7.5

- Add 121.14 g Tris ultrapure 99.9% to a beaker
- Adjust volume to 800 mL with ddH2O
- Adjust pH to 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) 8

- Add 186.12 g EDTA disodium salt to a beaker
- Adjust volume to 1 L with ddH2O
- Adjust pH to 8 with sodium hydroxide
- Sterilize by filtering and store at Room temperature

1 L wash buffer stock solution ([M] 50 millimolar (mM) Tris) 7.5

- Add 50 mL Tris stock solution 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 ([M] 2.5 Molarity (M) NaCl , [M] 20 Mass / % volume PEG 8000 , [M] 10 millimolar (mM) Tris , [M] 1 millimolar (mM) EDTA , [M] 0.05 % (v/v) Tween 20) 8

- Add 200 g PEG 8000 to a beaker

- Add 146.2 g NaCl
- 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 ([M] 10 millimolar (mM) Tris , [M] 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 ([M] 10 millimolar (mM) Tris , [M] 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 ([M] 10 millimolar (mM) Tris) pH 8.5

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

Troubleshooting

Safety warnings

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

Before start

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

Note

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

Note

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".

Note

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 [M] 2 Mass Percent .

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

Note

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 +31 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.

3 Add 9 µL of PCR product

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

> Note
>
> 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

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

> Note
>
> 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

10 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

Note

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

Note

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.