Automated 96-well PCR Purification

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ABSTRACT

This protocol describes a fully-automated PCR cleanup in a 96-well PCR plate format with AMPure XP beads on an Eppendorf epMotion. Multichannel pipetting with the 300 uL and 50 uL pipettes is used. It is designed to use the thermomixer module; however, this can be replaced by modifying the program to mix by pipetting. The bead magnet used in this program is a Alpaqua Low Elution Magnet Plate. We also use Eppendorf twin.tec PCR plates (LoBind, skirted, 150uL).

IMPORTANT: The program is currently set to add **22 µL** of water to each well followed by **65 µL** beads. This results in an approximately 1:1.5 bead ratio with a starting volume of **22 µL** PCR product. Modify the first step accordingly with your starting volume and desired bead ratio.

The manufacturer's instructions are found here:
https://www.beckmancoulter.com/wsrportal/techdocs?docname=B37419

The epMotion program is attached here.

ATTACHMENTS

Application_1x bead cleanup_210519_140208.export6

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PROTOCOL CITATION

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KEYWORDS

PCR purification, Eppendorf epMotion, bead cleanup, automatic

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MATERIALS TEXT

Agencourt AMPure XP beads **Contributed by users**

Eppendorf twin.tec® PCR 96-well plate, skirted **Eppendorf Catalog #951020401**

Molecular grade water nuclease-free **Contributed by users**

100% Molecular grade ethanol **Contributed by users**

LE Magnet Plate
Alpaqua **A000350**

1 Take aliquot of beads out of refrigerator and allow to warm to room temperature.

2 Start program and input the numer of samples you have. It will then tell you the minimum volumes for each reagent. The positions in the reservoir rack are:
1. Molecular grade H_2O
3. Beads
5. 70% EtOH
7. Elution buffer (e.g. H_2O, TRIS-Acetate (10 mM pH 8.0), or TE Buffer (10 mM Tris-Acetate pH

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8.0, 1 mM EDTA))

You can use the level sensor to detect volumes or measure them with a serological pipette. Do not start the program until all of your items are in the correct positions.

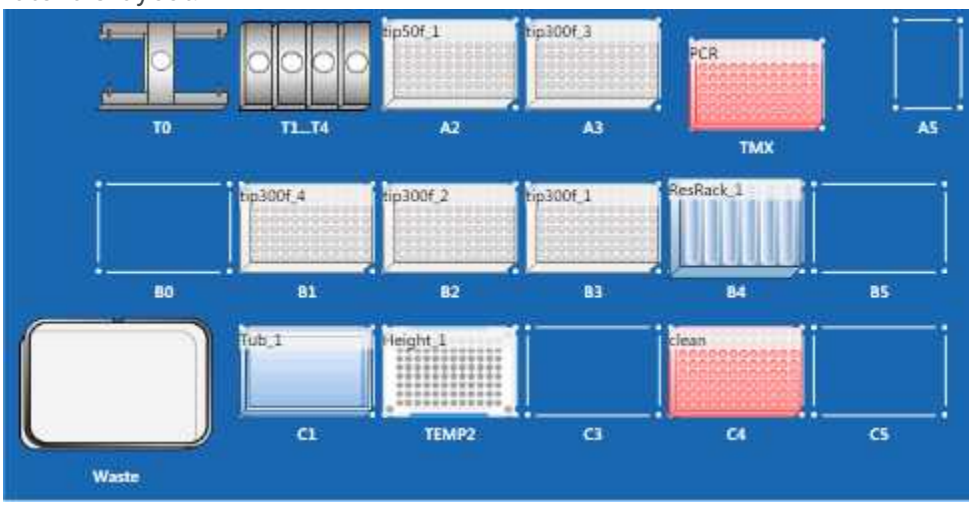
3 Mix beads well until they appear homogenous.

4 Prepare fresh 70% EtOH.

From the Agencourt XP beads manual: "Fresh 70% ethanol should be prepared for optimal results. There is also miscibility involved with ethanol and water. For example, measuring out 70 mL of ethanol and topping off to 100 mL with water will generate ~65% ethanol. Measuring 70 mL ethanol and 30 mL water separately, then combining them will generate ~95 mL of 70% ethanol."

5 Place reagents, your PCR plate, and a new clean plate on the epMotion.

Materials layout:



Run the program. It will take approximately 1 hour for a full plate.

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