NUCLEIC ACID PURIFICATION WITH THE MAGNETIC MODULE ON THE OT-2

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INTRODUCTION

Bead-based purification yields high quality nucleic acids without the use of centrifugation or vacuum separation. The OT-2 Magnetic Module, also known as the MagDeck, is designed for automated bead-based purification protocols without moving labware on and off a magnetic plate. Here we present a highly customizable protocol for the purification of PCR products and other nucleic acids.

REAGENTS AND LABWARE

Common reagents for this protocol are bead solution, ethanol and elution buffer (water, Tris, TE, etc.). We encourage users to choose from various magnetic bead products for nucleic acid purification. This protocol contains many parameters to customize protocols for many different magnetic beads. For example, customizing the amount of time that the module is engaged to pellet your particular beads can tailor the nucleic acid purification protocol for optimal results.

We recommend using the Bio-Rad Hard-Shell® low profile, thin wall, 96 well plate skirted on the Magnetic Module. This plate fits well in the bracket that secures labware on the module. We also support an array of labware as a reagent source for this experiment. The OT-2 4-in-1 tube rack or a 12-row-trough are great reservoirs for single and multi-channel pipettes, respectively. Optionally, the OT-2 Temperature Module and the 1.5 - 2 mL or the 96-well PCR plate aluminum block can be used to heat elution buffer to increase yield.

Figure 1. Magnetic Module with 0.2 mL PCR plate attached.

PROTOCOL SETUP

REQUIRED PARAMETERS

Pipette and Mount : Specify the pipette and mount, left or right, for your protocol.
We recommend the p50 and p300 single or multi-channel pipettes for the volumes of reagents in this experiment.
Sample number: Users can customize the number of samples to run per protocol. We recommend multiples of 8 if you are using a multi-channel pipette.
Sample volume: The starting volume of the input sample can be customized.
Sample volumes above 10 µL are recommended.
Bead Ratio: The ratio of beads can be customized for left or right side size-selection of fragments.
The default bead ratio is 1.8x the input sample volume.
Elution Volume: Users can choose the final volume to elute the purified nucleic acid. The Magnetic Module supports elution volumes above 10 µL.

ADVANCED PARAMETERS

Incubation Time: The incubation time is the amount of time that the bead solution and input sample interact. The default incubation time is 5 minutes.
Settling Time: This parameter allows the beads to pellet for specified amount of time.
Higher volumes may require a longer settling time to pellet beads. The default settling time is 2 minutes.
Drying Time: The drying time after the wash steps can vary depending on your bead products.

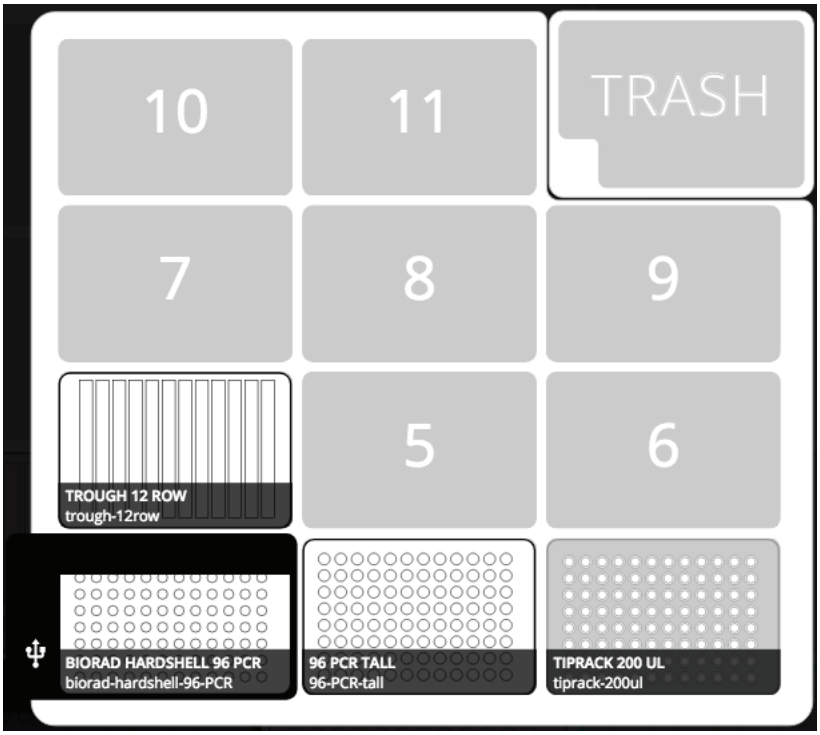

Figure 2. Simple Deck Layout for Nucleic Acid Purification. The Magentic Module needs to be connected to power source and via USB to the back left of the OT-2. Therefore, we suggest placing the module in the outer slots of the deck to avoid cords interfering with labware on the deck.

TIPS FOR REAGENT HANDLING

When preparing reagents, automation may require at least a 10% excess of reagents in your source labware. Be mindful of the minimum and maximum volume of the labware. For example, we suggest using at least 5 mL of liquid in the 12-row trough.

Magnetic beads can easily settle to the bottom of labware without mixing.
It is important to mix the beads before aspirating the bead solution and dispensing it to samples. Once the beads have been added to the sample, pipette mixing 10 times is suggested in lieu of vortexing the sample. Dispensing beads at the the top of the well containing the sample helps resuspend the beads more easily.

The low density of 70% ethanol can cause dripping from the pipette tip.
We include both an air gap and a short delay when aspirating ethanol in this protocol.

When aspirating the supernatant from the bead solution, we aspirate 1 mm from bottom of the well and aspirate slowly (~ 25 µL/s). This will prevent aspirating the beads with the supernatant at this step.

Optionally, heating elution buffer can increase the percent recovery of your purified product.
We recommend using the Temperature Module (or TempDeck) to actively heat your elution buffer to improve yield.