



FULLY AUTOMATED NGS CLEAN UP AND SIZE SELECTION WITH OMEGA BIO-TEK MAG-BIND® TOTALPURE NGS ON THE OT-2

Chris Lee¹, Travis Butts¹, Julie Baggs¹, Frank Yuan¹, and Kinnari Watson²

Omega Bio-tek¹ and Opentrons²

INTRODUCTION

DNA clean-up and size selection steps for next-generation sequencing (NGS) workflows require precise and accurate pipetting, which can be labor intensive for large scale experiments. Omega Bio-tek's Mag-Bind® TotalPure NGS Kit provides a fully automatable, highly flexible solution for both DNA and RNA clean-up and size selection. The Omega Bio-tek Mag-Bind® TotalPure NGS Kit is widely used in NGS clean-up for its affordability and simplicity. It is also well adapted for nucleic acid size selection by varying bead ratios for the isolation of a wide array of fragment sizes¹. Since size selection is dependent on the volume ratio of sample and magnetic beads, accurate pipetting is essential for reproducible, consistent results. In addition, complete ethanol removal from samples following clean-up or size selection is often critical for downstream applications. Here we present a fully automatable system for DNA and RNA clean-up and size selection on an Opentrons OT-2 liquid handling robot using the Omega Bio-tek Mag-Bind® TotalPure NGS kit. The Opentrons OT-2 is a flexible and affordable option for liquid handling that can be equipped with two pipettes, a single-channel and/or 8-channel in any combination, to accurately dispense volumes of 1-300 µL. Here we demonstrate accurate size recovery and high purity of indexed 16S metagenomics samples following clean-up and size selection on the OT-2. This application demonstrates that pairing Omega Bio-tek's reagents with Opentrons automation can provide a complete and reliable solution for NGS workflows. An optimized protocol for using the Opentrons OT-2 and Magnetic Module with the Omega Bio-tek Mag-Bind® TotalPure NGS kit can be downloaded directly from the Opentrons Protocol Library.

WORKFLOW

An Opentrons OT-2 equipped with a Magnetic Module and 8-channel and single pipette was used for these experiments. The Opentrons Magnetic Module utilizes a unique two-position system consisting of a raised position for beads to magnetize and a lowered position where the magnetic beads remain in solution.

The Magnetic Module eliminates the need for time-consuming plate transfers between the deck and magnetic stand. The OT-2 electronic pipettes have been tested to dispense accurate and precise liquid volumes². The high-strength bar magnets on the Magnetic Module enable short settling times for magnetizing and pelleting the magnetic beads.

Clean-up and size selection of indexed 16S metagenomic PCR samples and a 50 bp DNA ladder were performed on the Opentrons OT-2. The deck layout is illustrated in Figure 1. Both 1.8X and 1X bead ratios were added to 20 μ L of either sample and mixed on the liquid handler. Following magnetization and aspiration of supernatant, beads were washed twice with 70% ethanol then dried for 5 minutes. DNA was eluted with 20 μ L 10 mM Tris-HCl, pH 8.5. All experiments were performed in Bio-Rad HardShell[®] 0.2 mL PCR plates. Eluted samples were analyzed on an Agilent TapeStation using a High Sensitivity D1000 tape. Recovery rate was calculated by comparing input DNA against DNA recovered following 1X or 1.8X purification with Mag-Bind[®] TotalPure NGS. Eight or sixteen samples were processed for each run.

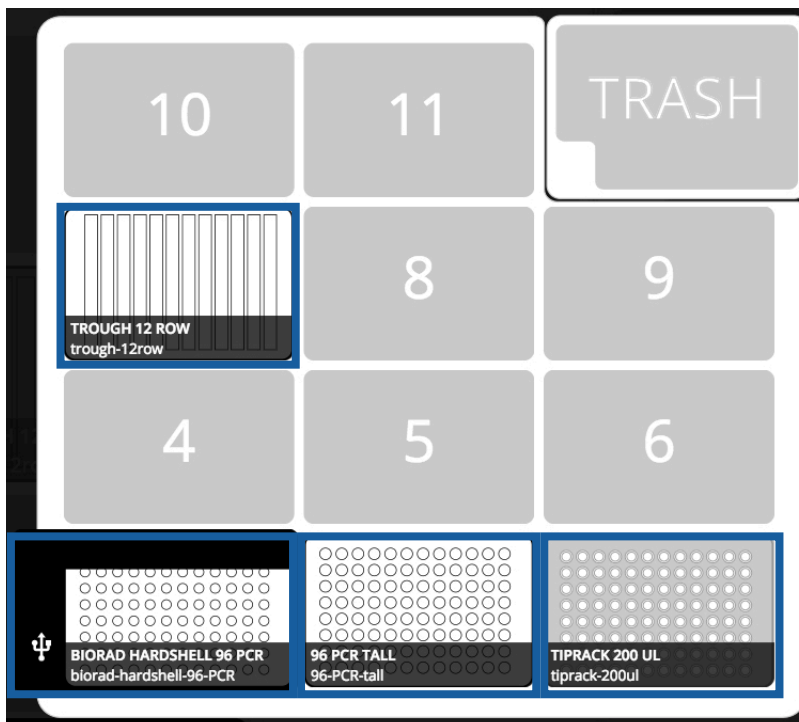


Figure 1. Deck layout for NGS clean-up with Omega Bio-Tek Mag-Bind[®] TotalPure NGS kit. The Magnetic Module and the Bio-Rad 0.2 mL PCR plate, the output plate for purified samples and the 300 μ L tip rack are shown placed in the slots in the front of the deck. The reagent reservoir, referred to as the 12-row trough, is shown in slot 7 on the deck. Blue rectangles are shown around labware and modules that need to be placed on the deck for this experiment.

RESULTS

To assess DNA recovery after clean-up and size selection of NGS libraries, we used bead ratios of 1X and 1.8X that are commonly used to remove adapter dimers and small fragments. Purification of 20 μ L indexed 16S metagenomic PCR sample resulted in high purity samples (Figure 2). We ran parallel experiments showing the size selection process using a 50 bp ladder.

Smaller DNA fragments were removed after 1X and 1.8X clean ups, indicating successful size selection procedures. Samples eluted in 20 μ L elution buffer maintained high concentrations. The calculated average recovery rate was over 90%. The A260/A280 values of the samples were approximately 2, indicating high-quality DNA samples (Table 1).

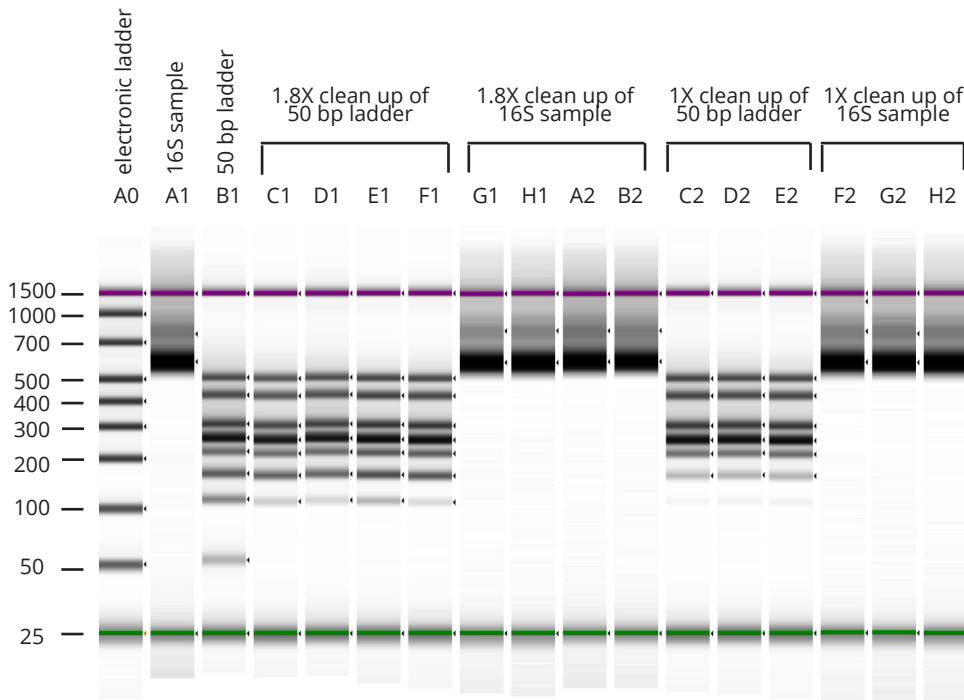


Figure 2 Purification of 20 μ L of indexed 16S Metagenomic and ladder samples with 1X and 1.8X bead volumes.

Agilent TapeStation High Sensitivity D1000 ScreenTape® of purifications of the 16S metagenomic sample and a 50 bp ladder to illustrate size-selection. 1X ratio means beads suspension volume is 1 time the sample volume; 1.8X ratio means beads suspension volume is 1.8 times the sample volume.

Table 1. Purity and recovery of 16S indexed metagenomic samples processed using the OT-2 Magnetic Module and the Omega Bio-tek Mag-Bind® TotalPure NGS kit.

Sample	Bead Proportion	A260/280	Recovery (%)
A1(Input)	-	1.87	-
G1	1.8X	1.94	92.31
H1	1.8X	2.21	92.31
A2	1.8X	2.02	92.31
B2	1.8X	2.08	92.31
E2	1X	1.99	92.31
G2	1X	2	100
H2	1X	2.1	92.31

CONCLUSIONS

Here we demonstrate a fully automated workflow for Mag-Bind® TotalPure NGS on the Opentrons OT-2 pipetting robot equipped with a Magnetic Module. Both the reagent and robot are flexible allowing the size selection range and throughput to be easily adjusted to meet individual requirements. The precise pipetting of the OT-2 robot generates highly reproducible results across multiple samples, thereby reducing the amount of sample repeats required due to pipetting errors and variability. The OT-2 Magnetic Module also allows elution volumes to be as low as 20 µL to maintain high concentrations of NGS libraries. Instrument scripts are available at no cost in the Opentrons Protocol Library. The combination of Omega Bio-tek's Mag-Bind® TotalPure NGS and Opentrons OT-2 pipetting robot provides a complete, flexible, and user-friendly automated solution for the clean-up and size selection of NGS libraries. High-quality DNA can be recovered at a rate of over 90% for a fraction of the cost of comparable solutions currently on the market. With Omega Bio-tek's Mag-Bind® TotalPure NGS Kit offered at 40% below equivalent magnetic bead products and Opentrons OT-2 starting at \$4,000, this automated solution is also an economical choice for any NGS application.

PRODUCT INFORMATION

The following products are available for purchase directly from the [OT Shop](#).

Product	Description
Mag-Bind TotalPure NGS kit	Beads for purification and size selection of DNA and RNA
OT-2 Pipetting Robot	Liquid handling robot
OT-2 8-channel Pipette (p300)	High performance electronic pipette compatible with the OT-2
OT-2 300 µL Tip Rack	Universal tips optimized for pipetting 10 -300 µL
OT-2 Magnetic Module	Module to engage and disengage with labware for bead-based purification on the OT-2

REFERENCES

1. Evaluation of Omega Mag-Bind® TotalPure NGS Beads for DNA Size Selection. By Maggie Weitzman, M.Sc. (University of Oregon / GC3F). http://omegabiotek.com/store/wp-content/uploads/2018/06/Evaluation_of_Omega_Mag-Bind_TotalPure_NGS_Beads_MWeitzman_April2018.pdf
2. Opentrons. Intro to Opentrons Electronic Pipettes Precision, Accuracy, Design, and Testing Methods. <https://s3.amazonaws.com/opentrons-landing-img/pipettes/OT-2-Pipette-White-Paper.pdf>



20 Jay St #528
Brooklyn, NY 11201

www.opentrons.com
info@opentrons.com



400 Pinnacle Way, Suite 450
Norcross, GA 30071

www.omegabiotek.com
info@omegabiotek.com