NucleoMag® NGS Clean-up and Size Select

Automated Clean-up and size selection for NGS Library preps on the Opentrons OT-2

Application benefits

The combination of the NucleoMag® NGS Clean-up and Size Select kit with the OT-2 has several advantages that streamline your enzymatic reaction clean-up and fragment size selection:

- Verified method for fully automated clean-up and size selection
- Efficient clean-up of NGS library preparation reactions
- Tunable size selection and reaction volume to be easily adjusted to meet individual requirements
- Processing of up to 48 samples in parallel
- Protocols available in the Opentrons Protocol Library or via MACHEREY-NAGEL technical automation support: automation-bio@mn-net.com

Keywords

NGS Clean-up and Size Selection, magnetic beads, Opentrons OT-2

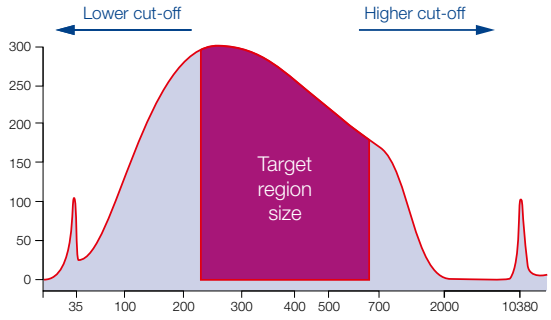
A verified method for PCR Clean-up and size selection of NGS library preparation reactions

Most next-generation sequencing workflows require DNA clean-up and size selection steps for library preparations. The NucleoMag® NGS Clean-up and Size Select kit is designed for the clean-up of reaction mixtures, as well as for single or double size selection for library construction processes in next generation sequencing applications (NGS).

In this Application Note we demonstrate automated clean-up processes using the NucleoMag® NGS Clean-up and Size Selection kit on the Opentrons OT-2 equipped with the Opentrons Magnetic Module, with the Single-Channel P1000 and with the 8-Channel P300 Pipettes.

Both the reagent and robot are flexible, allowing the size selection range and throughput to be easily adjusted to meet individual requirements.

An optimized protocol using the Opentrons OT-2 with the NucleoMag® NGS Clean-up and Size Selection kit can be downloaded directly from the Opentrons Protocol Library.



NucleoMag® NGS Clean-up and Size Select	
Technology	Magnetic beads
Application	Clean-up of enzymatic reaction mixtures or size selection for next-generation sequencing workflows
Typical recovery	≥ 80 %
Fragment size	~150bp to ~800bp (size selection)
Max. sample number on OT-2	48 samples

Material and Methods

NucleoMag® NGS paramagnetic beads selectively bind DNA fragments based on the volume ratio of bead suspension and sample. In general, increasing the volume ratio will favor the adsorption of shorter fragments to the paramagnetic beads. For single size selection (left side selection), fragmented plant DNA or linearized DNA fragments were mixed with the beads in predetermined ratios for the desired exclusion of smaller fragments and contaminants. For the double size selection, two binding steps were performed to exclude larger fragments above the cut-off and smaller fragments below the lower cut-off.

All liquid handling pipetting steps and magnetic bead separations were carried out by the OT-2 and Opentrons Magnetic Module.

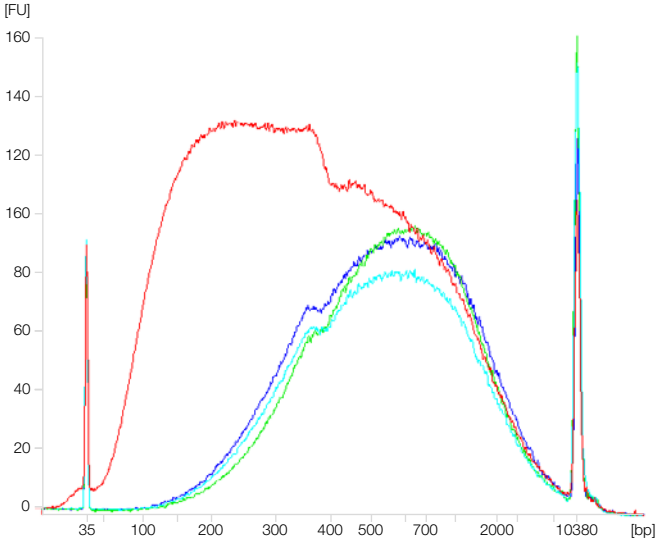
NucleoMag® NGS Clean-up and Size Select

Figure 1: The Opentrons OT-2 is equipped with the Opentrons Magnetic Module and Opentrons GEN2 Pipettes for NGS clean-up. The Magnetic Module uses high-strength magnetic bars that can be engaged to magnetize magnetic beads, and disengaged to allow magnetic beads to remain in solution.

Opentrons OT-2	
Technology	Automated liquid handling platform equipped with electronic pipettes and Magnetic Module (further modules are available for different applications)
Sample numbers	1 – 96 samples
Deck positions	Configurable platform with 11 deck slots
Pipetting volume	20 – 300 µL (P300 8-Channel Pipette) 100 – 1000 µL (P1000 Single-Channel Pipette) (Further Single-Channel and 8-Channel pipettes with different ranges are available for other applications)

Application data

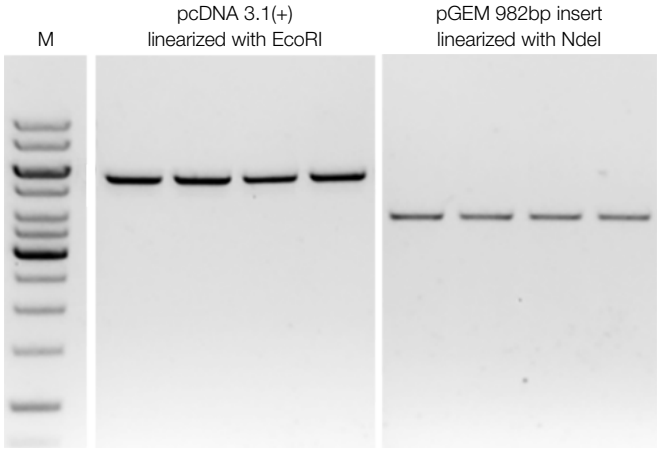
A



Fragment size analysis of fragmented plant DNA (wheat)

Genomic DNA from plant (wheat leaves) was fragmented using NEBNext® dsDNA Fragmentase® and purified via double size selection using the NucleoMag® NGS Clean-up and Size Select kit on the Opentrons OT-2. 30 µL fragmented DNA was used as input for the clean-up reaction (~600 ng), while elution was done in 15 µL. Fragment analysis was performed using the Bioanalyzer 2100. The results demonstrate reliable recovery of the expected fragment sizes of 600 bp. Purified DNA: light, blue, green lines; Input DNA: red

B



Reliable reproducibility in DNA clean-up procedures

To assess reproducibility of DNA clean-up, *Eco*RI-linearized pcDNA 3.1(+) and *Nde*I-linearized pGEM insert were purified in four replicates each. For DNA purification, a sample to NGS clean-up suspension-ratio of 1.0 was used. The parallel clean-up experiments show high reproducibility and recovery of the target DNA.

Ordering information

Product	Specifications	Pack of	REF
NucleoMag® NGS Clean-up and Size Select	NucleoMag® NGS Bead suspension	5 mL 50 mL 500 mL	744970.5 744970.50 744970.500
OT-2 pipetting robot	Automated liquid handling platform with Magnetic Module and electronic pipettes	OT-2 Pipetting Robot Single-Channel P1000 Pipette 8-Channel P300 Pipette Magnetic Module	999-00111 * 999-00004 * 999-00006 * 999-00098 *

NucleoMag® is a registered trademark of MACHEREY-NAGEL; Bioanalyzer® is a registered trademark of Agilent; * For more detailed information, please visit www.opentrons.com. To contact Opentrons Sales or to schedule a demo, please email info@opentrons.com.