One-Step NGS Cleanup Kit

Next-generation sequencing (NGS), also called massively parallel sequencing, is a high-throughput, quick, and scalable method that can replace first-generation Sanger sequencing. It enables the discovery and analysis of various types of genomic features in a single sequencing run, ranging from single nucleotide variants (SNVs) to copy number and structural variants, making it a quick and cost-effective tool for genomic research.

The NGS workflow consists of four steps: nucleic acid extraction, library preparation, sequencing, and analysis. Each step is essential to the success of your experiment.

Several processing procedures are required to prepare a library. Physical shearing or enzyme digestion is used to fragment DNA (or cDNA) samples into a small, homogeneous piece of DNA. The resultant DNA fragments are first end-polished and subsequently ligated to sequencing adapters. These adapter sequences are used to amplify the insert DNA by PCR to generate a fragment library.

Next Generation Sequencing (NGS) libraries require high-quality nucleic acid inputs in variable volumes, concentrations, and sizes depending on the library preparation procedures and sequencing platforms utilized. Despite these differences, traditional hands-on approaches such as magnetic beads, columns, or agarose gel electrophoresis are typically used as part of the entire lab technique attempting to assess the quality of determinants. These techniques are classified into two types based on their function:

Size Selection: Removes undesired nucleic acid fragments or library molecules that are larger or smaller than a specific size range that is ideal for the downstream sequencing platform.

Sample Cleanup: removes sequencing adaptors or PCR primers, dNTPs, enzymes, or undesirable buffer formulations from the sample.

Current technologies and chemistries have been in use for several years for the goals indicated above; nevertheless, they are used at the expense of performance and convenience. Many library preparation processes require repeated purifications, which might result in DNA loss. With each purification step, current methods can result in up to a 30%-50% loss. That may eventually need more starting material, which may not be possible with limited, valuable samples, or the addition of more PCR cycles, which may result in sequencing bias.

It is now possible to efficiently and precisely purify dsDNA for NGS, PCR, and general molecular biology applications. **BcMag™ One-Step NGS Cleanup Kit** is specially designed for ultrafast and efficient purification of DNA after adaptor ligation and PCR or possible replacement of size selection procedure after adaptor addition. The protocol is not only straightforward (one tube and one step, as shown in Fig.1) but also very flexible in removing different size DNA fragments by adjusting processing time, buffer's pH, and detergent concentration (table1). The magnetic Beads are added directly to the finished PCR reactions or other DNA reactions and mixed by a vortex mixer or pipetting to capture and remove the impurities (e.g., excess primer, dimer, adapter, salt, detergent, dNTPs, and enzyme). After mixing, the beads are magnetically removed, while the supernatant contains the purified and ready-to-run DNA. In just 1 minute, the purified DNA is ready for downstream applications, such as Sanger Sequencing, Restriction Digestion, Cloning, SNP Detection, or Library Preparation for NGS. The beads enable 96 samples to be processed simultaneously in less than 10 minutes.

Features and Advantages:

- Simple protocol: No liquid transfer, One-tube, One-step
- Ultrafast: One-minute protocol
- Higher purity and recovery > 90% DNA.
- Effective Cleanup: Removes excess primer (<100- Mer ssDNA), dimer, adapter, a salt such as Mg^{2+}, detergent, dNTPs, enzymes, and dye.
- Cost-effective: Eliminates columns, filters, laborious repeat pipetting, and ethanol

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- High throughput: Compatible with many different automated liquid handling systems

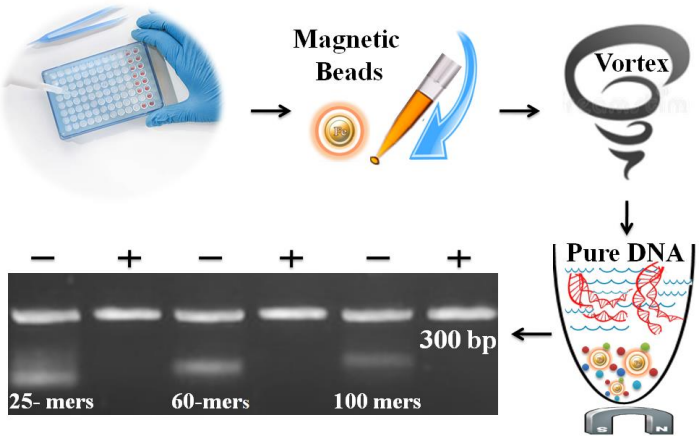

Fig.1 Workflow of One-Step NGS Cleanup

Products

Components	Storage	100 preps, Cat #: AO-101	250 preps, Cat #: AO-102
BcMag™ One-Step NGS Cleanup Kit	4°C	100 preps	250 preps

PROTOCOL

A. Materials Required by the User

- 18.2 MΩ.cm, DNase/RNase-Free Ultrapure Water
- Triton™ X-100, Sigma, Catalog # T8787
- Others

Item	Source
Magnetic Rack for centrifuge tube ** Based on sample volume, the user can choose one of the following magnetic Racks	• BcMag Rack-2 for holding two individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-01) • BcMag Rack-6 for holding six individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-02) • BcMag Rack-24 for holding twenty-four individual 1.5-2.0 ml centrifuge tubes (Bioclone, Cat. # MS-03) • BcMag Rack-50 for holding one 50 ml centrifuge tube, one 15 ml centrifuge tube, and four individual 1.5 ml centrifuge tubes (Bioclone, Cat. # MS-04)
BcMag 96-well Plate Magnetic Rack.	• BcMag 96-well Plate Magnetic Rack (side-pull) compatible with 96-well PCR plate and 96-well microplate or other compatible Racks (Blioclone, Cat#: MS-06)
Adjustable Single and Multichannel pipettes	
Centrifuge with swinging bucket	
Addition items are required if using 96-well PCR plates/tubes	
Vortex Mixer ** The user can also use other compatible vortex mixers. However, the time and speed should be optimized, and the mixer should be: Orbit ≥1.5 mm-4 mm, Speed ≥ 2000 rpm	
Eppendorf™ MixMate™	Eppendorf, Cat#:5353000529
Tube Holder PCR 96	Eppendorf, Cat#: 022674005
Tube Holder 1.5/2.0 mL, for 24 × 1.5 mL or 2.0 mL	Eppendorf, Cat#: 022674048
Smart Mixer, Multi Shaker	BenchTop Lab Systems, Cat#:5353000529
1.5/2.0 mL centrifuge tube	
96-well PCR Plates or 8-Strip PCR Tubes	
PCR plates/tubes ** ***IMPORTANT!*** Using other tubes or PCR plates, ensure that the well diameter at the bottom of the conical section of PCR Tubes or PCR plates has to be ≥2.5mm.	

B. Procedure

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Important!

1. The following protocol is optimized for the efficient cleanup of 10µl DNA sample. The procedure may need to be optimized if an alternative reaction scale is used.
2. Shake or vortex the bottle to completely resuspend the magnetic beads before using.
3. Do not allow the magnetic beads to sit for more than two minutes before dispensing.
4. Based on applications, the user should choose buffer conditions based on table1. For example, if the sample does not contain detergent, add 1 µL of 1% Triton™ X-100 solution to a 10 µL sample (final concentration is 0.1%).
5. Quantification of the nucleic acids: Use only fluorescence methods such as qPCR, Qubit, and Pico Green.

Table 1

DNA Fragment Removal						
Buffer / DNA	+ 0.1% Triton x-100, pH7.5	- 0.1% Triton x-100 pH7.5	+ 0.1% Triton x-100 pH 8.0	- 0.1% Triton x-100 pH 8.0	+ 0.1% Triton x-100 pH 8.8	- 0.1% Triton x-100 pH 8.8
dsDNA (100 bp)	No removal	removal	removal	removal	No removal	removal
dsDNA (150 bp)	No removal	removal	No removal	removal	No removal	removal
dsDNA (200 bp)	No removal	removal	No removal	removal	No removal	removal
dsDNA (300 bp)	No removal	No removal	No removal	No removal	No removal	No removal
ssDNA 100 mer	removal	removal	removal	removal	removal	removal

dsDNA- Double-Stranded DNA; ssDNA- Single-stranded DNA
The assay was done by using the following conditions:
1. 10 mM Tris-HCl with or without 0.1% triton (final concentration) and three different: pH 7.5, pH 8.0 and pH 8.8

1. Add 5 µL magnetic beads to the 10 µLDNA sample.

2. If necessary, briefly centrifuge at 2500 rpm for 30 seconds to bring all contents to the bottom of the tube.
3. Mix thoroughly for 1 minute by slowly pipetting up and down 25 times (one minute) or by vortex mixer for 5 minutes at 2500 rpm.
4. If necessary, briefly centrifuge at 2500 rpm for 30 seconds to bring all contents to the bottom of the tube.
5. Place the sample plate on the magnetic separation plate for 30 seconds or until the solution is clear to separate beads from the solution.
6. Transfer the supernatant to a clean plate while the sample plate remains on the magnetic separation plate for downstream applications.

C. Troubleshooting

Problem	Probable cause	Suggestion
Low DNA Recovery	Vertexing speed is too fast Vertexing time is too long.	• Reducing either the speed or time • If using other digital vortex mixers, the vortex condition, such as speed and time, has to be optimized.
	Using too many magnetic beads	Thoroughly resuspend the magnetic beads and use the correct amounts of the beads.
Failure to remove impurities.	Used inappropriate PCR tubes or PCR plates	Make sure that the well diameter at the bottom of the conical section of PCR Tubes or PCR plates is ≥2.5mm.
	Vortex speed is too slow, or vortex time is too short.	• Increasing either the speed or time • If using other digital vortex mixers, the vortex condition, such as speed and time, has to be optimized.
	Using fewer magnetic beads	Thoroughly resuspend the magnetic beads and use the correct amounts of the beads.
	Strong secondary structure of DNA fragments (<50bp dsDNA or < 100 mer ssDNA)	Denature the sample by heating it at 95°C for 2 min.

	Too much primer, dimer, adaptor, free dye, and detergent	• Use more magnetic beads. • Perform the second round of purification by following the same protocol.

Related Product

Products and Catalog Number	
Genomic DNA and RNA Purification	
One-Step Mammalian Cell DNA Purification Kit, Cat. No. AA101	One-Step Saliva Viral RNA-DNA Purification Kit, Cat. No. AR101
Cell-Free DNA Purification Kit, Cat. No AC101	Bone-Teeth DNA Purification Kit, Cat. No. AB101
One-Step FFPE & FNA DNA purification Kit, Cat. No. AJ-101	Rootless Hair DNA Purification Kit**,** Cat. No. AD101
One-Step Bacteria DNA Purification Kit, Cat. No. AE101	One-Step Buccal Cell DNA Purification Kit, Cat. No. AG101
One-Step Blood DNA Purification Kit, Cat. No. AF101	One-Step Touch DNA Purification Kit, Cat. No. AS101
One-Step Fungi &Yeast DNA Purification Kit, Cat. No. AL101	Sexual Assault Casework DNA Purification Kit, Cat. No. AT101
One-Step Insect DNA Purification Kit, Cat. No. AM101	One-Step Fingerprint DNA Purification Kit, Cat. No. AZ101
One-Step Mouse Tail DNA Purification Kit, Cat. No. AN101	One-Step Dandruff DNA Purification Kit, Cat. No. AAA101
One-Step Plant DNA Purification Kit, Cat. No. AQ101	Quick mRNA Purification Kit, Cat. No. MMS101
DNA & RNA Sample Preparation	
One-Step NGS Cleanup Kit, Cat. No. AO101	One-Step DNA-RNA Removal Kit, Cat. No. CA103
One-Step RNA Removal Kit, Cat. No. AU101	One-Step DNA/RNA Cleanup Kit, Cat. No. AH101
One-Step PCR Cleanup Kit, Cat. No. AP101	One-Step Sequencing Cleanup Kit, Cat. No. AI101
Quick Oligo-DNA Conjugation Kit, Cat. No. CA101	One-Step Fluorescent Labeling Cleanup Kit, Cat. No. AK101
One-Step DNA-RNA Removal Kit, Cat. No. AV101	One-Step Single-Stranded DNA Removal Kit, Cat. No. AW101
One-Step PCR Inhibitor Removal Kit, Cat. No. AX101	Pure Miniprep Plasmid DNA Purification Kit, Cat. No. AY101

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