Use of ExoSAP-IT PCR Product Cleanup Reagent in NGS

Cited in over 10,000 publications, Applied Biosystems™ ExoSAP-IT™ PCR Product Cleanup Reagent is widely used for enzymatic PCR cleanup. The one-tube, one-step ExoSAP-IT method has many advantages over using spin columns or magnetic beads for PCR cleanup. With its simple protocol and 100% recovery of both short and long amplicons, ExoSAP-IT reagent enables researchers to conserve limited samples and improve workflow efficiency. While Sanger sequencing–based methods remain popular for validation and long contiguous DNA sequence reads (>500 nucleotides), many genomic analysis applications are transitioning to next-generation sequencing (NGS) technology for its scalability and affordability for sequencing a large number of targets [1].

In addition to its routine use in Sanger sequencing, ExoSAP-IT reagent is proving beneficial in library preparation workflows across a broad range of NGS applications and platforms for Thermo Fisher Scientific, Illumina, and PacBio, among others (Table 1). This application note reviews NGS applications by platform and highlights the utility of ExoSAP-IT reagent and its benefits for each workflow.

Ion Torrent™ NGS instrument

The Ion Torrent™ Personal Genome Machine™ (PGM™) System uses semiconductor technology to sequence Ion Torrent™ DNA libraries like those prepared using the Ion Xpress™ Plus Fragment Library Kit. This technology works by detecting the positively charged hydrogen ion (H^+) that is released when a nucleotide is incorporated. By stepwise addition of one specific nucleotide after another, each position of a DNA template can be determined. Ion Torrent libraries comprise DNA fragments (~200 bp) ligated to blunt-ended adapters that enable sequencing on the Ion PGM platform.

Enzymatic cleanup applications

As shown in Table 1, ExoSAP-IT reagent for PCR cleanup is used in Ion Torrent workflows across a range of NGS applications, including species identification, genotyping, and targeted sequencing.

Table 1. Improved NGS workflows using ExoSAP-IT reagent across a broad range of applications and platforms.

Application	Platform	Workflow	ExoSAP-IT reagent benefits	Relevance	Ref.
Species identification	Ion PGM	Mitochondrial DNA PCR ⇨ ExoSAP-IT reagent ⇨ Ion Xpress kit	Efficient PCR cleanup prior to library construction using Ion Xpress kit	Identify species of meat	2
	Ion PGM	16S PCR ⇨ ExoSAP-IT reagent ⇨ PCR2 (addition of adapters)	PCR2 efficiency	Identify bacterial population in water	3
	Ion PGM	16S PCR ⇨ ExoSAP-IT reagent ⇨ Ion Xpress kit	Efficient PCR cleanup prior to library construction using Ion Xpress kit	Identify bacterial population in water	4, 5
Mutation analysis	Illumina MiSeq	Long range (LR) PCR ⇨ ExoSAP-IT reagent ⇨ Nextera library prep kit	Efficient PCR cleanup prior to library construction using Nextera kit	Detect markers for chronic fatigue syndrome	6
	Illumina MiSeq	LR-PCR ⇨ ExoSAP-IT reagent ⇨ column purification ⇨ TruSeq library prep	Improved purification efficiency	Validate indels from whole-genome sequencing (WGS)	7
	Illumina MiSeq	LR-PCR ⇨ ExoSAP-IT reagent ⇨ column purification ⇨ TruSeq library prep kit	Improved purification efficiency	Validate indels from WGS	8
	Illumina MiSeq	Multiplex PCR ⇨ ExoSAP-IT reagent ⇨ PCR2 (addition of adapters)	PCR2 efficiency	Tumor profiling for cancer mutations	9
Genotyping	Ion PGM	Human leukocyte antigen (HLA) PCR ⇨ ExoSAP-IT reagent ⇨ Ion Xpress kit	Removal of ssDNA for improved enzymatic shearing by Ion Shear Plus Reagent	HLA genotyping research	10
	Illumina HiSeq	Shear gDNA ⇨ ExoSAP-IT reagent ⇨ PCR2 (addition of adapters)	Increase in percent on-target reads	Genotyping-in-thousands by sequencing (GT-Seq)	11
	Illumina Genome Analyzer$_{IIx}$	Add adapters with PCR ⇨ ExoSAP-IT reagent ⇨ direct sequencing	Removal of primers that would bind to the surface of the flow cell	Antenatal SNP genotyping	12
	NEBNext reagent set for 454 library prep	HLA PCR ⇨ ExoSAP-IT reagent ⇨ 454 library prep	Efficient PCR cleanup prior to library prep	HLA genotyping research	13
Targeted DNA/RNA sequencing	Ion PGM	PCR ⇨ ExoSAP-IT reagent ⇨ Ion Xpress kit	Removal of ssDNA for improved enzymatic shearing by Ion Shear Plus Reagent	Identify growth-rate genes involved in development of pigs	14
	Ion PGM	RT-PCR ⇨ ExoSAP-IT reagent ⇨ Ion Xpress kit	Efficient PCR cleanup prior to library construction using Ion Xpress kit	Rotavirus in South African children presenting diarrhea	15
	Illumina HiSeq	RT ⇨ ExoSAP-IT reagent ⇨ poly(A) tailing ⇨ PCR ⇨ TruSeq library prep	Poly(A) tailing efficiency	Identify circular (circ)RNAs involved in development	16
	Illumina HiSeq	IP ⇨ adapter ligation ⇨ RT ⇨ ExoSAP-IT reagent ⇨ RNase H ⇨ adapter ligation ⇨ PCR	Downstream ligation and PCR efficiency	ID RNA-binding protein binding sites	17
	Illumina MiSeq	PCR ⇨ ExoSAP-IT reagent ⇨ PCR2 (addition of adapters)	PCR2 efficiency	Detect nitrogenase gene diversity in trees	18
	Illumina MiSeq	PCR ⇨ ExoSAP-IT reagent ⇨ PCR2/3 (addition of indices/adapters)	PCR2/3 efficiency	Examine leaf mycobiome	19, 20
Epigenetics	PacBio SMRTbell	SMRTbell prep ⇨ exonuclease III, VII ⇨ direct single-molecule real-time (SMRT) sequencing	Removal of incomplete SMRTbell templates	Direct sequencing of intergenic modified sites	21

In a study by Bertolini et al. [2], mitochondrial DNA (mtDNA) extracted from meat samples was amplified by PCR to enrich the species-specific mtDNA loci. PCR products were then cleaned with ExoSAP-IT reagent prior to library prep using the Ion Xpress Plus kit and sequenced to identify the species of the meat samples. Similar research workflows were applied in studies using 16S sequencing to identify bacterial populations in water [3-5] and in HLA genotyping assays [10]. The utility of ExoSAP-IT PCR cleanup reagent has also been demonstrated in targeted sequencing workflows in studies of growth-rate genes in pigs [14] and RNA rotavirus in South African children presenting diarrhea [15]. Several of these research workflows benefited from the ability of ExoSAP-IT reagent to efficiently remove excess primers that can interfere with the enzymatic shearing by Ion Shear™ Plus Reagent, which is part of the Ion Xpress Plus Fragment Library Kit (Figure 1).

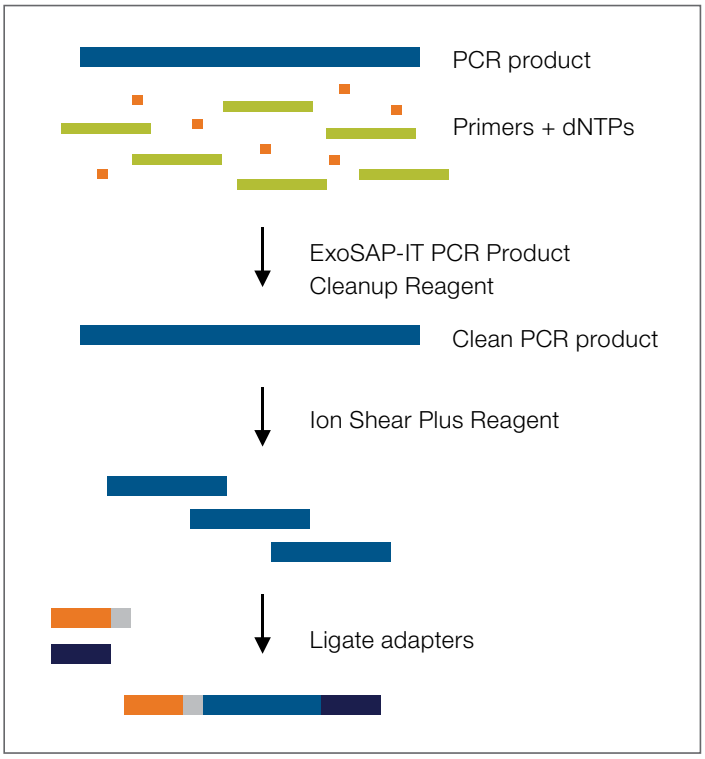

Figure 1. Ion Xpress Plus fragment library prep workflow. Example of Ion Xpress Plus Fragment Library Kit workflow starting with PCR-amplified DNA. ExoSAP-IT PCR reagent removes ssDNA and dNTPs, enabling more efficient fragmentation by Ion Shear Plus Reagent.

Illumina instruments

Illumina offers a range of instruments that vary in read length, sequencing depth, and throughput capacity. Illumina NGS technology is based on sequencing-by-synthesis (SBS) chemistry, which incorporates fluorescent nucleotides stepwise for base calling at each position of a DNA template. There are several methods available for preparing Illumina libraries, including a fragmentation approach using an Illumina Nextera™ kit, ligating T-tailed adapters with an Illumina TruSeq™ kit, and amplicon-based methods to add sequencing adapters.

Enzymatic cleanup applications

As shown in Table 1, ExoSAP-IT reagent for PCR cleanup is being used in Illumina workflows across a range of NGS applications, including mutation analysis, genotyping, and targeted sequencing.

In a study by Billing et al. [6], LR-PCR was used to amplify mtDNA to assess markers associated with chronic fatigue syndrome. LR-PCR products were cleaned with ExoSAP-IT reagent prior to Nextera library prep and sequenced for mutation analysis. This study demonstrated an efficient workflow for quantifying cleaned PCR products using ExoSAP-IT reagent with Invitrogen™ Quant-iT™ PicoGreen™ assay prior to pooling at equimolar ratios for Nextera library prep. ExoSAP-IT reagent has been cited in several publications involving amplification steps prior to TruSeq library prep. Studies by Fang et al. [7] and Narzisi et al. [8] describe a similar workflow where LR-PCR products were pooled, cleaned with ExoSAP-IT reagent, and then passed through a purification column prior to TruSeq library prep and NGS confirmation of indels. In this workflow, ExoSAP-IT reagent removes the large population of primers and nucleotides in the PCR pool, which is important for spin column efficiency and yield. In a study by Fan et al. [16], RNA from single cells was reverse-transcribed and cleaned with ExoSAP-IT reagent to enable poly(A) tailing in a sample prep workflow upstream of TruSeq library prep. Targeted NGS was then performed to sequence circular (circ)RNAs to investigate their regulatory function during development. An article by Van Nostrand et al. [17] describes another example of using ExoSAP-IT reagent to clean up after reverse transcription (RT) in a targeted NGS workflow for identifying binding sites for RNA-binding proteins with enhanced UV crosslinking and immunoprecipitation (eCLIP).

Based on recent publications, ExoSAP-IT reagent is proving to be very useful in workflows that add sequencing adapters using amplicon-based methods (Figure 2). Examples include a study by Doty et al. [18] that describes an amplicon-based library prep workflow incorporating ExoSAP-IT reagent in experiments investigating the plant microbiome, and similar studies by Siddique et al. [19] and Unterseher et al. [20] investigating the mycobiome. ExoSAP-IT reagent was also used to clean up multiplex PCR products in tumor profiling assays [9] and sheared DNA in GT-Seq [11] prior to amplicon-based library prep. In a study by Rieneck et al. [12], adapter sequences were included in PCR primers to amplify a single-nucleotide polymorphism (SNP) position, enabling direct sequencing of the SNP-containing PCR product in an antenatal genotyping assay. In this workflow, ExoSAP-IT PCR Product Cleanup Reagent effectively removed unincorporated primers that would otherwise bind to the Illumina flow cell and decrease NGS efficiency.

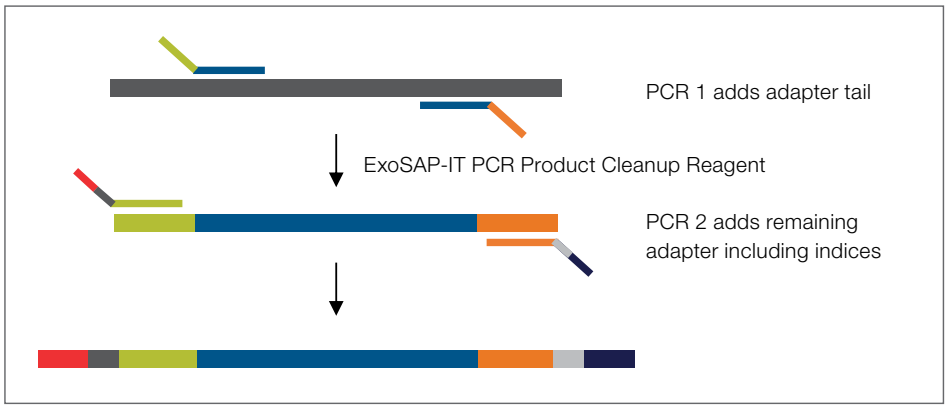

Figure 2. Amplicon-based library prep. Amplicon-based methods use PCR to target specific genes and add sequencing adapters. ExoSAP-IT PCR Product Cleanup Reagent can be used in between PCR steps to increase workflow efficiency.

PacBio platform

The Ion Torrent and Illumina platforms are similar in that the sequencing workflows include a clonal amplification step to augment the library to be sequenced. PacBio offers a third-generation sequencing platform that uses the novel SMRT sequencing technology to directly sequence single molecules in real-time (SMRT) using uniquely colored nucleotides. The SMRTbell™ Template Prep Kit is used to construct libraries of large DNA fragments (500 bp to >20 kb) flanked by SMRTbell hairpin adapters. During this process, exonuclease treatment is necessary to remove incomplete SMRTbell templates that cause inefficiencies in the direct sequencing reaction.

Enzymatic cleanup applications

Although only exonuclease III is used in this workflow, the PacBio technology is another example of how enzymatic cleanup is being applied in NGS. In a study by Seib et al. [21] that characterizes intergenic modified sites involved in gene regulation, the PacBio workflow includes use of USB™ Exonuclease VII for SMRTbell template purification.

Summary

The prevalence of ExoSAP-IT reagent usage in Sanger sequencing–based methods is reflective of its ease of use and ability to increase the efficiency and quality of traditional DNA sequencing results. As genomic research moves toward NGS applications run on platforms developed by Thermo Fisher Scientific, Illumina, and PacBio, the utility of ExoSAP-IT reagent in NGS library preparation protocols is becoming increasingly evident. Recent additions to the ExoSAP-IT reagent family (Table 2), including the fastest cleanup reagent on the market, Invitrogen™ ExoSAP-IT™ *Express* PCR Product Cleanup Reagent, are making it easier than ever to incorporate ExoSAP-IT PCR cleanup steps in NGS workflows for improved efficiency and more consistent results.

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Table 2. Selection guide for ExoSAP-IT reagents.

	ExoSAP-IT *Express* reagent	ExoSAP-IT reagent (original formulation)	HT ExoSAP-IT *Fast* High-Throughput reagent (for automated liquid handlers)
Protocol time	5 min	30 min	14 min
Format	Single tube 8-tube strips	Single tube	Single tube 8-tube strips 96-well plate
Throughput level	Low to high; recommended for processing any sample size	Low to medium; recommended for processing 1–96 samples at a time	High; recommended for processing ≥96 samples at a time
Platform	Single- or multichannel pipette, automated liquid handling platforms	Single-channel pipette	Automated liquid handling platforms
Freezes at –20°C	No	No	Yes
Stability	At –20°C for up to 2 years	At –20°C for up to 2 years	At –20°C for up to 2 years; once thawed, stable at 4°C for 1 month and room temperature for 2 days

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