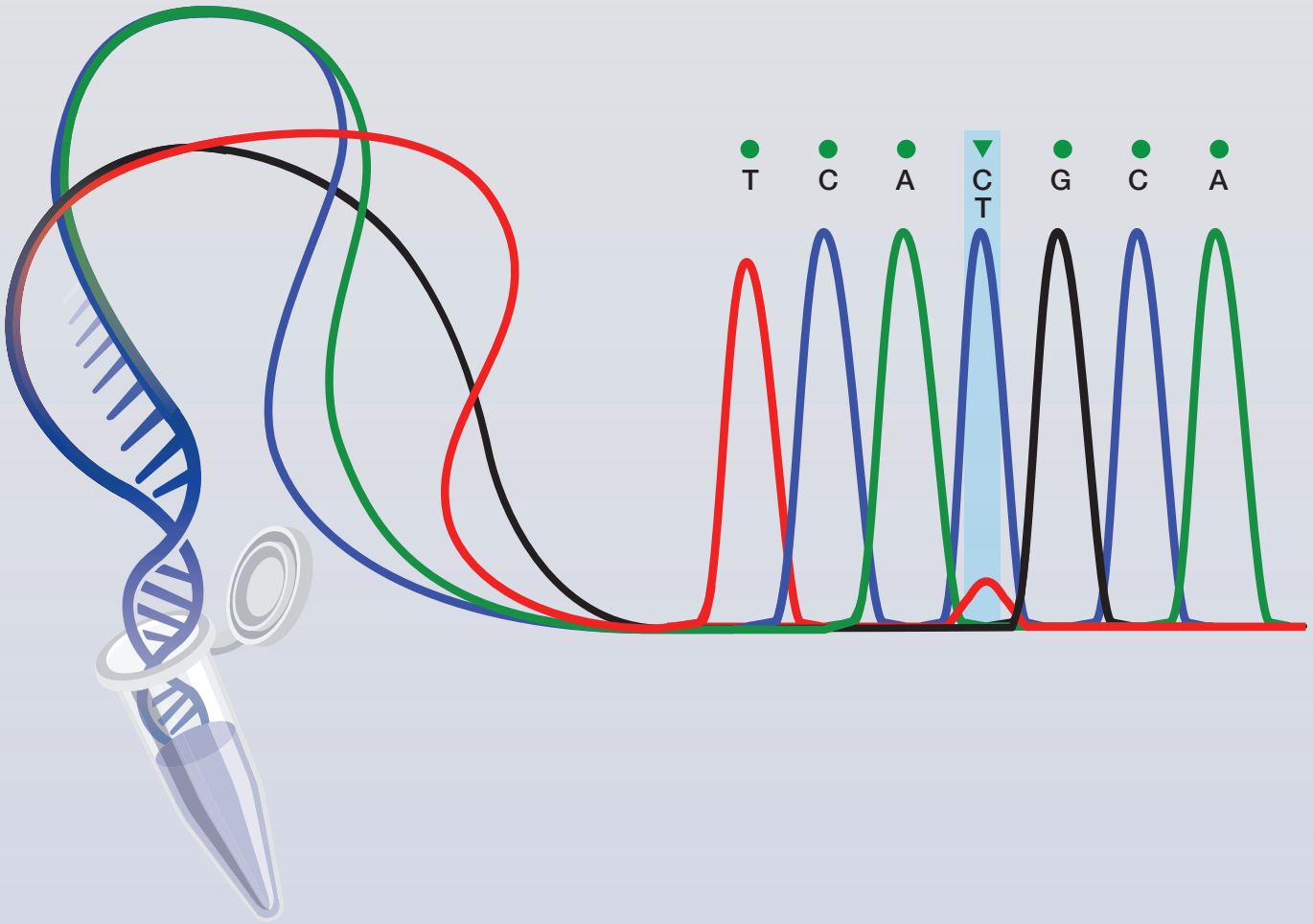
applied biosystems



Sanger sequencing

Generate high-quality data with our proven workflow

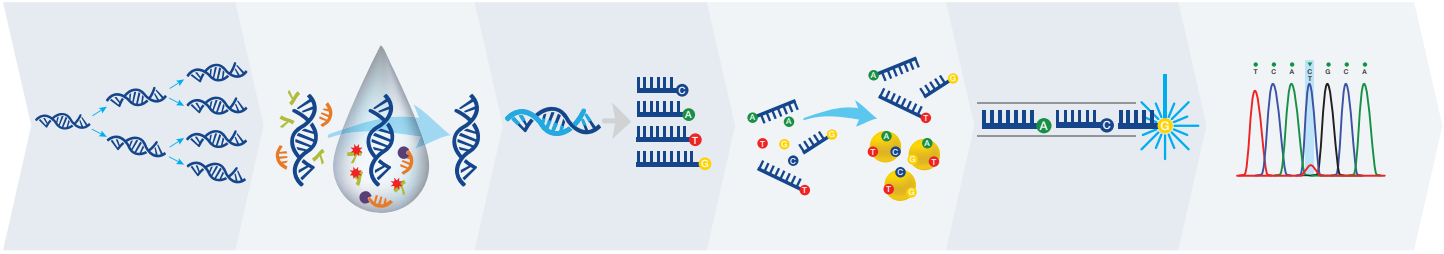
ThermoFisher SCIENTIFIC

Comprehensive solutions for your Sanger sequencing workflow

Proven through decades of results, Sanger sequencing is the gold-standard technology to:

- Study diseases with clearly defined phenotypes
- Sequence 1–2 genes or up to 96 targets
- Sequence 1–96 samples at a time without barcoding
- Confirm next-generation sequencing (NGS) variants with up to 99.99% accuracy
- Get longer read lengths (up to 1,000 bp)

The Sanger sequencing workflow



PCR amplification **Page 3**

PCR cleanup **Page 4**

Cycle sequencing **Page 5**

Sequencing cleanup **Page 6**

Capillary electrophoresis **Page 7**

Data analysis **Page 8**

We commit to quality to help ensure results you can rely on

The term “gold standard” represents quality and dependability. Sanger sequencing is the gold-standard DNA sequencing method that powered the Human Genome Project and continues to generate highly accurate, reliable sequencing. We know that reliable results are important to you. That’s why we have dedicated teams and individuals focusing on quality across our supply chain so that you can continue to rely on the trusted gold-standard sequencing technology.

Learn more at **thermofisher.com/abquality**

“A gold-standard product is a product that is consistent and meets or exceeds customer expectations every time.”

—Justin, Manufacturing Supervisor

PCR amplification

Primer Designer tool for PCR and Sanger sequencing

Use our online Invitrogen™ Primer Designer™ Tool to search for the right PCR or Sanger sequencing primer pair from a database of ~650,000 predesigned primer pairs for resequencing the human exome and human mitochondrial genome. Choose from different amplicon lengths to accommodate various research applications and biological sample types.

- Our primers are free of known single-nucleotide polymorphisms (SNPs) and primer-dimers, highly target-specific, and used under universal PCR conditions
- Full primer coverage for Ion AmpliSeq™ Exome Panel and Ion AmpliSeq™ Cancer Hotspot Panel v.2 Sanger confirmation workflow
- Flexible primer configurations to meet your research needs: primers can be ordered unmodified, M13-tailed, HPLC-purified, or desalted
- All the primers have been checked by mass spectrometry and have passed stringent bioinformatics metrics; lab bench verification tests have shown >95% success rate

Access the tool at **thermofisher.com/primerdesigner**

Platinum II *Taq* Hot-Start DNA Polymerase

Invitrogen™ Platinum™ II *Taq* Hot-Start DNA Polymerase helps you get to sequencing reactions faster. A unique combination of innovative buffer, high-performance *Taq* DNA polymerase, and superior Invitrogen™ Platinum™ hot-start technology helps enable exceptional PCR results, even in the toughest applications.

- Universal primer annealing at 60°C enables co-cycling of all assays, reducing tedious optimization steps
- An engineered *Taq* polymerase enables 4x faster DNA synthesis, inhibitor resistance, and robust amplification
- Platinum hot-start technology offers superior specificity, sensitivity, and yields, and allows for room temperature reaction setup
- 2X master mix formats help reduce pipetting errors with fewer pipetting steps

More formats and information are available at
thermofisher.com/platinumiitaq

Ordering information

Product	Quantity	Cat. No.
Platinum II Hot-Start PCR Master Mix (2X)	50 rxn	14000012
	200 rxn	14000013
	1,000 rxn	14000014

PCR cleanup

ExoSAP-IT *Express* reagent: fastest PCR cleanup method

The Applied Biosystems™ ExoSAP-IT™ *Express* reagent offers rapid turnaround times and improved efficiency of resource use while delivering the same superior cleanup as the original Applied Biosystems™ ExoSAP-IT™ reagent. The novel technology allows for a significant reduction in sample cleanup time with minimal steps, providing the simplest workflow (Figure 1).

- 5-minute protocol
- One-tube, one-step PCR cleanup
- 100% recovery of PCR products

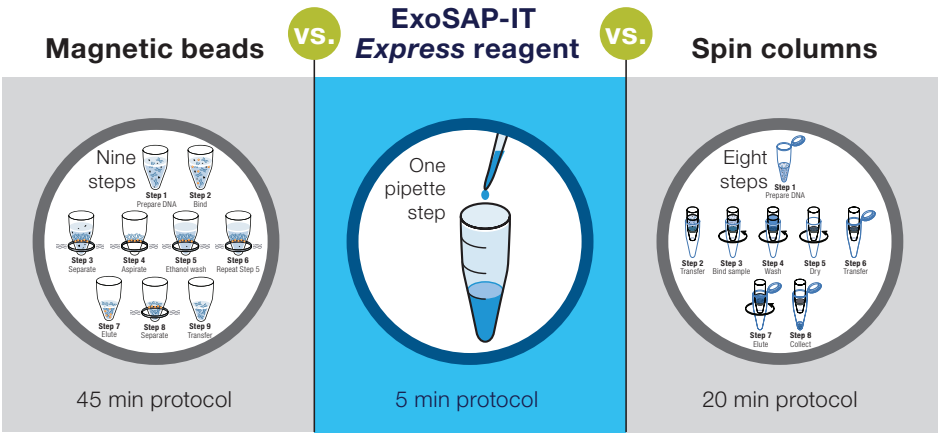


Figure 1. Use of ExoSAP-IT *Express* reagent eliminates spin columns, magnetic beads, centrifugation, filtration, and gel purification. With a 5-minute protocol, ExoSAP-IT *Express* reagent is the fastest and easiest method for PCR cleanup, minimizing pipetting errors and contamination.

Protocol

Treat 5 µL of PCR product with 2 µL of ExoSAP-IT *Express* reagent. The treatment is carried out at 37°C for 4 minutes, followed by an incubation at 80°C for 1 minute to irreversibly inactivate both enzymes. Once enzyme inactivation is complete, your PCR products are ready for downstream applications such as sequencing (Sanger/NGS), fragment analysis, SNP analysis, *in vitro* transcription, or single-base extension (Figure 2).

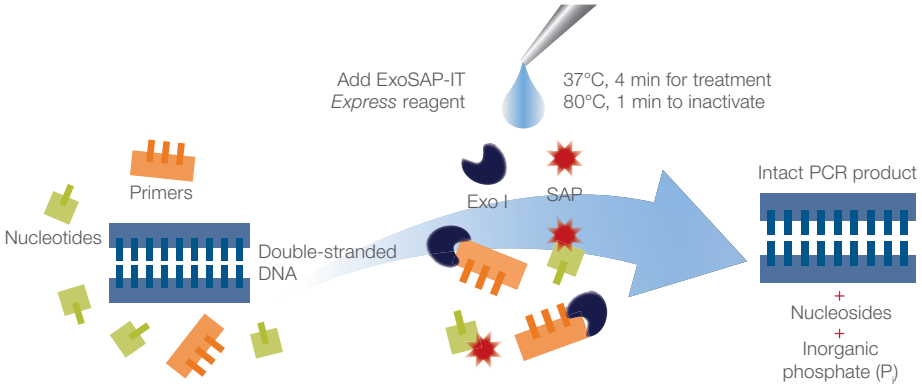


Figure 2. Enzymatic PCR cleanup using ExoSAP-IT *Express* reagent.

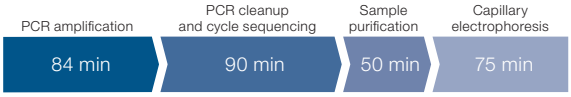
Ordering information

Product	Quantity	Cat. No.
ExoSAP-IT *Express* PCR Product Cleanup Reagent	100 rxn	75001.200.UL
	500 rxn	75001.1.ML
	2,000 rxn	75001.4X.1.ML
	5,000 rxn	75001.10.ML

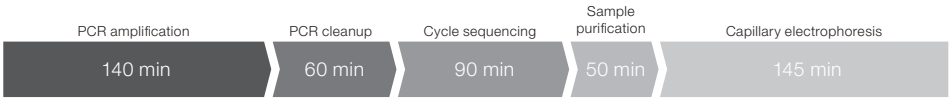
Cycle sequencing

BigDye Direct Cycle Sequencing Kit

The Applied Biosystems™ BigDye™ Direct Cycle Sequencing Kit provides a streamlined workflow by eliminating the PCR cleanup step, and improves resolution of sequencing data at the 5′ end. Moreover, the BigDye direct PCR and sequencing workflow requires use of only one plate, without having to transfer between steps. This helps reduce hands-on time and improves accuracy by reducing the possibility of pipetting errors.



BigDye Direct Cycle Sequencing Kit workflow, run with Applied Biosystems™ POP-7™ Polymer, takes 4 steps in approximately 5 process hr.



A traditional cycle sequencing workflow, run with Applied Biosystems™ POP-6™ Polymer, takes 5 steps in approximately 8 process hr.

Figure 3. The BigDye Direct kit delivers significant efficiency compared to standard sequencing. Traditional sequencing workflows can require more than 8 hours of process time and 5 steps to complete. In contrast, the BigDye Direct workflow typically requires only 5 hours and 4 steps, producing sequence reads up to 40% faster and with less hands-on time.

Ordering information

Product	Quantity	Cat. No.
BigDye Direct Cycle Sequencing Kit	24 rxn	4458689
	100 rxn	4458687
	1,000 rxn	4458688

BigDye Terminator v3.1 Cycle Sequencing Kit

The Applied Biosystems™ BigDye™ Terminator v3.1 Cycle Sequencing Kit has robust, highly flexible chemistry for *de novo* sequencing, resequencing, and finishing with PCR product, plasmid, fosmid, and BAC templates.

Ordering information

Product	Quantity	Cat. No.
BigDye Terminator v3.1 Cycle Sequencing Kit	24 rxn	4337454
	100 rxn	4337455
	1,000 rxn	4337456
	5,000 rxn	4337457
	25,000 rxn	4337458

BigDye Terminator v1.1 Cycle Sequencing Kit

The Applied Biosystems™ BigDye™ Terminator v1.1 Cycle Sequencing Kit is designed for specialty applications that require optimal basecalling adjacent to the primer, and for sequencing short PCR products with rapid electrophoresis.

Ordering information

Product	Quantity	Cat. No.
BigDye Terminator v1.1 Cycle Sequencing Kit	24 rxn	4337449
	100 rxn	4337450
	1,000 rxn	4337451
	5,000 rxn	4337452

Sequencing cleanup

BigDye XTerminator Purification Kit

Correctly cleaning up your sequencing reactions is an integral part of the Sanger sequencing workflow. If the sequencing reaction cleanup step is skipped or not performed properly, the residual dye in the reaction can compete with the labeled amplicons for entry into the capillary and can cause reduced signal intensity, which can interfere with the instrument's ability to make clear base calls. As a result, the data generated will be of poor quality.

The Applied Biosystems™ BigDye™ XTerminator Purification Kit provides a fast, simple purification method for removing unincorporated Applied Biosystems™ BigDye™ terminators and salts from DNA sequencing reactions; it also eliminates dye blobs in your reaction. Cleanup is complete in under 40 minutes and typically requires less than 10 minutes of hands-on labor.

Protocol

Dispense 55 µL of the SAM/BigDye XTerminator bead working solution to each sample well. Vortex for 20 minutes at 1,800 rpm, followed by centrifugation at 1,000 x *g* for 2 minutes.

Ordering information

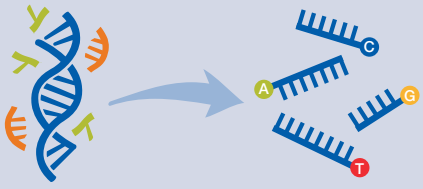
Product	Quantity	Cat. No.
BigDye XTerminator Purification Kit	100 preps	4376486
	1,000 preps	4376487
	2,500 preps	4376484
	40,000 preps	4376485

Simplify your sample prep workflow with the Sanger Sequencing Kit

The Applied Biosystems™ Sanger Sequencing Kit offers a convenient and affordable solution for preparing sequencing reactions. It provides all the reagents needed for 200 reactions of PCR cleanup, cycle sequencing, and sequencing product cleanup. The kit includes:

- ExoSAP-IT *Express* PCR Product Cleanup Reagent
- BigDye Terminator v3.1 Cycle Sequencing Kit
- BigDye XTerminator Purification Kit

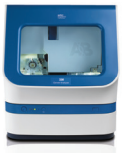
From PCR to purified sequencing reactions



Ordering information

Product	Quantity	Cat. No.
Sanger Sequencing Kit	200 rxn	A38073

Capillary electrophoresis

	Applied Biosystems™ SeqStudio™ Genetic Analyzer	Applied Biosystems™ 3500 Series Genetic Analyzers	Applied Biosystems™ 3730 Series Genetic Analyzers
	Easy-to-use, flexible system 	Meets the needs of verified and process-controlled environments 	Maximum throughput, scalability, and flexibility UPGRADED 
Number of capillaries	4	8 (3500), 24 (3500xL)	48- and 96-well plate compatible
Number of dyes	6	6	6
Capillary array length (cm)	28	36, 50	36, 50
RFID	Yes	Yes	No
Polymer type	POP-1, integrated into click-in cartridge	POP-6, POP-7, POP-4	POP-6, POP-7, conformational analysis polymer
Sample capacity	12 standard 8-strip tubes 1 standard 96-well plate	2 sample plates (96- or 384-well)	16 sample plates (96- or 384-well)
Integrated plate stacker	No	No	Yes
Applications	Sequencing and fragment analysis in same run	Sequencing, fragment analysis	Sequencing, fragment analysis
Minimum run time	30 minutes	30 minutes	20 minutes
Sequencing read length (bp)	At least 600	At least 650	At least 900
Maximum sequencing throughput (base pair reads/day)	67K	138K (3500), 403K (3500xL)	1.38M (3730), 2.76M (3730*xl*)
Maximum fragment throughput (samples/day)	192	384 (3500), 1,152 (3500xL)	3,456 (3730), 6,912 (3730*xl*)

SeqStudio Cartridge

The Applied Biosystems™ SeqStudio™ Cartridge is an easy-to-use reagent cartridge that includes capillaries, POP-1 universal polymer, buffer, and pump. The POP-1 universal polymer allows for flexibility to perform Sanger sequencing and fragment analysis on the SeqStudio Genetic Analyzer System with one cartridge. Just load your samples, click in the cartridge, and go.

On-instrument consumables

Using the right Applied Biosystems™ polymers can help reduce your re-run rate and enables high-quality, reproducible data.

Different polymer chemistries for different needs:

POP-4™ Polymer

- Primarily used for human identification applications, other fragment analysis applications, and for sequencing short DNA fragments (<500 bp)

POP-6™ Polymer

- Excellent resolution of nucleotides close to sequencing primer. Primarily used in conjunction with the BigDye Terminator v1.1 Cycle Sequencing Kit, for sequencing short PCR products

POP-7™ Polymer

- Mostly used for sequencing of read lengths up to ~1,000 bp in conjunction with the BigDye Terminator v3.1 Cycle Sequencing Kit. Also used for fragment analysis applications

applied biosystems

Data analysis

We offer a wide range of Applied Biosystems™ software solutions for viewing and interpreting your Sanger sequencing results.

Minor Variant Finder Software

The improved sensitivity achieved using Applied Biosystems™ Minor Variant Finder Software makes Sanger sequencing the ideal choice for oncologists and pathologists to call low-frequency somatic variants (5% or below) where the number of relevant targets is often limited. The software requires no change to your current Sanger sequencing workflow.

Features of MVF Software enable you to:

- Call minor variants at detection levels as low as 5%
- Sequence a moderate number of targets at low cost
- Confirm NGS findings in alignment view and Venn diagram

Sanger analysis modules—free cloud-based tools

Applied Biosystems™ Sanger analysis modules are innovative cloud-based secondary data analysis tools that bring together multiple data sets in one convenient place. This free solution makes it easier to view, store, and analyze Sanger sequencing data.

- **NGS confirmation**—confirm your NGS variants from one central location
- **Variant reporting in absolute genomic coordinates**—eliminate the need to calculate from references
- **Automated database search**—automatically report genomic annotations for SNPs
- **.vcf output for downstream analysis**—search multiple databases with the Ion Reporter™ annotation workflow

Additional software

Applied Biosystems™ Sequencing Analysis Software
Enables user to basecall, trim, display, edit, and print data from our entire line of capillary DNA sequencing instruments for data analysis and quality control.

Applied Biosystems™ SeqScape™ Software
Resequencing package designed for mutation detection and analysis, SNP discovery and verification, pathogen subtyping, allele identification, and sequence confirmation. It provides library functions for comparison to a known group of sequences, as well as functionalities to assist with 21 CFR Part 11 compliance.

Applied Biosystems™ Variant Reporter™ Software
Designed for reference-based and non–reference-based analysis such as mutation detection and analysis, SNP discovery and verification, and sequence confirmation. The software can call SNPs, insertions, deletions, and heterozygous insertions/deletions.

Applied Biosystems™ Sequence Scanner Software:
Free sequencing viewer software enables you to view, edit, print, and export sequence data from Applied Biosystems™ genetic analyzers. The software generates graphically expressive reports on results.

Learn more at **thermofisher.com/sangersoftware**

Find out more at **thermofisher.com/sangerworkflow**

ThermoFisher SCIENTIFIC