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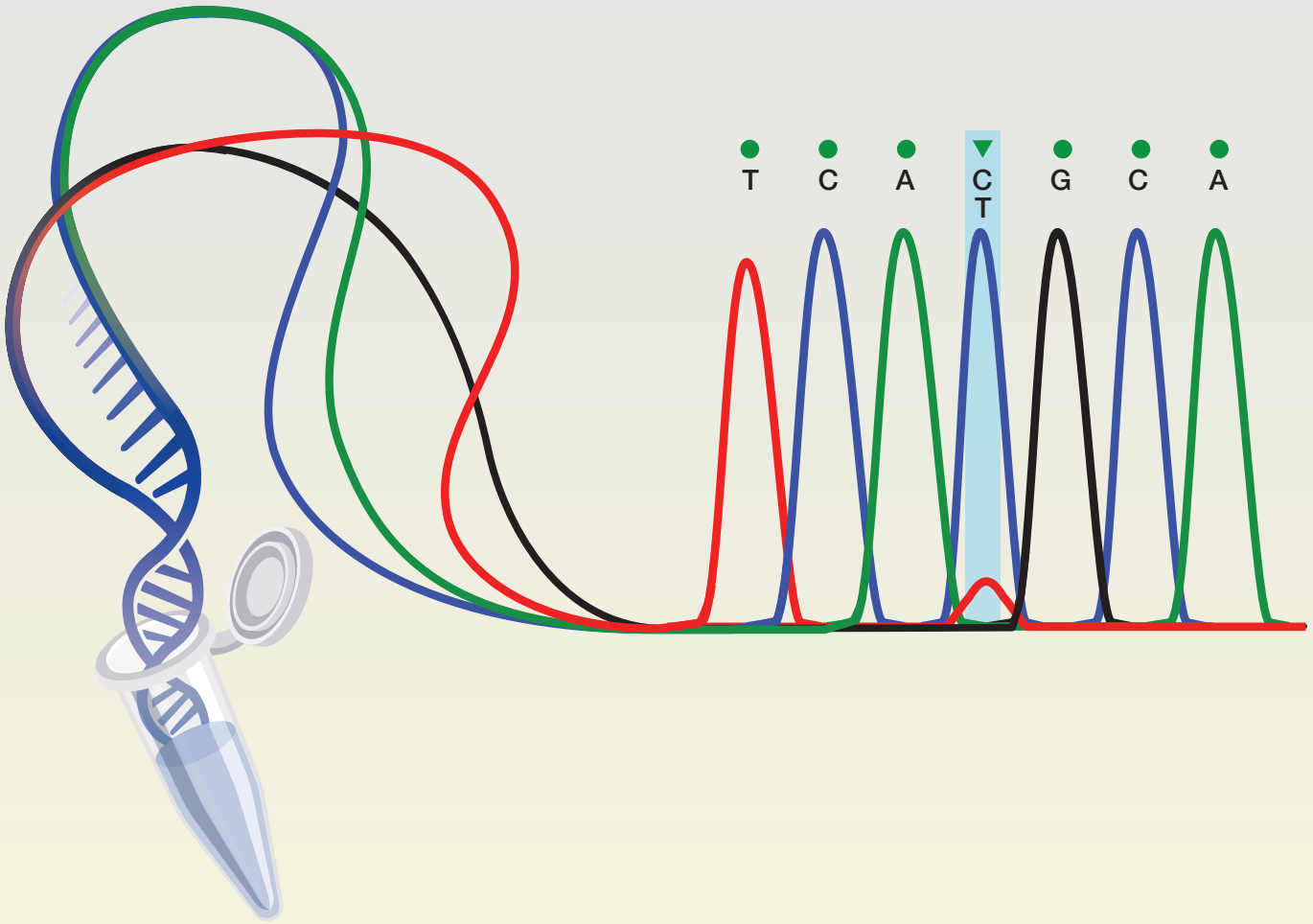
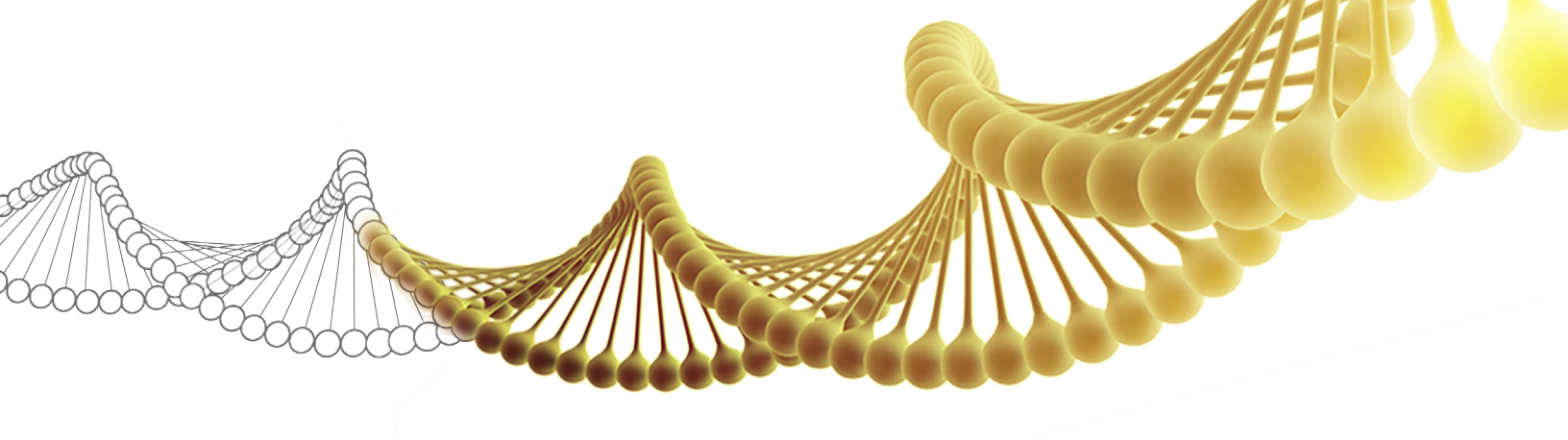


Sanger sequencing

Generate high-quality data with our proven workflow

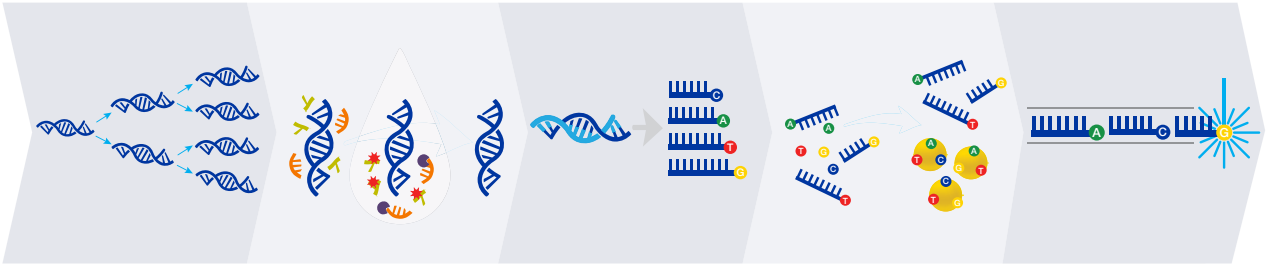
fisher scientific
part of Thermo Fisher Scientific



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



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Learn about our simple and fast Sanger sequencing workflow and the products that enable each step of the sequencing workflow. From PCR amplification to data analysis, we offer high-performance tools and technologies to make the process quick and easy.

PCR amplification

Invitrogen Primer Designer tool for PCR and Sanger sequencing

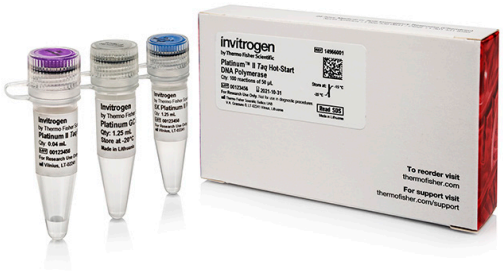
Use our online Invitrogen™ Primer Designer™ Tool to search for the right PCR/Sanger sequencing primer pair from a database of ~650,000 predesigned primer pairs for resequencing the human exome and human mitochondrial genome. Choose from different amplicon lengths to accommodate various research applications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

- Our primers are free of known SNPs and primer-dimers, highly target-specific, and used under universal PCR conditions
- Full primer coverage for Ion Torrent™ AmpliSeq™ Exome Panel and Ion Torrent™ AmpliSeq™ Cancer Hotspot Panel v.2 Sanger confirmation workflow
- Flexible primer configuration 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 validation tests have shown >95% success rate

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

Platinum II Hot-Start PCR Master Mix

Designed to deliver exceptional PCR results, even with the toughest templates. A universal primer annealing feature reduces optimization steps and allows for co-cycling of all assays. A unique combination of innovative buffer, high-performance Taq DNA polymerase, and superior hot-start technology enables highly sensitive, fast, and specific amplification of a full range of targets.



- Universal 60° C annealing temperature for any primer pair
- Up to 384 different PCR assays co-cycled using same PCR protocol
- 2X shorter time to PCR product
- Robust amplification of versatile range of targets, from AT-rich to GC-rich

Ordering information

Product	Size	Cat. No.
Invitrogen™ Platinum™ II Hot-Start PCR Master Mix (2x)	50 reactions	14000012
	200 reactions	14000013
	1,000 reactions	14000014

PCR clean-up

ExoSAP-IT *Express* reagent: fastest PCR clean-up method

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

- 5-min protocol
- One-tube, one-step PCR clean-up
- 100% recovery of PCR products

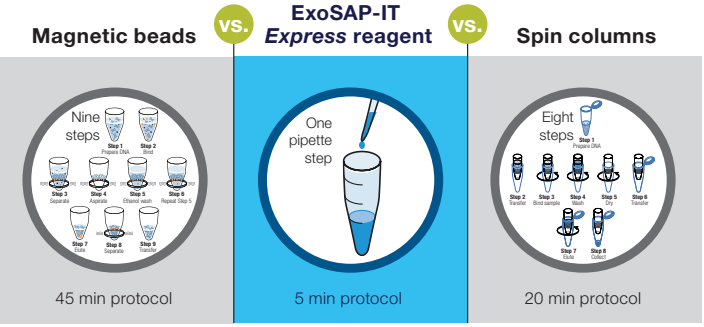


Figure 1. Use of ExoSAP-IT Express reagent eliminates spin columns, magnetic beads, sedimentations, filtrations, and gel purifications. With a 5 minute protocol, ExoSAP-IT Express reagent is the fastest and easiest method for PCR clean-up, minimizing pipetting errors or contamination.

Protocol

Treat 5 µL of PCR product with 2 µl of ExoSAP-IT *Express* reagent. Treatment is carried out at 37°C for 4 minutes followed by an incubation period 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).

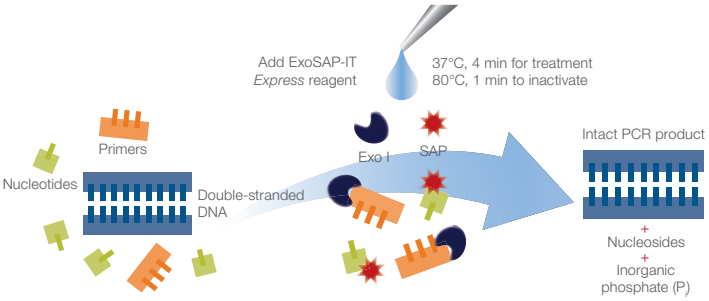


Figure 2. Workflow diagram for enzymatic PCR clean-up using ExoSAP-IT Express reagent.

Ordering information

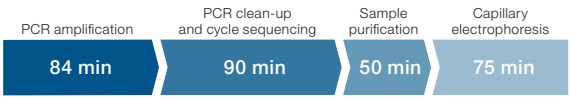
Product	Size	Cat. No.
ExoSAP-IT *Express* PCR Product Cleanup Reagent	100 reactions	75001.200.UL
	500 reactions	75001.1.ML
	2,000 reactions	75001.4X.1.ML
	5,000 reactions	75001.10.ML

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 Four steps in approximately five process hours



A traditional cycle sequencing workflow, run with Applied Biosystems™ POP-6™ Polymer Five steps in approximately eight process hours

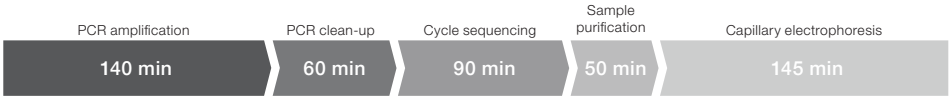


Figure 3. The BigDye Direct Kit delivers significant efficiencies compared to standard sequencing. Traditional sequencing workflows can require more than 8 hours of process time and 5 steps to complete. In contrast, the BigDye® Direct workflow typically requires only 5 hours and 4 steps, producing sequence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

Ordering information

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

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	Size	Cat. No.
BigDye Terminator v3.1 Cycle Sequencing Kit	24 reactions	4337454
	100 reactions	4337455
	1,000 reactions	4337456
	5,000 reactions	4337457
	25,000 reactions	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 product templates with rapid electrophoresis.

Ordering information

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

Sequencing clean-up

BigDye XTerminator Purification Kit

Correctly cleaning up your sequencing reactions is an integral part of the Sanger sequencing workflow. If the sequencing reaction clean-up step is missed or not performed properly, the residual dye in the reaction can out-compete the labelled amplicons for entry into the capillary and can cause reduced signal intensity, which can interfere with the instruments 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 DNA sequencing reactions to remove unincorporated Applied Biosystems™ BigDye™ terminators and salts. It also eliminates dye blobs in your reaction. Clean-up is complete in under 40 minutes and typically requires less than 10 minutes of labor.

Protocol

Dispense 55 µL/well of the SAM/BigDye XTerminator bead working solution to each sample. Vortex for 20 minutes at 1,800 rpm (for the Digital Vortex Genie™ 2 instrument), followed by centrifugation at 1,000 × g for 2 minutes.

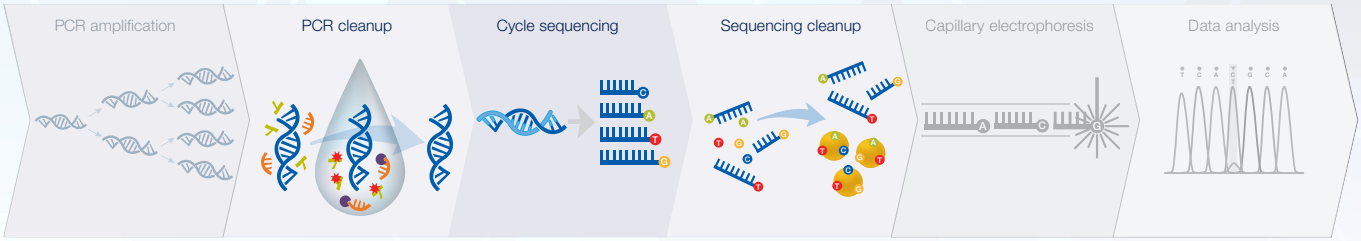
Ordering information

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

Generate high-quality Sanger sequencing data with our simplified workflow

Our Sanger Sequencing Kit provides all the reagents needed for PCR cleanup, cycle sequencing, and sequencing product cleanup

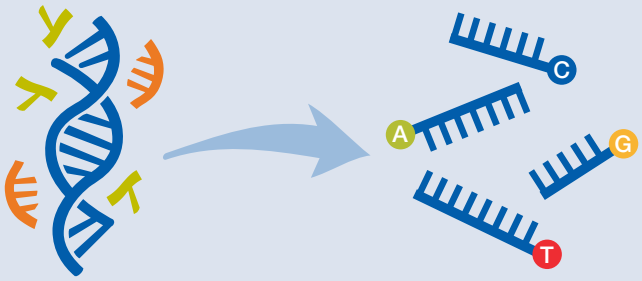
- Convenient all-in-one package with the optimal quantity for each component
- Affordable sample preparation for Sanger sequencing with built-in cost savings
- Optimal workflow for Applied Biosystems™ SeqStudio™, 3500 series, and 3730 series genetic analyzers



PCR cleanup, cycle sequencing, and sequencing cleanup with the all-in-one Sanger Sequencing Kit

Save time and enjoy the convenience of our Sanger Sequencing Kit

The Applied Biosystems™ Sanger Sequencing Kit provides a convenient and affordable solution for preparing sequencing reactions.



Ordering information

Product	Cat. No.
Sanger Sequencing Kit	A38073

Find out more about the Sanger sequencing kit and order at **thermofisher.com/sangerkit**

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Capillary electrophoresis and data analysis

	Applied Biosystems™ SeqStudio™ Genetic Analyzer	Applied Biosystems™ 3500 Series Genetic Analyzer	Applied Biosystems™ 3730 Series Genetic Analyzer
	Easy-to-use, flexible system 	Meets the needs of validated and process controlled environments 	Maximum throughput and scalability 
Number of capillaries	4	8 (3500), 24 (3500xL)	48 (3730), 96 (3730xl)
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
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 + fragment analysis on 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)	1.38 M (3730), 2.76 M (3730xL)
Maximum fragment throughput (samples/day)	192	384 (3500), 1,152 (3500xL)	3,456 (3730), 6,912 (3730xl)

We offer a wide range of Applied Biosystems™ software solutions for viewing and interpreting your Sanger sequencing results. Speak to your sales representative to find out more.

For more information, contact Fisher Scientific

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