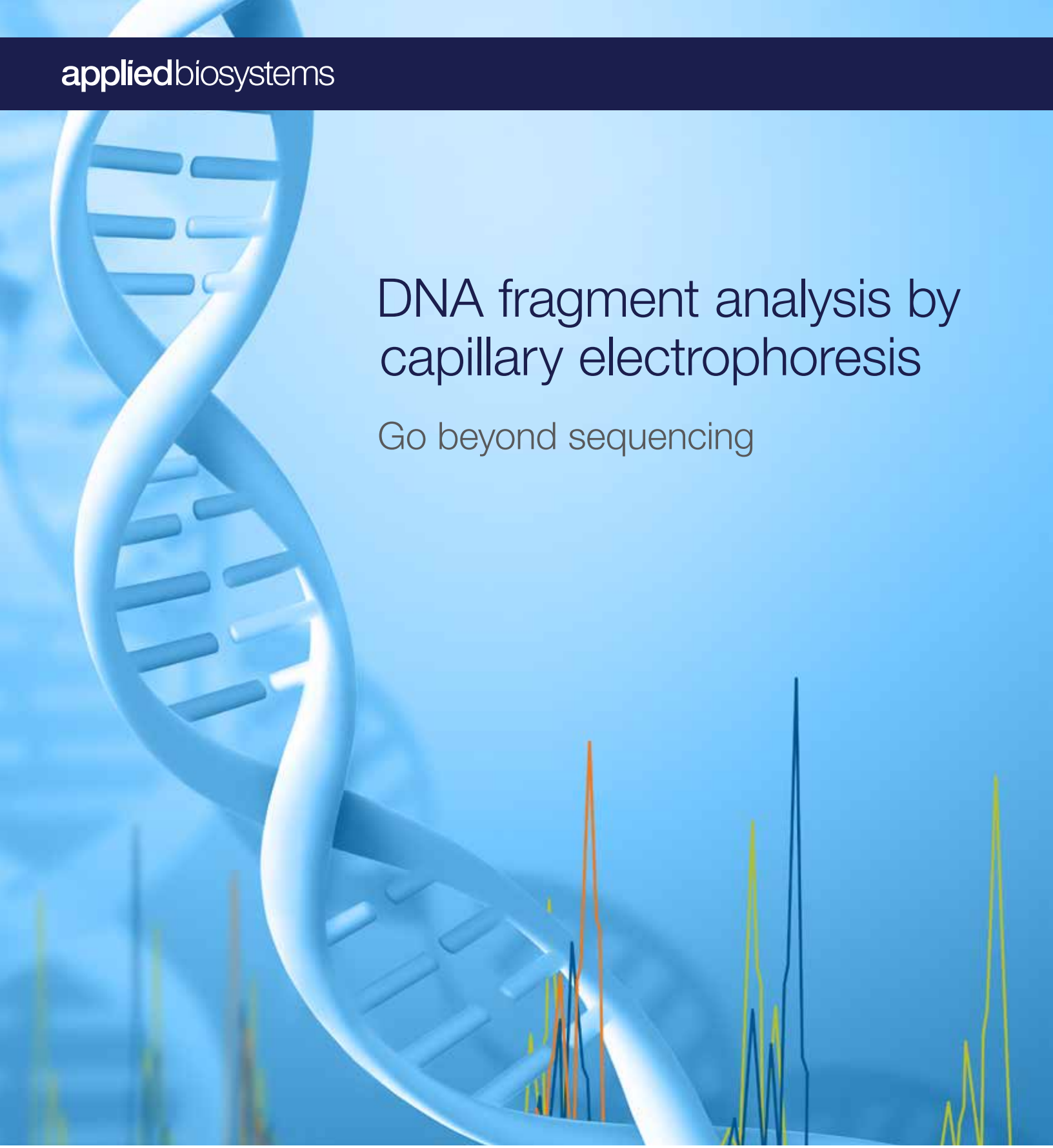
applied biosystems

DNA fragment analysis by capillary electrophoresis

Go beyond sequencing



Quality and dependability

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

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 gold-standard products and technologies you trust.

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

—Justin, Manufacturing Supervisor

- More choices, better results
- Superior support
- Focused innovation
- Proven quality
- Trust

Fragment analysis

Taking DNA analysis further

Analysis of DNA fragments enables a variety of applications, from cell line authentication to detection of aneuploidy. Fragment analysis comprises a series of techniques in which DNA fragments are fluorescently labeled, separated by capillary electrophoresis (CE), and sized by comparison to an internal standard. While DNA sequencing by CE is used to determine the specific base sequence of a particular fragment or gene segment, fragment analysis can provide sizing, relative quantitation, and genotyping information for fluorescently labeled DNA fragments produced by PCR using primers designed for a specific DNA target.

Simple

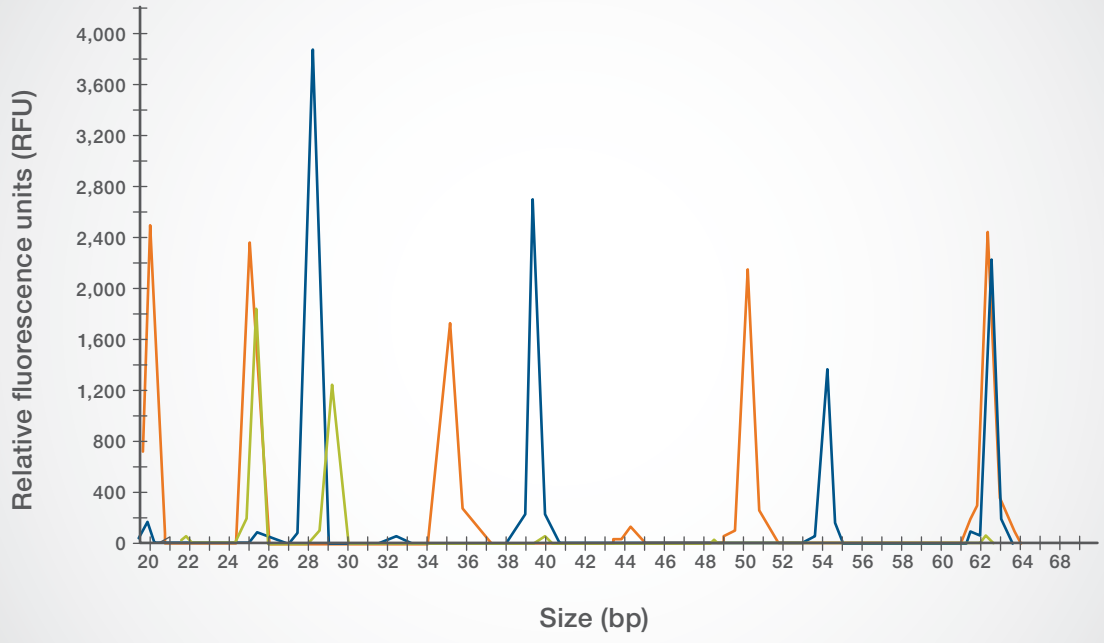
From straightforward sample preparation to peak analysis, fragment analysis applications use simplified workflows.

Fast turnaround time

Data are delivered in hours instead of days.

Cost-effective

- Fewer steps and less reagent per action are required, compared to sequencing.
- Multiple loci can be analyzed in a single run.



What can you do with fragment analysis?

Fragment analysis delivers the results you need. Generate highly accurate and reliable data using off-the-shelf or customizable solutions for a wide range of applications.

Cell line authentication

Misidentification of cell lines produces misleading results, confusion, and added costs to research. Many journals and funding agencies now require researchers to ascertain that the cell lines they use are authentic. Short tandem repeat (STR) genotyping by fragment analysis can provide a simple, inexpensive, and highly specific genetic "fingerprint" of a cell line.

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

Gene editing

Genome editing is poised to revolutionize our understanding of diverse biological systems. The CRISPR-Cas9 system, the easiest, most precise, and most widely adopted genome editing technology, is based on the components of a simple bacterial immune system. In any genome editing experiment, the repair process is not completely efficient or accurate. Fragment analysis can be used to evaluate primary transformed cultures to determine editing efficiency.

Learn more at
thermofisher.com/genomeeditconfirmce

Microsatellite marker analysis

Microsatellite markers are codominant, polymorphic DNA loci containing repeated nucleotide sequences, typically with 2 to 7 nucleotides per repeat unit. The number of nucleotides in the repeated unit is the same for the majority of the repeats within an individual microsatellite locus, but the number of repeats for a specific locus may differ, resulting in alleles of varying length, which can be analyzed by capillary electrophoresis. Because they are Mendelian inherited, analysis of variations in their length is a widely accepted tool for applications such as microsatellite instability (MSI), which is often used in inherited disease and oncology research.

Learn more at
thermofisher.com/microsatellite

Repeat DNA expansion

Repeat DNA expansion is the term given to a DNA mutation comprising any number of multi-nucleotide repeats. Often, these regions are challenging to amplify—and thus, characterize—because of high GC content. Short tandem repeats (STRs) are one category of repetitive DNA. They contain bursts of 1 to 6 nucleotides repeated over long stretches of genomic DNA and are crucial in understanding more than 30 debilitating genetic diseases. Fragment analysis is the technology of choice for these genetically "difficult" samples.

Learn more at
thermofisher.com/seqstudioapplications

QF-PCR

Relative fluorescent quantitation, or quantitative fluorescence PCR (QF-PCR), is a technique used in a variety of fragment analysis applications that require accurate peak height or peak area comparisons across multiple samples. Research applications that utilize this technique include evaluating loss of heterozygosity (LOH), aneuploidy assays, and detecting large chromosomal deletions.

Learn more at
thermofisher.com/qfpcr

MLPA

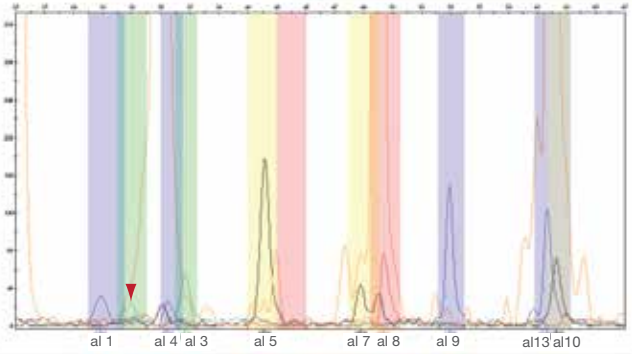
One widely used method for studying inherited human diseases and cancers arising from variations in copy number of a locus is the multiplex ligation-dependent probe amplification (MLPA™) assay. This fragment analysis method, developed and commercialized by MRC Holland, can analyze up to 50 multiplexed pairs of adjacently located probes hybridizing to the loci of interest. Researchers choose it because it is a high-throughput, cost-effective way to look for duplications and deletions.

Learn more at
thermofisher.com/seqstudioapplications

SNP genotyping

Single-nucleotide polymorphism (SNP) genotyping is a way to measure genetic variation. SNP genotyping is used to evaluate differences in genetic traits, susceptibility to disease, and response to drug therapies. A robust tool for SNP analysis is the Applied Biosystems™ SNaPshot™ Multiplex Kit. The kit allows multiplexing during single base extensions of up to 10 primer–template combinations in a single-tube, single-capillary format.

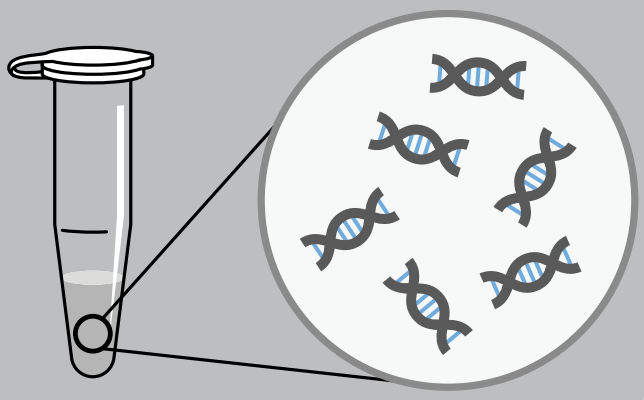
Learn more at **thermofisher.com/snp**



Comprehensive solutions for your fragment analysis workflow

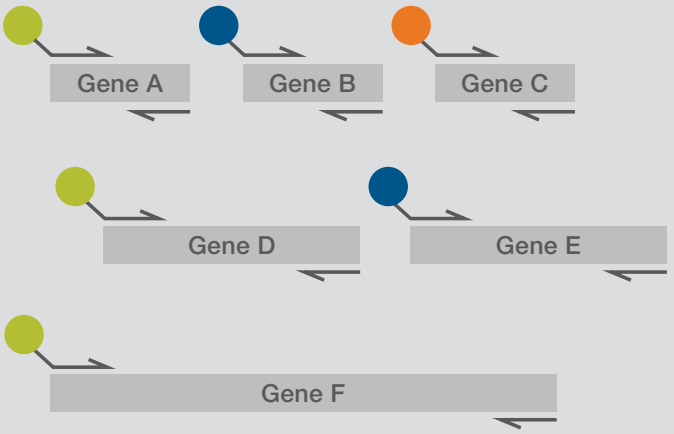
We offer a wide range of products and services to simplify each step of the fragment analysis workflow.

DNA extraction



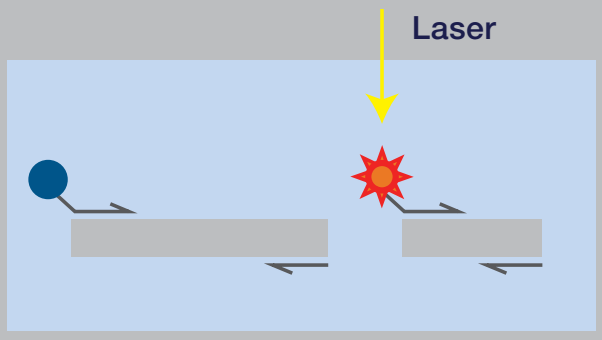
Choose from a range of genomic DNA extraction kits for sensitive, scalable purification (page 8).

PCR amplification

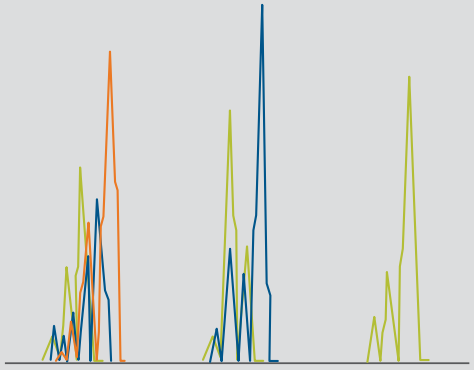


Choose from Applied Biosystems™ 5′ fluorescently labeled primers and primer pairs (page 9).

Capillary electrophoresis



Data analysis

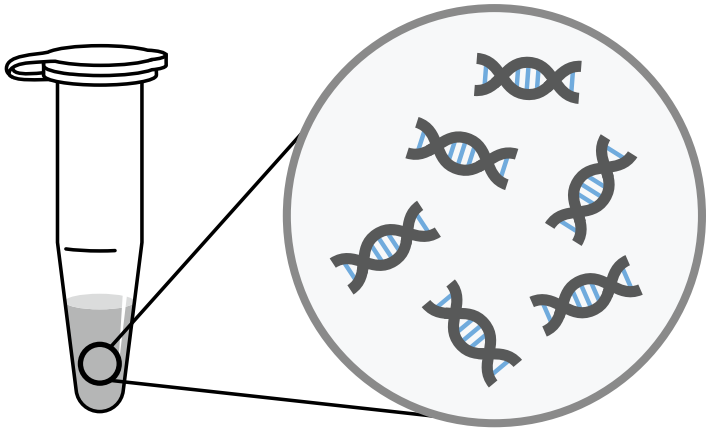


The Applied Biosystems™ SeqStudio™ Genetic Analyzer is optimized for fragment analysis (page 10).

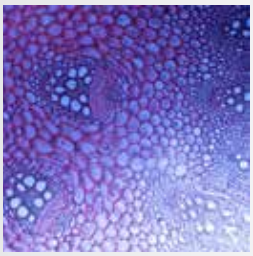
We offer a portfolio of Applied Biosystems™ software solutions for viewing and interpreting your fragment analysis results (page 13).

DNA extraction

DNA extraction is a critical first step in the experimental workflow of DNA fragment analysis. The overall efficiency, quality, and size of PCR products can be significantly affected by characteristics of the sample itself, and the method chosen for nucleic acid extraction and purification. Ideal methods will vary depending on the source or tissue type, how it was obtained from its source, and how the sample was handled or stored prior to extraction.

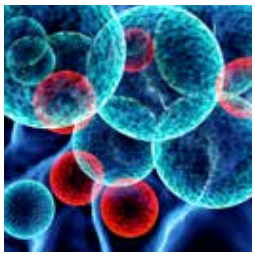


Maximize process efficiency and downstream performance with genomic DNA extraction kits for sensitive, scalable purification from a variety of samples, including tissue, cells, blood, serum, plants, forensic samples, and more.



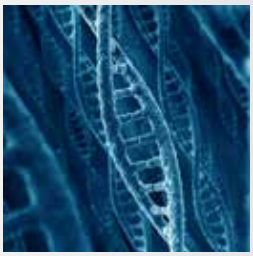
Tissue DNA extraction kits

Blood DNA extraction kits



Cellular DNA extraction kits

Plant DNA extraction kits



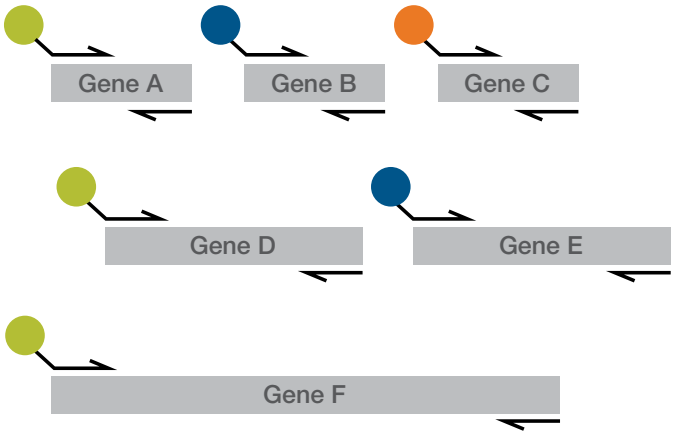
Cell-free DNA extraction kits

See the complete portfolio of genomic DNA isolation products at **thermofisher.com/gdnaprep**

PCR amplification

Primers

To perform fragment analysis on a CE system, primers must be designed that flank the region of interest. Typically, fluorescent dyes are attached to the primers, and the fragments are amplified by PCR before electrophoresis. We offer Applied Biosystems 5´ fluorescently labeled primers and primer pairs that have been validated for use in fragment analysis.



Key features:

- Custom 5´ fluorescently labeled primers
- Choice of label includes 6-FAM™, TET™, VIC™, HEX™, NED™, or PET™ dye
- All oligos are desalted; larger scales of labeled primers have an option for HPLC purification
- Choice of delivered scales

Design your primers at **thermofisher.com/faprimers**

PCR master mix

Invitrogen™ Platinum™ II Hot-Start PCR Master Mix helps you get to your fragment analysis run, faster. A unique combination of an innovative buffer, high-performance *Taq* DNA polymerase, and Invitrogen™ Platinum™ hot-start technology enables exceptional PCR results, even in the toughest applications.

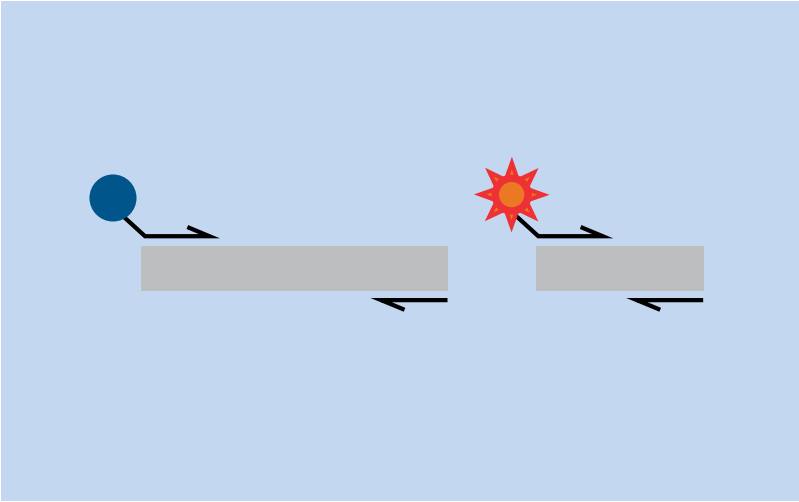
Key features:

- 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 tolerance, and robust amplification
- Platinum hot-start technology allows for room temperature reaction setup
- A 2X master mix format helps reduce pipetting errors with fewer pipetting steps

Capillary electrophoresis

Matrix and size standards

To prepare for capillary electrophoresis, a spectral calibration with the matrix standard corresponding to the dye set you select for labeling your DNA fragments must be performed on your genetic analyzer to accurately detect the dye labels on your primers. Each unknown sample is mixed with the size standard and Applied Biosystems™ Hi-Di™ Formamide before proceeding with electrophoresis. Size standards allow sizing of sample peaks and correct for injection variations. Refer to the following table for the recommended Applied Biosystems™ matrix standard and Applied Biosystems™ GeneScan™ size standard for your workflow.



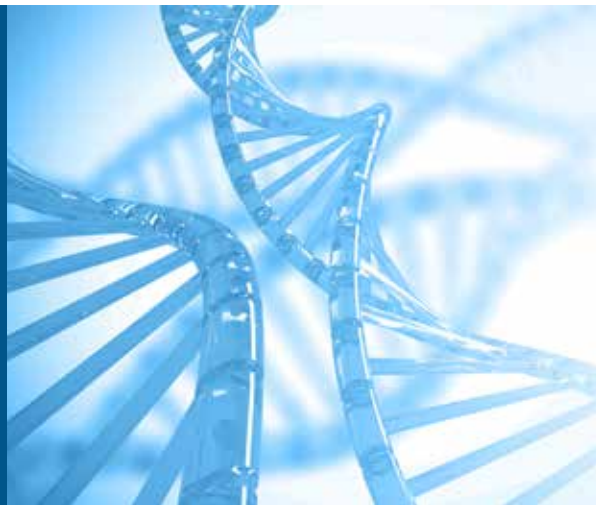
Selecting the right matrix and size standards

Dye set used to label DNA fragments	Corresponding matrix standard	Recommended size standard	Possible applications
Dye set E5: dR110 (blue), dR6G (green), dTAMRA (yellow), dROX (red)	DS-02	GeneScan 120 LIZ Size Standard (orange)	SNaPshot multiplexing
		GeneScan 600 LIZ Size Standard v2.0 (orange)	Microsatellite, LOH, chimerism, inter-simple sequence repeat (ISSR), restriction fragment length polymorphism (RFLP), terminal-RFLP (T-RFLP), methylation-sensitive mobility shift assay (MSMSA)
Dye set D: 6-FAM (blue), HEX (green), NED (yellow)	DS-30	GeneScan 500 ROX Size Standard (red)	Custom fragment analysis
Dye set D: 6-FAM (blue), VIC (green), NED (yellow)	DS-31	GeneScan 500 ROX Size Standard (red)	Custom fragment analysis
Dye set F: 5-FAM (blue), JOE (green), NED (yellow)	DS-32	GeneScan 500 ROX Size Standard (red)	Microsatellite
			Amplified fragment length polymorphism (AFLP)
Dye set G5: 6-FAM (blue), VIC (green), NED (yellow), PET (red)	DS-33	GeneScan 600 LIZ Size Standard v2.0 (orange)	Microsatellite
			Custom fragment analysis
Dye set J6: 6-FAM (blue), VIC (green), NED (yellow), SID (violet), TAZ (red)	DS-36	GeneScan 600 LIZ Size Standard v2.0 (orange)	Custom fragment analysis

SeqStudio Genetic Analyzer

The SeqStudio Genetic Analyzer is a low-throughput, easy-to-use, and convenient benchtop system that makes running CE experiments easier with minimal instrument hands-on time due to an all-in-one cartridge. There is no need to do prerun calibrations or change the polymer, buffer, or arrays. It is the only genetic analyzer that performs simultaneous sequencing and fragment analysis runs on the same plate. The system is integrated with Connect, our cloud-based scientific analysis and peer collaboration platform, so you can set up your run, check its progress, and access your data anytime, anywhere.*

* Internet connection and Connect account required.



Genetic analyzers and on-instrument reagents

	SeqStudio Genetic Analyzer	Applied Biosystems™ 3500 Series Genetic Analyzers	Applied Biosystems™ 3730xl Genetic Analyzer
	Easy to use, versatile, and flexible 	Meets the needs of validated and process-controlled environments 	Maximum throughput, scalability, and flexibility **Refreshed** 
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
Radio frequency identification (RFID)	Yes	Yes	No
Polymer type	POP-1, integrated into click-in cartridge	POP-6, POP-7, POP-4*	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 plus fragment analysis on same run	Sequencing, fragment analysis	Sequencing, fragment analysis
Minimum run time	30 minutes	30 minutes	20 minutes
Maximum fragment throughput (samples/day)	192	384 (3500), 1,152 (3500xL)	3,936

* POP-4 is generally only used with HID applications and requires Applied Biosystems™ GeneMapper™ ID-X Software for analysis.

** 96 capillaries are not recommended for fragment analysis.

On-instrument consumables

Universal all-in-one cartridge

The Applied Biosystems™ SeqStudio™ cartridge has an integrated system design that includes a capillary array, POP-1™ universal polymer, buffer, and pump. The POP-1 universal polymer allows for flexibility to perform simultaneous Sanger sequencing and fragment analysis with one cartridge on the same plate in a single run. The cartridge is removable and can be stored on the instrument up to 6 months. Just load your samples, insert the cartridge, and start the run.

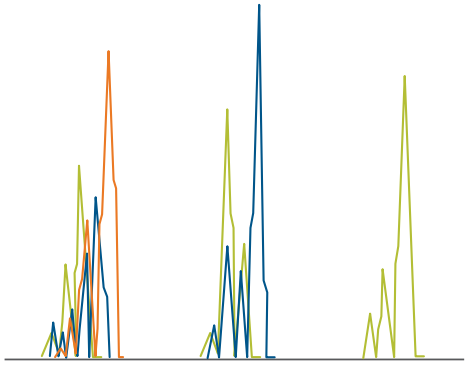
Polymer

Using the right Applied Biosystems™ polymer can help reduce your re-run rate and enables high-quality, reproducible data. We offer different polymer chemistries for different needs:

POP-4™	Human identification applications, other fragment analysis applications, short DNA fragments (<500 bp)
POP-6™	Mid-sized DNA fragments (500–700 bp)
POP-7™	Long DNA fragments (>700 bp)

Data analysis

We offer a broad portfolio of Applied Biosystems™ software solutions for viewing and analyzing your fragment analysis results. Access, analyze, and share data anytime, anywhere, with Connect.



Available on Connect

Peak Scanner Module
Offers peak identification and fragment sizing.

Microsatellite Analysis Module
Enables analysis of a mixture of DNA fragments separated by size. This analysis provides a profile of the separation, precisely calculates the sizes of the fragments, and determines the microsatellite alleles present in the sample.

Downloadable

Gene Mapper Software
A flexible genotyping software package that provides DNA sizing and quality allele calls. Specializes in multi-application functionality, including AFLP, LOH, and microsatellite and SNP genotyping analysis.

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

Service plans and education services

Our service plans maximize system uptime, reduce overall repair costs, extend the life of your instrument, and help keep it running at peak performance. Choose from a variety of service options, including Digital Service Innovations with remote support, that balance your budget, productivity, and uptime.

To help you look ahead, we offer a combination of virtual and in-person classroom instruction, and hands-on learning in your lab to match your schedule, budget, and learning preferences. Whichever course style you choose, you'll learn from one of our highly skilled application scientists who are available to lead sessions online, at your location, or at one of our 12 training centers located worldwide.

- **Instrument service plans**
- **Qualification services**
- **Analytical validation consulting services**
- **Education services**
- **Data management services**

Explore our services and support solutions at **thermofisher.com/instrumentservices**

Notes

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ThermoFisher SCIENTIFIC

Ordering information

Product	Quantity	Cat. No.
DNA extraction		
PureLink Genomic DNA Mini Kit	50 preps	K182001
	250 preps	K182002
MagMAX-96 DNA Multi-Sample Kit	96 preps	4413021
	5 x 96 preps	4413022
PureLink *Pro* 96 Genomic DNA Purification Kit	4 x 96 preps	K182104A
PCR amplification		
Platinum II Hot-Start PCR Master Mix (2X)	50 reactions	14000012
	200 reactions	14000013
	1,000 reactions	14000014
Capillary electrophoresis		
CLA IdentiFiler Direct PCR Amplification Kit	200 reactions	A44661
CLA IdentiFiler Plus PCR Amplification Kit	200 reactions	A44660
CLA GlobalFiler PCR Amplification Kit	200 reactions	A44662
TrueMark MSI Assay	100 reactions	A45295
GeneScan 120 LIZ Dye Size Standard	800 reactions	4324287
GeneScan 600 LIZ Dye Size Standard v2.0	800 reactions	4408399
GeneScan 1200 LIZ Dye Size Standard	800 reactions	4379950
GeneScan 500 ROX Dye Size Standard	800 reactions	401734
DS-02 Matrix Standard Kit for the 31xx, 3500/3500xl, and 3730/3730xl DNA Analyzers	8 runs	4323014
DS-30 Matrix Standard Kit (Dye Set D)	8 runs	4345827
DS-31 Matrix Standard Kit (Dye Set D)	8 runs	4345829
DS-32 Matrix Standard Kit (Dye Set F)	8 runs	4345831
DS-33 GeneScan Installation Standards with GeneScan 600 LIZ Size Standard v2.0	1 kit	4376911
DS-36 Matrix Standard (Dye Set J6)	8 runs	4425042
Hi-Di Formamide	25 mL	4311320
	4 x 5 mL	4440753
RecoverAll Total Nucleic Acid Isolation Kit for FFPE	40 preps	AM1975
SNaPshot Multiplex Kit, with protocol	100 reactions	4323151
	1,000 reactions	4323161
	5,000 reactions	4323163
SeqStudio Genetic Analyzer System with SmartStart	1 system	A35644
SeqStudio Cartridge v2	1,000 reactions	A41331
3500 Genetic Analyzer for Fragment Analysis	1 system	A30468
3500xL Genetic Analyzer for Fragment Analysis	1 system	A30469
Data analysis		
GeneMapper Software 6, full installation	1 license	A38888