SNP genotyping

SNaPshot Multiplex System for SNP genotyping

One system, many applications

Features of the SNaPshot Multiplex System

- Useful for a multitude of applications—SNP and methylation analysis, fingerprinting, quantitative allele frequency, validating NGS data, and more
- Customized for your target
- Offers multiplexing capability (up to 10-plex)
- Sensitive allele-frequency detection (typically 5%)
- Compatible with all Applied Biosystems™ CE instruments
- Automated analysis using a specific Applied Biosystems™ GeneMapper™ Software data analysis module

The Applied Biosystems™ SNaPshot™ Multiplex System is a primer extension–based method developed for the analysis of single-nucleotide polymorphisms (SNPs) (Figure 1). Through its multiplexing capability, up to 10 SNPs can be analyzed in a single reaction, regardless of their positions on the chromosome or the amount of separation from neighboring SNP loci. The ability to use unlabeled, user-defined primers allows researchers to incorporate SNPs of interest cost-effectively. The Applied Biosystems™ SNaPshot™ Multiplex Ready Reaction Mix (included in the system) helps ensure robust, reproducible analyses of multiplexed samples. Researchers can analyze more than 40,000 SNP genotypes per day on just one Applied Biosystems™ 3730xl Genetic Analyzer (Table 1).

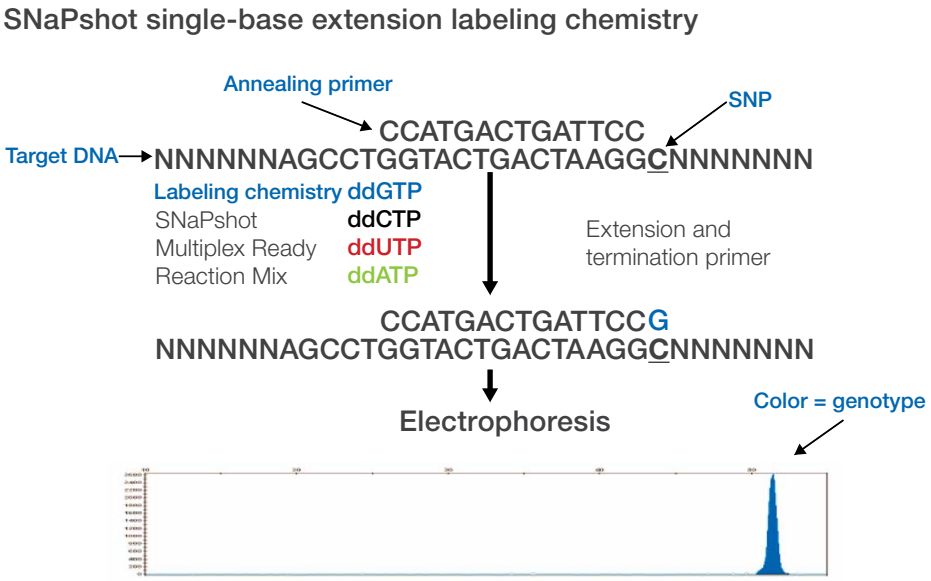

Figure 1. SNaPshot labeling chemistry relies on single-base extension and termination. The Applied Biosystems™ SNaPshot™ Multiplex Kit uses a single-tube reaction to interrogate SNPs at known locations. The chemistry is based on the dideoxy single-base extension of an unlabeled oligonucleotide primer (or primers). Each primer binds to a complementary template in the presence of fluorescently labeled ddNTPs and DNA polymerase. The polymerase extends the primer by one nucleotide, adding a single ddNTP to its 3′ end. The fluorescence color readout reports about the specific base that was added.

SNP analysis

SNPs have been identified in all genomes and can be used for a multitude of analyses, including studying mutations implicated in various cancers, genetic disease research, mitochondrial DNA investigations, scrapie susceptibility in sheep, loss of heterozygosity, assessing performance in food animal production, and even differentiating drug and non-drug forms of cannabis. Table 2 lists some of the many ways our customers use the SNaPshot Multiplex System in their research. A more extensive list of publications demonstrating the utility of the SNaPshot Multiplex System is available at thermofisher.com/snp.

Table 1. SNP analysis throughput on various Applied Biosystems™ genetic analyzers. Numbers stated are an approximation based on 24-hour operation and 10 SNPs per capillary, to show application and throughput scale across Applied Biosystems CE Instruments.

Applied Biosystems genetic analyzer	SeqStudio	SeqStudio Flex (8-capillary) and 3500	SeqStudio Flex (24-capillary) and 3500xL	3730	3730xl
SNP throughput per day	1,900	3,700	11,000	21,000	42,000

Table 2. Selected published articles that cite the SNaPshot Multiplex System. Find more publications at thermofisher.com/snp.

Type of analysis	Application	Title	Journal
SNP analysis	Differentiating drug and non-drug forms of cannabis	Differentiation of drug and non-drug cannabis using a single nucleotide polymorphism (SNP) assay	*Forensic Sci Int* 207:193–197 (2011)
	Antiretroviral resistance mutation	Pre-screening HIV-1 reverse transcriptase resistance mutations in subtype B patients using a novel multiplex primer extension assay	*Curr HIV Res* 7:398–409 (2009)
	Arabidopsis markers	Establishment of a high-efficiency SNP-based framework marker set for *Arabidopsis*	*Plant J* 36:122–140 (2003)
	Assess meat performance in pigs	SNaPshot minisequencing and a panel of candidate genes for complex routine testing of meat performance traits in pigs	*Anim Biotechnol* 18:109–115 (2007)
	Blood typing	Detection of blood group genes using multiplex SNaPshot method	*Transfusion* 49:740–749 (2009)
	Method for degraded samples	Developing multiplexed SNP assays with special reference to degraded DNA templates	*Nat Protoc* 1:1370–1378 (2006)
	Heteroplasmy validation after NGS analysis	Detecting heteroplasmy from high-throughput sequencing of complete human mitochondrial DNA genomes	*Am J Hum Genet* 87:237–249 (2010)
	Identifying species in *M. tuberculosis* complex	Identification and genotyping of *Mycobacterium tuberculosis* complex species by use of a SNaPshot Minisequencing-based assay	*J Clin Microbiol* 48:1758–1766 (2010)
	Loss of heterozygosity (LOH)	Multiplex SNaPshot genotyping for detecting loss of heterozygosity in the mismatch-repair genes *MLH1* and *MSH2* in microsatellite-unstable tumors	*Clin Chem* 54:1844–1854 (2008)
	Mitochondrial variants	Mitochondrial variants in schizophrenia, bipolar disorder, and major depressive disorder	*PLoS One* 4:e4913 (2009)
	Pathogen resistance	Single nucleotide polymorphisms and haplotypes in the IL10 region associated with HCV clearance	*Genes Immun* 6:347–357 (2005)
	Scrapie susceptibility screening	Primer extension assay for prion protein genotype determination in sheep	*Mol Cell Probes* 18:33–37 (2004)
	Selective breeding in horses	Identification of horse chestnut coat color genotype using SNaPshot	*BMC Res Notes* 2:255 (2009)
	Species identification within Lactobacillus casei isolates	Application of the SNaPshot minisequencing assay to species identification in the *Lactobacillus casei* group	*Mol Cell Probes* 25:153–157 (2011)
	Subspecies identification in tigers	The development and validation of a single SNaPshot multiplex for tiger species and subspecies identification-Implications for forensic purposes	*Forensic Sci Int Genet* 6:250–257 (2011)
	Tracking cows and their products	SNPmplexViewer—toward a cost-effective traceability system	*BMC Res Notes* 4:146 (2011)
	Wheat breeding	Insertion site-based polymorphism markers open new perspectives for genome saturation and marker-assisted selection in wheat	*Plant Biotechnol J* 8:196–210 (2010)

Type of analysis	Application	Title	Journal
SNP analysis	Species identification in pines	Development of a SNaPshot assay for the genotyping of organellar SNPs in four closely related pines	*Dendrobiology* 90:76-85 (2023)
	SNP marker methods	A new approach to detect a set of SNP-SNP markers: combining ARMS-PCR with SNaPshot technology	*Electrophoresis* 41:1189–1197 (2020)
	Historical genetic insights of Mongolians	Insights into the paternal admixture history of Chinese Mongolians via high-resolution customized Y-SNP SNaPshot panels	*Forensic Sci Int Genet* 54:102565 (2021)
	Forensic genotyping methods	HRM and SNaPshot as alternative forensic SNP genotyping methods	*Forensic Sci Med Pathol* 13:293–301 (2017)
	Detecting sex-related genes in *Cynoglossus semilaevis*	A combination of genome-wide association study screening and SNaPshot for detecting sex-related SNPs and genes in *Cynoglossus semilaevis*	*Comp Biochem Physiol Part D: Genomics and Proteomics* 35:100711 (2020)
	Y-chromosome SNP loci	Exploring of new Y-chromosome SNP loci using pyrosequencing and the SNaPshot methods	*Int J Legal Med* 126:825–833. (2011)
SNP quantification	Quantitative mutant measurement	Putative precursor cancer cells in human colorectal cancer tissue	*Int J Clin Exp Pathol* 2:154–162 (2009)
	Quantitative SNP allele frequency measurement	Universal, robust, highly quantitative SNP allele frequency measurement in DNA pools	*Hum Genet* 110:471–478 (2002)
	Allele-specific gene expression	Allelic variation in human gene expression	*Science* 297:1143 (2002)
		Cis-acting variation in the expression of a high proportion of genes in human brain	*Hum Genet* 113:149–153 (2003)
BAC fingerprinting	High-throughput fingerprinting of bacterial artificial chromosomes	High-throughput fingerprinting of bacterial artificial chromosomes using the snapshot labeling kit and sizing of restriction fragments by capillary electrophoresis	*Genomics* 82:378–389 (2003)
Methylation	Methylation status	Single nucleotide extension technology for quantitative site-specific evaluation of metC/C in GC-rich regions	*Nucleic Acids Res* 33:e95 (2005)
		Capillary electrophoretic analysis of methylation status in CpG-rich regions by single-base extension of primers modified with N6-methoxy-2,6-diaminopurine	*Anal Biochem* 380:13–20 (2008)

Methylation

The study of epigenetic effects, including methylation, is emerging as an important component of genetic research. In a typical assay to detect methylation, bisulfite treatment of DNA deaminates unmethylated cytosines (converting them to uracils) and leaves methylated cytosines unchanged. The subsequent PCR amplification step converts uracil bases to thymines. Researchers can use the SNaPshot Multiplex System to quantify the cytosine-to-thymine changes in treated and untreated samples to determine the methylation status.

BAC fingerprinting

Fingerprinting of large-insert genomic fragment libraries, also known as bacterial artificial chromosomes (BAC) clones, leads to the construction of a genome-wide physical map. These maps are critical to genome sequencing, positional cloning, and understanding of the relative organization of genes and markers. When BAC libraries are arranged into maps that reflect the DNA sequence in a chromosome, they enable maximal information and utility. The SNaPshot Multiplex Kit is a widely used, efficient method to label BAC fragments. The labeled fragments can then be separated and detected on any Applied Biosystems CE instrument. Sizing information from GeneMapper Software is imported into subsequent editing and contig-assembly programs. The high-quality results you get from the SNaPshot Multiplex Kit provide an easy-to-use and cost-effective solution for high-throughput BAC fingerprinting.

A complete solution

Across the SNP analysis workflow, Thermo Fisher Scientific offers solutions to help you get the research data you need. Key components include:

SNaPshot Multiplex System

The system supplies the SNaPshot Multiplex Ready Reaction Mix, control primer mix, control template, and protocol.

Applied Biosystems™ GeneScan™ 120 LIZ™ Size Standard

This is a LIZ dye–labeled size standard that is designed for reproducible sizing of small-fragment analysis data generated with the SNaPshot Multiplex System. It accurately sizes samples ranging from 20 to 120 nucleotides. When used with GeneMapper Software, the GeneScan 120 LIZ Size Standard significantly reduces the need for manual allele calls.

Applied Biosystems™ DS-02 Matrix Standard Kit

This standard set is used to generate the "multicomponent matrix" needed to detect the dyes (dR110, dR6G, dTAMRA™, dROX™, and LIZ dyes), critical for successful five-dye SNP analysis on all Applied Biosystems genetic analyzers.

GeneMapper Software

GeneMapper Software specializes in multi-application functionality, including SNP genotyping analysis for data generated with the SNaPshot Multiplex System.

Data analysis simplified using GeneMapper Software

GeneMapper Software is a flexible genotyping software package that provides DNA sizing and quality allele calls for all electrophoresis-based genotyping systems. This software specializes in multi-application functionality, including SNP genotyping analysis (Figure 2). GeneMapper Software can help users increase data processing efficiency with remote auto-analysis and command line operation, and allows for multi-user, client-server deployment. The software uses process quality values (PQVs) for automated identification that reduces data review time for high-throughput genotyping. In addition, the security and audit features help users meet 21 CFR 11 requirements. Applied Biosystems™ Peak Scanner™ Software v1.0 is an alternative free analysis software for low-complexity analysis. Download for free at **thermofisher.com**.

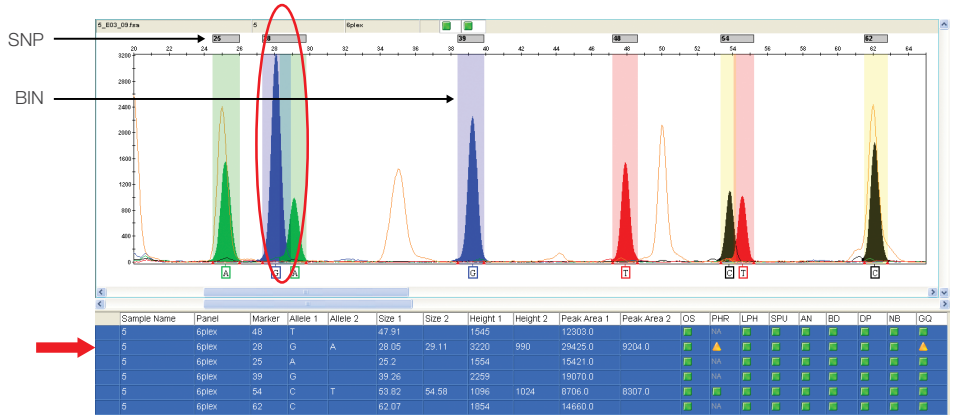

Figure 2. Example of SNP genotyping results obtained from GeneMapper Software. The vertical colored boxes are bins created automatically by the software using a sample or an artificial extension product created using the Applied Biosystems™ SNaPshot™ Primer Focus™ Kit. Each bin defines the minimum and maximum allowable size for each allele. GeneMapper Software identifies each peak and assigns the corresponding allele. In this example, the sample is heterozygous G/A for SNP 28. GeneMapper Software generates a customized report with genotype result (including size, height, peak area) and provides information about the quality of the sample (PQVs in columns OS through GQ), facilitating review by the operator. For example, a yellow triangle in the peak height ratio column (PHR) indicates that the ratio between the 2 alleles is outside what is defined in the default analysis settings (50% for each allele). Unfilled peaks (outlined only) represent the GeneScan 120 LIZ Size Standard positions.

Ordering information

Description	Quantity	Cat. No.
SNaPshot Multiplex Kit	100 reactions	4323159
	1,000 reactions	4323161
	5,000 reactions	4323163
GeneScan 120 LIZ Size Standard	800 analyses	4324287
DS-02 Matrix Standard Kit, for 3500/3730/SeqStudio/SeqStudio Flex	8 analyses	4323014
GeneMapper Software	1	4366925
Peak Scanner Software v1.0 (free download)	1	4381867
SNaPshot primers—custom DNA oligo synthesis service	NA	Custom
SeqStudio 8 Flex Genetic Analyzer	1	A53627

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