Capillary electrophoresis

Gene editing detection and confirmation

Sanger sequencing and fragment analysis solutions

applied biosystems

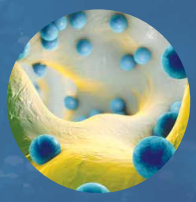
Introduction

Advances in genome editing have changed the way we detect, prevent, treat, and cure diseases. Continued evolution of available genome editing tools helps advance our ability to recreate more biologically relevant disease models, including stem cells, tissues, and animal systems.

Insights from these models can provide a better understanding of life's most challenging biological questions, so that we can uncover answers to complex diseases and develop treatments, such as cell and gene therapies, to help improve the human condition. These critical insights, however, hinge on the precision of genome engineering. Thus, confirmation of genetic alterations during the gene editing process is important to the successful interpretation of experiment results and the safety and efficacies of downstream therapies.

A variety of technologies are available for detecting and verifying gene edits. Here we discuss the use of capillary electrophoresis (CE), Sanger sequencing, and fragment analysis for gene editing confirmation.

Animal disease models



Tissue disease models

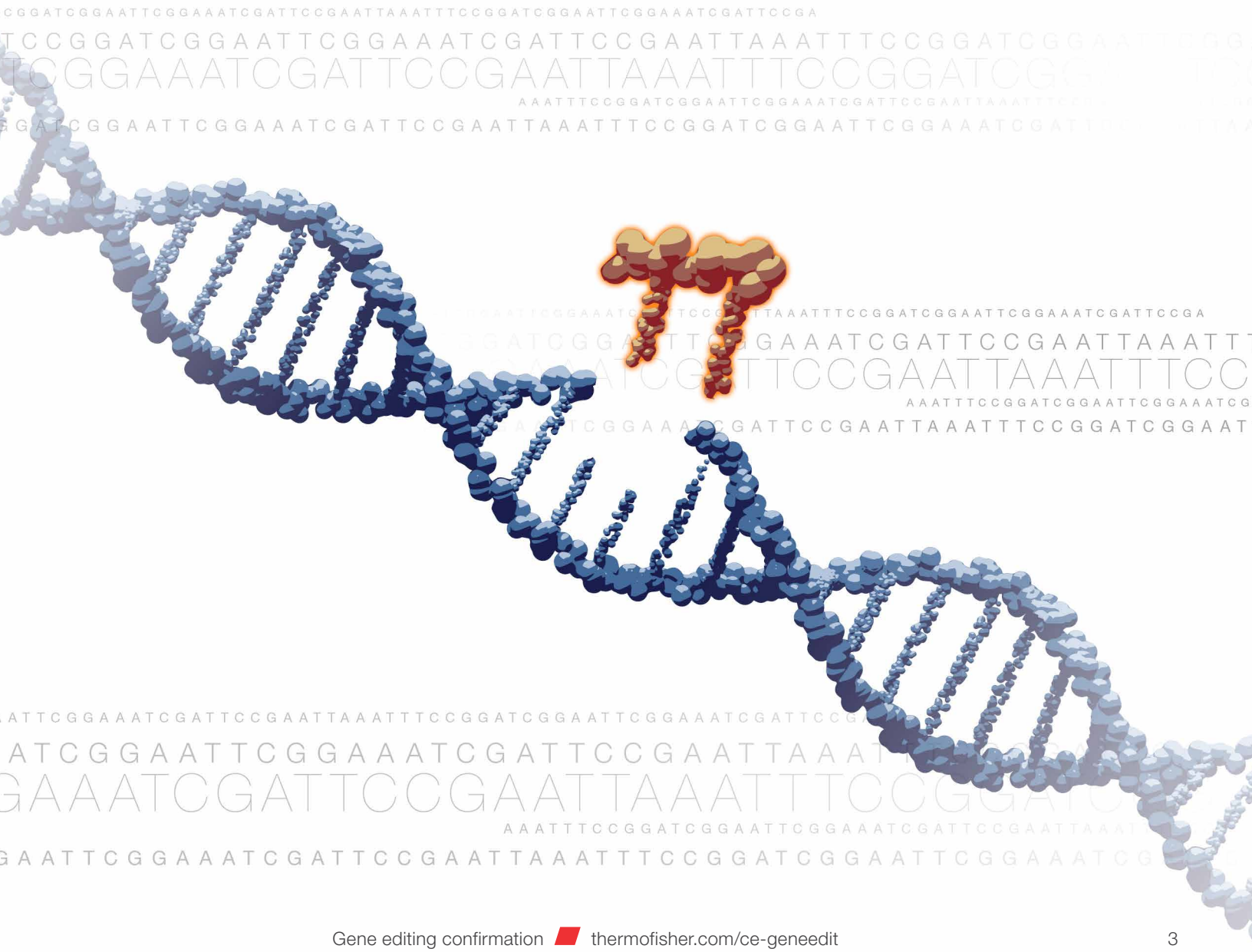
Stem cell engineering

Gene therapy

Capillary electrophoresis

When you can't afford to get it wrong …

… because efficient, accurate gene editing is critical to your scientific insights.



Thermo Fisher Scientific offers a portfolio of products to design, deliver, and detect gene edits, so you can engineer your cells—all from one place.

Efficient and precise gene editing is critical to gaining insights derived from engineered cells and animal models. Genome editing technologies, such as the CRISPR-Cas9 system and transcription activator-like effector nucleases (TALEN™ nucleases), provide precise and efficient methods for manipulating genomic DNA sequences. However, in any genome editing experiment, the repair process is not completely efficient or accurate. Thus, determining gene editing efficiency and confirming genomic alterations are essential steps of the gene editing workflow.

Gene editing workflow solutions

Design and build

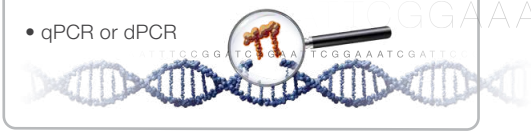
- TrueDesign Genome Editor
- TrueCut Cas9 Proteins
- TrueGuide Synthetic gRNA
- TrueTag Donor DNA Kits
- TALEN mRNA

Culture and deliver

- Gibco cell growth media, supplements, and reagents
- Invitrogen transfection reagents and instruments

Determine efficiency

- GeneArt Genomic Cleavage Detection Kit
- TOPO cloning and Sanger sequencing
- Sanger sequencing and SeqScreener app
- Ion Torrent NGS
- qPCR or dPCR



Establish clones or animal models

- Gibco media

Confirm gene edits

- TOPO cloning and Sanger sequencing
- Sanger sequencing and SeqScreener app
- Ion Torrent targeted sequencing
- qPCR or dPCR

Characterize edits

- TaqMan Assays
- Attune NxT Flow Cytometer
- EVOS imaging systems
- Invitrogen antibodies
- Mass spectrometry

Validation is critical to help ensure efficient, accurate gene editing and to help provide confidence in the engineered cells or animal model prior to use in experiments.

Trust the accuracy and simplicity of capillary electrophoresis for gene editing confirmation

Whether using the CRISPR-Cas9 system or TALEN nucleases, you will want to verify the gene editing efficiency and genomic alterations at different points of your gene engineering workflow:

- gRNA design and optimization
- Primary (pool) screening
- Secondary (clonal) screening
- Targeted confirmation of cell or animal model

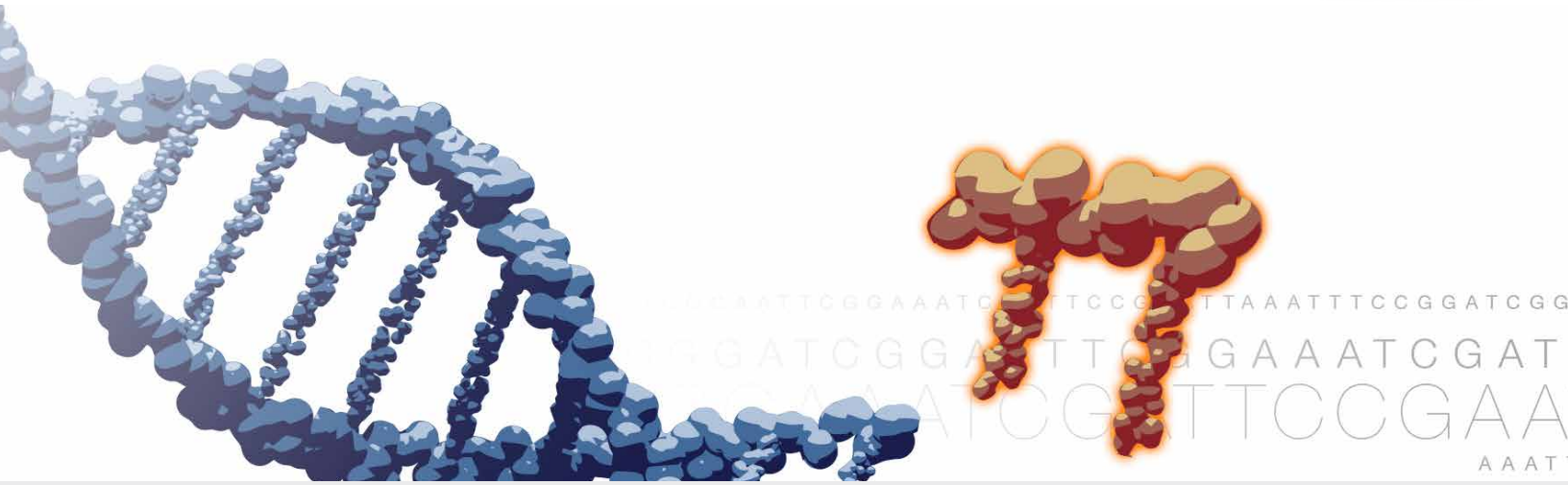
A number of cleavage assays and capillary electrophoresis–based methods are available to confirm gene edits and to help meet your speed, throughput, and budgetary needs.

Assays and methods for gene editing confirmation

	Efficiency and primary (pool) screening	Secondary (clonal) screening	Targeted confirmation of cell or animal model
Cleavage assay—gel	✓ (manual)	–	–
Cleavage assay—CE	✓ (automated)	–	–
TOPO cloning and Sanger sequencing	✓	–	–
Sanger sequencing	✓	✓	✓

GeneArt Genomic Cleavage Detection Kit

The Invitrogen™ GeneArt™ Genomic Cleavage Detection (GCD) Kit provides a quick, simple, and reliable assay that assesses the editing efficiency at a given locus. A sample of the edited cell population is used as a direct PCR template for amplification with primers specific to the targeted region. The PCR product is denatured and reannealed so that mismatches are generated as strands with an indel reanneal to strands with no indel or a different indel. The mismatches are recognized and cleaved by the detection enzyme. The cleavage products can be separated using standard gel electrophoresis or capillary electrophoresis–based fragment analysis on an Applied Biosystems™ genetic analyzer.



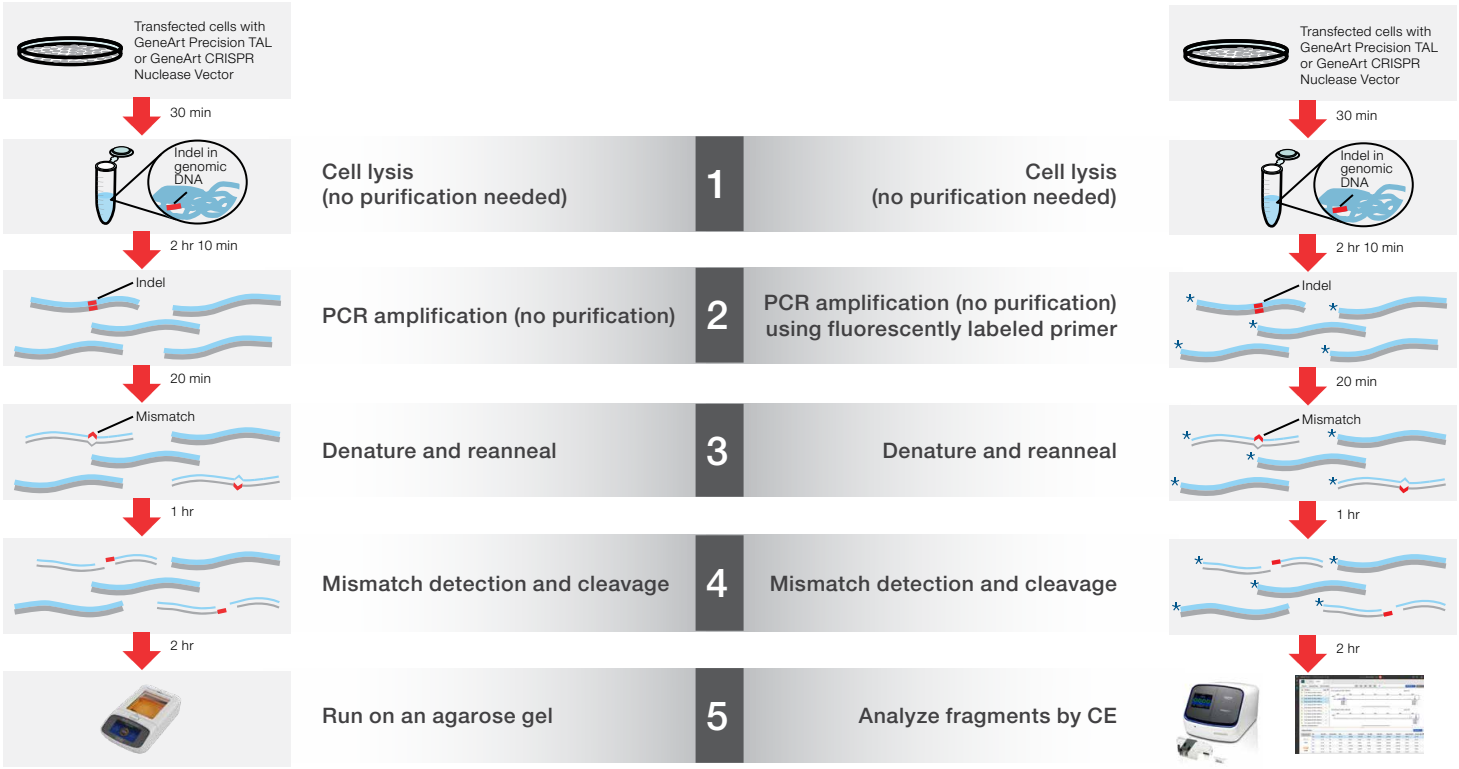
GeneArt GCD analysis on agarose gels:

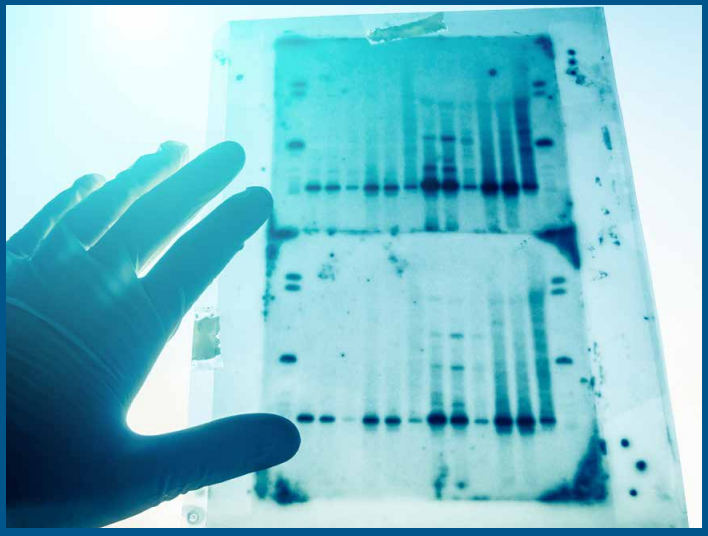
- Simple mechanism for examining editing efficiencies
- Low size resolution only provides size estimates of cleavage products
- Quantification requires densitometry
- Lower throughput due to manual process of loading and analyzing agarose gels

GeneArt GCD analysis by CE:

- Single-base resolution
- Peak heights provide more accurate quantification
- High throughput due to the automation capability of genetic analyzers
- Simplified analysis using Applied Biosystems™ Peak Scanner™ Software

Cells to answers in 6 hours





While the GeneArt Genomic Cleavage Detection assay provides a rapid method for evaluating the efficiency of indel formation following an editing experiment, it does not provide information about the type of indels. Sanger sequencing followed by analysis using the Applied Biosystems™ SeqScreener™ Gene Edit Confirmation Application can be used to accurately calculate editing efficiency in primary pools and determine the range and percentages of indel lengths.

Sanger sequencing

Sanger sequencing by CE is a simple and well-established method for analyzing the results of genome editing workflows. From determining the efficiency of edits in a primary transfected culture to verifying an edit at a locus in a purified secondary culture or animal tissues, Sanger sequencing remains the gold standard for analyzing sequences with single-base resolution.

Genetic analyzers for gene editing confirmation

Applied Biosystems™ genetic analyzers feature proven CE technology for all your Sanger sequencing and fragment analysis applications and throughput needs.

BigDye reagents for sequencing

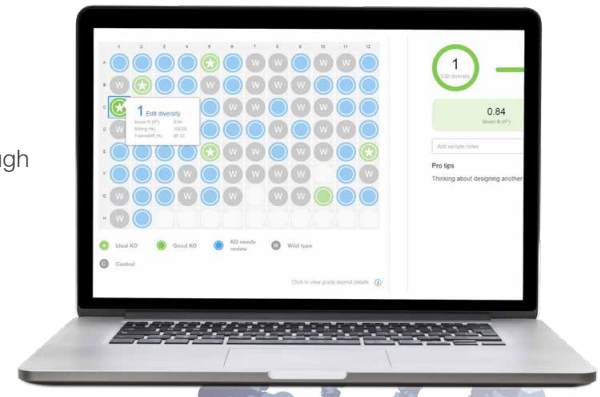
The Applied Biosystems™ BigDye™ family of reagents provides quality results, long read lengths, and optimal base calling for a multitude of Sanger sequencing applications.

Analysis of Sanger sequencing data

- Sanger sequencing followed by analysis with the SeqScreener app offers a useful way to get information from primary pools of edited cells.
- Well-established sequencing workflows can provide a straightforward method for determining the efficiency and types of deletions to expect in clonally expanded cells.
- Since homology-directed repair (HDR) happens less frequently than double-strand break (DSB) repair, any SNPs or other directed-edits may be lost among the random deletions present in the primary pool. The SeqScreener app is designed to identify and analyze the frequency of SNPs.

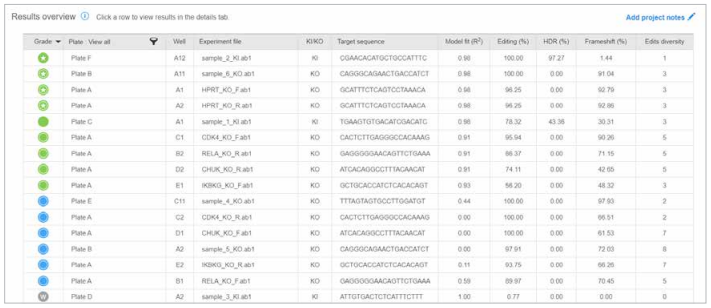
SeqScreener Gene Edit Confirmation Application

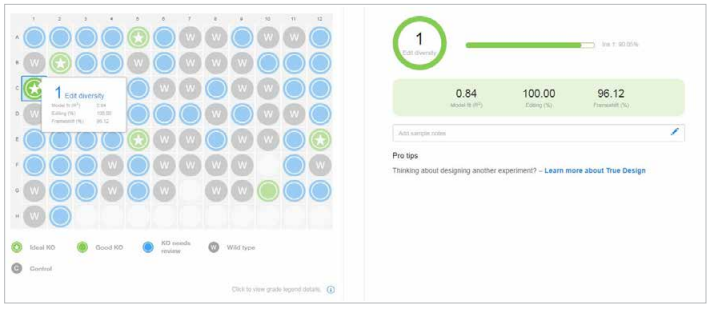
The SeqScreener Gene Edit Confirmation Application is a free app available through the Thermo Fisher™ Connect Platform, for analyzing Sanger sequencing data to determine gene editing efficiency and validating engineered sequences.

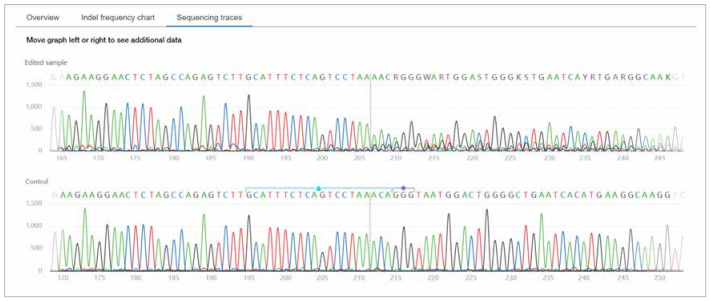


Intuitive, fast, and accurate gene editing analysis

Primary (pool) screening	✓
Secondary (clonal) screening	✓
Batch mode processing	✓
Analysis in seconds	✓
Plate view of results	✓
Gene editing design feedback	✓
Product recommendation	✓
Quick retrieval of historical data	✓
Video tutorials	✓
Indel frequency charts	✓
Donor-to-control alignments	✓
Sequencing traces annotated with cut site and target region	✓
Exportable results with publication-quality images	✓







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Resources

- Access our genome editing resource guide here
- Access our white paper about genetic analysis tools for genome editing workflows here

Genome Editing Learning Center

- Includes educational articles, videos, webinars, and more, covering the latest CRISPR and TALEN nuclease methods and best practices
- Access the learning center here

Ordering information

Product	Quantity	Cat. No.
GeneArt Genomic Cleavage Detection Kit	20 reactions	A24372
SeqStudio Genetic Analyzer	1 system	A35644
SeqStudio 8 Flex Genetic Analyzer	1 system	A53627
SeqStudio 24 Flex Genetic Analyzer	1 system	A53630
BigDye Terminator v3.1 Cycle Sequencing Kit	100 reactions	4337455
BigDye Direct Cycle Sequencing Kit	100 reactions	4458687
GeneScan 500 LIZ Dye Size Standard	800 reactions	4322682
SeqScreener Gene Edit Confirmation App	–	–

Ensure accurate editing at **thermofisher.com/ce-geneedit**

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