#### c g g a t c g g a a t t c g a a t t c g a a t t a a a t t t c c g g a t t c g g a a t c g g a a t c g g a a t c T C C G G A T C G G A A T T C G G A A A T C G A T T C C G A A T T A A A T T C C G G A T C G G A A T T C G G A A A T C G A T T C C G A A T T A A A T T

GATE G G A ATTE G G A A ATE G ATTE C G A ATTA A ATTE C G G ATTE G G A ATE G

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A A A A C G A T T C C G A A T T A A A T T T C C G G A T C G G A A

GATEGAAATCGATTCCGAATTAAATTTCCGGATCGGAATTCGGAAATCGATTCCG GATEGGAAATTCGGAATTCGGAATTCGGAATCGAATTCCGAATTCCG GAAATCGATTCCGAATTCCGAATTCCGAATTCCGAATTCCG GAAATCGATTCCCGAATTCCCGAATTCCCGAATTCCCC

G A ATTC G G A A ATC G A TTC C G A ATTA A ATTTC C G G A TC G G A ATTC G G A A AT

**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

A A T T C G G A A A T C C G A A T T A A A T T T C C G G A T C G G A A T C G G A A T C G A T T C C G

#### Capillary electrophoresis

When you can't afford to get it wrong ...

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



# GAAATCGATTCCGAATT

## G A A T T C G G A A A T C G A T T C C G A A T T A A A T T

# a a t t c g g a a t t c g g a a a t c g a t t c c g a a t t a a a t t t c c g g a t c g g a t t c g g a a t t c g g a a a t c g a a t t c c g a a t t c c g g a t c g g a t t c g g a a t t c g g a a t t c g g a a t t c g g a a t t c g g a a t t c g g a t c g g a t c g g a t t c g g a a t t c g g a a t t c g g a a t t c g g a a t t c g g a a t t c g g a a t t c g g a a t t c g g a a t t c g g a a t t c g g a a t t c g g a a t t c g g a a t t c g g a t c g g a t c g g a t c g g a t t c g g a a t t c g g a a t t c g g a a t t c g g a a t t c g g a a t t c g g a a t t c g g a a t t c g g a a t t c g g a a t t c g g a a t t c g g a a t t c g g a t c g g a t c g g a t t c g g a a t t c g g a a t t c g g a a t t c g g a a t t c g g a a t t c g g a t c g g a t t c g g a t t c g g a t t c g g a a t t c g g a a t t c g g a a t t c g g a t t c g g a t t c g g a t t c g g a t t c g g a t t c g g a t t c g g a t t c g g a a t t c g g a a t t c g g a t t c g g a t t c g g a t t c g g a t t c g g a t t c g g a t t c g g a a t t c g g a t

### 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<sup>™</sup> 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



# AAATTTCCGGAATCCGAATTAAATTCG GGATCGGAATTCGGAATTAAATTCGA TCCGGATCGGAATTCGGAATTCGA

# A A A T T C C G G A A T C G G A A T C C G A A T C C G G A A T C G G A A T C C G A A T C C G G A A T C C G G A A T C C G G A A T T C C G G A A T T C C G G A A T T C C G G A A T T C C G G A A T T C C G G A A T T C C G G A A T T C C G G A A T T C C G G A A T T C C G G A A T T C C G G A A T C G G A A T T C C G G A A T T C C G G A A T C G G A A T T C C G G A A T T C C G G A A T C G G G A A T C G G A A T C G G G A A T C G G A A T C G G G A A T C G G A A T C G G G G A A T C G G G A A T

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.

#### **GATCGGATTCGGAATCGATTCCGAATTAAATTTCCGGATCGCAATTODDAA** GAAATCGATTCCGAATTAAATTTCCGGATOGGAAA

# 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	$\checkmark$	~	✓

## **GeneArt Genomic Cleavage Detection Kit**

The Invitrogen<sup>™</sup> GeneArt<sup>™</sup> 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<sup>™</sup> 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<sup>™</sup> Peak Scanner<sup>™</sup> Software

# 

#### Cells to answers in 6 hours

Transfected cells with GeneArt Precision TAL or GeneArt CRISPR Nuclease Vector				Transfected cells with GeneArt Precision TAL or GeneArt CRISPR Nuclease Vector
30 min				30 min
Indel in genomic DNA	Cell lysis (no purification needed)	1	Cell lysis (no purification needed)	Indel in genomic DNA
2 hr 10 min				2 hr 10 min
Indel	PCR amplification (no purification)	2 PC us	CR amplification (no purification) sing fluorescently labeled primer	* * *
20 min				20 min
Mismatch	Denature and reanneal	3	Denature and reanneal	* * * *
1 hr				1 hr
	Mismatch detection and cleavage	4 м	lismatch detection and cleavage	* * *
2 hr				2 hr
<b>S</b>	Run on an agarose gel	5	Analyze fragments by CE	



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<sup>™</sup> SeqScreener<sup>™</sup> 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<sup>™</sup> 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<sup>™</sup> BigDye<sup>™</sup> 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<sup>™</sup> Connect Platform, for analyzing Sanger sequencing data to determine gene editing efficiency and validating engineered sequences.



#### GATCGGAATTCGGAAATCGATTCCGAATTAAAT GAAATCGGAATCGGAATCGGAATCGGAAATCGG GAATTCGGGAAATCGGAATCGGGAATCGGGAATCGGGAAATCGG GAATTCGGGAAATCGGATCCGGAATCGGGAATCGGGAATCGGGAATCGGGAAATCGGAATCGGAA

#### Intuitive, fast, and accurate gene editing analysis

Primary (pool) screening	<b>~</b>
Secondary (clonal) screening	<b>~</b>
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	<b>~</b>
Sequencing traces annotated with cut site and target region	<b>~</b>
Exportable results with publication-quality images	<b>~</b>

Grade 🕶	Plate View all	Well	Experiment file	KIKO	Target sequence	Model fit (R <sup>2</sup> )	Editing (%)	HDR (%)	Frameshift (%)	Edits diversity
0	Plate F	A12	sample_2_KLab1	10	CGAACACATGCTGCCATTTC	0.98	100.00	97.27	1.44	1
0	Plate B	A11	sample_6_KO ab1	ко	CAGGGCAGAACTGACCATCT	0.98	100.00	0.00	91.04	3
0	Plate A	A1	HPRT_KO_Fab1	ко	GCATTTCTCAGTCCTAAACA	0.98	98.25	0.00	92.79	3
0	Plate A	A2	HPRT_KO_R.ab1	ко	GCATTTCTCAGTCCTAAACA	0.98	95.25	0.00	92.86	3
•	Plate C	A1	sample_1_NLab1	10	TGAAGTGTGACATCGACATC	0.98	78.32	43.35	30.31	3
	Plate A	C1.	CDK4_KO_Fab1	ко	CACTCTTGAGGGCCACAAAG	0.91	95.94	0.00	90.26	5
	Plate A	82	RELA_KO_R.ab1	ко	GAGGGGGGAACAGTTCTGAAA	0.91	66.37	0.00	71.15	5
	Plate A	02	CHUK_KO_R.ab1	ко	ATCACAGGCCTTTAGAAGAT	0.91	74.11	0.00	42.65	5
	Plate A	E1	IKBKG_KO_Fab1	ко	GCTGCACCATCTCACACAGT	0.93	58.20	0.00	48.32	3
۲	Plate E	C11	sample_4_KO ab1	ко	TTTAGTAGTGCCTTGGATGT	0.44	100.00	0.00	97.93	2
۲	Plate A	C2	CDK4_KO_R ab1	ко	CACTCTTGAGGGGCCACAAAG	0.00	100.00	0.00	05.51	2
	Plate A	D1	CHUK_KO_Fab1	ко	ATCACAGGCCTTTACAACAT	0.00	100.00	0.00	61.53	7
	Plate B	A2	sample_5_KO ab1	ко	CAGGGCAGAACTGACCATCT	0.00	97.91	0.00	72.03	8
	Plate A	E2	IKEKG_KO_R ab1	ко	OCTOCACCATCTCACACAGT	0.11	93.75	0.00	66.26	7
	Plate A	81	RELA_KO_Fab1	ко	GAGGGGGGAACAGTTCTGAAA	0.59	89.97	0.00	70.45	5
0	Plate D	A2	sample_3_KLab1	K	ATTGTGACTCTCATTICTTT	1.00	0.77	0.00	0.00	0



and
Move graph left or right to see additional data
Edited sample
1500 GAAGAAGGAACTCTAGCCAGAGTCTTGCATTTCTCAGTCCTAAAACRGGGWARTGGASTGGGKSTGAATCAYRTGARGGCAAKG 1
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## Resources

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



# 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

# GGAATTCGGAATCGATTCCGA AATCGATTCCGAATTAA



#### ATCGATTCCGAATTAA AAATTTCCGGATC CGGAAATCGATTCCGAATTAAATTTCC

#### 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

# applied biosystems

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