USER GUIDE

invitrogen

GeneArt™ CRISPR Nuclease mRNA

Ready-to-transfect wild-type Cas9 mRNA

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About this guide

Revision history

Revision	Date	Description
A.0	September 2015	New document

Product information

Product description

GeneArt™ CRISPR Nuclease mRNA

The GeneArt™ CRISPR Nuclease mRNA is the ready-to-transfect wild-type Cas9 mRNA for performing CRISPR/Cas9-mediated genome editing.

The CRISPR Nuclease mRNA can be used in experiments in two different formats:

- **Complete RNA format:** mRNA is co-transfected with *in vitro* transcribed gRNA
- **Ready-to-transfect format:** mRNA is co-transfected directly with custom GeneArt™ CRISPR U6 Strings™ DNA or other synthetic gRNA expression cassettes

For more information on selecting the experimental format appropriate for your needs, see "Select a suitable CRISPR system", page 6.

Kit contents and storage

Reagent	Concentration	Amount	Storage*
GeneArt™ Platinum™ Cas9 Nuclease	1.0 µg/µL in 0.1 mM EDTA, pH 8	15 µL	–80°C

* Working stock can be stored at –20°C for short term (no longer than 1 month)

Materials required but included

The following materials necessary to perform CRISPR/Cas9-mediated genome editing are not included with GeneArt™ CRISPR Nuclease mRNA. See page 27 for ordering information.

- GeneArt™ Precision gRNA Synthesis Kit (complete RNA format) (Cat. no. A29377)

  or
- *in vitro* transcribed gRNA (complete RNA format):
  - GeneArt™ CRISPR T7 Strings™ DNA (contact **geneartsupport@thermofisher.com**)

    or
  - Ready-to-use *in vitro* transcribed gRNA (contact **customservices@thermofisher.com**)

  or
- GeneArt™ CRISPR U6 Strings™ DNA (ready-to-transfect format) (contact **geneartsupport@thermofisher.com**)
- TranscriptAid™ T7 High yield Transcription Kit (Cat. no. K0441) **or** MEGAshortscript™ T7 Transcription Kit (Cat. no. AM1354) (for complete RNA format using GeneArt™ CRISPR T7 Strings™ DNA)
- GeneJET™ RNA Cleanup and Concentration Micro Kit (Cat. no. K0841) **or** MEGAClear™ Transcription Clean-Up Kit (Cat. no. AM1908) (for complete RNA format using GeneArt™ CRISPR T7 Strings™ DNA)
- Lipofectamine™ MessengerMAX™ Transfection Reagent (for most cell lines) (Cat. no. LMRNA003) or other recommended transfection reagent

  or

  Neon™ Transfection System (Cat. no. MPK5000) (for suspension cell lines such as Jurkat)
- GeneArt™ Genomic Cleavage Detection Kit (Cat. no. A24372)

Description of the system

Overview of CRISPR/Cas9-mediated DNA cleavage

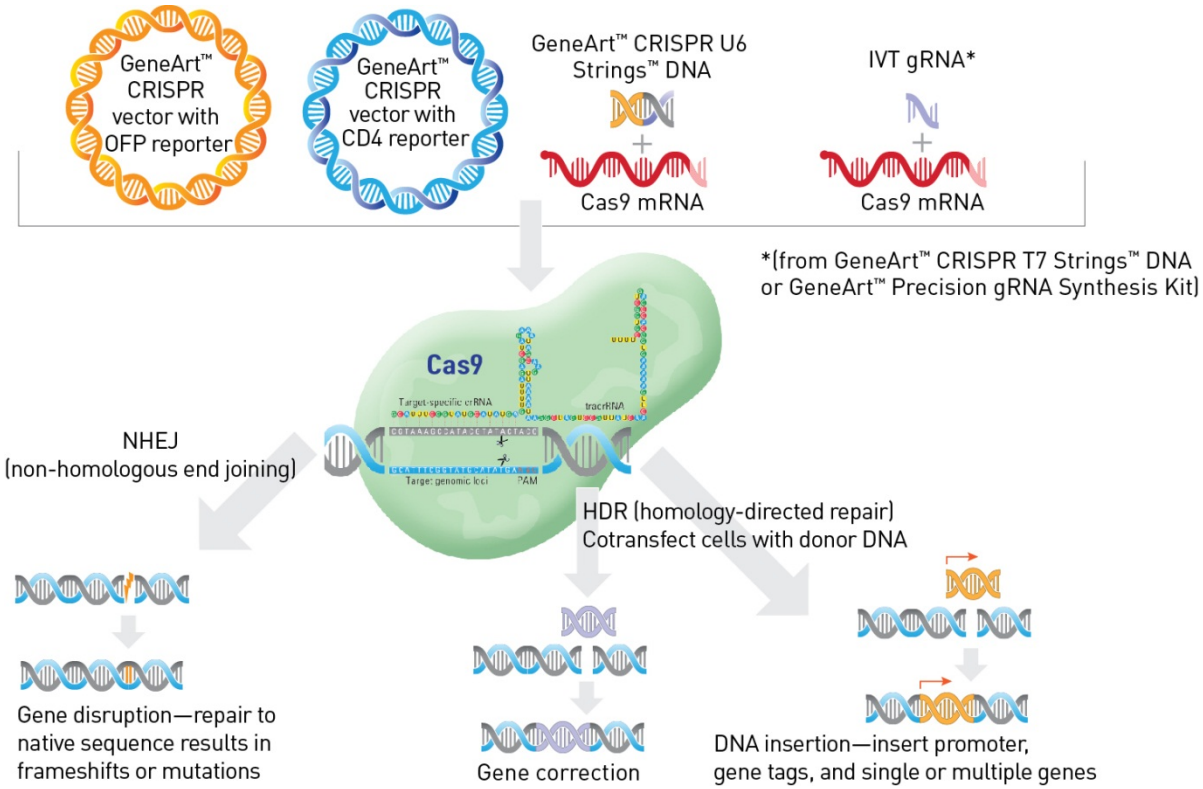
The CRISPR (clustered regularly interspaced short palindromic repeats) system is a prokaryotic adaptive immune system that uses the RNA-guided DNA nuclease Cas9 to silence viral nucleic acids (Jinek et al., 2012), and it has been shown to function as a gene editing tool in various organisms including mammalian cells. (Mali et al., 2013; Cong et al., 2013).

The CRISPR system consists of a short non-coding guide RNA (gRNA) made up of a target complementary CRISPR RNA (crRNA) and an auxiliary trans-activating crRNA (tracrRNA). The gRNA guides the Cas9 endonuclease to a specific genomic locus via base pairing between the crRNA sequence and the target sequence, and cleaves the DNA to create a double-strand break (Figure 1).

In bacteria CRISPR loci are composed of a series of repeats separated by segments of exogenous DNA (of ~30 bp in length), called spacers. The repeat-spacer array is transcribed as a long precursor and processed within repeat sequences to generate small crRNAs that specify the target sequences (also known as protospacers) cleaved by Cas9 protein, the nuclease component of CRISPR system. CRISPR spacers are then used to recognize and silence exogenous genetic elements at the DNA level. Essential for cleavage is a three-nucleotide sequence motif (NGG) immediately downstream on the 3′ end of the target region, known as the protospacer-adjacent motif (PAM). The PAM is present in the target DNA, but not the crRNA that targets it.

Following DNA cleavage, the break is repaired by cellular repair machinery through non-homologous end joining (NHEJ) or homology-directed repair (HDR) mechanisms.

Figure 1 Schematic representation of CRISPR/Cas9-mediated target DNA cleavage.



GeneArt™ CRISPR Nuclease mRNA

The GeneArt™ CRISPR Nuclease mRNA is the ready-to-transfect wild-type Cas9 mRNA for performing CRISPR/Cas9-mediated genome editing. The mRNA format allows the experiment to proceed without the need for time consuming cloning steps required when using CRISPR vector systems.

The GeneArt™ CRISPR Nuclease mRNA can be used in two different experimental workflows:

- **Complete RNA format:** mRNA is co-transfected with *in vitro* transcribed gRNA, which can be generated from DNA oligonucleotide templates using the GeneArt™ Precision gRNA Synthesis Kit or from GeneArt™ CRISPR T7 Strings™ DNA or other custom templates (see page 8).
- **Ready-to-transfect format:** mRNA is co-transfected directly with custom GeneArt™ CRISPR U6 Strings™ DNA or other synthetic gRNA expression cassettes (see page 10).

Following co-transfection, the Cas9 protein generated from the GeneArt™ CRISPR Nuclease mRNA is directed by the crRNA sequence of the gRNA to the encoded genomic locus to perform the desired genome editing.

The GeneArt™ CRISPR Nuclease mRNA allows easy optimization of Cas9 to gRNA ratio for optimal genome targeting efficiency. Cas9 mRNA and *in vitro* transcribed gRNA are especially useful for microinjection applications or for circumventing promoter restrictions.

Note: Use of the GeneArt™ CRISPR Nuclease mRNA for genome editing assumes that users are familiar with the principles of the CRISPR system, handling RNA, transfection in mammalian systems, and downstream processing of cells for detection of genomic cleavage.

Methods

Before you begin

Guidelines for handling RNA

- Always use RNase-free reagents and plasticware.
- Clean workspace/benches/laminar hood surfaces using RNAse decontaminating reagents like RNaseZap™ Solution (see page 27).
- Decontaminate pipets with RNaseZap™ Solution or similar reagent.
- Change gloves after touching any potentially contaminated object or surface.

Select a suitable CRISPR system

Based upon the requirements of your experiment, select a CRISPR system from the following table. Depending on your choice, you will have different options for generating the gRNA required for CRISPR/Cas9-mediated genome editing.

	GeneArt™ CRISPR Nuclease mRNA + IVT gRNA[1]	GeneArt™ CRISPR Nuclease mRNA + U6 Strings™ DNA[2]
Ready to transfect	—	✓
Shows high efficiency with broad cell type application	✓	—
Avoids random integration associated with DNA	✓	—
Suitable for microinjections[3]	✓	—

[1] *In vitro* transcribed (IVT) gRNA can be prepared using the GeneArt™ Precision gRNA Synthesis Kit or from a synthetic DNA template such as GeneArt™ CRISPR T7 Strings™ DNA or a user-defined template. Ready to use IVT gRNA can also be ordered through custom services by contacting **customservices@thermofisher.com**.

[2] GeneArt™ CRISPR U6 Strings™ DNA is directly introduced into the cells along with Cas9 mRNA, where the U6 promoter drives expression of CRISPR gRNA for complexing with the Cas9 protein generated from the Cas9 mRNA.

[3] Potential applications of Cas9 mRNA include generation of transgenic model systems, but microinjection and other *in vivo* delivery methods have not been tested with the GeneArt™ CRISPR Nuclease mRNA. However, a significant number of articles have described Cas9 mRNA use for *in vivo* applications in a wide variety of organisms including mouse, rat, zebrafish, and *Drosophila* (Haoyi et. al., 2013; Ma et. al., 2014; Ota et. al., 2014; Andrew et. al., 2013).

Select a CRISPR target sequence

The first step in performing CRISPR/Cas9-mediated genome editing is the analysis of your sequence of interest to identify potential CRISPR target sequences. For this, we recommend using the Invitrogen™ GeneArt™ CRISPR Search and Design Tool (see below). For general guidelines on choosing a target sequence, see "Appendix B: CRISPR target sequences", page 18.

Note: The cleavage efficiency of a CRISPR sequence at its target depends upon many factors that are not well understood. Therefore, we recommend that you choose **at least three CRISPR sequences** against a gene of interest and identify the CRISPR sequence with the best cleavage efficiency. For knocking out a gene, we recommend targets within the first 3 exons.

GeneArt™ CRISPR Search and Design Tool

For best results, use the Invitrogen™ GeneArt™ CRISPR Search and Design Tool, available at **www.thermofisher.com/crisprdesign**, to analyze your sequence of interest for potential CRISPR target sequences or search our database of >600,000 pre-designed gRNA sequences using a gene name, symbol, or accession number, and to design and order primers for gRNA template assembly.

Based on your input, the CRISPR design tool identifies the top six CRISPR target sequences with PAM sites, provides recommendations based on potential off-target effects for each CRISPR sequence, displays exon maps with gRNA binding sites, and allows one-click online ordering for custom primers used in gRNA template assembly (for countries with enabled online ordering).

For instructions on using the GeneArt™ CRISPR Search and Design Tool, see page 19.

Proceed with CRISPR/Cas9-mediated genome editing

- If you are using the GeneArt™ CRISPR Nuclease mRNA with *in vitro* transcribed gRNA, follow the "Complete RNA workflow" on page 8.
- If you are using the GeneArt™ CRISPR Nuclease mRNA with GeneArt™ CRISPR U6 Strings™ DNA, follow the "Ready-to-transfect workflow" on page 10.

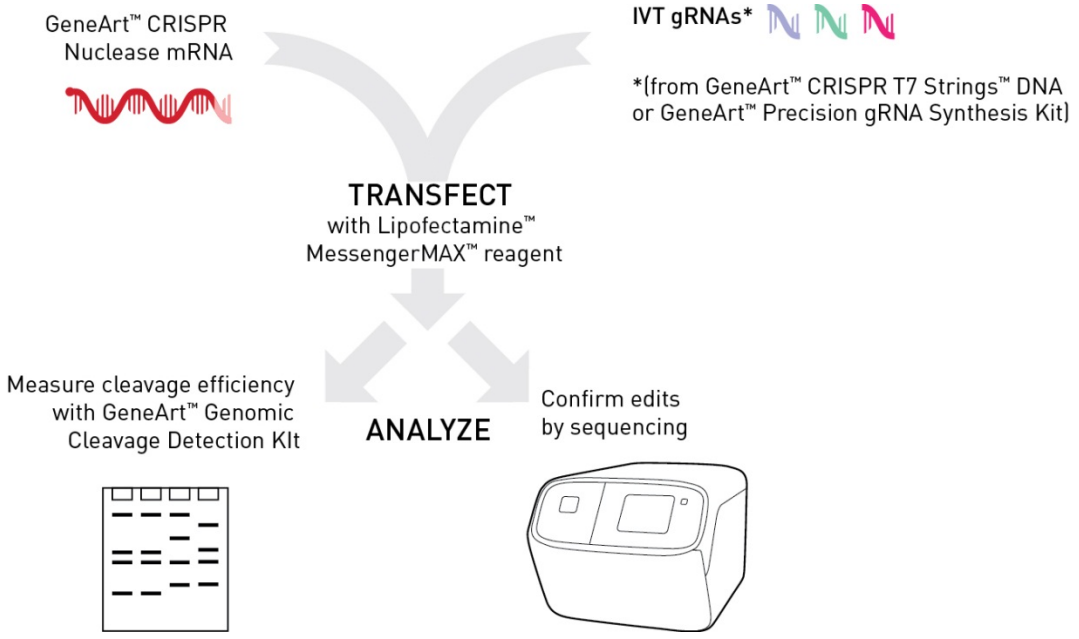
Complete RNA workflow

Experimental outline

The complete RNA protocol involves the co-transfection of GeneArt™ CRISPR Nuclease mRNA and *in vitro* transcribed (IVT) gRNA into your cell line of interest. The procedure requires the production (or ordering) of IVT gRNA using the GeneArt™ Precision gRNA Synthesis Kit or GeneArt™ CRISPR T7 Strings™ DNA (or other gRNA encoding sequence) as a template.

Step	Action	Page
1	Select a target CRISPR sequence	7
2	Generate gRNA by *in vitro* transcription or order ready-to-transfect IVT gRNA	9
3	Co-transfect IVT gRNA with GeneArt™ CRISPR Nuclease mRNA	12
4	Determine the genomic cleavage efficiency	15

Figure 3. Complete RNA workflow for CRISPR/Cas9-mediated genome editing using the GeneArt™ CRISPR Nuclease mRNA and *in vitro* transcribed gRNA.



Materials required

Unless otherwise indicated, all materials are available from **www.thermofisher.com**. See page 27 for ordering information.

- GeneArt™ CRISPR Nuclease mRNA
- IVT gRNA; see "Options for producing gRNA (complete RNA workflow)", page 9
- Lipofectamine™ MessengerMAX™ Transfection Reagent (for most cell lines)
  or
  Neon™ Transfection System or equivalent (for suspension cells such as Jurkat)

  Note: For more information on choosing the appropriate transfection method, see "Transfection methods", page 12.
- Cell line of interest

Options for producing gRNA (complete RNA workflow)

After you have selected your CRISPR target sequence, choose from the following three options to produce your gRNA for the complete RNA workflow:

1. Use the GeneArt™ Precision gRNA Synthesis Kit to synthesize transfection-ready gRNA. You can order the oligonucleotide primers used for the gRNA DNA template assembly via the GeneArt™ CRISPR Search and Design Tool.
2. Purchase GeneArt™ CRISPR Strings™ T7 DNA, a synthetic DNA template comprising the gRNA with a T7 promoter for *in vitro* transcription (IVT), using the GeneArt™ CRISPR Search and Design Tool.

   You can also complete the GeneArt™ CRISPR Strings™ DNA order form, which can be downloaded from our website and submitted to **geneartsupport@thermofisher.com**.
3. Order ready-to-use IVT gRNA. Save time and effort and have our GeneArt™ custom services team design, synthesize, and purify IVT gRNA sequences for you. To obtain a services quotation, or to order, contact our Custom Services department by calling 1-800-955-6288 ext 45682 or sending an email to **custom.services@thermofisher.com**.

Produce IVT gRNA

If you have ordered ready-to-use *in vitro* transcribed gRNA, proceed directly to transfection (page 12).

- For instructions on generating IVT gRNA using the GeneArt™ Precision gRNA Synthesis Kit, refer to the GeneArt™ Precision gRNA Synthesis Kit user guide available at **www.thermofisher.com**.
- For instructions on generating IVT gRNA from GeneArt™ CRISPR Strings™ T7 DNA, see "Appendix D: Produce IVT gRNA from GeneArt™ CRISPR Strings™ T7 DNA", page 23.
- After you have generated your gRNA by IVT and removed the DNA template by DNAse I digestion, purify the gRNA using the gRNA Clean Up Kit (included in the GeneArt™ Precision gRNA Synthesis Kit), the GeneJET™ RNA Cleanup and Concentration Micro Kit (Cat. no. K0841), or the MEGAClear™ Transcription Clean-Up Kit (Cat. no. AM1908), following the instructions provided in the respective user guides.

Note: A white precipitate will form after the IVT reaction. The precipitate contains pyrophosphate and smaller amounts of RNA; it does not affect downstream steps (purification). Resuspending the precipitate and including it in your purification will increase the amount of RNA recovered.

Determine the purified gRNA concentration and quality

- Determine the concentration of the purified gRNA using the Qubit™ RNA BR Assay Kit (Cat. no. Q10210). NanoDrop™ spectrophotometer or an equivalent system can also be used, but we have seen up to a 2X variation in concentration estimation.
- Check the quality of the gRNA with electrophoresis on a 2% E-Gel™ EX Agarose Gel against a known control or an RNA ladder that has a 100-base band (e.g., Century™ RNA Markers, Cat. no. AM7140). The expected gRNA transcript size is 100 bases. A discreet band at 100 bases indicates intact RNA. Usually >90% of IVT gRNA will be full length.

  Note: You can also check the gRNA quality by running it on 10% Novex™ TBE-Urea Gel or an equivalent against a known control or an RNA ladder.

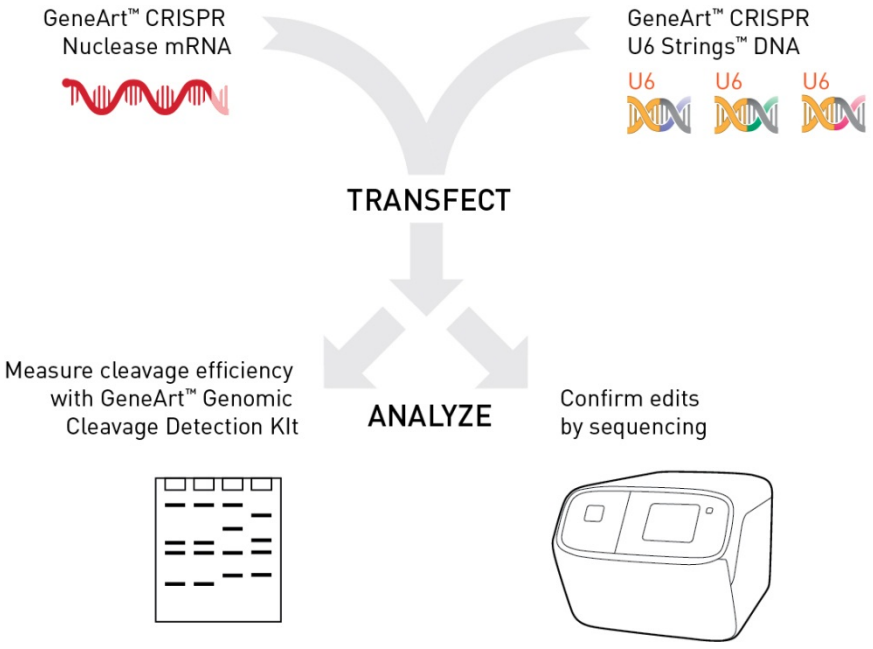
Ready-to-transfect workflow

Experimental outline

The ready-to-transfect workflow involves the direct co-transfection of GeneArt™ CRISPR Nuclease mRNA and GeneArt™ CRISPR U6 Strings™ DNA (or other synthetic gRNA expression cassette) into your cell line of interest.

Step	Action	Page
1	Select a target CRISPR sequence	7
2	Place an order for the appropriate GeneArt™ CRISPR U6 Strings™ DNA	11
3	Co-transfect the GeneArt™ CRISPR Nuclease mRNA with the GeneArt™ CRISPR U6 Strings™ DNA	12
4	Determine the genomic cleavage efficiency	15

Figure 4. Ready-to-transfect workflow for CRISPR/Cas9-mediated genome editing using the GeneArt™ CRISPR Nuclease mRNA and GeneArt™ CRISPR U6 Strings™ DNA.



Materials required

Unless otherwise indicated, all materials are available from **www.thermofisher.com**. See page 27 for ordering information.

- GeneArt™ CRISPR Nuclease mRNA
- GeneArt™ CRISPR U6 Strings™ DNA or other synthetic expression cassette; see "Options for producing gRNA (ready-to-transfect workflow)", page 11.
- Lipofectamine™ MessengerMAX™ Transfection Reagent (for most cell lines)

  or

  Neon™ Transfection System or equivalent (for suspension cells such as Jurkat)

  Note: For more information on choosing the appropriate transfection method, see "Transfection methods", page 12.
- Cell line of interest

Options for producing gRNA (ready-to-transfect workflow)

Once you have selected your CRISPR target sequence, choose from the following two options to produce to your gRNA:

1. Purchase GeneArt™ CRISPR Strings™ U6 DNA, a synthetic DNA template comprising the gRNA with a U6 promoter that can be directly co-transfected with Cas9 mRNA, via the GeneArt™ CRISPR Search and Design Tool.

   You can also complete the GeneArt™ CRISPR Strings™ DNA order form, which can be downloaded from our website and submitted to **geneartsupport@thermofisher.com**.

2. Create your own synthetic gRNA expression cassette. If you have your own system for expressing gRNA, it is not necessary to order GeneArt™ CRISPR Strings™ DNA.

   For more information on creating your own synthetic gRNA expression cassette or template, see "Appendix F: Cloning GeneArt™ CRISPR Strings™ DNA", page 26.

Prepare GeneArt™ CRISPR Strings™ U6 DNA for transfection

1. Pellet the lyophilized GeneArt™ CRISPR Strings™ U6 DNA by centrifugation at room temperature for 30 seconds.
2. Resuspend GeneArt™ CRISPR Strings™ U6 DNA in 20 µL of nuclease-free water.
3. Centrifuge the resuspended DNA at high speed for 30 seconds to collect contents at the bottom of the tube.
4. Determine the DNA concentration using a NanoDrop™ spectrophotometer or an equivalent system.
5. Record the concentration and dilute to the required working concentration. For assays using a 24-well plate format, we recommend diluting the U6 Strings™ DNA to ≥20–50 ng/µL.
6. Proceed to transfection (page 12).

Perform CRISPR transfections

Transfection methods

- For lipid-mediated transfections, we recommend using the Lipofectamine™ MessengerMAX™ Transfection Reagent (Cat. no. LMRNA003).

  Lipofectamine™ MessengerMax™ Reagent can be used for transfecting GeneArt™ CRISPR Nuclease mRNA into a wide range of eukaryotic cells including difficult-to-transfect cell lines, and results in high genome editing efficiency when used in conjunction with the complete RNA format.

  However, we recommend electroporation using the Neon™ Transfection System (Cat. no. MPK5000) or equivalent for suspension cell lines like Jurkat.

- The delivery reagent is critical for transfection and gene editing efficiency, and the results will vary based on the cell type being used. For transfection reagent recommendations, see **www.thermofisher.com/transfection**.

  Additional information on cell lines and recommended transfection reagents are listed in "Appendix E: Cell-specific transfection conditions", page 23.

Guidelines for CRISPR transfections

- Various factors including cell seeding density, cell health, media, nucleic acid quality, and delivery method affect the transfection efficiency, which in turn determine the efficiency of genome editing. For an overview of the factors influencing transfection efficiency, refer to the "Transfection Basics" chapter of the Gibco™ Cell Culture Basic Handbook, which is available at **www.thermofisher.com/cellculturebasics**.
- To maximize transfection efficiency, take special care while handling and processing the cells, and strictly follow the recommended cell culture and transfection protocols.
- Perform the transfections using standard 24-well culture plates. This plate size is convenient when screening different CRISPR sequences to identify the most suitable and effective candidate for genome editing.
- Seed cells 24 hours prior to transfection so that they are 70–90% confluent on the day of transfection.

  Note: Seeding density varies with cell type (see "Appendix E: Cell-specific transfection conditions", page 23).

- Perform transfection using the appropriate transfection reagent. Use the amounts of nucleic acid in the following table as a starting point (optimization may be required).

  For multiplex experiments, up to three target sequences can be combined at the same time.

Component	Singleplex	Multiplex
GeneArt™ CRISPR Nuclease mRNA	0.5 µg	1.0 µg
GeneArt™ CRISPR U6 Strings™ DNA/IVT gRNA	50 ng	50 ng/target

- If using different well formats, scale the recommended amounts based on the transfection plate format. In cases where the amount of GeneArt™ CRISPR Strings™ DNA is limiting, the GeneArt™ CRISPR Strings™ DNA can be cloned into a plasmid vector. For more information, see "Appendix F: Cloning GeneArt™ CRISPR Strings™ DNA", page 26.

Controls

- Include controls in the experiment to evaluate your results. A negative control can be mock/untransfected cells.
- For a positive control, use a gRNA whose efficiency at the genomic level is already known. In the absence of such a control, you can use the following CRISPR sequence targeting human HPRT gene, which has been validated for cleavage efficiency using the GeneArt™ Genomic Cleavage Detection Kit in 293 FT cell lines.

  5′–CAT TTC TCA GTC CTA AAC A–3′

  The corresponding target in the genomic DNA for this CRISPR sequence is:

  CATTTCTCAGTCCTAAACA**GGG**

  The PAM (underlined) is a feature of the genomic locus and not included in the CRISPR RNA coding sequence.
- The primer sequences to perform PCR when using the GeneArt™ Genomic Cleavage Detection Kit are:

  Forward: 5′–ACATCAGCAGCTGTTCTG–3′

  Reverse: 5′–GGCTGAAAGGAGAGAACT–3′
- You can purchase ready-to-transfect controls and validated IVT gRNA targeting the HPRT gene through our Custom Services department by calling 1-800-955-6288 ext 45682 or by sending an e-mail to **custom.services@thermofisher.com**.

Transfect cells using the Lipofectamine™ MessengerMAX™ Reagent

The following example protocol describes the transfection of 293 FT cells using the Lipofectamine™ MessengerMAX™ Reagent. Optimization of conditions is required when using other cell lines or transfection reagents.

Note: For an example transfection protocol using the Neon™ Transfection System, see page 14.

1. Seed 0.2×10^6 cells/well in a 24-well tissue culture plate one day prior to transfection.
2. Label and prepare tubes for appropriate experimental and controls samples.
3. Dilute the GeneArt™ CRISPR Nuclease mRNA by combining the following components and mix gently.

Opti-MEM™ I Reduced Serum Medium	25 µL
GeneArt™ CRISPR Nuclease mRNA	500 ng
IVT gRNA **or** GeneArt™ CRISPR U6 Strings™ DNA	50 ng

4. Dilute 1.5 µL of the Lipofectamine™ MessengerMAX™ Reagent in 25 µL of Opti-MEM™ I Reduce Serum Medium. Mix gently.
5. Add the diluted nucleic acid solution to the diluted transfection reagent and mix gently.
6. Incubate the nucleic acid/transfection reagent solution at room temperature for 5 minutes to allow the formation of RNA-lipid complexes.
7. Add the RNA-lipid complexes to the cells to be transfected. Swirl the plate gently to allow the mixing of the transfection mixture with the culture medium.
8. Incubate the cells in a humidified 37°C, 5% CO_2 incubator for 48–72 hours.
9. Harvest the cells and measure the CRISPR/Cas9-mediated cleavage efficiency using the GeneArt™ Genomic Cleavage Detection Kit or by sequencing (page 15).

Transfect cells using the Neon™ Transfection System

The following example protocol describes the electroporation of Jurkat cells in suspension using the Neon™ Transfection System. Optimization of conditions is required when using other cell lines. For detailed instructions on using the Neon™ Transfection System, refer to the Neon™ Transfection System user guide available at **www.thermofisher.com**.

1. Cultivate the required number of cells. (see below).
2. One to two days prior to electroporation, transfer the cells into flask with fresh growth medium such that the cells are at 0.8×10^6–1.0×10^6 cells/mL on the day of the experiment.

   Note: For the Jurkat cell line, you will need 1×10^5 cells per electroporation using the 10 µL Neon™ Tip. For optimized protocols using other cell lines, refer to "Neon™ Protocols" at **www.thermofisher.com/neon**.
3. Pre-warm aliquots of PBS without Ca^{2+} and Mg^{2+} and cell culture medium containing serum to 37ºC.
4. Add 0.5 mL of culture medium containing serum and supplements without antibiotics (pre-warmed to 37ºC) per well of a 24-well culture plate and place them in a humidified 37°C, 5% CO_2 incubator.

   Note: Each electroporation requires one well.
5. Take an aliquot of cell culture and count the cells to determine the cell density.
6. Calculate the volume of the cell culture needed.

   For example, if you wish to perform 6 electroporations using the Jurkat cell line, then you will need a total of 8.0×10^5 cells, which are enough for 8 electroporations (always include enough cells for 2 additional electroporations as a precaution). If the cell concentration is 1.0×10^6 cells/mL, this will require 800 µL of cell culture.
7. Transfer the cells in growth medium with serum to a 1.5-mL microfuge tube and pellet the cells by centrifugation at 100–400 × *g* for 5 minutes at room temperature.
8. Aspirate the medium and wash the cells with PBS without Ca^{2+} and Mg^{2+} (pre-warmed to 37ºC) by centrifugation at 100–400 × g for 5 minutes at room temperature.
9. Aspirate the PBS and resuspend the cell pellet in Resuspension Buffer R to a final concentration of 1.0×10^7 cells/mL. Gently pipette the cells up and down to obtain a single cell suspension.

   Note: Avoid storing the cell suspension for more than 15–30 minutes at room temperature, which can reduce cell viability and transfection efficiency.
10. Set up a Neon™ Tube with 3 mL of Electrolytic Buffer (use Buffer E for 10 µL Neon™ Tip and Buffer E2 for 100 µL Neon™ Tip) into the Neon™ Pipette Station.
11. Transfer 500 ng of GeneArt™ CRISPR Nuclease mRNA and 100 ng of IVT gRNA into a sterile, 1.5-mL microcentrifuge tube.
12. Add 10 µL of the cells suspension to the tube containing the GeneArt™ CRISPR Nuclease mRNA/IVT gRNA solution and gently mix.

    Note: The total volume of GeneArt™ CRISPR Nuclease mRNA and IVT gRNA must be ≤1 µL.

13. Aspirate the cell-RNA mixture into the Neon™ Tip.

    Note: Avoid air bubbles during pipetting. Air bubbles can cause arcing during electroporation, which lead to lowered or failed transfection. If you notice air bubbles in the tip, discard the sample and carefully aspirate a fresh sample into the tip without introducing any air bubbles.

14. Electroporate the cells using the Protocol #24 (1600 V, 10 ms, and 3 pulses) on the Neon™ device (optimized mRNA electroporation parameters for Jurkat cells).
15. Immediately transfer the electroporated cells to a 24-well plate containing 0.5 mL of the appropriate growth medium.
16. Incubate the cells in a humidified 37°C, 5% CO2 incubator for 48–72 hours.
17. Harvest the cells and measure cleavage efficiency using the GeneArt™ Genomic Cleavage Detection Kit or by sequencing.

Note: To optimize the electroporation conditions for a particular cell line, follow the Neon™ transfection 24-well optimization protocol described in the Neon™ Transfection System user guide. Each of the 24 optimized conditions are varied in pulse voltage, pulse width, and the number of pulses to determine which condition works best with your cell line. Alternative electroporation systems may be used, but optimal conditions will vary.

Determine the genomic cleavage efficiency

Perform GeneArt™ Genomic Cleavage Detection assay

48–72 hours after from transfection, perform a genomic cleavage detection assay. This technique leverages mismatch detection endonucleases to detect genomic insertions or deletions (indels) incorporated during cellular NHEJ (non-homologous end joining) repair mechanisms.

We recommend the GeneArt™ Genomic Cleavage Detection Kit (Cat. no. A24372) for performing cleavage efficiency analysis. See the GeneArt™ Genomic Cleavage Detection Kit User Guide for detailed instructions. For this analysis you need to design PCR primers that are around 150–300 bases away from the target cleavage site on each side and amplify the genomic target of 400–600 bases. The GeneArt™ Genomic Cleavage Detection assay will cleave the amplified genomic PCR product if a modification has occurred, which can then be visualized on a gel.

Appendix A: Troubleshooting

Issues with *in vitro* transcription of gRNA

When performing experiments using the complete RNA format, it is important to follow proper RNA handling procedures (see page 6). Refer to the GeneArt™ Precision gRNA Synthesis Kit or the MEGAshortscript™ T7 Transcription Kit user guide for detailed instructions on performing the IVT procedure.

Observation	Possible cause	Solution
No or poor IVT gRNA yield	RNase contamination	Ensure that the water used for preparing the gRNA or reconstituting the GeneArt™ CRISPR Strings™ DNA is RNase-free.
		Clean all the work areas with an RNase decontaminating agent such as the RNaseZap™ Solution.
		Ensure that all the tubes and tips used are RNase-free.
		If using a PCR amplified or plasmid-based template, ensure that these reagents are clean and RNAse-free. Plasmid purification kits frequently introduce ribonucleases into the purified plasmids.
	Problem with the IVT reaction	Run the positive control reaction included with the GeneArt™ Precision gRNA Synthesis Kit or the MEGAshortscript™ T7 Transcription Kit to ensure the kit is performing well. • The positive control in the GeneArt™ Precision gRNA Synthesis Kit generally yields >20 µg of IVT gRNA. Yield of IVT gRNA generated using the kit should be >10 µg (typically 20–70 µg); however, the gRNA yield varies based on the target sequences. • The yield of IVT gRNA produced using the TranscriptAid™ T7 High Yield Transcription Kit should be >10 µg (typically 20–55 µg); however, the gRNA yield varies based on the target sequences. • The positive control in MEGAshortscript™ T7 Transcription Kit generally yields 90 µg of RNA; however, the yield of gRNA produced using the kit is not expected to be high (typically 10–40 µg).
		If the positive control has worked, but the experimental sample has not produce gRNA, collect 1 µL aliquots at different stages of the process (e.g., when setting up the reaction, before and after the DNase treatment, etc.) and run them on a gel to identify the problematic step.

No detectable cleavage of the target region

There are many factors that can result in no detectable cleavage in the target region. Make sure to follow proper RNA handling procedures (see page 6). Refer to the GeneArt™ Genomic Cleavage Detection Kit user guide for detailed instructions on performing the cleavage assay.

Observation	Possible cause	Solution
No detectable cleavage of target region	Suboptimal transfection	Check all the transfection conditions.
		Check if the transfection reagent used was suitable for the cell line (refer to the transfection reagent recommendations at **www.thermofisher.com/transfection)**.
		Perform a positive control reaction. A commercially available mRNA for a fluorescent protein (e.g., GFP) can be used to assess transfection efficiency.
	mRNA was degraded	Check the integrity by running 0.5–1.0 µg of mRNA on a gel.
	gRNA was degraded or suboptimal amount used	Check the integrity of gRNA by confirming it on a 1% E-Gel™ agarose gel.
		Ensure that an adequate amount of gRNA is used for the transfection.
	Cleavage detection assay protocol did not work	Make sure that the PCR of the target region generates a discreet (single) DNA band. If it does not, optimize the PCR conditions. Refer to the GeneArt™ Genomic Cleavage Detection Kit user guide for more details.
		Perform the positive control provided with the GeneArt™ Genomic Cleavage Detection Kit. If no cleavage is observed with the control, contact Technical Support (page 30).
	Target region is inaccessible to the Cas9/CRISPR complex	More than one CRISPR sequence may be needed to identify the target that yields the best CRISPR cleavage efficiency. Little or no cleavage can be detected if the Cas9/CRISPR complex cannot access the target region. We recommend selecting at least 3 different CRISPR target sequences in the gene of interest to ensure that at least one sequence works well.

Appendix B: CRISPR target sequences

General guidelines for CRISPR target sequence selection

When performing CRISPR/Cas9-mediated DNA double-strand cleavage, your choice of the target sequence can significantly affect the degree of cleavage observed. We recommend following the guidelines below when choosing your target sequence. Note that these are general recommendations only; exceptions may occur.

- **Length:** Choose a target sequence 20 nucleotides in length that is adjacent to an NGG proto-spacer adjacent motif (PAM) on the 3′ end of the target sequence.

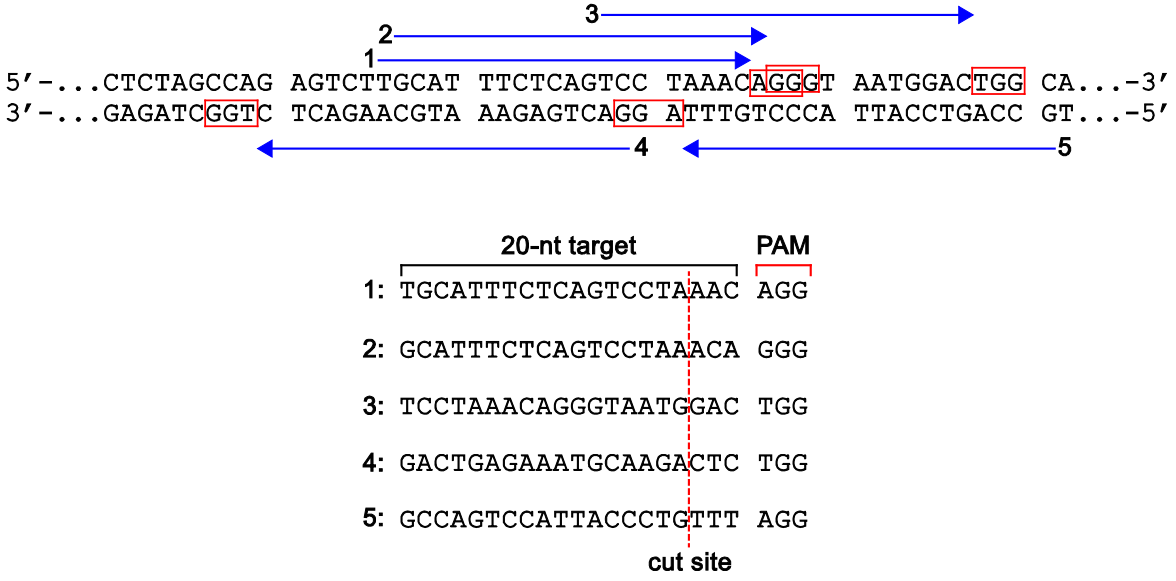
  Note: In the example below (Figure 5), PAMs are depicted as red boxes and potential targets as blue lines.

- **Homology:** Make sure that the target sequence does **not** contain significant homology to other genomic sequences as this can increase off-target cleavage. Recently published work has shown that gRNA-Cas9 nuclease complexes can potentially tolerate up to 1–4 mismatches. Refer to published articles for more insights into choosing target sequence (Fu et al., 2013; Mali et al., 2013).

- **Direction:** You may choose a target sequence encoding the **sense** or the **antisense** sequence of the target locus. Thus, you can generate CRISPR RNA in two possible orientations, provided that it meets the PAM requirements on the 3′ ends.

- **Cleavage:** Cas9 nuclease will generally make a dsDNA cut 3–4 bases upstream from the PAM site.

Figure 5. Identifying potential CRISPR targets



IMPORTANT! Do not include the PAM sequence in the oligonucleotide primers.

Appendix C: GeneArt™ CRISPR Search and Design Tool

Introduction

The GeneArt™ CRISPR Search and Design Tool allows you to search our database of >600,000 pre-designed CRISPR gRNA sequences or to analyze your sequence of interest for *de novo* gRNA design.

Follow the instructions below to analyze your sequence of interest for potential CRISPR target sequences and optimized gRNA design, and to directly order oligonucleotide primers for gRNA synthesis using the GeneArt™ Precision gRNA Synthesis Kit.

Analyze your sequence for potential CRISPR targets

1. Go to **www.thermofisher.com/crisprdesign** and log into Thermo Fisher Cloud using your existing username and password. If you do not have an account, follow the instructions to create one.

   Note: You will be asked to verify your e-mail address the first time you log in. Future visits to the GeneArt™ CRISPR Search and Design Tool will not require a Thermo Fisher Cloud login.

2. To search our predesigned CRISPR database, enter up to 10 NCBI synonyms or RefSeq accession numbers (see Figure 6, below).

   Alternatively, click **Custom Design** and enter the gene name and up to 500 bp in FASTA format in the search text box (i.e., >GeneName followed by the sequence in the next line; see Figure 7, page 20).

3. For the CRISPR-Cas9 expression format, select **mRNA – GeneArt™ CRISPR Cas9 mRNA**.

4. For the gRNA format, select **gRNA Synthesis Kit**, **T7 Strings**, or **U6 Strings**.

5. For the host genome, select **Homo sapiens** or **Mus musculus**.

   Note: Species selection is for predicting off-target effects.

7. Click **Search** to initiate *de novo* design of CRISPR gRNAs.

Figure 6. Example search query for predesigned CRISPR sequences for the human BRCA2 gene

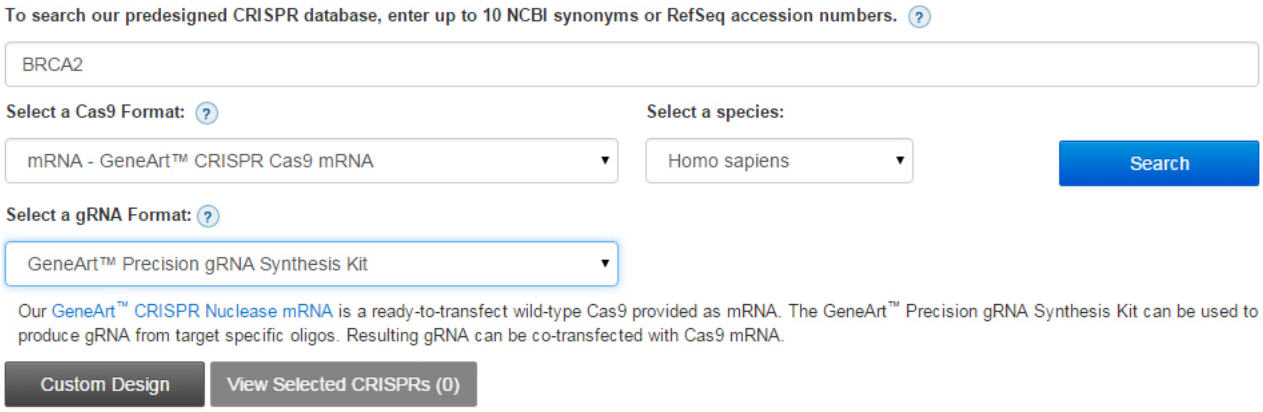
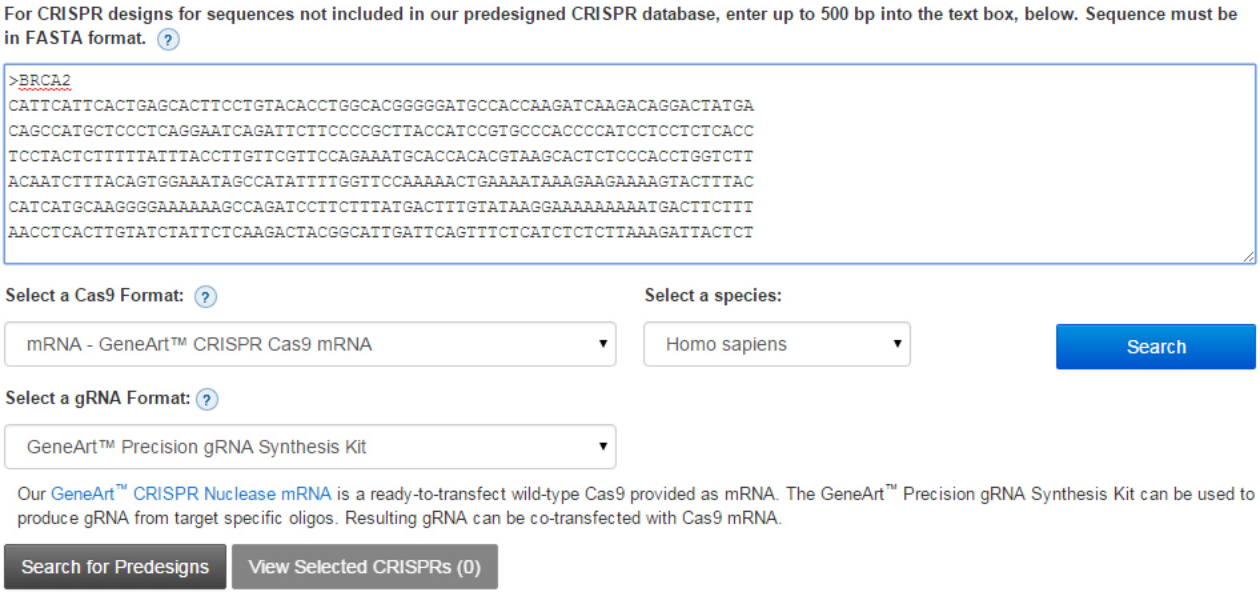


Figure 7. Example search query for custom CRISPR sequences using the first 420 bp of the human BRCA2 gene in FASTA format

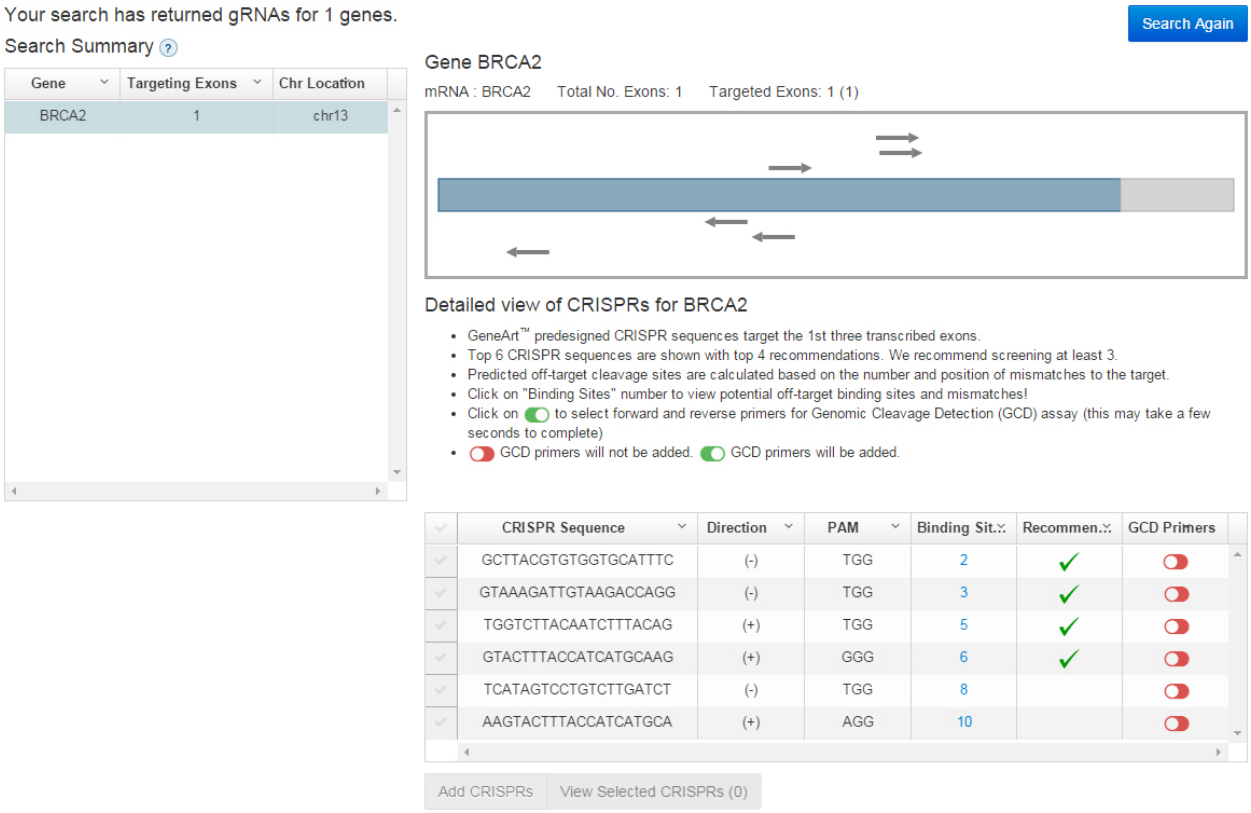


Output example from the GeneArt™ CRISPR Search and Design Tool

The example below (Figure 8, below) shows the output from the GeneArt™ CRISPR Search and Design Tool for a Custom CRISPR oligonucleotide design using the first 420 bp of the human BRCA2 gene.

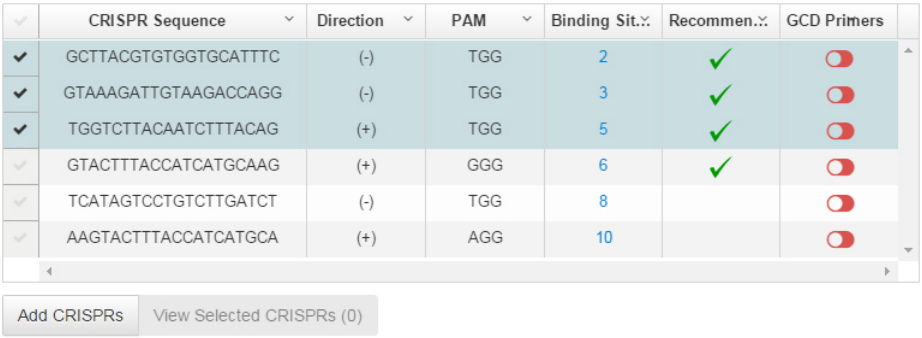
- Search Summary panel on the left shows the name of the gene analyzed, the exons targeted by the CRISPR sequences suggested by the design tool, and the chromosome on which the target sequences reside.
- Search Results panel on the right shows the exon map with gRNA binding sites and the top six CRISPR target sequences. The top four recommended CRISPR sequences are identified by green check marks.
- Hover over a CRISPR sequence to see where it falls in the gene representation at the top. An arrow will appear for each sequence in the table.
- For each CRISPR sequence, the design tool provides the direction (sense or antisense), the PAM sequence, and the predicted off-target binding sites (calculated based on the number and position of mismatches to the target).
- Click on the number in the Binding Sites column to view potential off-target binding sites and mismatches for the CRISPR sequence.

Figure 8. Example output from search query for custom CRISPR sequences using the first 420 bp of the human BRCA2 gene in FASTA format



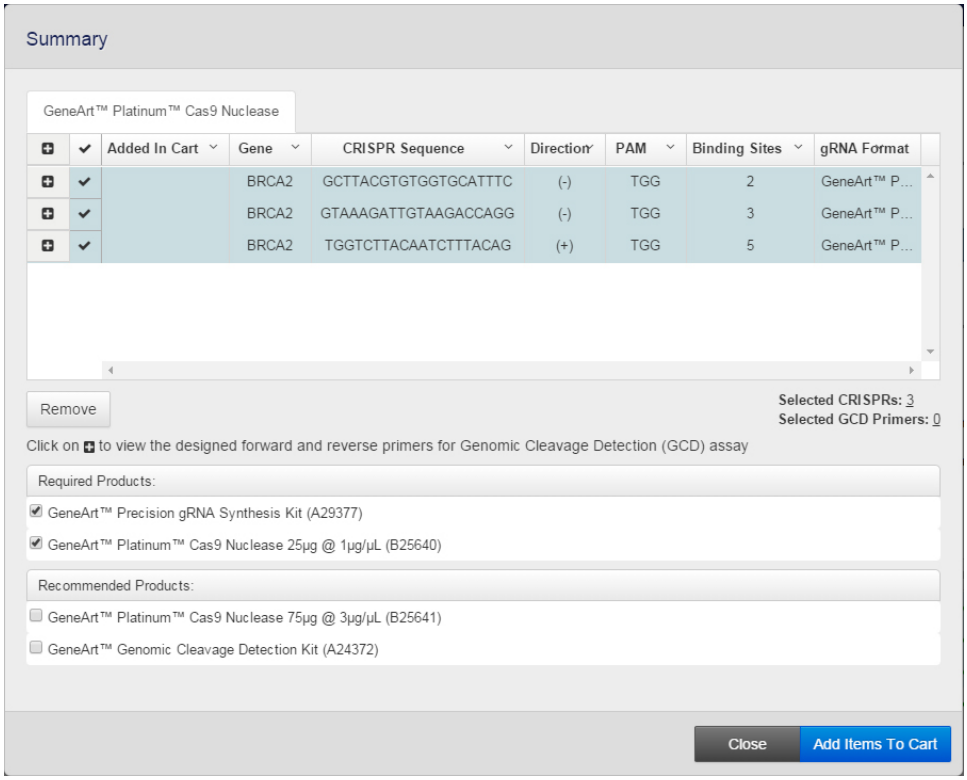
Select CRISPR sequences and place an order

1. To select the desired CRISPR sequences, click the **check** button to the left of the sequence. The selected CRISPR sequences will be highlighted.



2. *Optional*: Click the **GCD Primers** toggle switch to add forward and reverse primers for the Genomic Cleavage Detection (GCD) assay using the GeneArt™ Genomic Cleavage Detection Kit (Cat. no. A24372).
3. Click **Add CRISPRs** to add the selected sequences to your project.
4. Click **View Selected CRISPRs** to view the Summary window for your project and to confirm the CRISPR sequences and any recommended products you would like to include in your order.

   Note: Carefully review the CRISPR sequences before adding to cart. Once you add the sequences to the cart, you cannot apply any edits. Should you require different gRNA designs, delete your project from the cart and return to the web tool for additional designs.



5. Click **Add Items to Cart** to place your order.

   Note: Ensure that the pop-up blocker on your browser is turned off. If the pop-up blocker is enabled, the shopping cart will not open.

Appendix D: Produce IVT gRNA from GeneArt™ CRISPR Strings™ T7 DNA

Introduction

The following protocols describe the generation of IVT gRNA from GeneArt™ CRISPR Strings™ T7 DNA using the TranscriptAid™ T7 High Yield Transcription Kit (Cat. no. K0441) or the MEGAshortscript™ T7 Transcription Kit (Cat. no. AM1354). For detailed instructions, refer to the respective user guides for the kits.

Note: You can use any T7 *in vitro* transcription kit to generate IVT gRNA from GeneArt™ CRISPR Strings™ T7 DNA.

Prepare GeneArt™ CRISPR Strings™ T7 DNA for *in vitro* transcription

1. Pellet the lyophilized GeneArt™ CRISPR Strings™ T7 DNA by centrifugation for 30 seconds at room temperature.
2. Resuspend the DNA in 10 µL of nuclease-free water.
3. Centrifuge the resuspended DNA briefly to collect the contents at the bottom of the tube.
4. Determine the DNA concentration using a NanoDrop™ spectrophotometer or an equivalent system.
5. Record the concentration and dilute to the required working concentration. We recommend diluting the GeneArt™ CRISPR Strings™ T7 DNA to 50–100 ng/µL.

Generate IVT gRNA using the TranscriptAid™ T7 High Yield Transcription Kit

1. Set up the following IVT reaction in a 20-µL volume using 100–200 ng of template DNA.

   Note: Higher amounts of template DNA results in increased IVT gRNA yields.

Component	Amount
GeneArt™ CRISPR T7 Strings™ DNA	100–200 ng (≤6 µL)
5X TranscriptAid™ Reaction Buffer	4 µL
ATP/CTP/GTP/UTP mix*	8 µL
TranscriptAid™ Enzyme Mix	2 µL
DEPC-treated water	to 20 µL final volume

*Combine equal volumes of the four NTP solutions provided in one tube.

2. Mix thoroughly, centrifuge briefly to collect the contents at the bottom of the reaction tube, and incubate at 37°C for 2–4 hours.
3. Add 2 µL of DNase I to the reaction and incubate at 37°C for 15 minutes.
4. Purify IVT gRNA using the MEGAclear™ Transcription Clean-Up Kit (Cat. no. AM1908) or the GeneJET™ RNA Cleanup and Concentration Micro Kit (Cat. no. K0841). For detailed instructions, refer to the respective user guides.
5. Determine the concentration of the eluate (gRNA) using a NanoDrop™ spectrophotometer or an equivalent system. The typical yield from 200 ng template DNA is 20–55 µg of IVT gRNA.
6. Check the quality of the gRNA by electrophoresis on a denaturing acrylamide gel. The expected gRNA transcript size is 100 bases. A discreet band at 100 bases indicates intact RNA.

   Note: Quality can also be checked on a 4% agarose gel, but a control RNA of known size is required to compare the size of RNA bands with this method.

Generate IVT gRNA using the MEGAshortscript™ T7 Transcription Kit

1. Set up the following IVT reaction in a 20-µL volume using using 100 ng of template DNA.

Component	Amount
GeneArt™ CRISPR T7 Strings™ DNA	100 ng (≤8 µL)
T7 10X Reaction Buffer	2 µL
T7 ATP Solution (75 mM)	2 µL
T7 CTP Solution (75 mM)	2 µL
T7 GTP Solution (75 mM)	2 µL
T7 UTP Solution (75 mM)	2 µL
Optional: Labeled ribonucleotide	~1 µL
T7 Enzyme Mix	2 µL
Water (Nuclease-free)	to 20 µL final volume

2. Incubate the reaction at 37°C for 3–4 hours.
3. Add 1 µL of Turbo DNase to the reaction and incubate at 37°C for 30 minutes.
4. Purify IVT gRNA using the MEGAclear™ Transcription Clean-Up Kit (Cat. no. AM1908) or the GeneJET RNA Cleanup and Concentration Micro Kit (Cat. no. K0841). For detailed instructions, refer to the respective user guides.
5. Determine the concentration of the eluate (gRNA) using a NanoDrop™ spectrophotometer or an equivalent system. The typical yield is 10–40 µg of gRNA.
6. Check the quality of the gRNA by electrophoresis on a denaturing acrylamide gel. The expected gRNA transcript size is 100 bases. A discreet band at 100 bases indicates intact RNA.

   Note: Quality can also be checked on a 4% agarose gel, but a control RNA of known size is required to compare the size of RNA bands with this method.

Appendix E: Cell-specific transfection conditions

Seeding densities for tested cell lines

The GeneArt™ CRISPR Nuclease mRNA system has been optimized for 24-well tissue culture plates. If using different well formats, the seeding density must be scaled accordingly.

Cell line	Seeding density	Viability (at time of seeding)	Transfection reagents tested
293 FT	0.2 × 10^6 cells/well	>90%	• Lipofectamine™ MessengerMAX™ Reagent • Lipofectamine™ RNAiMAX Reagent • Lipofectamine™ 2000 Reagent
HeLa	0.1 × 10^6 cells/well	>85%	• Lipofectamine™ MessengerMAX™ Reagent • Lipofectamine™ RNAiMAX Reagent • Lipofectamine™ 2000 Reagent
U2OS	0.1 × 10^6 cells/well	>90%	• Lipofectamine™ MessengerMAX™ Reagent • Lipofectamine™ RNAiMAX Reagent
A549	0.1 × 10^6 cells/well	>85%	• Lipofectamine™ MessengerMAX™ Reagent
HCT116	0.1 × 10^6 cells/well	>90%	• Lipofectamine™ MessengerMAX™ Reagent • Lipofectamine™ RNAiMAX Reagent
Hep-G2	0.1 × 10^6 cells/well	>90%	• Lipofectamine™ MessengerMAX™ Reagent • Lipofectamine™ RNAiMAX Reagent
Mouse Neuro2A (N2)	0.8 × 10^5 cells/well	>85%	• Lipofectamine™ MessengerMAX™ Reagent • Lipofectamine™ RNAiMAX Reagent • Lipofectamine™ 2000 Reagent

Appendix F: Cloning GeneArt™ CRISPR Strings™ DNA

Generation of a sequence verified gRNA expression plasmid

GeneArt™ CRISPR Strings™ DNA fragments can be cloned into a vector if a sequence verified expression plasmid is desired. This procedure can also be used in cases where the amount of GeneArt™ CRISPR Strings™ DNA becomes a limiting factor such as when larger transfection well or plate formats are desired.

- Use the Zero Blunt™ TOPO™ PCR Cloning Kit (Cat. no. K2800-20) to clone GeneArt™ CRISPR Strings™ DNA fragments.
- Sequence the resulting clones using the SP6 primer from the vector (we do not recommend using M13 primers for sequencing GeneArt™ CRISPR Strings™ DNA).
- Use the sequence verified clones as a template to PCR amplify the appropriate gRNA cassette and promoter with the following primers.
  - **T7 Strings™ DNA primers**:

    Forward: `5′–AGAGGCGGTTTGCGTATTG–3′`

    Reverse: `5′–AAAAAAGCACCGACTCGGTG–3′`
  - **U6 Strings™ DNA primers**:

    Forward: `5′–AATTAAGGTCGGGCAGGAAG–3′`

    Reverse: `5′–ACAGCTATGACCATGATTACGCC–3′`

Note: If you already have your CRISPR sequence of interest cloned in a GeneArt™ CRISPR Nuclease vector (Cat. no. A21177 or A21178), the same set of **U6 Strings™ DNA primers** can be used to perform PCR and generate a U6 expression cassette.

To generate a T7-based expression template, the following primers can be used with these vectors.

Forward: `5′–TAATACGACTCACTATAGGNNNNNNNNNNNNNNNNNN–3′`

Reverse: `5′–AAAAAAGCACCGACTCGGTG–3′`

In the forward primer, the T7 promoter sequence is underlined. The subsequent run of 19–20 nucleotides should be replaced by the CRISPR target sequence.

Appendix G: Ordering information

Introduction

The products listed in this section may be used with the GeneArt™ CRISPR Nuclease mRNA (Cat. no. A29378). For more information, refer to our website (**www.thermofisher.com**) or contact Technical Support (see page 30).

Ordering oligonucleotides

Custom oligonucleotides for use with the GeneArt™ CRISPR Nuclease Vectors can be ordered from Thermo Fisher Scientific. For additional information, visit our website at **www.thermofisher.com/oligos**.

Accessory products

The following accessory products are available separately from Thermo Fisher Scientific. Ordering information is provided below. For more information, refer to our website (**www.thermofisher.com**) or contact Technical Support (see page 30).

Product	Amount	Catalog no.
GeneArt™ Precision gRNA Synthesis Kit	1 kit	A29377
TranscriptAid™ T7 High Yield Transcription Kit	50 reactions	K0441
MEGAshortscript™ T7 Transcription Kit	25 reactions	AM1354
GeneJET™ RNA Cleanup and Concentration Micro Kit	50 preps	K0841
MEGAclear™ Transcription Clean up kit	20 preps	AM1908
Lipofectamine™ MessengerMAX™ Reagent	0.3 mL	LMRNA003
Lipofectamine™ RNAiMAX™ Transfection Reagent	0.75 mL	13778-075
Lipofectamine™ 2000 Reagent	1.5 mL	11668-019
GeneArt™ Genomic Cleavage Detection Kit	20 reactions	A24372
GeneArt™ Genomic Cleavage Selection Kit	10 reactions	A27663
Opti-MEM™ I Reduced Serum Medium	100 mL	31985-062
Qubit™ RNA BR Assay Kit	100 assays	Q10210
E-Gel™ EX Agarose Gels, 2X	10 gels	G4010-02
RNA Gel Loading Dye, 2X	1 mL	R0641
RNA Century™ Markers (1 µg/µL)	50 µg	AM7140
Novex™ TBE-Urea Gels, 10%	1 box	EC68752BOX
DNase/RNase-free Microfuge Tubes	500 tubes	AM12400
RNaseZap™ Solution	250 mL	AM9780
Neon™ Transfection System	1 each	MPK5000
Zero Blunt™ TOPO™ Cloning Kit	25 reactions	K2800-20
One Shot™ Top10 Competent *E. coli*	20 reactions	C40404-03
Purelink™ Quick Plasmid Miniprep Kit	50 preps	K2100-10

Appendix H: Safety

General safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc.). To obtain SDSs, see the "Documentation and support" section in this document (page 30).

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and support" section in this document (page 30).
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! **BIOHAZARD.** Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:

  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf

- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:

  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Obtaining support

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- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**.

If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support.**

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