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TrueDesign Genome Editor

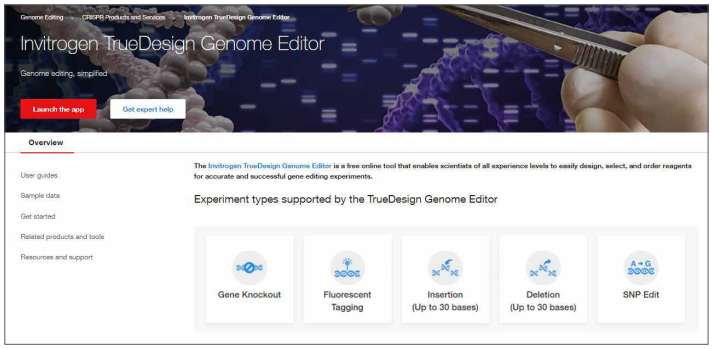
Workflow guide for creating a gene knockout by causing frameshift indels

The Invitrogen™ TrueDesign™ Genome Editor is easy-to-use, free online software for designing and ordering the reagents needed for precise genome editing by homology-directed repair with RNA-guided nucleases and single-stranded DNA donors. This workflow guide will walk through the steps for creating a gene knockout using imperfect repair following a double-stranded break by a nuclease. The resulting insertions or deletions (indels) can cause frameshifts or premature stop codons to cause functional knockout of the target gene.

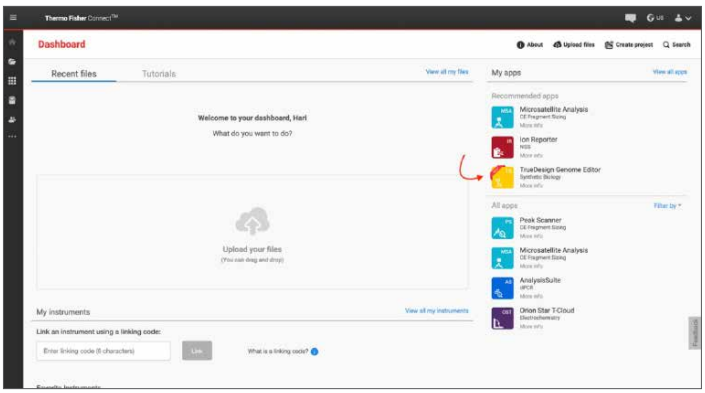
Please note: Algorithm-designed CRISPR gRNAs optimized for functional knockouts are available for all human and mouse genes, and may be ordered directly from **thermofisher.com/trueguide**.

Step 1:

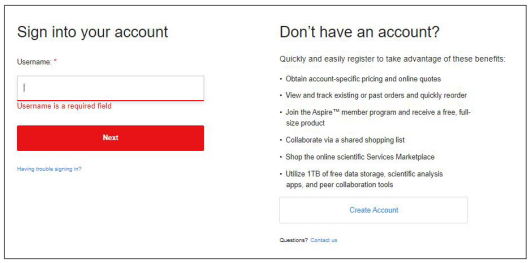
Go to **thermofisher.com/truedesign**. Select one of the links to launch the software.



Alternatively, go directly to the **Thermo Fisher™ Connect Platform** and navigate to the TrueDesign Genome Editor.



You may be prompted to sign in. Use your existing credentials, or simply provide an email address to register as a new user.



A "Terms of Use" window may pop up. Read the content, scroll to the bottom of the screen, and click "Accept".

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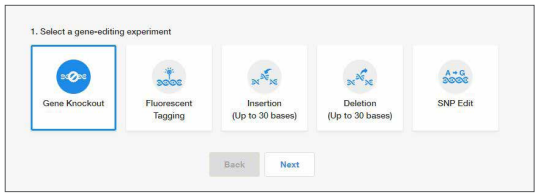
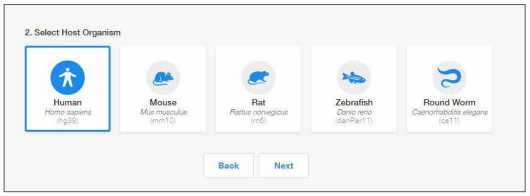
Step 2:

In the TrueDesign software, choose **Gene Knockout** as your experiment type, and click “Next”.



Step 3:

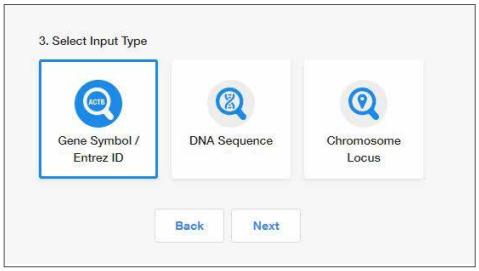
Select the host organism for your knockout experiment and click “Next”.



Step 4:

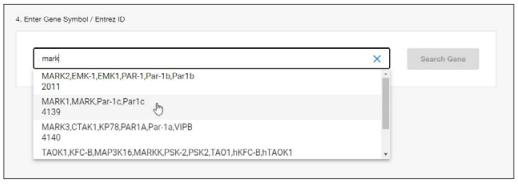
Select **Gene Symbol/Entrez ID** to identify your gene of interest. You may also identify a transcript of interest by entering a DNA sequence or chromosome location.

Click “Next”.



Step 5:

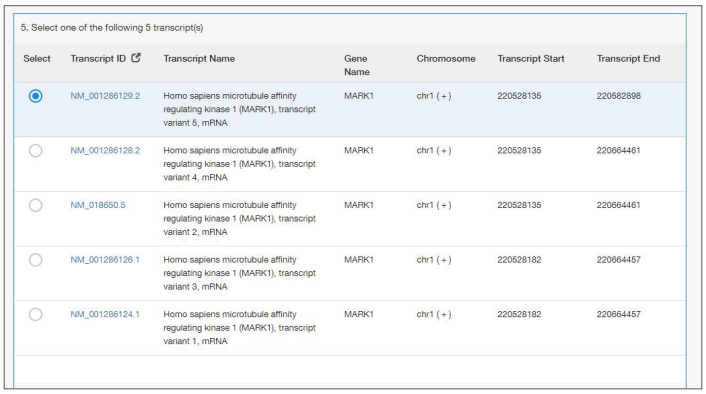
Begin typing the gene symbol or Entrez ID in the gene identifier box. A filtered drop-down list will appear. Select your gene of interest and click “Search Gene”.



Step 6:

All of the protein-coding transcripts for your selected gene will be displayed. If there is more than one protein-coding transcript and you are unsure of which one to select, click the transcript ID hyperlink to be taken to the NCBI website, where you can better view the transcript maps.

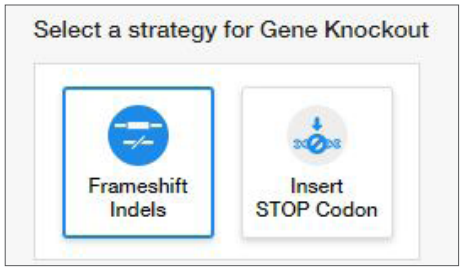
After you make a selection, click “Edit”.



Step 7:

The next screen will display the entire transcript’s topology along the top of the screen, with a zoomed-in sequence-level view below.

You will be prompted to choose **Frameshift Indels** or **Insert STOP Codon.**



- Frameshift indel: The most common approach for gene knockout, this process relies on imperfect repair following a double-stranded DNA break. You will be required to select a region on the transcript to search for custom CRISPR and TALEN™ designs.
- Insert stop codon: A more precise method for knockout of gene expression, this process uses a DNA donor to insert multiple stop codons to prevent translation. By default, this insertion will be designed adjacent to the transcriptional start site.

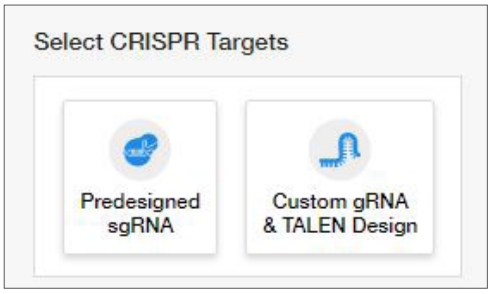
Step 7: (continued)

For this workflow example, select **Frameshift Indels**. Please refer to the workflow guide for insertion of stop codons for details on that experiment type.

Step 8:

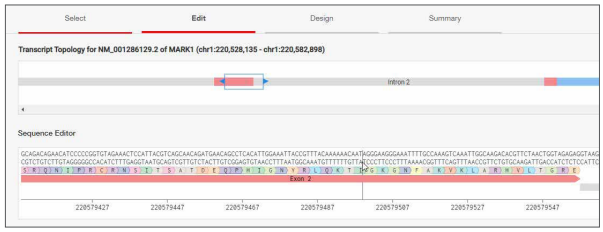
If your selected species is human or mouse, you will be prompted to choose between predesigned synthetic gRNA (sgRNA) or custom designs. Selecting the **Predesigned sgRNA** option will retrieve algorithm-designed Invitrogen™ TrueGuide™ Synthetic gRNAs for your gene, let you view them along the transcript, and give you the chance to order genomic cleavage detection and sequencing primers. For more information on TrueGuide Synthetic gRNA, go to **thermofisher.com/trueguide**.

For all other species, or for custom human or mouse designs targeting a specific region of interest, choose **Custom gRNA & TALEN Design**.



Step 9:

You will be prompted to select a location on the sequence editor. Click and drag the blue box on the transcript topology to navigate to the region where you wish to create an indel. This region will be displayed at the DNA level in the sequence editor. Click on the sequence editor to indicate the indel site. A blinking cursor will indicate your selection. The software will use this location as the anchor point for designing custom CRISPR gRNAs and TALEN pairs. Typically, designs are made to target an early exon or may be directed to a known functional region of your gene.



Step 10:

An edit list will appear, displaying the type and selected location of the edit. You can undo this edit by clicking the blue “undo” arrow in the edit list.

When you are satisfied with your changes, click “Design”. This will initiate the design process for the software to find and analyze available TALEN pairs and CRISPR gRNA target regions, and check them for specificity.

Step 11:

When complete, you will see a table with two tabs: CRISPR and TALEN targets. View the target location on the sequence editor display by clicking anywhere in the row.

Each row in the CRISPR results will display:

- The CRISPR gRNA design; green checkmarks indicate recommended gRNAs due to score and proximity to the insertion site
- The gRNA PAM site
- The gRNA score, which is a weighted score for efficiency and specificity
- The number of predicted off-targets; click the hyperlink to display a pop-up window with detailed location and mismatch information for the potential off-targets
- The edit site’s distance from the cut site

To view the TALEN targets, click on the TALEN tab of the table and similar information will be displayed for each TALEN pair. TAL effector nuclease (TALEN) pairs are recommended when there are no PAM sites within 10 bp of the designated target location, or if the efficiency and specificity of the gRNAs are not optimal. Green checkmarks in the design results table will indicate the recommended technology. Learn more about TALEN technology at **thermofisher.com/tal**.

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Step 12:

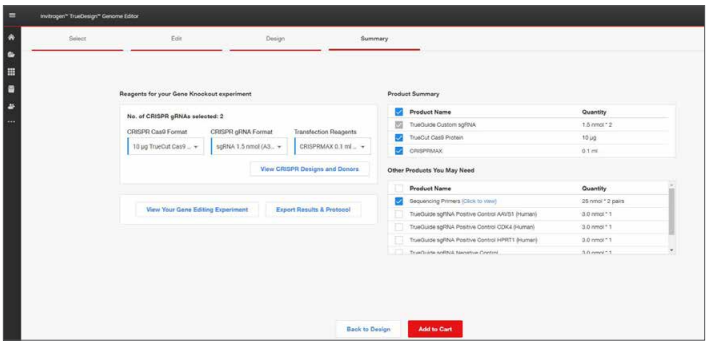
To select one or more CRISPR gRNAs or TALEN pairs to add to your experiment, use the checkboxes in the table and click “Summary”.

Step 13:

The summary page will display all the reagents needed for your knockout experiment and give you the opportunity to add additional products to complete your workflow.

For an indel frameshift experiment using CRISPR technology, the gRNAs selected in the design step are automatically included. The tool will also add Invitrogen™ TrueCut™ Cas9 Protein v2 and Invitrogen™ Lipofectamine™ CRISPRMAX™ Cas9 Transfection Reagent.

Products may be deselected from the product summary area, or a different quantity may be selected from the drop-down lists.



Step 14:

You may use the checkboxes to select additional items such as sequencing primers, to verify your indel, or positive and negative experimental controls.

When you have completed your product selections, click “Add to Cart” for easy one-step ordering of all selected reagents.

If “Add to Cart” is not enabled in your region or you want to send the list of reagents to your purchasing agent, you can download and save a detailed report of your experiment by clicking “Export Results & Protocol”. The resulting Microsoft™ Excel™ file contains multiple tabs that include all the designs generated by the software, plus all of the gene-specific experimental details and ordering information.

TrueDesign Report

Get started at **thermofisher.com/truedesign**

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