# TrueDesign Genome Editor

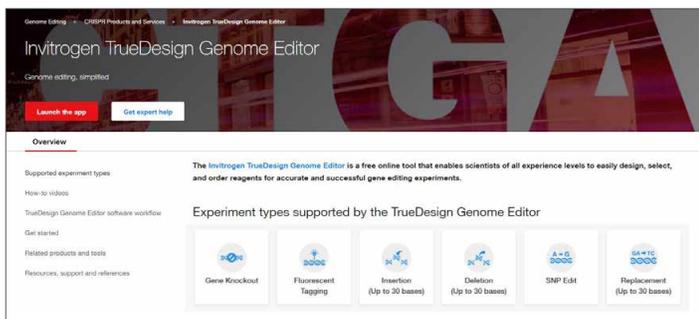## Workflow guide for creating a gene knockout using insertion of stop codons

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 by insertion of stop codons. To accomplish gene knockout using imperfect repair following a double-strand break, **see our workflow guide** for creating a gene knockout by causing frameshift indels.
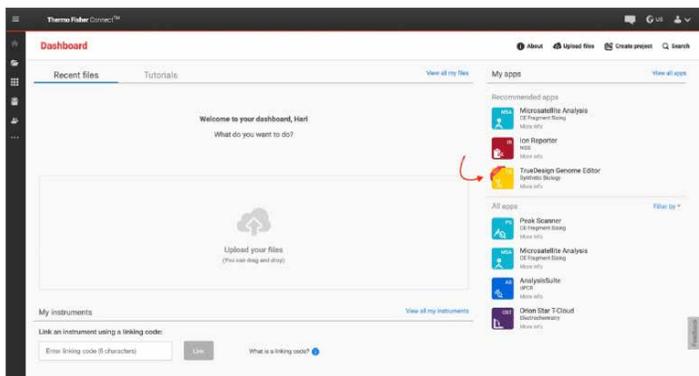
Please note that algorithm-designed CRISPR gRNAs optimized for functional knockout are available for all human and mouse genes and may be ordered directly from **thermofisher.com/trueguide**.
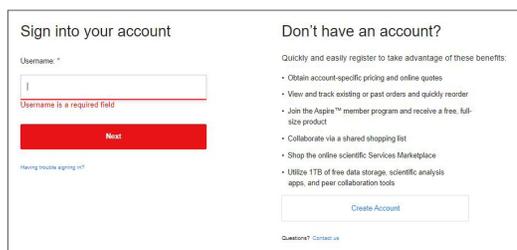
### Step 1:

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.
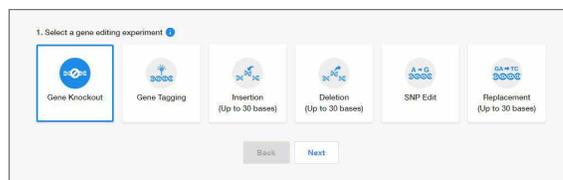


You may be prompted to sign in. Use your existing credentials, or simply provide an email address to register as a new user.



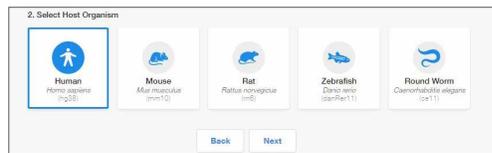A "Terms of Use" window may pop up. Read the content, scroll to the bottom of the screen, and click "Accept."

### Step 2:

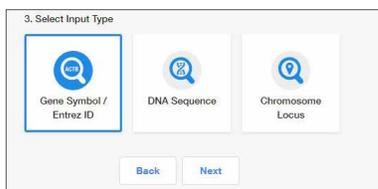In the TrueDesign software, choose **Gene Knockout** as your experiment type and click "Next."



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S C I E N T I F I C

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

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

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."

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 "Gene Symbol" hyperlink to be taken to the NCBI website, where you can better visualize the transcript maps. After you make a selection, click "Next."
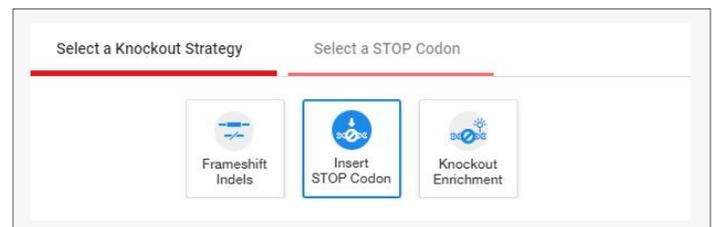
The next screen will display the entire transcript's topology along the top of the screen, with a zoomed-in sequence-level view below.

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

- 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. Predesigned gRNAs for human and mouse are available with the **Invitrogen™ TrueGuide™ Synthetic gRNA** product line.

- 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.

- Knockout enrichment: The optimal method for generating a knockout cell line, this process uses a DNA donor template from the Invitrogen™ TrueTag™ Knockout Enrichment Donor DNA Kit to insert multiple stop codons to prevent translation, as well as resistance markers for enrichment and fluorescent tags for visualization and cell sorting to help ensure biallelic knockout.

For the workflow example in this document, select **Insert STOP Codon**. Please refer to the workflow guide for **causing frameshift indels** or **knockout enrichment** for details on those experiment types.

## Step 8:

The view will update and prompt you to click on the stop codon you wish to insert. The relative frequency of each codon's usage within your selected species will be displayed.

Next, choose to add either 3 or 4 stop codons from the drop-down list. Click "OK."



## Step 9:

The view will update to show "Select a gRNA option." Click on the sgRNA option that fits your need—either "Predesigned sgRNA" (human and mouse genes only) or "Custom gRNA and TALEN design." If you do not have a preference for the region in which to insert your stop codons and are working in human or mouse cells, "Predesigned sgRNA" is recommended. Otherwise, select "Custom gRNA and TALEN design." Most steps shown in this document will be similar for both workflows.



## Step 10:

The sequence editor will update and prompt you to select a region on the transcript in which to insert the stop codons (this step will not occur if the "Predesigned sgRNA" option was selected). You may now easily navigate to different exons using the transcript topology at the top of the view. Click on the sequence editor to choose a region for insertion.



An edit list will appear, displaying the type and location of the edit. If you want to change the location, you can undo this edit by clicking the blue "undo" arrow in the edit list.

When you are satisfied with your changes, click "Next." 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 for each design result on the sequence editor display by clicking anywhere in the row.

Each row in the CRISPR table displays:

- 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 link to open a pop-up window that displays the location and mismatch information for each potential off-target
- The edit site's distance from the cut site
- The DNA oligo required as a donor template for the insertion of the stop codons



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 knock-in site, 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 a stop codon insertion experiment using CRISPR technology, the gRNAs and donor oligos 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 different quantities may be selected from the drop-down lists.



## Step 14:

You may use the checkboxes to add additional items such as sequencing primers 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.



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

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