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TrueDesign Genome Editor

Workflow guide for using the TrueTag Knockout Enrichment kit for knockout cell line generation

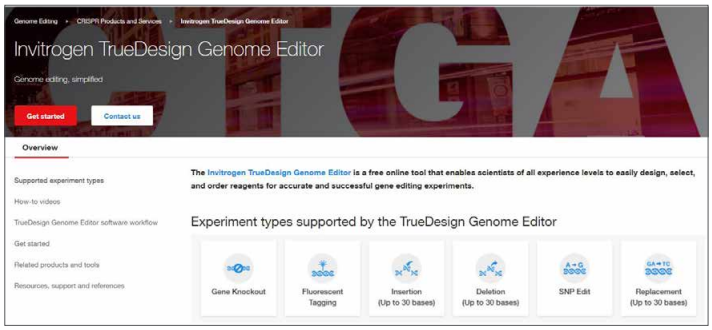
The Invitrogen™ TrueDesign™ Genome Editor is easy-to-use, free online software for designing and ordering the reagents needed for precise genome editing by homology-directed repair with RNA-guided nucleases and single-stranded DNA donors.

This workflow guide will walk through the steps for creating a gene knockout and enabling enrichment of edited cells with the Invitrogen™ TrueTag™ Knockout Enrichment Donor DNA Kit. This kit provides a template for insertion of a cassette with stop codons, GFP/RFP reporters, and blasticidin and puromycin resistance markers.

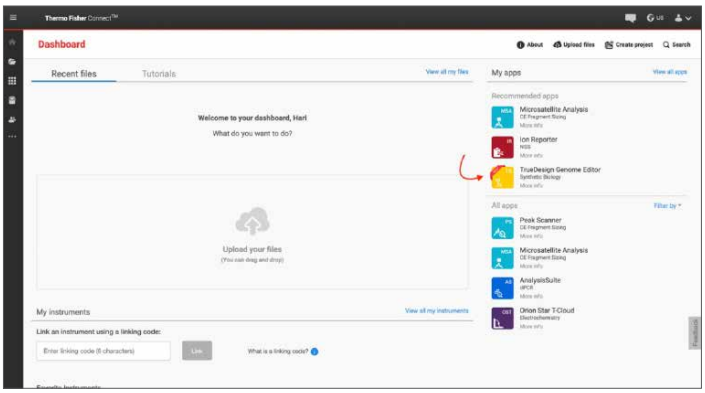
Please note that algorithm-designed CRISPR gRNAs optimized for functional knockout are available for all human and mouse genes and may be ordered directly from **thermofisher.com/trueguide**.

Step 1:

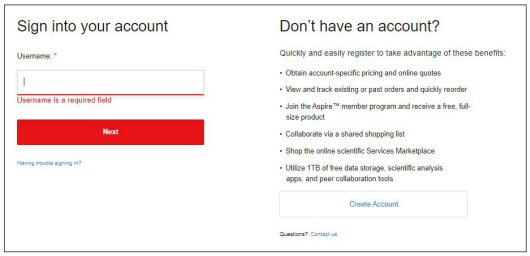
Go to **thermofisher.com/truedesign**. Select one of the links to launch the software.



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."

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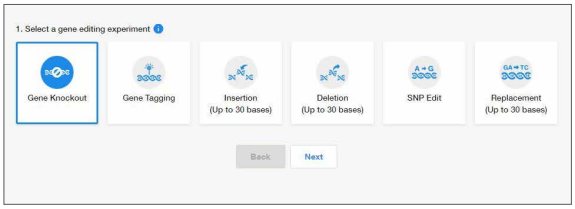
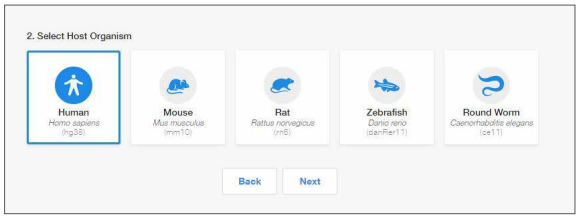
Step 2:

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



Step 3:

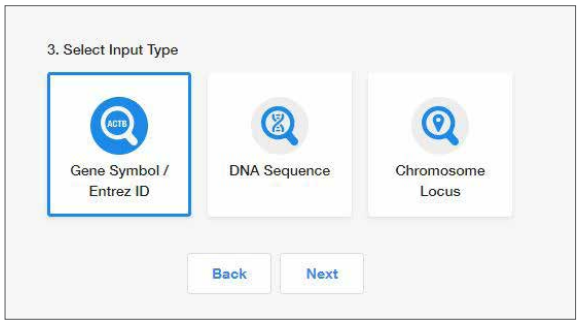
Select the host organism for your knockout experiment and click “Next.”



Step 4:

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

Click “Next.”



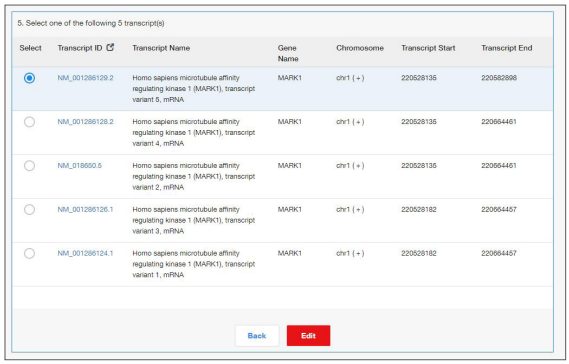
Step 5:

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 visualize 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.

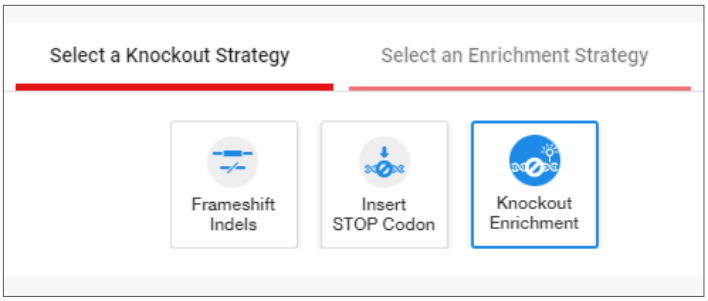
You will be prompted to choose **Frameshift Indels**, **Insert STOP Codon,** or **Knockout Enrichment**.

- 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.

Step 7 (continued)

- 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.
- Knockout enrichment: The optimal method for generating a knockout cell line, this process uses a DNA donor template from the TrueTag Knockout Enrichment 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 bi-allelic knockout.

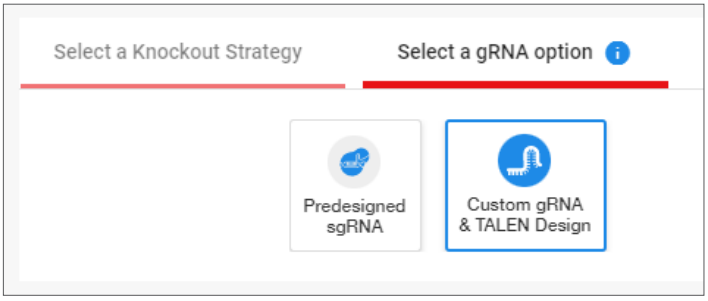
For this workflow example, select **Knockout Enrichment**. Please refer to the workflow guide for causing frameshift indels or for insertion of stop codons for details on those experiment types.



Step 8:

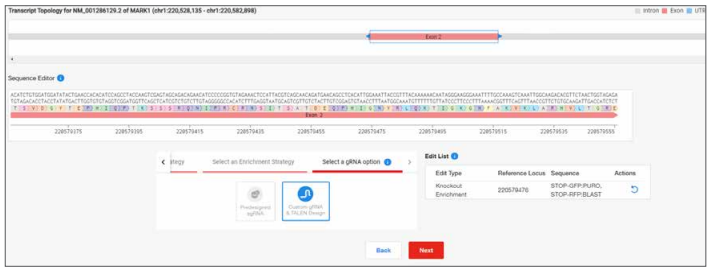
The view will update to **Select a gRNA option**. Click on the sgRNA option that fits your need, either **Predesigned sgRNA** (human and mouse genes only) or **Custom gRNA and TALEN Design**.

If you do not have a preference for the region to insert your knockout enrichment cassette and are working in human or mouse cells, Predesigned sgRNA is recommended. Otherwise, select Custom gRNA and TALEN Design. Most steps will be similar for both workflows.



Step 9:

For custom designs, you will be prompted to select a location on the sequence editor. You can navigate within the transcript from the Topology view by dragging the blue box, using the magnifying glass icons to zoom in and out as needed.



Select a region by clicking on the Sequence Editor view within an exon. The software will search the entire visible 200 bp region for optimal gRNAs.

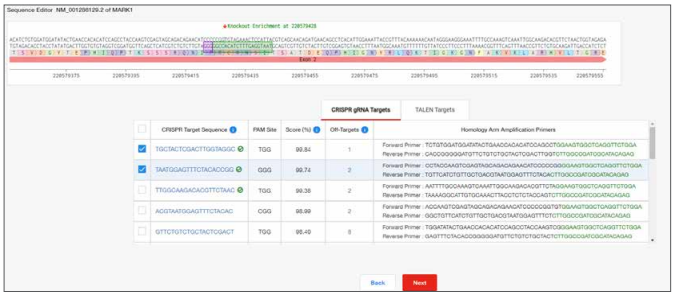
Click “Next” to begin the design process.

Step 10:

When complete, you will see a results 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 table displays:

- The CRISPR gRNA design; green checkmarks indicate recommended gRNAs due to score and specificity
- 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 primers required for PCR amplification and homology arm addition to the Knockout Enrichment donor template



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Step 10 (continued)

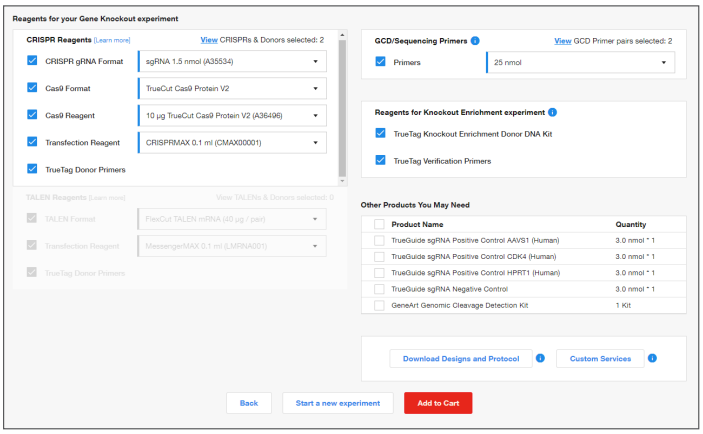
To view the TALEN targets, click on the TALEN tab of the table, and similar information will be displayed for each TALEN pair. TAL effector nuclease (TALEN) pairs are recommended when there are no PAM sites within 10 bp of the knock-in site, or if the efficiency and specificity of the gRNAs are not optimal.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

Step 11:

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

Step 12:

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



For a knockout enrichment experiment using CRISPR technology, the gRNAs and Invitrogen™ TrueTag™ donor primers selected in the design step are automatically included. The tool will also add the TrueTag Knockout Enrichment Donor DNA Kit, which includes the required donor templates, PCR reagents, and cleanup kit to generate your transfection-ready donor DNA. Also included are Invitrogen™ TrueTag™ verification primers to do junction analysis to ensure proper insertion of the donor template.

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

Step 13:

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 “Download Designs.” 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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