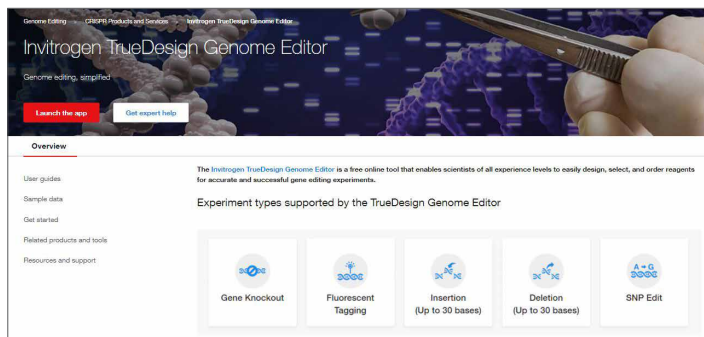# invitrogen

TrueDesign Genome Editor

Workflow guide for creating a SNP edit

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.
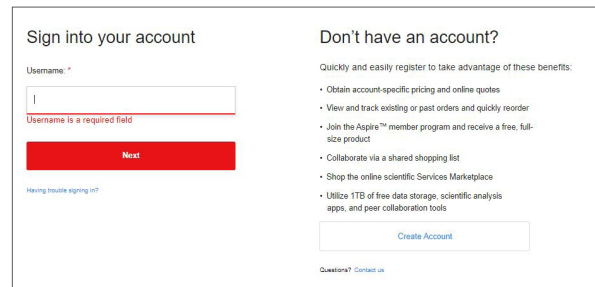
This workflow guide will walk through the steps for creating a single-nucleotide polymorphism (SNP) in a gene transcript of interest.
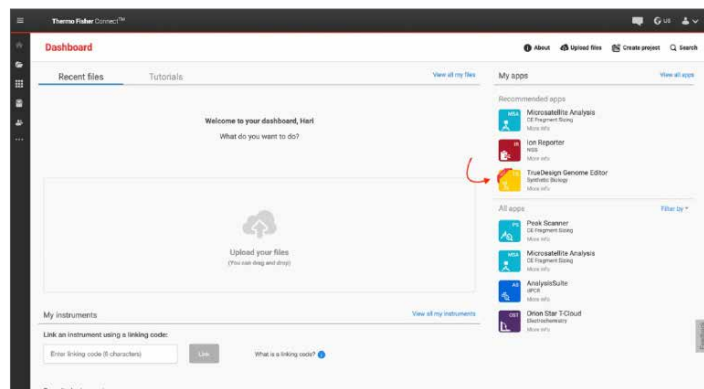
Step 1:

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.
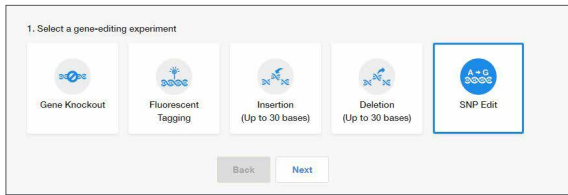

A "Terms of Use" window may pop up. Read the content, scroll to the bottom of the screen, and click "Accept".
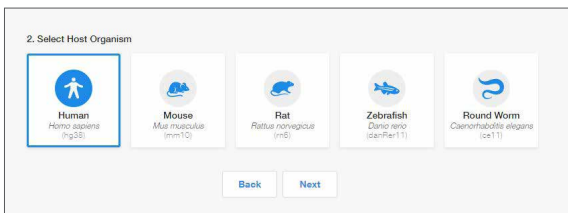
Thermo Fisher
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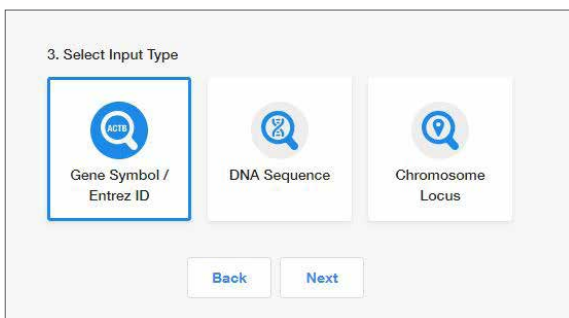In the TrueDesign software, choose **SNP Edit** as your experiment type and click "Next".

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

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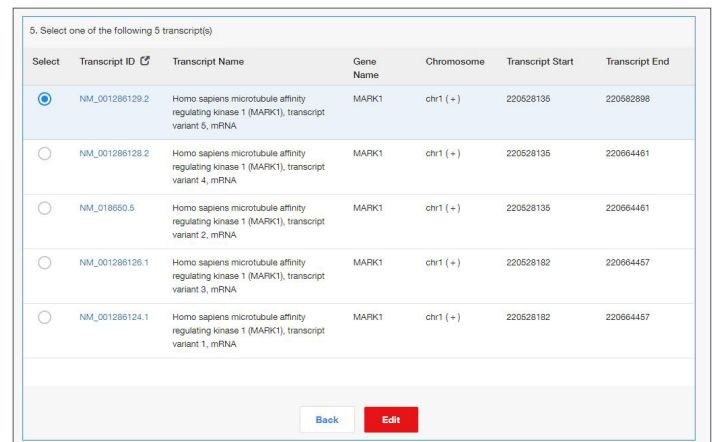
Click "Next".

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 transcript ID hyperlink to be taken to the NCBI website, where you can better visualize the transcript maps.

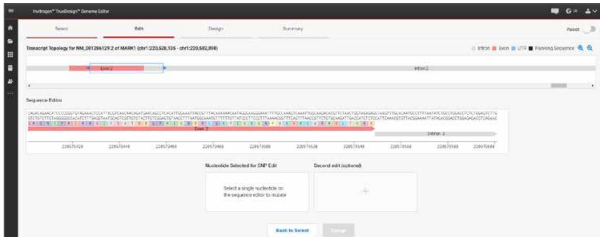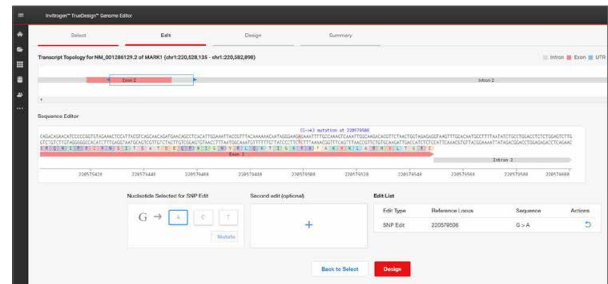After you make a selection, click "Edit".

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. Click and drag the blue box on the transcript topology to navigate to the region where you will create your SNP edit. You may zoom in and out by clicking the magnifying glass icons.
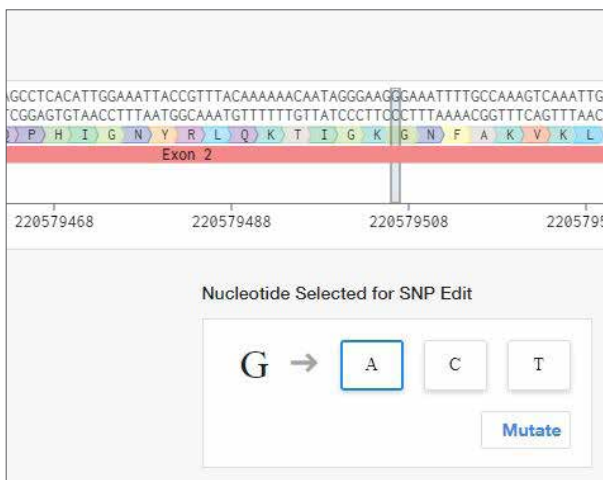

Step 8:

In the sequence editor, click on the single nucleotide you wish to replace. Choose the replacement nucleotide from the selection box.

Click "Mutate".

Step 9:

The view will update to display the nucleotide change, and an edit list will appear to indicate the type of edit and its position. You can undo the change by clicking the blue "undo" arrow in the edit list.

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 10:

When the design step is 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 target region; green checkmarks indicate recommended gRNAs due to score and proximity to the edit site

• The gRNA PAM site

• The gRNA score, which is a weighted algorithm score for efficiency and specificity

• The number of predicted off-targets

• The edit site's distance from the cut site

• The DNA oligo designed as a donor template for the SNP edit

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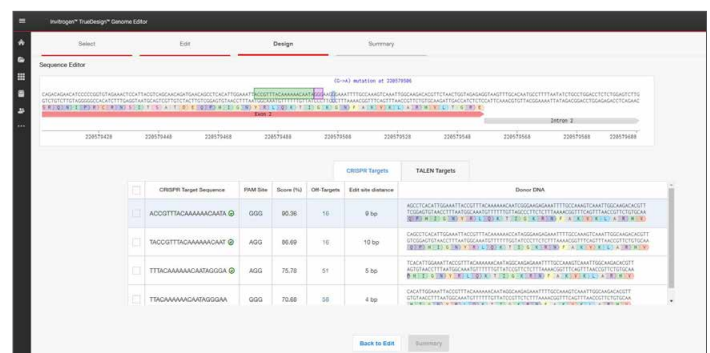
To view the TALEN targets, click on the TALEN tab of the table and similar information will be displayed for each TALEN pair. TAL effector nuclease (TALEN) pairs are recommended when there are no PAM sites within 10 bp of the knock-in site, or if the efficiency and specificity of the gRNAs are not optimal. Green checkmarks in the design results table will indicate the recommended technology. Learn more about TALEN technology at **thermofisher.com/tal**.
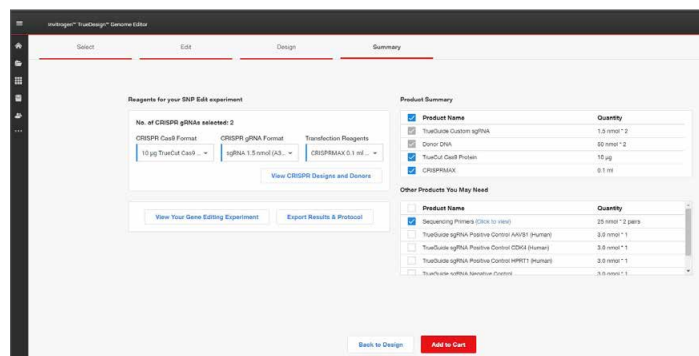
Step 11:

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

Step 12:

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

For a SNP editing 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 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 "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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