

TrueDesign Genome Editor

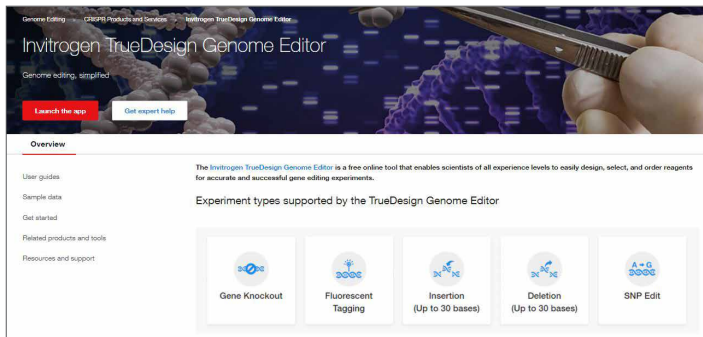
Workflow guide for creating a deletion

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.

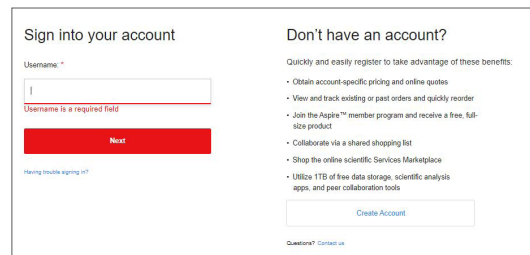
This workflow guide will walk through the steps for creating a deletion of up to 30 bp in a gene of interest. If you want to create a larger deletion, please contact our team at GEMServices@thermofisher.com.

Step 1:

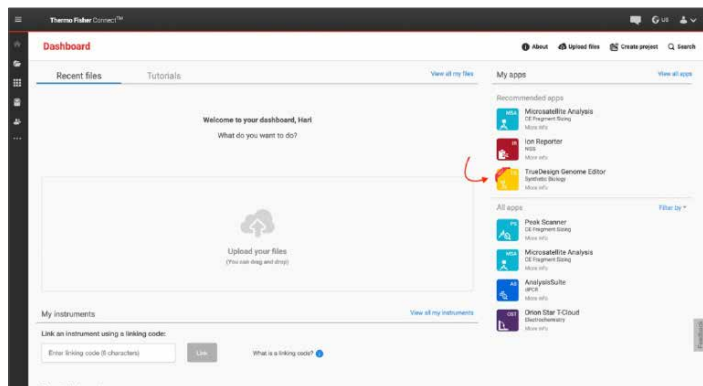
Go to thermofisher.com/truedesign. Select one of the links to launch the software.



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



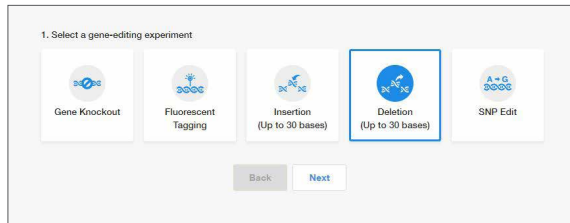
Alternatively, go directly to the **Thermo Fisher™ Connect Platform** and navigate to the TrueDesign Genome Editor.



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

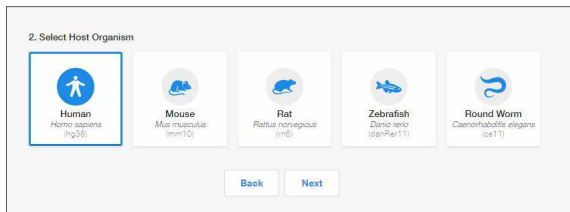
Step 2:

In the TrueDesign software, choose **Deletion** as your experiment type, which allows for deletion of up to 30 bp of DNA, and click “Next”.



Step 3:

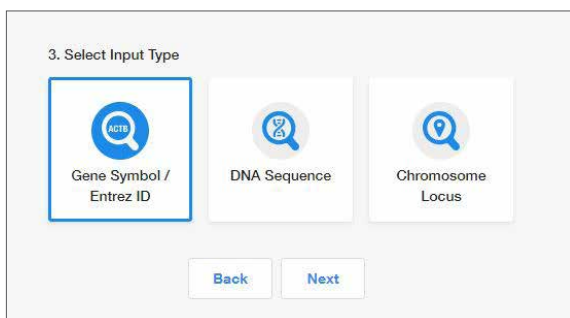
Select the host organism for your deletion experiment and click “Next”.



Step 4:

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”.



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:

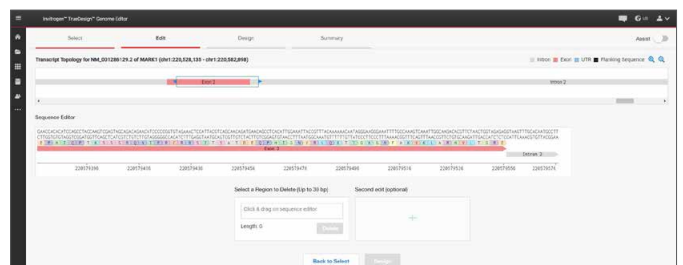
All of the protein-coding transcripts for your selected gene will be displayed. If there is more than one protein-coding transcript and you are unsure of which one to select, click the transcript ID hyperlink to be taken to the NCBI website, where you can better view the transcript maps.

After you make a selection, click “Edit”.

Select	Transcript ID	Transcript Name	Gene Name	Chromosome	Transcript Start	Transcript End
<input checked="" type="radio"/>	NM_001286128.2	Homo sapiens microtubule affinity regulating kinase 1 (MARK1), transcript variant 5, mRNA	MARK1	chr1 (+)	220528135	220682898
<input type="radio"/>	NM_001286128.2	Homo sapiens microtubule affinity regulating kinase 1 (MARK1), transcript variant 4, mRNA	MARK1	chr1 (+)	220528135	220664481
<input type="radio"/>	NM_018600.5	Homo sapiens microtubule affinity regulating kinase 1 (MARK1), transcript variant 2, mRNA	MARK1	chr1 (+)	220528135	220664481
<input type="radio"/>	NM_001286126.1	Homo sapiens microtubule affinity regulating kinase 1 (MARK1), transcript variant 3, mRNA	MARK1	chr1 (+)	220528182	220664467
<input type="radio"/>	NM_001286124.1	Homo sapiens microtubule affinity regulating kinase 1 (MARK1), transcript variant 1, mRNA	MARK1	chr1 (+)	220528182	220664467

Step 7:

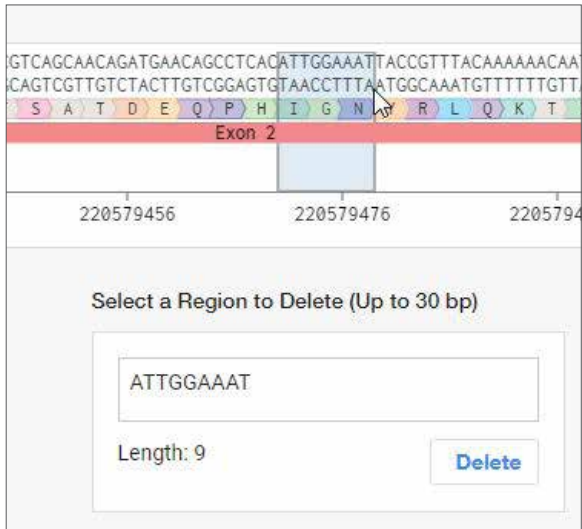
The next screen will display the entire transcript’s topology along the top of the screen, with a zoomed-in sequence-level view below. Click and drag the blue box on the transcript topology to navigate to the region where you will create your edit. You may also zoom in and out by clicking the magnifying glass icons.



Step 8:

In the sequence editor, click and drag, as you would in a text document, the region of genomic sequence to delete. The selected region and its length will be displayed below the sequence editor.

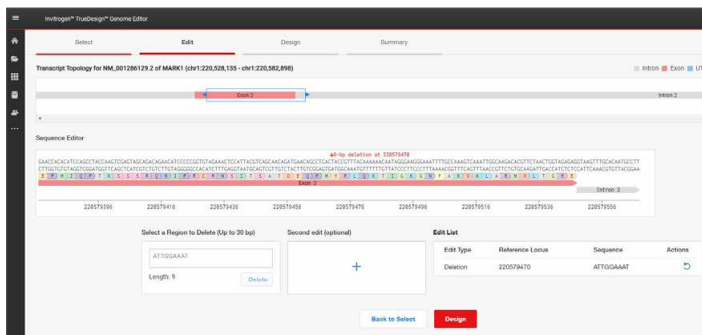
Click “Delete”.



Step 9:

The view will update to show the resulting DNA and amino acid sequence with the location of the deletion indicated by a red arrow. An edit list will also appear to indicate the type of edit, the location, and the sequence of the deleted DNA. 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.

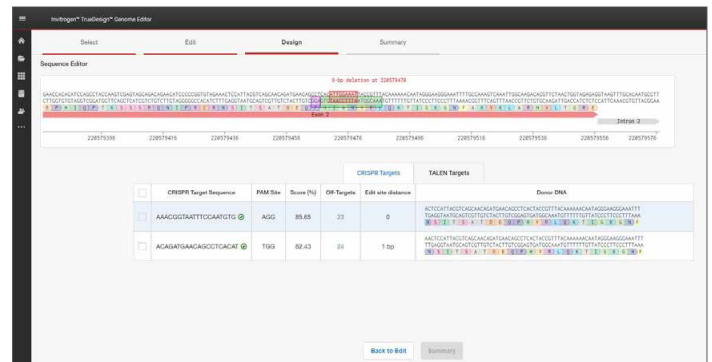


Step 10:

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 deletion site
- The gRNA PAM site
- The gRNA score, which is a weighted algorithm 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 designed as a donor template for the deletion



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 gene editing experiment and give you the opportunity to add additional products to complete your workflow.

For a deletion 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.

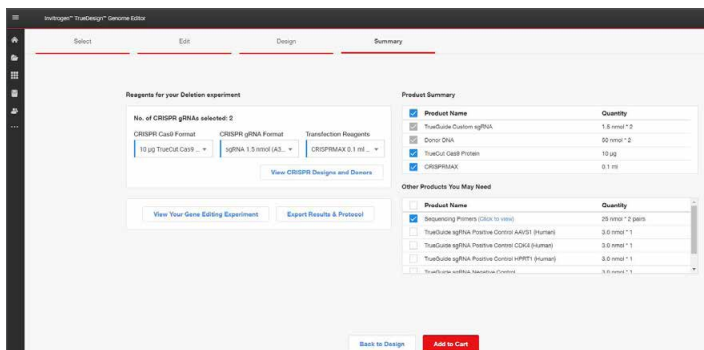
Products may be deselected from the product summary area, or a different quantity may be selected from the drop-down lists.

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.



Get started at thermofisher.com/truedesign