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TrueDesign Genome Editor

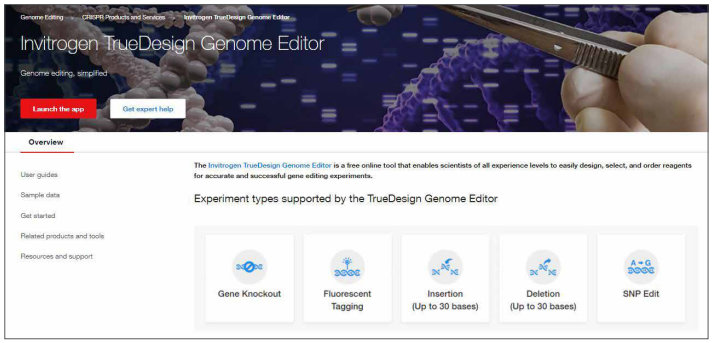
Workflow guide for creating a fluorescent tag

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.

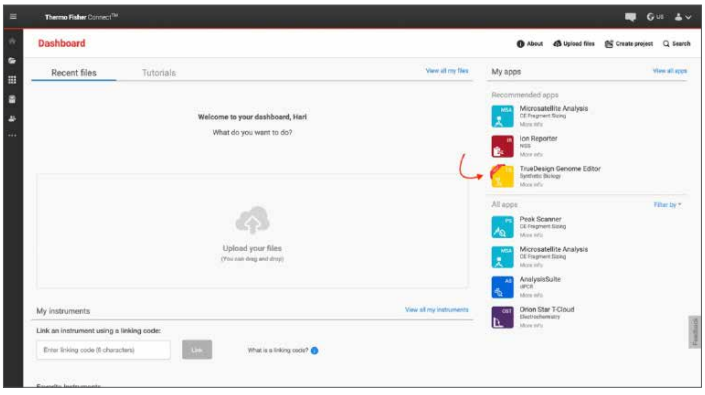
This workflow guide will walk through the steps for adding a fluorescent tag to a gene of interest using Invitrogen™ TrueTag™ DNA Donor Kits. Learn how these kits simplify the process of knocking in a fluorescent tag and enriching tagged cells at **thermofisher.com/truetag**.

Step 1:

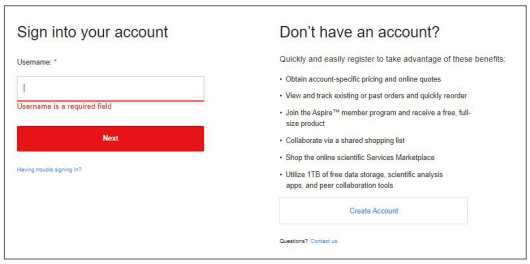
Go to **thermofisher.com/truedesign**. Select one of the links to launch the software.



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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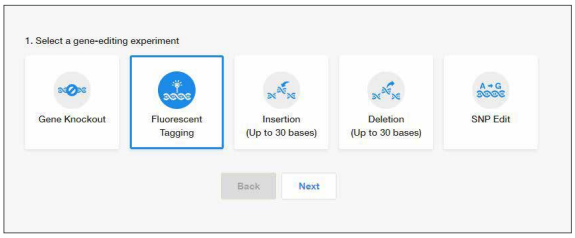
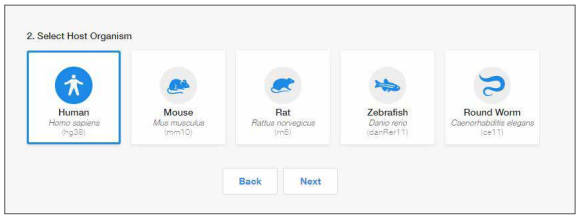
Step 2:

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



Step 3:

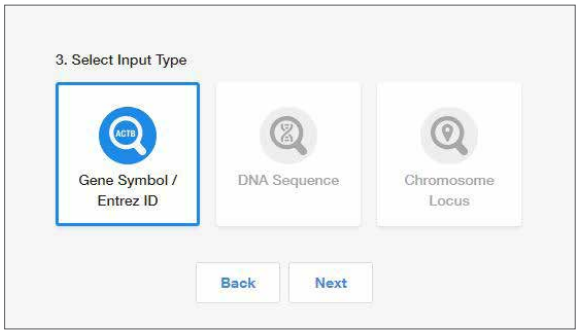
Select the host organism for your tagging experiment and click “Next”.



Step 4:

Select **Gene Symbol/Entrez ID** to identify your gene of interest.

Click “Next”.



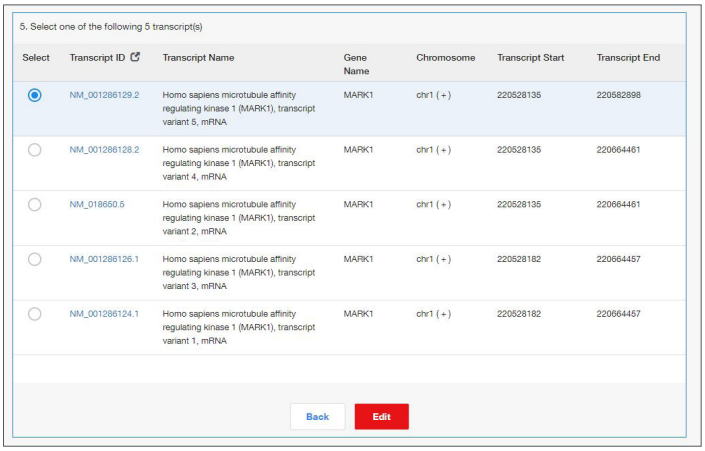
Step 5:

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:

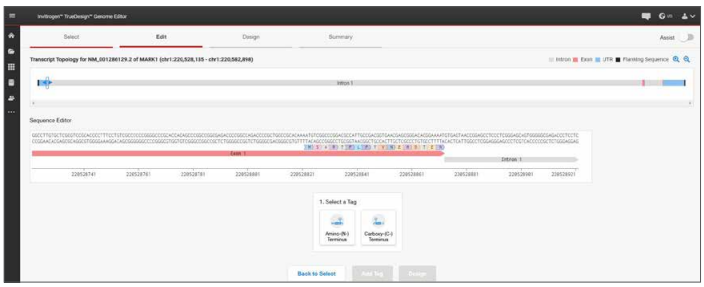
All of the protein-coding transcripts for your selected gene will be displayed. If there is more than one protein-coding transcript and you are unsure of which one to select, click the transcript ID hyperlink to be taken to the NCBI website, where you can better view the transcript maps.

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.

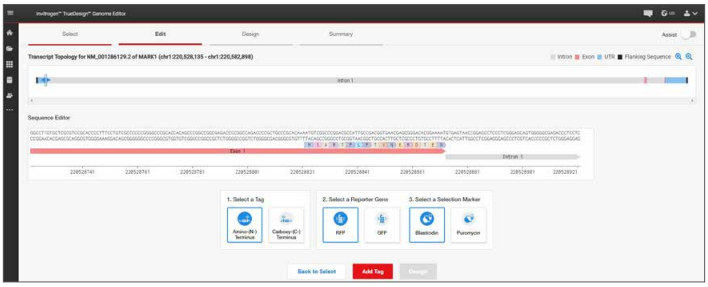


Step 8:

Configure your fluorescent tag by selecting:

- N- or C-terminus
- Red Fluorescent Protein (RFP) or Green Fluorescent Protein (GFP) as the tag
- Blasticidin or puromycin as the antibiotic resistance marker

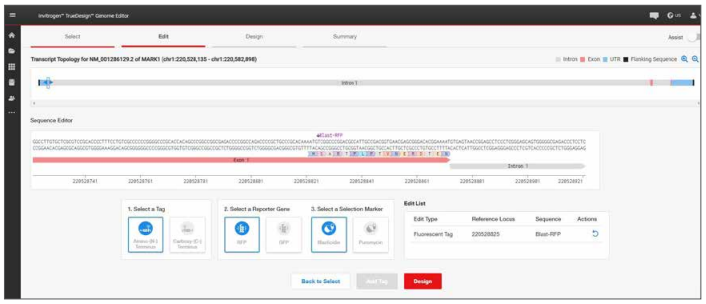
When your selections are complete, click “Add Tag”.



Step 9:

The sequence editor display will update to indicate where your tag will be inserted, and an edit list will appear to indicate the configured insertion. If you wish to change any of the parameters, click the blue “undo” arrow in the edit list. If everything is correct, 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.

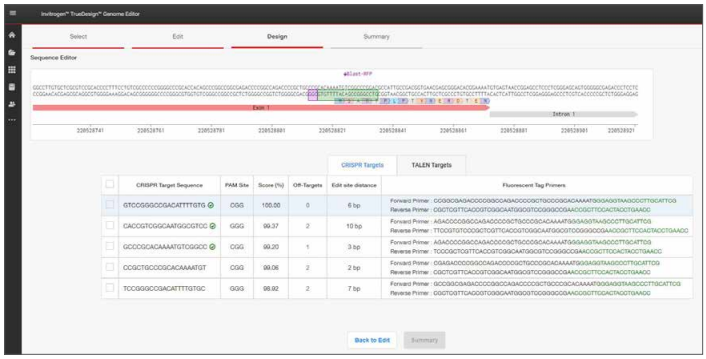


Step 10:

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 insertion 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 primers with homology arms required to generate a full-length donor DNA using the template and components included in the TrueTag Donor DNA Kit
  - Learn more about TrueTag Donor DNA Kits at **thermofisher.com/truetag**



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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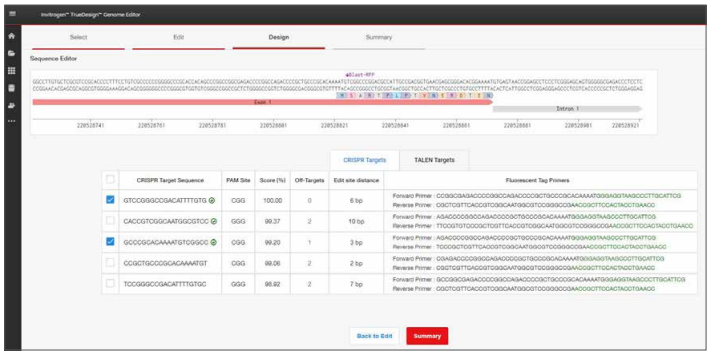
Step 11:

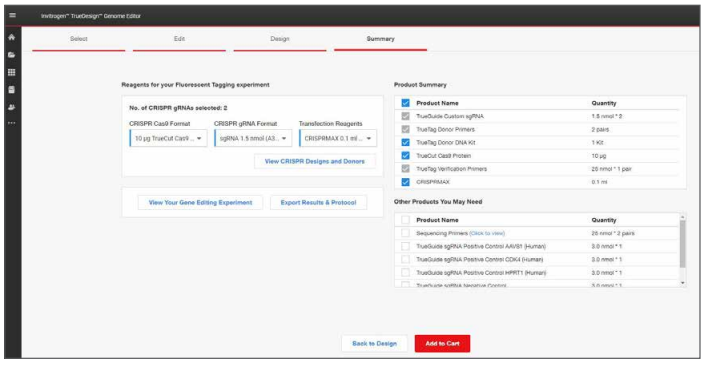
To select one or more CRISPR gRNAs or TALEN pairs to add to your experiment, use the checkboxes in the table and click “Summary”.



Step 12:

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

For a tagging experiment, the appropriate TrueTag Donor DNA Kit, Invitrogen™ TrueCut™ Cas9 Protein v2, Invitrogen™ Lipofectamine™ CRISPRMAX™ Cas9 Transfection Reagent, gRNA, and all required primers are added.



Step 13:

Use the checkboxes and add additional items such as sequencing primers (to assess the CRISPR-Cas9 cutting efficiency of your gRNA) 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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