# TrueDesign Genome Editor

### Workflow guide for creating a fluorescent tag

The Invitrogen<sup>™</sup> TrueDesign<sup>™</sup> 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<sup>™</sup> TrueTag<sup>™</sup> 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.



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

Sign into your account	Don't have an account?
Username: *	Quickly and easily register to take advantage of these benefit
	<ul> <li>Obtain account-specific pricing and online quotes</li> </ul>
	View and track existing or past orders and quickly reorder
Username is a required field	<ul> <li>Join the Aspire<sup>™</sup> member program and receive a free, full- size product</li> </ul>
Next	<ul> <li>Collaborate via a shared shopping list</li> </ul>
	<ul> <li>Shop the online scientific Services Marketplace</li> </ul>
Having trouble signing in?	<ul> <li>Utilize 1TB of free data storage, scientific analysis apps, and peer collaboration tools</li> </ul>
	Create Account
	Destruct Colors

Alternatively, go directly to the **Thermo Fisher<sup>™</sup> 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".



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".

	8	Q
Gene Symbol / Entrez ID	DNA Sequence	Chromosome Locus

#### 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".

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MARK2,EMK-1,EMK1,PAR-1,Par-1b,Par1b 2011		-
MARK1,MARK,Par-1c,Par1c		
MARK3,CTAK1,KP78,PAR1A,Par-1a,VIPB 4140		
TAOK1 KEC-B MAP3K16 MARKK PSK-2 PSK2 TAO1 bKEC-B bTAOK1		

#### 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
۲	NM_001286129.2	Homo sapiens microtubule affinity regulating kinase 1 (MARK1), transcript variant 5, mRNA	MARK1	chr1 (+)	220528135	220582898
	NM_001286128.2	Homo sapiens microtubule affinity regulating kinase 1 (MARK1), transcript variant 4, mRNA	MARK1	chr1 (+)	220528135	220664461
	NM_018650.5	Homo sapiens microtubule affinity regulating kinase 1 (MARK1), transcript variant 2, mRNA	MARK1	chr1 (+)	220528135	220664461
	NM_001286126.1	Homo sapiens microtubule affinity regulating kinase 1 (MARK1), transcript variant 3, mRNA	MARK1	chr1 (+)	220528182	220664457
	NM_001286124.1	Homo sapiens microtubule affinity regulating kinase 1 (MARK1), transcript variant 1, mRNA	MARK1	chr1 (+)	220528182	220664457

#### Step 7:

The next screen will display the entire transcript's topology along the top of the screen, with a zoomed-in sequencelevel view below.

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#### 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".

		Design	Summary			Assis
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#### 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<sup>™</sup> pairs and CRISPR gRNA target regions and check them for specificity.

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#### 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

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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**.

## invitrogen

#### 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".

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#### 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<sup>™</sup> TrueCut<sup>™</sup> Cas9 Protein v2, Invitrogen<sup>™</sup> Lipofectamine<sup>™</sup> CRISPRMAX<sup>™</sup> Cas9 Transfection Reagent, gRNA, and all required primers are added.

bened	Edit Design	Sum	nary		
	Reagents for your Fluorescent Tagging experiment		Product Surr	mary	
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#### 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<sup>™</sup> Excel<sup>™</sup> file contains multiple tabs that include all the designs generated by the software, plus all of the genespecific experimental details and ordering information.





### Get started at thermofisher.com/truedesign

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