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TrueDesign Genome Editor

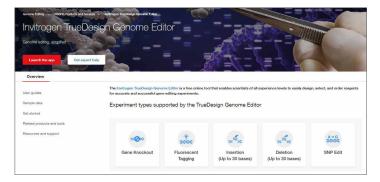
Workflow guide for creating a SNP edit

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.



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 benefits
	Obtain account-specific pricing and online quotes
1	 View and track existing or past orders and quickly reorder
Username is a required field	 Join the Aspire[™] member program and receive a free, full- size product
Next	 Collaborate via a shared shopping list
	 Shop the online scientific Services Marketplace
Having trouble signing in?	 Utilize 1TB of free data storage, scientific analysis apps, and peer collaboration tools
	Create Account
	Quantora? Contact us

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

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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 **SNP Edit** as your experiment type and click "Next".



Step 3:

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

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*	2				2
Human Homo sapiens (hg38)	Mouse Mus musculus (mm10)	Rattus /	Rat norvegicus m6)	Zebrafish Danio rerio (danRer11)	Round Worm Caenorhabolitis elegans (ce11)
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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".

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Gene Symbol / Entrez ID		equence	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".

mark	×	Search Gen
MARK2,EMK-1,EMK1,PAR-1,Par-1b,Par1b 2011	*	
MARK1,MARK,Par-1c,Par1c		
MARK3,CTAK1,KP78,PAR1A,Par-1a,VIPB 4140		
TAOK1,KFC-B,MAP3K16,MARKK,PSK-2,PSK2,TAO1,hKFC-B,hTAOK1	-	

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".

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 saplens 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 saplens 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. 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.

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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".

CGGAGTGTAACCTTT	AATGGCAAATGTTTTTTGTT	TAGGGAAGGGAAATTTTGC ATCCCTTCCCTTTAAAACG	GTTTCAGTTTA
/ F / H / J / G / N	Exon 2	I O K O N F A	K V/KI
220579468	220579488	220579508	22057
	Nucleotide Se	lected for SNP Edit	
	$G \rightarrow$	A C	T

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.

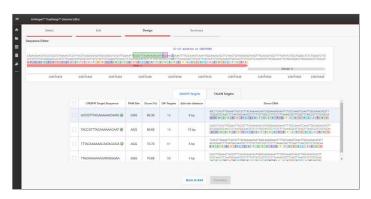


Step 10:

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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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 "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 dropdown lists.

Select	Edit Design	Sum	navy	C	
	Reagents for your SNP Edit experiment		Produ	at Summary	
	No. of CRISPR gRNAs selected: 2			Product Name	Quantity
	CRISPR Case Format CRISPR gRNA Format	Transfection Reagents	82	TrueOurce Custom sgRNA	1.5 nmol * 2
			8	Donor DNA	50 nmol * 2
	10 µg TrueCut Cas9 _ = sgRNA 1.5 nmol (A3. =	CRISPRMAX 0.1 ml +		TrueCut Casil Protein	10 µg
	View CBIS	PR Designs and Denore		CRISPRMAX	0.1 ml
			Other	Products You May Need	
				Product Name	Quantity
	View Your Gene Editing Experiment Expo	rt Results & Protocol		Sequencing Primers (Click to view)	25 nmol * 2 pers
				TrueGuide sgRNA Positive Control AAV31 (Human)	3.0 nmol * 1
				TrueGuide sgRNA Positive Control CDK4 (Hamar)	3.0 cmol * 1
				TrueDuide sgRNA Positive Control HPRT1 (Human)	3.0 mmol * 1
				Truefessie softblik Neostrue Control	3.0 onoi 1 1

Get started at thermofisher.com/truedesign

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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 genespecific experimental details and ordering information.



