TrueDesign Genome Editor

Workflow guide for creating a gene knockout by causing frameshift indels

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 gene knockout using imperfect repair following a double-stranded break by a nuclease. The resulting insertions or deletions (indels) can cause frameshifts or premature stop codons to cause functional knockout of the target gene.

Please note: Algorithm-designed CRISPR gRNAs optimized for functional knockouts are available for all human and mouse genes, and may be ordered directly from **thermofisher.com/trueguide**.

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?
Usemame: *	Quickly and easily register to take advantage of these benefits
T	Obtain account-specific pricing and online quotes
A	 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 algoing in?	 Utilize 1TB of free data storage, scientific analysis apps, and peer collaboration tools
	Create Account
	Disation? Contact in

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

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





Step 2:

In the TrueDesign software, choose **Gene Knockout** as your experiment type, and click "Next".

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Gene Knockout	Fluorescent	Insertion (Up to 30 bases)	Deletion (Up to 30 bases)	SNP Edit

Step 3:

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



Step 4:

Select **Gene Symbol/Entrez ID** to identify your gene of interest. You may also identify 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".

mark	×	Search Gene
MARK2,EMK-1,EMK1,PAR-1,Par-1b,Par1b 2011	1	
MARK1,MARK,Par-1c,Par1c		
MARK3,CTAK1,KP78,PAR1A,Par-1a,VIPB 4140		
TADK1 KEC-B MAP3K16 MARKK PSK-2 PSK2 TAD1 5KEC-B 5TADK1		

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 saplens 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 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.

You will be prompted to choose Frameshift Indels or Insert STOP Codon.



- Frameshift indel: The most common approach for gene knockout, this process relies on imperfect repair following a double-stranded DNA break. You will be required to select a region on the transcript to search for custom CRISPR and TALEN[™] designs.
- Insert stop codon: A more precise method for knockout of gene expression, this process uses a DNA donor to insert multiple stop codons to prevent translation. By default, this insertion will be designed adjacent to the transcriptional start site.

Step 7: (continued)

For this workflow example, select **Frameshift Indels**. Please refer to the workflow guide for insertion of stop codons for details on that experiment type.

Step 8:

If your selected species is human or mouse, you will be prompted to choose between predesigned synthetic gRNA (sgRNA) or custom designs. Selecting the **Predesigned sgRNA** option will retrieve algorithm-designed Invitrogen[™] TrueGuide[™] Synthetic gRNAs for your gene, let you view them along the transcript, and give you the chance to order genomic cleavage detection and sequencing primers. For more information on TrueGuide Synthetic gRNA, go to **thermofisher.com/trueguide**.

For all other species, or for custom human or mouse designs targeting a specific region of interest, choose **Custom gRNA & TALEN Design**.



Step 9:

You will be prompted to select a location on the sequence editor. Click and drag the blue box on the transcript topology to navigate to the region where you wish to create an indel. This region will be displayed at the DNA level in the sequence editor. Click on the sequence editor to indicate the indel site. A blinking cursor will indicate your selection. The software will use this location as the anchor point for designing custom CRISPR gRNAs and TALEN pairs. Typically, designs are made to target an early exon or may be directed to a known functional region of your gene.

Select	Edit	Design	Summary	
Transcript Topology for NM_001286	129.2 of MARK1 (chr1:220,528,135	- chr1:220,582,898)		
		}	Infron 2	-
e.				
Sequence Editor				
GCAGACAGAACATCCCCCGGTGTAGAAACTCC CGTCTGTCTTGTAGGGGGGCCACATCTTTGAGG S RIQINII PIRICIRINIS	ATTACGTCAGCAACAGATGAACAGCCTCACA TAATGCAGTCGTTGTCTACTTGTCGGAGTGT I T S A T O E QIP)H	TTGGAAATTACCGTTTACAAAAAAAAAAAAAAAAAAAAA	GAAGGGAAATTTTGCCAAAGTCAAATTGGCAAGAC CTTCCCTTTAAAAGGGTTTCAGTTTAACCGTTCTG K) G N) F A K) Y K) L) A R)	ACGTTCTAACTGGTAGAGAGAGGTAA TGCAAGATTGACCATCTCTCCATT
220579427	220579447 220579467	220579487	220579507 220579527	220579547

Step 10:

An edit list will appear, displaying the type and selected location of the edit. You can undo this edit by clicking the blue "undo" arrow in the edit list.

When you are satisfied with your changes, 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 11:

When 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 design; 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 score for efficiency and specificity
- The number of predicted off-targets; click the hyperlink to display a pop-up window with detailed location and mismatch information for the potential off-targets
- The edit site's distance from the cut site

20579	9466 2.	i6 220579486 220579506			220579526	220579546	
		CRISPR Ta	rgets	TALEN Ta	rgets		
	CRISPR Targ	et Sequence	PAM Site	Score (%)	Off-Targets	Edit site distance	
	ACCGTTTACAA		GGG	90.36	16	3 bp	
	TACCGTTTACA		AGG	86.69	16	4 bp	

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 designated target location, 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 12:

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 13:

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

For an indel frameshift experiment using CRISPR technology, the gRNAs 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 dropdown lists.

Respects for your Scow Rockets experiment Product Summary Ne. of CHSM gRNAs assesses 2 USPECTATION USPECTATIO USPECTA	ry Neme Quantity & Calton sg/tkk 1.5 mma* 2
Ns. of CHSPR gRNA selence 2 CRSPR Graf Forest CRSPR Graf Forest Upg TheOLEGET+ gRNA 1.5mm(J.O. + CRSPR Graf Designs of Designs Vesc CRSPR Designs of Designs CRSPR CRSPR CR	Name Quantity © Custom sgRNA 1.5 nmol * 2
CHEFF Up Case Farmer CHEFF Up Case Farmer Up processor Up	e Custom sg/RNA 1.5 rmol * 2
Castral Castral yound Castral of MAX some Instantion Insuperti 18 gp Trecht Castral + spSNA 15 mml (AL. + Castral -	2212 C
13 lig Truccut Cerely v spBNA 1.5 mmt (AS v CRUPPBAX 0.1 mt v CRUPPBAX 0.1 mt v CRUPPBAX 0.1 mt v Other Products Ye	ase Protein To µg
View CRIGPRI Designs and Denters Other Products Ye	NAX 0.1 ml
	You May Need
Preduct Na	Name Quantity
Sequencing	ng Primera (Click to view) 25 mmol * 2 pairs
View Your Gene Editing Experiment Export Results & Protocol TrueOutors	e sg/INA Positive Control A42/51 (Human) 3.0 mitol * 1
ToeOutde to	e sgRNA Positive Control CDK4 (Human) 3.0 mmol * 1
ToeGude to	e sgRNA Positive Control HPRTT (Human) 3.0 nmol * 1
Tradicise	a solitică Navativa Control 3.0 ovori * 1

Step 14:

You may use the checkboxes to select additional items such as sequencing primers, to verify your indel, 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.





Get started at thermofisher.com/truedesign

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