



OmniGuide RNA Design Tool Protocol

Enabling Easy & Precise Design

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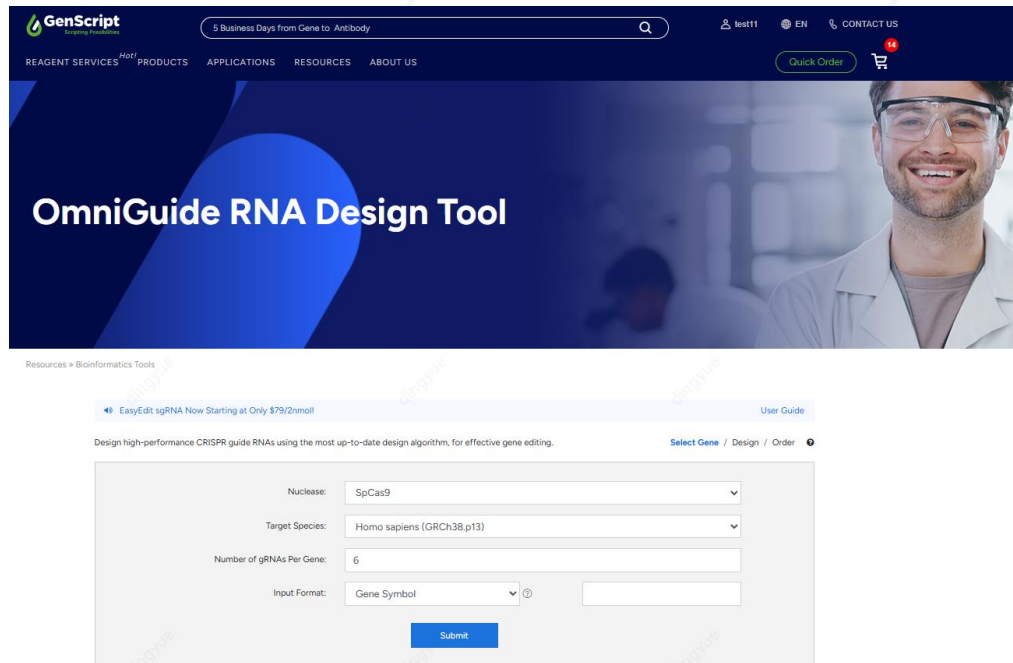
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Please send an Email to crispr@genscript.com for additional support

Applications & Advantages – OmniGuide RNA Design Tool

What is OmniGuide RNA design tool?

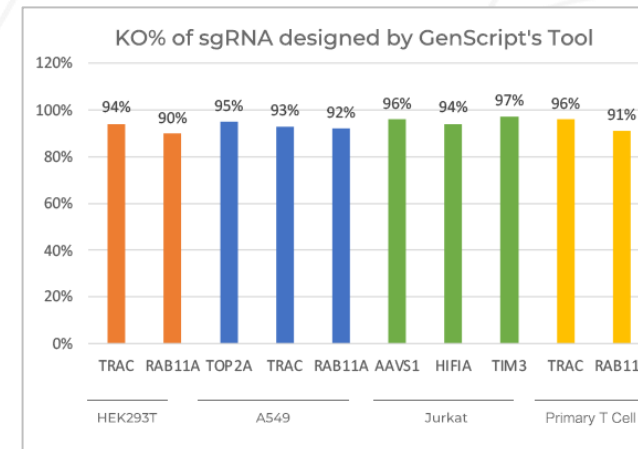
- Design gRNA sequences for CRISPR knockout experiments, provide gRNA parameters, and enable downstream OmniGuide RNA ordering



The screenshot shows the GenScript website's OmniGuide RNA Design Tool interface. The header includes the GenScript logo, a search bar, and navigation links. The main banner features the text "OmniGuide RNA Design Tool" and a smiling scientist. Below the banner, there's a section for "EasyEdit sgRNA" with a price of \$79/2nmol. The design tool form includes fields for "Nuclease" (set to SpCas9), "Target Species" (set to Homo sapiens), "Number of gRNAs Per Gene" (set to 6), and "Input Format" (set to Gene Symbol). A "Submit" button is at the bottom of the form.

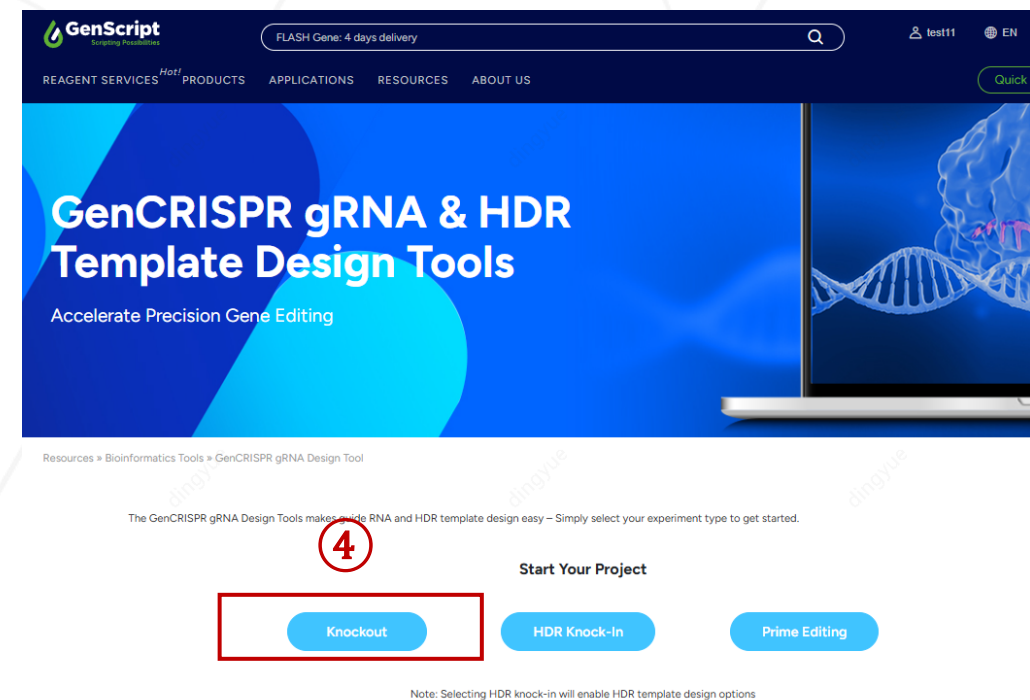
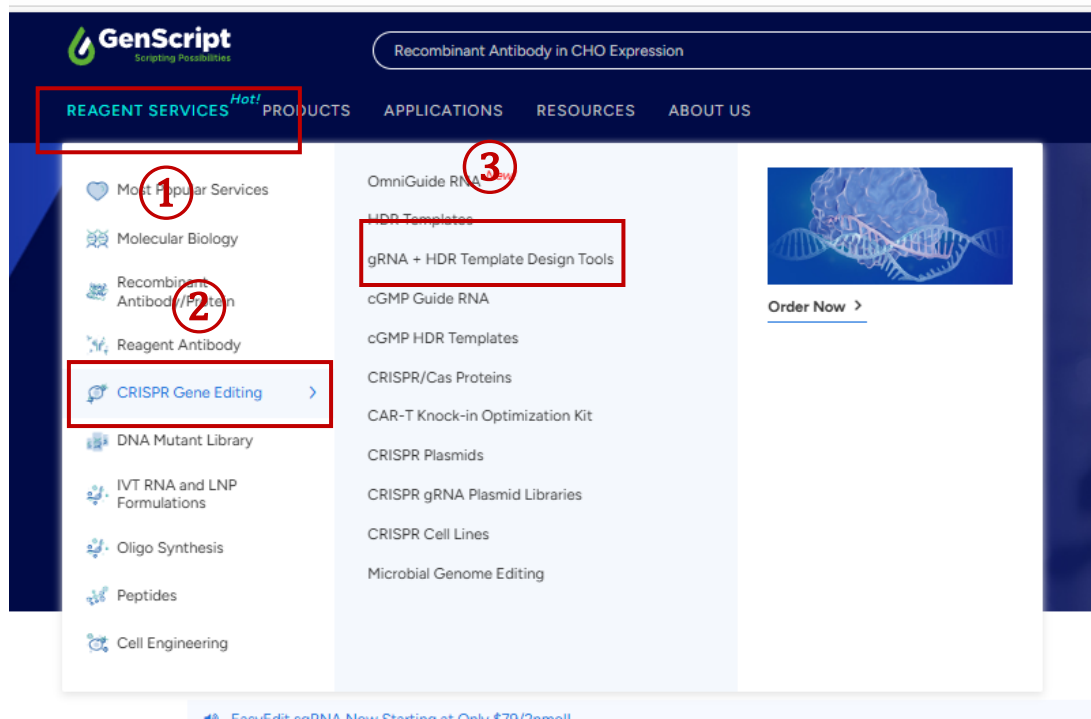
Advantages of OmniGuide RNA design tool

1. **Comprehensive applications:** Support 14 species, Cas9, Cas12a base editing, and prime editing
2. **More precise design:** updated on-target and off-target scores
3. **Enhance editing efficiency:**
 - Designs target early exons to avoid truncated functional proteins
 - Higher transcripts coverage
 - Ideal GC% for gRNA
4. **Validated efficiency:** Indel% up to 97% validated by experiments

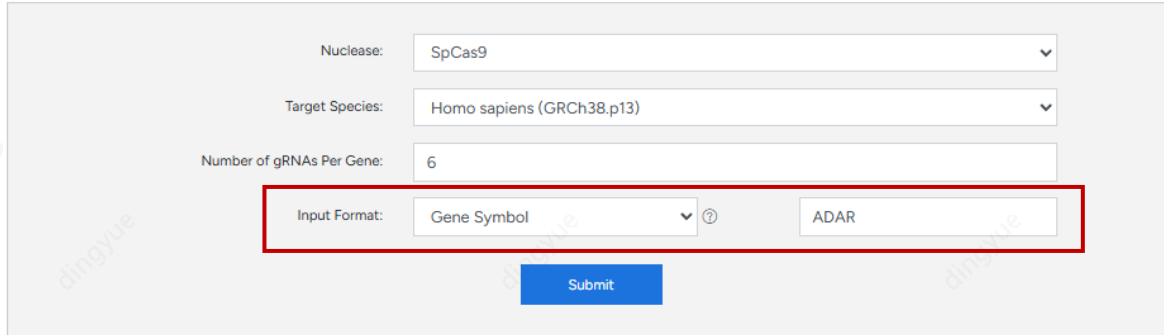


Where can we find OmniGuide RNA Design Tool?

1. Visit the address: <https://www.genscript.com/tools/gRNA-design-tool>
2. Find the tool in [GenScript website](#):



Cas9 / Cas12 Design Processes



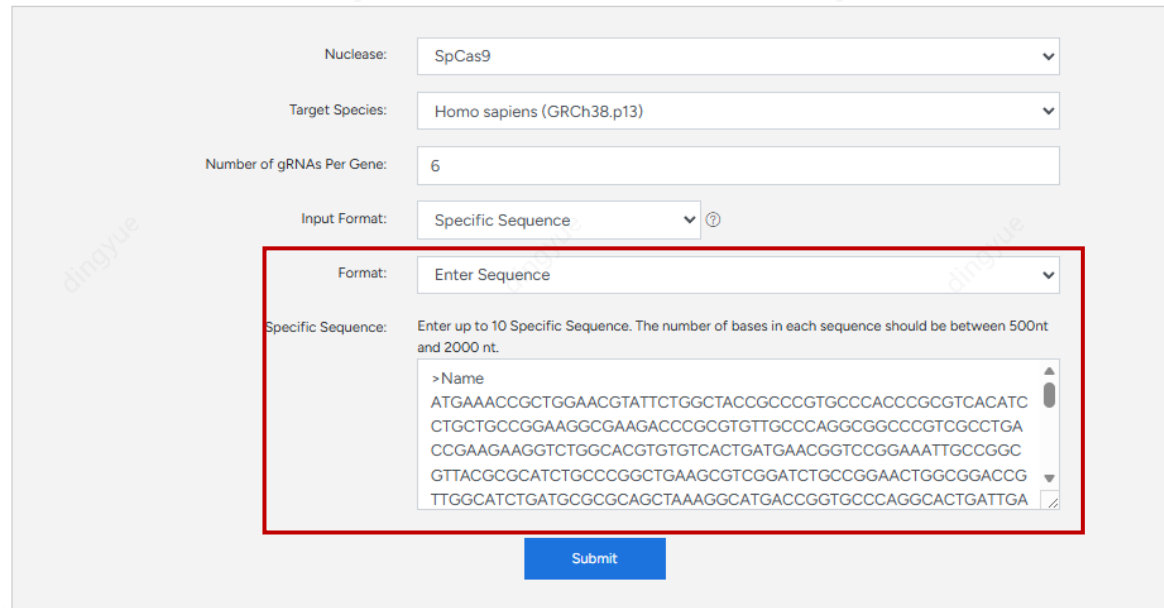
Nuclease: SpCas9

Target Species: Homo sapiens (GRCh38.p13)

Number of gRNAs Per Gene: 6

Input Format: Gene Symbol

Submit



Nuclease: SpCas9

Target Species: Homo sapiens (GRCh38.p13)

Number of gRNAs Per Gene: 6

Input Format: Specific Sequence

Format: Enter Sequence

Specific Sequence: Enter up to 10 Specific Sequence. The number of bases in each sequence should be between 500nt and 2000 nt.

> Name
ATGAAACCGCTGGAACGTATTCTGGCTACCGCCCGTGCCACCCGCGTCACATC
CTGCTGCCGGAAGGCGAAGACCCCGCTGTTGCCAGGCGGCCGTCGCCTGA
CCGAAGAAGGTCTGGCACGTGTCTACTGATGAACGGTCCGGAAATTGCCGGC
GTTACGCGCATCTGCCCGGCTGAAGCGTCGGATCTGCCGGAACGGCGGACCG
TTGGCATCTGATGCGCGCAGCTAAAGGCATGACCGGTGCCAGGCACTGATTGA

Submit

Step 1. Enter your request

1. Select Nuclease / Species

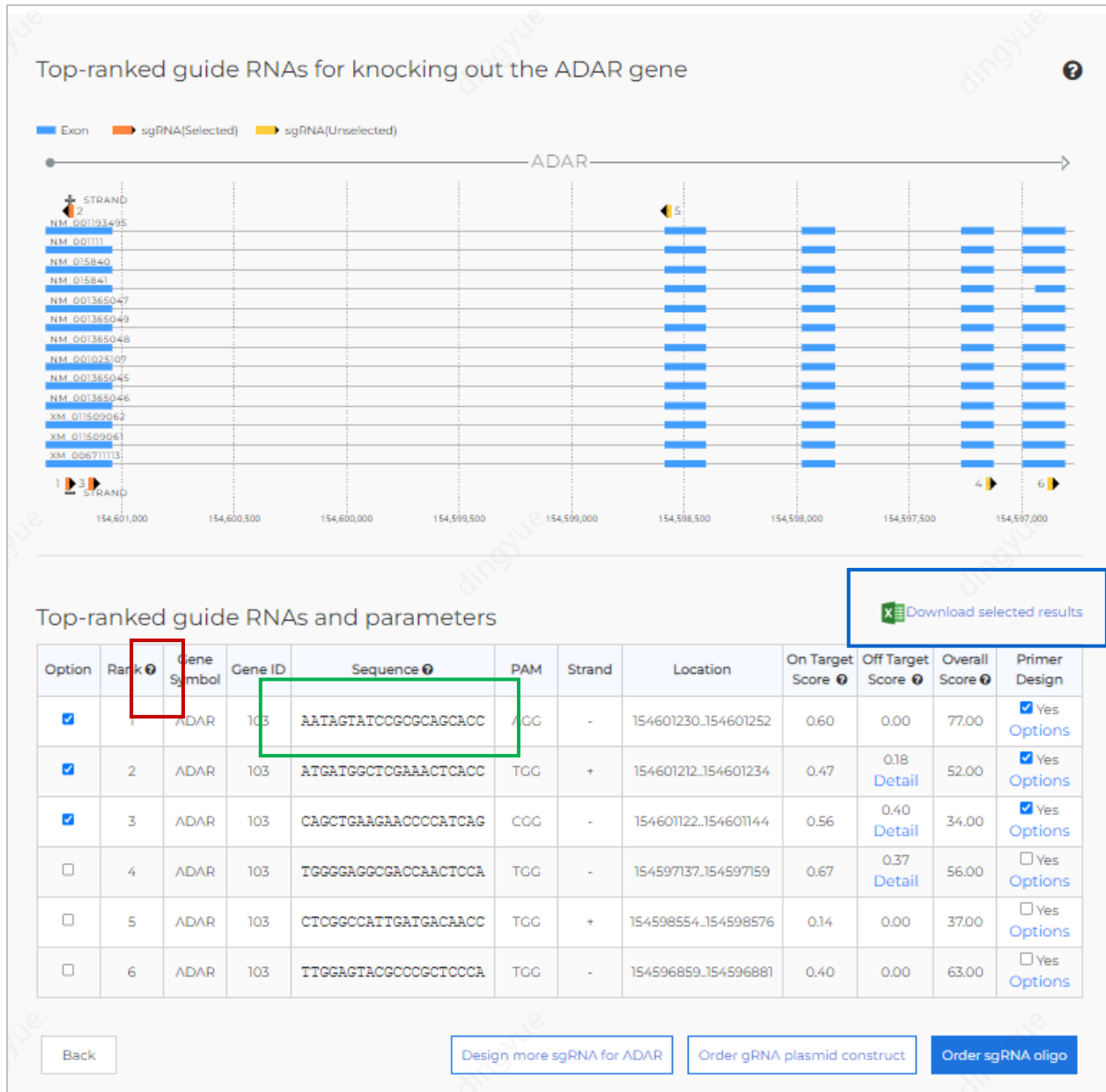
2. Choose Input Format

- Gene Symbol / Gene ID (the image above)
- Specific Sequence (the image below)
 - 500-2000 nt
 - Sequence format (enter directly or upload file)
>Name
ATCGATGAGATGA
 - Up to 10 specific sequence, more sequences lead to slower designs

3. Click “submit”



Cas9 / Cas12 Design Processes (for gene symbol / ID)



Step 2. Select your sequences

1. We recommend top 3 sequences for one gene
2. Selected desired sgRNA and click “order sgRNA oligo” for chemical synthetic sgRNA or “order gRNA plasmid construct”

Parameter's introduction

- On target score (from -1.5 to 1.5): higher score means higher editing efficiency. Some gRNAs have penalty factors that lead to negative On-Target Scores. Below 0.2 will be marked in red, typically indicating low editing efficiency. Consider using them only if no better options are available
- Off target score (from 0 to 1): lower score means lower off target effects
- Over all score: higher score means higher on target score, lower off target score and cover more transcripts
- Primer design: we provide primer design for verifying editing efficiency. Click "Option" to select or deselect primers
- **Ranking (most comprehensive evaluation)**: Higher over all score and target earlier exon to avoid truncated functional protein

Notes:

- Click the black question marks to see the explanations (red labeled box)
- Click the sequence to view its position in sequence map (green labeled box)
- Click “download selected results” to download the sequences (blue labeled box)

Cas9 / Cas12 Design Processes (for Specific Sequences)

Top-ranked guide RNAs for knocking out

Jump to Gene: Select a gene...

Gene: Gene 1

Target Sequence sgRNA (Selected) sgRNA (Unselected)

Gene 1

Top-ranked guide RNAs and parameters

Option	Rank	Sequence Name	Sequence	PAM	Strand	Location	On Target Score	Off Target Score	Crossed Score	Primer Design
☒	1	Gene 1	AGGAGCTGGCCCGAGGGTCA	GGG	-	8,30	0.81	0.29 Detail	42/50	
☒	2	Gene 1	GCCCGGAGAGGCTTGGGCA	TGG	-	26,57	0.96	0.00	67/50	
☒	3	Gene 1	AGCGGCAGCCGTTGGGGA	CGG	-	62,84	0.92	0.07 Detail	60/50	

Show More >>

Gene 2: Gene 2

Target Sequence sgRNA (Selected) sgRNA (Unselected)

Gene 2

Top-ranked guide RNAs and parameters

Option	Rank	Sequence Name	Sequence	PAM	Strand	Location	On Target Score	Off Target Score	Crossed Score	Primer Design
☒	1	Gene 2	GCGGATCAACGGAGCCGG	CGG	-	21,42	0.77	0.13 Detail	70/50	
☒	2	Gene 2	ACAGCGATCAACGGAGCC	GGG	-	26,46	0.97	0.04 Detail	56/50	
☒	3	Gene 2	TCCGCGATGATCGGATGA	CGG	+	28,47	1.13	0.00	106/50	

Show More >>

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[Design more sgRNAs](#) [Order gRNA plasmid construct](#) [Order sgRNA oligo](#)

Step 2. Select your sequences

1. We recommend top 3 sequences for one gene
2. Selected desired sgRNA and click “order sgRNA oligo” for chemical synthetic sgRNA or “order gRNA plasmid construct”

Parameter's introduction & Notes

- Parameter meanings, comments, and result downloads are the same as on the previous page ([Page 5](#))

How to find multiple design results for multiple target sequences?

- Check target sequence names and design results. (red labeled box)
- Select a name from the dropdown for quick access to design results. (green labeled box)
- Click "Show More" for additional gRNA designs. (blue labeled box)

Cas9 / Cas12 Design Processes

 **GenScript**
Make Research Easy

test11 [CONTACT US](#) [MY ORDER](#)  12

sgRNA Ordering (* Required Fields) [Information](#) [Cart](#) [Confirm Order](#) [Result Feedback](#)

* Delivery Format: Dry Powder

* Format: Single Tubes

Enter the sgRNA sequence(s) into the spreadsheet below. [Clear Table](#)

	* Name	* Input Sequence	Final sgRNA Sequence	Length	*Quantity	*Purity	*Aliquoting Into
1	ADAR-1	AATAGTATCCCGCAGCACC	mA*mA*mU*rArGrUrArCrCrGrCrGrCrArGrC	20 nt	2 nmol	EasyEdit	1
2	ADAR-2	ATGATGGCTCGAACTCACC	mA*mU*mG*rArGrCrUrCrGrArArArCrUrC	20 nt	2 nmol	EasyEdit	1
3	ADAR-3	CAGCTGAAGAACCCATCAG	mC*mA*mG*rCrUrGrArArGrArArCrCrArL	20 nt	2 nmol	EasyEdit	1
4							
5							
6							
7							
8							
9							
10							

Add rows [Apply](#)

Custom Primer for Assessing Editing Efficiency

Enter the primer sequence(s) into the spreadsheet below. [Clear Table](#)

	* Primer Name	* Primer Sequence(5'->3')	Length	Quantity
1	ADAR-1 Pr1 LeftPrimer	AAAGAAACGACAGTTCCTC	20 nt	2 nmol
2	ADAR-1 Pr1 RightPrimer	ATATTCTACAGCCCCCTGA	20 nt	2 nmol
3	ADAR-2 Pr1 LeftPrimer	TCACCTGTATATACCCACA	20 nt	2 nmol
4	ADAR-2 Pr1 RightPrimer	TTGACTAGCGAACTGGGCAT	20 nt	2 nmol
5	ADAR-3 Pr1 LeftPrimer	AGAAACAGGCAAGAGGCCA	20 nt	2 nmol

Add rows [Apply](#)

[Add-on Items \(Cas9 Protein/mRNA and positive control\)](#) ^

[Add To Cart](#)

Step 3. Order your gRNA

1. Select quantity, purity, aliquoting tubes
2. Click Add-On Item section, you can find nuclease, nuclease mRNA, and positive control gRNA and order them as needed. (green labeled box)
3. Click “Add to cart”
4. Click “Continue” → “Get a quote” → “Thank you for your Quotation!”

Notes:

- Click “Clear Table” if you do not need product in the table. (red labeled box)

Base Editing gRNA Design Processes

Base Editing Design Tool

① Target Species: Homo sapiens (GRCh38.p13) ▼

① Nuclease: ABE (A to G) ▼

PAM: Cas9-NGG ▼

② Editing Window: 4 to 8 ?

③ * Select CDS frame: +0 ▼ ?

④ Target Sequence: Please input a sequence containing ATCG, supporting 40-1000 nt.

> Name
Sequence
It is recommended to input a 40–50 nt target sequence (sense strand) that contains the desired editing site, keeping about 20 nt on each side of the site. Excessively long sequences may result in too many design outputs.

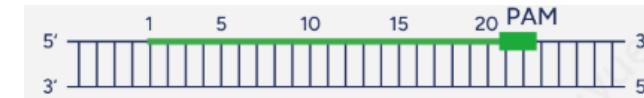
Submit

Step 1. Choose Editing System

ABE (A to G) or CBE (C to T)

Sept 2. Select Editing Window

The specific region on target DNA where base conversion occurs



- Recommend 4–8, and no wider than 2–12 to maintain editing efficiency.
- If you need a window beyond 2–12, consider switching to prime editing or HDR knock-in.

Step 3. Select CDS frame

- +0: Start codons from base 1
- +1: Start codons from base 2
- +2: Start codons from base 3

Step 4. Input Target Sequence

40-50 nt target sequence (sense strand) that contains the desired editing site, keeping about 20 nt on each side of the site.

Base Editing gRNA Design Processes

Base Editing guide RNAs Design Results and Distribution

Original sequence ATCG/N | Edited sequence ATCG/N | Original amino acid A | Edited amino acid A

W R L A Q S *
 TTGG CGG CTA GCT CAG TCC TAG G
 GAAT TCG CGG CCG CTT CTA GAT TGA CGG CTA GCT CAG TCC TAG GTA CAG TGC TAG CTA CTA GAG AAA GAG GAG
 N S R P L L D * R L A Q S * V Q C * L L E K E E
 AAA TAC TAG ATG AGC GGC AAC CGT GGT GTG GTT TAC CTG GGT GCG GGC AAG GTT GAG GTG CAG AAA ATC GAC TAT
 K Y * M S G N R G V V Y L G A G K V E V Q K I D Y
 CCG AAG ATG CAA GAT CCG CGT GGT AAG AAA ATC GAA CAC GGC GTG ATT CTG AAA GTG GTT AGC ACC AAC ATT TGC
 P K M Q D P R G K K I E H G V I L K V V S T N I C
 GGT AGC GAT CAG CAC ATG GTT CGT GGC ACC AAC CAC ACA CAC CAC AAC CAC ACA CAC ACA CAA CAC CAC ACA CAA
 G S D Q H M V R G T N H T H H N H T H T Q H H T Q
 AA

Base Editing guide RNAs and Parameters

Selecting a gRNA sequence highlights it in blue and shows its editing effect on the map. Tick the checkbox to use the sequence for ordering.

Option	gRNA Sequence	Editing Window Sequence	Target Sequence	Position ⑦	Direction	GC% ⑦	On Target Score ⑦	Off Target Score ⑦	Validation Primer
☐	GCTAGCCGCTCAATCTAGAAG	<u>AGC</u> CG	<u>CGG</u> <u>CCA</u> <u>GCT</u> A C A	11	-	0.5	1.06	0.00	
☐	TTGACGGCTAGCTCAGTCTCT	<u>AC</u> <u>GGC</u>	<u>GA</u> <u>TTG</u> <u>GCG</u> <u>G</u> L A	22	+	0.55	0.25	0.22 Detail	
☐	TAGTAGCTAGCACTGTACCT	T <u>AGC</u> T	T <u>AGC</u> <u>CAC</u> <u>TA</u> S H	39	-	0.45	0.57	0.42 Detail	

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Order gRNA oligo

Step 1. Choose desired gRNA

- The tool shows nucleotide and amino acid changes before and after gRNA editing
- Click different gRNAs sequence to find those that meet your needs. (Green box)

Step 2. Review related parameters

On-target, off-target, and validation primers are provided.

(If on-target score <0 or off-target score >0.95, and these parameters are critical for your application, you may try base editing, or switch to prime editing, or an HDR knock-in system for better efficiency.)

Step 3. Get sequence and order

Select a gRNA, then click the Excel icon to download or "Order gRNA oligo" to order online.

Base Editing gRNA Design Processes



test11 [CONTACT US](#) [MY ORDER](#) 

OmniGuide RNA Online Order (* Required Fields) [Information](#) > [Cart](#) > [Confirm Order](#) > [Result Feedback](#)

* Editing System 

Base Editing 

* Type:

Conventiona 

* Delivery Format:

Dry Powder 

* Container:

Single Tubes 

96-well plate display area
(shown when 96-well plates are selected)

[Download Template](#) [Upload Template](#)

 Drag & Drop or Click [Choose Files](#)

Form instructions: Bulk copy/paste from an Excel sheet here  Number in sequence  Fill according to first row  Clear all values [Clear Table](#) [User Guide](#)

	* Name 	* Input Sequence  	Length	* Final sequence 	* Purification 	* Quantity 	* Aliquoting Into 	Price	Option
1	BaseEditing-2	UUGACGGCUAGCUCAGUCCU	20 nt	mU*mU*mG*ACGGCUAGCUCAGI 	Desalt 	2 nmol 	1 	\$79.00	
2									
3									
4									
5									
6									
7									
8									
9									
10									

Add rows [Apply](#)

If you have any special requirements, for example adding any modifications to the RNA sequence, please describe them here.

 Contract Save: \$0.00  Coupon Save: \$0.00 [Discount Details](#)

Production Time: 4 Business Days Total Amount: \$79.00 

Add-on Items (Custom Primer for Assessing Editing Efficiency) 

Order your gRNA

1. Select quantity, purity, aliquoting tubes
2. Click “Add to cart”
3. Click “Continue” → “Get a quote” → “Thank you for your Quotation!”

Prime Editing gRNA Design Processes

Step 1. Select Gene

Option 1: 'Enter Gene Symbol' tab

- **Required:** gene symbol or transcript ID of interest, editing system
- **Optional:** Edit locations (base or amino acid), design nicking sgRNA ^[1], 3' extension ^[2] (added by default), add gRNA spacer sequence

Option 2: 'Enter Sequence' tab

- **Required:** wildtype & desired sequence, CDS frame, editing system
- **Optional:** 3' extension (added by default), design nicking sgRNA

[1] Nicking sgRNA induces a single-strand break on the opposite DNA strand to promote repair, enhancing editing efficiency.

[2] Add 3'Extension: an extension sequence at 3'end protecting the 3' end from degradation which will improve prime editing efficiency. Reference: Nelson,J.W. et al, 2022.

Prime Editing gRNA Design Processes

Step 2. Edit Mutations

- Select the editing site:** The green bar in the sequence map indicates the editing site. Click the 'Zoom to Edit' button to display the bases in the target sequence. Enter the desired deletion length in the 'Deletion Length' box or left-click the green bar and hold to drag it across the nucleotide/sequence to be modified.
 - For a deletion edit: Delete the original sequence in the 'Mutation' box.
 - For a nucleotide substitution: Delete the original sequence and rewrite the desired sequence in the 'Mutation' box.
 - For a sequence insertion: Enter the insertion sequence in the 'Mutation' box.Click the left mouse button in any area outside the Mutation Box to see the effective editing request on the sequence map.
- Optional- Insert a tag:** Nine commonly used tags can be selected and inserted during the pegRNA design process. Find the desired tag in the dropdown menu and click the 'Insert' button to add it.

Note: If the arrow in the sequence map points to the left, indicating the gene is on the - strand, choose the (- strand) tag option. If the arrow points to the right, choose the (+ strand) tag option.
- Optional- Add a silent mutation** to the RTT region to prevent Cas9 recutting while preserving the translated amino acid sequence.
- Click 'Submit'

pegRNA design tool

Select Gene > Mutation > Design Results

Exon (negative direction) Exon (positive direction) Deletion Insertion sgRNA Translation Previous translation

3' 5'

154,001,720 154,001,730 154,001,740 154,001,750 154,001,760 154,001,770 154,001,780

Start Edit Deletion Length

154607754 0 Zoom to Edit

< Previous Next >

Mutation Sequence Flip Tool

Select Tag (optional)

Choose... Insert

☒ Add silent mutations

Back Submit

pegRNA design tool

5' 3'

114,044,105 114,044,110 114,044,115 114,044,120 114,044,125 114,044,130 114,044,135 114,044,140

Start Edit Deletion Length

114044136 0 Zoom to Edit

< Previous Next >

Mutation Sequence Flip Tool

ATC

☒ Add silent mutations

Back Submit

FLAG (- strand)
FLAG (+ strand)
HA (- strand)
HA (+ strand)
Myc (- strand)
Myc (+ strand)
SwGS (- strand)
SwGS (+ strand)
SwGS (- strand)
SwGS (+ strand)
HIBIT (- strand)
HIBIT (+ strand)
V5 (- strand)
V5 (+ strand)
Kozak (- strand)
Kozak (+ strand)

Choose... Insert

Prime Editing gRNA Design Processes

Step 3. Select Your Sequence

1. Select sgRNA (spacer) sequence

Parameters:

- On-target score (from -1.5 to 1.5): A higher score indicates higher editing efficiency. Some gRNAs have penalty factors that lead to negative On-Target Scores. Below 0.2 will be marked in red, typically indicating low editing efficiency. Consider using them only if no better options are available. [1]
- Off-target score (from 0 to 1): A lower score indicates fewer off-target effects [2]
- Distance to mutation: The distance from actual cutting site to your desired cutting site. Generally, the smaller the distance, the better.
- Ranking: Sequences with a smaller 'Distance to mutation' will have a higher ranking. (If the 'Distance to mutation' is within 20, we recommend sequences with a high on-target score and 40-80% GC content. If experimental editing efficiency is poor, please consider another design).

2. Select pegRNA:

Click "Show more" to select pegRNAs with different PBS and RTT lengths. Authoritative literature recommends testing several pegRNA to determine the best choice.

- Click the black question marks to view each full sequence (red-labeled box)
- Click 'Download Results' to download the sequences (green-labeled box)

2. Select Nicking sgRNA:

Nicking sgRNA will help enhance editing efficiency in a PE3 system. Please choose a pegRNA and nicking sgRNA from the same group.

3. Click 'Place Order'

pegRNA design tool

Select Gene > Mutation > Design Results ?

Output for Gene Symbol ADAR (Transcript ID NM_001025107) ?

Download Result

Design #1

Component	Sequence (5'→3')	Length	On-target Score	Off-target Score	Distance to mutation	GC%
sgRNA (Spacer)	UCCUCUUGAGUUUUUAGACA	20	1.17	0.5	4	35

Show More >

Select	Item ?	RTT Sequence ?	RTT Length	PBS Sequence	PBS Length	Final Sequence ?	Final Sequence Length	GC%
☐	pegRNA1_RTT38nt_PBS10nt	CUCCUUGAUCUCGGCAGGGUG...	38	CUAAAAACUC	10	UCCUCUUGAGUUUUUAGACA...	195	42.05
☐	pegRNA2_RTT42nt_PBS13nt	UUUUUCCUUGAUCUCGGCAU...	42	CUAAAAACUCAAAG	13	UCCUCUUGAGUUUUUAGACA...	202	41.09
☐	pegRNA3_RTT48nt_PBS16nt	CGCAGAUUUUCCUUGAUCU...	48	CUAAAAACUCAAAGG	16	UCCUCUUGAGUUUUUAGACA...	211	42.18

Show More >

Design #2

Component	Sequence (5'→3')	Length	On-target Score	Off-target Score	Distance to mutation	GC%
sgRNA (Spacer)	GCAGAUUUUCCUUGAUCU	20	-0.03	0.42	25	40

Show More >

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Place Order

Reference

[1] Doench's Rule Set 3 <https://pubmed.ncbi.nlm.nih.gov/36068235/>

[2] Doench's CFD score
<https://pubmed.ncbi.nlm.nih.gov/26780180/>

Prime Editing gRNA Design Processes

Step 4. Select Specifications

- a) Select the desired delivery format and container
- b) Select the quantity and purity for pegRNA and nicking sgRNA
- c) Add PE mRNA / protein or PE Positive control if needed (green labeled box)
- d) Click "Add to Cart"
- e) Select the desired items in the cart, then click "Continue" → "Get a Quote" → "Thank you for your quotation!"

OmniGuide RNA Online Order (* Required Fields)

1 Information > 2 Cart > 3 Confirm Order > 4 Result Feedback

Order Information

* Editing System ⓘ: Prime Editing gRNA (pegRN ⓘ)

* Type: PE2/PE3/PE4/PE5/PE Max ⓘ

* Delivery Format: Dry Powder ⓘ

* Container: Single Tubes ⓘ

☒ Input spacer sequence ⓘ

☐ Input full-length sequence ⓘ

96-well plate display area
(shown when 96-well plates are selected)

Bulk Order

Download Template ⓘ

Upload Template ⓘ

Drag & Drop or Click [Choose Files](#)

Form instructions: Bulk copy/paste from an Excel sheet here ⓘ ↓ Number in sequence ⓘ Fill according to first row ⓘ Clear all values ⓘ [How to Order?](#) [Clear Table](#) [User Guide](#)

	* Name ⓘ ⓘ	* Input protospacer sequence ⓘ ⓘ ⓘ	* Input RTT+Edit+PBS sequence ⓘ ⓘ ⓘ	* 3' Extension ⓘ ⓘ ⓘ	* Default Modification ⓘ ⓘ ⓘ	Final sequence ⓘ	Length	*Purification ⓘ ⓘ	*Quantity ⓘ ⓘ	*Aliquoting Into ⓘ ⓘ	Price	Option
1	pegRNA1_RTT20nt_PBS	CUGAAUUAGCUGUAUCGUCA	CGUAGGCAAGUGUGCUUUGACGAUACGCU	Yes ⓘ	Yes ⓘ	mC*mU*mG*AAUUAGCUGUAUCC ⓘ	177 nt	⌵	⌵	1		🗑
2				Yes ⓘ	Yes ⓘ			⌵	⌵			🗑
3				Yes ⓘ	Yes ⓘ			⌵	⌵			🗑

Add-on Items Nicking sgRNA ⌵

Add-on Items (Custom Primer for Assessing Editing Efficiency) ⌵

PE mRNA/Protein and Positive control OTS products ⌵

Add To Cart

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