



GenScript

OmniGuide RNA Design Tool Protocol

Enabling Easy & Precise Design

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

Enter Gene Symbol

Enter Sequence

* Target Species: Homo sapiens (GRCh38.p13)

* Editing System: PE2/PE3/PE4/PE5/PE Max

* Gene Symbol: ADAR

* Transcript ID: NM_001025107

* Select CRISPR Nuclease: Cas9-NGG

* Design nicking sgRNA: Yes

* Max nick distance to edit site: 90

* Add 3' Extension: Yes

* Export Design Number: 1

Edit Location: Base Position

gRNA(spacer) sequence within pegRNA(optional): Sequence length between 17nt to 23nt.

Submit

Click the black question mark marks for detailed explanations of each option.

Option 2: 'Enter Sequence' tab

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

Enter Gene Symbol

Enter Sequence

* Target Species: Homo sapiens (GRCh38.p13)

* Editing System: PE2/PE3/PE4/PE5/PE Max

* Enter sequence:

* Wildtype sequence before editing: 5' → 3'

Please fill in the sequence according to the following rules:

1. The wildtype sequence and the desired sequence must be different and both should exceed 102 nt and include the PAM sequence.
2. The maximum insertion length is 100 nt, so the desired sequence can be up to 100 nt longer than the wildtype sequence.
3. There must be a 51 nt sequence on both the left and right sides of the edit site.

* Desired sequence after editing: 5' → 3'

Please fill in the sequence according to the following rules:

1. The wildtype sequence and the desired sequence must be different and both should exceed 102 nt and include the PAM sequence.
2. The maximum insertion length is 100 nt, so the desired sequence can be up to 100 nt longer than the wildtype sequence.
3. There must be a 51 nt sequence on both the left and right sides of the edit site.

* Select CDS frame: +0

* Select CRISPR Nuclease: Cas9-NGG

* Design nicking sgRNA: Yes

* Max nick distance to edit site: 90

* Add 3' Extension: Yes

* Export Design Number: 1

Submit

Input sequence rules:

1. The wildtype sequence and desired sequence must be different and both should exceed 102 nt.
2. Both the wildtype sequence and desired sequence must include the PAM sequence.
3. There must be a 51 nt sequence on both the left and right sides of the edit site.

Please choose your open reading frames as shown below:

CDS+0

Base: ACA TTT GCT TCT ...

amino acid: T FAS ...

CDS+1

Base: A CAT TTG CTT CT ...

amino acid: H LL ...

CDS+2

Base: AC ATT TGC TTC T ...

amino acid: I CF ...

Click the black question mark marks for detailed explanations of each option.

[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'

The top screenshot shows the 'pegRNA design tool' interface. At the top, there's a navigation bar with 'Select Gene > Mutation > Design Results'. Below this is a legend for the sequence map: Exon (negative direction), Exon (positive direction), Deletion, Insertion, sgRNA, Translation, and Previous translation. The sequence map shows the 'ada' gene with a green bar indicating the editing site. Below the map, there are input fields for 'Start Edit' (154601754) and 'Deletion Length' (0), and a 'Zoom to Edit' button. A 'Mutation' box is empty, and a 'Select Tag (optional)' dropdown is set to 'Choose...'. The bottom screenshot shows the same tool with a different sequence map. A dropdown menu is open, showing various tags like FLAG, HA, Myc, 3xGS, and HiBIT, with 'HiBIT (+ strand)' selected. The 'Mutation' box now contains 'ATC'.

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	CUCUUGACUCGCGCAGGGG...	38	CUAAAAACUC	10	UCCUCUUGAGUUUUUAGACA...	195	42.05
☐	pegRNA2_RTT42nt_PBS13nt	UUUUCUCCUUGACUCGCGCAU...	42	CUAAAAACUCAAAG	13	UCCUCUUGAGUUUUUAGACA...	202	41.09
☐	pegRNA3_RTT48nt_PBS16nt	CGCAGAUUUUCCUUGACUCU...	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)	GCAGAUUUUCCUUGACUCU	20	-0.03	0.42	25	40

Show More >

Back

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
- d) Click "Add to Cart"
- e) Select the desired items in the cart, then click "Continue" → "Get a Quote" → "Thank you for your quotation!"

pegRNA Ordering

* Delivery Format: Dry Powder

* Editing System: Prime Editing gRNA (pegRNA)

* Format: Single Tubes

Enter the RNA sequence(s) into the spreadsheet below. How to add customized modification?

Clear Table

	Extension	* Default Modification	* Final sequence	Length	*Quantity	*Purity	*Aliquoting Into
1		Yes	mU ^m mC ^m mC ^U UUGAGUUUUUAGACAGUUUUAGACCUAGA	219 nt			

Add rows

Apply

Comments: Comments

sgRNA Ordering

* 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	Nicking_sgRNA_1_adar	AGGGGUUUGAGUCUGGGUCC	m ^A mC ^m mC ^G GrUUrGrArGrUUrCUrGrGr	20 nt		EasyEdit	1

Add rows

Apply

Comments: Comments

Add-On Items

Cat. No.	Name	Quantity	Price	Numbers
SC2346	PE2/PE3 mRNA	0.2 mg	\$350	0
		1 mg	\$1300	0

14