GenScript

OmniGuide RNA Design Tool Protocol

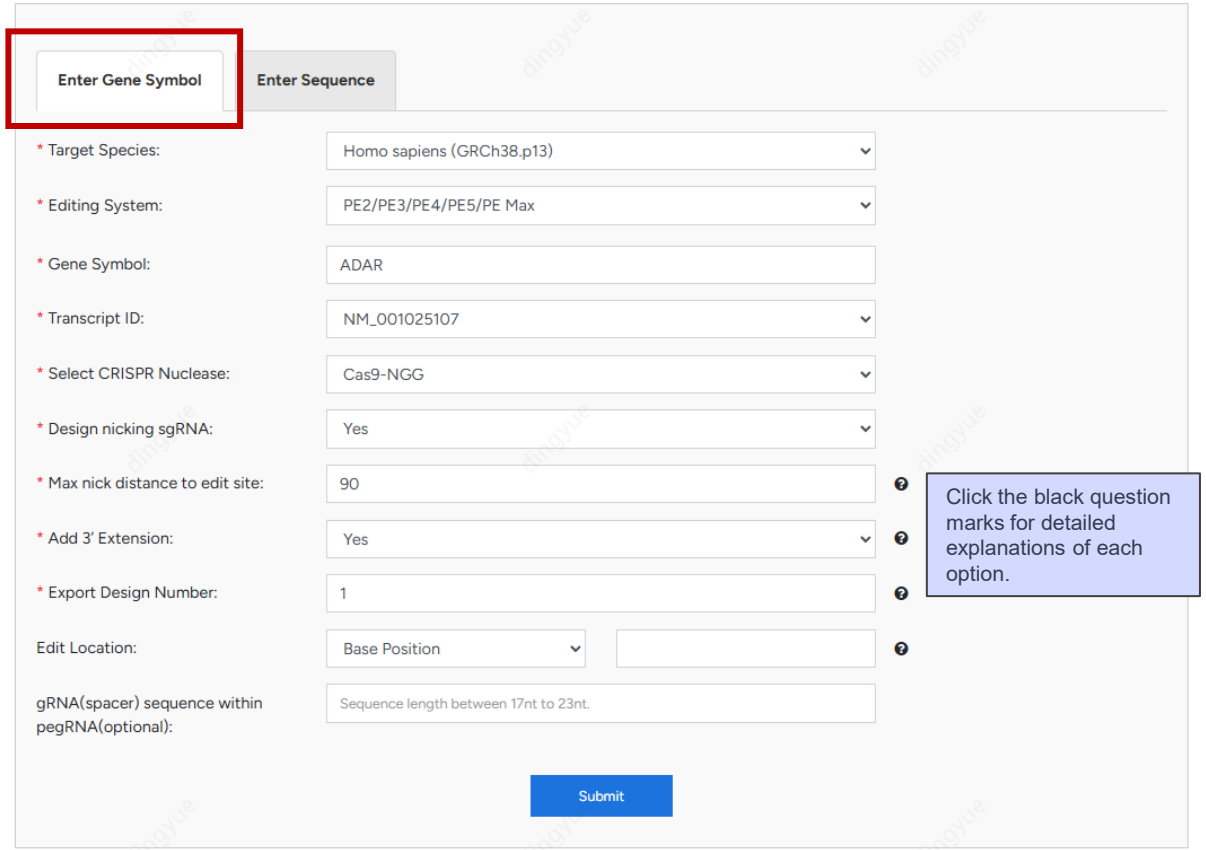
Enabling Easy & Precise Design

Prime Editing gRNA Design Processes

Step 1. Select Gene

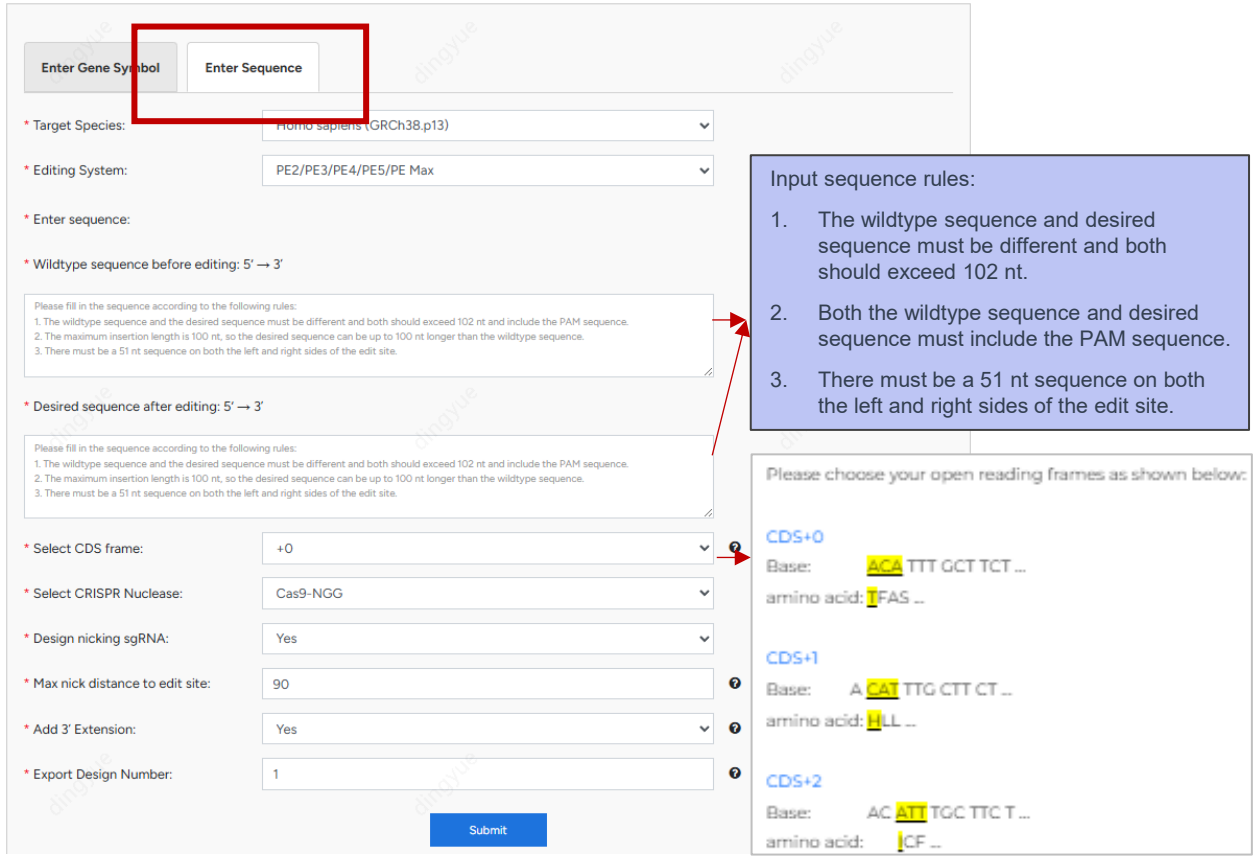
Option 1: 'Enter Gene Symbol' tab

- **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



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.

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Step 2. Edit Mutations

1. **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.
   a) For a deletion edit: Delete the original sequence in the ‘Mutation’ box.
   b) For a nucleotide substitution: Delete the original sequence and rewrite the desired sequence in the ‘Mutation’ box.
   c) For a sequence insertion: Enter the insertion sequence in the ‘Mutation’ box.

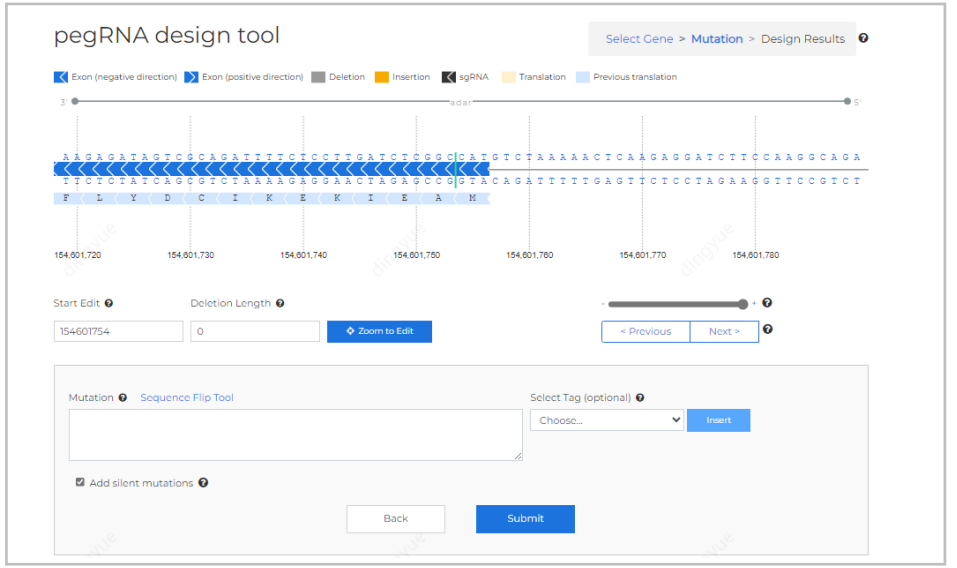
   Click the left mouse button in any area outside the Mutation Box to see the effective editing request on the sequence map.

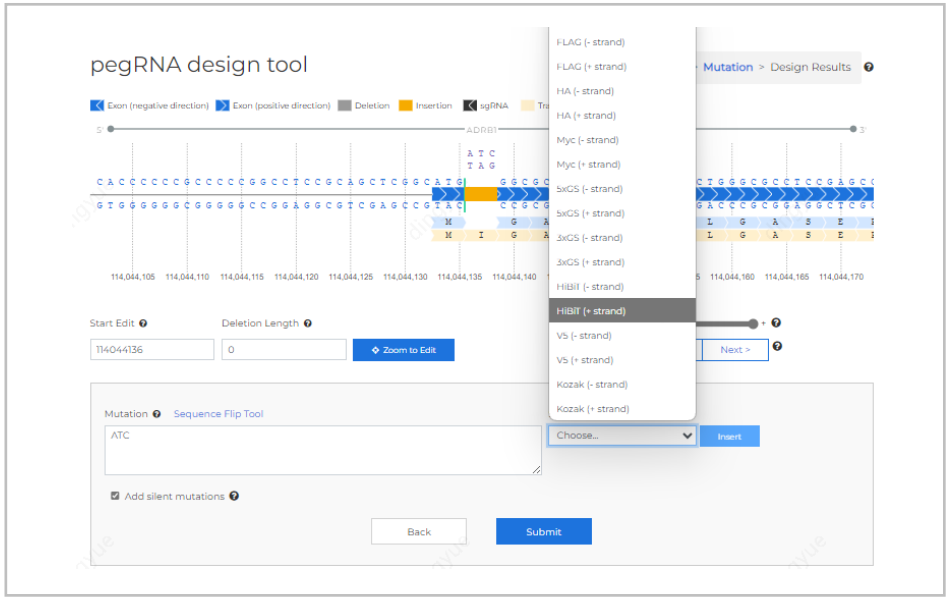
2. ***Optional*- Insert a tag:** Nine commonly used tags can be selected and inserted during the pegRNA design process. Find the desired tag in the dropdown menu and click the ‘Insert’ button to add it.

   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.

3. ***Optional*- Add a silent mutation** to the RTT region to prevent Cas9 recutting while preserving the translated amino acid sequence.

4. **Click ‘Submit’**





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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).

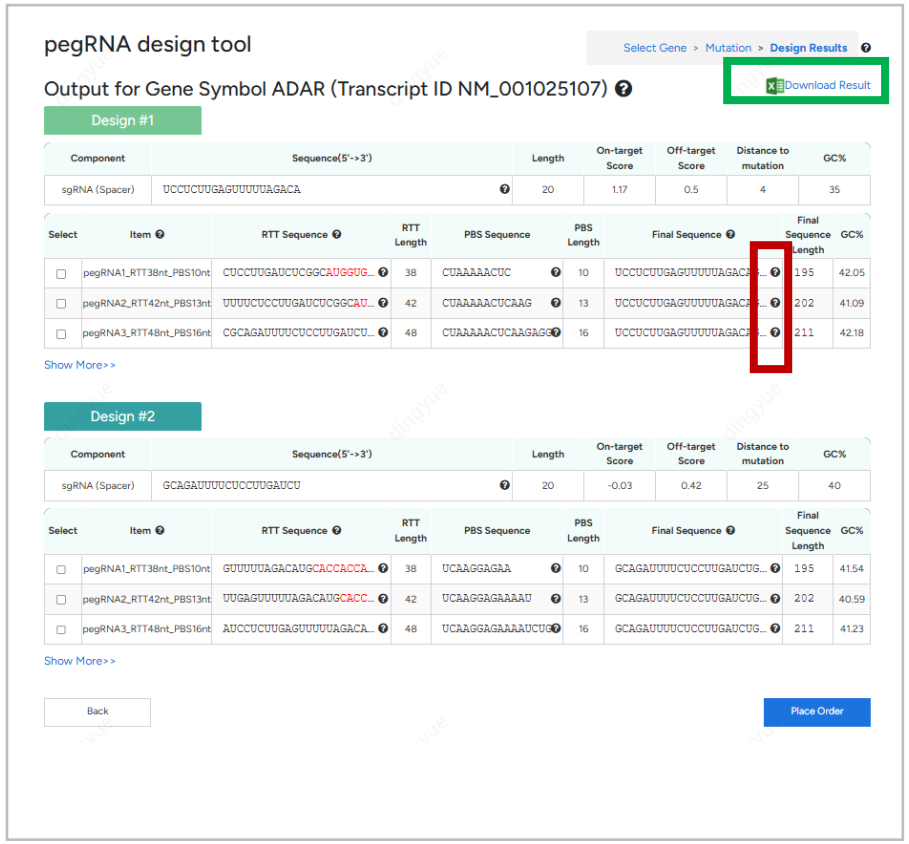
2. **Select pegRNA:**

   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'**



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/

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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!"