GenScript

OmniGuide RNA Design Tool Protocol

Enabling Easy & Precise Design

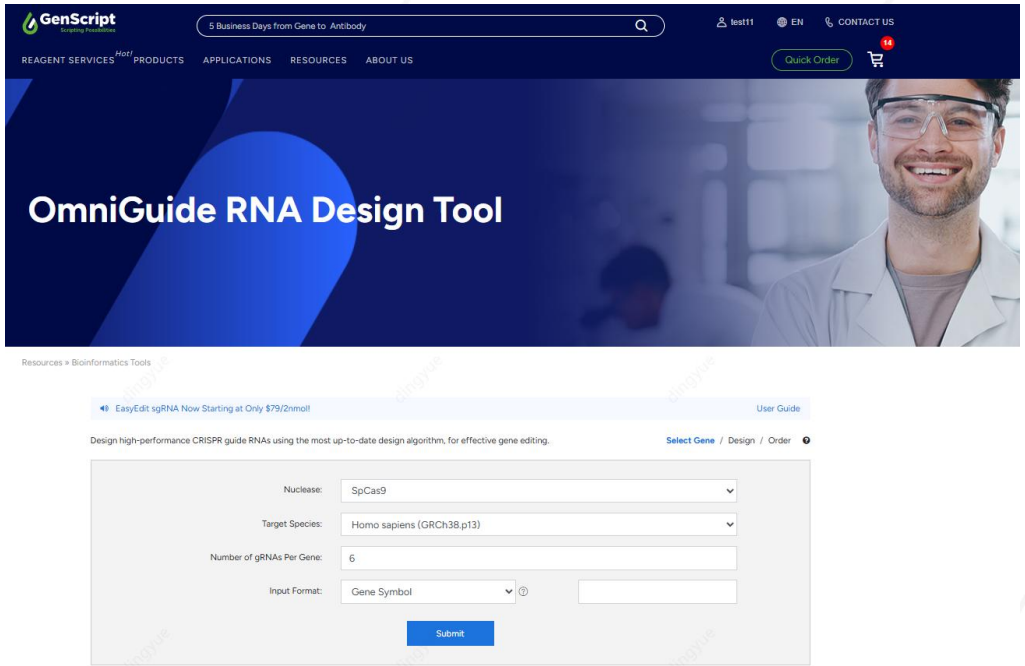
Content

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

Applications & Advantages – OmniGuide RNA Design Tool

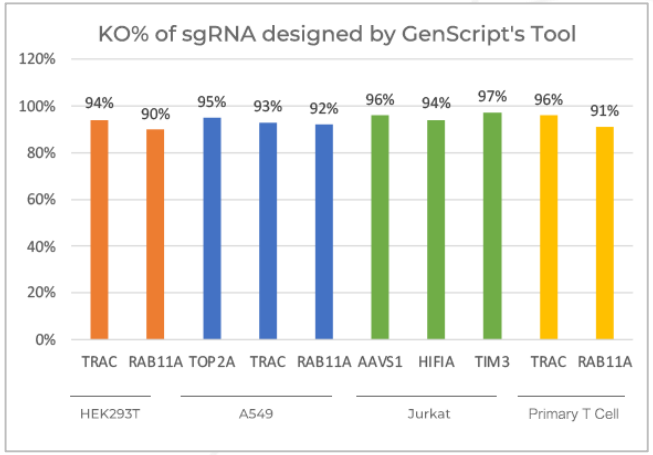
What is OmniGuide RNA design tool?

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



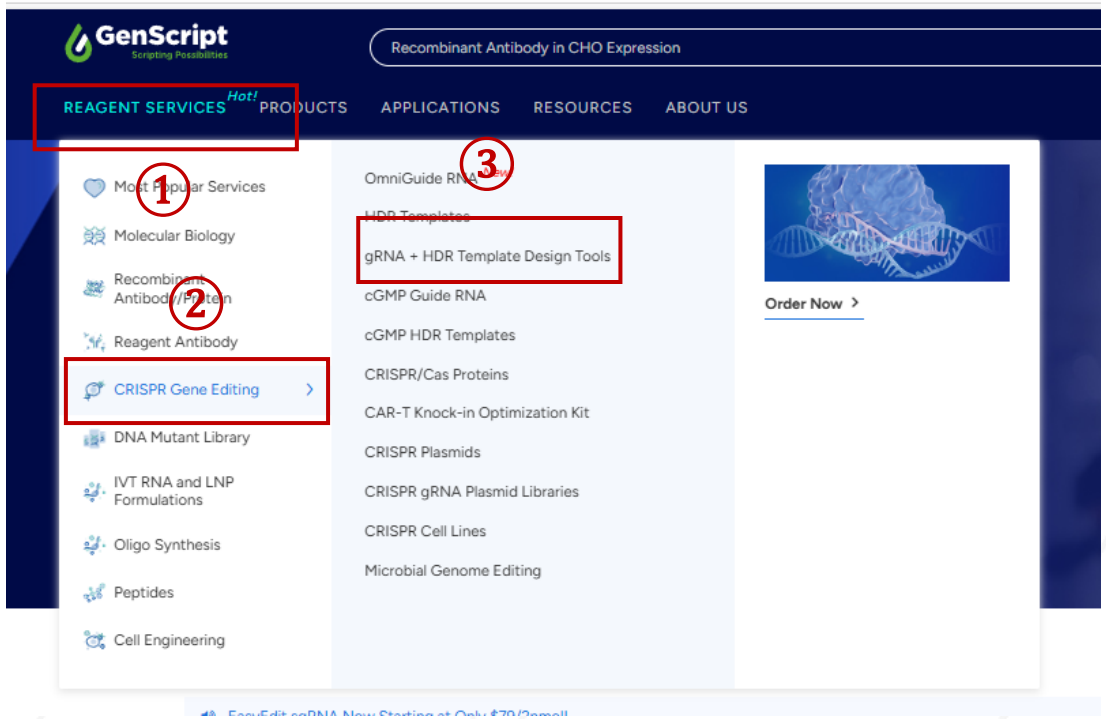
Advantages of OmniGuide RNA design tool

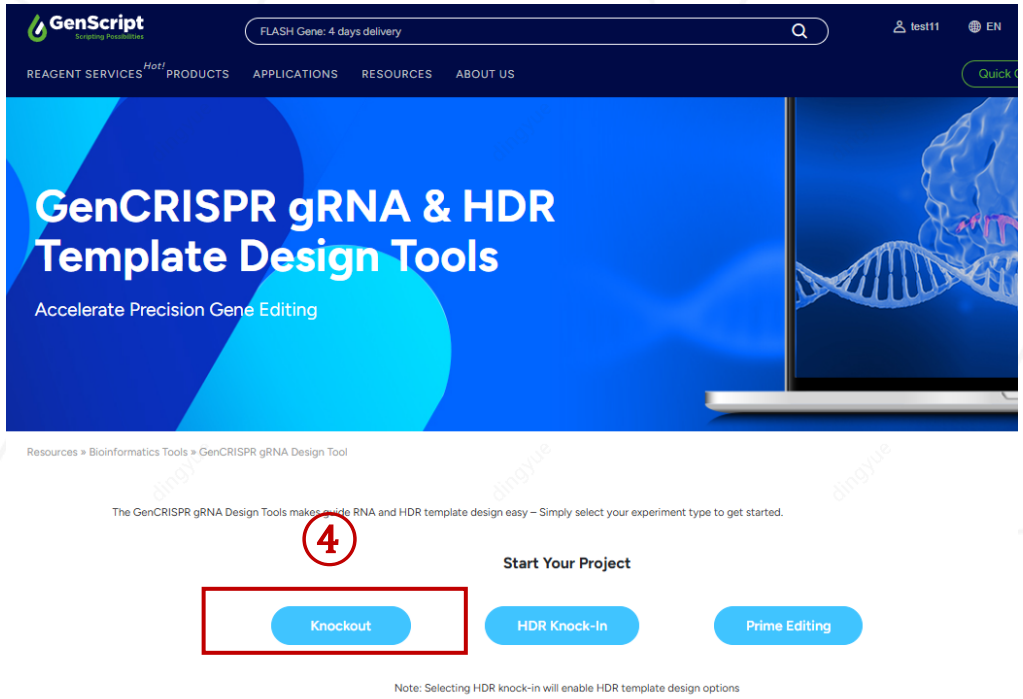
1. **Comprehensive applications**: Support 14 species, Cas9, Cas12a base editing, and prime editing
2. **More prices design:** updated on-target and off-target scores
3. **Enhance editing efficiency:**
   - Designs target early exons to avoid truncated functional proteins
   - Higher transcripts coverage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



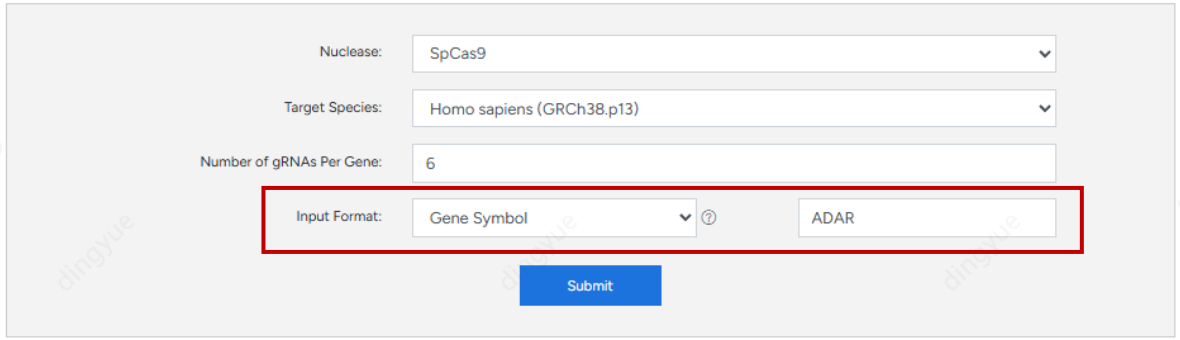
Where can we find OmniGuide RNA Design Tool?

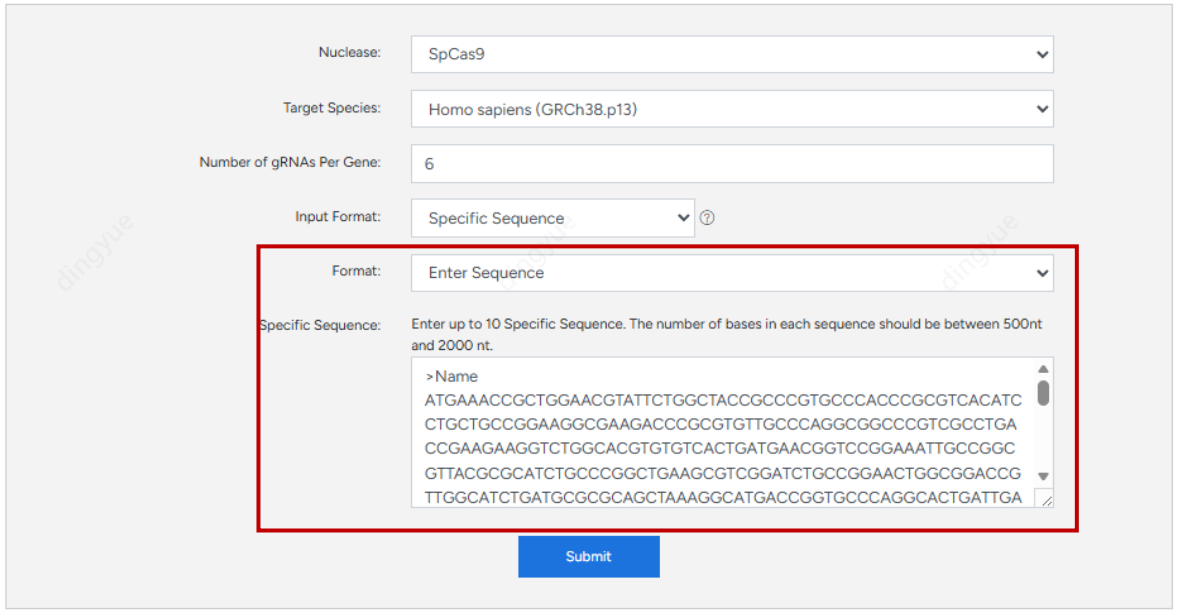
1. Visit the address: https://www.genscript.com/tools/gRNA-design-tool
2. Find the tool in GenScript website:





Cas9 / Cas12 Design Processes





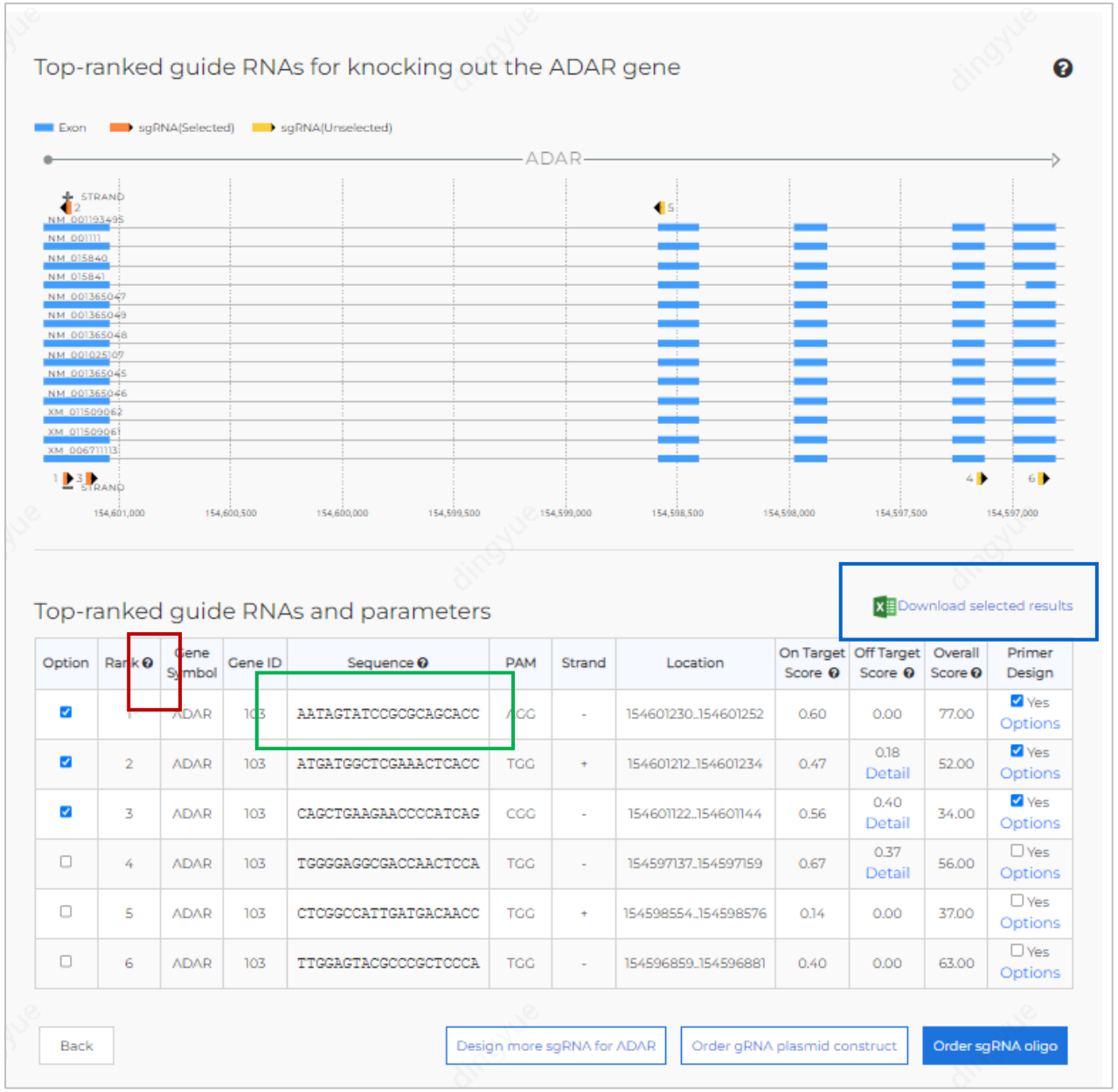
Step 1. Enter your request

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)

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”

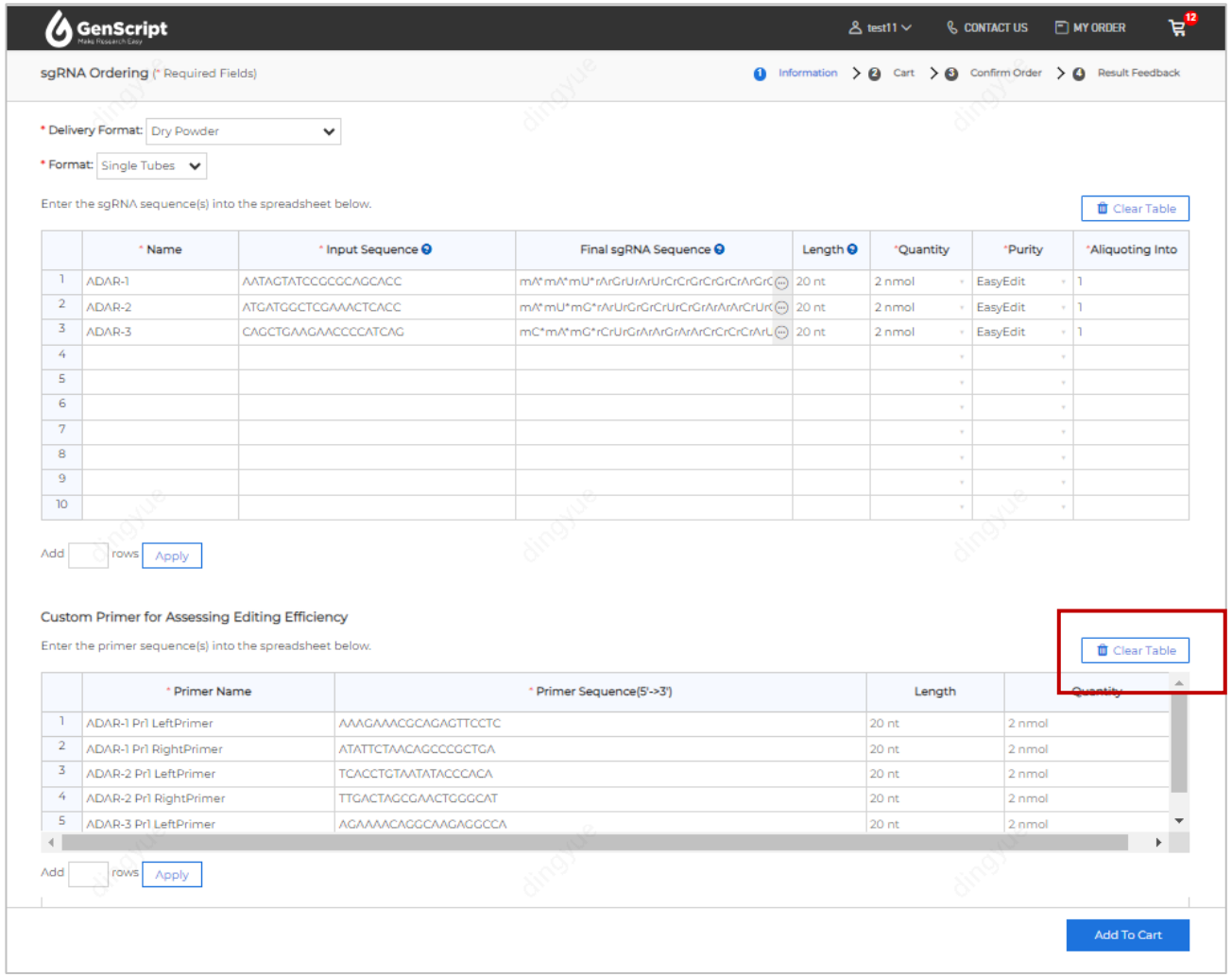
Parameter's introduction & Notes

- 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



Step 3. 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!”

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

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.

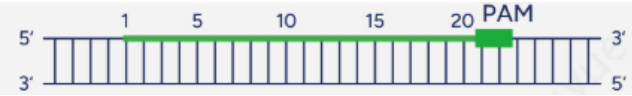
Submit

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.

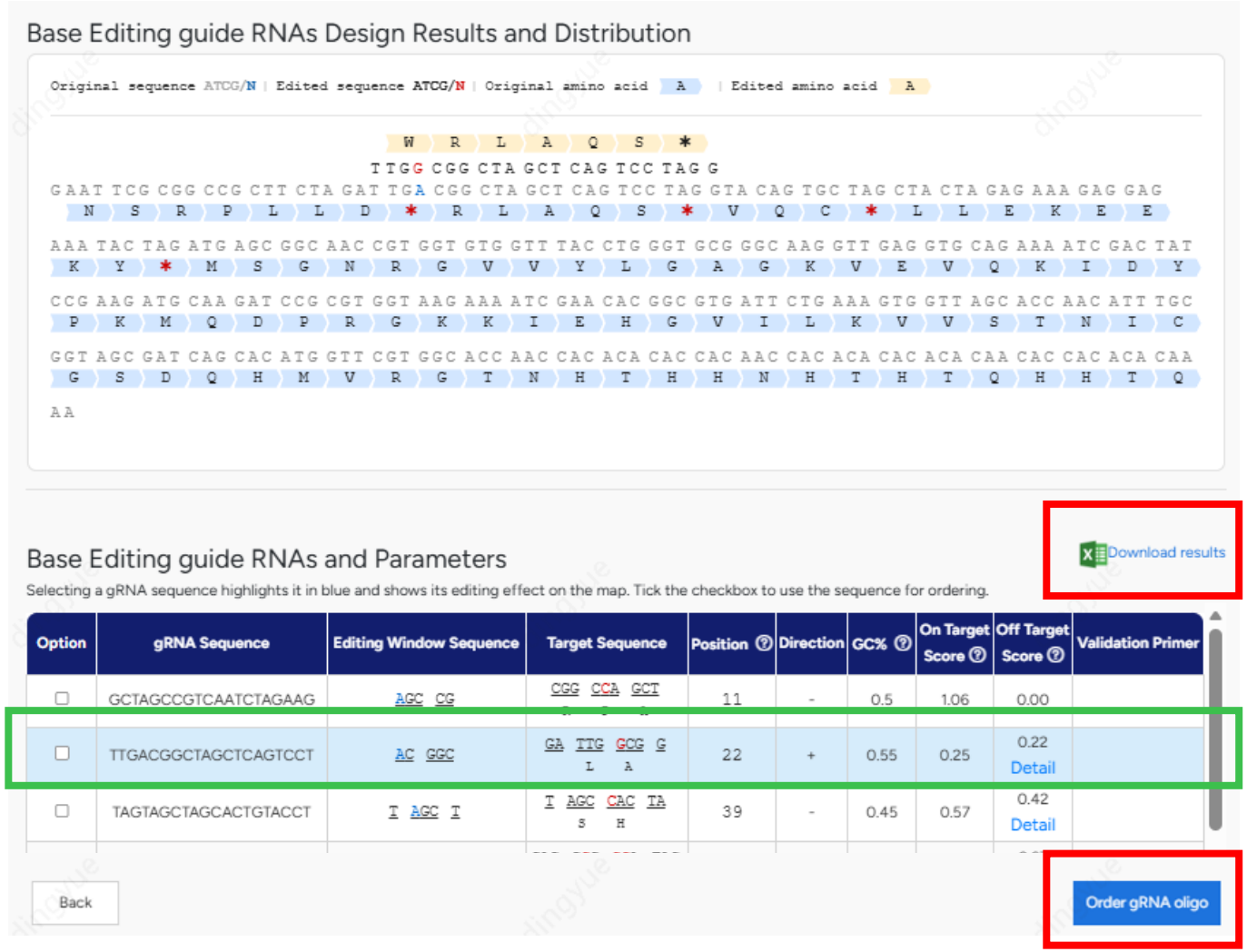
Step 3. Select CDS frame

- +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



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

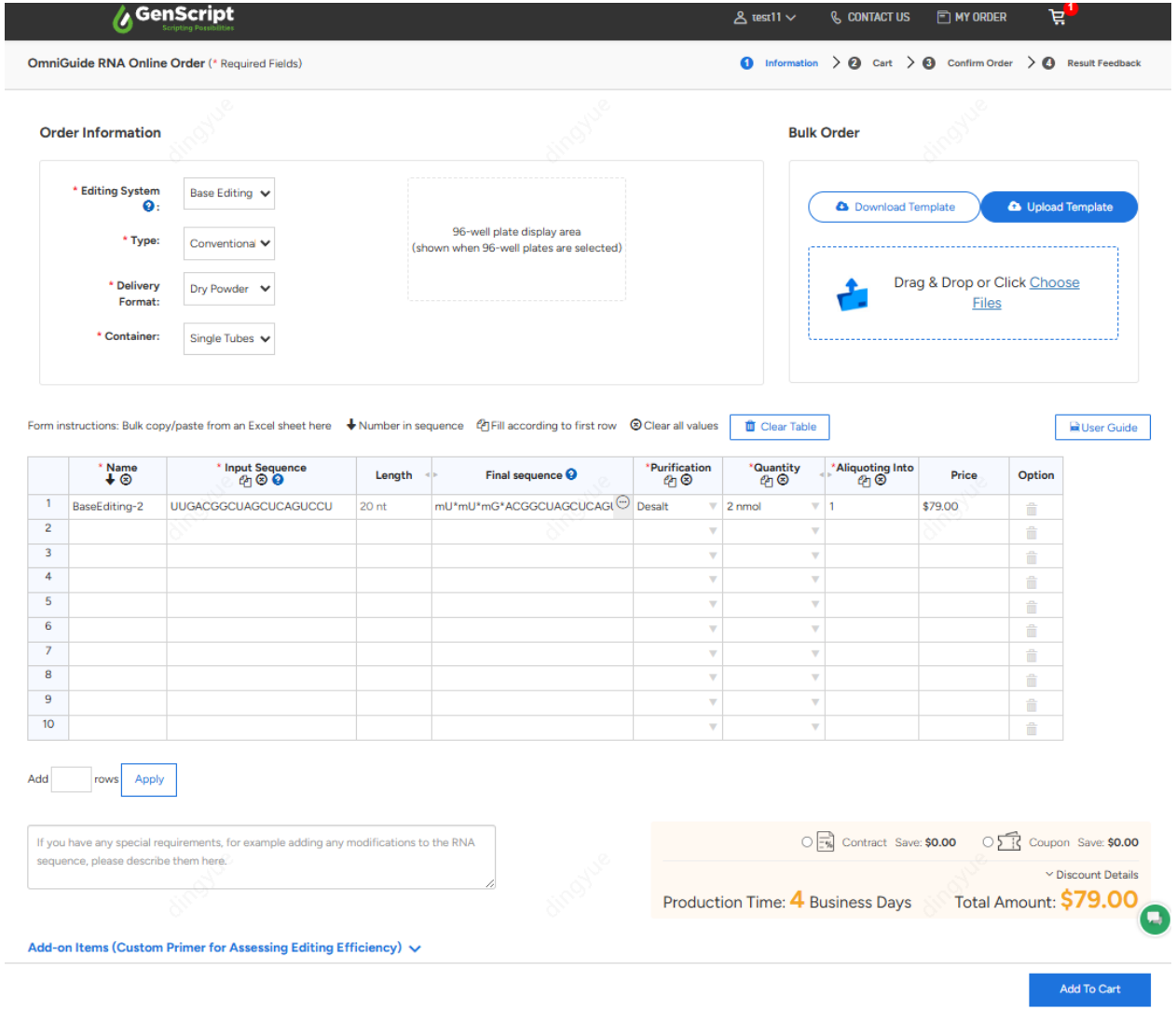
On-target, off-target, and validation primers are provided.

(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



Order your gRNA

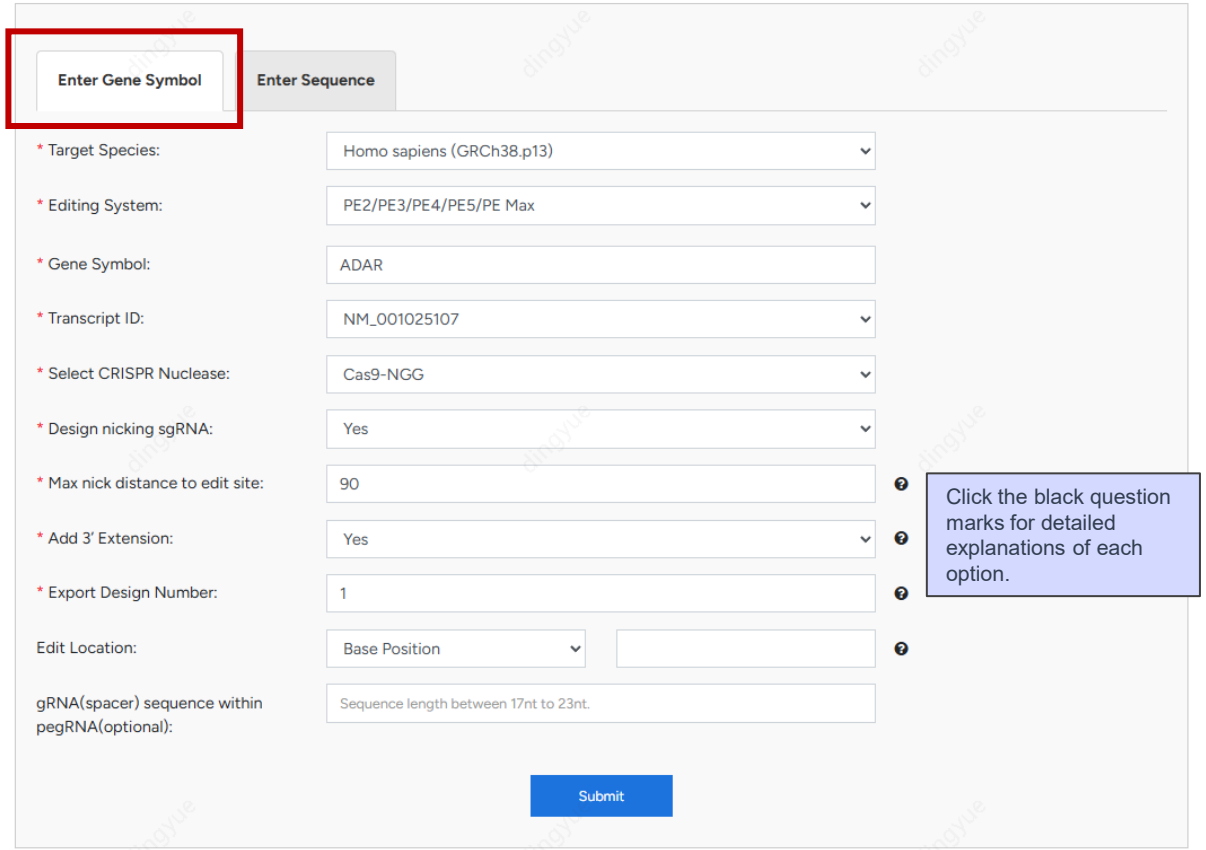
1. Select quantity, purity, aliquoting tubes
2. Click “Add to cart”
3. Click “Continue” → “Get a quote”→” Thank you for your Quotation!”

Prime Editing gRNA Design Processes

Step 1. Select Gene

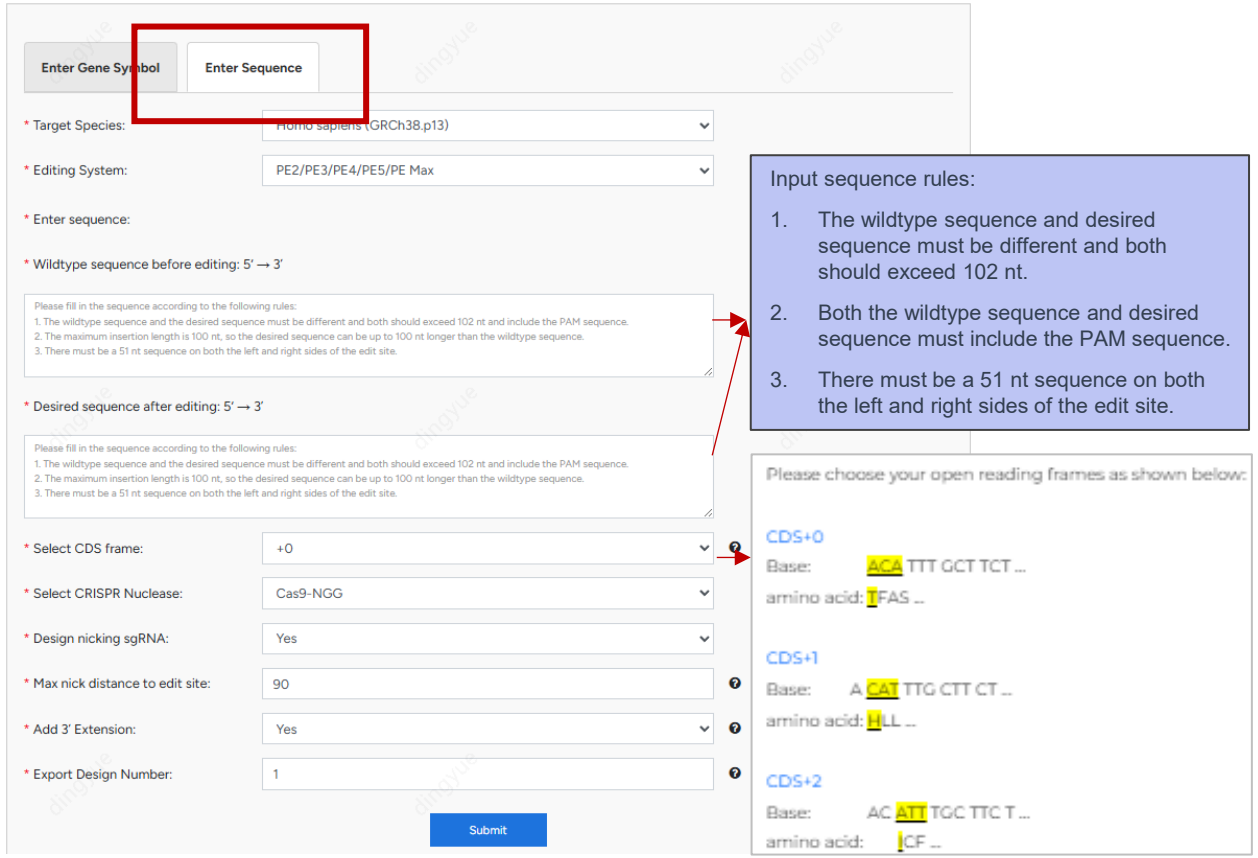
Option 1: 'Enter Gene Symbol' tab

- **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

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.

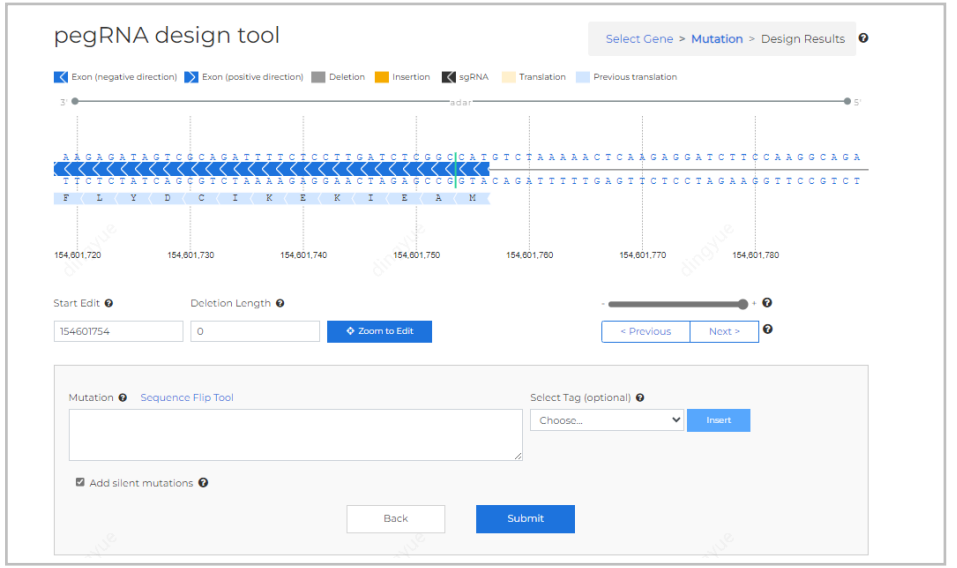
   Click the left mouse button in any area outside the Mutation Box to see the effective editing request on the sequence map.

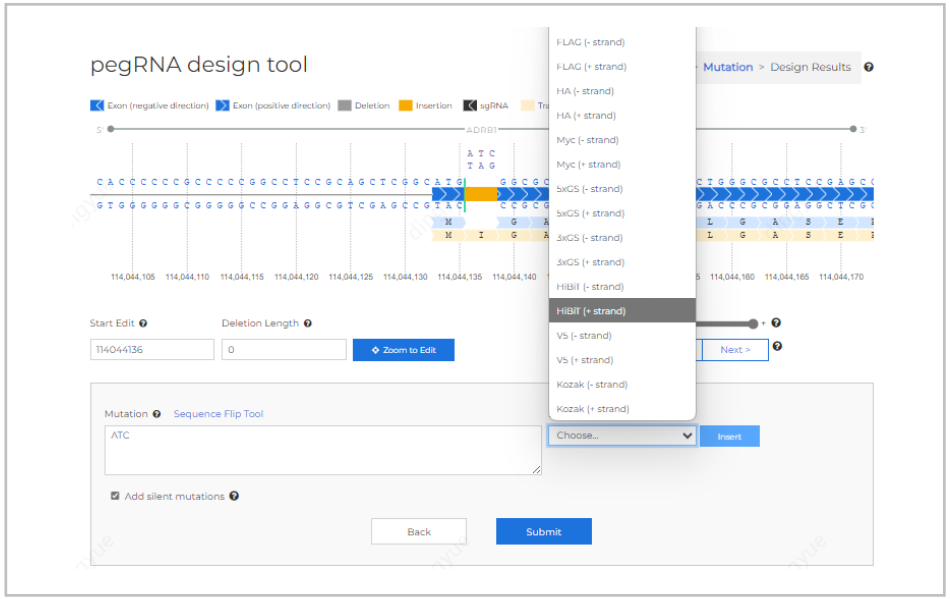
2. ***Optional*- Insert a tag:** Nine commonly used tags can be selected and inserted during the pegRNA design process. Find the desired tag in the dropdown menu and click the ‘Insert’ button to add it.

   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’**





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).

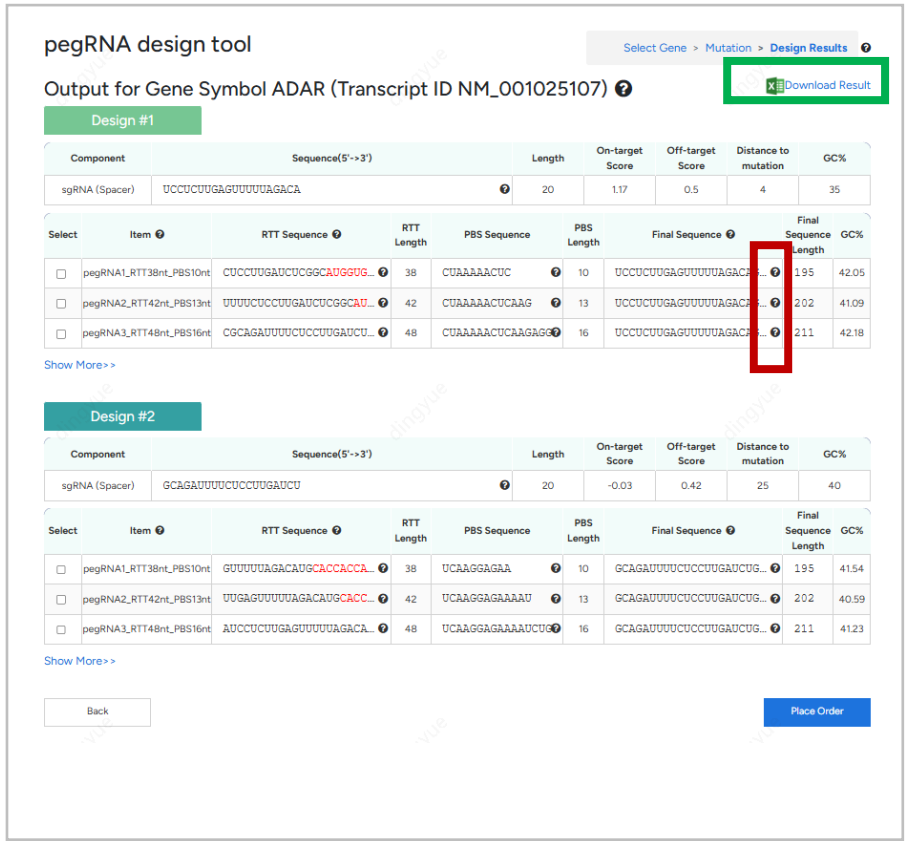
2. **Select pegRNA:**

   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'**



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!"