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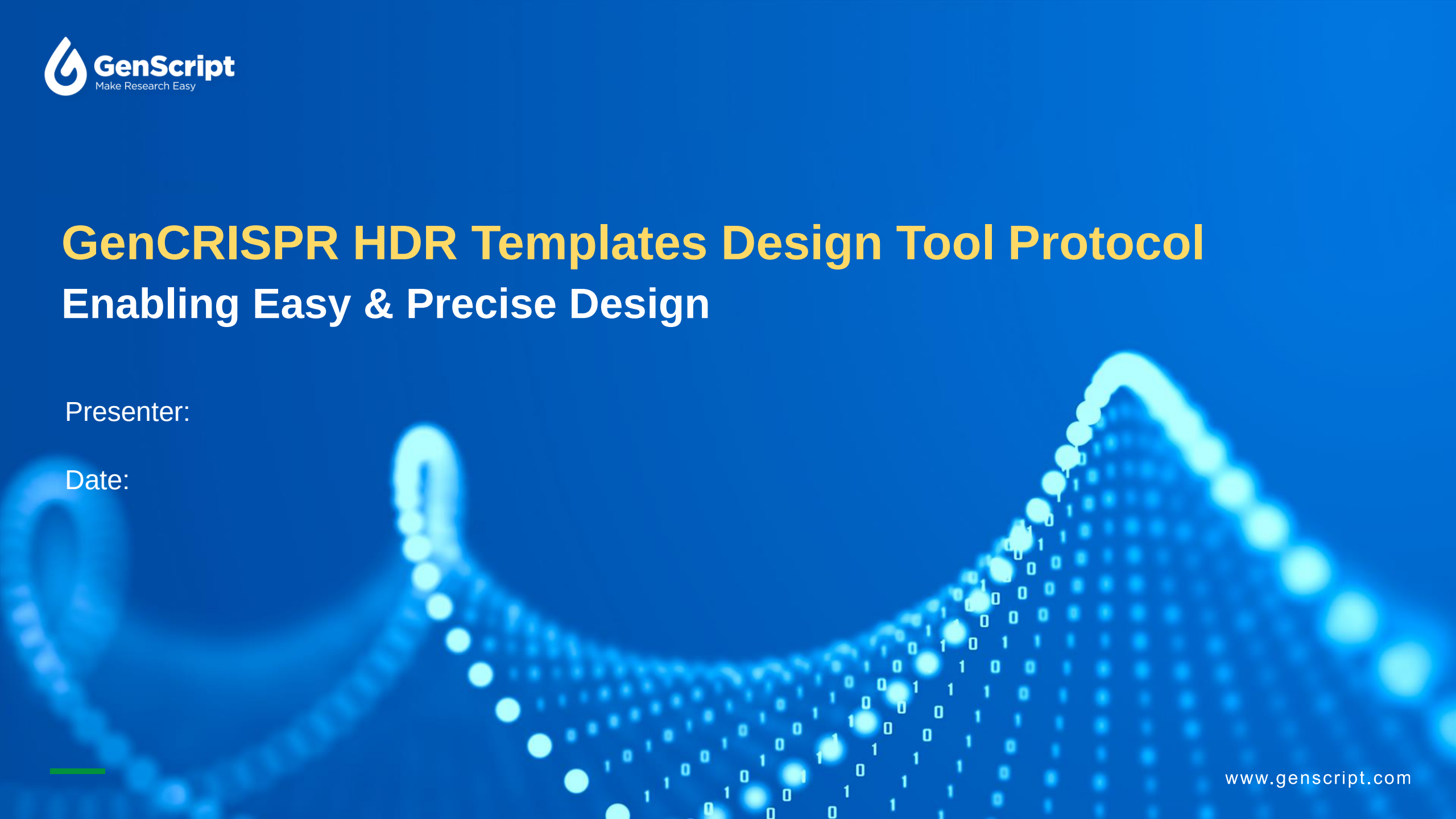
GenCRISPR HDR Templates Design Tool Protocol

Enabling Easy & Precise Design

Presenter:

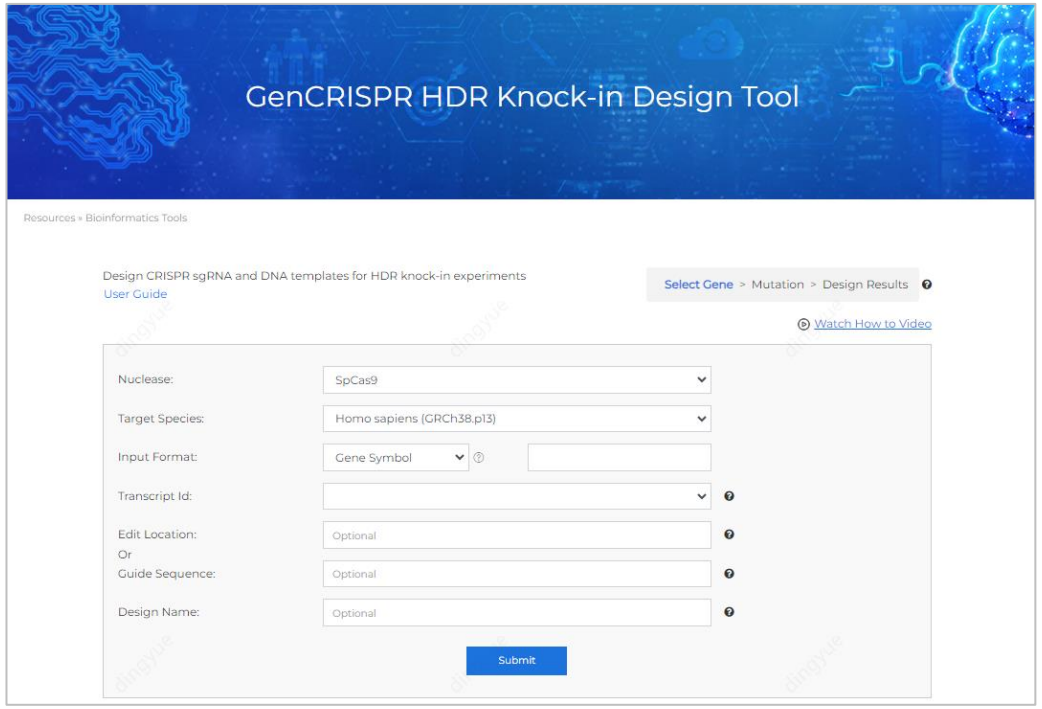
Date:



Applications & Advantages – HDR template design tool

What is HDR template design tool?

- Design sgRNA sequences and HDR template sequence for knock-in experiment, downstream order **GenExact ssDNA / GenWand dsDNA / GenCircle dsDNA / EasyEdit sgRNA / SafeEdit sgRNA**



Advantages of HDR template design tool

1. **Comprehensive applications:**
   - Supports 1-1500bp deletions, 1bp-20kb insertions
   - Support ssDNA / dsDNA / GenCircle dsDNA templates
   - Support 10 species, Cas9 and Cas12a,
2. **More prices design:** updated on-target and off-target scores
3. **Enhance editing efficiency:** silent mutation function & CTS option
4. **Multiple functions**
   - Multiple tags
   - Optimized or customized HA length
   - Sequence flip tool
   - Multiple sequence check functions help avoid mistakes
5. **Validated design:** the tool has been double checked by bioinformatics scientist & FAS to ensure the accuracy

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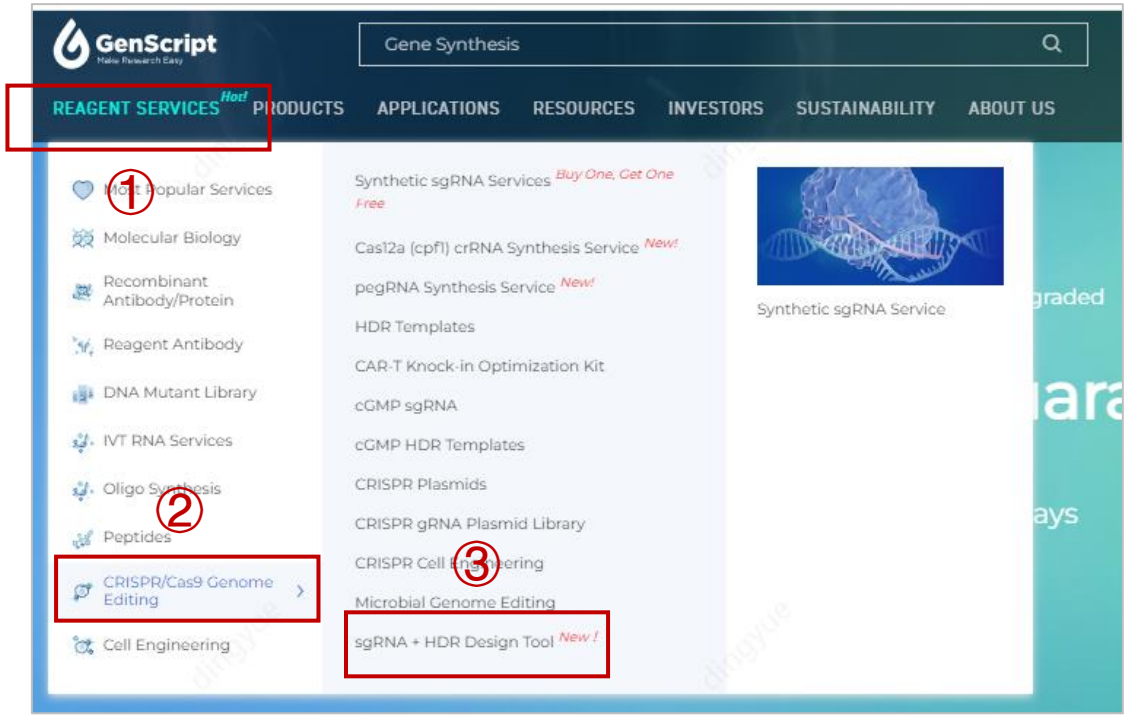
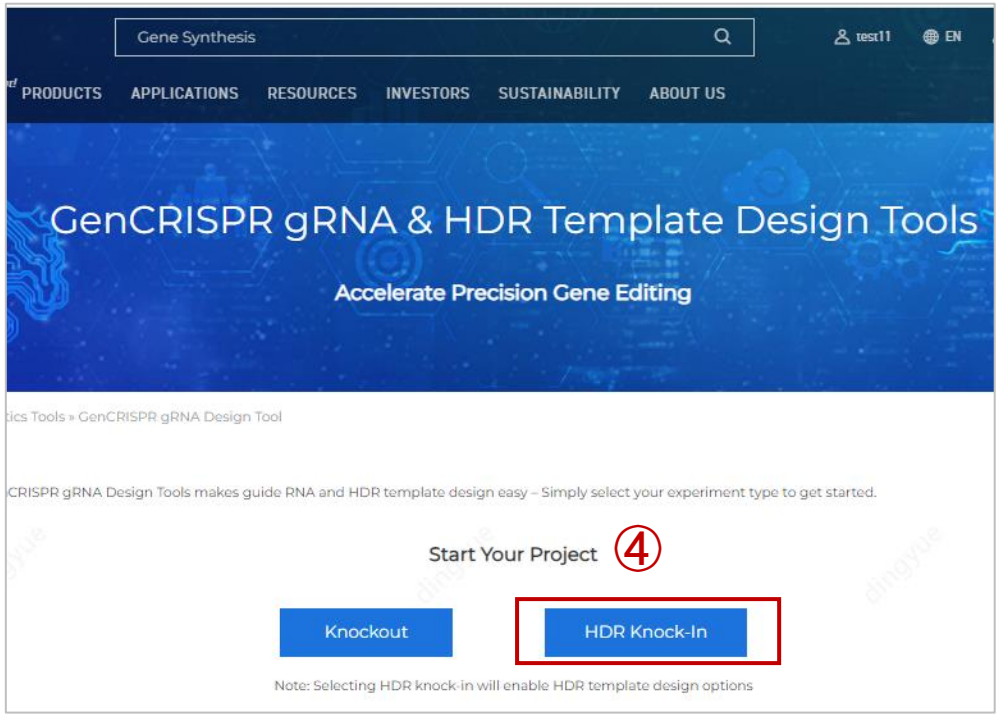
Design Process - HDR template design tool

Where can we find HDR template design tool?

1. Visit the address: https://www.genscript.com/tools/gRNA-design-tool/hdr_knock_in

1. Find the tool in official site:





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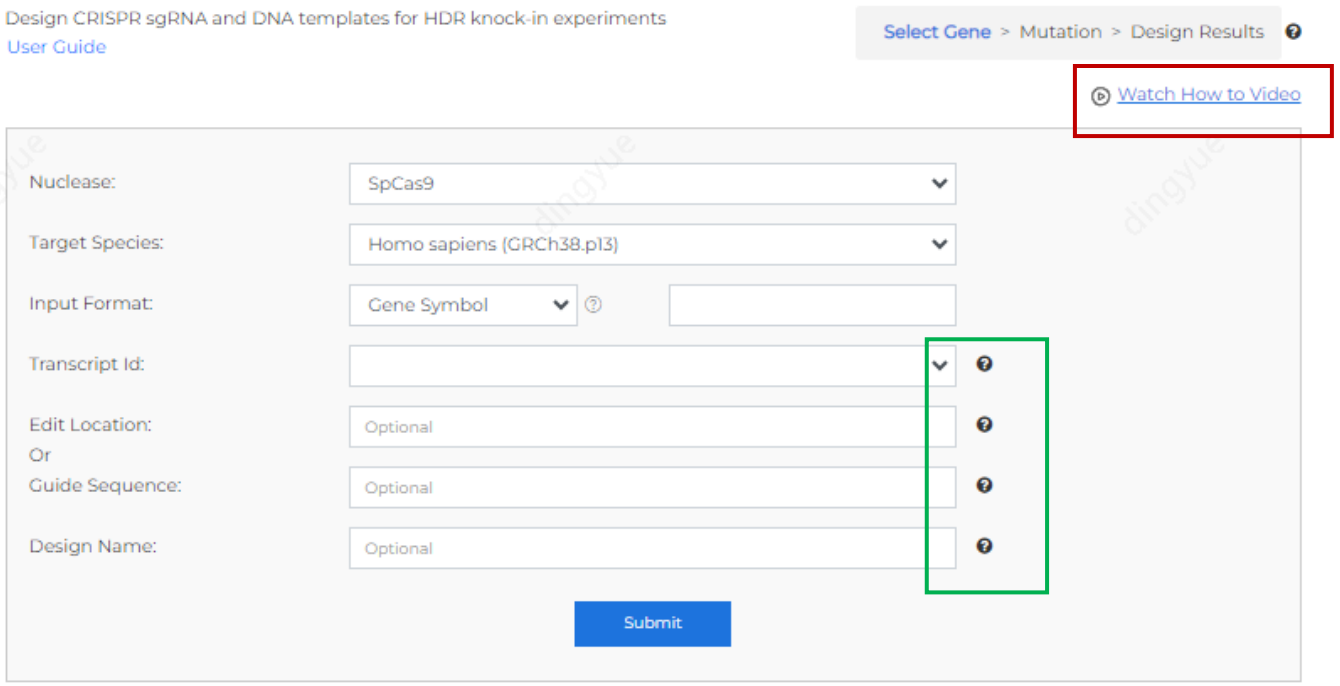
Design Process - HDR template design tool



Step 1. Select Gene

1. Select Nuclease / Select species / Enter Gene symbol
2. Please enter the editing location if you have a desired one (optional) - Input: 28725099; do not include the chr #
3. Please enter the guide sequence if you want to use it in knock-in experiment (optional)
4. Please enter the design name if you want, then you will get the sequence download file containing this design name (optional)
5. Click “submit”

Notes:

- Click “Watch How to Video” to see the design process (red labeled box)
- Click the black question marks to see the explanations (green labeled box)

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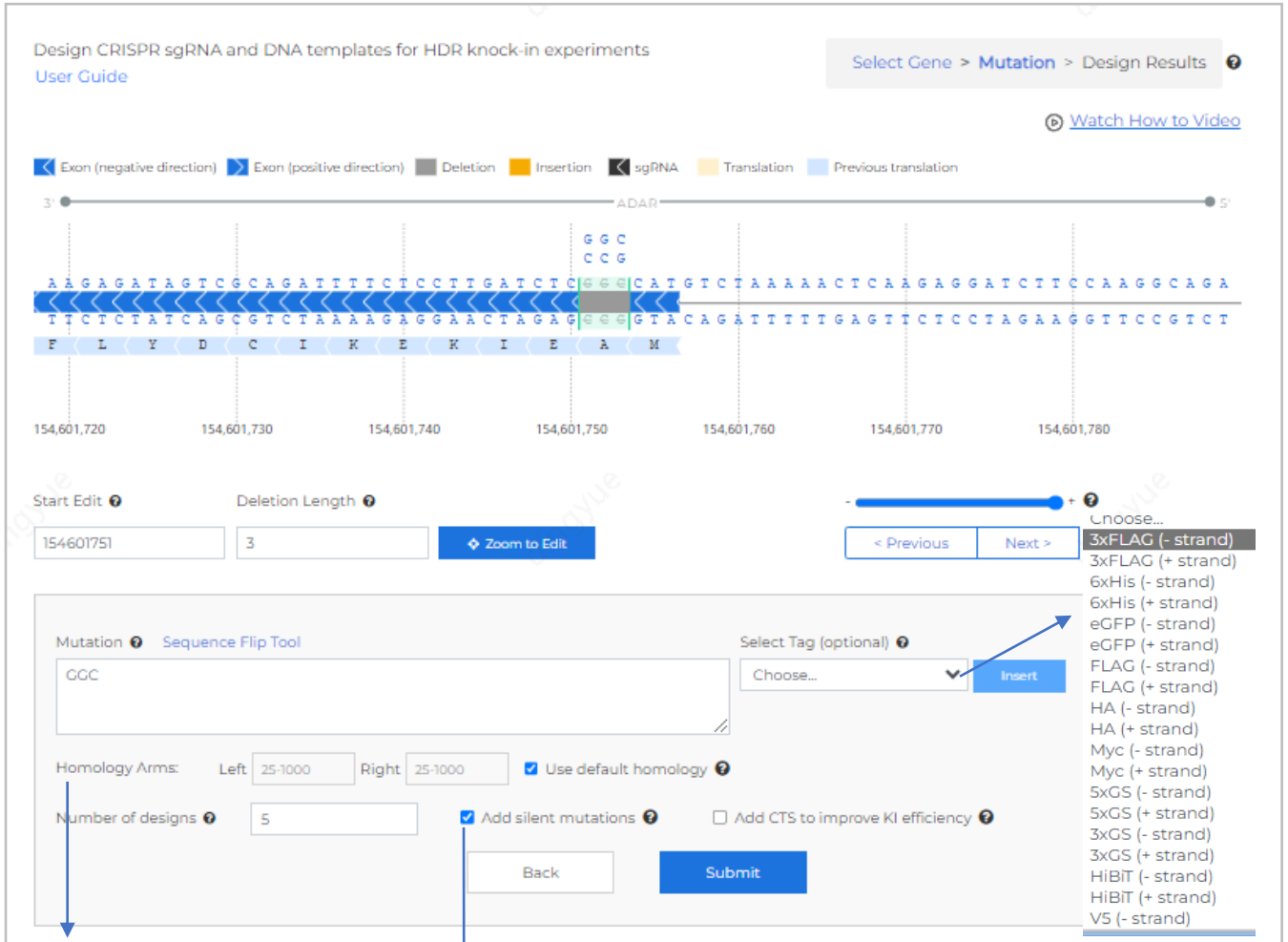
Design Process - HDR template design tool



- **HA length:** Recommend default HA length which is optimized according to deletion & insertion length (If you want to use customized HA length please unclick the "use default homology" and enter the desired length.)
- **Silent mutation:** Recommend to avoid recutting after knock-in

Step 2. Edit mutation

1. **Select editing site:** The green bar in the sequence map indicates the editing site. Click "Zoom to edit" to show the bases in the target sequence. Enter "Deletion Length" or drag the green bar across the nucleotide/sequence to be modified.
   a) For deletion edit, delete the original sequence in the "Mutation" Box
   b) For nucleotide substitution, rewrite the desired sequence in the "Mutation" box
   c) For a sequence insertion, enter the insertion sequence in the "Mutation" box
2. **Insertion of a common Tag:** 24 commonly used tags can be selected and inserted conveniently during the HDR design, click the "Insert" button to add the desired tag.

   Note: If the arrow in the sequence map points to the left, it indicates the gene is on the – strand, choose the tag option with the (-strand) sign; If the arrow points to the right, choose the tag with the (+ strand) sign.
3. **Choose CTS**: Increase editing efficiency, available for ssDNA / dsDNA
4. **Click "Submit"**

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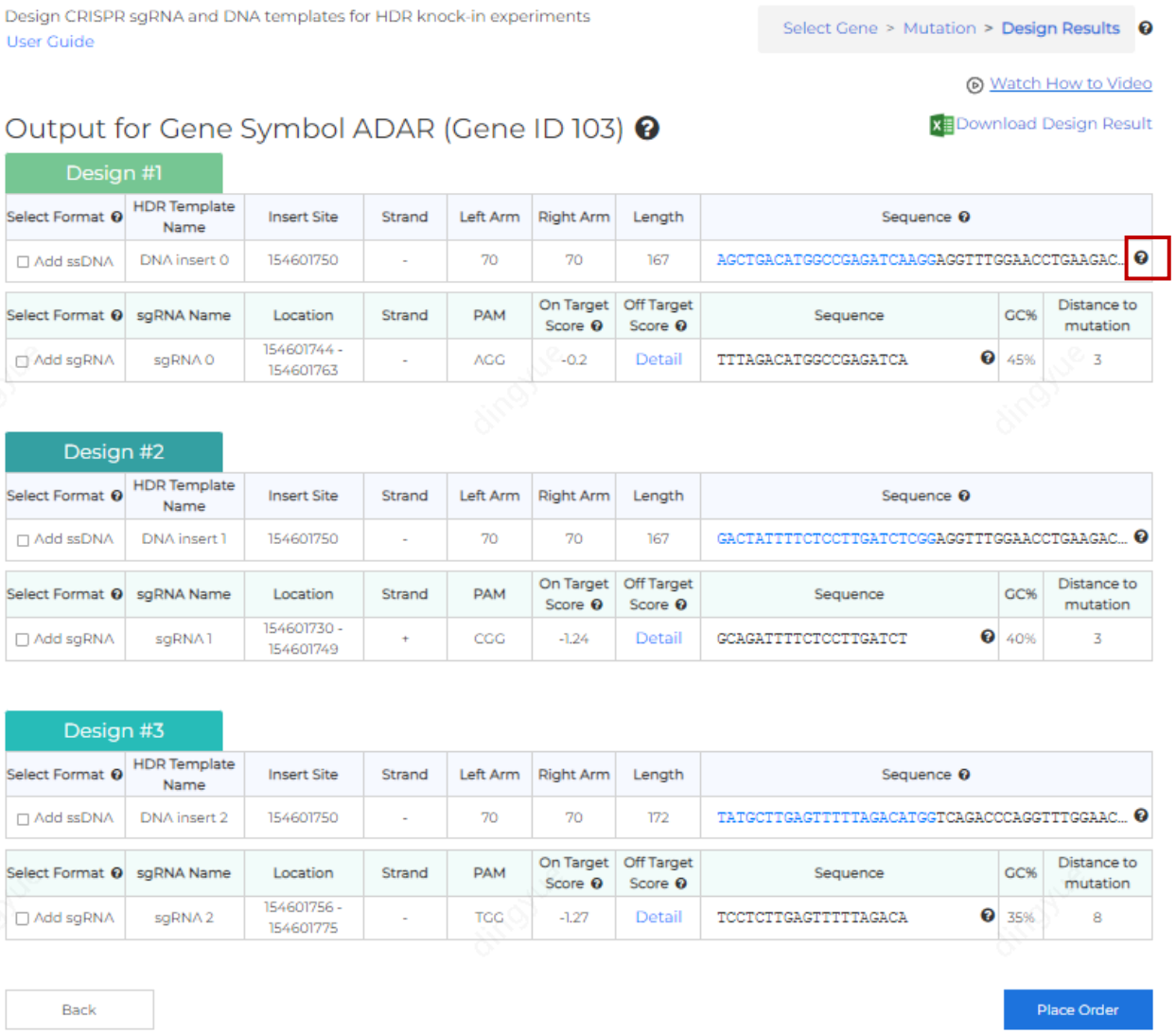
Design Process - HDR template design tool



Step 3. Select your sequence

1. **Select sgRNA and HDR template in the same design group**

   Parameter introduction

   - On target score: higher score means higher editing efficiency
   - Off target score: lower score means lower off target effects
   - Distance to mutation: the distance form actual cutting site to your desired cutting site, usually the smaller the better
   - Ranking: the smaller "Distance to mutation" will have higher ranking (If the Distance to mutation is within 20, we recommend sequence with high on target score and 40-80% GC%)

   Notes:

   (1) Different length has different HDR template type.

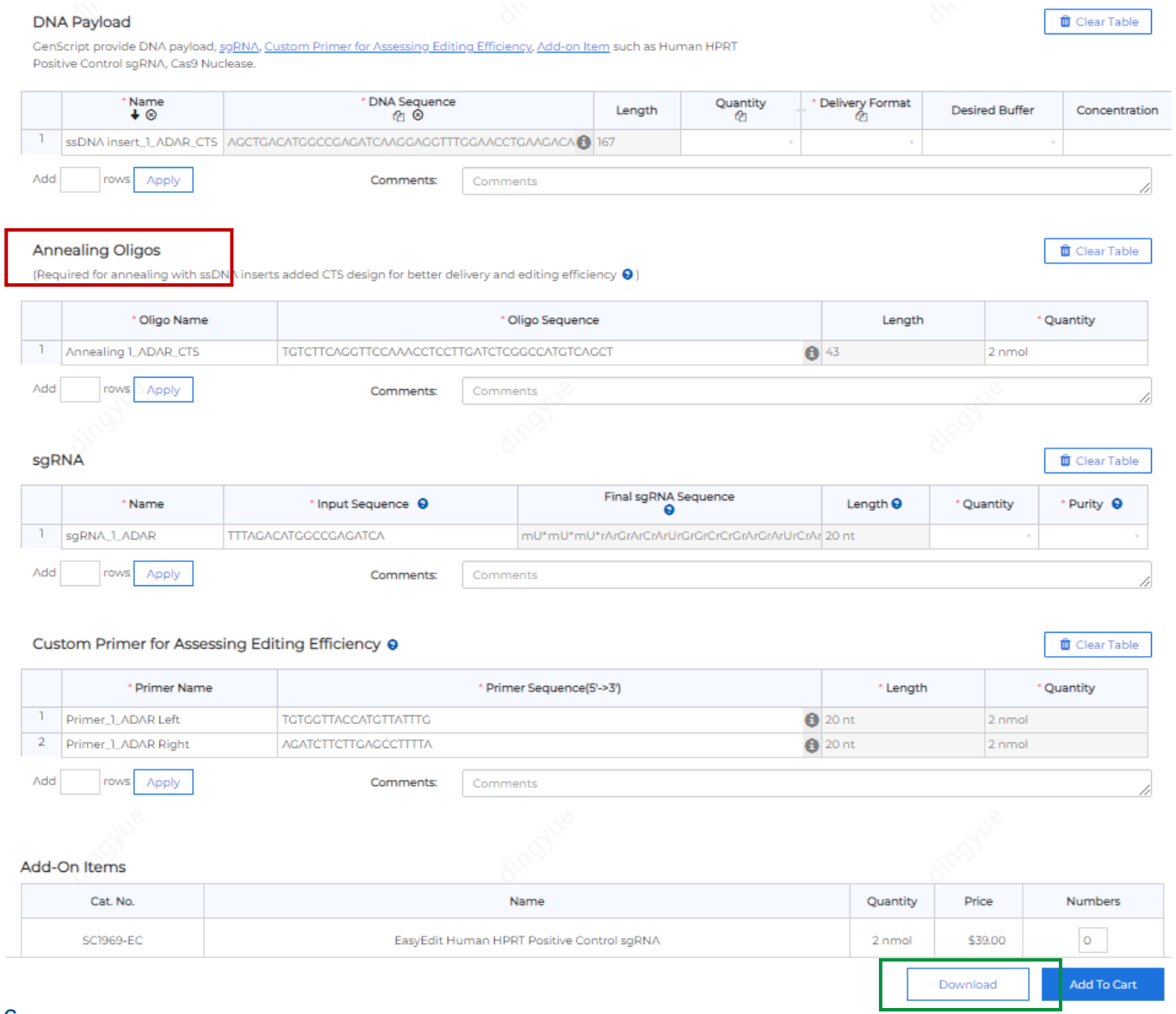
   ssDNA: 150nt – 5kb / dsDNA: 1-10kb / GenCircle dsDNA: 1- 20kb

   (2) Click black question marks to view sequence (red labeled box) blue bases present CTS sequence, orange bases present mutation sequence

   (3) Click "Download Design Results" to download the sequences

2. **Click "Place Order"**

Design Process - HDR template design tool



Step 4. Order your sequence

1. Select the quantity / deliver format for the HDR template

   Note: ssDNA with CTS needs an additional annealing oligo (red labeled box)

2. Select quantity/purity for sgRNA

3. Click “Download” to download the service specifications you just entered (green labeled box)

4. Click “Add to cart”

5. Click “Continue” → “Get a quote”→” Thank you for your Quotation!”

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