

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**

Resources > Bioinformatics Tools

Design CRISPR sgRNA and DNA templates for HDR knock-in experiments
User Guide

Select Gene > Mutation > Design Results

Watch How to Video

Nuclease: SpCas9

Target Species: Homo sapiens (GRCh38.p13)

Input Format: Gene Symbol

Transcript Id:

Edit Location: Optional

Or Guide Sequence: Optional

Design Name: Optional

Submit

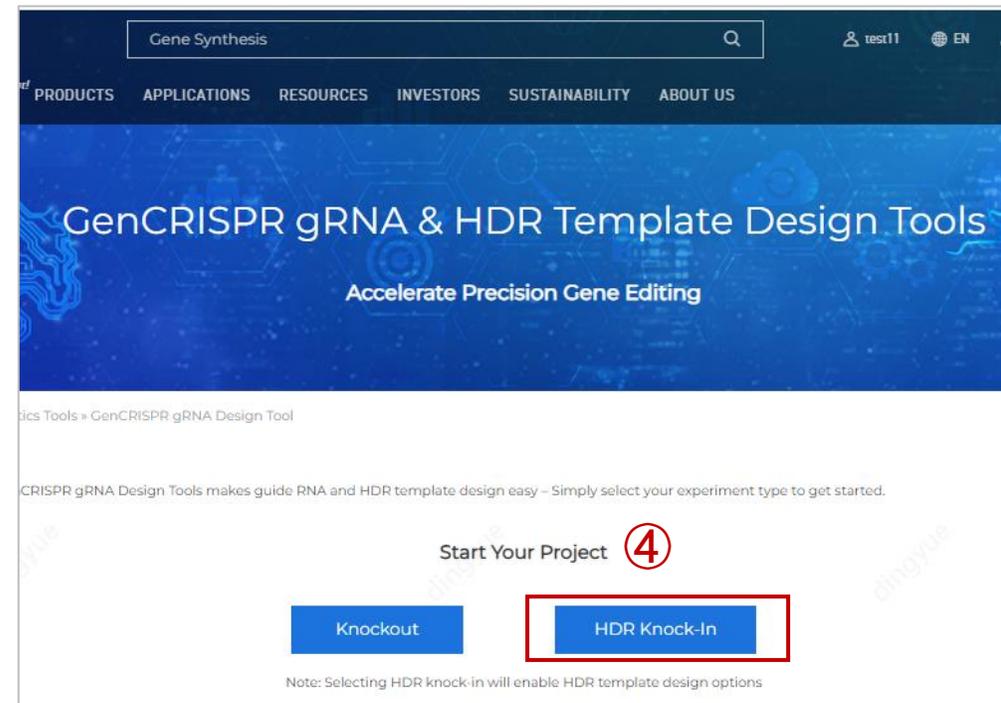
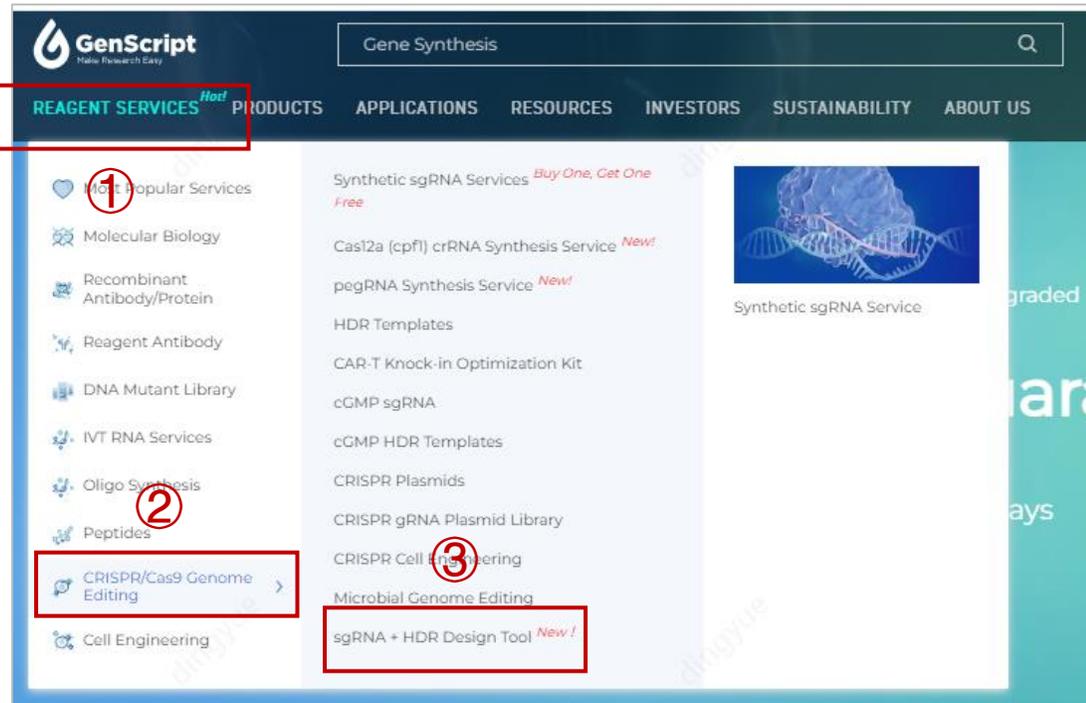
Advantages of HDR template design tool

1. **Comprehensive applications:**
 - Supports 1-1500bp deletions, 1bp-20kb insertions
 - Support ssDNA / dsDNA / GenCircle dsDNA templates
 - Support 10 species, Cas9 and Cas12a,
2. **More precise 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

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:



Design Process - HDR template design tool

Design CRISPR sgRNA and DNA templates for HDR knock-in experiments
[User Guide](#)

[Select Gene](#) > [Mutation](#) > [Design Results](#) ⓘ

Ⓢ [Watch How to Video](#)

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Transcript Id: [] ⓘ

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Or

Guide Sequence: Optional ⓘ

Design Name: Optional ⓘ

Submit

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)

Design Process - HDR template design tool

Design CRISPR sgRNA and DNA templates for HDR knock-in experiments
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Exon (negative direction) Exon (positive direction) Deletion Insertion sgRNA Translation Previous translation

3' ADAR 5'

AAGAGATAGTTCGCAGATTTTCTCCTTGATCTCCGTCATGTCTAAAAAAGGAGGATCTTCCAAGGCAGA
TTCTCTATCAGCGTCTAAAAGAGGAACTAGAGGGCTACAGATTTTGTAGTTCTCCTAGAAAGGTTCCGCTCT

F L Y D C I K E K I E A M

154,601,720 154,601,730 154,601,740 154,601,750 154,601,760 154,601,770 154,601,780

Start Edit Deletion Length

154601751 3 Zoom to Edit

Undo...
3xFLAG (- strand)
3xFLAG (+ strand)
6xHis (- strand)
6xHis (+ strand)
eGFP (- strand)
eGFP (+ strand)
FLAG (- strand)
FLAG (+ strand)
HA (- strand)
HA (+ strand)
Myc (- strand)
Myc (+ strand)
5xGS (- strand)
5xGS (+ strand)
3xGS (- strand)
3xGS (+ strand)
HIBIT (- strand)
HIBIT (+ strand)
V5 (- strand)

Mutation Sequence Flip Tool

GGC

Select Tag (optional) Choose... Insert

Homology Arms: Left 25-1000 Right 25-1000 Use default homology

Number of designs 5 Add silent mutations Add CTS to improve KI efficiency

Back Submit

- **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”**

Design Process - HDR template design tool

Design CRISPR sgRNA and DNA templates for HDR knock-in experiments
[User Guide](#)

Select Gene > Mutation > Design Results

[Watch How to Video](#)

[Download Design Result](#)

Output for Gene Symbol ADAR (Gene ID 103)

Design #1

Select Format	HDR Template Name	Insert Site	Strand	Left Arm	Right Arm	Length	Sequence
<input type="checkbox"/> Add ssDNA	DNA insert 0	154601750	-	70	70	167	AGCTGACATGGCCGAGATCAAGGAGGTTTGGAACTGAAGAC...

Select Format	sgRNA Name	Location	Strand	PAM	On Target Score	Off Target Score	Sequence	GC%	Distance to mutation
<input type="checkbox"/> Add sgRNA	sgRNA 0	154601744 - 154601763	-	AGG	-0.2	Detail	TTTAGACATGGCCGAGATCA	45%	3

Design #2

Select Format	HDR Template Name	Insert Site	Strand	Left Arm	Right Arm	Length	Sequence
<input type="checkbox"/> Add ssDNA	DNA insert 1	154601750	-	70	70	167	GACTATTTTCTCCTTGAATCTCGGAGGTTTGGAACTGAAGAC...

Select Format	sgRNA Name	Location	Strand	PAM	On Target Score	Off Target Score	Sequence	GC%	Distance to mutation
<input type="checkbox"/> Add sgRNA	sgRNA 1	154601730 - 154601749	+	CCG	-1.24	Detail	GCAGATTTTCTCCTTGAATCT	40%	3

Design #3

Select Format	HDR Template Name	Insert Site	Strand	Left Arm	Right Arm	Length	Sequence
<input type="checkbox"/> Add ssDNA	DNA insert 2	154601750	-	70	70	172	TATGCTTGAATTTTGTAGACATGTCAGACCCAGGTTTGGAACT...

Select Format	sgRNA Name	Location	Strand	PAM	On Target Score	Off Target Score	Sequence	GC%	Distance to mutation
<input type="checkbox"/> Add sgRNA	sgRNA 2	154601756 - 154601775	-	TCG	-1.27	Detail	TCCTCTTGAATTTTGTAGACA	35%	8

[Back](#)

[Place Order](#)

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 from 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

DNA Payload

GenScript provide DNA payload, [sgRNA](#), [Custom Primer for Assessing Editing Efficiency](#), [Add-on Item](#) such as Human HPRT Positive Control sgRNA, Cas9 Nuclease.

Clear Table

	* Name	* DNA Sequence	Length	Quantity	* Delivery Format	Desired Buffer	Concentration
1	ssDNA insert_1_ADAR_CTS	AGCTGACATGCCCGAGATCAAGGAGGTTGGACCTGAAGACA	167	*	*	*	*

Add rows Comments:

Annealing Oligos

(Required for annealing with ssDNA inserts added CTS design for better delivery and editing efficiency)

Clear Table

	* Oligo Name	* Oligo Sequence	Length	* Quantity
1	Annealing_1_ADAR_CTS	TGCTTCAGGTTCCAAACCTCCTTGATCTCGGCCATGTCAGCT	43	2 nmol

Add rows Comments:

sgRNA

Clear Table

	* Name	* Input Sequence	Final sgRNA Sequence	Length	* Quantity	* Purity
1	sgRNA_1_ADAR	TTTAGACATGCCCGAGATCA	mU*mU*mU*rArGrArCrArUrGrGrCrGrArGrArUrCrAr	20 nt	*	*

Add rows Comments:

Custom Primer for Assessing Editing Efficiency

Clear Table

	* Primer Name	* Primer Sequence(5'->3')	* Length	* Quantity
1	Primer_1_ADAR Left	TGTGCTTACCATGTTATTTG	20 nt	2 nmol
2	Primer_1_ADAR Right	AGATCTTCTTGAGCCCTTTTA	20 nt	2 nmol

Add rows Comments:

Add-On Items

Cat. No.	Name	Quantity	Price	Numbers
SC1969-EC	EasyEdit Human HPRT Positive Control sgRNA	2 nmol	\$39.00	<input type="text" value="0"/>

Download

Add To Cart

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!"