TECHNICAL MANUAL

horizon™
INSPIRED CELL SOLUTIONS

Dharmacon™ Edit-R™ HDR plasmid donor kit for fluorescent reporter knock-in

Contents

1 Introduction to the CRISPR-Cas9 system for gene engineering

CRISPR-Cas: an adaptive immunity defense mechanism in bacteria and archaea

The CRISPR (clustered regularly interspaced palindromic repeats)-Cas (CRISPR-associated proteins) system is an adaptive bacterial and archaeal defense mechanism that serves to recognize and silence incoming foreign nucleic acids. Upon infection by bacteriophage or other foreign DNA elements, a host organism can incorporate short sequences from the invading genetic material, called *protospacers*, into a specific region of its genome (the CRISPR locus) between short palindromic DNA repeats of variable lengths. Multiple spacer-repeat units are clustered at the CRISPR locus to form the CRISPR array. The entire locus, including the CRISPR array, is transcribed by RNA polymerase into a primary transcript, the pre-CRISPR RNA (pre-crRNA). The pre-crRNA is then processed into small, mature CRISPR RNAs (crRNAs) such that they include sequences complementary to the foreign, invading DNA. crRNAs guide a multifunctional protein or protein complex (the CRISPR-associated or Cas proteins) to cleave complementary target DNA that is adjacent to short sequences known as protospacer-adjacent motifs (PAMs). Thus, the organism acquires a way to protect itself from subsequent infection (Bhaya, 2011).

A CRISPR-Cas9 platform for mammalian genome editing

Many bacterial and archaeal CRISPR-Cas systems have been identified with diverse sets of mechanisms, Cas proteins and multi-subunit complexes. In particular, the processes and key components of the *Streptococcus pyogenes* CRISPR-Cas9 system have been well-studied and adapted for genome engineering in mammalian cells. In *S. pyogenes*, only three components are required for targeted DNA cleavage at specific target sites adjacent to a protospacer adjacent motiff (PAM; Jinek, 2012): (1) The endonuclease Cas9, programmed by (2) a mature crRNA processed from transcription of the CRISPR locus/array which complexes with (3) another CRISPR locus-encoded RNA, the *trans*-activating CRISPR RNA (tracrRNA; Figure 1A; Deltcheva, 2011). Alternatively, the crRNA can be fused to the tracrRNA creating a chimeric structure termed a single guide RNA (sgRNA; Figure 1B: Jinek, 2012). Upon site-specific double-stranded DNA cleavage, a mammalian cell can repair such a break through either non-homologous end joining (NHEJ) or homologous recombination (HR). NHEJ is often imperfect, resulting in insertions and deletions (indels) that can cause nonsense mutations resulting in gene disruptions to produce gene knockouts (Mali, 2014; Sampson, 2014). This endogenous DNA break repair process, coupled with the highly tractable *S. pyogenes* CRISPR-Cas9 system, allows for a readily engineered platform to permanently disrupt gene function.

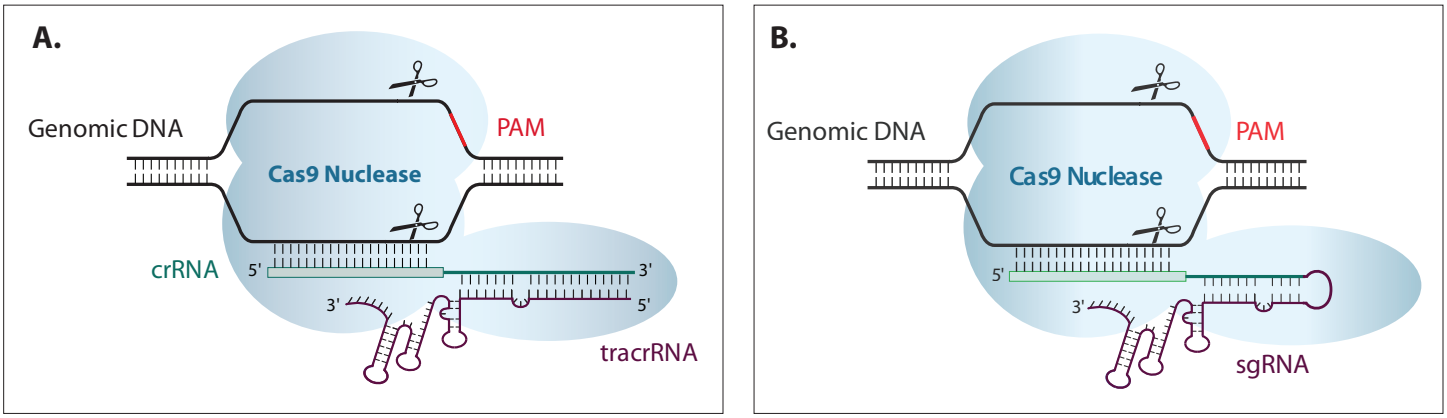


Figure 1. Illustration of CRISPR-Cas9 system. Cas9 nuclease (light blue), programmed by the crRNA (green):tracrRNA (blue) complex (A) or the sgRNA (B), cutting both strands of genomic DNA 5' of the PAM (red).

Homologous recombination is the exchange of DNA sequence information using sequence homology. Homology-directed repair (HDR) is a process of homologous recombination whereby a DNA template provides the homology necessary for precise repair of a double-strand break (DSB). This DNA template can come from within the cell during late S phase and G2 phase of the cell cycle, when sister chromatids are available, prior to the completion of mitosis. Importantly, exogenous repair templates can be delivered into a cell, most often in the form of a DNA plasmid donor or a synthetic single-stranded DNA (ssDNA) donor oligonucleotide (oligo), to generate a precise change in the genome. (See Figure 2 for an illustration).

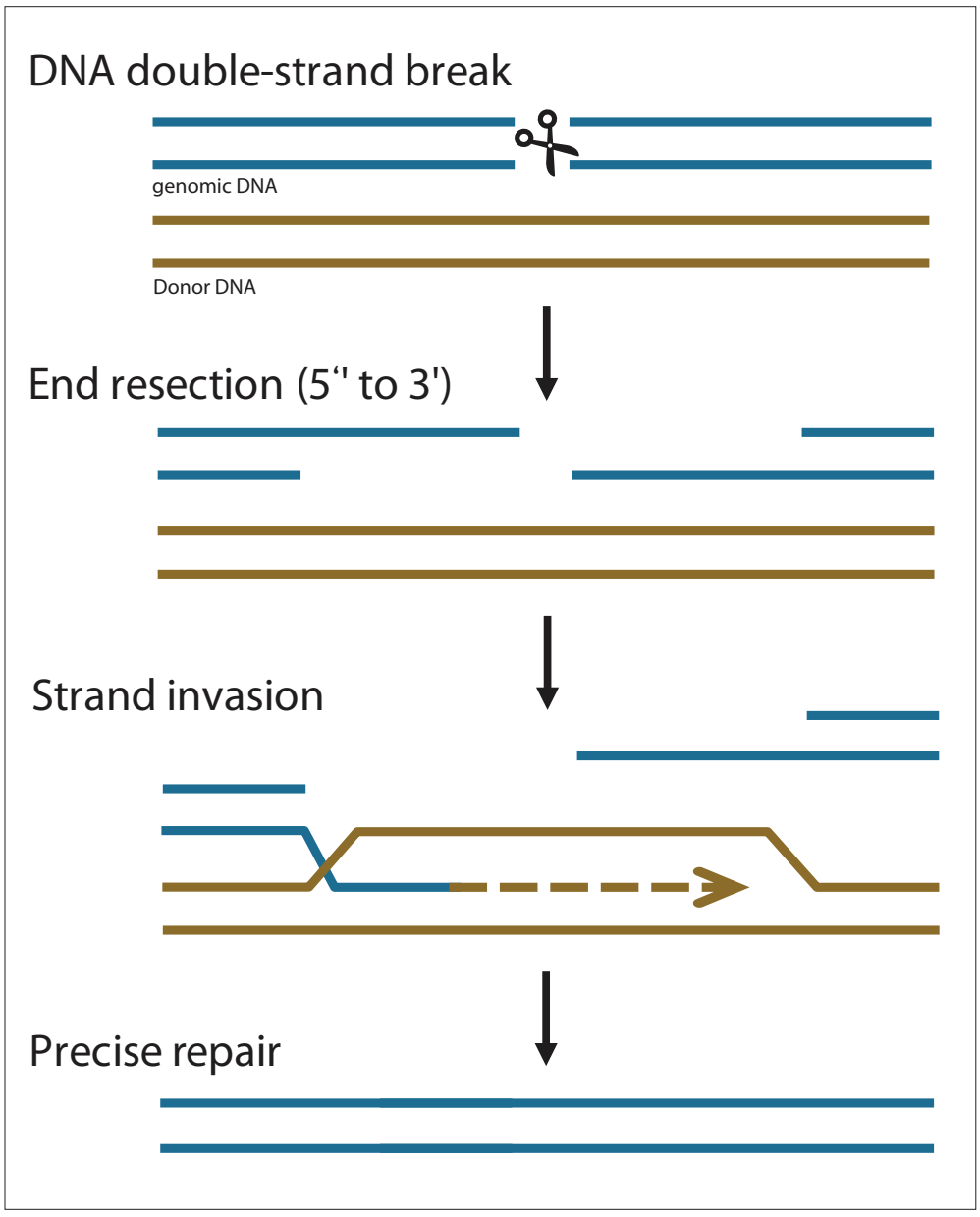


Figure 2. Diagram of DSB repair using the homology-directed repair (HDR) pathway.

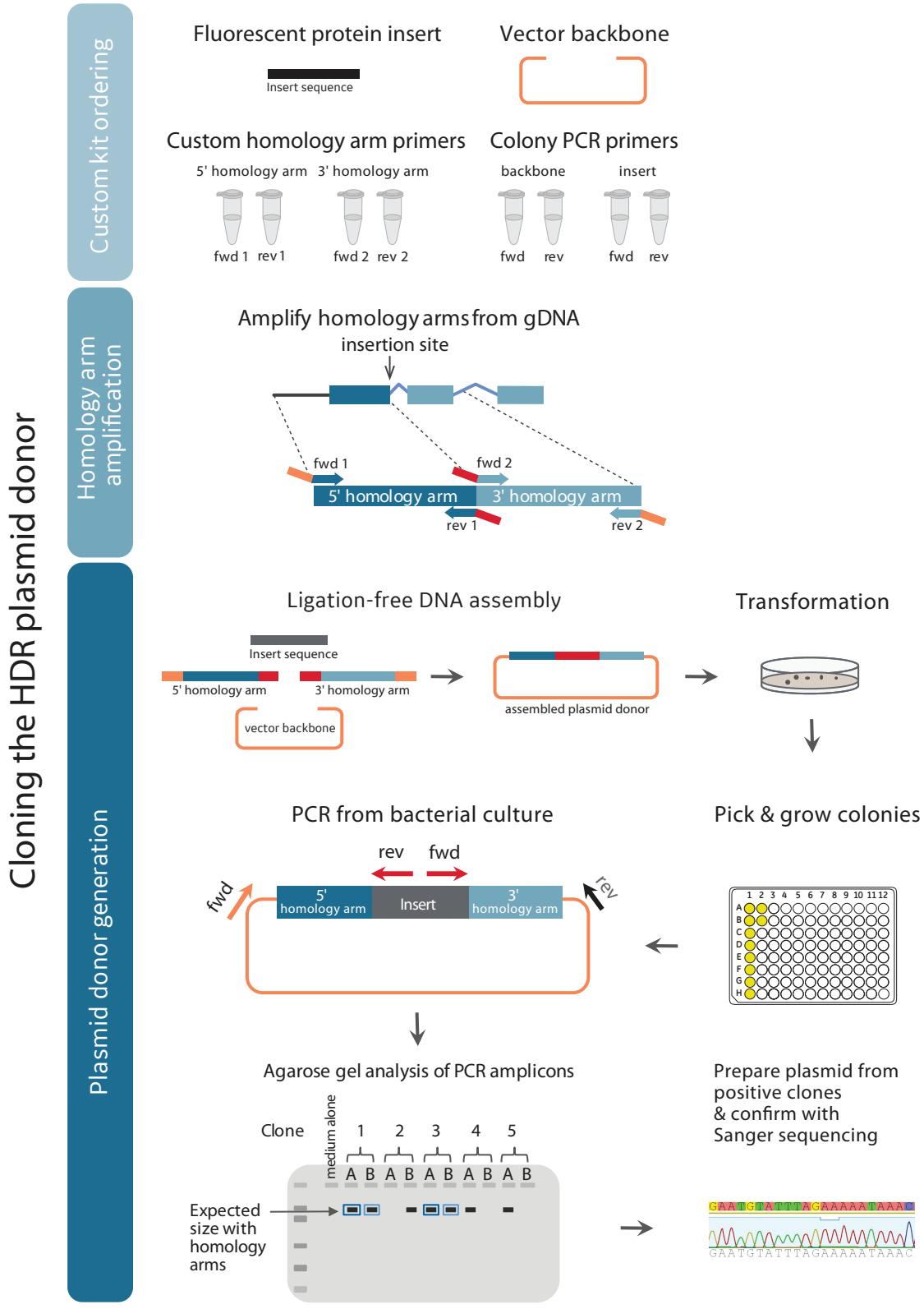


Figure 3. Diagram of the plasmid donor cloning workflow including component ordering, homology arm amplification, and plasmid donor generation. This product supports the use of EGFP, mKate2, and custom sequence inserts. For illustrative purposes, mKate2 will be used. Colors on the diagram indicate the origin of the DNA (dark blue = 5' homology arm, light blue = 3' homology arm, red = mKate2 fluorescent reporter sequence, orange = plasmid backbone). medium alone = media-only negative PCR control.

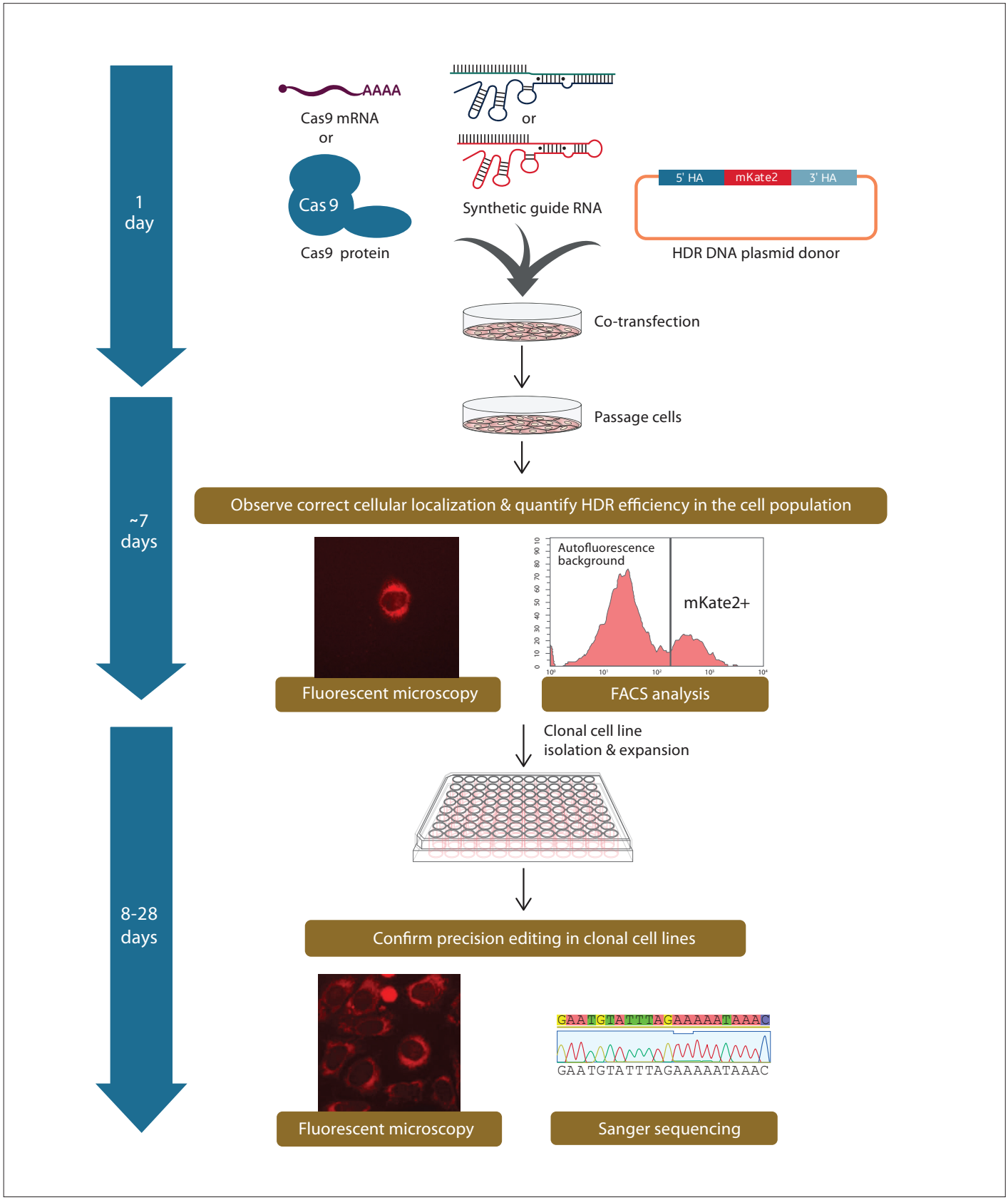


Figure 4. Workflow to acquire desired mKate2 knock-in clonal cell line. A similar workflow will be followed if using EGFP or a custom insert sequence.

The Edit-R HDR plasmid donor kit contains the components to construct a plasmid donor for insertion of monomeric EGFP (University of California, San Diego), mKate2 (Evrogen, Moscow, Russia), or a custom, user specified insert sequence in a desired endogenous genomic target location. The final plasmid donor (generated with the kit) will have the desired insert flanked by two homology arms that are required for HDR-mediated knock-in. The workflow involves: (1) order gene-specific homology arm primers and the Edit-R HDR donor kit (using the Edit-R HDR Donor Designer), (2) amplify the homology arms surrounding the insertion site in your target gene, (3) assemble and verify the sequence of the HDR plasmid donor (Figure 3), and (4) co-transfect the assembled plasmid donor with the Edit-R CRISPR-Cas9 components: Cas9 mRNA or protein, synthetic guide RNA (crRNA:tracrRNA complex or sgRNA), followed by confirming the HDR-mediated knock-in in a population as well as generating a clonal cell line (Figure 4).

This technical manual provides protocols and guidelines optimized for efficient plasmid donor generation and HDR-mediated knock-in for precise genome engineering using the Edit-R CRISPR-Cas9 platform.

Edit-R Cas9 Nuclease

The Edit-R Cas9 Nuclease mRNA (Cat #CAS11195) contains a human codon-optimized version of the *S. pyogenes* Cas9 (*Csn1*) gene with a 5' and 3' nuclear localization signal (NLS). The Cas9 mRNA is *in vitro* transcribed, 5' capped and polyadenylated for translation and nuclear localization of the Cas9 protein. The Edit-R Cas9 Nuclease protein NLS (Cat #CAS11200) contains the *S. pyogenes* Cas9 (*Csn1*) gene with a C-terminal nuclear localization signal (NLS). The Cas9 mRNA and Cas9 protein are ready for direct transfection or electroporation into cells.

Edit-R synthetic guide RNA

Edit-R *trans*-activiating CRISPR RNA (tracrRNA)

Edit-R tracrRNA is a synthetic, HPLC-purified, long RNA required for use with Edit-R crRNA to form the complex that programs Cas9 nuclease. It is modified for nuclease resistance and can be used with modified or unmodified Edit-R crRNA.

Edit-R CRISPR RNA (crRNA)

The Edit-R crRNA is a synthetic RNA, comprised of 20 nucleotides identical to the genomic DNA (gDNA) target site, or protospacer, followed by a fixed *S. pyogenes* repeat sequence that interacts with the tracrRNA. The chosen protospacer sequence in the target gDNA must be immediately upstream of a PAM sequence (NGG for *S. pyogenes*). Edit-R Predesigned crRNAs are available for human, mouse and rat coding genes on and custom crRNA (for specific applications including HDR) can be designed and ordered using the Dharmacon CRISPR Design Tool.

Edit-R synthetic single guide RNA (sgRNA)

Edit-R synthetic sgRNA is a 100 nucleotide chimera fusing the crRNA and tracrRNA sequences with a 4 nt tetraloop sequence (Jinek 2012). It is modified for nuclease resistance on both 5′ and 3′ ends of the molecule. Edit-R synthetic sgRNAs can be ordered through the Dharmacon CRISPR Design Tool.

Edit-R HDR plasmid donor kit

The Edit-R HDR plasmid donor kit contains all the DNA components to build and quality check a custom DNA plasmid donor to introduce a fluorescent tag to any genomic location when coupled with Edit-R HDR DNA plasmid donor homology arm (HA) custom primer pairs. Specifically, the kit comes with custom-designed HA PCR primers and three static components: the Edit-R HDR plasmid donor backbone, the Edit-R HDR plasmid donor insert, and Edit-R HDR plasmid donor colony PCR primer pairs specific to verify the sequence of mKate2 fluorescent reporters. The HA PCR primers and kit components are designed and ordered using the HDR Donor Designer. The HA PCR primers are designed to amplify HAs with lengths ranging from 500 to 1000 bp, with an adapter sequence appended, which is necessary for compatibility with the HDR plasmid donor backbone. The HDR Donor Designer additionally designs the HA primers to introduce silent mutations to disrupt the intact CRISPR targeting sequence to avoid unintentional cutting of gDNA repaired by HDR.

3 Guidelines for cloning HDR plasmid donors

To knock-in DNA sequence such as a fluorescent tag into specified gene target, an HDR plasmid donor needs to be constructed to contain the intended DNA sequence or fluorescent tag (EGFP/mKate2) flanked by two HAs of 500 to 1000 bp in length. This protocol provides instructions for ligation-free cloning of the HDR plasmid donor.

Materials required for cloning HDR plasmid donors

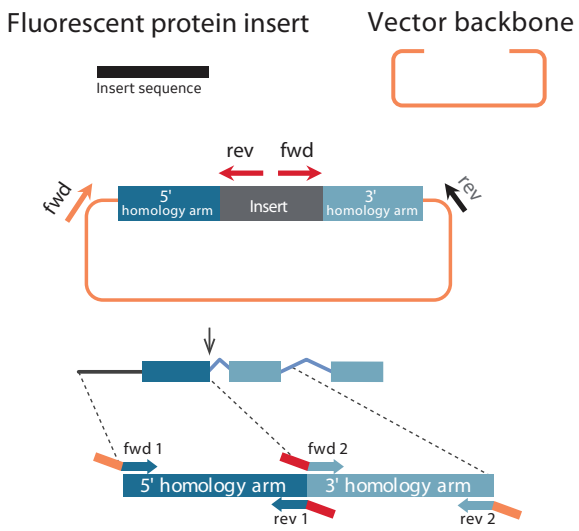
- » Edit-R HDR EGFP, mKate2, or custom insert
- » Edit-R HDR plasmid donor backbone

- » Edit-R colony PCR primer backbone forward, 5 nmoles
- » Edit-R colony PCR primer EGFP or mKate2 reverse, 5 nmoles
- » Edit-R colony PCR primer EGFP or mKate2 forward, 5 nmoles
- » Edit-R colony PCR primer backbone reverse, 5 nmoles

Custom PCR primers for homology arm amplification

5' homology arm primers:
- » Edit-R custom HA primer forward 1, 20 nmoles
- » Edit-R custom HA primer reverse 1, 20 nmoles

3' homology arm primers:
- » Edit-R custom HA primer forward 2, 20 nmoles
- » Edit-R custom HA primer reverse 2, 20 nmoles



Reagents to be supplied by researcher

This cloning protocol uses common molecular biology reagents and instruments. The following protocol is provided for illustrative purposes, but substitutions with other similar products are acceptable. For further protocol information or troubleshooting please consult the product's manufacturer. The following additional materials are required:

- High-fidelity DNA polymerase [such as Thermo Scientific Phusion Hot Start II DNA Polymerase (Cat #F549S) or NEB Q5® High-Fidelity DNA Polymerase (Cat #M0491S)]
- *Taq* DNA polymerase [such as Thermo Scientific DreamTaq Green PCR Master Mix 2x (Cat #K0801)]
- 1x TAE buffer
- 1x PBS buffer
- DNA Assembly cloning kit [such as NEB Gibson Assembly® Cloning Kit (Cat #E5510S)]
- Chemically competent *E. coli* cells with a transformation efficiency of at least 1×10^9 cfu/µg [such as NEB 5-alpha Competent *E. coli* (High Efficiency) Cat #C2987I)]
- NZYM plus broth [such as Teknova (Cat #N1215)]
- LB Agar plates with carbenicillin (100 µg/mL)
- Thermal cycler
- Endotoxin-free plasmid prep kit [such as Clontech NucleoBond® Xtra Midi Plus EF (Cat #740422.10)]
- Tris buffer: 10 mM Tris-HCl pH 7.4, nuclease-free buffer [such as 10 mM Tris-HCl Buffer pH 7.4 (Cat # B-006000-100)] or prepare with the recipe here
- DNA extraction kit [such as Thermo Scientific™ GeneJET Gel Extraction Kit (Cat #K0691)]
- Genomic DNA isolation kit [such as Thermo Scientific™ GeneJET Genomic DNA Purification Kit (Cat #K0712)]
- Blue light transilluminater [such as the Thermo Scientific™ Safe Imager 2.0 blue light transilluminator (Cat #G6600)]
- Fluorometric assay [such as Life Technologies Qubit® dsDNA HA Assay Kit (Cat #Q32851)]

Cloning HDR plasmid donors

1. Design and order custom HA primers and the Edit-R HDR plasmid donor kit
   a. Use the Edit-R HDR Donor Designer webtool to custom design HA primers by defining the genomic location for HDR insertion.
   b. Add all desired primers to the cart and ensure the static kit components are added to the cart as well.
   c. The static kit components may also be ordered independently for use with previously designed HA primer sets.
2. Generate homology arms
   a. Isolate gDNA following the manufacturer's instructions to use as template for homology arm PCR.
   b. Adjust concentration of isolated gDNA to 25 ng/µL.

gDNA from the cell line where will be performed is required, as cell line-specific SNPs can affect knock-in efficiency

   c. PCR amplify 5' and 3' homology arms
      i. Resuspend Edit-R custom HA PCR primers to 50 µM. Verify the concentration using UV spectrophotometry at 260 nm, and adjust volume accordingly.
      ii. Set up a 50 µL PCR for each homology arm to be assembled into the plasmid donor (Tables 1 and 2) and perform PCR (Table 3). Conditions for Phusion Hot Start II High-Fidelity DNA polymerase are shown (Table 3).

Multiple 50 µL reaction replicates (3-6) are recommended for each homology arm to acquire enough material after gel purification (minimum of 20 ng/µL purified homology arm).

Additives such as DMSO or high GC buffer may be necessary for difficult gDNA context as recommended by polymerase manufacturer.

Table 1. PCR setup for 5' homology arm for use generation of HDR plasmid donor

Reagent	Volume	Final concentration
5x Phusion HF buffer	10 µL	1x
Edit-R custom HA primer forward 1 (50 µM)	0.5 µL	500 nM
Edit-R custom HA primer reverse 1 (50 µM)	0.5 µL	500 nM
dNTPs (10 mM)	1 µL	200 µM each
Phusion Hot Start II High-Fidelity DNA polymerase (2 U/µL)	1 µL	0.04 U/µL
Water, nuclease free	33 µL	N/A
gDNA (25 ng/µL)	4 µL	2 ng/µL

Table 2. PCR setup for 3' homology arm for generation of HDR plasmid donor

Reagent	Volume	Final concentration
5x Phusion HF buffer	10 µL	1x
Edit-R custom HA primer forward 2 (50 µM)	0.5 µL	500 nM
Edit-R custom HA primer reverse 2 (50 µM)	0.5 µL	500 nM
dNTPs (10 mM)	1 µL	200 µM each
Phusion Hot Start II High-Fidelity DNA polymerase (2 U/µL)	1 µL	0.04 U/µL
Water, nuclease free	33 µL	N/A
gDNA (25 ng/µL)	4 µL	2 ng/µL

Table 3. Thermal cycling conditions

Reagent	Temperture	Time	Cycle(s)
Initial denaturation	98 °C	30 s	1
Denature Annealing Extension	98 °C Tm + 3 °C 72 °C	10 s 20 s 40 s	35
Final extension	72 °C	10 min	1

When calculating melting temperature (Tm) the adapter sequences should not be included even though the Tm will change after the first few cycles. Refer to Table 4 for the adapter sequences.

Table 4. Edit-R custom HA primer components

HA primers	Adapter sequence (5' to 3')	Gene specific
Edit-R custom HA primer forward 1	ACAGAGTGATATTATTGACACGCCC	custom
Edit-R custom HA primer reverse 1	TCGCCCTTGCTCACGCTGCCACCAGC (EGFP) ATCAGCTCGCTCACGCTGCCACCAGC (mKate2)	custom
Edit-R custom HA primer forward 2	GGACGAGCTGTACAAGGGAGGTAGC (EGFP) CAAACTGGGGCACAGAGGAGGTAGC (mKate2)	custom
Edit-R custom HA primer reverse 2	ATAACGGAGACCGGCACACTGGCCAT	custom

3. Purification of amplified homology arms
   a. Load homology arm PCR, or pooled replicate reactions, on a 2% TAE agarose gel with SYBR green (1:10,000 dilution).
   b. Use a blue light transilluminator to visualize and cut out the appropriate size DNA band.

Use of blue light (~470 nM) is required over ultraviolet (UV) light to avoid DNA damage and therefore improve overall cloning efficiency.

   c. Extract DNA from the gel using a gel extraction kit.
      i. Elute in Tris buffer for stability of PCR products through freeze-thaw cycles.
   d. Quantify DNA, using an agarose gel or a fluorometric assay such as the Qubit® dsDNA HA Assay Kit (Life Technologies Cat #Q32851).

Quantification using UV-Vis spectrophotometry is not recommended for this step, because accurate quantification of dsDNA is required.

4. Ligation-free cloning of the Edit-R HDR plasmid donor
   a. Resuspend the HDR insert and Edit-R HDR plasmid donor backbone components:
      i. Resuspend insert to 20 ng/µL by adding 12.5 µL Tris buffer.
      ii. Resuspend Edit-R HDR plasmid donor backbone to 30 ng/µL by adding 40 µL Tris buffer.
      iii. Adjust concentration of PCR amplicons of homology arms to 20 ng/µL using Tris buffer.
      iv. For all components (insert, backbone and PCR amplicons) verify concentration by agarose gel or fluorometric assay and adjust if necessary.
   b. Set up a Gibson Assembly reaction according to manufacturer's instructions. We have found that 0.2 pmol total reaction at a 1:1:1:1 molar ratio of (Edit-R HDR plasmid donor: backbone insert:5' homology arm:3' homology arm) works well for this four-part assembly. Table 5 below contains example reaction setup for 1000 bp homology arms. Adjust the volumes as necessary to accommodate different length homology arms, while still maintaining the 0.2 pmol Gibson Assembly reaction.
      i. Incubate at 50 °C for 60 minutes then store on ice until ready for transformation.

The Edit-R HDR plasmid donor kit contains material for 10 assembly reactions of 0.2 pmol total DNA as shown in Table 5. If the amount of total DNA for assembly is adjusted, the number of reactions will differ accordingly.

We recommend storing single use aliquots of the Gibson Assembly master mix to avoid multiple freeze-thaw cycles.

Table 5. Gibson Assembly reaction setup with total DNA input of 0.2 pmol using homology arms with length of 1000 bp and mKate2 insert.

Reagent	Size (kb)	Molecular Weight (g/mol)	Concentration (ng/µL)	DNA (pmol)	Volume (µL)
Edit-R HDR plasmid donor backbone	3.449	2,131,482	30	0.05	3.6
Edit-R HDR mKate2 insert	0.714	441,252	20	0.05	1.1
5' homology arm	1	618,000	20	0.05	1.5
3' homology arm	1	618,000	20	0.05	1.5
water, nuclease free	N/A	N/A	N/A	N/A	2.3
Gibson Master Mix	N/A	N/A	N/A	N/A	10
				Total Volume	**20**

5. Transformation
   a. Transform 1 × 10^9 competent cells.
      i. Protocol for NEB® 5-alpha Competent *E. coli* (High Efficiency) (Cat # C2987I)—please refer to manufacturer's instructions for specific competent cells.
         1. Warm LB Agar plates with Carbenicillin (100 µg/mL) in 37 °C incubator.
         2. Thaw 50 µL of competent cells per assembly reaction on ice.
         3. Add 2 µL Gibson Assembly reaction to the thawed cells.
         4. Mix gently by pipetting the reaction 4-5 times.
         5. Place on ice for 30 minutes.
         6. Heat shock at 42 °C for 30 seconds without any mixing.
         7. Return to ice for 5 minutes.
         8. Add 100 µL SOC medium to each tube.
         9. Spread up to 150 µL onto each prewarmed selection plate.
         10. Incubate plates overnight at 37 °C.

6. Colony PCR
   a. Any colony PCR protocol can be used based on typical lab protocols. Below is an optional and optimized fast colony PCR protocol that will allow for quick growing of colonies and colony PCR analysis in only one day.
      i. Pick 10 colonies per assembly reaction and grow in 100 µL of NZYM plus medium for 6 hours at 37 °C.

After 6 hours, the 100 µL should be turbid by visual inspection. If not, continue growing at 37 °C for another 2 hours or until turbid.

      ii. Set up colony PCR in a 96-well plate as shown in Table 6 below. Refer to Table 7 for specific primer pairs to amplify both the 5' and 3' regions for the intended insert. We recommend including a no template control reaction (add NYZM plus medium alone instead of bacterial culture)

Table 6. Specific primer pairs needed for colony PCR QC of amplification regions, including the expected positive product sizes after electrophoresis.

Insert	Primer pairs	Amplification region	Positive product size (bp)
EGFP	Edit-R Colony PCR Primer Backbone Forward—Universal and Edit-R colony PCR primer EGFP reverse	5' EGFP homology arm	289 + 5' homology arm length
	Edit-R Colony PCR Primer Backbone Reverse—Universal and Edit-R colony PCR primer EGFP forward	3' EGFP homology arm	563 + 3' homology arm length
mKate2	Edit-R Colony PCR Primer Backbone Forward—Universal and Edit-R colony PCR primer mKate2 reverse	5' mKate2 homology arm	215 + 5' homology arm length
	Edit-R Colony PCR Primer Backbone Reverse—Universal and Edit-R colony PCR primer EGFP forward	3' mKate2 homology arm	415 + 3' homology arm length
Custom insert	Edit-R Colony PCR Primer Backbone Forward—Universal and Edit-R colony PCR primer EGFP reverse	5' homology arm, insert, and 3' homology arm	464 + 5' homology arm length, insert length, and 3' homology arm length

Table 7. Example Colony PCR setup to detect the 5', mKate2 homology arms. (For EGFP or custom inserts, use the colony PCR primers specified in Table 6.)

Reagent	1 reaction volume (µL)
DreamTaq 2x master mix	12.5
Edit-R Colony PCR Primer Backbone Forward - Universal (10 µM)	2.5
Edit-R colony PCR primer mKate2 reverse (10 µM)	2.5
Water, nuclease free	4.25
DMSO (5%)	1.25
Template (diluted 4 fold)	2
Total volume	25

iii. Make a master mix of all components except the template according to Table 7.
iv. Add 23 µL of the master mix to each well of the 96-well plate.
v. Dilute growing cultures from step 6a. (template) into a new 96-well plate by adding 2 µL culture to 6 µL of nuclease-free water and pipette up and down 3-4 times.
vi. Add 2 µL of the diluted template to one well in the colony PCR master mix plate. Add 2 µL NYZM plus medium alone to one reaction for the no template control sample.
vii. Pipette up and down 3-4 times to mix the reactions.
viii. Seal the PCR plate carefully, spin down all volume to the bottom of the plate and run in a thermal cycler according to these optimized conditions (Table 8). For a custom insert, adjust the PCR cycling conditions to match insert length, according to manufactures instructions.
ix. Store the remaining cultures at 4 °C to use as starter culture for an overnight growth and from positive resulting colony PCR.

Table 8. Optimized cycling conditions for colony PCR

Cycle step	Temperature	Time	Cycle(s)
Initial denaturation	95 °C	10 min	1
Denature	95 °C	15 s	
Annealing	61 °C	50 s	35
Extension	72 °C	30 s*	
Final extension	72 °C	5 min	1

Custom inserts will require a longer extension time. For DreamTaq polymerase an extension time of 30 s per kb is recommended.

x. Load 5 µL on an agarose gel to confirm amplicon length is as expected for homology arm insertions into the HDR plasmid donor.
xi. Expected sizes for negative controls are shown in Table 7. Product size for positive clones will run higher based on the size of the homology arms.

b. Grow 1-3 positive colonies (containing the expected sized 5'and 3' homology arm) and prepare the plasmids using a plasmid DNA miniprep kit.
c. Sanger sequence plasmid DNA using the Edit-R HDR colony PCR and sequencing primers to confirm correct insertion into the plasmid donor.
   i. Align Sanger sequencing reads to either the provided EGFP or mKate2 insert sequence, or custom insert sequence as proved to us during the design process. Successful cloning is defined as in frame addition of the intended insert sequence between the defined homology arms.
d. Grow cultures containing sequence-confirmed plasmid and prepare using a transfection-quality plasmid DNA kit.

Our EGFP and mKate2 insert sequences contain a four amino acid flexible linker at the beginning of the fluorescent protein sequence, and a three amino acid flexible linker at the end of the fluorescent protein sequence. See section 6 Frequently asked questions (FAQs) for more detail.

4 Guidelines for knock-in co-transfection using the Edit-R platform with HDR DNA plasmid donors

The following are example protocols for co-transfection of Edit-R components (Cas9 nuclease, synthetic guide RNA, and HDR plasmid donor) required for HDR-mediated knock-in in a 96-well format. The first protocol is for Cas9 nuclease mRNA, synthetic guide RNA, and HDR DNA plasmid donor and the second protocol is for Cas9 nuclease protein, synthetic guide RNA, and HDR DNA plasmid donor. See the Appendix for additional recommendations on cell plating and optimizing lipid co-transfection conditions and HDR DNA plasmid donor design recommendations.

We recommend DNA-free reagents, Cas9 protein or Cas9 mRNA and synthetic guide RNA, to reduce the potential for unwanted sequences integrating into the gDNA instead of your specific DNA donor.

Materials required for knock-in co-transfection

Edit-R CRISPR-Cas9 materials for gene editing can be ordered at https://dharmacon.horizondiscovery.com/.

- Cas9 nuclease (choose one):
  - » Edit-R Cas9 Nuclease mRNA, 20 µg (1 µg/µL; Cat #CAS11195)

    or
  - » Edit-R Cas9 Nuclease protein NLS, 500 pmol (20 µM; Cat #CAS11200)
- Synthetic targeting guide RNA (choose one):
  - » Edit-R synthetic crRNA and tracrRNA oligos:
    a. crRNA, predesigned for your gene of interest in a variety of sizes, or designed and ordered using the Dharmacon CRISPR Design Tool
    b. tracrRNA, 5, 20 or 50 nmol (Cat #U-002005-XX)

    or
  - » Edit-R synthetic sgRNA, custom ordering using the Dharmacon CRISPR Design Tool

For the most efficient knock-in, we recommend testing three to five guide RNA designs per knock-in experiment to identify the one with highest editing efficiency. It has been demonstrated that knock-in is most efficient when the DSB is less than 10 bp from the desired insertion site, so choosing a crRNA close to the DSB with highest function is desirable.

- Plasmid donor (constructed by the researcher from the Edit-R HDR plasmid donor kit) and utilizes the Edit-R HDR Donor Designer webtool.
- DharmaFECT™ Duo transfection reagent [Cat #T-2010-01 (0.2 mL); T-2010-02 (0.75 mL); T-2010-03 (1.5 mL); or T-2010-04 (1.5 mL × 5 tubes)]

Additional reagents to be supplied by researcher

Transfection experiments require standard cell culture reagents and instruments appropriate for maintenance of cells. The following additional materials are required but not supplied:

- Multi-well tissue culture plates or tissue culture dishes
- Antibiotic-free complete medium: Cell culture medium (including serum and/or supplements) recommended for maintenance and passaging of the cells of interest without antibiotic
- Assay for assessing cell viability (such as Resazurin cell viability reagent)
- Assay(s) for detecting gene editing events in a cell population

Co-transfection of HDR plasmid donor with Edit-R Cas9 Nuclease mRNA and Edit-R synthetic guide RNAs

The following is a general protocol using DharmaFECT Duo transfection reagent to deliver Edit-R Cas9 Nuclease mRNA, synthetic guide RNA, and HDR plasmid donor into cultured mammalian cells. Exact reagent amounts and parameters for co-transfection should be empirically determined through careful optimization in your cells prior to experimentation (see the transfection optimization section of the Edit-R CRISPR-Cas9 Genome Engineering with Cas9 Nuclease mRNA and Synthetic RNAs technical manual. The protocol below describes delivery conditions in U2OS cells in one well of a 96-well plate and is given for illustrative purposes only. Reagent volumes should be calculated to include replicate samples and pipetting overage as necessary.

Day 1

1. Plate cells in 96-well plates using growth medium at a cell density so that the cells are 70 to 90% confluent the next day.
2. Incubate cells at 37 °C in a humidified CO_2 incubator overnight.

Day 2

3. Prepare a 100 ng/µL Cas9 mRNA working solution by thawing Cas9 mRNA on ice and adding 2 µL of 1 µg/µL stock solution of Cas9 mRNA to 18 µL of Tris buffer. Verify the Cas9 mRNA concentration using UV spectrophotometry at 260 nm and adjust the volume if necessary to obtain 100 ng/µL.

On initial use, aliquot remaining Cas9 mRNA stock or working solution into convenient volumes and store all aliquots at -80 °C to minimize the number of freeze-thaw cycles.

4. Prepare 100 ng/µL HDR plasmid donor working solution (*e.g.*, Tris buffer).
5. Prepare 2 µM synthetic guide RNA transfection complex from previously prepared 10 µM stocks (see Appendix)
   - » For crRNA:tracrRNA add 2 µL of 10 µM crRNA and 2 µL of 10 µM tracrRNA to 6 µL of Tris buffer (total volume is 10 µL).

     or
   - » For synthetic sgRNA add 2 µL of 10 µM synthetic sgRNA to 8 µL of Tris buffer (total volume is 10 µL).
6. In a 1.7 mL tube (or deep-well plate) prepare for each sample to be transfected as described in Table 9 (columns 2-5) for a final 25 nM concentration of the synthetic guide RNA, 200 ng/well of Cas9 mRNA and 200 ng/well of the HDR plasmid donor in the final transfection mixture.

We strongly recommend including three controls: 1) an untransfected control as a negative control and to assess cell viability, 2) a gene editing control to confirm base editing and transfection efficiency, and 3) an HDR plasmid donor only control to normalize for potential nonspecific-fluorescence from the plasmid donor alone.

We have observe background fluorescence from expression of the HDR DNA plasmid donor alone. This typically occurs when promoter sequences or transcription initiation components are part of the 5' homology arm when the fluorescent protein insertion is at the 5' end of the target gene.

7. Prepare a 30 µg/mL DharmaFECT Duo working solution by diluting 3 µL of 1 mg/mL stock DharmaFECT Duo transfection reagent in 97 µL serum-free medium and mix gently; this volume is sufficient for 10 wells with 0.3 µL/well in 96-well format. Incubate for 5 minutes at room temperature.

Table 9. Preparing transfection samples for gene editing experiment in a 96-well plate format

Sample	Serum-free medium	Synthetic guide RNA transfection complex (2 µM)	Cas9 mRNA working solution (100 ng/µL)	HDR DNA plasmid donor (100 ng/µL)	DharmaFECT Duo working solution (30 µg/mL)	Growth medium	Total volume per well
Cas9 nuclease mRNA with HDR plasmid donor and gene-specific synthetic guide RNA **(HDR knock-in)**	4.75	1.25	2	2	10	80	100
Cas9 nuclease mRNA with gene-specific synthetic guide RNA **(gene editing control)**	6.75	1.25	2	0	10	80	100
HDR plasmid donor only (No Cas9 mRNA and synthetic guide RNA **(nonspecific-fluorescence control)**	8	0	0	2	10	80	100
Untransfected **(negative control)**	20	0	0	0	0	80	100

Volumes (µL) are for a single well of a 96-well plate of U2OS cells to be transfected. It is recommended to prepare sufficient sample volumes for the total number of replicates and to account for pipetting error. Exact reagent amounts for co-transfection in other cell lines of interest should be empirically determined through careful optimization prior to experimentation (see the Transfection optimization section in the Edit-R CRISPR-Cas9 Genome Engineering with Cas9 Nuclease mRNA and Synthetic guide RNAs technical manual.

8. Add 10 µL DharmaFECT Duo working solution to each sample tube as shown in Table 9 (column 6); this will result in 0.3 µg/well final concentration. DO NOT add DharmaFECT Duo working solution to the untransfected control, which should contain serum-free medium only. This brings the total volume to 20 µL in each tube. Mix by pipetting gently up and down and incubate for 20 minutes at room temperature.
9. Prepare transfection mixture by adding 80 µL antibiotic-free complete medium to each sample to bring the total volume in each tube to 100 µL as shown in Table 9 (columns 7 and 8).
10. Remove medium from the wells of the 96-well plate containing cells and replace with 100 µL of the appropriate transfection mixture to each well.
11. Incubate cells at 37 °C with 5% CO_2 for 72 hours.
12. At 72 hours:
    a. Image cells using fluorescent microscopy.
        i. Observe cellular localization of the inserted fluorescent reporter in the HDR knock-in transfected sample.
        ii. Confirm no fluorescence expression in the controls.
            » If the nonspecific-fluorescence control exhibits fluorescence, passage cells until the plasmid (and fluorescence) is diluted out of the cells. At this point, an aliquot of the HDR-transfected cells can be assayed for knock-in on a cell population level (see the Detect HDR integration of a fluorescent protein with several assays section in the Appendix).
    b. Retain an aliquot of the gene editing control and negative control samples for analysis (see the Detect base gene editing with mismatch detection assay section in the Appendix).
13. Expand HDR-transfected cells for clonal cell line generation and assay clonal populations to confirm the desired insertion (see Detect HDR integration of a fluorescent protein with several assays in the Appendix).

Co-transfection of HDR plasmid donor with Edit-R Cas9 Nuclease protein and Edit-R synthetic RNAs

The following is a general protocol using DharmaFECT Duo transfection reagent to deliver Edit-R Cas9 Nuclease protein, synthetic guide RNA, and plasmid donor into cultured mammalian cells. Exact reagent amounts and parameters for co-transfection should be empirically determined through careful optimization in your cells of interest prior to experimentation (see the Transfection optimization section of the Dharmacon Edit-R CRISPR-Cas9 Genome Engineering with Cas9 protein and synthetic guide RNAs technical manual. The protocol below describes delivery conditions in U2OS cells in one well of a 96-well plate and is given for illustrative purposes only. Reagent volumes should be calculated to include replicate samples and pipetting overage as necessary.

Day 1

1. Plate cells in 96-well plates using growth medium at cell density so that the cells are 70 to 90% confluent the next day.
2. Incubate cells at 37 °C in a humidified CO_2 incubator overnight.

Day 2

3. Prepare a 2.5 µM Cas9 protein working solution by adding 0.5 µL of Cas9 protein (20 µM stock solution) to 3.5 µL of serum-free medium.

It is advised that the minimum volume of Cas9 protein working solution required for the experiment be prepared, as storage of diluted Cas9 protein is not recommended.

4. Prepare 100 ng/µL HDR plasmid donor working solution (*e.g.*, Tris buffer).
5. Prepare 2 µM synthetic guide RNA, transfection complex from previously prepared 10 µM stocks (see Appendix).
   - » For crRNA:tracrRNA add 2 µL of 10 µM crRNA and 2 µL of 10 µM tracrRNA to 6 µL of Tris buffer (total volume is 10 µL).

     or
   - » For synthetic sgRNA add 2 µL of 10 µM synthetic sgRNA to 8 µL of Tris buffer (total volume is 10 µL).
6. In a 1.7 mL tube (or deep-well plate) prepare for each sample to be transfected as described in Table 10 (columns 2-5) for a final 50 nM concentration of the synthetic guide RNA, 25 nM of Cas9 protein and 200 ng/well in the final transfection mixture.

We strongly recommend including three controls: 1) an untransfected control as a negative control and to assess cell viability, 2) a gene editing control to confirm base editing and transfection efficiency and 3) an HDR plasmid donor only control to normalize for potential nonspecific fluorescence from the plasmid donor alone.

We have observe background fluorescence from expression of the HDR DNA plasmid donor alone. This typically occurs when promoter sequences or transcription initiation components are part of the 5' homology arm when the fluorescent protein insertion is at the 5' end of the target gene.

Table 10. Preparing transfection samples for gene editing experiment in a 96-well plate format

Sample	Serum-free medium	Synthetic guide RNA transfection complex (2 µM)	Cas9 protein working solution (2.5 µM)	HDR DNA Plasmid donor (100 ng/µL)	DharmaFECT Duo working solution (6 µg/mL)	Total volume per well
Cas9 nuclease protein with HDR plasmid donor and gene-specific synthetic guide RNA **(HDR knock-in)**	45.5	2.5	1	1	50	100
Cas9 nuclease mRNA with and gene-specific synthetic guide RNA **(gene editing control)**	46.5	2.	1	0	50	100
HDR plasmid donor only (nuclease protein and synthetic guide RNA) **(nonspecific-fluorescence control)**	49	0	0	1	50	100
Untransfected **(negative control)**	100	0	0	0	0	100

Volumes (µL) are for a single well of a 96-well plate of U2OS cells to be transfected. It is recommended to prepare sufficient sample volumes for the total number of replicates and to account for pipetting error. Exact reagent amounts for co-transfection in other cell lines of interest should be empirically determined through careful optimization prior to experimentation (see the Transfection optimization section in the Edit-R CRISPR-Cas9 Genome Engineering with Cas9 protein and synthetic guide RNAs technical manual).

7. Prepare a 6 µg/mL DharmaFECT Duo working solution by diluting 3 µL of 1 mg/mL stock DharmaFECT Duo transfection reagent in 497 µL serum-free medium and mix gently; this volume is sufficient for 10 wells with 0.3 µL/well in 96-well format. Incubate for 5 minutes at room temperature.
8. Add 50 µL DharmaFECT Duo working solution to each sample tube as shown in Table 10 (column 6); this will result in 0.3 µg/well final concentration. DO NOT add DharmaFECT Duo working solution to the untransfected control, which should contain serum-free medium only. This brings the total volume to 100 µL in each tube. Mix by pipetting gently up and down and incubate for 20 minutes at room temperature.
9. Remove growth medium from the wells of the 96-well plate containing cells and replace with 100 µL of the appropriate transfection mixture to each well.
10. Incubate cells at 37 °C with 5% CO_2 for 14-18 hours; replace transfection mixture on the cells with growth medium without antibiotics.
11. Continue to incubate cells at 37 °C with 5% CO_2 until 48-72 hours post-transfection.
12. At 48-72 hours post-transfection:
    a. Image cells using fluorescent microscopy.
        i. Observe cellular localization of the inserted fluorescent reporter in the HDR knock-in transfected sample.
        ii. Confirm no fluorescence expression in the controls.
            » If the nonspecific-fluorescence control exhibits fluorescence, passage cells until the plasmid (and fluorescence) is diluted out of the cells. At this point, an aliquot of the HDR-transfected cells can be assayed for knock-in on a cell population level (see the Detect HDR integration of a fluorescent protein with several assays section in the Appendix).
    b. Retain an aliquot of the gene editing control and negative control samples for analysis (see the Detect base gene editing with mismatch detection assay section in the Appendix).
13. Expand HDR-transfected cells for clonal cell line generation and assay clonal populations to confirm the desired

5 Appendix

Additional resources

For general recommendations on cell plating and optimizing lipid co-transfection conditions, see the Edit-R CRISPR-Cas9 Genome Engineering with Cas9 Nuclease mRNA and Synthetic guide RNAs technical manual; and/or the Edit-R CRISPR-Cas9 Genome Engineering with Cas9 protein and synthetic guide RNAs technical manual. For a convenient benchtop version of the HDR co-transfection protocol, see the Edit-R Cas9 mRNA, synthetic guide RNA and HDR donor template transfection protocol. We recommend optimizing lipid co-transfections with the Edit-R components (Cas9 nuclease and synthetic guide RNA) before performing lipid co-transfection with a HDR plasmid donor. This maximizes the number of DSBs available to the cell to repair through the HDR pathway.

For general HDR plasmid donor design recommendations see this useful guide . The tips included in the guide are used in the HDR Donor Designer.

For a protocol to make 10 µM concentrated stock solutions of synthetic guide RNAs see (the Edit-R synthetic guide RNA resuspension protocol.

Gene editing assay recommendations

Detect NHEJ-mediated gene editing with mismatch detection assay

The most commonly used method for detection of indels in a cell population is a DNA mismatch detection assay (Guschin, 2010; Reyon, 2012; Cong, 2013). This assay can be performed on either purified genomic DNA or whole cell lysate using T7 Endonuclease I (T7EI) . For HDR-mediated knock-in experiments, mismatch detection should be performed on the gene editing control (Cas9 nuclease and synthetic guide RNA) and compared to the negative control (untransfected) sample to obtain the "base" editing or NHEJ-mediated editing of the transfection experiment. This is typically performed at 72 hours post-transfection. Including this analysis is useful to confirm efficient editing in the transfection. If the NHEJ-mediated editing on the control sample is significantly less than the previously optimized transfections, then the experiment may need to be repeated or further optimized because the HDR-mediated knock-in efficiency is always lower than the NHEJ-mediated editing (and might not be detectable when the transfection is low efficiency).

Detect integration of a fluorescent protein with several assays

The most commonly used methods for detecting HDR-mediated integration and expression of a fluorescent protein in a population of cells are fluorescence microscopy and flow cytometry. Additionally, PCR-based assays [*e.g.*, restriction fragment length polymorphism (RFLP) assay] and Sanger sequencing are utilized. For more details about gene editing assays to detect HDR-mediated knock-in on a population level, please see the following Application Note: Homology-directed repair with Edit-R CRISPR-Cas9 reagents and single-stranded DNA oligos.

After analysis at a population level we recommend isolating several fluorescently tagged clonal cell lines and performing Sanger sequencing across the insertion site to verify correct insertion. For more details about clonal cell line generation or detection of fluorescent protein knock-in in a clonal population, please see the following Application Notes: A CRISPR-Cas9 gene engineering workflow: generating functional knockouts using Edit-R Cas9 and synthetic crRNA and tracrRNA and Fluorescent tagging of an endogenous gene by homology-directed repair using Dharmacon Edit-R CRISPR-Cas9 reagents.

6 Frequently asked questions (FAQs)

Is the inserted florescent protein a direct fusion or is there a linker?

We have designed our donor template to contain a flexible linker of 4 and 3 amino acids, respectively, before and after the fluorescent protein insertion.

Do I have to add in a start or stop codon for the fluorescent protein? No, this system is designed to utilize the start and stop codon of the endogenous gene transcript. When designing the custom homology arm PCR primers, the insertion location should be chosen to be within the endogenous gene transcript. Ideally, the insertion location should be precisely after the start codon for an N-terminal insertion, or conversely, immediately before the stop codon for a C-terminal insertion. Please note that cells may express an alternative transcript from the one you design to and that may impact expression of the inserted fluorescent reporter.

Can I use the HDR donor kit with my own homology arms (without ordering the custom HA primers from your website)?

No, the HDR Donor Designer appends an adapter sequence to the homology arm primers that is necessary for compatibility with the HDR plasmid donor backbone and the mKate2 kit components.

How should I choose the homology arm lengths?

The HDR Donor Designer will design optimal PCR primer pairs that are ranked based on a quality score provided by Primer3 with options of homology length ranging between 500 and 1000 bp. Depending on the chosen insertion site and the corresponding sequence composition, the insert-adjacent primers may be non-ideal and require PCR optimization. For this reason we suggest testing 2 or 3 pairs for each HA, representing different lengths, to ensure optimal success.

Do I need to gel purify the PCR-amplified homology arms or can I just do a column clean up?

We recommend gel purification of homology arms, because it results in the highest cloning efficiency. Gel purification is absolutely required when PCR optimization is challenging (when multiple amplification bands or primer dimers are present).

Is there a way to check that the assembly reaction worked?

Yes, if you are using the Gibson Assembly method or another *in vitro* assembly method you can confirm assembly with PCR; use 1 µL of the assembly reaction as template and the colony PCR primers provided in the kit in a PCR and then run it out on a 2% agarose gel to confirm presence of the assembled junctions.

What should I do if I have multiple bands from my PCR-amplified homology arm reaction?

If there are multiple bands, including the desired amplicon length, the PCR should be optimized further to remove non-specific product(s). PCR can be optimized by running a gradient thermal cycling protocol, adding DMSO for high GC content, or using a different polymerase. If, after optimization, multiple bands still exist, separate on an agarose gel and extract the band of expected size. In this case, multiple PCRs can be pooled together and then purified to ensure enough material for assembly (at least 1.5 µL of 20 ng/µL).

What should I do if I have multiple bands from my colony PCR?

Because colony PCR is a crude method, multiple bands may result from potential contamination (inhibition from bacterial cells or culture medium). If there is a band at the expected size of a positive clone and another band as well, in most cases this indicates a positive clone and Sanger sequencing should be performed to confirm.

Can I use the colony PCR primers for Sanger sequencing?

Yes, the colony PCR primers are intended to be used for both colony PCR and the Sanger sequencing.

What are the sequences of the colony PCR/Sanger sequencing primers?

Primer name	Primer sequence 5'- 3'
Edit-R Colony PCR Primer EGFP Forward	AGGTGAACTTCAAGATCCGCCACA
Edit-R Colony PCR Primer EGFP Reverse	AACTTCAGGGTCAGCTTGC
Edit-R Colony PCR Primer mKate2 Forward	GGCCGACAAAGAGACCTACG
Edit-R Colony PCR Primer mKate2 Reverse	GTTGTTCACGGTGCCCTCCA
Edit-R Colony PCR Primer Backbone Forward	TCGCCCGGTTTATTGAAATG
Edit-R Colony PCR Primer Backbone Reverse	TTCGCCACCTCTGACTTGAGC

What are good stopping points during the plasmid donor cloning protocol?

We recommend that the PCR purification and assembly reaction are completed on the same day to avoid freeze-thaw cycles of purified PCR products. Appropriate stopping points are before PCR (step 2b.) or gel purification (step 3c) where DNA in agarose gel or PCRs can be frozen at -20 °C before proceeding with protocol.

How should I store my DNA assembly components?

Assembly components should be eluted and/or resuspended in 10 mM Tris-HCL pH 7.5, aliquoted into single-use tubes and stored at -20 °C or -80 °C to minimize freeze-thaw cycles.

Can I use another bacterial growth medium instead of NYZM plus?

NZYM plus medium is a rich broth that supplies bacteria with amino acids, vitamins and metabolites that in turn do not need to be synthesized by the bacteria allowing for faster growth. This is part of our optional, quick colony PCR protocol so that growth and colony PCR can be completed in one day. Other medium can be substituted for the quick protocol, but growth time will need to be optimized for consistent colony PCR results.

What is the benefit to using Cas9 protein or Cas9 mRNA instead of Cas9 plasmid or a Cas9-integrated cell line for HDR experiments?

Specifically, for HDR-mediated knock-in experiments, Cas9 protein and Cas9 mRNA are preferred as they typically give higher gene editing efficiencies compared to Cas9 plasmid. This is important because HDR-mediated knock-in will only occur in a fraction of DSBs at the target sites. Additionally, Cas9 protein and Cas9 mRNA are DNA-free options so there is no concern of DNA integration resulting from the Cas9 source. Similarly, a Cas9-integrated cell line is not ideal for HDR-mediated knock-in because continuous expression of the Cas9 nuclease increases potential for undesired off-targets and mutations.

What is the stability of the Edit-R synthetic guide RNA?

Dried RNA oligonucleotide pellets are stable at room temperature for two to four weeks, but should be placed at -20 °C or -80 °C for long-term storage. Under these conditions, the dried synthetic guide RNA will be stable for at least one year. Maintaining sterile, RNase- and DNase-free conditions is always recommended as a critical precaution.

I see a fair amount of cell death after transfection of my cells. What can I do about this?

Extensive cell death following transfection is an indication that delivery conditions need to be further optimized. Basic parameters to consider when optimizing transfection include transfection reagent and cell-specific conditions such as the amount of transfection reagent, the lot/batch of transfection reagent, duration of transfection, cell passage number and cell density at transfection. Often decreasing the amount of lipid present during transfection and/or the total duration of transfection will help minimize the toxic effect to the cells. Additionally, it is not uncommon to observe some variability

from one tube of transfection reagent to another, and this may also represent a source of experimental variability. If the problem persists, we recommend that other transfection reagents be considered or contact Technical Support (dharmacon.horizondiscovery.com/service-and-support) for additional troubleshooting help.

How many freeze-thaw cycles can the Edit-R Cas9 Nuclease mRNA be subjected to?

We recommend not exceeding two to three freeze-thaw cycles for Cas9 mRNA. On initial use, thaw the Cas9 mRNA on ice, aliquot remaining stock into convenient volumes and store all aliquots at -80 °C to minimize the number freeze-thaws cycles prior to each experiment.

How many freeze-thaw cycles can the synthetic sgRNA, crRNA, or tracrRNA be subjected to?

We recommend not exceeding four to five freeze-thaw cycles for synthetic guide RNA to ensure reagent integrity.

How should I store my synthetic guide RNA?

RNA oligonucleotides should be stored at -20 °C or -80 °C in a non-frost-free freezer, either as a dried pellet or resuspended in an RNase-free solution buffered to pH ~ 7.4 to help with stability during freeze-thaw cycles. We recommend that RNA oligonucleotides be resuspended to a convenient stock concentration and stored in small aliquots to avoid multiple freeze-thaw cycles. RNA oligonucleotides should not go through more than four to five freeze thaw cycles. If degradation is a concern, the integrity of the RNA oligonucleotides can be evaluated on an analytical PAGE gel. For a resuspension protocol see the Dharmacon crRNA and tracrRNA Resuspension protocol.

What is the formula for spectrophotometric quantification of synthetic crRNA, tracrRNA and sgRNA?

To quantify RNA, use Beer's Law: Absorbance (260 nm) = (ε)(concentration)(path length in cm), where ε,epsilon, is the molar extinction coefficient (provided on the Product Transfer Form supplied with the crRNA or synthetic sgRNA order; the extinction coefficient for tracrRNA Cat# U-002005 is 757800). When solved for the unknown, the equation becomes: Concentration = (Absorbance, 260 nm) / [(ε)(path length in cm)]. When a standard 10 mm cuvette is used, the path length variable in this equation is 1. If a different size of cuvette is used, e.g., a 2 mm microcuvette, then the path length variable is 0.2

Can I use a different transfection reagent other than DharmaFECT Duo to deliver the Edit-R components into my cells for HDR experiments?

We have validated our protocols and demonstrated successful gene editing using DharmaFECT Duo transfection reagent to efficiently deliver the Edit-R components for HDR (both ssDNA oligos and HDR DNA plasmids). We cannot predict the co-transfection ability of other transfection reagents, nor can we troubleshoot experiments performed with any reagent other than DharmaFECT Duo transfection reagent for this application. However, other suitable transfection reagents could be utilized provided co-transfection conditions are carefully optimized for each cell line.

Can the ploidy of my cell line affect the results of my CRISPR-Cas9 gene editing experiment?

When using a CRISPR-Cas9 system to disrupt gene function, it is important to know as much as you can about the gene you are targeting and your cells. In particular, the ploidy of your cells, the gene copy number and the presence of any SNPs are important considerations. In normal diploid cells, obtaining mutations on both alleles may be required for a complete knockout and, an observable phenotype. Virtually all cancer lines and many immortalized cell lines exhibit aneuploidy and, as such, mutation of multiple alleles may be necessary. The presence of SNPs and multiple genomic locations should also be taken into account when designing crRNAs, as they can affect whether a complete knockout can be achieved. Expansion of clones from single cells followed by sequence validation to verify the desired mutation on all alleles is the most rigorous approach for confirming complete gene knockout.

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8 Limited use licenses

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