


# HOMOLOGY-DIRECTED REPAIR USING THE ALT-R CRISPR-Cas9 SYSTEM AND MEGAMER ssDNA FRAGMENTS

Simultaneous delivery of RNP complexes and ssDNA repair templates using the Nucleofector™ System

## Prepare CRISPR reagents

Resuspend your oligos in Nuclease-Free IDTE, pH 7.5.


Component	Final concentration
Alt-R crRNA with tracrRNA, or Alt-R sgRNA	100 $\mu$ M
Alt-R Cas9 Electroporation Enhancer	100 $\mu$ M
Megamer ssDNA fragment	0.5 $\mu$ g/ $\mu$ L, or an optimal concentration for your planned experiment (See <b>Tips</b> below)

 **Note:** For assistance, use the IDT Resuspension Calculator at [www.idtdna.com/SciTools](http://www.idtdna.com/SciTools).

### Tips:

- Always store CRISPR reagents at  $-20^{\circ}\text{C}$ .
- When preparing your Megamer donor, dilute donor in Nuclease-Free IDTE, or water, so that your desired dose is delivered in an 8  $\mu$ L volume.
- The Megamer dose may need to be optimized for your cell type. (See the protocol [Homology-directed repair using the Alt-R CRISPR-Cas9 System and Megamer ssDNA Fragments](#)).

## Prepare the gRNA complex


 **Note:** If you are preparing a sgRNA, no annealing step is required. Simply dilute the sgRNA to the desired concentration in Nuclease-Free IDTE.

Prepare a two-part gRNA complex (combining crRNA and tracrRNA), which anneals the oligos to form a guide complex.

1. Combine the following components to make the gRNA complex at a final concentration of 50  $\mu$ M.


Component	Amount ( $\mu$ L)
100 $\mu$ M Alt-R CRISPR-Cas9 crRNA	5
100 $\mu$ M Alt-R CRISPR-Cas9 tracrRNA	5
IDT Duplex Buffer (to final volume)	As needed
<b>Total volume</b>	<b>10</b>

2. Heat the mixture at  $95^{\circ}\text{C}$  for 5 minutes.
3. Cool to room temperature ( $15$ – $25^{\circ}\text{C}$ ) on the bench top.

 **Stopping point (optional):** gRNA complexes can be stored at  $-20^{\circ}\text{C}$  up to 1 year.

## Prepare the RNP complex


Combining the gRNA and Cas9 Nuclease allows an RNP complex to form. Prepare the delivery mix to yield a Cas9:gRNA RNP final concentration of 2:2.4  $\mu$ M.

 **Note:** You can optimize the final RNP concentration for each guide. In general, a 1–4  $\mu$ M RNP concentration allows for maximal editing.

1. Combine the following components per each electroporation well:


Component	Amount ( $\mu$ L)
PBS	0.6
gRNA (50 $\mu$ M)	1.4 (72 pmol)
Alt-R Cas9 enzyme (61 $\mu$ M)	1.0 (60 pmol)
<b>Total volume</b>	<b>3</b>

2. Incubate at room temperature for 10–20 minutes.


 **Stopping point (optional):** RNP complexes can be stored at  $4^{\circ}\text{C}$  up to 1 month, or at  $-80^{\circ}\text{C}$  up to 2 years in single-use aliquots.

## Prepare the cell culture media


Prepare cell culture media with and without HDR Enhancer and prewarm to 37°C for use after nucleofection.

1. Prewarm 75  $\mu$ L of cell culture media per nucleofection sample.
  -  **Note:** This media will be added to cells in the 96-well Nucleocuvette module after nucleofection.

2. If using Alt-R HDR Enhancer, mix HDR Enhancer with cell culture media, then aliquot in a 96-well plate.

 **Tip:** To reduce sample-to-sample variability, make a stock solution of cell culture media with HDR Enhancer, then aliquot to the final culture plate.

3. Prewarm the plate in a tissue culture incubator.

 **Note:** This media will be used for culturing cells for 12–24 hr after nucleofection. If desired, plate triplicate wells for each nucleofection sample.

The following table demonstrates the amounts needed per well, for a final concentration of 30  $\mu$ M HDR Enhancer after the nucleofected cells are added. If needed, scale up according to the number of samples you have.

Component	Sample ( $\mu$ L)	Negative control, no HDR Enhancer ( $\mu$ L)	Negative control, DMSO only ( $\mu$ L)
3 mM Alt-R HDR Enhancer	2*	—	—
DMSO	—	—	2
Cell culture media	173	175	173
<b>Total volume</b>	<b>175</b>	<b>175</b>	<b>175</b>

\* The final concentration of the HDR Enhancer may need to be optimized for your cell type (see [Minimize cytotoxicity when using Alt-R HDR Enhancer](#)).

## Transfect cells

Prepare cells as you would for a standard CRISPR-Cas9 nucleofection experiment, ensuring the cells are washed with PBS before nucleofection to remove any residual nucleases.

1. Suspend cells in 18  $\mu$ L of the required Nucleofection Buffer.

2. Make the final transfection mix by combining the following components:

Component	Amount ( $\mu$ L)
RNP complex	3
Megamer ssDNA donor	8
100 $\mu$ M Alt-R Cas9 Electroporation Enhancer or Nucleofection Buffer*	0.6
Cell suspension	18
PBS (to final volume)	0.4
<b>Total volume</b>	<b>30</b>

\* The final concentration of Alt-R Cas9 Electroporation Enhancer may need to be optimized for your cell type (see [Minimize cytotoxicity when using Megamer ssDNA donor templates](#)).


3. After mixing the transfection mix, transfer 25  $\mu$ L to a 96-well Nucleocuvette module. Gently tap to remove any air bubbles that may be present.
4. Transfect cells according to the manufacturer's specifications.
5. After electroporation, add 75  $\mu$ L of prewarmed culture media (without HDR Enhancer) per well and gently resuspend cells.
6. Transfer 25  $\mu$ L of resuspended cells to the culture plates containing the prewarmed 175  $\mu$ L of culture media containing HDR Enhancer (if applicable).
7. Incubate cells in a tissue culture incubator.

## Change media

After 12–24 hours, remove the media from the cells, and replace with fresh media without HDR Enhancer.

## Isolate gDNA

You can perform genomic DNA isolation and detect mutations 48–72 hours after nucleofection.

 **Stopping point (optional):** gDNA can be stored for future use by following the recommendations of the gDNA isolation protocol used.

See [Homology-directed repair using the Alt-R CRISPR-Cas9 System and Megamer ssDNA Fragments](#) for the comprehensive protocol.

## Technical support: [applicationsupport@idtdna.com](mailto:applicationsupport@idtdna.com)

For more than 30 years, IDT's innovative tools and solutions for genomics applications have been driving advances that inspire scientists to dream big and achieve their next breakthroughs. IDT develops, manufactures, and markets nucleic acid products that support the life sciences industry in the areas of academic and commercial research, agriculture, medical diagnostics, and pharmaceutical development. We have a global reach with personalized customer service.

> SEE WHAT MORE WE CAN DO FOR YOU AT [WWW.IDTDNA.COM](http://WWW.IDTDNA.COM).

### For Research Use Only.

© 2020 Integrated DNA Technologies, Inc. All rights reserved. Alt-R and Megamer are trademarks of Integrated DNA Technologies, Inc., and are registered in the USA. Nucleofector and Nucleofection are trademarks of Lonza Cologne GmbH. All other marks are the property of their respective owners. For specific trademark and licensing information, see [www.idtdna.com/trademarks](http://www.idtdna.com/trademarks). CRS-10203-QR 5/2020