PROTOCOL

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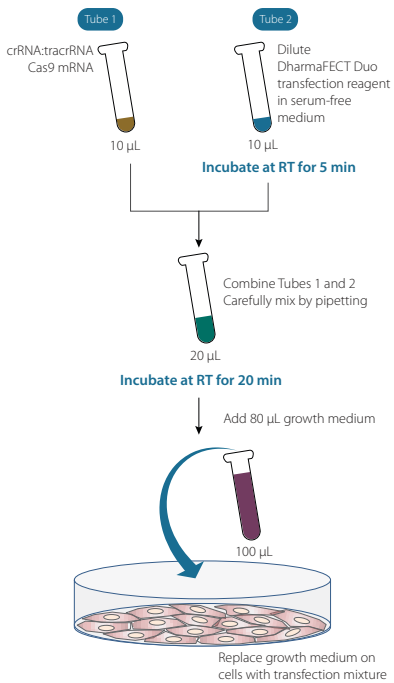
INSPIRED CELL SOLUTIONS

Edit-R™ Cas9 mRNA, synthetic guide RNA, and HDR donor template transfection protocol

The following is a protocol for transfecting Edit-R™ Cas9 mRNA with synthetic guide RNA and homology-directed repair (HDR) donor template (ssDNA oligonucleotide or plasmid) into cultured mammalian cells using DharmaFECT™ Duo transfection reagent. Synthetic guide RNA can be either Edit-R synthetic tracrRNA complexed with crRNA (predesigned or custom) or Edit-R synthetic single guide RNA (sgRNA, custom). For a more detailed protocol please see this protocol and technical manual.



96–well protocol			
Day 1			
Cell plating	Seed cells at a density that gives 70-90% confluency on the next day		
Day 2			
Prepare working solutions of reagents for transfection	Synthetic guide RNA	Dilute and mix crRNA and tracrRNA to a working concentration of 2 µM in 10 mM Tris-HCl pH 7.4 **or** Dilute synthetic sgRNA to a working concentration of 2 µM in 10 mM Tris-HCl pH 7.4	
	Donor template	Dilute donor oligo to a working concentration of 1.0 µM in 10 mM Tris-HCl pH 7.4 **or** Dilute donor plasmid to a working concentration of 100 ng/µL in 10 mM Tris-HCl pH 7.4	
	Cas9 mRNA	Dilute Cas9 mRNA to a working concentration of 100 ng/µL in serum-free medium	
Combine working solutions for transfection mixture		For one well	For mulitple wells
	Tube 1		
	Synthetic guide RNA	1.25 µL	_ µL
	Donor template	ssDNA donor oligo1 µL / Donor plasmid 2 µL	_ µL
	Cas9 mRNA	2 µL	_ µL
	Serum-free medium	To 10 µL	_ µL
Prepare working solution of DharmaFECT Duo for transfection	Tube 2		
	DharmaFECT Duo transfection reagent	0.1–0.8 µL	_ µL
	Serum-free medium	To 10 µL	_ µL
	Incubate at room temperature for 5 minutes before next step		
Combine transfection mixture	Combine Tube 1 and Tube 2 and carefully mix by pipeting		
	Incubate at room temperature for 20 minutes before next step		
	Add full growth medium	80 µL	_ µL
	Total	100 µL	_ µL
Transfect cells	Replace growth medium on cells with 100 µL of transfection mixture		

For more information

To find the contact information in your country for your technology of interest, please visit us at **horizondiscovery.com/contact-us**

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