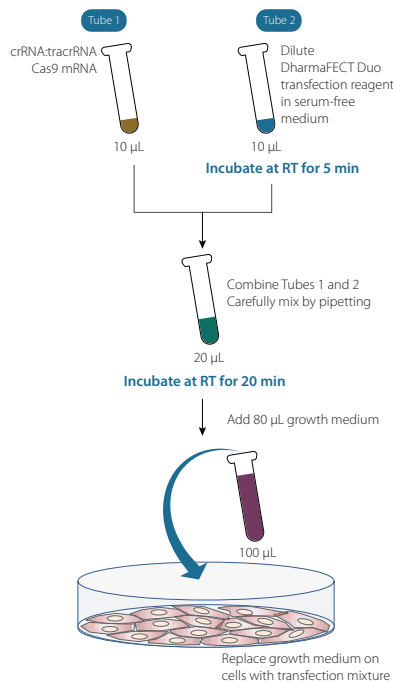


Dharmacon™ Edit-R™ Cas9 mRNA, synthetic guide RNA, and HDR donor template transfection protocol

The following is a protocol for transfecting Dharmacon™ Edit-R™ Cas9 mRNA (Cat #CAS11195, #CAS11859, or #CAS11860) with synthetic guide RNA and homology-directed repair (HDR) donor template (ssDNA oligonucleotide or plasmid) into cultured mammalian cells using **DharmaFECT™ Duo transfection reagent** (Cat #T-2010-xx). Synthetic guide RNA can be either Edit-R synthetic tracrRNA (Cat #U-002005-xx) 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 multiple wells
	Tube 1		
	synthetic guide RNA	1.25 µL	– µL
	Donor template	ssDNA donor oligo 1 µL	Donor plasmid 2 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 pipetting		
	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		

If you have any questions, contact

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