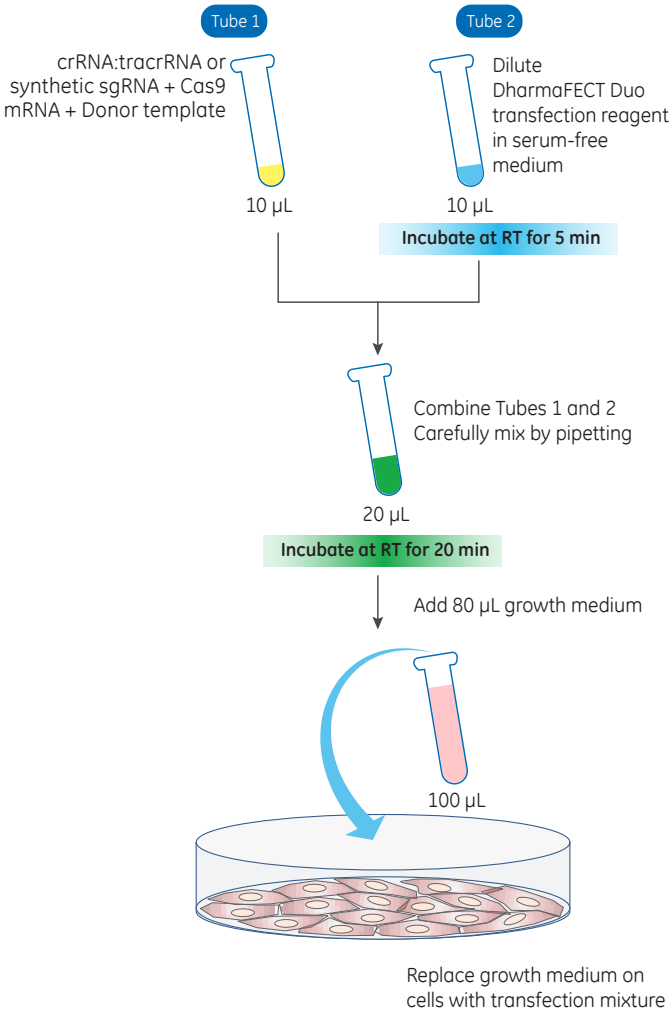
GE Healthcare

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) 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.

The protocol is written for transfection into 96-well tissue culture plates (100 µL final volume).



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 7.4-7.5 or Dilute synthetic sgRNA to a working concentration of 2 µM in 10 mM Tris-HCl pH 7.4-7.5		
	Donor template	Dilute donor oligo to a working concentration of 1.0 µM in 10 mM Tris-HCl pH 7.4-7.5; or Dilute donor plasmid to a working concentration of 100 ng/µL in 10 mM Tris-HCl pH 7.4-7.5		
	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 oligo 1 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**			

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