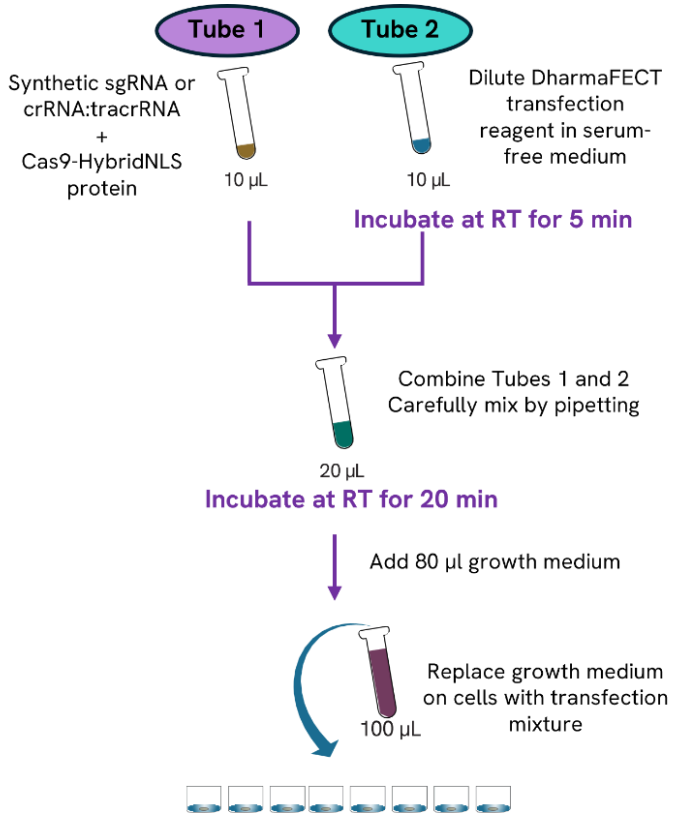
Dharmacon™ Edit-R™ Synthetic guide RNA and Cas9 Protein quick transfection protocol

The following is a protocol for transfecting Dharmacon™ Edit-R™ synthetic guide RNA with Cas9 Protein (Cat# CAS135XX or CAS122XX) into cultured mammalian cells using DharmaFECT™ transfection reagents. Synthetic guide RNA can be either Edit-R predesigned or custom synthetic single guide RNA (sgRNA), or Edit-R synthetic tracrRNA (Cat #U-002005-xx) complexed with Edit-R predesigned or custom crRNA. This protocol is intended for use after transfection conditions have been optimized for your cell line of interest. For full details, as well as optimization guidelines please see the full technical manual .



96-well protocol				
Day 1	Plate cells	Seed cells at a density that generates 70-90% confluency on the next day		
Day 2	Prepare working solutions of reagents for transfection	Synthetic guide RNA	Dilute synthetic sgRNA to a working concentration of 2.5 µM in 10 mM Tris-HCl pH 7.4 or dilute and mix crRNA and tracrRNA to a working concentration of 2.5 µM in 10 mM Tris-HCl pH 7.4	
		Cas protein	Dilute Cas9 protein to a working concentration of 2.5 µM in serum-free medium	
	Combine working solutions for transfection mixture		For one well	For multiple wells
		Tube 1		
		Synthetic guide RNA	1 µl	__ µl
		Cas9 protein	1 µl	__ µl
		Serum-free medium	To 10 µl	__ µl
	Prepare working solution of DharmaFECT for transfection	Tube 2		
		DharmaFECT 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 volume	100 µl	__ µl
	Transfect cells	Replace growth medium on cells with 100 µl of transfection mixture		

If you have any questions, please visit us at
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