PROTOCOL

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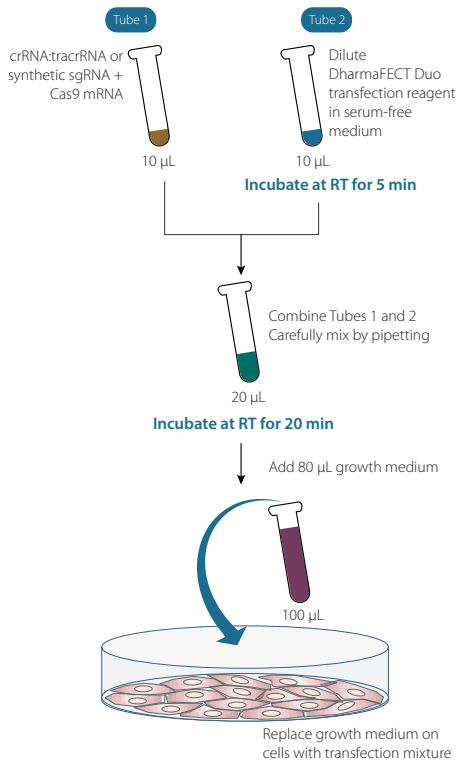
Dharmacon™ Edit-R™ Cas9 mRNA and synthetic guide RNA transfection protocol

The following is an abbreviated protocol for transfecting Dharmacon™ Edit-R™ Cas9 mRNA (Cat #CAS11195, #CAS11859, or #CAS11860) with synthetic guide RNA 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). Intended for use after optimization for your cell line has been completed. For full details, as well as optimization guidelines please see the 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	
	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
	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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24–well protocol			
Day 1			
Cell plating	Seed cells at a density that gives 70-90% confluency on the next day		
Day 2			
Prepare working concentration solutions of materials for transfection	synthetic guide RNA	Dilute and mix crRNA and tracrRNA to a working concentration of 2.5 µM in 10 mM Tris-HCl (pH7.4) **or** Dilute synthetic sgRNA to a working concentration of 2 µM in 10 mM Tris-HCl pH 7.4	
	Cas9 mRNA	Dilute Edit-R CRISPR-Cas9 mRNA to a working concentration of 100 ng/µL in serum-free medium	
Combine working concentration solutions for transfection mixture		For one well	For mulitple wells
	Tube 1		
	synthetic guide RNA	5 µL	_ µL
	Cas9 mRNA	10 µL	_ µL
	Serum-free medium	To 50 µL	_ µL
Prepare working concentration DharmaFECT Duo for transfection	Tube 2		
	DharmaFECT Duo transfection reagent	0.5–0.8 µL	_ µL
	Serum-free medium	To 50 µL	_ µL
	Incubate at room temperature for 5 minutes before next step		
Prepare 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	400 µL	_ µL
	Total	500 µL	_ µL
Transfect cells	Replace growth medium on cells with 100 µL of transfection mixture		

6–well protocol			
Day 1			
Cell plating	Seed cells at a density that gives 70-90% confluency on the next day		
Day 2			
Prepare working concentration solutions of materials for transfection	synthetic guide RNA	Dilute and mix crRNA and tracrRNA to a working concentration of 2.5 µM in 10 mM Tris-HCl (pH7.4) **or** Dilute synthetic sgRNA to a working concentration of 2 µM in 10 mM Tris-HCl pH 7.4	
	Cas9 mRNA	Dilute Edit-R CRISPR-Cas9 mRNA to a working concentration of 100 ng/µL in serum-free medium	
Combine working concentration solutions for transfection mixture		For one well	For mulitple wells
	Tube 1		
	synthetic guide RNA	25 µL	_ µL
	Cas9 mRNA	50 µL	_ µL
	Serum-free medium	To 250 µL	_ µL
Prepare working concentration DharmaFECT Duo for transfection	Tube 2		
	DharmaFECT Duo transfection reagent	2.5–20 µL	_ µL
	Serum-free medium	To 250 µL	_ µL
	Incubate at room temperature for 5 minutes before next step		
Prepare 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	2,000 µL	_ µL
	Total	2,500 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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