PROTOCOL

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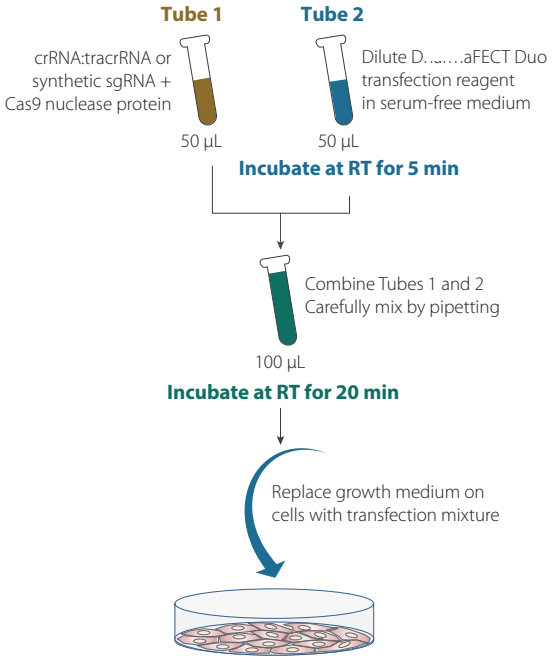
Edit-R™ Cas9 nuclease protein and synthetic guide RNA transfection protocol

The following is a protocol for transfecting Edit-R™ Cas9 Nuclease protein NLS, (Cat #CAS11XXX) with synthetic guide RNA into cultured mammalian cells using DharmaFECT™ transfection reagents (Cat #T-20XX-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 sgRNA (custom). For a more detailed protocol please see the 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 **or** Dilute synthetic sgRNA to a working concentration of 2 µM in 10 mM Tris-HCl pH 7.4	
	Cas9 nuclease protein	Dilute Cas9 nuclease protein to a working concentration of 2.5 µM in serum-free medium	
Combine working solutions for transfection mixture		For one well	For mulitple wells
	Tube 1		
	Synthetic guide RNA	2.5 µL	_ µL
	Cas9 nuclease protein	1 µL	_ µL
	Serum-free medium	To 50 µL	_ µL
Prepare working solution of DharmaFECT for transfection	Tube 2		
	DharmaFECT transfection reagent	0.1–0.8 µL	_ µL
	Serum-free medium	To 50 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		
	Total	100 µL	_ µL
Transfect cells	Replace growth medium on cells with 100 µL of transfection mixture		
Return to full media	After 14-18 hours; replace transfection mixture on the cells with typical cellular growth medium		

If you have any questions, contact

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V7-0418

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	crRNA:tracrRNA	Dilute and mix crRNA and tracrRNA to a working concentration of 2.5 µM in 10 mM Tris-HCl (pH7.5)	
	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		
	crRNA:tracrRNA	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	crRNA:tracrRNA	Dilute and mix crRNA and tracrRNA to a working concentration of 2.5 µM in 10 mM Tris-HCl (pH7.5)	
	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		
	crRNA:tracrRNA	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**		

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