PROTOCOL

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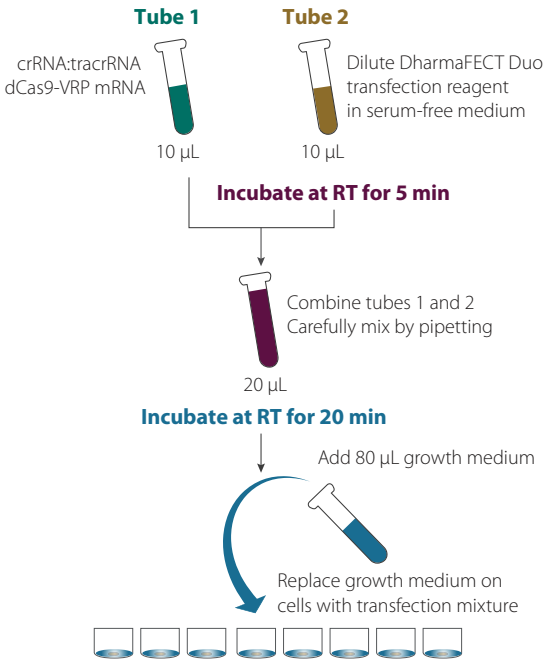
INSPIRED CELL SOLUTIONS

Dharmacon™ Edit-R™ dCas9-VPR mRNA and synthetic guide RNA transfection protocol

The following is a protocol for transfecting Dharmacon™ Edit-R™ dCas9-VPR mRNA with synthetic tracrRNA and crRNA into cultured mammalian cells using DharmaFECT™ Duo transfection reagent (Cat #T-2010-xx).

The protocol is written for transfection into 96-well tissue culture plates.



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 materials for transfection	crRNA:tracrRNA	Dilute and mix crRNA and tracrRNA to a working concentration of 2.5 µM in 10 mM Tris-HCl (pH7.4)	
	dCas9-VPR mRNA	Dilute Edit-R dCas9-VPR mRNA to a working concentration of 100 ng/µL in serum-free medium	
Combine working solutions for transfection mix		For one well	For mulitple wells
	Tube 1		
	crRNA:tracrRNA	1 µL	_ µL
	dCas9-VPR 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		

If you have any questions, contact

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