PROTOCOL

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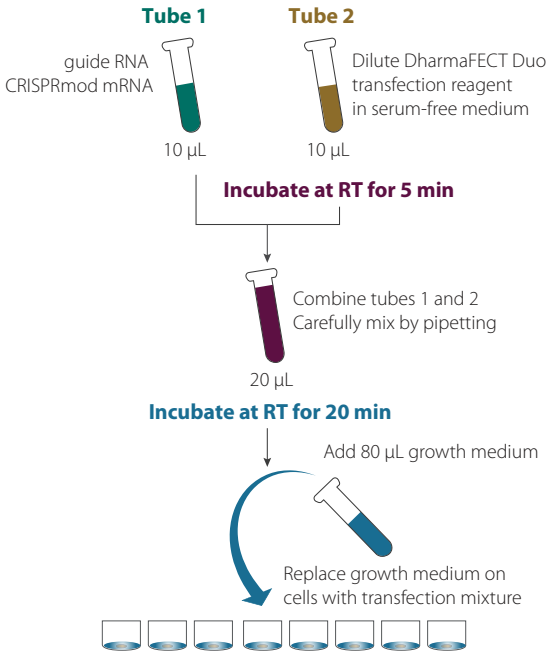
INSPIRED CELL SOLUTIONS

CRISPRmod dCas9-VPR or dCas9-SALL1-SDS3 mRNA and synthetic guide RNA transfection protocol

The following is a protocol for transfecting CRISPRmod dCas9-VPR or dCas9-SALL1-SDS3 mRNA with synthetic guide RNA 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 or sgRNA	Dilute and mix crRNA and tracrRNA to a working concentration of 2.5 µM in 10 mM Tris-HCl (pH7.4 Dilute sgRNA to a working concentration of 2.5 µM in 10 mM Tris-HCl (pH7.4)	
	CRISPRmod mRNA	Dilute CRISPRmod 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 or sgRNA	1 µL	_ µL
	CRISPRmod 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		

For more information

To find the contact information in your country for your technology of interest, please visit us at **horizondiscovery.com/contact-us**

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