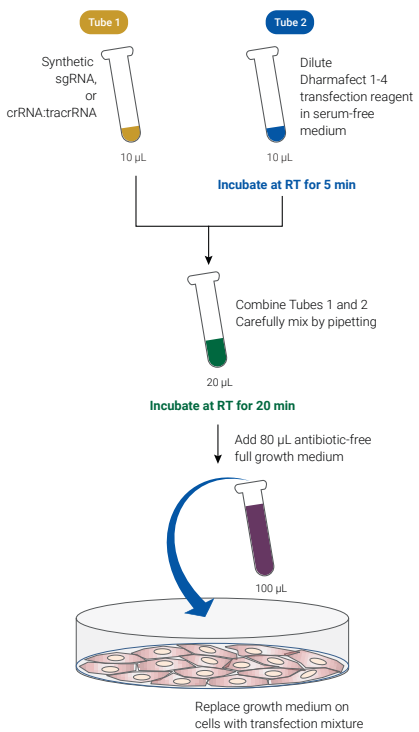
PROTOCOL

CRISPRmod synthetic guide RNA transfection protocol for dCas9-VPR or dCas9-SALL1-SDS3 expressing cells

The following is an abbreviated protocol for transfecting CRISPRmod CRISPRa/CRISPRi synthetic guide RNA into cultured mammalian cells expressing dCas9-VPR or dCas9-SALL1-SDS3 using DharmaFECT™ 1-4 transfection reagent (Cat. T-2001, T-2002, T-2003, T-2004). Synthetic guide RNA can be either synthetic single guide RNA, or synthetic crRNA complexed with tracrRNA. Intended for use after optimization for your cell line has been completed. For full details, as well as optimization guidelines please see the appropriate CRISPRmod technical manual CRISPRa tech manual and CRISPRi technical manual).

This protocol is written for transfection of dCAS9 expressing cells into 96, 24, or 6-well tissue culture plates at 25 nM final concentration of synthetic guide RNA.



96–well protocol

Day 1

Cell plating	Seed cells at a density that is optimal for specific downstream phenotypic assay(s)	

Day 2

Prepare working solutions of reagents for transfection	Synthetic guide RNA	Dilute sgRNA to a working concentration of 1 µM in 10 mM Tris-HCl, pH 7.4 **or** Dilute and mix crRNA and tracrRNA to a working concentration of 1 µM in 10 mM Tris-HCl, pH 7.4	
Combine working solutions for transfection mixture		For one well	For multiple wells
	Tube 1		
	Synthetic guide RNA	2.5 µL	_ µL
	Serum-free medium	To 10 µL	_ µL
Prepare working solution of Dharmafect 1-4 for transfection	**Tube 2**		
	DharmaFECT 1-4 transfection reagent	0.05-0.8 µL	__uL
	Serum-free medium	To 10 µL	_ µL
	Incubate at room temperature for 5 minutes before next step		
	Combine Tube 1 and Tube 2 and carefully mix by pipeting		
	Incubate at room temperature for 20 minutes before next step		
Combine transfection mixture	Add full growth medium	80 µL	_ µL
	Total	100 µL	_ µL
Transfect cells	**Replace growth medium on cells with 100 µL of transfection mixture**		
	Incubate cells for 48-72 hours before performing downstream phenotypic assay(s) or gene expression analysis.		

24–well protocol

Day 1

Cell plating	Seed cells at a density that is optimal for specific downstream phenotypic assay(s)	

Day 2

Prepare working solutions of reagents for transfection	Synthetic guide RNA	Dilute sgRNA to a working concentration of 1 µM in 10 mM Tris-HCl, pH 7.4 **or** Dilute and mix crRNA and tracrRNA to a working concentration of 1 µM in 10 mM Tris-HCl, pH7.4	
Combine working solutions for transfection mixture		For one well	For multiple wells
	Tube 1		
	Synthetic guide RNA	12.5 uL	_ µL
	Serum-free medium	To 50 µL	_ µL
Prepare working solution of Dharmafect 1-4 for transfection	**Tube 2**		
	DharmaFECT 1-4	0.24 - 4 uL	_ µL
	Serum-free medium	To 50 µL	_ µL
	Incubate at room temperature for 5 minutes before next step		
	Combine Tube 1 and Tube 2 and carefully mix by pipeting		
Combine transfection mixture	**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 500 uL of transfection mixture**		
	Incubate cells for 48-72 hours before performing downstream phenotypic assay(s) or gene expression analysis.		

6–well protocol

Day 1

Cell plating	Seed cells at a density that is optimal for specific downstream phenotypic assay(s)	

Day 2

Prepare working solutions of reagents for transfection	Synthetic guide RNA	Dilute sgRNA to a working concentration of 1 µM in 10 mM Tris-HCl, pH 7.4 **or** Dilute and mix crRNA and tracrRNA to a working concentration of 1 µM in 10 mM Tris-HCl, pH7.4	
Combine working solutions for transfection mixture		For one well	For multiple wells
	Tube 1		
	Synthetic guide RNA	50 uL	_ µL
	Serum-free medium	To 200 uL	_ µL
Prepare working solution of Dharmafect 1-4 for transfection	**Tube 2**		
	DharmaFECT 1-4	1-20 uL	_ µL
	Serum-free medium	To 250 µL	_ µL
	Incubate at room temperature for 5 minutes before next step		
	Combine Tube 1 and Tube 2 and carefully mix by pipeting		
Combine transfection mixture	**Incubate at room temperature for 20 minutes before next step**		
	Add full growth medium	1600 uL	_ µL
	Total	2000 uL	_ µL
Transfect cells	**Replace growth medium on cells with 2000 uL of transfection mixture**		
	Incubate cells for 48-72 hours before performing downstream phenotypic assay(s) or gene expression analysis.		

For more information

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

Horizon Discovery, 8100 Cambridge Research Park, Waterbeach, Cambridge, CB25 9TL, United Kingdom

horizon

V10822