PROTOCOL

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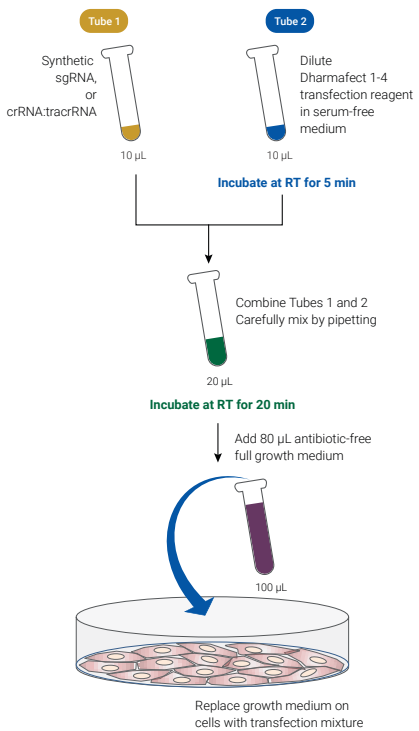
Edit-R synthetic guide RNA transfection protocol for Cas9 expressing cells

The following is an abbreviated protocol for transfecting synthetic guide RNA into cultured mammalian cells expressing Cas9 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 Technical Manual.

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



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 2 µM in 10 mM Tris-HCl, pH 7.4 or Dilute and mix crRNA and tracrRNA to a working concentration of 2 µM in 10 mM Tris-HCl, pH 7.4

		For one well	For multiple wells
Combine working solutions for transfection mixture	**Tube 1**		
	Synthetic guide RNA	1.25 µL	_ µL
	Serum-free medium	To 10 µL	_ µL
Prepare working solution of Dharmafect 1-4 for transfection	**Tube**		
	DharmaFECT 1-4	0.05-0.8 µL	
	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 72-96 hours before performing downstream phenotypic assay(s) or gene editing 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 2 µM in 10 mM Tris-HCl, pH 7.4 **or** Dilute and mix crRNA and tracrRNA to a working concentration of 2 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	6.25 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 72-96 hours before performing downstream phenotypic assay(s) or gene editing 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 2 µM in 10 mM Tris-HCl, pH 7.4 **or** Dilute and mix crRNA and tracrRNA to a working concentration of 2 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	25 uL	_ µL
	Serum-free medium	To 200 µL	_ 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 µL	_ µL
	Total	2000 µL	_ µL
Transfect cells	**Replace growth medium on cells with 2000 uL of transfection mixture**		
	Incubate cells for 72-96 hours before performing downstream phenotypic assay(s) or gene editing 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**

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