GENProtocols

Protocol

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

DRUG DISCOVERY GENOME EDITING

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Aug 10, 2021

Abstract

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.

Introduction

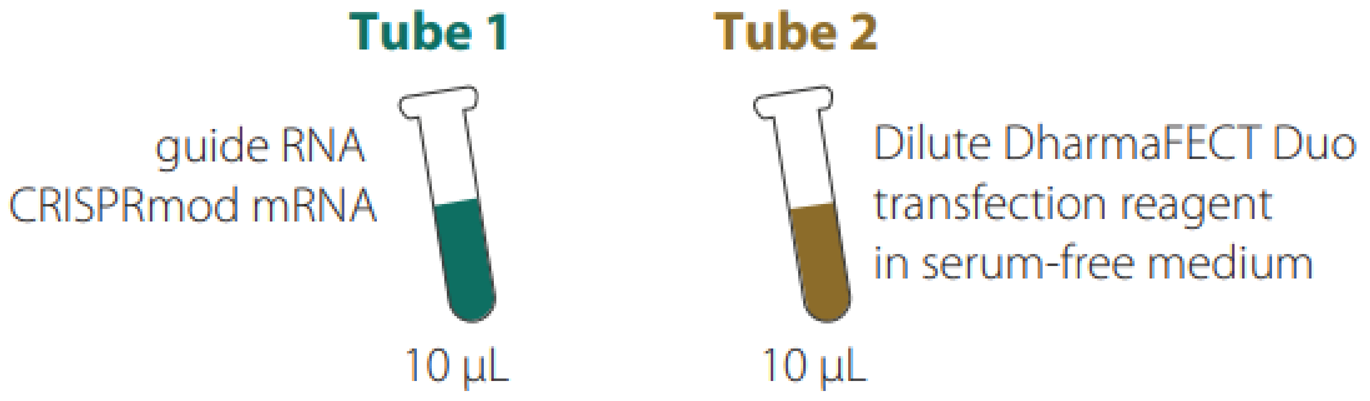
The CRISPR activation (CRISPRa) system is a variation of the canonical CRISPR-Cas9 used for creation of double-strand breaks in genomic DNA. It utilizes a nuclease-deactivated *S. pyogenes* Cas9 (dCas9), often called "dead Cas9", that is fused to one or more transcriptional activators. When paired with a well-designed guide RNA that targets a gene near a promoter region, the gene's native transcription start site is activated

dCas9-VPR mRNA expresses a human codon-optimized version of the nuclease-deactivated *S. pyogenes* Cas9 gene, the three transcriptional activators (VP64, p65 and Rta), and two nuclear localization signals (NLS). The dCas9-VPR mRNA is available in a form that co-expresses either EGFP or puromycin for transfection optimization or enrichment using fluorescence-activated cell sorting (FACS) or antibiotic selection.

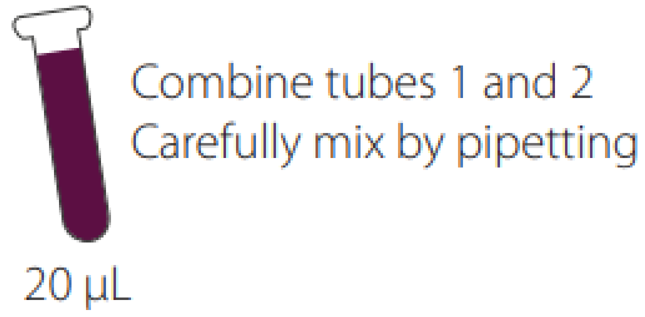
Reagents and Equipment

DharmaFECT™ Duo transfection reagent (Cat #T-2010-xx)

Procedure

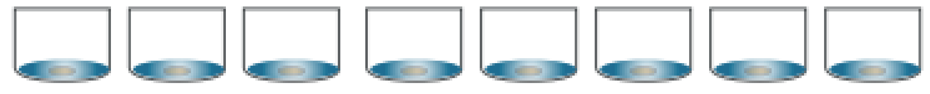


Incubate at RT for 5 min



Incubate at RT for 20 min

Add 80 µL growth medium



<table>
<tr><th colspan="4">96–well protocol</th></tr>
<tr><td colspan="4">Day 1</td></tr>
<tr><td>Cell plating</td><td colspan="3">Seed cells at a density that gives 70-90% confluency on the next day</td></tr>
<tr><td colspan="4">Day 2</td></tr>
<tr><td rowspan="2">Prepare working solutions of materials for transfection</td><td>crRNA:tracrRNA or sgRNA</td><td colspan="2">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)</td></tr>
<tr><td>CRISPRmod mRNA</td><td colspan="2">Dilute CRISPRmod mRNA to a working concentration of 100 ng/µL in serum-free medium</td></tr>
<tr><td rowspan="5">Combine working solutions for transfection mix</td><td></td><td>For one well</td><td>For mulitple wells</td></tr>
<tr><td colspan="3">Tube 1</td></tr>
<tr><td>crRNA:tracrRNA or sgRNA</td><td>1 µL</td><td>_ µL</td></tr>
<tr><td>CRISPRmod mRNA</td><td>2 µL</td><td>_ µL</td></tr>
<tr><td>Serum-free medium</td><td>To 10 µL</td><td>_ µL</td></tr>
<tr><td rowspan="4">Prepare working solution of DharmaFECT Duo for transfection</td><td colspan="3">Tube 2</td></tr>
<tr><td>DharmaFECT Duo transfection reagent</td><td>0.1-0.8 µL</td><td>_ µL</td></tr>
<tr><td>Serum-free medium</td><td>To 10 µL</td><td>_ µL</td></tr>
<tr><td colspan="3">Incubate at room temperature for 5 minutes before next step</td></tr>
<tr><td rowspan="4">Combine transfection mixture</td><td colspan="3">Combine tube 1 and tube 2 and carefully mix by pipeting</td></tr>
<tr><td colspan="3">Incubate at room temperature for 20 minutes before next step</td></tr>
<tr><td>Add full growth medium</td><td>80 µL</td><td>_ µL</td></tr>
<tr><td>Total</td><td>100 µL</td><td>_ µL</td></tr>
<tr><td>Transfect cells</td><td colspan="3">Replace growth medium on cells with 100 µL of transfection mixture</td></tr>
</table>

Time Taken

1 hour