Tranfection of sgRNA using SpCas9 containing plasmids to generate cell lines with Cas9 V.1

Binnypreet Kaur

1 Institute of Parasitology, Biology Centre, Czech Academy of Sciences; 2 Faculty of Sciences, University of South Bohemia, České Budějovice, Czech Republic; 3 Canadian Institute for Advanced Research, Toronto, Canada

Abstract

This protocol describes transfection of ribonucleoprotein (RNP) complexes that consist of purified Cas9 nuclease duplexed with synthetic guide RNA in cultured cells using the AMAXA Nucleo AMAXA Nucleofector II Electroporation system using Program X-001. RNPs complex have only transient expression inside the cell, this allows for the highest levels of editing efficiency and greatly reduces the chances of possible off target and toxic effects of integration of foreign DNA in host genome.

Guidelines

Pre-Electroporation

Subculture cells 2-3 days before electroporation and seed cells in the appropriately sized vessel so that they are 70-80% confluent on the day of transfection. Each transfection reaction will require approximately 5x 10^7 cells.

Materials

1. Synthetic guide RNA (sgRNA or crRNA:tracrRNA)
2. 2NLS-Cas9 nuclease
3. AMAXA Nucleoporator
4. AMAXA T-cell solution
5. AMAXA T-supplement
6. Cell counter
7. Normal growth medium
8. Tissue culture plates
Protocol status: In development
We are still developing and optimizing this protocol

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1 Step1: Assemble RNP complexes with (30pmol/μl) of gRNA and (20pmol/μl) of Cas9 in the ratio of (Cas9:gRNA) 1:9 in resuspension buffer before electroporation and keep it at room temperature for 10 min and then transfer it to ice until use.

2 Step2: Follow the protocol 7 for transfection