VERSION 1

FEB 13, 2019

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.x37fqrn

Protocol Citation: Binnypreet Kaur, 2, Drahomíra Faktorová^{1, 2}, and Julius Lukeš^{1, 2, 3}, 2019. Transfection of sgRNA using SpCas9 containing plasmids to generate cell lines with Cas9. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.x37fqrn>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: In development
 We are still developing and optimizing this protocol

Created: Feb 13, 2019

Last Modified: Feb 13, 2019

PROTOCOL integer ID:
 20319

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

Binnypreet Kaur^{1, 2}, Drahomíra Faktorová^{1, 2}, and Julius Lukeš^{1, 2, 3}

¹Institute of Parasitology, Czech Academy of Sciences;

²Faculty of Sciences, University of South Bohemia, České Budějovice, Czech Republic;

³Canadian Institute for Advanced Research, Toronto, Canada

Binnypreet Kaur

1 Institute of Parasitology, Biology Centre, Czech Academy o...

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 foreignDNA 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 5×10^7 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

- 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