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

Version 3

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dx.doi.org/10.17504/protocols.io.x39fqr6

Protist Research to Optimize Tools in Genetics (PROT-G) Julius Lukes

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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

PROTOCOL STATUS

In development

We are still developing and optimizing this protocol

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 TEXT

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 untill use.

2 Step2: Follow the protocol 7 for transfection

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