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Determination of edits in CRISPR-edited cell lines by sequencing

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ABSTRACT

This protocol details the procedure of determination of edits in CRISPR-edited cell lines by sequencing.

ATTACHMENTS

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CRISPR, Genomic DNA , Qiagen PCR

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MATERIALS TEXT

Buffers and reagents:

Zymo genomic DNA isolation kit (D3025)
QIAquick PCR Purification
Kit **Qiagen Catalog #28104**
T4 DNA Ligase - 20,000 units **New England**
Biolabs Catalog #M0202S
BamHI-HF - 10,000 units **New England**
Biolabs Catalog #R3136S and
HindIII-HF - 10,000 units **New England**
Biolabs Catalog #R3104S
NEB 5-alpha Competent E. coli (NEB #C2987).

Procedure

1 Harvest the CRISPR-edited cells that need to be sequenced and the control parental cells.

2 Isolate genomic DNA using according to manufacturer's instructions.

3

Amplify the region of interest (CRISPR-target region) via PCR using primers obtained when designing CRISPR construct.

See "Generation of CRISPR constructs" protocol.

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4 Run a 1 % DNA agarose gel to check if the PCR has worked.

5 If PCR products are present, clean them up with Qiagen PCR cleanup kit.

6 Send the cleaned-up PCR products to sequencing service with a sequencing primer.

I normally choose a 15 bp DNA sequence (less than 60 % of GC content) within the region of interest at least 100 bp away from CRIPR target site as the sequencing primer.

7 Analyze the sequencing data using this website https://ice.synthego.com/#/.

8 Sometimes, if the sequencing service provider(s) have trouble sequence the PCR products, it might be worth trying to clone these PCR products into a small non-expression plasmid such as pGEM4Z prior to sequencing:

8.1

Incorporate BamHI site (GCGC**GGATCC**; BamHI site is highlighted in grey, the rest is overhang) and HindIII site (GCGC**AAGCTT**; HindIII site is highlighted in green, the rest is overhang) into the primers mentioned in step 3.

8.2

Amplify the region of interest from genomic DNA isolated from the CRISPR-edited cells with these primers via PCR.

8.3 Cut the amplified PCR products and pGEM4Z with BamHI and HindIII.

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8.4 Clean up the cut PCR products and pGEM4Z with Qiagen PCR cleanup kit.

8.5 Ligate the PCR products and pGEM4Z together using T4 DNA ligase.

8.6 Transform the ligated product mix into E. coli competent cells and plate on an Ampicillin agar plate.

8.7 Screen for colonies with pGEM4Z ligated with the PCR products.

8.8 Send them for sequencing with M13 forward or reverse primer.

8.9 Align the sequencing data with the reference sequence to determine the edits.

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