One-step generation of triple knockout CHO cell lines using CRISPR/Cas9 and fluorescent enrichment.

The CRISPR/Cas9 genome editing technology has previously been shown to be a highly efficient tool for generating gene disruptions in CHO cells. In this study we further demonstrate the applicability and efficiency of CRISPR/Cas9 genome editing by disrupting FUT8, BAK and BAX simultaneously in a multiplexing setup in CHO cells. To isolate Cas9-expressing cells from transfected cell pools, GFP was linked to the Cas9 nuclease via a 2A peptide. With this method, the average indel frequencies generated at the three genomic loci were increased from 11% before enrichment to 68% after enrichment. Despite the high number of genome editing events in the enriched cell pools, no significant off-target effects were observed from off-target prediction followed by deep sequencing. Single cell sorting of enriched multiplexed cells and deep sequencing of 97 clones revealed the presence of four single, 23 double and 34 triple gene-disrupted cell lines. Further characterization of selected potential triple knockout clones confirmed the removal of Bak and Bax protein and disrupted fucosylation activity as expected. The knockout cell lines showed improved resistance to apoptosis compared to wild-type CHO-S cells. Taken together, multiplexing with CRISPR/Cas9 can accelerate genome engineering efforts in CHO cells even further.

General information
State: Published
Organisations: Department of Systems Biology, Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design, CHO Core, Glyco-Engineering of CHO, Big Data 2 Knowledge, iLoop, Network Reconstruction in Silico Biology, Korean Advanced Institute of Science and Technology (KAIST)
Number of pages: 11
Pages: 1446-1456
Publication date: 2015
Peer-reviewed: Yes
Early online date: 2015

Publication information
Journal: Biotechnology Journal
Volume: 10
Issue number: 9
ISSN (Print): 1860-6768
Ratings:
BFI (2019): BFI-level 1
Web of Science (2019): Indexed yes
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): CiteScore 3.12
Web of Science (2017): Impact factor 3.507
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.2 SJR 1.29 SNIP 0.969
Web of Science (2016): Impact factor 3.649
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): CiteScore 2.91 SJR 1.172 SNIP 0.874
Web of Science (2015): Impact factor 3.781
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): CiteScore 2.98 SJR 1.189 SNIP 1.062
Web of Science (2014): Impact factor 3.49
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): CiteScore 3.01 SJR 1.136 SNIP 1.093
Web of Science (2013): Impact factor 3.708
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): CiteScore 2.4 SJR 0.944 SNIP 0.957
Web of Science (2012): Impact factor 3.446
ISI indexed (2012): ISI indexed no
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): CiteScore 1.94 SJR 0.785 SNIP 0.726
ISI indexed (2011): ISI indexed no
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.787 SNIP 0.798
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.695 SNIP 0.749
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 0.581 SNIP 0.806
Scopus rating (2007): SJR 0.568 SNIP 0.709
Web of Science (2007): Indexed yes
Original language: English
Keywords: Chinese hamster ovary cells, CRISPR/Cas9, Deep sequencing, Genome editing, Multiplexing
DOI: 10.1002/biot.201500027
Source: PublicationPreSubmission
Source-ID: 110809197
Research output: Research - peer-review ; Journal article – Annual report year: 2015