Enhanced genome editing tools for multi-gene deletion knock-out approaches using paired CRISPR sgRNAs in CHO cells

Since the establishment of clustered regularly interspaced short palindromic repeat (CRISPR)/Cas9, powerful strategies for engineering of CHO cell lines have emerged. Nevertheless, there is still room to expand the scope of the CRISPR tool box for further applications to improve CHO cell factories. Here, we demonstrate activity of the alternative CRISPR endonuclease Cpf1 in CHO-K1 for the first time and that it can be used in parallel to CRISPR/Cas9 without any interference. Both, Cas9 and Cpf1, can be effectively used for multi-gene engineering with a strategy based on paired single guide RNAs (sgRNAs) for full gene deletions. This strategy also enables the targeting of regulatory regions, which would not respond to the conventional frameshift mutations, as shown by deleting the α-1,6-Fucosyltransferase 8 (FUT8) promoter resulting in a functional knock-out. Fut8 also served as model to verify that deletion efficiency is size-independent (2 - 150 kb). To test the efficiency of multi-gene approaches in combination with gene deletion, clones harboring triple deletions in β-1,4-Galactosyltransferase (B4GALT) isozymes were identified using solely conventional PCR/qPCR. In addition two bicistronic transcription strategies were implemented to enable unequivocal pairing of sgRNAs: a CHO-derived tRNA linker that works for both, Cas9 and Cpf1, as well as paired sgRNAs in an array format, which can be used with Cpf1 due to its RNA processing ability. These strategies broaden the range of application of CRISPR for novel gene editing approaches in CHO cells and also enable the efficient realization of a genome-wide deletion library.

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