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CRISPR/Cas9-Mediated knock-in of large DNA in human embryonic stem cells and somatic cells

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CRISPR/Cas9-induced site-specific DNA double-strand breaks (DSBs) can be repaired by homology-directed repair (HDR) or non-homologous end joining (NHEJ) pathways. Extensive efforts have been made to knock-in exogenous DNA to a selected genomic locus in human cells; which, however, has focused on HDR-based strategies and was proven inefficient. Here, we report that NHEJ pathway mediates efficient rejoining of genome and plasmids following CRISPR/Cas9-induced DNA DSBs, and promotes high-efficiency DNA integration in various human cell types. With this homology-independent knock-in strategy, integration of a 4.6 kb promoterless ires-eGFP fragment into the *GAPDH* locus yielded up to 20% GFP+ cells in somatic LO2 cells, and 1.70% GFP+ cells in human embryonic stem cells (ESCs). Quantitative comparison further demonstrated that the NHEJ-based knock-in is more efficient than HDR-mediated gene targeting in all human cell types examined. These data support that CRISPR/Cas9-induced NHEJ provides a valuable new path for efficient genome editing in human ESCs and somatic cells.

Biography

Bo Feng is an Assistant Professor at the School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong. She is an active Staff Member in the Stem Cell and Regeneration program, MOE key laboratory for Regenerative Medicine and CUHK-GIBH joint laboratory on Stem Cell and Regenerative Medicine. She graduated from Nankai University with BSc (1993) and MSc (1996), and received her Ph.D (2006) from National University of Singapore. After graduation, she joined Prof. Ng Huck Hui's lab in Genome Institute of Singapore as a Post-doc. She worked on stem cells and reprogramming and published her works in Nature Cell Biology, Cell Stem Cell and Nature. In Nov 2010, she joined CUHK and her current research interest lies within the molecular mechanism that controls pluripotency and differentiation of ESCs/iPSCs, as well as development of new tools for stem cell research and applications.

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