Efficient simultaneous knock-in of multiple long DNA sequences in mouse embryonic stem cells by CRISPR/Cas9 for generating gene-edited mouse models.

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ABSTRACT

Gene targeting of embryonic stem (ES) cells followed by chimera production has been conventionally used for developing gene-manipulated mice. Although direct knock-in (KI) using murine zygote via CRISPR/Cas9-mediated genome editing has been reported, ES cell targeting still has merits, e.g., high throughput work can be performed in vitro. In this study, we first compared the KI efficiency of mouse ES cells with CRISPR/Cas9 expression vector and ribonucleoprotein (RNP), and confirmed that KI efficiency was significantly increased by using RNP. Using CRISPR/Cas9 RNP and circular plasmid with homologous arms as a targeting vector, knock-in within ES cell clones could be obtained efficiently (~50%) without drug selection, thus potentially shortening the vector construction or cell culture period. Moreover, by incorporating a drug-resistant cassette into the targeting vectors, KI of multiple DNA fragments (over several kilo-base-pairs) at independent

genomic loci can be simultaneously achieved at high efficiency (up to 100%) by a single electroporation. This technique will help facilitate the production of genetically modified mouse models that are fundamental for exploring topics related to human and mammalian biology, including reproductive biology.