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Tools and techniques

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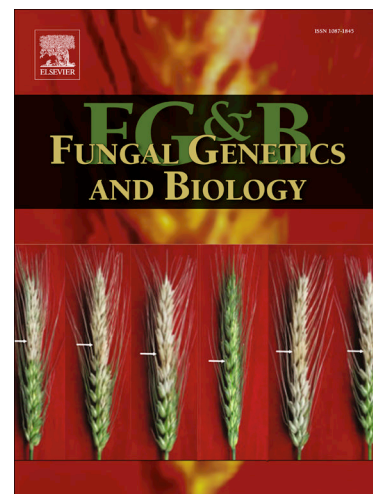
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Efficient Oligo nucleotide mediated CRISPR-Cas9 Gene Editing in *Aspergilli*

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ABSTRACT

CRISPR-Cas9 technologies are revolutionizing fungal gene editing. Here we show that survival of specific Cas9/sgRNA mediated DNA double strand breaks (DSBs) depends on the non-homologous end-joining, NHEJ, DNA repair pathway and we use this observation to develop a tool to assess protospacer efficiency in *Aspergillus nidulans*. Moreover, we show that in NHEJ deficient strains, highly efficient marker-free gene targeting can be performed. Indeed, we show that even single-stranded oligo nucleotides efficiently works as repair templates of specific Cas9/sgRNA induced DNA DSBs in *A. nidulans*, *A. niger*, and in *A. oryzae* indicating that this type of repair may be wide spread in filamentous fungi. Importantly, we demonstrate that by using single-stranded oligo nucleotides for CRISPR-Cas9 mediated gene editing it is possible to introduce specific point mutations as well gene deletions at efficiencies approaching 100%. The efficiency of the system invites for multiplexing and we have designed a vector system with the capacity of delivering Cas9 and multiple sgRNAs based on polymerase III promoters and tRNA spacers. We show that it is possible to introduce two point mutations and one gene insertion in one transformation experiment with a very high efficiency. Our system is compatible with future high-throughput gene-editing experiments.

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