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A CRISPR/Cas9 system for genetic engineering of filamentous fungi

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Many fungi are both excellent degraders of biomass and natural producers of industrially interesting compounds, making them good candidates for cell factories. Several members of the genus Aspergillus are successfully used as industrial cell factories for production of organic acids, enzymes and other primary or secondary metabolites, and many other Aspergilli are currently being sequenced and might possess traits making them similar suitable as potential cell factories. Yields from such cell factories can be greatly enhanced by employing genetic engineering strategies, however there are several obstacles such as low gene targeting frequencies, slowing down the process.

The harnessing of the prokaryotic and archaeal immune mechanism CRISPR (clustered regularly interspaced short palindromic repeats) as a tool for genetic engineering in eukaryotes, has proved to be a powerful technology. CRISPR/Cas9 introduces specific DNA double strand breaks (DSB) with high precision, which in turn can be employed to efficiently stimulate gene targeting. Consisting of two components, an RNA guided nuclease Cas9 and a chimeric guide RNA (gRNA), a specific DSB can be produced in the host organism. The cleavage target site is determined by 20 base pairs (bp) in the gRNA, and by exchanging those 20 bp, Cas9 can be programmed to target a specific chromosomal location with few constraints. The technology has had a huge impact on genetic engineering of organisms, such as plants or mammalian cells where gene targeting is notoriously inefficient, but has only recently been adapted to filamentous fungi.

Low gene targeting frequencies is a common problem when attempting to do gene editing in filamentous fungi. A common strategy to circumvent this problem is to delete or disable one of the key genes in the non-homologous end-joining (NHEJ) pathway to greatly enhance gene-targeting frequencies. However, for fungi where a genetic toolbox is not in place, the initial establishment of genetic markers and NHEJ-deficiency can be laborious. Here we present a CRISPR/Cas9 system adapted for filamentous fungi and show that it can be efficiently used to introduce specific genomic modifications. Considering that the number of fully sequenced fungi is dramatically increasing, and that the vast majority of these fungi does not possess a genetic toolbox, our system will be a highly useful in developing the initial marker- and NHEJ gene mutations to establish such a toolbox. To this end, we have also developed a gRNA design software that facilitates identification of gRNA sequences that can target a desired gene in several different species, hence, reducing the plasmid construction workload. Together, we envision that our tools can be used to rapidly expand the repertoire of fungi where genetic engineering is possible and therefore greatly accelerate the exploration of fungal biology and design of fungal cell factories.