

High throughput gene replacement in *Aspergillus fumigatus*.

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Abstract

Aspergillus fumigatus is a human pathogen and the principal etiologic agent of invasive and chronic aspergillosis leading to several hundreds of thousands of deaths every year. Very few antifungals are available to treat infections caused by *A. fumigatus*, and resistance is developing to those we have. Our understanding of the molecular mechanisms that drive pathogenicity and drug resistance have been hampered by the lack of large mutant collections, which limits our ability to perform functional genomics analysis. Here we present a high-throughput gene knockout method that combines a highly reproducible fusion PCR method to enable generation of gene replacement cassettes with a multiwell format transformation procedure. This process can be used to generate 96 null mutants within 5 days by a single person at a cost of less than £18 (\$24) per mutant and is being employed in our laboratory to generate a barcoded genome-wide knockout library in *A.*

fumigatus.

Involved units

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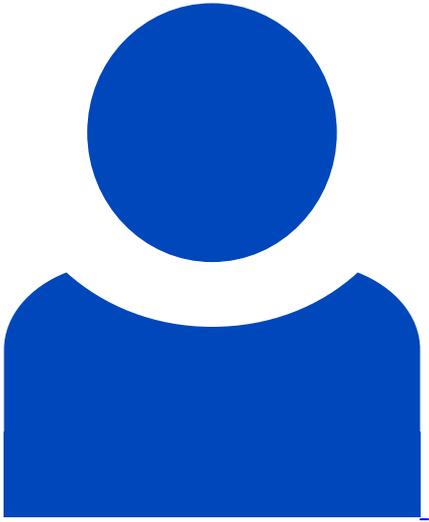
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