

Modular Synthetic Biology Toolkit for Filamentous Fungi

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Supporting Information

Fungal strain	Genotype	Transformed donor DNA
<i>P. rubens</i> DS68530	Δ hdfA (Δ Ku70), Δ pen-BGC	n/a
DS68530_pLM-AMA002	Δ hdfA (Δ Ku70), Δ pen-BGC	pLM-AMA002
DS68530_pLM-AMA002_P40s-dSpCas9-eGFP-NLS-Tif35	Δ hdfA (Δ Ku70), Δ pen-BGC	pLM-AMA002_P40s-dSpCas9-eGFP-NLS-Tif35

Table S1. List of fungal strains used in this study and created strains with their corresponding transformed donor DNA

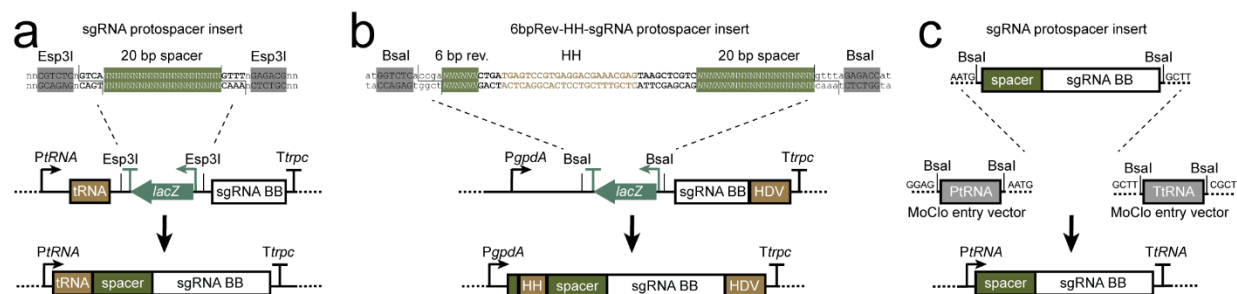


Figure S1. Representation of different sgRNA transcription unit assembly methods. (a) Ligation of the sgRNA target sequence carrying double-stranded-DNA into sgRNA recipient cloning site, using *Esp31* restriction sites for *PtrRNA* driven and tRNA-linked transcripts, (b) Ligation of HH-sgRNA (and 6 bp inverted repeat of the spacer sequence) using *BsaI* sites for *PgpdA* driven HH-sgRNA-HDV-based transcription (c) Ligation of sgRNA sequence using entry vectors and *BsaI* restriction-ligation-based assembly for creating functional sgRNA transcription units.

Supplementary File S1 Elements of the Fungal Toolkit for Modular Cloning (FTK) as Genebank files