Rapid methods for plasmid based deletion construction in fungi
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ABSTRACT:
We describe DelsGate and OSCAR, novel methods for the rapid generation of plasmid based gene deletion constructs for protoplast mediated (PMT) and Agrobacterium tumefaciens-mediated (ATMT) transformation in fungi, respectively. For DelsGate, plasmid vectors for use in Ascomycota and Ustilago maydis are available. DelsGate involves PCR of the flanks of the gene of interest with primers 1 and 2 for the 5’ flank and 3 and 4 for the 3’ flank. These primers have gene specific 3’ ends and universal 5’ sequences that allow application to any gene in the fungal genome. These flanks are as follows: primer 1, I-SceI; primer 2, attP1; primer 3, attB2; and primer 4, I-SceIR. A single BP-clonase reaction with the amplified flanks and the vector generates the deletion plasmid. Digestion with I-SceI readsies the plasmid construct for PMT. For OSCAR we designed i) a marker vector with an Ascomycota functional hygromycin B resistance cassette, flanked by the attP1r and attP4 recombination sites and ii) a modified binary vector containing the ccdB gene flanked by attP2r and attP3 recombination sites. 5’ and 3’ gene flanks with attB2r/attB1r and attB4/attB3 recombination sites, respectively are generated by PCR. A single BP clonase reaction containing both the above vectors and PCR amplified flanks results in a deletion plasmid containing the resistance cassette surrounded by the upstream and downstream gene flanks, all contained between the T-DNA borders. Both DelsGate and OSCAR have been used to delete several fungal genes. Additionally, due to their relative rapidity and robust nature the methods have been used in an undergraduate course at UGA with considerable success. In summary, DelsGate and OSCAR combine PCR and Gateway technology, to rapidly and robustly generate precise deletion constructs for PMT or ATMT based homologous gene replacement.

INTRODUCTION:
The increasing availability of fungal genome sequences and genome wide examination tools, such as global transcriptome and multispecies comparative analyses is leading to the identification of large sets of genes whose roles are worthy of functional exploration. This situation elevates the need for highly efficient systems for gene deletion, a critical tool for functional analyses.

Recently, we reported development of DelsGate, a rapid and efficient method for generating gene deletion plasmid constructs for protoplast-mediated transformation of fungi (Garcia-Pedrajas et al., 2008; Garcia-Pedrajas et al., 2010). DelsGate employs Gateway® technology in combination with the homing endonuclease I-SceI allowing a universal methodology for generation of deletion constructs for any gene in the fungal genome. Additionally, Agrobacterium tumefaciens-mediated transformation (ATMT) is now an established method of choice for both targeted as well as random gene mutation in an increasing number of fungal species, including plant pathogens such as Magnaporthe grisea, Fusarium oxysporum (Khang et al., 2005) and Verticillium dahliae (Dobinson et al., 2004). Therefore, in addition to DelsGate for protoplast transformation, we devised OSCAR to rapidly produce deletion constructs for use with ATMT in fungi. We named this method “OSCAR” for One Step Construction of Agrobacterium-Recombination-ready-plasmids. A publication describing OSCAR is in press at Fungal Genetics and Biology.

METHODS and RESULTS:
Figure 1: Schematic of the DelsGate method for construction of deletion plasmids for protoplast based fungal transformation.
Figure 2: Schematic of the OSCAR method for construction of deletion plasmids for fungal ATMT.
Figure 3: Structure of a final OSCAR deletion construct.
Table 1: Efficiency of the OSCAR deletion construction system as measured for 8 genes.

DISCUSSION:
We have tested DelsGate’s and OSCAR’s efficiency by producing deletion constructs for numerous genes. DelsGate has been used successfully in the deletion numerous U. maydis and ascomycete genes. OSCAR has been used by our lab and others to delete several genes in Verticillium dahliae. DelsGate and OSCAR provide rapid, robust and cost-effective approaches for generation of gene deletion constructs for protoplast and ATMT based fungal transformation systems, respectively. These methodologies should be widely applicable to fungal species employing their preferred genetic transformation method.

We have used both DelsGate and OSCAR successfully in an undergraduate laboratory class were students are each assigned a gene of interest at random. Each student designed and constructed a deletion construct for a different U. maydis gene (DelsGate) or for a V. dahliae gene (OSCAR). See “OSCAR Nominees” image at top right.

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