Efficient four fragment cloning for the construction of vectors for targeted gene replacement in filamentous fungi

Background: The rapid increase in whole genome fungal sequence information allows large scale functional analyses of target genes. Efficient transformation methods to obtain site-directed gene replacement, targeted over-expression by promoter replacement, in-frame epitope tagging or fusion of coding sequences with fluorescent markers such as GFP are essential for this process. Construction of vectors for these experiments depends on the directional cloning of two homologous recombination sequences on each side of a selection marker gene. Results: Here, we present a USER Friendly cloning based technique that allows single step cloning of the two required homologous recombination sequences into different sites of a recipient vector. The advantages are: A simple experimental design, free choice of target sequence, few procedures and user convenience. The vectors are intended for Agrobacterium tumefaciens and protoplast based transformation technologies. The system has been tested by the construction of vectors for targeted replacement of 17 genes and overexpression of 12 genes in Fusarium graminearum. The results show that four fragment vectors can be constructed in a single cloning step with an average efficiency of 84% for gene replacement and 80% for targeted overexpression. Conclusion: The new vectors designed for USER Friendly cloning provided a fast reliable method to construct vectors for targeted gene manipulations in fungi.

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