

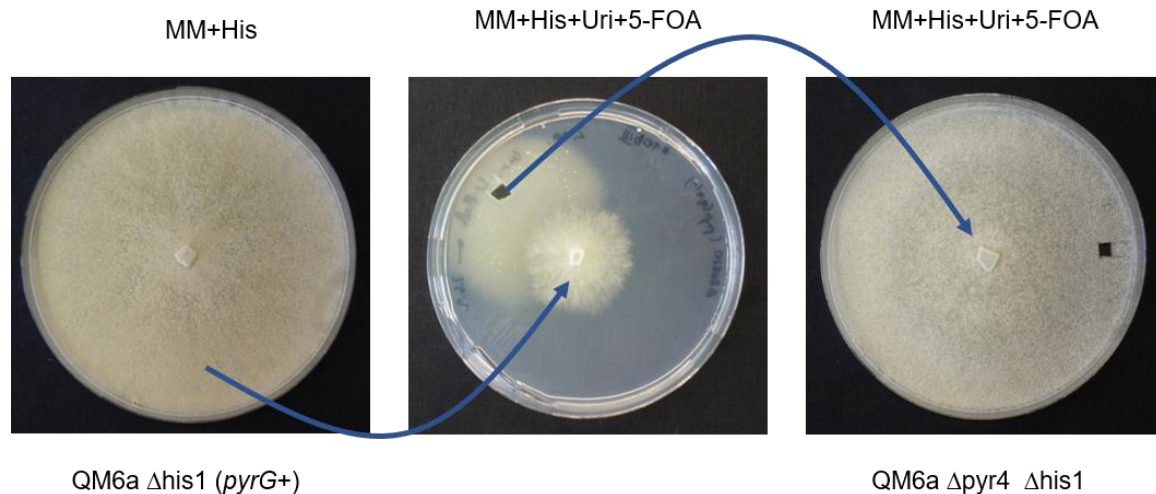
Expanding the toolbox: another auxotrophic marker for targeted gene integrations in *Trichoderma reesei*

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Additional File 2 - Marker recycling due to a spontaneous internal recombination leading to the loss of the *pyrG* gene.



The strain *T. reesei* QM6a Δ his1(*pyrG*⁺) was incubated on MAM plates without peptone (MM) containing uridine, histidine, and 5-FOA. The plate was incubated at 30°C for up to 4 weeks, until the *pyrG* marker was lost due to a random internal homologous recombination (Fig. 1B) and the fungus gained 5-FOA tolerance.