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Note

Development of a *URA5* integrative cassette for gene disruption in the *Candida guilliermondii* ATCC 6260 strain

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ABSTRACT

We designed an efficient transformation system for *Candida guilliermondii* based on a *ura5* ATCC 6260 derived recipient strain and a *URA5* recyclable selection marker. This "*URA5* blaster" disruption system represents a powerful tool to study the function of a large pallet of genes in this yeast of clinical and biotechnological interest.

Candida guilliermondii is an opportunistic emerging fungal agent of candidiasis often associated with oncology patients (Pfaller et al., 2006; Girmenia et al., 2006). This yeast also remains an attractive biotechnological model for the industrial production of valuable metabolites (Mussatto et al., 2005; Boretsky et al., 2007a; Schirmer-Michel et al., 2008; Sibirny and Boretsky, 2009). The recent whole genome sequencing of the *C. guilliermondii* ATCC 6260 reference strain (Butler et al., 2009) provides an interesting resource for elucidating new molecular events supporting pathogenicity, antifungal resistance and for exploring the potential of yeast metabolic engineering. For this reason, the establishment of a convenient genetic transformation system becomes indispensable to evaluate the function of a large number of genes in this species.

We initiated this work by isolating a *C. guilliermondii* uracil auxotroph strain following a 5-fluoroorotic acid (FOA) counter-selection method (Boeke et al., 1984). A total of 1500 spontaneous FOA resistant colonies derived from the ATCC 6260 strain were obtained on minimal medium (YCS) containing uracil, uridine and FOA (supplementary data: experimental procedures). All of these clones were uracil prototroph except one, NP566U, which displayed strong uracil deficiency (Fig. 1). The ATCC 6260 and NP566U strains exhibited similar doubling times in liquid YPS medium (supplementary data: Table S1). The NP566U auxotrophic mutant displayed a very low reversion frequency (up to 10^{-9}) as revealed by plating a stationary phase culture onto YCS. Streaking NP566U cells onto YCS supplemented with either uracil or uridine showed that this *C. guilliermondii* auxotroph could metabolize uridine but not uracil (Fig. 1).

In the yeast *S. cerevisiae*, L-glutamine is converted to uridine monophosphate in sequential reactions catalyzed by five enzymes encoded by *URA2*, *URA4*, *URA1*, *URA5* and *URA3* genes. Mutations in *ura2*, *ura4* and *ura1* in *S. cerevisiae* leave cells sensitive to FOA but lead to a requirement for uracil in the medium. However, *ura3* and *ura5* mutants are resistant to FOA as well as being uracil auxotroph (Dave and Chattoo, 1997). This indicates that the *C. guilliermondii* NP566U mutant may be defective in either *ura3* or *ura5* since it is resistant to FOA as well as being uridine-requiring. Therefore, the sequences of the *C. guilliermondii URA3* (PGUG_01920.1) and *URA5* (PGUG_01426.1) genes were directly identified from the annotated *Candida* database of the Broad Institute (http://www.broad.mit.edu/). We then constructed two backbone plasmids, pG-RU3R and pG-RU5R, consisting of the *C. guilliermondii URA3* and *URA5* genes, respectively, flanked on both sides by a 327-bp repeat (fragment REP) obtained by amplification from the prokaryotic *NPTI* gene (Fig. 2A). In a preliminary experiment, these plasmids were each used to transform NP566U cells by electroporation (supplementary data: experimental procedures). Only one or two Ura^+ colonies developed on YCS medium when yeast cells were transformed with either sterile water or pG-RU3R whereas up to 300 colonies were obtained after electroporation in the presence of pG-RU5R (Fig. 2B). Additional experiments shown in the

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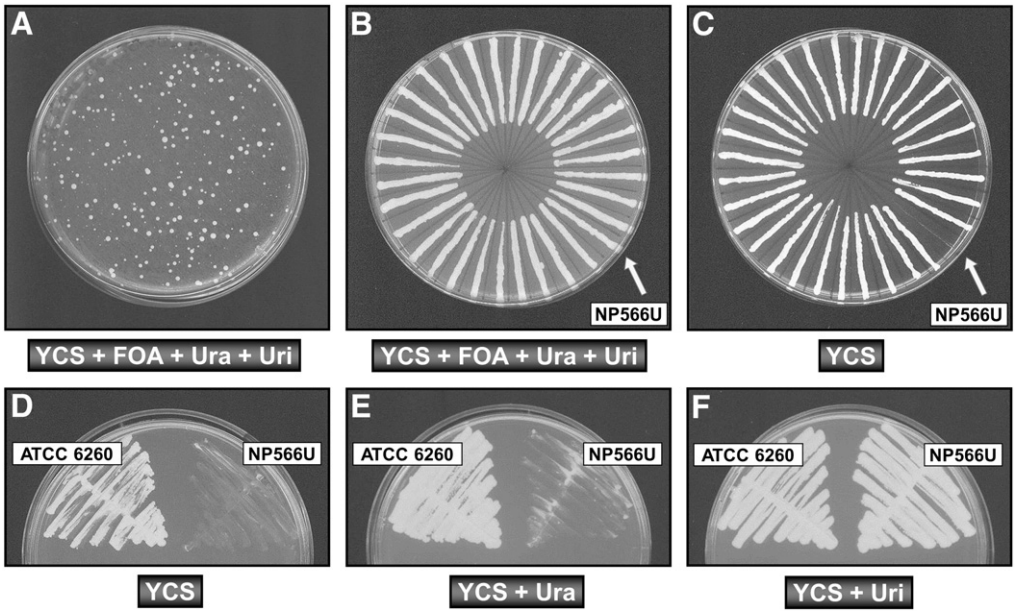

Fig. 1. Selection and characterization of the *C. guilliermondii* uracil biosynthesis deficient strain. A. Cells from 3 days old YPS-grown streaks of ATCC 6260 were plated onto ten YCS petri dishes (approximately 10^8 cells per dish) supplemented with 50 μg/ml uracil, 50 μg/ml uridine and 1 mg/ml FOA. After 7–10 days of growth, 150–200 colonies developed per plate. B. Individual clones were isolated by streaking (32 per dish) onto the same selection medium to confirm FOA resistance (the petri dish containing the NP566U strain is shown). C. FOA-resistant clones were then replicated onto YCS plates to screen for uracil auxotrophy (the petri dish containing the NP566U strain is shown). D. The reference strain ATCC 6260 and the NP566U mutant were grown for two days at 30 °C on YCS plates. E. ATCC 6260 and NP566U were grown for two days at 30 °C on YCS plates containing 50 μg/ml uracil. F. ATCC 6260 and NP566U were grown for two days at 30 °C on YCS plates containing 50 μg/ml uridine.

supplemental information (supplementary data: Table S2) indicate that early log-phase cells and high amounts of DNA were needed to efficiently recover transformants. These results allowed us to attribute the genotype *ura5* to NP566U. Furthermore, these experiments demonstrate the ability to use the *C. guilliermondii URA5* wild-type allele as a selectable marker in the NP566U strain.

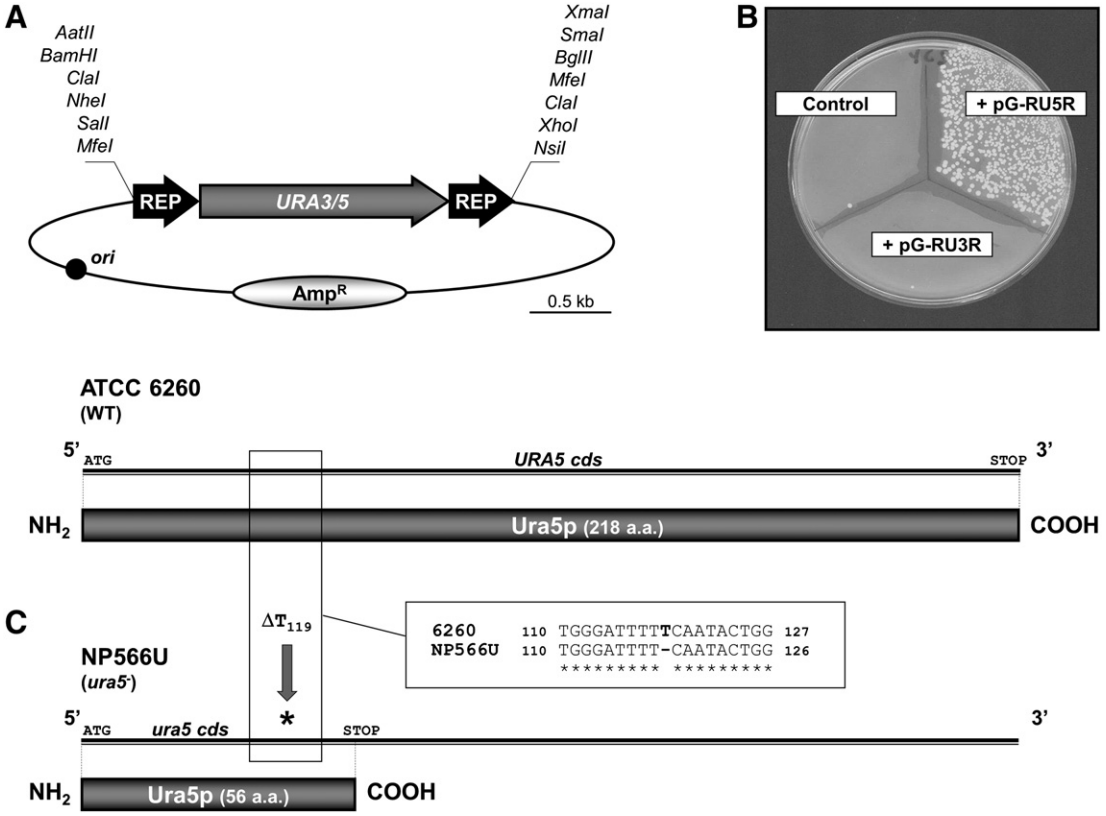

Fig. 2. A. Schematic representation of pG-RU3R/pG-RU5R plasmids. B. Transformation by electroporation of log-phase lithium acetate pre-treated NP566U cells in the presence of sterile water (control), 2 μg pG-RU3R or 2 μg pG-RU5R. The YCS petri dish was photographed after 3 days of growth at 30 °C. C. Comparison of the *URA5* nucleotide sequence from ATCC 6260 and NP566U.