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# STRAINS AND PLASMIDS FOR MAKING HOMOZYGOUS KNOCKOUTS IN C. ALBICANS

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## BRIEF DESCRIPTON

Researchers at UCSF have developed new *C. albicans* strains that use different auxotrophic markers that do not affect the virulence of *C. albicans* in a mouse model. Furthermore, these researchers have cloned complementing markers to be used in selection of knockout mutants from *Candida* strains other than *C. albicans*, thereby greatly reducing misintegration of DNA gene disruption fragments into the *Candida* auxotrophic marker site instead of the knockout target site. Combining these strains and markers with a fusion PCR technique allows for quick and efficient disruption of both alleles of the target gene in *C. albicans*. Generating homozygous knockouts is improved from 2% to 70% efficiency for knocking out the more difficult second allele.

## FULL DESCRIPTION

BACKGROUND:*Candida* pathogenic fungal infections has become an increasing threat to humans, particularly in immuno-compromised patients. *Candida albicans* accounts for >50% of fungal infections and thus is a severe concern to clinicians. The search for novel drugs has become urgent as the common *Candida* strains acquire multi-drug resistance to antifungals, thereby hampering successful therapy. One approach to developing new antifungal agents is to identify and target virulence genes important in the pathogenesis of *C. albicans*. Many conventional genetic techniques used to study bacterial pathogens cannot be applied to *C. albicans* due to its asexual life cycle and diploid genome. Current genetic knockout technologies in *C. albicans*, adapted from techniques developed for the haploid yeast *S. cerevisiae*, is time-consuming with a low efficiency for generating homozygous knockout strains. Furthermore, these methods rely on use of auxotrophic selectable markers, such as URA3, that complement nutritional requirements to study virulence. The major disadvantage of using URA3 is that it is also a virulence factor; URA3 gene disruption strongly reduces virulence and impacts morphology, which complicates interpretation of virulence studies of other targeted genetic disruptions. Consequently, *C. albicans* strains containing genetic markers that do not influence virulence are highly desirable.

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### OTHER INFORMATION

#### KEYWORDS

novel candida strains

#### CATEGORIZED AS

- ▶ **Medical**
  - ▶ **Disease: Infectious Diseases**
  - ▶ **Research Tools**
- ▶ **Research Tools**
  - ▶ **Other**

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2005-050-0

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FEATURES/BENEFITS

- ▶ Provides efficient method for knocking out one or both alleles of genes of interest in C. albicans.
- ▶ Improved 70% efficiency in knocking out the second allele.
- ▶ Mutants are suitable for virulence analysis.
- ▶ Reduced misintegration of DNA gene disruption fragment into knockout target site.

OTHER INFORMATION

- ▶ Noble, et al. (2005) Eukaryotic Cell, Vol. 4 (2), pp. 298-309.

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