

DEVELOPMENT OF NEW TRANSPOSONS FOR GFP PROTEIN TAGGING IN
CANDIDA ALBICANS

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Transposons are important tools for gene function analysis, for example they can be used to easily mutagenize individual genes or genomic libraries and also to insert sequences coding for protein tags. We developed two new transposons that incorporate a GFP (green fluorescent protein) coding sequence useful for generating GFP-fusion proteins in the diploid fungus *Candida albicans*. In the *CaTn5-GFP-FL* transposon, the bacterial and yeast markers, *KanR* and *URA3* respectively, are flanked by a rare-cutting enzyme. After *in vitro* transposition into a plasmid-borne target gene, the markers are eliminated by restriction digestion and religation, resulting in a construct coding for full-length GFP-fusion proteins. This transposon may be used to generate plasmid libraries of GFP insertions in proteins where N- or C-terminal tagging may alter localization. Besides a GFP coding sequence, a *URA-flipper* recycling cassette was incorporated in the transposon *CaTn5-GFP-FLP*. After *in vitro* transposition, the insertional allele is transferred to the chromosomal locus by homologous recombination. Then the *URA-flipper* cassette can be excised by inducing the Flip recombinase. A second round of transformation results in the substitution of the second wild type allele. This strategy is useful to obtain homozygous strains carrying GFP truncated genes. The transposons described here represent new tools to obtain global subcellular or cellular expression data in *Candida albicans*.

Key words: *Candida albicans*, GFP, transposon

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