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ABSTRACT

In recent years, the Bioluminescence Resonance Energy Transfer (BRET) technology has undergone significant improvements with the introduction of two novel protein tags, NanoLuc and HaloTag. These two tags offer notably improved sensitivity and modularity. Unfortunately, the use of this system is currently limited to mammalian expression systems. In this study, the heterologous expression system consisting of the genetically modified *Saccharomyces cerevisiae* host strain, ADΔΔ, and plasmid pABC3 was modified so that this NanoBRET technology can also be used in arguably the best studied eukaryotic model host, *S. cerevisiae*. Furthermore, to allow the stable integration of two heterologous ORFs in tandem into the genomic *PDR5* locus of *S. cerevisiae* ADΔΔ, a step required for protein-protein interaction studies, two additional sets of pABC3 derivative plasmids with a recyclable *URA3* selection marker were created. One set of plasmids (pABC3A and pABC3A derivatives) contains a *URA3* blaster cassette with the *URA3* selection marker flanked by two identical 196 bp *PDR5* terminators and the other set of plasmids (pABC3B and pABC3B derivatives) contains a *URA3* blaster cassette that has two identical 35 bp synthetic terminators flanking the *URA3* selection marker. These modifications will pave the way for superior protein-protein interaction studies and for metabolic engineering in the genetically modified *S. cerevisiae* host strain, ADΔΔ.

Keywords: Plasmid, pABC3, pABC3XL, pABC3A, pABC3B, synthetic terminator, *URA3* blaster, NanoBRET, NanoLuc, HaloTag