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INDUCIBLE PIGGYBAC TRANSPOSASE CONSTRUCTS

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Chemically inducible PiggyBac transposase constructs. The plasmids that contain mutated destabilized domains, FKBP and DHFR, were purchased from Addgene with ID 31763 and 29326, respectively. The PBase-ERT2 plasmid (mPBase-L3-ERT2) was a kind gift from Dr. Allen Bradley . The coding sequence of mPBase-L3-ERT2 was sub-cloned into a yeast shuttle vector pRS314 containing CEN6, ARS and TRP1 to use ‘gap repair’ cloning technique in yeast cells (Strathern and Higgins 1991). This engineered yeast shuttle vector was used as a ‘parental’ plasmid (pRM1056) to derive other variants of PBase constructs by gap repair method. Briefly, PCR-generated sequences were cloned into linearized vectors by recognizing a 40 bp overlap at their ends. This 40 bp overlap can be engineered by designing primers for amplification of the desired sequences. For example, to replace the ERT2 sequence with the FKBP sequence, the pRM1056 plasmid was linearized by restriction digestion that cut the plasmid within the ERT2 sequence. The FKBP sequence was amplified with a pair of primers that have a 40 bp sequence that is homologous to pRM1056. The FKBP PCR products and linearized pBM1056 were cotransformed into yeast cells and the yeast cells were selected for Trp+ colonies. DNA extracted from Trp+ yeast colonies was introduced into E. coli. Finally, the plasmid was isolated by QIAprep Spin Miniprep Kit (QIAGEN) and was confirmed by Sanger sequencing.