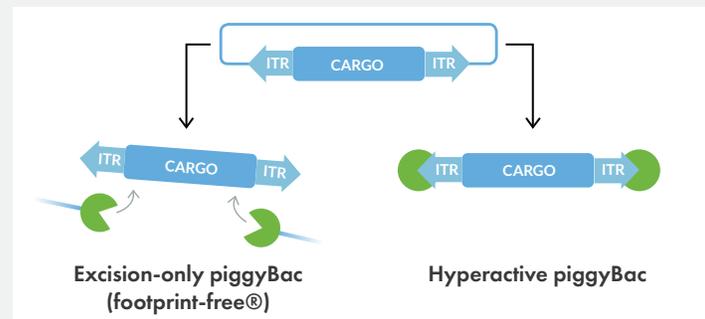


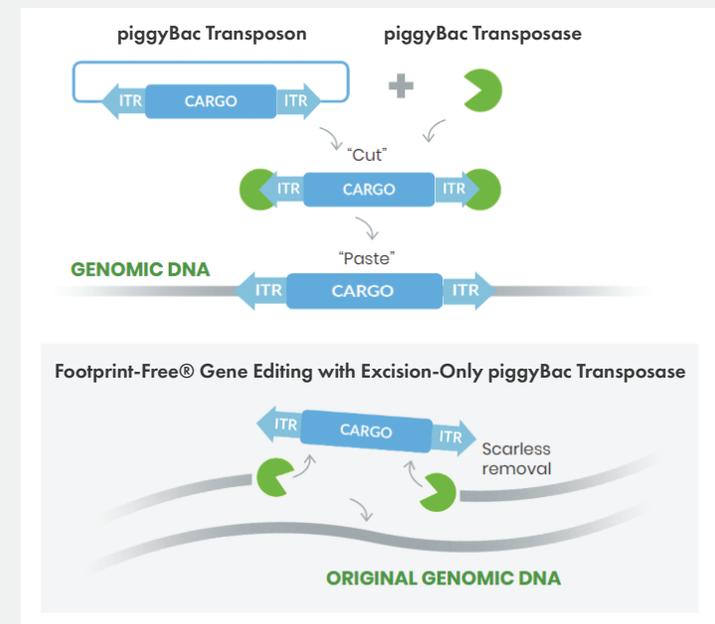


piggyBac: A Simple, Versatile & Efficient Tool For Yeast Strain Development

piggyBac is a transposase + transposon system for the introduction of small to large genetic cargo and removal of genetic cargo in a scarless manner in transgenic and stable yeast lines. A proven technology, piggyBac has been published over 750 times. piggyBac has demonstrated effective gene integration & expression capabilities across species, including those with less robust genetic tools.

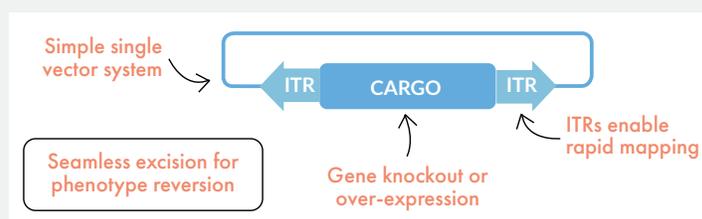


As opposed to other gene editing tools, one piggyBac vector can rapidly develop mutant libraries for loss (gene-trapping) and gain of function (enhancer-trapping) across species. With piggyBac, genes are easily mapped, and phenotype reversion is seamless.

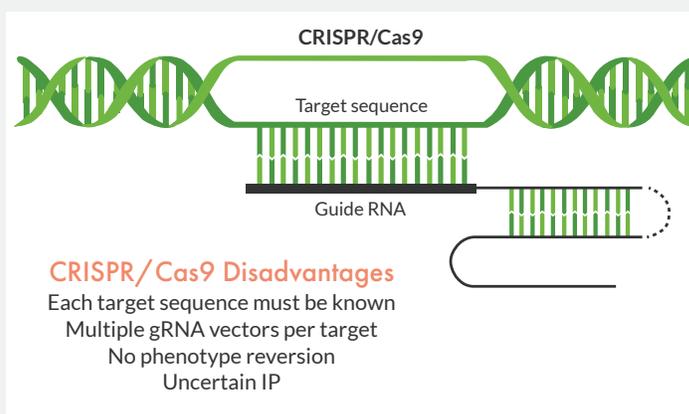


How it works

- Step 1:** Clone cargo into transposon vector.
- Step 2:** Co-transfect transposon and transposase into cells.
- Step 3:** Transposase cuts out cargo and pastes into random TTAA sites.
- Step 4:** Screen clones for desired insert.
- Step 5 (optional):** Use excision-only piggyBac for footprint-free gene editing



The piggyBac system features high efficiency, expression and stability. It does not have many of the disadvantages of the CRISPR system. With small to large gene integration (200Kb+), you can engineer any metabolic pathway. The piggyBac system also includes scarless removal for phenotype reversion.



Uses for piggyBac

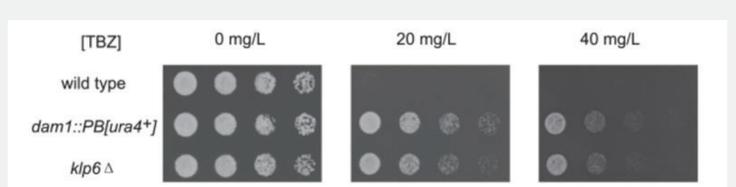
- Robust genetic tools for trait development
- Metabolic engineering
- Biotherapeutic & industrial protein production
- Mutagenesis & phenotype reversion
- Pathogen drug discovery & resistance studies

Validated in Yeast

piggyBac is potentially active in all yeast strains, including difficult to engineer yeasts. Another key advantage is that the IP is issued and freedom to operate is clear. Below are some published examples of piggyBac uses in yeast strain development and industrial biotechnology.

S. pombe mutagenesis

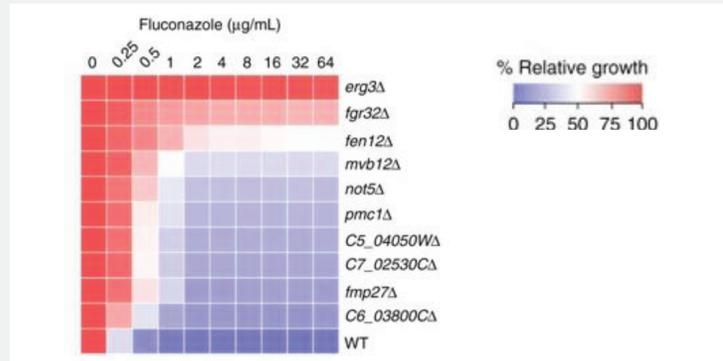
Although *S. pombe* is a powerful genetic system, chemical mutagenesis requires extensive mapping and CRISPR requires design and testing of individual guide RNA's for each target. piggyBac on the other hand integrates efficiently with rapid genetic mapping to the ITRs (1).



TBZ drug resistant strains identified by piggyBac mutagenesis

Candida albicans: a human pathogenic yeast

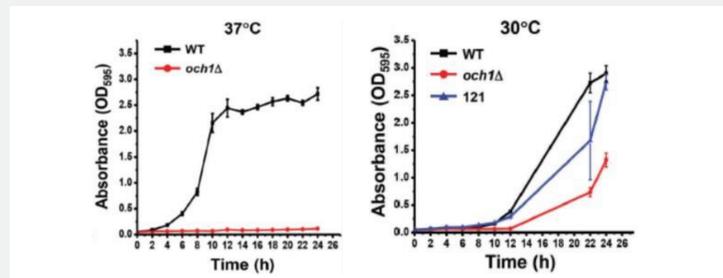
C. albicans kills hundreds of thousands worldwide annually. While rapid emergence of multidrug-resistant strains increases, the mechanisms are not well understood. piggyBac genome-wide mutagenesis screens identified candidate genes for antifungal drug fluconazole resistance (2).



Confirmation of increased resistance to fluconazole in mutants

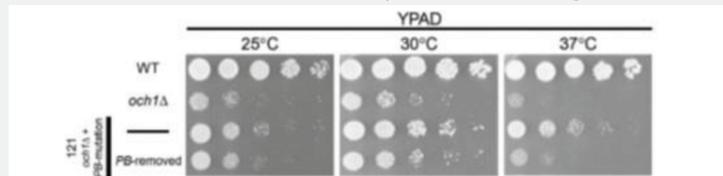
Glyco-engineering of yeasts for biotherapeutics

Biotherapeutics are produced in mammalian cells with high yields of correctly folded proteins. Immunogenicity is a factor when yeasts are used due to different N-glycan structures produced. Disruption of och1 results in proper N-glycan profile in yeast, however, the mutation causes growth defects. piggyBac transposition was used to discover BEM4 overexpression (enhancer-trapping) which rescued the growth defects of an och1 deficient strain (3).



Slow growth in och1

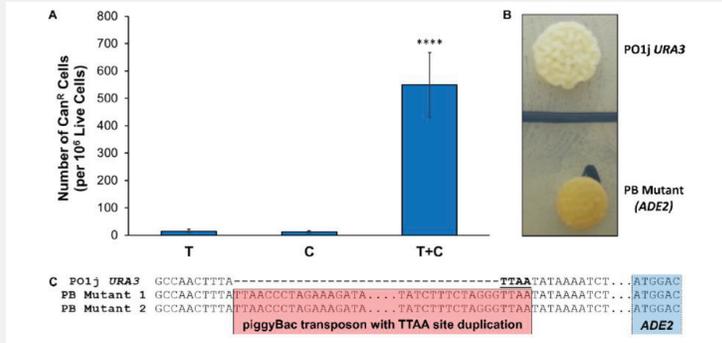
PB insertion into bem4 promoter restores growth



PB phenotype reversion confirms results

Yarrowia lipolytica screening

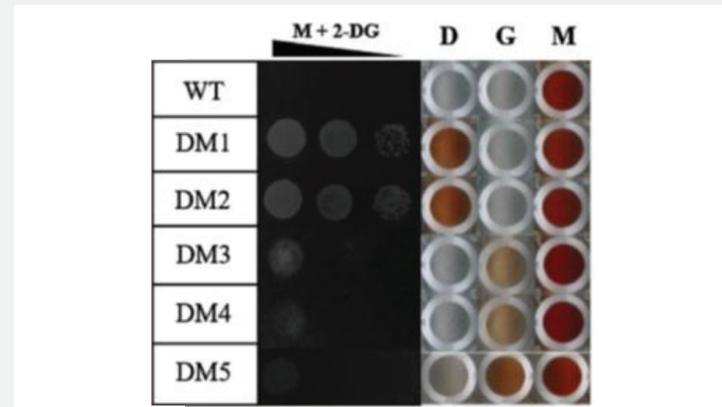
Y. lipolytica lacks robust genetic tools but is attractive to produce FDA GRAS designated molecules. CRISPR mutant libraries are cost prohibitive. In contrast, piggyBac is applied rapidly to genome-wide insertional mutagenesis (4).



(A) piggyBac (T+C) significantly increases colonies compared to cargo only (C) or hyPBase only (T) controls. (B) Visible brown colony phenotype, without adenine auxotrophy on SD-Ade plates. (C) Rapid identification of ADE2 piggyBac insertions

Komagataella phaffii heterologous protein expression

Pichia pastoris (*P. pastoris*) is the most widely used yeast system for heterologous protein expression. The function of 25% *K. phaffii* genes are still unknown and this important strain lacks powerful genetic tools (5).



piggyBac efficiently integrates into the genome and was used to select for growth rate mutants. Methanol plates containing 2-DG, and Aox activities (shown by colorimetric assay) of several mutants. The deeper color suggests higher Aox activity.

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Demeetra holds the exclusive licenses for this simple and powerful tool in synthetic biology, and offers accessible and flexible licensing terms for research and commercial applications. Unlike CRISPR, piggyBac IP is issued and straightforward. Contact us to learn more about how piggyBac can help your research at:

visit.demeetra.com/piggybac-for-industrial-biotechnology

2. Gao et al. Nature Communications (2018) Candida albicans gains azole resistance by altering sphingolipid composition.
 3. Mutumwinka et al. (2018) PiggyBac-based screening identified BEM4 as a suppressor to rescue growth defects in och1- disrupted yeast cells
 4. Wagner et al. Biotech J. (2018) Developing a piggyBac transposon system and compatible selection markers for insertional mutagenesis and genome engineering in Yarrowia lipolytica
 5. Zhu et al. (2018) PiggyBac transposon-mediated mutagenesis and application in yeast Komagataella phaffii