Supporting Information

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SI Text

Plasmids and Strains for *piggyBat* Transposition in *Saccharomyces cerevisiae*. BY4711 is *MATa trp1Δ63* and BY4727 is *MATa his3Δ200 leu2Δ0 lys2Δ0 met15Δ0 trp1Δ63 ura3Δ0* (1).

To make the yeast *piggyBat* helper plasmid, the *piggyBat* transposase ORF + C-terminal MYC-His6 tag was PCR amplified from *piggyBat* ORF in pBAD-MYC-His6 (Invitrogen) with a primer from the 5′ end of the gene (5′-GCAT*CCCGGG*-ATGGCGCAACACTCAGATTAC; the XmaI site is in italics, the start codon is underlined) and the 3′ end of the gene with a primer containing an XhoI site (5′-GCAT*CTCGAG*CTCA-ATGATGATGATGATGATGG; the XhoI site is in italics, the stop codon is underlined). The PCR product was digested with XmaI and XhoI and cloned between the XmaI and XhoI sites downstream of the *GALS* promoter in p414*GALS* (2). The plasmid also carries *TRP1*.

To generate the *piggyBat* donor plasmid used in Ura$^-$ to Ura$^+$ excision assays, a G418 resistance cassette fragment (3) was PCR amplified using SpeI-containing primers from both ends of cassette (5′-GATC*ACTAGT*CGTACGCTGCAGGTCGACGG and 5′-GATC*ACTAGT*TTCGACACTGGATGGCGGCG); the SpeI sites are in italics, digested with SpeI, and cloned into the SpeI site of plasmid-*piggyBat* terminal inverted repeat plasmid from DNA2.0. The plasmid was digested with XhoI, and the XhoI-bounded *piggyBat*-G418^R fragment was cloned into the XhoI site in the actin intron of the *URA3*::actin intron (4) in pRX1 (5) such that the transposon is flanked by XhoI (bold) and *piggyBac*'s characteristic insertion site TTAA: **CTCGAG**TTAA-*piggyBatL*$_{end}$-G418-*piggyBatR*$_{end}$-TTAA**CTCGAG.** The plasmid also carries *HIS3*.

To make the donor plasmid used in the *piggyBat* integration assays, the XhoI-TTAA-*piggyBat*-G418^R-TTAA-XhoI fragment described above was cloned into the single XhoI site of pRS416. The plasmid backbone also contains *URA3.*

To generate the chromosomal *URA3*::actin intron::*piggyBat*-G418^R, the *URA3*::actin intron::*piggyBat*-G418^R segment was PCR amplified from the *URA3*::actin intron::*piggyBat*-G418^R donor plasmid using the *URA3* forward 5′-ATGTCGAAAGCTACA-TATAAGGAACGTGCT and *URA3* reverse 5′-GCTGGCCGC-ATCTTCTCAAATATGCTTCCCAG primers and was introduced into the *URA3* gene of BY4711 by homologous recombination, selecting for G418R resistance (200 μg/mL) after transformation of the DNA fragment.

piggyBat Excision in *Saccharomyces cerevisiae*. To assay excision in BY4727 containing pRX1-*URA3*::actin intron::*piggyBat*-G418^R and pRS414-*GALS piggyBat* transposase, colonies were streaked onto synthetic complete (SC) media lacking tryptophan and histidine (SC-Trp-His) plates containing 2% galactose for transposase induction and incubated for 5 d at 30 °C. Single colonies were then resuspended in water, serially diluted, and plated on SC-Ura to determine the number of cells in which excision occurred and on SC to determine the total number of cells. The frequency of excision is the ratio of the number of colonies on SC-Ura plates to the total number of cells on SC plates.

Integration from a Plasmid Donor. For the integration assay in which we measured the chromosomal acquisition of a *piggyBat* encoding G418 resistance after exclusion of the donor plasmid with 5-fluoroorotic acid (5-FOA), the *piggyBat* donor plasmid contained the same *piggyBat* transposon cassette in the XhoI site of pRS416 whose backbone contains *URA3,* and the transposase plasmid was the p*GALS* derivative described above. For the assay, cells were streaked on SC-Ura plates containing 2% galactose for transposase induction and incubated for 5 d at 30 °C. Single colonies were then resuspended in water, serially diluted, and plated on SC + 5-FOA (1 mg/mL) to determine the total number of plasmid-free cells and on SC + 5-FOA + G418 (200 μg/mL) to determine the number of integrants. The frequency of integration is the ratio of the number of colonies on SC + 5-FOA + G418 plates to the total number of cells on SC + 5-FOA plates.

Measuring the Frequency of Reintegration Following Excision. Two methods were used to measure the frequency of *piggyBat* reintegration after excision. In one method, the frequency of *piggyBat* reintegration was measured by determining what fraction of Ura$^+$ cells obtained from *piggyBat* excision at chromosomal *URA3*::actin intron:: *piggyBat*-G418^R following transposase expression was still G418^R. Cells containing the p*GALS* transposase plasmid were induced on SC-Trp + Gal plates for 5 d. Single colonies were plated on SC-Trp-Ura and SC-Trp-Ura + G418 plates. Reintegration frequency was calculated by the number of colonies on SC-Trp-Ura + G418 plates compared with the number of colonies on SC-Trp-Ura plates. The observed integration frequency was 2.5×10^{-4} G418^R cells/total cells compared with the excision frequency of 6×10^{-4} Ura$^+$ cells/total cells, i.e., 42% reintegration. In the other method, cells containing the chromosomal *URA3*::actin intron:: *piggyBat-G418*^{R} and p*GALS* transposase plasmid were induced by growth in SC-Trp+Gal liquid for 2 d and then plated on SC-Trp-Ura plates. Single colonies were picked and then tested for growth on yeast extract peptone-dextrose and yeast extract peptone-dextrose + G418 plates. Of 108 colonies tested, 40 were G418^R, giving a reintegration frequency of 37%.

Analysis of Repair at the Donor Site Following Excision. Excision products were isolated using several different methods and then sequenced. The actin intron portion of the *URA3*::actin intron site in 10 independent Ura$^+$ excisants was isolated by colony PCR using primers from the actin intron ActI_f 5′-GTTCTAGCG-CTTGCACCATCC and ActI_r 5′-GTGGTTATTACAGATCA-GTCAATATAG; 10 of 10 were XhoI-TTAA-XhoI. The *URA3* genes in 25 independent Ura$^+$ Can^R excisants were sequenced from the chromosomal *URA3*::actin intron::*piggyBat*-G418^R donor sites; 24 of 25 were XhoI-TTAA-XhoI, and 1 was XhoI. The *URA3* genes in 15 independent Can^R products isolated from the chromosomal *URA3*::actin intron::*piggyBat*-G418^R donor site were sequenced; 14 of 15 were XhoI-TTAA-XhoI, and 1 was XhoI.

Sequence Analysis of Yeast *piggyBat* Insertions. The target site duplications at 20 independent *piggyBac* insertions into *CAN1* were determined. Insertions were isolated in cells containing the chromosomal donor site *URA3*::actin intron::*piggyBat-G418* and the p414*GALS*/*piggyBat* helper plasmid. Single colonies were grown at 30 °C for 2 d in SC-Trp + Gal liquid medium for transposase induction. Insertions in *CAN1* were identified by plating on SC-Arg-Ura + Can plates. To capture insertions in *CAN1* in either orientation, colony PCR was performed with four primers sets: *CAN1* forward 5′-GACAAATTCAAAAGAAGACGCCG and *piggyBat* left 5′-CCGAGTTAAGACGGCTCCC; *CAN1* forward and *piggyBat* right 5′-GCGCGGGAAACCTAAATAATTGC; *CAN1* reverse 5′-GATGTCGACATCTCCAATCTTCC and *piggyBat* left, and *CAN1* reverse and *piggyBat* right. PCR products were gel purified with the QIAQuick gel extraction kit (Qiagen) and sequenced to determine the target sequences flanking the inserted transposon.

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Other Supporting Information Files

Table S1 (DOC)
Table S2 (DOCX)
Table S3 (DOC)
Table S4 (DOCX)
Dataset S1 (XLSX)