**Supplementary note: optimizing the barcoded yeast piggyBac system**

With the goal of maximizing transposition efficiency in barcoded yeast piggyBac, we optimized two facets of the system by making changes to the piggyBac vector (Figure S2A; all cloning by Genscript, Inc.) and assaying transposition as follows.

We reasoned that we could optimize transposition efficiency independent of barcode location by making codon and/or amino acid changes to the transposase coding region. For this purpose, in the un-barcoded, BbsI-ready piggyBac plasmid pCW328 (Figure S2A), we codon-optimized the transposase coding region for expression in *S. cerevisiae* (using a proprietary method from Genscript), yielding the test plasmid pJC10 (Figure S2A, top right). We then used the latter plasmid as a backbone for the introduction of amino acid changes to the transposon coding region (I30V, S130P, S165S, M282V,  S509G, N538K, N571S) which (Yusa et al. 2011) reported to result in a hyperactive piggyBacin mammalian cells, resulting in the test plasmid pJC11 (Figure S2A, center right). For these two plasmids, we assessed transposition efficiency with test transformations as in Methods, but at one tenth the scale, using only one tenth of the cell pellet of each 50 mL log-phase hybrid culture at OD 0.9. Approximately 100 individual 5FOA+ colonies were patched onto YPD+ G418 300ug/ml plates and incubated overnight at 28oC. Transposition efficiency was calculated as the proportion of G418 patches that grew on 5FOA media out of the total patches on the G418 plate. Results revealed no evidence for improvement in transposition by either manipulation (Figure S2B).

Separately, to explore the potential position of barcodes in piggyBac, we inserted a test barcode in the transposon of the un-barcoded, BbsI-ready piggyBac plasmid pCW328 (Figure S2A) in each of two locations. In one scheme, a 64-nucleotide segment, containing a single barcode flanked by universal priming regions and custom two-nucleotide overhang sequences, was inserted between the 3’ end of the left arm of the transposon and the 5’ end of the TEF promoter of the kanamycin resistance cassette, nucleotides 353-354 of the pCW328 vector, resulting in pJC4 (Figure S2A, bottom left). This position of insertion was chosen to avoid the two internal binding sites in the left arm of piggyBac (Morellet et al. 2018). In a second scheme, the 64-nucleotide segment replaced 64 endogenous nucleotides inside the end of the right arm of the transposon (nucleotides 1984-2047 of the pCW328 vector), resulting in pJC9 (Figure S2A, bottom right). The latter was chosen to avoid compromising the region which (Morellet et al. 2018) suggest functions as a C-terminal DNA-binding domain in thetransposon based on DNAse I footprinting data, and which has previously been shown to constitute a minimal transposable element (Mitra et al. 2008; Meir et al. 2011; Solodushko et al. 2014). Transposition assays results revealed that the piggyBac bearing the replacement in the right arm of the transposon, pJC9, performed best (Figure S2B), and we used this scheme for the final barcoding of piggyBac for RH-seq at production scale as detailed in Methods.