## Genome-Wide Determination of Gene Essentiality by Transposon Insertion Sequencing in Yeast *Pichia pastoris*

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Figure S1



**Figure S1. High-efficiency thermal asymmetric interlaced PCR (hiTAIL-PCR) analysis of His+ transformants.** (A) Amplification of TcBTIRsHis flanking sequences from host genome by hiTAIL-PCR. (B) Amplification of SBTIRsHis flanking sequences from host genome by hiTAIL-PCR.





**Figure S2.** Flow cytometric analysis of the ratio of GS115 and GS115-*GFP-HIS4* cells at different time points.



Figure S3. (A) Distributions of TcB (top panel) and SB (bottom panel) insertions in intergenic regions, exons and introns of *P. pastoris*. (B) Saturation analysis of our Tn-seq data sets. The blue bars show the saturation level of the individual subsets. Cumulative saturation levels of insertions are represented by the red line.





**Figure S4.** (A) The overall correlation between insertions per gene and gene length. (B) The overall correlation between insertions per gene and TA number.

## Figure S5



**Figure S5.** The transposon insertion map and domain positions of *PAS\_chr1-1\_0015* (A), *PAS\_chr1-1\_0035* (B) and *PAS\_chr1-3\_0239* (C). Motifs were predicted by SSDB motif search tool on KEGG website.

Figure S6



Figure S6. The training accuracy of insertion subsets with different saturation.