Supplemental Data

Supplemental Table S1. Complete list of plasmids used in this study

Plasmids	Description	Reference
pKN3050	Ty3-*HIS3*-ppt (galactose inducible Ty3 tagged downstream of *POL3* with a *HIS3* gene flanked by 60 bp unique sequences and followed by an extra copy of ppt and LTR sequences present in the POL3 open reading frame)	This study
pKN3097	Ty3-ppt (as above but lacking the *HIS3* insertion and one of two unique 60 bp tag sequences)	This study
pXP622	Shuttle vector, Amp^R, 2µ, loxP-*LEU2*-loxP, used to construct plasmid-borne targets	(Fang, Salmon et al. 2010)
pFA6a-3HA-KanMX6	Template for 3HA tag.	(Bähler, Wu et al. 1998)
pKN3481	pXP622 with loxP-*LEU2*-loxP replaced by loxP-*HIS3*-loxP	This study
pCR2.1	TA-cloning vector	Invitrogen, Inc. (Carlsbad, CA)
pXQ3617	pCR2.1, Ty3 U5 insertion at *tK(CUU)F*; qPCR standard for chromosomal integration at *tK(CUU)F*	This study
pXQ3618	pCR2.1, Ty3 U5 insertion at *tG(GCC)G2*; qPCR standard	This study
pXQ3619	pCR2.1, Ty3 U5 insertion at *tA(UGC)E*; qPCR standard	This study
pXQ3620	pCR2.1, Ty3 U5 insertion at *tT(UGU)G1*; qPCR standard	This study
pXQ3621	pCR2.1, Ty3 U5 insertion at *tG(CCC)D*; qPCR standard	This study
pXQ3622	pCR2.1, Ty3 U5 insertion at *tG(GCC)F2*; qPCR standard	This study
pXQ3623	pCR2.1, Ty3 U5 insertion at *tV(AAC)G1*; qPCR standard	This study
pXQ3624	pCR2.1, Ty3 U5 insertion at *tD(GUC)K*; qPCR standard	This study
pXQ3625	pCR2.1, Ty3 U5 insertion at *tQ(UUG)D1*; qPCR standard	This study
pXQ3626	pCR2.1, Ty3 U5 insertion at *tV(AAC)G3*; qPCR standard	This study
pXQ3627	pCR2.1, mimicked Ty3 U5 insertion at *ETC1*, amplified with primers XQ3398 and XQ3397; qPCR standard	This study
pXQ3628	pCR2.1, Ty3 U3 insertion at *tK(CUU)F*; qPCR standard	This study
pXQ3629	pCR2.1, Ty3 U3 insertion at *tG(GCC)G2*; qPCR standard	This study
pXQ3630	pCR2.1, Ty3 U3 insertion at *tA(UGC)E*; qPCR standard	This study
pXQ3631	pCR2.1, Ty3 U3 insertion at *tT(UGU)G1*; qPCR standard	This study
pXQ3632	pCR2.1, Ty3 U3 insertion at *tG(CCC)D*; qPCR standard	This study
pXQ3633	pCR2.1, Ty3 U3 insertion at *tG(GCC)F2*; qPCR standard	This study
pXQ3634	pCR2.1, Ty3 U3 insertion at *tV(AAC)G1*; qPCR standard	This study
pXQ3635	pCR2.1, Ty3 U3 insertion at *tD(GUC)K*; qPCR standard	This study
pXQ3636	pCR2.1, Ty3 U3 insertion at *tQ(UUG)D1*; qPCR standard	This study
pXQ3637	pCR2.1, Ty3 U3 insertion at *tV(AAC)G3*; qPCR standard	This study
pXQ3638	pCR2.1, mimicked Ty3 U3 insertion at *ETC1*, amplified with primers XQ3399 and XQ3397, and subsequently with primers XQ3418 and XQ3397; qPCR standard	This study

Plasmids	**Description**	**Reference**
pKN3525	pXP622, *tK(CUU)F* with 120 bp flanking sequence, amplified with primers KN2727 and KN2728; target	This study
pKN3540	pXP622, *tG(GCC)G2* with 120 bp flanking sequence, amplified with primers KN3102 and KN3122; target	This study
pJY3611	pXP622, *tA(UGC)E* with 120 bp flanking sequence, amplified with primers JY3400 and JY3401; target	This study
pJY3613	pXP622, *tT(UGU)G1* with 120 bp flanking sequence, amplified with primers JY3402 and JY3403; target	This study
pKN3471	pXP622, *tG(CCC)D* with 120 bp flanking sequence, amplified with primers KN2266 and KN2267; target	This study
pXQ3662	pXP622, *tG(GCC)F2* with 120 bp flanking sequence, amplified with primers JY3402 and JY3412; target	This study
pJY3615	pXP622, *tV(AAC)G1* with 120 bp flanking sequence, amplified with primers JY3406 and JY3407; target	This study
pJY3612	pXP622, *tD(GUC)K* with 120 bp flanking sequence, amplified with primers JY3408 and JY3409; target	This study
pKN3526	pXP622, *tQ(UUG)D1* with 120 bp flanking sequence, amplified with primers KN2731 and KN2732; target	This study
pJY3616	pXP622, *tV(AAC)G3* with 120 bp flanking sequence, amplified with primers JY3410 and JY3411; target	This study
pXQ3674	pXP622, 330 bp fragment containing *ETC1*, amplified with primers XQ3507 and XQ3508; target	This study
pXQ3675	pCR2.1, Ty3 U5 insertion on *tG(CCC)D* target plasmids; qPCR standard for integration on target plasmids using primers XQ2603 and XQ3313	This study
pXQ3639	pCR2.1, Ty3 U3 insertion on *tK(CUU)F* target plasmids; qPCR standard for integration on target plasmids using primers XQ3135 and XQ3582	This study
pXQ3689	pCR2.1, Ty3 U5 insertion at *tF(GAA)H1*; qPCR standard for chromosomal integration using primers XQ2603 and XQ3621	This study
pKD3400	pXP622, i*YIL100W*-flanking fragment (290 bp to the left and 312 bp to the right) amplified with primers KD3089 and KD3090	This study
pKD3401	pXP622, i*CLD1*-flanking fragment (236 bp to the left and 95 bp to the right) amplified with primers KD3103 and KD3104	This study
pKD3402	pXP622, i*SCS3*-flanking fragment (191 bp to the left and 213 bp to the right) amplified with primers KD3087 and KD3808	This study
pXQ3406	pXP622, i*RHB1*-flanking fragment (278 bp to the left and 278 bp to the right) amplified with primers XQ3291 and XQ3292	This study
pXQ3670	pXP622, i*YJL206*-flanking fragment (253 bp to the left and 264 bp to the right) amplified with primers XQ3482 and XQ3483	This study
pJY3640	pKD3402, i*SCS3* fragment truncation, left half (191 bp)	This study
pJY3641	pKD3402, i*SCS3* fragment truncation, right half (213 bp)	This study
pXQ3671	pKD3402, i*SCS3* fragment mutation, left-side Box B	This study
pXQ3672	pKD3402, *iSCS3* fragment mutation, right-side Box B	This study
pXQ3676	pKD3400, i*YIL100W* fragment Box B mutation	This study

Supplemental Table S2. Oligonucleotides

Oligo	Sequence (5' - 3')	Description
KN1606	CGGTTCAAGGGTGAGAGTATGTGTCGAATC ATCGCCTAGACCGAAATGCC	Unique tag flanked by *Sac*I/*Afl*II introduced in pKN3050
KN1607	TTAAGGCATTTCGGTCTAGGCGATGATTCG ACACATACTCTCACCCTTGAACCGAGCT	Unique tag flanked by *Sac*I/*Afl*II introduced in pKN3050. Rev
KN1608	GGTCCTAGTCGCACGCTTGTAGTGGATCGT CTGCATAATATCGTGTGAC	Unique tag flanked by *Sbf*I/*Xma*I introduced in pKN3050. For
KN1609	CCGGGTCACACGATATTATGCAGACGATCC ACTACAAGCGTGCGACTAGGACCTGCA	Unique tag flanked by *Sbf*I/*Xma*I introduced in pKN3050. Rev
KN1939	CTTCCCGGGTGAGAGAGAGGAAGATGTTGT ATCTCAAAATGAGATATGTCAGTATGAC	Introduction of intact 3' LTR into pKN3050. For
KN1940	GTAGCGGCCGCCTGTTGTATTACGGGCTCG AGTAATACCG	Introduction of intact 3' LTR into pKN3050. Rev
KN2037	CTCCTAGTCGCACGCTTGTAGTGGATCGTCT GCATAATATCGTGTGAC	Unique tag flanked by *Sac*I/*Xma*I introduced in pKN3097. For
KN2038	CCGGGTCACACGATATTATGCAGACGATCC ACTACAAGCGTGCGACTAGGAGAGCT	Unique tag flanked by *Sac*I/*Xma*I introduced in pKN3097. Rev
XQ3627	CAGCTCAGTGTGCAAGTTGTT	*tM(CAU)J3* For
XQ3628	TGAGGCATACTGATCGAGAATTT	*tM(CAU)J3* Rev
XQ3629	TTGGAAGGCCATGATGTGTA	*tP(UGG)O1* For
XQ3630	TGCGTACACATGCTACATCG	*tP(UGG)O1* Rev
XQ3631	CATCTTGGAAGGACCGGATA	*tY(GUA)J2* For
XQ3632	TTGCAAGTCTGGGAAGTGAA	*tY(GUA)J2* Rev
XQ2603	TCGAGCCCGTAATACAACA	U5 insertion qPCR forward primer, also used in standard construction
XQ3135	GGGTGACGTATTGTCATACTG	U3 insertion qPCR reverse primer, also used in standard construction
XQ3376	GACATATCTCATTTTGAGATACAACA	U3 insertion forward primer, used in standard construction
XQ3418	GGGTGACGTATTGTCATACTGACATATCTC ATTTTGAGATACAACA	Extended U3 insertion forward primer, following XQ3376
XQ3377	CCTAGTTTTGCTGTATAAGAGCCGAG	*tK(CUU)F* integration qPCR Rev
XQ3378	GAGAGGTGATTTTCTAGAGCATGTC	*tG(GCC)G2* integration qPCR Rev
XQ3379	CTGTTTTCCTGTGGGGTGAG	*tA(UGC)E* integration qPCR Rev
XQ3380	GGCGCGATAATTCTATACCG	*tT(UGU)G1* integration qPCR Rev
XQ3381	GGCGTAGCGCTCCTGATCTTGTG	*tG(CCC)D* integration qPCR Rev
XQ3382	CTTGCTTATATGCGAAGTGACTACG	*tG(GCC)F2* integration qPCR Rev
XQ3383	TCGAGCCCGTAATACAACAAATAAGCGCAA GTGGTTTAGTGGT	U5 LTR-*tG(GCC)F2* forward primer for standard construction

Oligo	Sequence (5’ - 3')	Description
XQ3384	GACATATCTCATTTTGAGATACAACAAATA AGCGCAAGTGGTTTAGTGGT	U3 LTR-*tG(GCC)F2* forward primer for standard construction
XQ3385	GAACGGAAAGTAGGACAACTACC	*tV(AAC)G1* integration qPCR Rev
XQ3386	TCGAGCCCGTAATACAACACGAAACATCAA AAAACTGGTTTCGTGGTC	U5 LTR-*tV(AAC)G1* forward primer for standard construction
XQ3387	GACATATCTCATTTTGAGATACAACACGAA ACATCAAAAAACTGGTTTCGTGGTC	U3 LTR-*tV(AAC)G1* forward primer for standard construction
XQ3388	ACGCTTGGGAATTCCCTTCACG	*tD(GUC)K* integration qPCR Rev
XQ3389	TCGAGCCCGTAATACAACACAACAAATAAC GTTCCGTGATAGTTTAATGG	U5 LTR-*tD(GUC)K* forward primer for standard construction
XQ3390	GACATATCTCATTTTGAGATACAACACAAC AAATAACGTTCCGTGATAGTTTAATGG	U3 LTR-*tD(GUC)K* forward primer for standard construction
XQ3391	GCGAGGGGCTGTGCAATTCAAAGC	*tQ(UUG)D1* integration qPCR Rev
XQ3392	TCGAGCCCGTAATACAACACCAATCGTCCA TTTTAGGGTCCTATAG	U5 LTR-*tQ(UUG)D1* forward primer for standard construction
XQ3393	GACATATCTCATTTTGAGATACAACACCAA TCGTCCATTTTAGGGTCCTATAG	U3 LTR-*tQ(UUG)D1* forward primer for standard construction
XQ3394	TGCGCTTGCGCAGAGTACGTTC	*tV(AAC)G3* integration qPCR Rev
XQ3395	TCGAGCCCGTAATACAACAATTTCAATTAA GTGGTTTCGTGGTCTAG	U5 LTR-*tV(AAC)G3* forward primer for standard construction
XQ3396	GACATATCTCATTTTGAGATACAACAATTTC AATTAAGTGGTTTCGTGGTCTAG	U3 LTR-*tV(AAC)G3* forward primer for standard construction
XQ3397	CTCGCTCTAAAATGGTAGTTCTAGTG	*ETC1* integration qPCR Rev
XQ3398	TCGAGCCCGTAATACAACACATCAACCTTG AGGCTAGGAG	U5 LTR-*ETC1* forward primer for standard construction
XQ3399	GACATATCTCATTTTGAGATACAACACATC AACCTTGAGGCTAGGAG	U3 LTR-*ETC1* forward primer for standard construction
JY3435	GTGCCACCTGACGTCTAAGAA	pXP622 backbone For
JY3436	GCTCTGATGCCGCATAGTTA	pXP622 backbone Rev
XQ3133	GGG GAT CCT CTA GAG TCG ACC	Target plasmids integration qPCR reverse primer, pair with XQ2603
XQ3582	GTCGACCTGCAGGCATGC	Target plasmids integration qPCR reverse primer, pair with XQ3135
KN2406	ATTAATTACGACGCCATTGACGGTTTGTTTA GGACTAGTTACCCATACGATGTTCCTGAC	flanking sequence upstream of stop codon of BRF1 and 5' end of 3HA
KN2407	GCGTGACATAACTAATTACATGACTCGAGG ATTCATTAAGCAGCGTAATCTGGAACGTC	3'end of 3HA, stop codon and 5' end of CYC1term
KN2408	GACGTTCCAGATTACGCTGCTTAATGAATC CTCGAGTCATGTAATTAGTTATGTCACGC	3'end of CYC1term and loxP-*HIS3*-loxP
KN2409	TTTCCTTCCTAGGGTTGATTACCTAAACGTT AGAATTCGAGCTCGGTACCCGGG	downstream region of loxP-*HIS3*-loxP into the template backbone and flanking sequence downstream of the stop codon of BRF1
KN2410	GATAGTGTCAAGAACATGTTGCAGAAGGCA AGTTTCTCCAAGAAGATTAATTACGACGCC	flanking sequence upstream of stop codon of BRF1 and 5' end of 3HA and overlapping sequence to the 5' end of PCR Fragment 1
KN2411	AGCCAAAACCAAATGTATTGCGTCTTTATTT	overlapping sequence to the 3' end of

Oligo	Sequence (5’ - 3')	Description
	CCGTTCCCTTTTTCCTTCCTAGGGTTGAT	PCR Fragment 1 and flanking sequence downstream of the stop codon of BRF1
KN2727	GGACATATGTATTCCTCTACTTTTGTGCAATGTGAAAGGACAAAGAGACAGG	PCR forward primer, 120 bp upstream of *tK(CUU)F*
KN2728	TGGAAGCTTATCTTTTTTATATGAACCTAGTTTTGCTGTATAAGAGCCGAGA	PCR *tK(CUU)F* coding sequence and 120 bp downstream sequence
KN3102	CCTCATATGGATTTAGAGTGCATAAATTAATTCCCTTCTAATGGGTACTCTTGC	PCR forward primer, 120 bp upstream of *tG(GCC)G2*
KN3122	GGGAAGCTTTTAAGAGGCTTTATAGATGTAAAAATGAACAATAAGAATAATTTAAATTTG	PCR reverse primer *tG(GCC)G2* coding sequence and 120 bp downstream sequence
JY3400	GGACATATGTAGGCATACTCAATCTTACCTTTT	PCR forward primer, 120 bp upstream of *tA(UGC)E*
JY3401	TGGAAGCTTAATAGTCATTTAGCAGCCGTTC	*tA(UGC)E* 120 flanking reverse
JY3402	GGACATATGCACTTTTCTTTAGAAAATTTGCAATTATAG	PCR forward primer, 120 bp upstream of *tT(UGU)G1*
JY3403	TGGAAGCTTACCTAAATCTTTACTCGGCCCTG	*tT(UGU)G1* 120 flanking reverse
KN2266	GGACATATGTAGTGAGCTTCAGTCTGTTTTTAGATGTAGTTAGCAATTACTATTTTAGG	PCR forward primer, 120 bp upstream of *tG(CCC)D*
KN2267	CAAGCTTTCTGTTGAAGTACGCAATTAGTGGATTGCTCATCCAAATATCTT	PCR reverse primer *tG(CCC)D* coding sequence and 120 bp downstream sequence
JY3404	GGACATATGCTTATTGAGAAAGATAAGAATACAGAGACA	PCR forward primer, 120 bp upstream of *tG(GCC)F2*
JY3412	CCCTGCAGGCATGCAAGCTTCTACGAAATATTAAGGGAAAATATACGA	*tG(GCC)F2* 120flanking reverse
JY3406	GGACATATGGTCTATAGATGGTGGTCGATTCTT	PCR forward primer, 120 bp upstream of *tV(AAC)G1*
JY3407	TGGAAGCTTAAAAGACGAAAAGAACGGAAAG	*tV(AAC)G1* 120flanking reverse
JY3408	GGACATATGGTTGATTCTTATTTAGACACAATAAA	PCR forward primer, 120 bp upstream of *tD(GUC)K*
JY3409	TGGAAGCTTAAAATATATGCATCGTTGCAC	*tD(GUC)K* 120flanking reverse
KN2731	GGACATATGTTTCTGTAAAGAAGAGTAAACCATAACCTAAGTCAATATCAAATTTAGG	PCR forward primer, 120 bp upstream of *tQ(UUG)D1*
KN2732	TGGAAGCTTAGAAATTGCCCTTATTTCTTCGCCTTTTTCCTTTTTTCAG	PCR *tQ(UUG)D1* coding sequence and 120 bp downstream sequence
JY3410	GGACATATGGTGCGCAGTAATGGCTGGTA	PCR forward primer, 120 bp upstream of *tV(AAC)G3*
JY3411	TGGAAGCTTAAAATTGGAAGCAACAATAGTATATATATATATGC	*tV(AAC)G3* 120flanking reverse
XQ3507	GTCATATGATACGACAATTCTGGCCATAGC	PCR forward primer, *ETC1* fragment
XQ3508	GTAAGCTTGAGGAACCTTCTGCTCGCTC	PCR reverse primer, *ETC1* fragment
KN2803	CCGGAATAATCAAAAAAGATTTAGAGTGCATAAATTAATTCCCTTCTAATGGGTACTCTT	*tG(GCC)G2*, ChIP-qPCR, Forward
KN2804	TTTAAATTTGTTTTATTGAGAGGTGATTTTCTAGAGCATGTCAAACTTTACTATTCAGGG	*tG(GCC)G2*, ChIP-qPCR, Reverse

Oligo	Sequence (5’ - 3')	Description
KN2560	TAGTGAGCTTCAGTCTGTTTTTAGATGTAGT TAGCAATTACTATTTTAGG	*tG(CCC)D*, ChIP-qPCR, Forward
KN2039	TCTGTTGAAGTACGCAATTAGTGGATTGCTC ATCCAAATATCTTG	*tG(CCC)D*, ChIP-qPCR, Reverse
KN2805	AGAACTCGGATGTAAACTAACTAATAAGTG CTTATTGAGAAAGATAAGAATACAGAGACA	*tG(GCC)F2*, ChIP-qPCR, Forward
KN2806	CTTGCTTATATGCGAAGTGACTACGAAATA TTAAGGGAAAATATACGACAATTTCGTG	*tG(GCC)F2*, ChIP-qPCR, Reverse
KN2566	GCATGCCTTTTGTATTTCTGTAAAGAAGAGT AAACCATAACCTAAGTC	*tQ(UUG)D1*, ChIP-qPCR, Forward
KN2215	CTGAGAAACATTTAAATACAGTTGAAAACT GGGAGGGGCGAGAGGGG	*tQ(UUG)D1*, ChIP-qPCR, Reverse
KN2617	ATACGACAATTCTGGCCATAGCTTTTGGAA AGATTATTGA	*ETC1*, ChIP-qPCR, Forward
KN2618	GAGGAACCTTCTGCTCGCTCTAAAATGGTA GTTCTAGTGA	*ETC1*, ChIP-qPCR, Reverse
XQ3621	TGCGAATTCTGTGGATCG	*tF(GAA)* family integration qPCR, Reverse
KD3087	ATTCATATGGTCACCCGGACTAATCGCGCG TTGGCG	*iSCS3* fragment, Forward
KD3088	AGGAAGCTTCTGCCATTTCTGCGCGTACAA GTGAGCCA	*iSCS3* fragment, Rerverse
KD3089	CCGCATATGGTTGTGTGGGGAGAAGAACTG GATGCG	*iYIL100W* fragment, Forward
KD3090	CGGAAGCTTGCGCAGGAGGAAACAGGGAA CCAA	*iYIL100W* fragment, Reverse
KD3103	CGCCATATGCTTCCCTAGCTGAACTACCCA AAG	*iCLD1* fragment, Forward
KD3104	CCGAAGCTTAGATGCTCATCACAGCTACTC C	*iCLD1* fragment, Reverse
XQ3291	GTCATATGGGATTCAACGAACCGCGATTC	*iRHB1* fragment, Forward
XQ3292	GTAAGCTTCCTTGCGTTTCAGCTTCCTCT	*iRHB1* fragment, Reverse
XQ3482	GTCATATGTGACGATACCGTATTGTGTC	*iYJL206C* fragment, Forward
XQ3483	CCCTGCAGGCATGCAAGCTTAGCAATGTTC AGCAAACTTGAAGGC	*iYJL206C* fragment, Reverse
JY3421	GTAAGCTTAAGTATTGATGGCAGCGG	*iSCS3* cloned fragment left-half, Reverse primer, pair with KD3087
JY3422	GTCATATGTTTATTCATGACCAGATGCA	*iSCS3* cloned fragment right-half, Forward primer, pair with KD3088
XQ3488	AACTCTGGGACCGCTCCATAGGGTCCATAC AGGGCGCCCTGCAGGCGACG	*iSCS3* left side Box B mutation, For
XQ3489	CGTCGCCTGCAGGGCGCCCTGTATGGACCC TATGGAGCGGTCCCAGAGTT	*iSCS3* left side Box B mutation, Rev
XQ3490	GCCATTGGGCTGGAGGACACGACCCTTCGG GGCACGTGTTCTTGGCCACC	*iSCS3* right side Box B mutation, For
XQ3491	GGTGGCCAAGAACACGTGCCCCGAGGGGTC GTGTCCTCCAGCCCAATGGC	*iSCS3* right side Box B mutation, Rev
XQ3512	GCTGTTGTTCAATGGATGCGcTTtGAGGCCC AGCGCAAGGGGG	*iYIL100W* Box B mutation Forward

Oligo	Sequence (5’ - 3')	Description
XQ3513	CCCCCTTGCGCTGGGCCTCAAAGCGCATCC ATTGAACAACAGC	*iYIL100W* Box B mutation Reverse
XQ3563	CTTTCAACGTTCCAGCCTTCTACG	*ACT1*-qPCR, Forward
XQ3564	GTTCGAAGTCCAAGGCGACG	*ACT1*-qPCR, Reverse

Supplemental Table S3A and 3B (applies to each). Analysis of aligned Ty3-genome joint reads (all sequences are available on NCBI Gene Expression Omnibus; http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSExxx **) A. Uniquely aligning sequence reads. B. Multiply aligning sequence reads.**

Column definitions

clusterid - unique ID for each cluster, starting at 1.

clusterwidth - cluster width in bp determined by junction sites

pos - cluster reads on the W (top) strand

neg - cluster reads on the C (bottom) strand

avg - average between both experiments (pKN3050YMA1322 + pKN3050YMA1356) / 2)

Nearest upstream and downstream features (e.g. PolIII txd, ORF, LTR, ARS). The cluster's "orientation" is always on the top Watson strand in chromosomal coordinates. Distance to features to the left of the cluster in chromosomal coordinates are positive (upstream) on the Watson strand and negative (downstream) on the Crick strand. Features to the right of the cluster are the reverse. Numbers represent the coordinate difference from the nearest edge of the feature to the nearest edge of the cluster. Where distance is “0”, feature and cluster overlap.

Orientation of features - Positive is on W strand; negative is on C strand

Feature density (Pol III) - average number of features in a window of +/-7 kbp (14 kbp total) around the cluster

Feature upstream/downstream - Closest feature 5’/3’ of cluster

BoxA(BoxB).BBLS - Bayesian Branch Length Score of the closest Box A(Box B) sequence, found from the MotifMap pipeline

BoxA_BoxB_pairs.source_BBLS - Bayesian Branch Length Score of the closest Box A sequence that is within 100 bp of a Box B on the same strand, found from the MotifMap

pipeline. The BBLS score estimates the probability that a motif is conserved across seven yeast species (including S. cerevisiae) (Xie, Rigor et al. 2009).

BoxA_BoxB_pairs.target_BBLS - Bayesian Branch Length Score of the closest Box B sequence that is within 100 bp of a Box A on the same strand, found from the MotifMap pipeline.

NLOD scores- Normalized Log Odds Scores of the closest Box A/Box B sequences, found from the MotifMap pipeline. The NLOD estimates the degree of relatedness to the consensus motif (Xie, Rigor et al. 2009).

Supplemental Table S4. Ty3 insertion reads not associated with a known Pol III-transcribed gene.

Cluster ID #	Location	HTS fraction	Vicinity Gene (Coordinates)	Relative location	Cloned fragment	Plasmids	Plasmid integration	Possible mechanism
89	chrIII: 169,065	7.9E-04	*RHB1*	Downstream (1000 bp)	168,448-169,343	pXQ3406	No	Sigma distal artifact
98	chrVII: 271,599	3.6E-04	*SCS3* 271,003-272,145	Inside ORF	271,408-271,812	pKD3402	Yes	Box A/B
117	chrVII: 712,550	6.6E-05	*CLD1*	Upstream (1150 bp)	712,314-712,645	pKD3401	No	Sigma distal artifact
72	chrIX: 177,569	3.6E-05	*YIL100W* 177,370-177,723 *YIL100C-A* 177,340-177,678	Inside both ORFs	177,279-177,881	pKD3400	Yes	Box A/B
99	chrVII: 287,305	2.9E-05	*tW(CCA)G1*	Downstream (45 bp)	N/A	N/A	N/A	Unknown
49	chrX: 48,796	5.9E-06	*YJL206C* 47,659-49,935	Inside ORF	48,543-49,060	pXQ3670	No	Unknown
169	chrIV: 1,023,475	5.6E-06	*YDR282C*	Downstream (34 bp)	N/A	N/A	N/A	Sigma distal artifact
172	chrIV: 1,118,706	1.6E-06	*YCG1* 1,117,122-1,120,229	Inside ORF	N/A	N/A	N/A	Unknown

Supplemental Table S5. Integration sites analyzed by qPCR.

Loci	U5 qPCR standard		U3 qPCR standard		Targets on plasmids		ChIP-qPCR primers
	Name	Cloning primers	Name	Cloning primers	Name	Cloning primers	
tK(CUU)F	pXQ3617	*2603/3377*	pXQ3628	*3135/3377*	pKN3525	*2727/2728*	*2727/2728*
tG(GCC)G2	pXQ3618	*2603/3378*	pXQ3629	*3135/3378*	pKN3540	*3102/3122*	*2803/2804*
tA(UGC)E	pXQ3619	*2603/3379*	pXQ3630	*3135/3379*	pJY3611	*3400/3401*	*3400/3401*
tT(UGU)G1	pXQ3620	*2603/3380*	pXQ3631	*3135/3380*	pJY3613	*3402/3403*	*3402/3403*
tG(CCC)D	pXQ3621	*2603/3381*	pXQ3632	*3135/3381*	pKN3471	*2266/2267*	*2560/2039*
tG(GCC)F2	pXQ3622	*3383/3382*	pXQ3633	*3384/3382**	pXQ3662	*3404/3412*	*2805/2806*
tV(AAC)G1	pXQ3623	*3386/3385*	pXQ3634	*3387/3385**	pJY3615	*3406/3407*	*3406/3407*
tD(GUC)K	pXQ3624	*3389/3388*	pXQ3635	*3390/3388**	pJY3612	*3408/3409*	*3408/3409*
tQ(UUG)D1	pXQ3625	*3392/3391*	pXQ3636	*3393/3391**	pKN3526	*2731/2732*	*2566/2215*
tV(AAC)G3	pXQ3626	*3395/3394*	pXQ3637	*3396/3394**	pJY3616	*3410/3411*	*3410/3411*
ETC1	pXQ3627	*3398/3397*	pXQ3638	*3399/3397**	pXQ3676	*3507/3508*	*2617/2618*

*PCR products were extended through another round of PCR using forward primer XQ3418, such that they could be bound by primer XQ3135.

Supplemental Table S6. Clusters close to existing chromosomal Ty3 LTRs (sigma elements). As a result of an iPCR artifact, double clusters were found at some integration sites. In these cases, clusters densities were combined from two clusters originating from the same target site, which were then used to generate the scatter plot in Fig. 3.

Single clusters		**Double clusters**		
Cluster ID #	Pol III	Cluster A ID #	Cluster B ID #	Pol III
14	*tE(UUC)E2*	10	11	*tQ(UUG)E1*
52	*tA(AGC)J*	16	17	*tH(GUG)E2*
75	*tI(AAU)I2*	29	30	*tG(GCC)B*
104	*tE(UUC)G2*	78	79	*tD(GUC)I1*
105	*tR(UCU)G1*	101	102	*tH(GUG)G2*
116	*tC(GCA)G*	119	120	*tR(UCU)G3*
117	*tC(GCA)G*	136	137	*tS(AGA)A*
127	*tG(GCC)G1*	160	161	*tI(AAU)D*
139	*tV(AAC)H*	164	165	*tY(GUA)D*
143	*tA(AGC)H*	192	193	*tL(UAA)N*
147	*tT(UGU)H*	204	205	*tK(UUU)P*
167	*tG(GCC)D2*	212	213	*tI(AAU)P2*
169	*tE(CUC)D*	219	220	*tK(CUU)K*
174	*tM(CAU)D*	271	272	*tA(UGC)O*
181	*tN(GUU)N1*	285	286	*tA(AGC)L*
185	*tP(UGG)N1*			
194	*tW(CCA)P*			
210	*tA(AGC)P*			
256	*tG(GCC)O1*			
262	*tT(AGU)O2*			
276	*tS(AGA)L*			
279	*tR(ACG)L*			

Supplemental Methods

Plasmid constructions. Ty3-ppt-*HIS3* was expressed under control of the *GAL1-10* UAS from a *URA3*-marked low-copy plasmid (pKN3050). Ty3 containing antisense insertion of *HIS3* derived from pNB2361 (Beliakova-Bethell, Terry et al. 2009) was cloned into YCplac33 (ATCC) via unique SacII and NotI sites. A SacI site was introduced at the end of *POL3*. Two unique tags flanked by SacI-AflII and SbfI-XmaI sites were inserted at 3'- and 5'-ends, respectively of *HIS3*. Because Ty3 *POL3* extends into the downstream LTR, insertion of the *HIS3* gene at the downstream end of *POL3* disrupted a key region for replication containing both the ppt and the downstream LTR. Therefore, the plasmid was linearized by digestion with XmaI and NotI downstream of the tagged *HIS3* gene. An intact copy of the 3'LTR was amplified from pDLC201 (Hansen, Chalker et al. 1988) using primer oligonucleotides KN1939 and KN1940 and was also cleaved with XmaI and NotI. The LTR fragment was then ligated into the linearized Ty3 plasmid regenerating Ty3 containing *HIS3*, a second copy of the polypurine tract (ppt), and an intact LTR, in that order (Ty3-ppt-*HIS3*). This Ty3 expression plasmid was designated pKN3050. Plasmid pKN3050 was cleaved with AflII and SbfI and religated thereby removing one tag and the *HIS3* gene and creating pKN3097 (Table 1A). Positive clones were verified by DNA sequencing. This and other sequence analysis was performed at Genewiz Inc. (La Jolla, CA).

In order to test specific loci for target activity 300- to 600-bp DNA fragments containing the loci were amplified in PCR reactions using primers with *Nde*I or *Hin*dIII restriction sites at the outside ends (Sup. Inform. Table S1). PCR products were digested with *Nde*I/*Hin*dIII and ligated into pXP622 restricted with *Nde*I/*Hin*dIII. In the cloning of the iRHB1 fragment, an internal *Nde*I site was used. In the cloning of tG(GCC)F2 and iYJL206C fragments, *Pst*I was used in place of *Hin*dIII because of the presence of internal *Hin*dIII sites. All plasmids were confirmed by DNA sequencing.

In order to test the requirement for box B in targets *iYIL100W* and *iSCS3*s, site-directed mutagenesis of plasmid-borne loci using primers described in Supplemental Information. Table S2 was substitute key conserved positions. Mutations were confirmed with DNA sequencing.

Construction of qPCR standard plasmids. To construct the plasmids used as qPCR standards for measurement of integration at specific chromosomal loci, tDNAs with a segment of flanking Ty3 insertion were amplified and cloned into the pCR2.1 vector (Invitrogen Inc. Carlsbad, CA) . For loci exhibiting high-frequency integration, Ty3-ppt was induced to transpose and loci together with integrated Ty3 were amplified using PCR primed with primers annealing to Ty3 and target loci. At loci exhibiting low or no integration frequency, the Ty3 associated product was amplified in a one or two-step reaction in which the locus was amplified and Ty3 ends were included in the PCR primer. Plasmids and qPCR standards for experiments in which target loci were plasmid-borne were constructed using an approach similar to that used for chromosomal targets. Amplicons were cloned into pCR2.1 using TA cloning and constructs were confirmed with DNA sequencing. Details of the constructions are provided in Supplemental Methods. Oligonucleotide primers are listed in Table S2. Product plasmids are listed in Table S1.

Ty3 transposition. For HTS analysis of Ty3-*HIS3* insertion sites, YMA1322 and YMA1356 transformed with the Ty3-*HIS3* expression plasmid pKN3050 were grown to log phase in SR -Ura, -His at 24^{o}C (RT). From each culture, 2 x 10^{6} transformants were transferred to each of ten 100 mm plates of SG -Ura, -His medium or on SD-Ura, -His medium as negative control and maintained for 3 d to allow Ty3 expression. Subsequent steps were performed for both tested conditions. Cultures were replica-plated onto YPD, and incubated at 30^{o}C for 24 h. Colonies were replica-plated onto SD -His medium supplemented with 0.1% 5-fluoro-orotic acid (5FOA) (Boeke, LaCroute et al. 1984) to select for cells that had lost the URA3-marked donor plasmid, but contained Ty3-*HIS*3 chromosomal integrants. On average, there were approximately 10^{3} integrants per plate for Ty3 induced samples and 20 to 40 colonies for negative control samples. Approximately 10,000 colonies each for YMA1322 and YMA1356 cultures were scraped from plates. Harvested cells were inoculated into 10 ml SD -His and incubated at 30^{o}C on a rotating wheel for 24 h. Genomic DNA was extracted according to standard procedures (Ausubel, Brent et al. 2007).

For quantitative comparison of transposition into different chromosomal target loci, yMA1322 transformed with Ty3-ppt donor plasmid pKN3097 was grown to log phase in SR-Ura medium, diluted into 25 ml SG-Ura medium to final concentration of 0.2 OD_{600}, and grown with shaking at 180 rpm, at RT for 24 h. As negative control, cultures were diluted into 5 ml SD-Ura medium to 0.2 OD_{600} and allowed to grow for 24 h at RT. Quantitative comparison of transposition into plasmid-borne targets was performed similarly except that -Ura -Leu, medium was used to select for donor and target plasmids. Slower growth necessitated extending the SG -Ura -Leu incubation from 24 h to 36 h.

Construction of Brf1-3HA yeast strain: Brf1 was tagged with three copies of the hemagglutinin epitope (3HA) by introducing 3HA in frame at the downstream end of the respective ORF in YMA1322 by homologous recombination using a modified procedure of Longtine (Wach, Brachat et al. 1997; Longtine, McKenzie et al. 1998). The tagging fragment was produced by two-step nested PCRs: (1) 3HA from templates pFA6a-3HA-KanMX6 using primers KN2406FN and KN2407R containing 45-bp homology arm to the 3'end of Brf1 coding sequence and CYC1-terminator (*CYC1*-T), respectively, (2) *CYC1*-T-loxP-*HIS3*-loxP from pKN3481 (unpublished) using primers KN2408 and KN2409 containing homology arms to the 5'end of the endogenous terminator and the 3'end of loxP-*HIS3*-loxP, (3) Fusion of 3HA and *CYC1*-T fragments by PCR using primers KN2410-KN2411R. The final fusion PCR amplicons (500 ng to 1 μg DNA) were used to transform yMA1322 strain, and transformants conferred His+ phenotype were screened by PCR using Brf1-specific primers KN2485F and KN2411R and verified by sequencing with KN2485F. Western blot analysis was performed using α-3HA to verify positive candidates from sequencing results. The resulting yeast strain is YKN1692 (Table 1A).

In order to monitor Brf1 occupancy at genomic loci, three copies of a sequence encoding the hemagglutinin epitope (3HA) were used as previously described for Brf1 ChIP (Harismendy, Gendrel et al. 2003; Roberts, Stewart et al. 2003). A fragment encoding the 3HA was introduced in frame at the downstream end of Brf1 in yeast strain

YMA1322 by homologous recombination using a modification of the procedure of Longtine et al. 1998 (Wach, Brachat et al. 1997; Longtine, McKenzie et al. 1998).

HTS sequence analysis of Ty3 insertion sites. One microgram of Ty3 ppt+ *HIS*3 integrant gDNA was subjected to one of three parallel digestions using four base cutters *Csp*6I, *Hae*III, and *Hpy*CH4V (5-10 units/each) (New England Biolabs, USA) for 3 h at 37^{o}C. Enzymes were inactivated for 20 min at 65^{o}C. Complete digestion products were fractionated by electrophoresis on a 0.7% agarose gel, resulting in a smear from 300 bp to 10 kb. Digested gDNAs were purified using QIAquick Spin Columns 50 (Qiagen Inc., Germany) and eluted with 30 µl of sterile water. One hundred nanograms of digested DNA was self-ligated using 18 Weiss units of T4 DNA ligase (New England Biolabs, USA) at a concentration of 1 ng/µl at 15^{o}C for more than 12 h in 10 µl of 10x ligase buffer (New England Biolabs, USA) diluted into a 100 µl reaction. Samples were ethanol precipitated.

Self-ligated DNA was amplified using forward (OM8763) and reverse (OM8764) primers (Table S2) with sequences complementary to Ty3 tag and U5 terminal sequences on the inside and to Illumina adapters on the outside ends (Fig. 1). The forward primer contained an additional sequence complementary to the Illumina sequencing primer. Five ng or one µg of circularized DNA was used as template for iPCR. The final concentration of all PCR components per 50-µl reaction was 0.5 x RedTaq buffer, 0.2 mM of each dNTPs, 1 M Betaine, 0.5 µM of each primer, 2 units or 4 units of RedTaq DNA polymerase (Sigma-Aldrich Inc.), 1 µg circularized DNA and water. Thermal cycler settings included 1 cycle at 93^{o}C for 3 min, 32 cycles (93^{o}C for 30 sec, 60^{o}C for 6 min), and 1 cycle holding indefinitely at 4^{o}C. Five µl of PCR products were fractionated by electrophoresis on a 0.7% agarose gel. Product size from iPCR was from 200 bp to 2 kb, but averaged approximately 400 bp. The PCR product was purified using a PCR purification kit (Qiagen Inc., Germany) and eluted with 30 µl of sterile water. Ten nM iPCR stock was prepared based on an estimation for a 200 bp DNA fragment (10nM is equivalent to 1.32 ng/ µl). Equal amounts of iPCR from three different restriction enzyme digests of the same sample were combined and submitted for 36 cycles of single-end sequencing in the GAIIx system.

Chromatin immunoprecipitation analysis of Brf1 binding to target loci. A 50-ml culture of OD_{600} = 0.6-1.0 was crosslinked at 24^{o}C for 15 min using 37% formaldehyde at a final concentration of 1%. The crosslinking reaction was quenched by adding 2.5 ml of 2.5 M glycine and incubating at 24^{o}C for 5 min. The culture was harvested and washed twice with 20 ml ice-cold 1x PBS buffer (0.14 M NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4 and 1.5 mM KH_2PO_4). Cells were resuspended in 400 µL ChIP lysis buffer [50 mM HEPES (pH 7.5), 140 mM NaCl, 1 % Triton x100, 0.1% Na-deoxycholate] supplemented with proteinase inhibitor mix: (w/v) 0.2% leupeptin, 0.2% aprotinin, 0.1% pepstatin, 7.5% PMSF and 0.6 g of glass beads. The cells were lysed by beating (speed setting: 4.5, 3x 40 sec) in a FastPrep-24 System (MP Biomedicals, LLC) at 4^{o}C. The cell extract was transferred into a 15 ml Falcon conical tube (BD Bioscience), brought to 2 ml with cold ChIP lysis buffer with proteinase inhibitors, and sonicated in Bioruptor (Diagenode Inc., Denville, NJ) at 4^{o}C for 15 min (Level: high; 30 s ON, 30 s OFF) to shear the majority of chromatin to 500-bp fragments. The extract was centrifuged for 30 min at 4^{o}C at 13K rpm, and cleared lysate was transferred to a fresh tube. Protein was quantified by Bradford assay (BioRad Inc, Richmond, CA; cat# 500-0006). Twenty microliter of lysate was added to 180 uL of ChIP elution buffer (50 mM Tris pH8.0, 1% SDS, 10 mM EDTA) and later to be reverse crossliked to produce input sample (INPUT).

Five milligrams of total protein were aliquoted for each immunoprecipitation and incubated overnight at 4oC on a Nutator mixer (BD-Diagnostic system, cat# 421105) with 50 µl of 50% slurry of n-ProteinA Sepharose 4 Fast Flow (GE Healthcare, cat# 17-5280-01) with either 2 µg of anti-HA antibody (Roche Inc., cat# 1583816, San Francisco, CA) and cold ChIP lysis buffer to a final volume of 500 µl. After 12-24 h of incubation, the IP reaction was centrifuged at 6K rpm for 5 min at 4^{o}C. The recovered beads were washed thoroughly: twice with 1 ml/each of cold ChIP lysis buffer, twice with 1 ml/each cold ChIP wash buffer (10 mM Tris-HCl pH8.0, 250 mM LiCl, 0.5% NP-40, 0.5% Na-deoxycholate, 1 mM EDTA), and twice with 1 ml/each of TE (10 mM Tris-HCl, pH8.0, 1 mM EDTA) at 24^{o}C. Washed beads were eluted with 100 µl ChIP elution buffer and incubated at 65^{o}C for 10 min. Eluate was transferred to a fresh microfuge tube (IP). The elution step was repeated once more and eluates were pooled. IP and INPUT samples

were incubated at 65^{o}C overnight to reverse crosslinking. DNA was extracted and precipitated using standard procedures. DNA was dissolved in 200 uL deionized water and stored at -20^{o}C.

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