Supplementary Figure 1. Parallel approaches used for TagModule synthesis.
(a) Uptags and downtags were individually synthesized as 76-mers in 96-well format and linked via PCR amplification. Cloning of the 175-base linked fragment into the Gateway entry vector pCR8/GW/TOPO was also performed in 96-well format, and unique clones were individually recovered and sequence verified.

(b) All remaining unused tags available on the TAG4 array were paired and synthesized as 135-mers on an Agilent microarray. Once dissolved off the array, the resulting pool of TagModules was PCR amplified and cloned as a pool. Individual colonies were then recovered and sequenced, and correct and unique clones were then archived.

Supplementary Figure 2. Improved performance of TagModules compared with current tags in use with the *S. cerevisiae* collection. In (a), raw intensity values of paired tags amplified from an *S. cerevisiae* heterozygous essential/homozygous deletion pool (n= 6077) were plotted. Pearson correlation of tags with hybridization intensities above 5X background in the upper left. In (b), TagModule performance from Fig. 2a is plotted in black, with performance of identical tag pairs from *S. cerevisiae* plotted in red.

Supplementary Figure 3. The workflow for using the TagModules for genome mutagenesis will differ from species to species.

Supplementary Figure 4. Construction of tagged transposon mutants in *S. oneidensis* MR-1.
TagModules were transferred via the Gateway LR reaction into two Gateway-compatible transposon delivery vectors, the Tn5-based pRL27-Dest and the *mariner*-based pMiniHIMAR_RB1-Dest. The tagged transposon vectors were transformed into an *E. coli* conjugation donor. Subsequent conjugation with *S. oneidensis* MR-1 yielded a collection of tagged transposon mutants. The transposon junction from individual kanamycin resistant clones was amplified via arbitrary PCR and sequenced to determine both the genome insertion location and the TagModule identity. Transposon inverted repeats are marked by a red bar, Kan^R encodes resistance to kanamycin, Cm^R encodes resistance to chloramphenicol, Spc^R encodes resistance to spectinomycin, *ori*T is the conjugation origin of transfer, R6K is a origin of replication conditional on the *pir* gene, and *att* sites are recombination sequences of the Gateway cloning system.

Supplementary Figure 5. Performance of TagModules in a pool of *S. oneidensis* MR-1 mutants is robust.

(a) Signal distribution of the $\log_2$ hybridization intensities of the 3522 tags amplified from a pool of 1761 *S. oneidensis* MR-1 mutants. In red are the signals of the expected

3522 tags; in blue are the signals of the 639 TagModules (1278 tags) that were used in making the overall collection of the 7387 transposon mutants but were not included in the pool.

(b) A comparison of minimal media relative fitness values for 1761 *S. oneidensis* MR-1 mutants calculated using only the uptag or downtag is illustrated. These results demonstrate that the two tags of a single TagModule produce similar relative fitness values independently. Pearson correlation is indicated in the upper left ($p<10^{-16}$).

(c) A comparison of LB relative fitness values for 1761 *S. oneidensis* MR-1 mutants from two separate timecourse experiments is illustrated. For each timecourse, both the uptag and downtag were used in the calculation of relative fitness. Pearson correlation is indicated in the upper left ($p<10^{-16}$).

Supplementary Figure 6. Construction of tagged transposon mutants in *C. albicans.*

(a) A commercial Tn5 was modified to contain a Gateway conversion cassette (with the *ccdB* selection gene and chloramphenicol resistance gene Cm^R), a kanamycin resistance gene (Kan^R), and the *UAU1* marker cassette at the multiple cloning site (MCS). A reaction with the modified Tn5, a pool of TagModules, and LR clonase induces recombination at the *att* sites, placing the TagModule within the transposon mosaic ends (Tn5L and Tn5R).

(b) To generate the heterozygous disruption strains, first a BWP17 genomic DNA library was constructed (see Online Methods for details) and then mutagenized *in vitro* with the pool of tagged Tn5 transposons from (a). Insertions were recovered in *E. coli* and sequenced (arrow) to determine the gene disrupted and the corresponding tag. Results were sorted and archived to maximize the unique gene insertions and tag pairs. The selected insertions were then amplified, the genomic DNA containing the insertion was excised and chemically transformed into BWP17, selecting for Arg+ mutants. Homologous recombination results in a gene disruption with a tagged Tn5 transposon.

Supplementary Figure 7. Performance of TagModules in a pool of *C. albicans* is robust.

(a) Independent pools of 1290 heterozygous disruption strains were grown for 20 generations in YPD + 1% DMSO. Tags were amplified from each pool and hybridized to a TAG4 array. Pearson correlation of unnormalized tags is in the top left.

(b) Uptags and downtags of an aliquot of the 1290-strain pool were amplified and hybridized to a TAG4 array. Pearson correlation of raw intensities is in the top left.

Supplementary Table 1. List of TagModules used in this study.

Supplementary Table 2*. List of mutagenized *S. oneidensis* MR-1 strains generated in this study.

Supplementary Table 3*. List of *S. oneidensis* MR-1 mutants selected for pooled growth.

Supplementary Table 4*. List of *S. oneidensis* MR-1 mutants with a fitness defect in minimal media.

Supplementary Table 5*. List of *S. oneidensis* MR-1 mutants with a fitness defect in LB + 300mM NaCl.

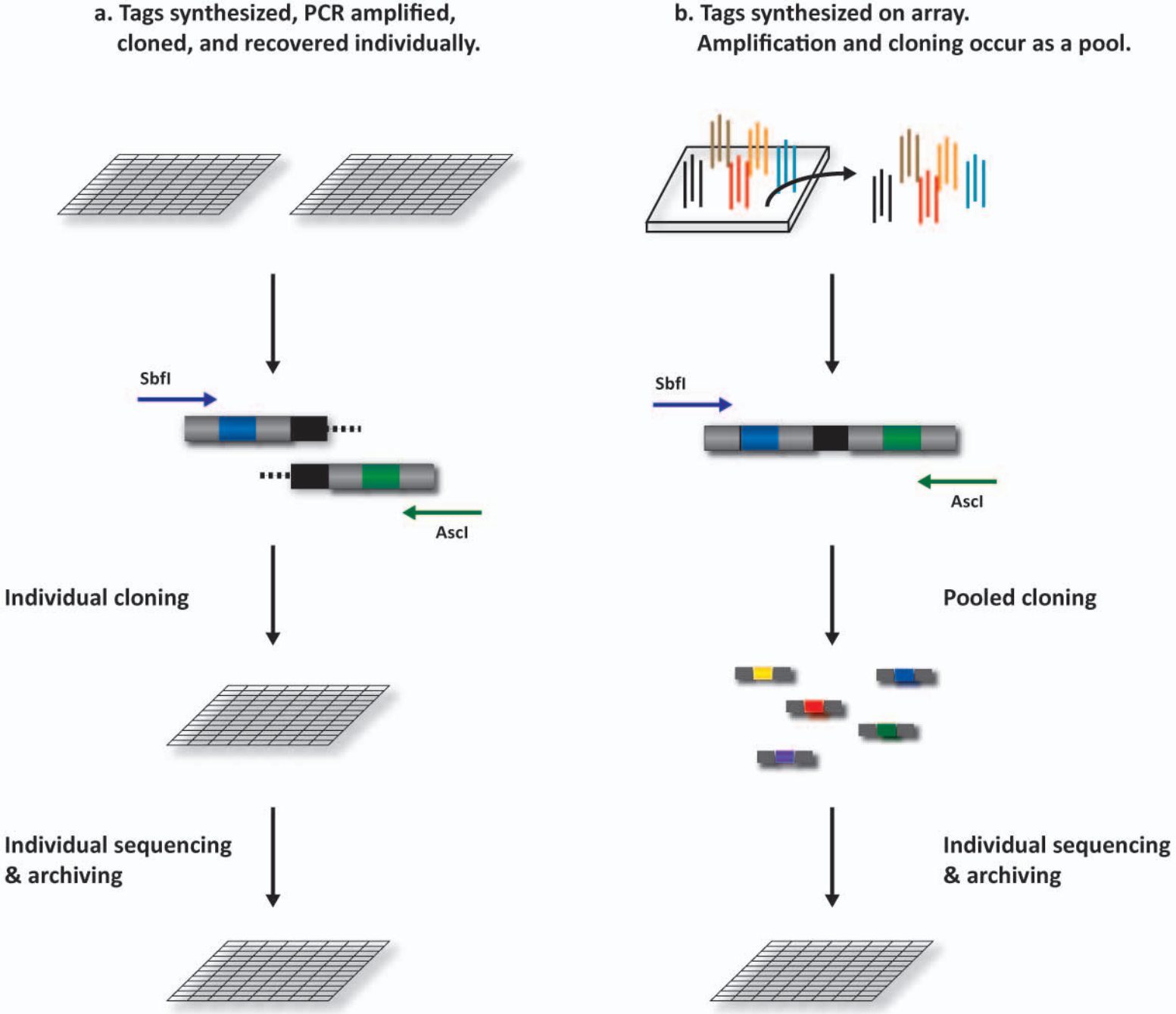
Supplementary Table 6. List of heterozygous disruption *C. albicans* mutants generated in this study.

Supplementary Table 7. Strains used in this study.

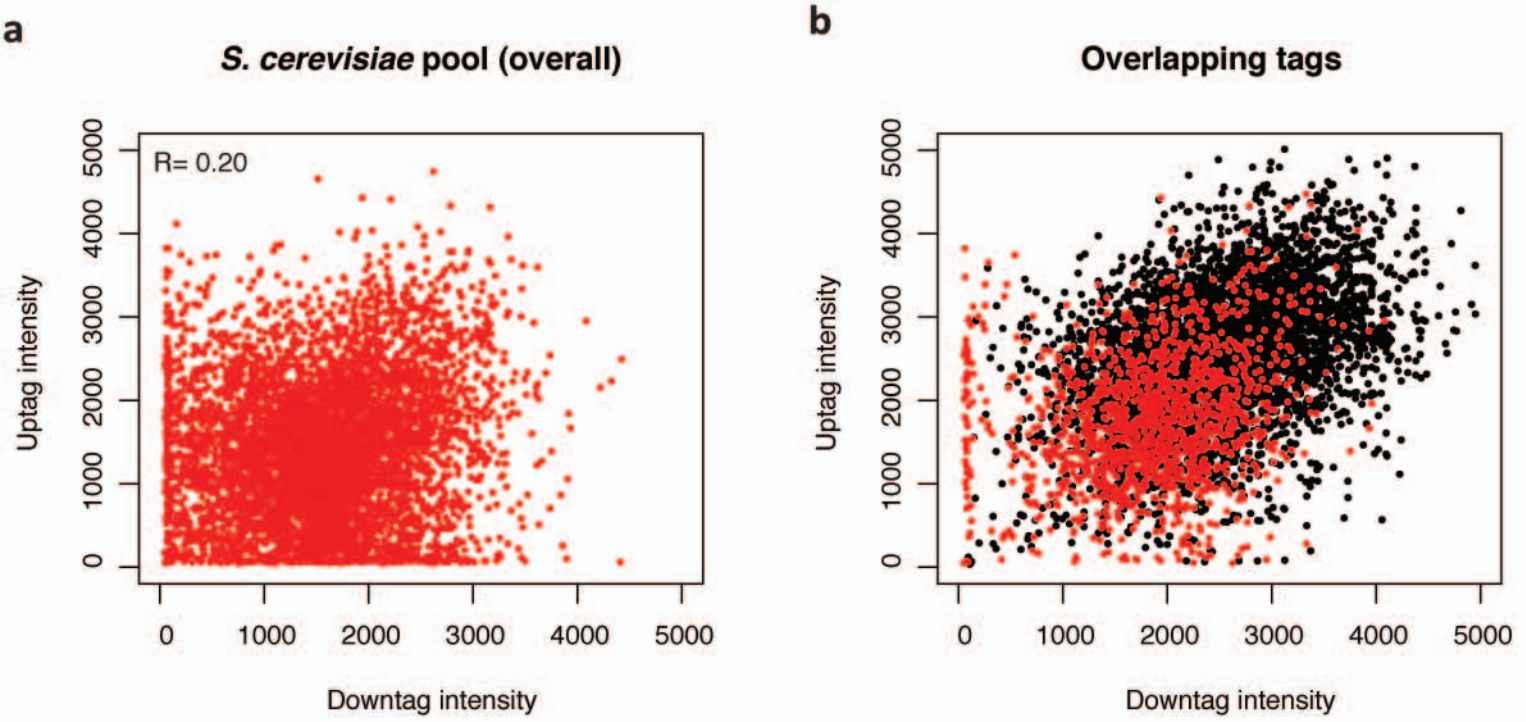
Supplementary Table 8. Primers used in this study.

Supplementary Table 9. Plasmids used in this study.

***** a readme file is available for these tables.

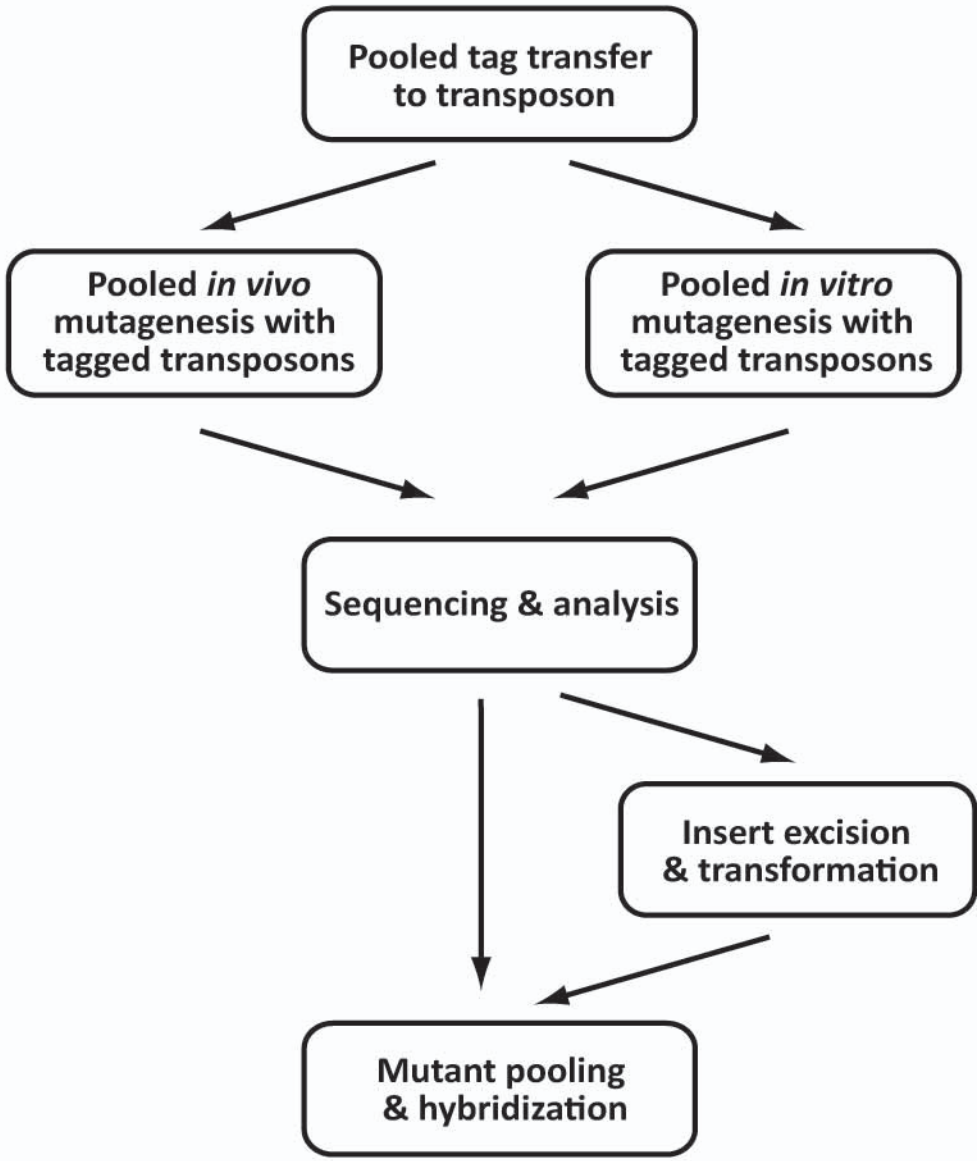


Supplementary Figure-1 (Deutschbauer)

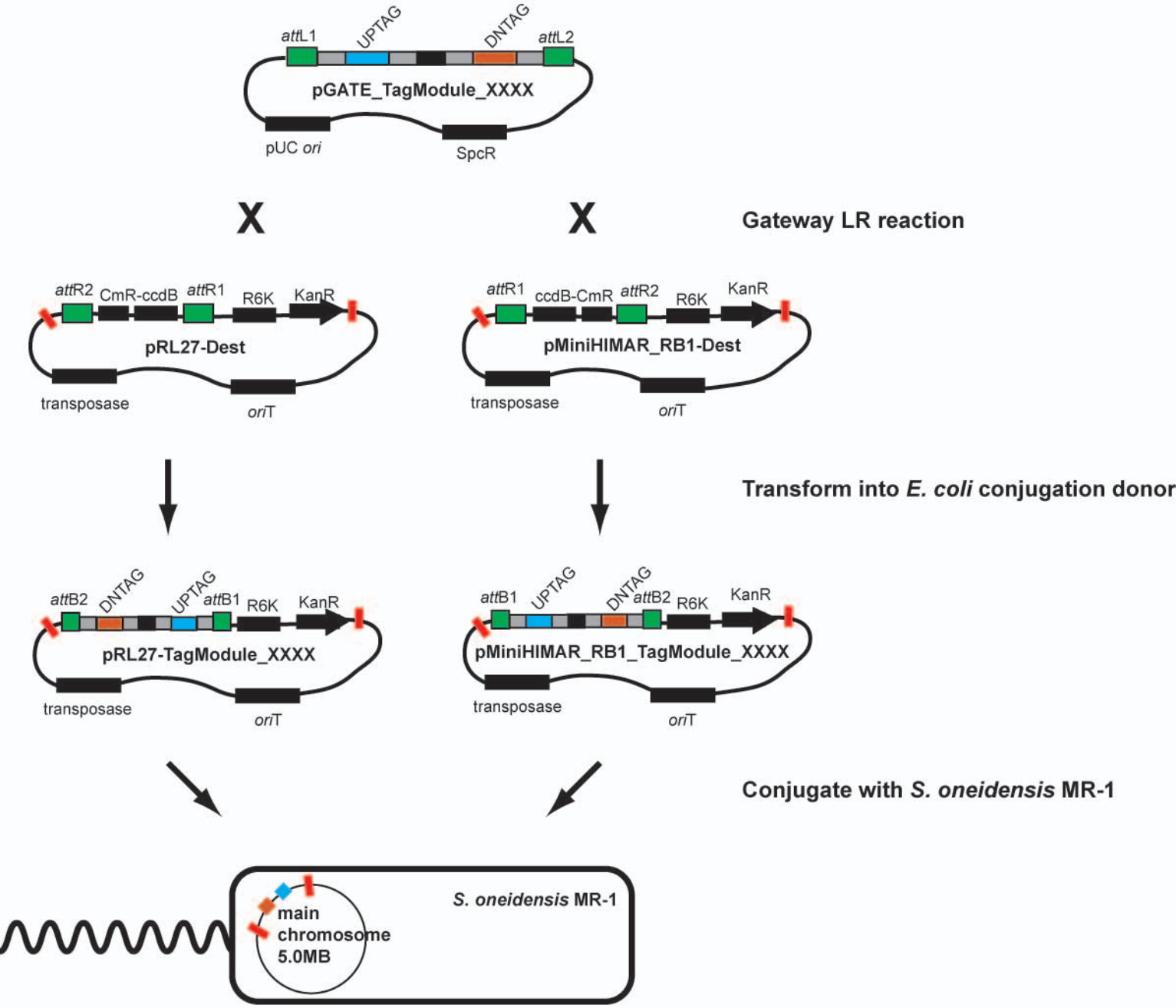


Supplementary Figure-2 (Deutschbauer)

Shewanella oneidensis MR-1 *Candida albicans*



Supplementary Figure-3 (Deutschbauer)

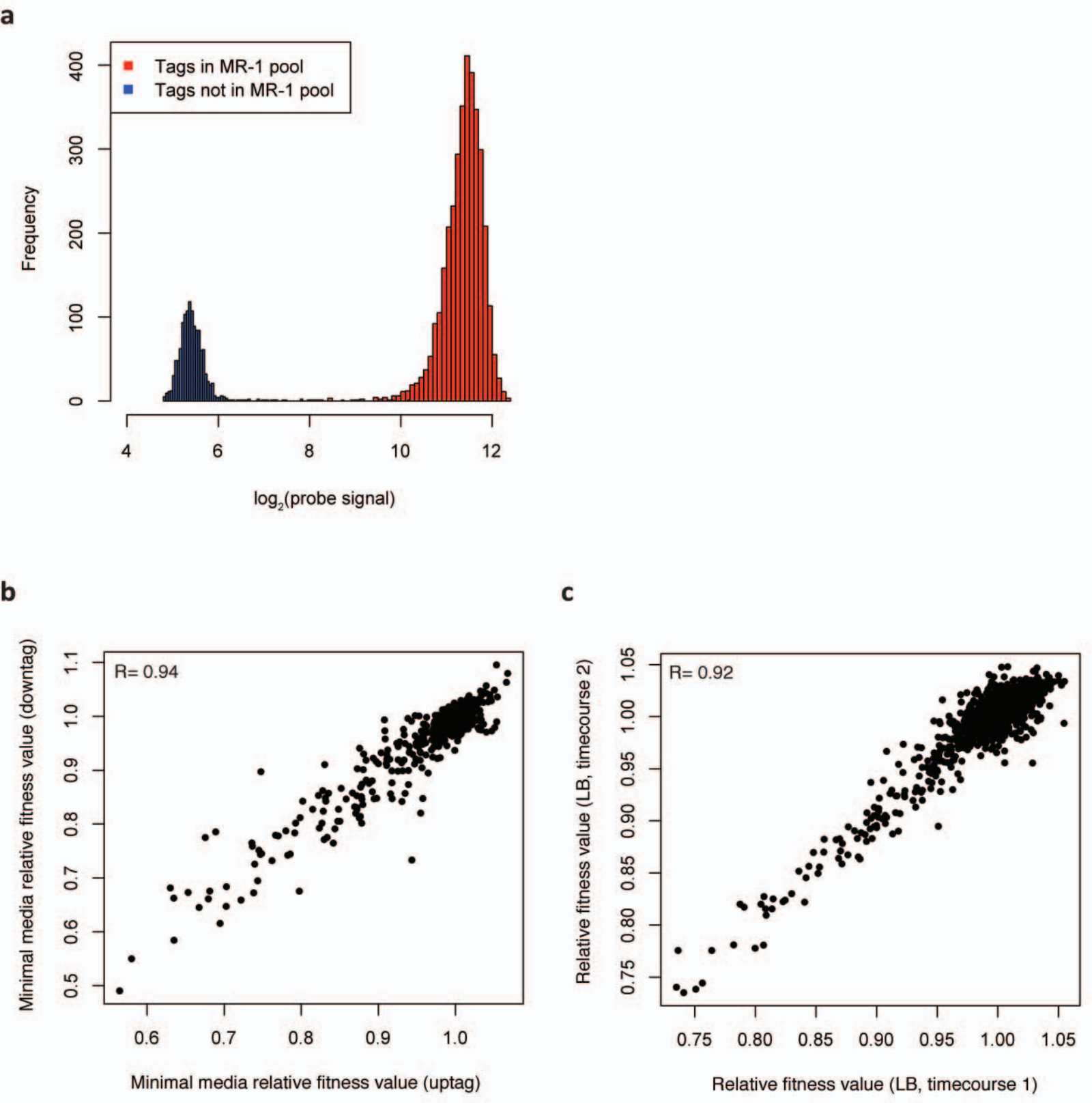


Map transposon insertion location and TagModule identity by arbitrary PCR and sequencing.

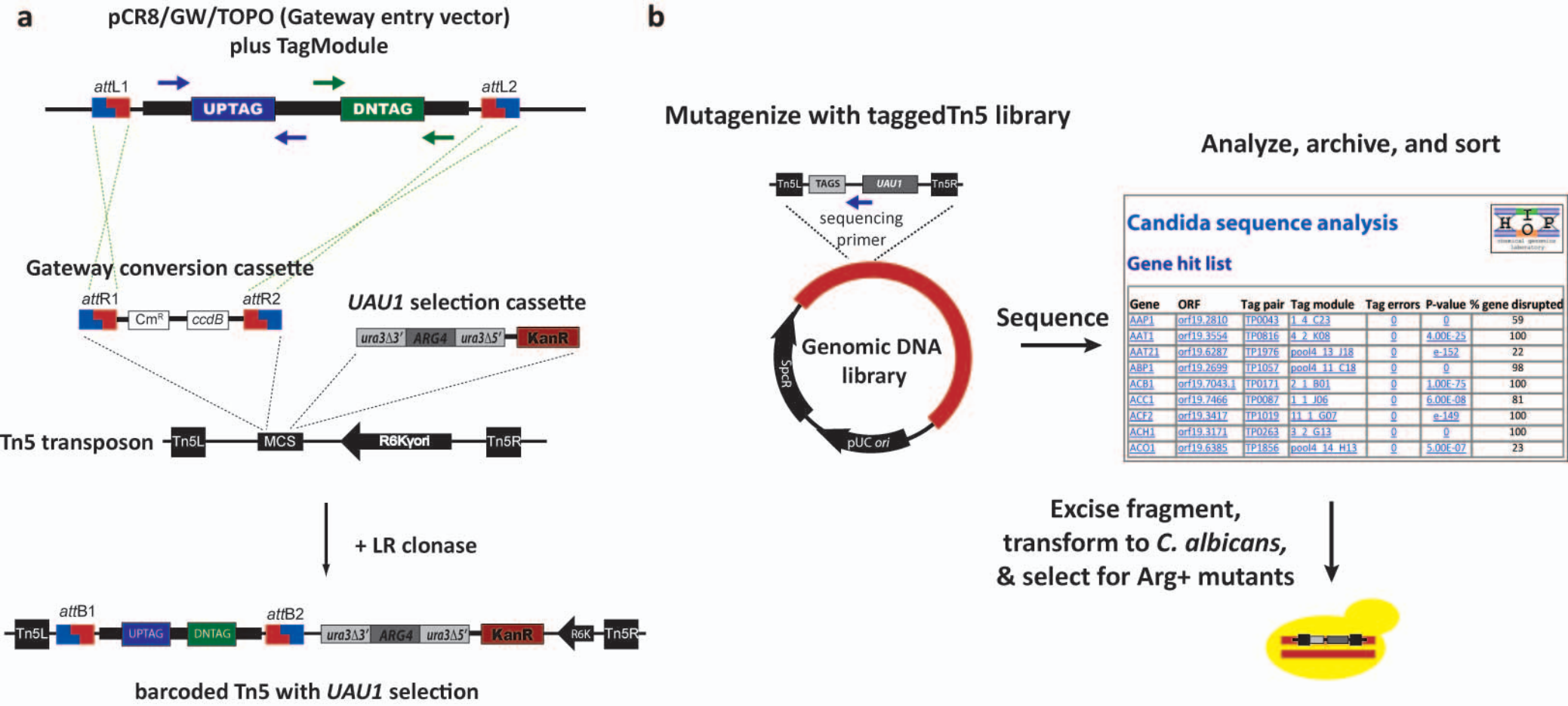
Archive all strains as single, clonal events.

Thousands of strains, each carrying a unique TagModule, can be pooled together for parallel analysis.

Supplementary Figure-4 (Deutschbauer)

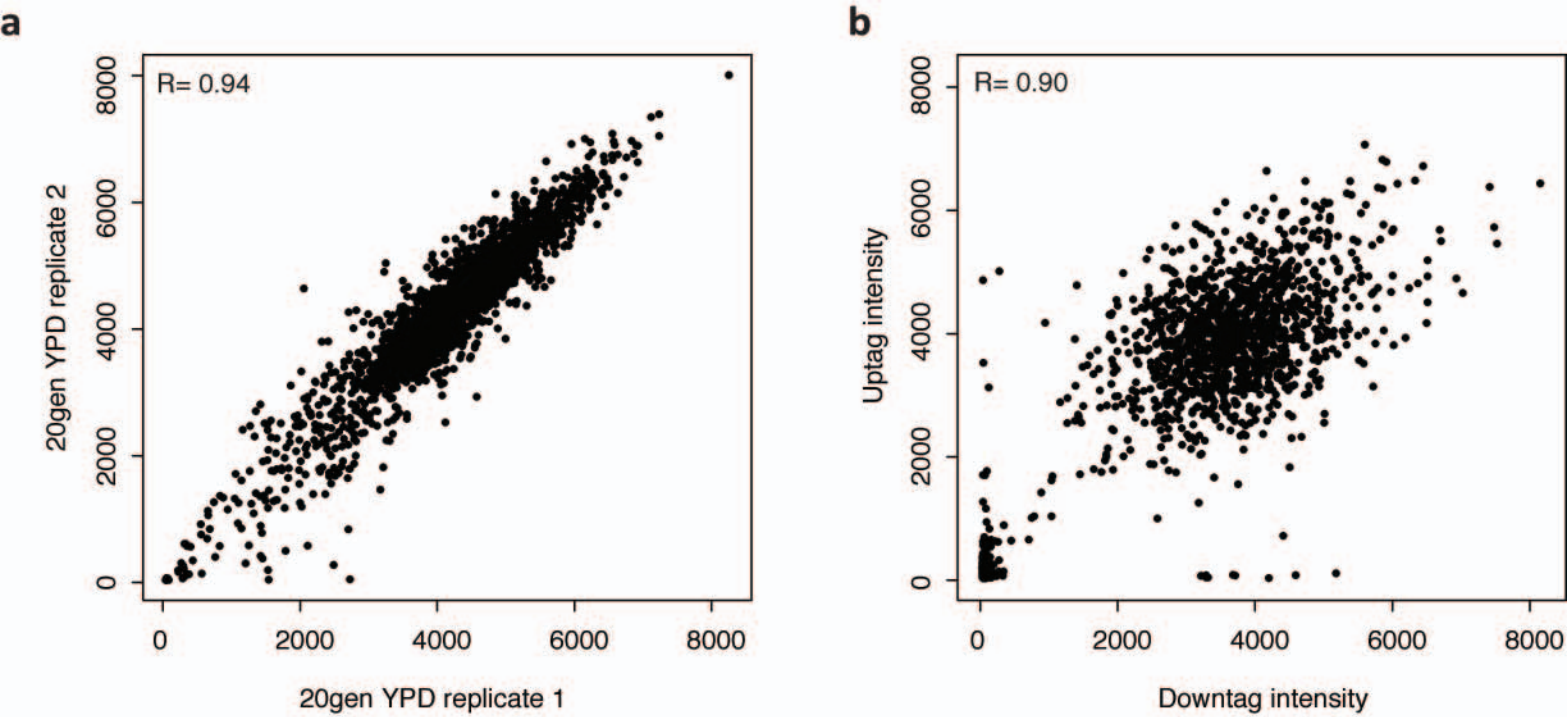


Supplementary Figure-5 (Deutschbauer)



Gene	ORF	Tag pair	Tag module	Tag errors	P-value	% gene disrupted
AAP1	orf19.2810	TP0043	1_4_C23	0	0	59
AAT1	orf19.3554	TP0816	4_2_K08	0	4.00E-25	100
AAT21	orf19.6287	TP1976	pool4_13_J18	0	e-152	22
ABP1	orf19.2699	TP1057	pool4_11_C18	0	0	98
ACB1	orf19.7043.1	TP0171	2_1_B01	0	1.00E-75	100
ACC1	orf19.7466	TP0087	1_1_J06	0	6.00E-08	81
ACF2	orf19.3417	TP1019	11_1_G07	0	e-149	100
ACH1	orf19.3171	TP0263	3_2_G13	0	0	100
ACO1	orf19.6385	TP1856	pool4_14_H13	0	5.00E-07	23

Supplementary Figure-6 (Deutschbauer)



Supplementary Figure-7 (Deutschbauer)