Stanyon, C.A., Limjindaporn, T., and Finley, Jr., R.L. Simultaneous transfer of open reading frames into several different expression vectors. Biotechniques, 35, 520-536, 2003.
http://www.biotechniques.com

Supplementary MATERIAL (at http://proteome.wayne.edu)

Supplemental Table 1. Sequencing/PCR Primers.

Cassette	Forward Primer	Name	Reverse Primer	Name	Product
*ccd*B/Cm^R	AAACGCCATTAACCTGATGTT	attR2-S	TTACGATGCCATTGGGATATA	attR1-AS	N/A
LexA	GCTGTGTGGCTGCCGGTGAAC	LexA-C-S	GCCGGTAGAGGTGTGGTCAAT	CAS_02+	652
B42AD	CCAGCCTCTTGCTGAGTGGAGATG	BC01	GGGAGGGCGTGAATGTAAGCGTG	JZ20	198
*GAL*10p	AAATAAATAGGGACCTAGACTT	CAS_04	GACAAGCCGACAACCTTGATTGGA	BC02	1048
TetR	GTATGCCGCCATTATTACGACAAGC	TetOFF-F1	GAGTCACTTTAAAATTTGTATACAC	JF16	301

Cassette denotes the ORFs expressed by the expression cassette targeted by the PCR primers used to detect insert presence, where "Product" is the length of vector DNA in base pairs amplified by the primers, in addition to the ORF present. Primer direction is relative to that of the attB1 fusion ORF of each destination or expression vector. The *ccd*B/Cm^R cassette primers allow sequencing of attR sites in all destination vectors, and are located with the cassette, oriented outwards: the forward primer enables sequencing of the attR2 (3') site, and the reverse primer, sequencing of the attR1 site (see Supplementary Figure 2 for primer locations).

Supplemental Table 2. Contamination by heterologous plasmids.

Expected Prototrophy	DNA Sample Location	Plating Media h/D	l/D	w/D	u/D	Exp'd	Cont'd	Ratio
his	**A09**	**402**	0	0	178	402	178	0.4428
	B03	**192**	10	0	1	192	11	0.0573
	B04	**186**	0	0	0	186	0	0.0000
	B08	**174**	1	0	0	174	1	0.0057
leu	**C02**	4	**750**	0	0	750	4	0.0053
	D01	0	**564**	11	0	564	11	0.0195
	D02	0	**852**	1	3	852	4	0.0047
	D10	8	**1056**	0	0	1056	8	0.0076
	D11	0	**990**	1	0	990	1	0.0010
	D12	9	**660**	2	0	660	11	0.0167
trp	**F03**	29	19	**522**	0	522	48	0.0920
	F04	0	0	**414**	26	414	26	0.0628
	F06	0	0	**552**	214	552	214	0.3877
	F09	0	4	**720**	8	720	12	0.0167
	F12	90	0	**282**	76	282	166	0.5887
ura	**G03**	0	0	0	**858**	858	0	0.0000
	G04	0	0	0	**396**	396	0	0.0000
	G05	0	0	2	**534**	534	2	0.0037
	G09	0	0	7	**840**	840	7	0.0083
	G11	0	0	321	**468**	468	321	0.6859
	H03	1	0	2	**750**	750	3	0.0040
	H09	7	0	0	**720**	720	7	0.0097

Yeast were transformed with 5□l of DNA isolated from KC8 E.coli transformed with the LR reaction containing 4 destination vectors and one entry clone; the plasmid DNA was isolated from single KC8 colonies selected on the indicated media (Expected Prototrophy). The numbers of yeast colonies growing on the expected plates (bold) or the other 3 plates are shown. Ratios are between heterologous and expected transformant numbers. On average, the ratio of expected to unexpected yeast transformants is ~8:1. When outliers (yellow highlights) and zero scores (purple) are omitted, the ratio is ~48:1, while amongst the outliers, the ratio is ~2:1. The highly contaminated DNA samples produce a co-transformation frequency of ~10% (data not shown).

Supplemental Figure 1. Method used for cloning ORFs into Gateway vectors. Linears amplified with gene-specific primers tailed with unique 5'- and 3'-specific sequences, common to all products, can be cloned into Gateway vectors by adding additional attB sequences. In our approach, the 5' recombination tag (5RT) includes 15bp of the attB1 site followed by two bases to maintain ORF and then the ATG of the gene specific primer (GSP; truncated attB1 emboldened: **AC AAA AAA GCA GGC T**TA-GSP), while the 3RT is a heterologous sequence derived from the pACT2 two-hybrid vector (GGG TTT TTC AGT ATC TAC GA-GSP; BD Biosciences), 3' of the vector MCS. For Gateway cloning, after the first round of amplification, raw PCR products are re-amplified using PAGE-purified tailing primers that extend the PCR products to include the complete attB sites (underlined: attB1 GGGG ACA AGT TTG TAC AAA AAA GCA GGC TTA ATG; attB2 GGGG ACCAC TTTGT ACAAG AAAGC TGGGT CGGGT TTTTC AGTAT CTACGA). The attB-tailed products are introduced into pDONR to create entry clones by the *in vitro* recombination BP reaction, which are sequence-verified before being cloned into expression vectors by the LR reaction.

Supplemental Figure 2. New Gateway destination vectors for expression of proteins in yeast. Linearised vectors and PCR-amplified *ccd*B/Cm^R cassettes, with ~20bp homology at the ends, were introduced into yeast. DNA was extracted from yeast and plasmid DNA rescued through KC8 bacteria under simultaneous selection for linerised vector-specific prototrophic marker presence, ampicillin and chloramphenicol resistance. Clones were sequenced to confirm fidelity of attR site reproduction. Plasmid representations were derived from VectorNTI (v5.2.1; Informax).

Supplemental Figure 3. Digestion of the DNA preparations used to transform yeast strains with XmnI shows that the four plasmid preparations each have bands at mobilities expected for the prototrophic marker used to select bacterial transformants (columns "O" – original DNA sample). Minor contaminating bands consistent with alternative plasmid presence are also visible in all samples. This presence was confirmed by re-transforming KC8 with the original DNA samples, differentially

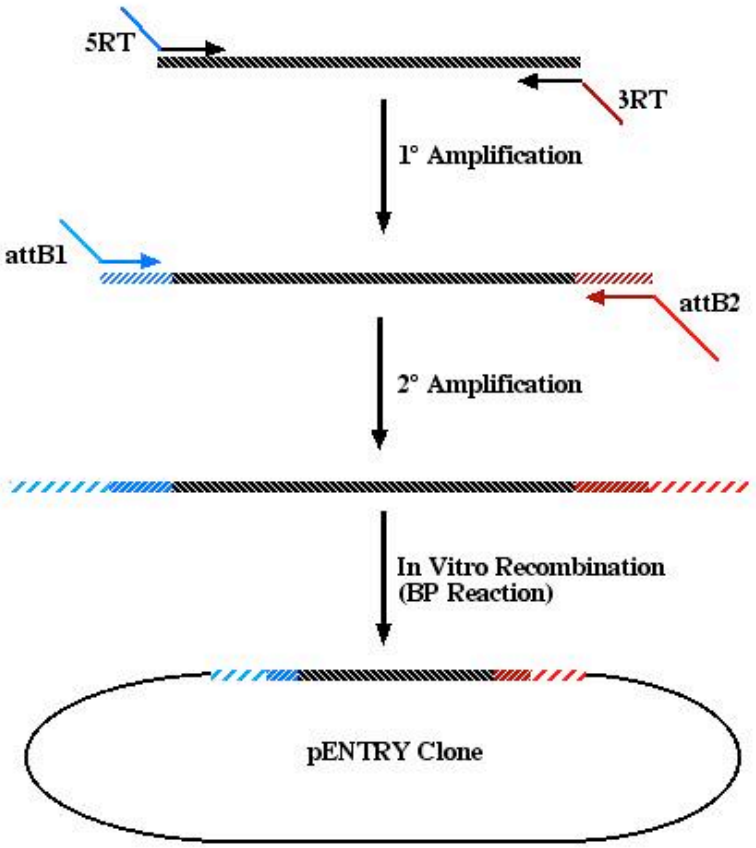
selecting for colony growth on –ura and –trp minimal media, and digesting extracted DNA with *Xmn*I. The U and W columns show the resultant profiles, which clearly correlate with the band patterns in the original preparations.

Supplemental Figure 4. Interaction profiles of expression clones constructed in multiple vector transfer reactions. Yeast two-hybrid interaction profiles for clones constructed by simultaneous ORF transfer. Strains expressing BD fusions contained the *lacZ* reporter plasmid pSH18-34, and one of four plasmids directing the synthesis of LexA fused to *C.jejuni* proteins, CheY, AnsA, DnaA, or Cj0036. The BD strains were mated with five AD strains containing plasmids directing the synthesis of B42AD fused to nothing (lane 1) or to *C.jejuni* proteins CheA (lane 2), Cj0036 (lane 3), DnaA (lane 4), or AnsA (lane 5). The diploids from the mating were replicated to four indicator plates: two that were –ura –his –trp –leu (leu^-), and two that were –ura –his –trp with 16 g/ml X-Gal (X-Gal). The plates on the left have 2% glucose, which represses expression of the AD fused protein, and the plates on the right have 0.5% raffinose plus 2% galactose to induce expression of the AD fusion. An interaction is indicated by growth of strains on the galactose –leu plate. For example, CheY interacts with CheA, AnsA interacts with AnsA, Dna A interacts with DnaA, and Cj0036 interacts with Cj0036. The strengths of interactions are suggested by the level *lacZ* activity, as indicated by the level of blue on the X-Gal plates. For example, the interaction between Cj0036 and Cj0036 activates *lacZ* strongly, and the other interactions activate *lacZ* weakly or not at all.

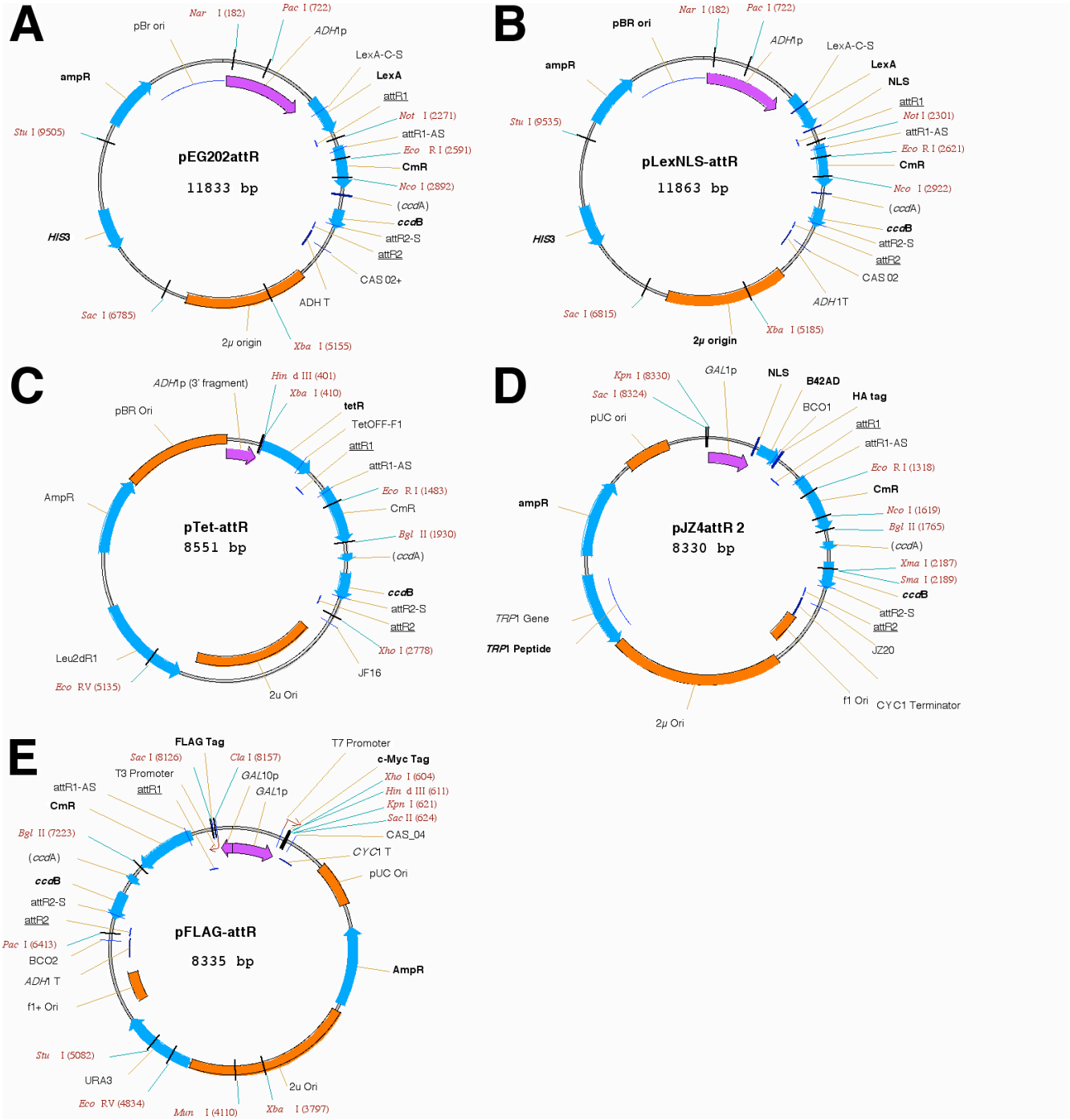
Supplemental Figure 5. Yeast transformed with KC8 bacterial DNA preparations. Yeast strains transformed by expression clones carrying each of the four markers – in order, two rows of 6 colonies for each of: *HIS*3 (H), *TRP*2 (W), *LEU*2 (L) and one row of five and one row of six colonies for *URA*3 (U) – were cultured on each of the four media (-his, -trp, -leu, -ura). Transformed yeast grew on the

media selective for their respective marker (eg, H plasmid on -his media). Four bacterial preparations also produced transformed yeast that grew strongly on one other media: three preparations from ura^+ bacteria produced trp^+ yeast transformants, and one from trp^+ bacteria gave ura^+ yeast colonies. Control transformations with no plasmid DNA resulted in no colonies on any of the media (not shown).

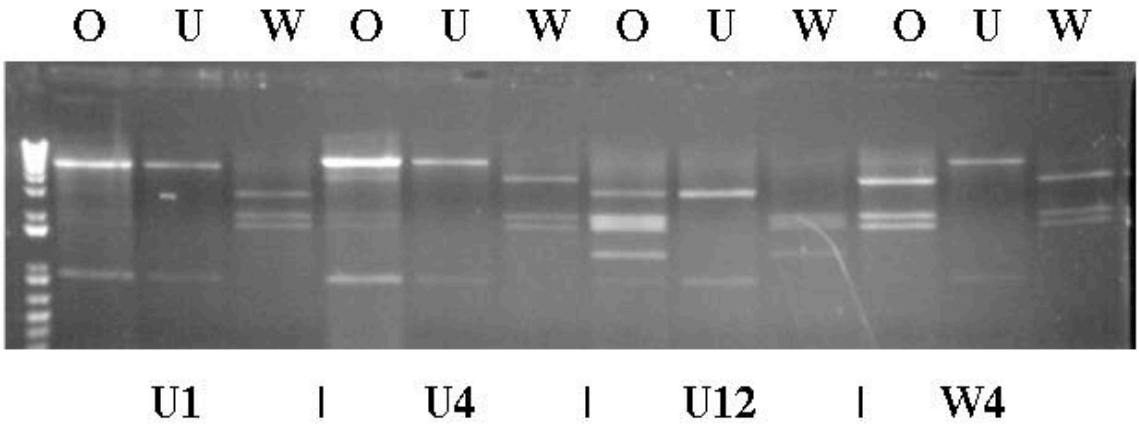
Supplemental Figure 1. Method used for cloning ORFs into Gateway vectors.



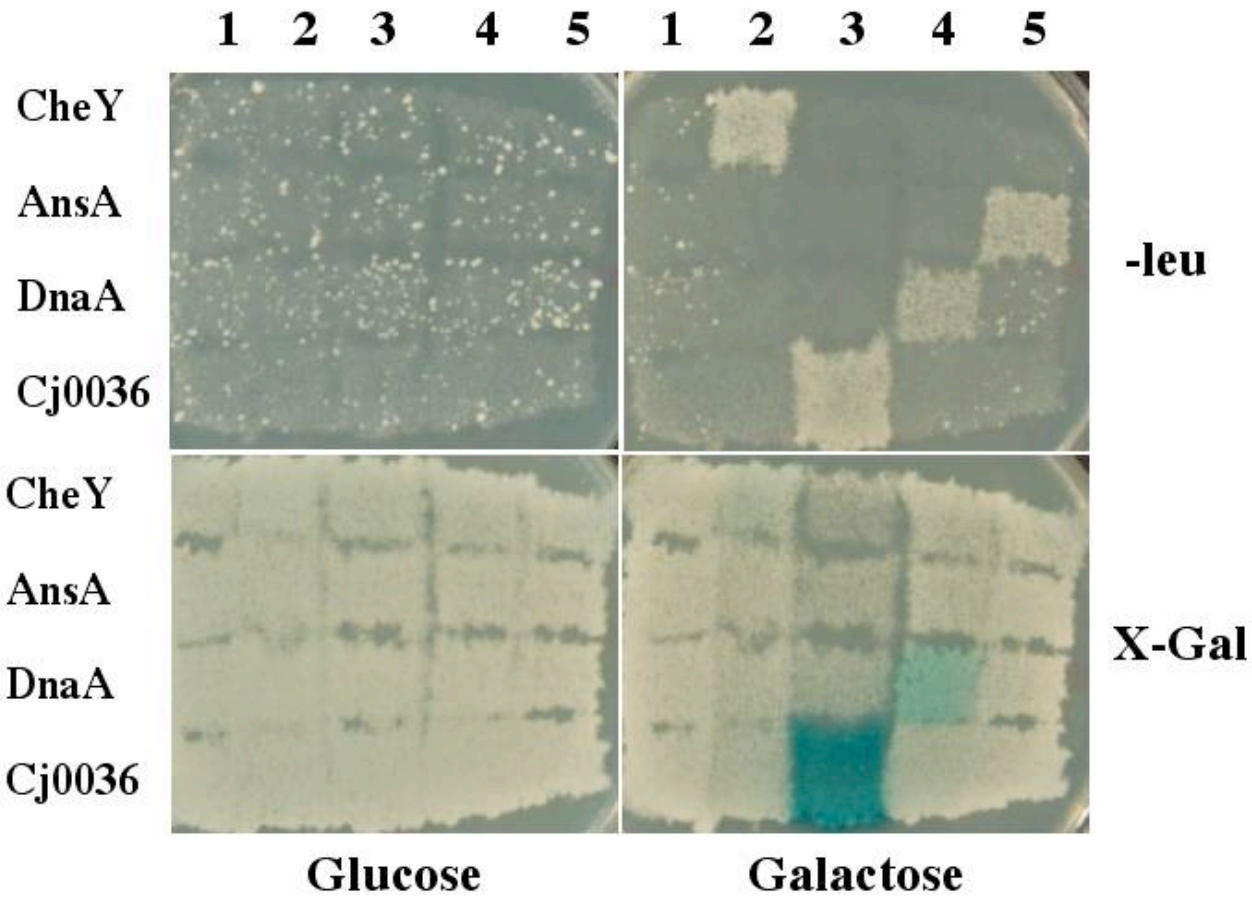
Supplemental Figure 2. New Gateway destination vectors for expression of proteins in yeast.



Supplemental Figure 3. Restriction profiles of four KC8 DNA preparations with contaminating plasmids.



Supplemental Figure 4. Interaction profiles of expression clones constructed in multiple vector transfer reactions.



Supplemental Figure 5. Yeast transformed with KC8 bacterial DNA preparations.