

Supplemental Table 1 Comparison of high throughput two-hybrid strategies^a

Strategy	Mating BD strains with AD strains (BD/AD)	Matings ^c	Sequencing reactions ^h	Quantify reporter activity	Transactivating BD fusions	Interactions with toxic proteins ^k
Library screen	Each BD/one pool of all AD ^b	9,216 ^d	184,320 ⁱ	no	removed	unlikely
Matrix screen	Each BD/ array of ADs	884,736 ^e	0	yes	removed ^j	yes
Two-phase matrix	BD array/AD pools (phase 1) Each BD/AD array subset (phase 2)	27,648 ^f	0	yes	Not removed	yes
3-D pooling matrix	BD array/orthogonal AD pools	16,960 ^g	0	yes	Not removed	yes

^a Based on two yeast arrays, each with 9,216 strains expressing different AD or BD-fused proteins. Each array is distributed over 96 x 96-well plates. The calculations assume that every BD will interact with an average of 2 ADs.

^b The AD pool could be constructed by combining all 9,216 members of the AD array, for example, as in Uetz et al., 2000.

^c Each mating involves one 96-well plate, in which BD and AD strains are mixed in each well.

^d Library screen matings are conducted at 96/plate. Denser configurations may lead to an insufficient number of mated diploids to represent an entire library.

^e BD strains x number of plates holding the AD array. If the AD array was distributed over 24 x 384-well plates, the number of matings would be 221,184.

^f In the two-phase approach, the number of matings in the first phase depends on the total number of strains in each array (N) and the chosen pool size; here we use a pool size of 96 corresponding to all the strains from one plate. The number of matings in the second phase depends on number of interactions that will be detected, which can be represented as the average number of AD interactors per BD strain ($I^{AD/BD}$) times the number of BD strains (N). Thus,

$$\text{Total Number of Matings on 96-well plates} = (N/96)^2 + N \times I^{AD/BD}$$

^g In the 3-D approach, the number of matings in the first phase depends on the number and size of the pools. Table 1 showed a method for making 72 pools, each with 192 yeast AD clones from N=4608 AD clones, where each clone is found in three orthogonal pools. Using this approach, the number of pools will equal $N/192 \times 3$, or $.016N$. This formula works for N which are whole multiples of 4608; for additional clones the two-phase matrix approach can be used. The number of matings for the 3-D approach also depends on the total number of interactions that will be detected ($N \times I^{AD/BD}$), because they need to be tested in confirmation matings. These can be performed at 96 interactions per plate, or 96 interactions per mating. Finally, the number of confirmation matings will be increased by a factor of f , where f depends on the frequency of finding more than one interactor in a pool and on the consequences of false addresses that would be generated in the 3-D pooling method; we estimate that f will be less than 10. Thus,

$$\text{Number of matings on 96-well plates} = .016N (N/96) + (fN \times I^{AD/BD})/96$$

^h In addition to the sequencing reactions needed to verify the arrays (9,216 x 2)

ⁱ Extrapolated from Uetz et al., 2000, in which 10 sequences had to be determined for each interaction detected.

^j Transactivators were removed in previous screens, though they could remain in the array using the system described here.

^k Requires use of regulated expression vectors, as used in the strategy outlined in this paper

Supplemental Table 2. List of plasmids used in this study

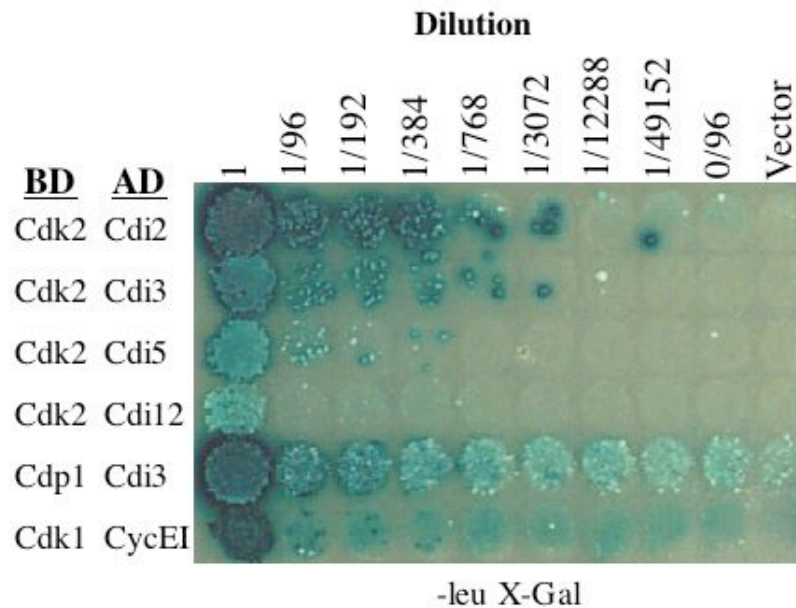
Name	Promoter	Fusion Motif	Other Key Features	References
pEG202	<i>ADH1p</i>	LexA	-	Gyuris, P. et al 1993
pRFHM12	<i>ADH1p</i>	LexA-DmCdk1	-	Finley and Brent, 1994;
pRFHM13	<i>ADH1p</i>	LexA-DmCdk2	-	Finley and Brent, 1994;
pEG202-DmCdp1	<i>ADH1p</i>	LexA-DmCdp1	-	Unpublished; see Methods
pNLexAattR2-DmCycEI	<i>ADH1p</i>	NLS-LexA-DmCycEI	NLS	Finley, 2002
pHZ5attR2-DmCycEI	<i>MAL62p</i>	NLS-LexA-DmCycEI	NLS	Finley 2002
pJG4-5	<i>GAL1p</i>	NLS-B42	NLS	Gyuris et al 1993
pJG4-5-Cdi2	<i>GAL1p</i>	NLS-B42-DmCdi2	NLS	Finley and Brent, 1994
pJG4-5-Cdi3	<i>GAL1p</i>	NLS-B42-DmCdi3	NLS	Finley and Brent, 1994
pJG4-5-Cdi5	<i>GAL1p</i>	NLS-B42-DmCdi5	NLS	Finley and Brent, 1994

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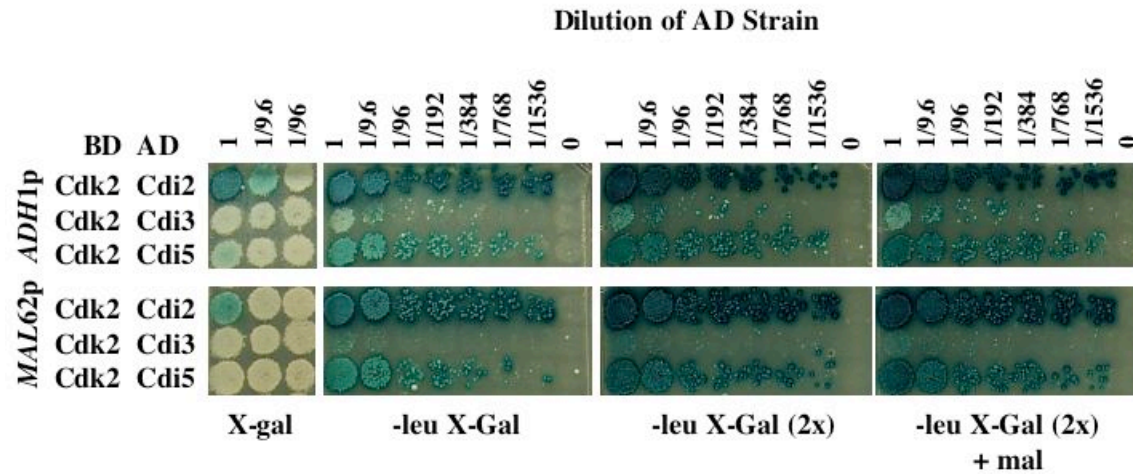
pJG4-5-Cdi12	<i>GAL1p</i>	NLS-B42-DmCdi12	NLS	Finley and Brent, 1994
pJG4-5-CycEI	<i>GAL1p</i>	NLS-B42-DmCycEI	NLS	Finley and Brent, 1994
pJG4-5-Cdi4	<i>GAL1p</i>	NLS-B42-DmCdi4	NLS	Finley and Brent, 1994
pJG4-5-Rux	ADH1p	NLS-B42-DmRux	NLS	Thomas et al., 1997

See also Supplemental Table 5

Supplemental Figure 1. Detection of interactions in pools of AD strains depends on the strength of reporter activation. Strains expressing the indicated interacting AD fusions were serially diluted with strains expressing non-interacting AD fusions. Diploids were replicated onto -leu X-Gal Gal/Raf plates. Interactions that strongly activate the reporters (eg. Cdk2-Cdi2 and Cdk2-Cdi3) can be detected when the AD is diluted more than 1/768, whereas weaker interactions (e.g. Cdk2-Cdi5) are not detected in AD pools diluted much greater than 1/192.



Supplemental Figure 2. Sensitivity of *LEU2* and *lacZ* reporter assays using DNA binding fusions expressed from *ADH1p* and *MAL62p*. Matings between a strain expressing LexA fused with *Drosophila* Cdk2 and strains expressing the AD fused with *Drosophila* Cdi2, Cdi3, or Cdi5. The BD fusions were expressed from either the constitutive *ADH1p* (top three rows) or the glucose-repressible *MAL62p* (lower three rows); all AD fusions were expressed from *GAL1p*. The AD fusion strains were serially diluted with a strain expressing only the AD. Mated yeast were transferred to media lacking leucine as indicated, and containing X-gal (40ug/ml, or 80ug/ml (2x)) galactose, and maltose. Reporter gene activation is strongest in diploids expressing BD-Cdk2/AD-Cdi2, followed by the /AD-Cdi5 then /Cdi3 combination. In the latter two diploid strains, blue colonies are observed from matings with AD fusion strains at a proportion of 1 in 1536.



Supplemental Table 3

	Position original	Position calculated	Plate Position	Plasmid name	LexA fused to	FlybaseID	Provided by	Reference	Endnote
A 01 07	A1	A01, A07	A01, A07	tra2(1475)	Dm tra2	FBgn0003742	Yujing Liu and J. Belote	Liu and Belote, 1995	{Liu, 1995 #1631}
A 02 08	A2	A02, A08	A02, A08	p205K	Dm faf (clone 205K)	FBgn0005632	J. Fischer and R. Lehman	J. Fischer and R. Lehman, unpublished	
A 03 09	A3	A03, A09	A03, A09	lex202-da	Dm daughterless da	FBgn0000413	Z. Paroush and D. Ish-Horowicz	Paroush et al., 1994	
A 04 10	A4	A04, A10	A04, A10	pRF HM1	Dm bicoid 2-160	FBgn0000166	R.L.F.	Finley and Brent, 1994	{Finley, 1994 #2599}
A 05 11	A5	A05, A11	A05, A11	pRF HM7-3	Dm ftz HD aa239-327	FBgn000107	R.L.F.	R.L.F. unpublished	
A 06 12	A6	A06, A12	A06, A12	pRF HM12	Dm cdc2	FBgn0004106	R.L.F.	Finley and Brent, 1994	{Finley, 1994 #2599}
B 01 07	B1	B01, B07	B01, B07	pRF HM13	Dm cdc2c	FBgn0004107	R.L.F.	Finley and Brent, 1994	{Finley, 1994 #2599}
B 02 08	B2	B02, B08	B02, B08	pRF HM2	Dm bicoid prd repeat aa2-95	FBgn0000166	R.L.F.	R.L.F. unpublished	
B 03 09	B3	B03, B09	B03, B09	pRF HM3	Dm bicoid hd aa50-160	FBgn0000166	R.L.F.	R.L.F. unpublished	
B 04 10	B4	B04, B10	B04, B10	pRF HM-Q	Dm Bcd polyQ aa254-213	FBgn0000166	R.L.F.	R.L.F. unpublished	
B 05 11	B5	B05, B11	B05, B11	pRF HM 202-CDI3	Dm Cdi3 cyclin D aa30-end	FBgn0010315	R.L.F.	Finley and Brent, 1994	{Finley, 1994 #2599}
B 06 12	B6	B06, B12	B06, B12	pRF HM 202-CDI5	Dm Cdi5 cyclin J	FBgn0010317	R.L.F.	Finley and Brent, 1994	{Finley, 1994 #2599}
C 01 07	C1	C01, C07	C01, C07	pRF HM 202-CDI11	Dm Cdi11(CG32226) aa1088-end	FBgn0052226	R.L.F.	R.L.F. unpublished	
C 02 08	C2	C02, C08	C02, C08	pRF HM 202-CDI12	Dm Cdi12 (CG3689) aa26-end	FBgn0035987	R.L.F.	R.L.F. unpublished	
C 03 09	C3	C03, C09	C03, C09	lex202-hairy	Dm hairy	FBgn0001168	Z. Paroush	Paroush et	

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C 04 10	C4 C04, C10	C04, C10	pB8GN	Dm PCNA (mus209) aa14-end	FBgn0005655	M. Axton and T. Orr-Weaver	and D. Ish-Horowicz M. Axton and T. Orr-Weaver, unpublished	al., 1994
C 05 11	C5 C05, C11	C05, C11	pLexA-Vasa	Dm Vasa	FBgn0003970	W. Breitwieser and A. Ephrussi	W. Breitwieser et al., 1995	{Breitwieser, 1996 #1331}
C 06 12	C6 C06, C12	C06, C12	pLexA-NLS-Oskar	Dm Oskar	FBgn0003015	W. Breitwieser and A. Ephrussi	W. Breitwieser et al., 1995	{Breitwieser, 1996 #1331}
D 01 07	D1 D01, D07	D01, D07	plexesc1	Dm esc	FBgn0000588	Y.Ma and J. Simon	Jones et al., 1998	{Jones, 1998 #2723}
D 02 08	D2 D02, D08	D02, D08	plexesc2	Dm esc c-Term 2/3	FBgn0000588	Y.Ma and J. Simon	Y. Ma and J. Simon, unpublished	
D 03 09	D3 D03, D09	D03, D09	pBD-ftz2	Dm ftz N-term HD aa236-362	FBgn000107	B. Dietrich	B. Dietrich, unpublished	
D 04 10	D4 D04, D10	D04, D10	pLTcd	Dm Toll cyto domain aa831-1097	FBgn0003717	C. Hashimoto	C. Hashimoto, unpublished	
D 05 11	D5 D05, D11	D05, D11	pfs(1)Ya	Dm fs(1)Ya	FBgn0000927	S. Turner and M. Wolfner	S. Turner and M. Wolfner, unpublished	
D 06 12	D6 D06, D12	D06, D12	pEG202-Rux	Dm roughex	FBgn0003302	K. Zavitz and S.L. Zipursky	Thomas et al., 1997	{Thomas, 1997 #2597}
E 01 07	E1 E01, E07	E01, E07	pEG202-ERK	Dm ERK (rolled)	FBgn0003256	K. Zavitz and S.L. Zipursky	K. Zavitz and S.L. Zipursky, unpublished	
E 02 08	E2 E02, E08	E02, E08	pEGLRR-3	Dm flightless (flii) leucine rich repeat (LRR) aa 1-147	FBgn0000709	K. Fong	Fong and de Couet, 1999	{Fong, 1999 #2724}
E 03 09	E3 E03, E09	E03, E09	pLex-Ben	Dm Bendless (Ben)	FBgn0000173	C. Oh	C. Oh,	

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E 04 10	E4 E04, E10	E04, E10	p202 Gap1 N	Dm Gap1 N-terminal region	FBgn0004390	T. Cutforth and G. Rubin	unpublished T. Cutforth and G. Rubin, unpublished	
E 05 11	E5 E05, E11	E05, E11	p202 Gap1 M	Dm Gap1 M central region	FBgn0004390	T. Cutforth and G. Rubin	T. Cutforth and G. Rubin, unpublished	
E 06 12	E6 E06, E12	E06, E12	p202 Gap1 C	Dm Gap1 C-terminal region	FBgn0004390	T. Cutforth and G. Rubin	T. Cutforth and G. Rubin, unpublished	
F 01 07	F1 F01, F07	F01, F07	pRF HM16	Dm PCNA	FBgn0005655	R.L.F.	R.L.F., unpublished	
F 02 08	F2 F02, F08	F02, F08	pRF pGIL-DmA	*GIL Dm CycA	FBgn0000404	M. Kolonin	Kolonin and Finley, 2000	{Kolonin, 2000 #2591}
F 03 09	F3 F03, F09	F03, F09	p202-K4i7	Dm K4i7 (CG3837) aa645 to end	FBgn0038279	R.L.F.	J.Z. and R.L.F., unpublished	
F 04 10	F4 F04, F10	F04, F10	pLexA PNR	Dm Pannier	FBgn0003117	P. Simpson	P. Simpson, unpublished	
F 05 11	F5 F05, F11	F05, F11	pEG202-DmCdk4/6	Dm Cdk4/6	FBgn0016131	M. Kolonin	Kolonin and Finley, 1998	{Kolonin, 1998 #2595}
F 06 12	F6 F06, F12	F06, F12	pEG202-DmCdk5	Dm Cdk5	FBgn0013762	M. Kolonin	Kolonin and Finley, 1998	{Kolonin, 1998 #2595}
G 01 07	G1 G01, G07	G01, G07	pEG202-Dip1	Dm Cdp1(CG15610) aa1386-end	FBgn0034170	R.L.F.	J. Lewis and R.L.F., unpublished	
G 02 08	G2 G02, G08	G02, G08	pEG202-Cdk4i2	Dm K4i2 (Cdc37)	FBgn0011573	R.L.F.	J.Z. and R.L.F., unpublished	
G 03 09	G3 G03, G09	G03, G09	pEG202-Cdk4i36	Dm K4i36 (CG32068) aa15-end	FBgn0052068	R.L.F.	J.Z. and R.L.F., unpublished	
G 04 10	G4 G04, G10	G04, G10	p205D	Dm Faf aa 1-400	FBgn0005632	J. Fischer	J. Fischer, unpublished	
G 05 11	G5 G05, G11	G05, G11	Arl2	Dm Arl2 (Arf84F)	FBgn0004908	M. Kolonin	Kolonin and	{Kolonin,

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G 06 12	G6 G06, G12	G06, G12	pEG / DRas1	Dm ras1	FBgn0003205	M. Therrien and G. Rubin	and J. Lewis Finley. 2000	2000 #2591}
H 01 07	H1 H01, H07	H01, H07	pEG / DRas1 v12	Dm ras1 v12 (valine 12)	FBgn0003205	M. Therrien and G. Rubin	M. Therrien and G. Rubin, unpublished	
H 02 08	H2 H02, H08	H02, H08	pEG / DRaf CR1	Dm Raf CR1 domain aa1-317	FBgn0003079	M. Therrien and G. Rubin	M. Therrien and G. Rubin, unpublished	
H 03 09	H3 H03, H09	H03, H09	pEG / DRas1 CR1+2	Dm ras1 CR1+2 domains aa1-418	FBgn0003205	M. Therrien and G. Rubin	M. Therrien and G. Rubin, unpublished	
H 04 10	H4 H04, H10	H04, H10	pEG / DRaf CR3	Dm Raf CR3 domain aa419-788	FBgn0003079	M. Therrien and G. Rubin	M. Therrien and G. Rubin, unpublished	
H 05 11	H5 H05, H11	H05, H11	pEG / DSor1 (DMapkk)	Dm Sor1 (DMapkk)	FBgn0010269	M. Therrien and G. Rubin	M. Therrien and G. Rubin, unpublished	
H 06 12	H6 H06, H12	H06, H12	Blank					

Unless otherwise noted, the full-length protein is fuse to the C-terminal end of LexA

Supplemental Table 4

Plate Position	Flybase ID	Gene Name	Synonym	Amino Acid	#of interactions out of 85 DBDs	Included in "AD-non-interactors"	Included in "AD Array I"	Included in "AD Array II"
1A01	FBgn0034289	CG10910	CG10910	216	0.0E+01	YES	YES	YES
1A02	FBgn0001090	CG7088	bnb	22	2	NO	NO	NO
1A03	FBgn0013676	mt:Colll	COXIII	8	0.0E+01	YES	YES	YES
1A04	FBgn0035422	CG12740	CG12740	17	0.0E+01	YES	YES	YES
1A05	FBgn0013678	mt:Cyt-b	cyto b	208	0.0E+01	YES	YES	YES
1A06	FBgn0013955	CG3969	PR2	1026	0.0E+01	YES	YES	YES
1A07	CG31762	CG31762	aret	229	0.0E+01	YES	YES	YES
1A08	FBgn0004922	CG10944	RpS6	141	0.0E+01	YES	YES	YES
1A09	FBgn0004867	CG5920	sop	1	0.0E+01	YES	YES	YES
1A10	FBgn0026261	CG4207	bonsai	1	0.0E+01	YES	YES	YES
1A11	FBgn0002543	CG5481	robo2	1351	0.0E+01	YES	YES	YES
1A12	FBgn0017545	CG2168	RpS3A	1	0.0E+01	YES	YES	YES
1B01	FBgn0004551	CG3725	Ca-P60A	191	0.0E+01	YES	YES	YES
1B02	FBgn0010808	CG1715	l(3)03670	155	0.0E+01	YES	YES	YES
1B03	FBgn0035887	CG7170		1	2	NO	NO	NO

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1B04	FBgn002631 6	CG5788	UbcD10	1	0.0E+01	YES	YES	YES
1B05	FBgn001121 1	CG3612	blw	509	0.0E+01	YES	YES	YES
1B06	FBgn000015 0	CG2210	awd	1	0.0E+01	YES	YES	YES
1B07	FBgn003475 1	CG3751		20	0.0E+01	YES	YES	YES
1B08	FBgn003732 8	CG2099		1	0.0E+01	YES	YES	YES
1B09	FBgn000260 7	CG2746	RpL19	115	0.0E+01	YES	YES	YES
1B10	FBgn000260 7	CG2746	RpL19	115	0.0E+01	YES	YES	YES
1B11	FBgn003352 7	CG11777		14	0.0E+01	YES	YES	YES
1B12	FBgn003985 7	CG11522		1	3	NO	NO	NO
1C01	FBgn003729 9	CG1115		1	0.0E+01	YES	YES	YES
1C02	FBgn000260 7	CG2746	RpL19	1	0.0E+01	YES	*	YES
1C03	FBgn003682 5	CG6846		1	0.0E+01	YES	YES	YES
1C04	FBgn001007 8	CG366	RpL17A	46	0.0E+01	YES	YES	YES
1C05	FBgn001367 4	mt:Col	COX1	6	0.0E+01	YES	YES	YES
1C06	FBgn002672	CG8730	drosha	1095	0.0E+01	YES	YES	YES
1C07	FBgn000278 1	CG32491	CG15500	199	2	NO	NO	NO
1C08	FBgn002994 1	CG1677		883	0.0E+01	YES	YES	YES
1C09	FBgn001367 2	mt:ATPase6	ATPase subunit 6	34	0.0E+01	YES	YES	YES
1C10	FBgn003251 8	CG9282		1	0.0E+01	YES	YES	YES

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1C11	FBgn001367 4	mt:Col	COX1	6	0.0E+01	YES	YES	YES
1C12	CG15010	CG15010	ago	120	0.0E+01	YES	YES	YES
1D01	FBgn002672	CG8730	drosha	1128	0.0E+01	YES	YES	YES
1D02	FBgn003610 6	CG6409		252	1	YES	YES	YES
1D03	FBgn003640 1	CG6513		34	0.0E+01	YES	YES	YES
1D04	FBgn001575 6	CG6141	RpL9	1	0.0E+01	YES	YES	YES
1D05	FBgn000004 2	CG4027	Act5C	unknown	1	YES	YES	YES
1D06	FBgn003177 6	CG13993		1	4	NO	NO	NO
1D07	FBgn002321 3	CG10811	eIF-4G	796	0.0E+01	YES	YES	YES
1D08					0.0E+01	YES	YES	YES
1D09	FBgn000442 7	CG9118	LysD	1	0.0E+01	YES	YES	YES
1D10	FBgn003542 2	CG12740		1	1	YES	YES	YES
1D11	FBgn003731 4	CG12000		92	0.0E+01	YES	YES	YES
1D12	FBgn003904 9	CG6726		244	0.0E+01	YES	YES	YES
1E01	FBgn003621 3	CG7283		1	0.0E+01	YES	YES	YES
1E02	FBgn003211 4	CG3752		232	0.0E+01	YES	YES	YES
1E03	FBgn001757 9	CG6253	RpL14	129	0.0E+01	YES	YES	YES
1E04	FBgn000386 3	CG18444	aTrypsin	1	0.0E+01	YES	YES	YES
1E05	FBgn000337 8	CG6132	Sgs8	1	2	NO	NO	NO
1E06	FBgn003446 2	CG15905		21	0.0E+01	YES	YES	YES

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1E07	FBgn002473 3	CG17521	Qm	1	0.0E+01	YES	YES	**
1E08	FBgn003935 9	CG4759		1	0.0E+01	YES	YES	YES
1E09	FBgn000122 4	CG4463	Hsp23	56	0.0E+01	YES	YES	YES
1E10	FBgn002641 5	CG1780	ldgf4	370	0.0E+01	YES	YES	YES
1E11	FBgn001007 8	CG3661	RpL17A	44	0.0E+01	YES	YES	YES
1E12	FBgn003390 6	CG8331		95	0.0E+01	YES	YES	YES
1F01	FBgn002672 2	CG8730	drosha	1071	2	NO	NO	NO
1F02	FBgn003695 2	CG6933		1	0.0E+01	YES	YES	YES
1F03	FBgn000442 7	CG9118	LysD	9	0.0E+01	YES	YES	YES
1F04	FBgn001575	CG6141	RpL9	1	0.0E+01	YES	YES	YES
1F05	FBgn003768 0	CG8121		206	1	YES	YES	YES
1F06	FBgn002640 1	CG40281	Nipped-B	1123	0.0E+01	YES	YES	YES
1F07	FBgn003308 1	CG3183	geminin	97	0.0E+01	YES	YES	YES
1F08	FBgn003646 7	CG12310		36	0.0E+01	YES	YES	YES
1F09	FBgn000397 7	CG3496	vir	1727	1	YES	YES	YES
1F10	FBgn000360 7	CG8409	Su(var)205	130	0.0E+01	YES	YES	YES
1F11	FBgn001754 5	CG2168	RpS3A	1	0.0E+01	YES	YES	YES
1F12	FBgn003894 7	CG7073	sar1	119	0.0E+01	YES	YES	YES
1G01	FBgn003165 3	CG8871		18	0.0E+01	YES	YES	YES

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1G02	FBgn001754 5	CG2168	RpS3A	125	0.0E+01	YES	YES	YES
1G03	FBgn000440	CG1524	Rps14a	1	0.0E+01	YES	YES	YES
1G04	FBgn000260 7	CG274	RpL19	1	0.0E+01	YES	YES	YES
1G05	FBgn002480 7	CG17686	DIP1	113	0.0E+01	YES	YES	YES
1G06	FBgn001367 6	mt:Coll	COXIII	19	0.0E+01	YES	YES	YES
1G07	FBgn003886 8	CG5862		64	0.0E+01	YES	YES	YES
1G08	FBgn003985 7	CG11522		1	0.0E+01	YES	YES	YES
1G09	FBgn000031 8	CG11024	clot	1	0.0E+01	YES	YES	YES
1G10	FBgn000260 7	CG2746	RpL19	1	0.0E+01	YES	YES	YES
1G11	FBgn005213 8	CG3213		1201	0.0E+01	YES	YES	YES
1G12	None	None	gypsy DMGYPF1A	unknown	0.0E+01	YES	YES	YES
1H01	FBgn003886 8	CG5862		64	0.0E+01	YES	YES	YES
1H02	FBgn003883 4	CG15697		1	0.0E+01	YES	YES	YES
1H03	FBgn000260 7	CG2746	RpL19	1	0.0E+01	YES	YES	YES
1H04	FBgn003390 2	CG8309		261	0.0E+01	YES	YES	YES
1H05	FBgn002754 1	CG3274	BcDNA:GH12 174	1467	4	NO	NO	NO
1H06	FBgn003496 7	CG3186	eIF-5A	54	0.0E+01	YES	YES	YES
1H07	FBgn003013 6	CG2998		1	0.0E+01	YES	YES	YES
1H08	FBgn003757 3	CG7483		357	0.0E+01	YES	YES	YES

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1H09	FBgn003391	CG8415		1	0.0E+01	YES	YES	YES
	2							
1H10	FBgn000057	CG1007	emc	3'UTR	0.0E+01	YES	YES	YES
	5							
1H11	FBgn000010	CG7490	RpO	1	0.0E+01	YES	YES	YES
	0							
1H12	FBgn000010	CG7490	RpO	269	0.0E+01	YES	YES	YES
	0							

1. "Flybase ID" corresponds to the record for this gene in Flybase (<http://flybase.bio.indiana.edu/>).
 2. "Gene Name" and "Synonym" are according to Flybase
 3. "Amino Acid" refers to the amino acid position in the protein where the cDNA starts, relative to the methionine at position 1.
 4. Note that "1" in the Amino Acid column means "includes aa1"; the clone may include additional amino acids from the 5'UTR
 5. "Trimmed sequence" is the sequence of the clone starting immediately after the cloning site.
 6. The remaining columns are as described in Methods
- * For AD Array I the clone at position C2 was replaced with a clone expressing AD-Cdi3
- ** For AD Array II the clone at position E7 was replaced with a strain expressing AD-Cdi5

Supplemental Table 5. Information posted at <http://proteome.wayne.edu>

Strains and vectors on this page are available by contacting Julie Hines (jhines@genetic.wayne.edu)

Vectors

The sequence files available here were assembled and annotated based on published plasmids and sequences available in Genbank. The .gb files are text files in the Genbank format. The pdf contains annotated sequences and the jpg file is a plasmid map. In most cases a VectorNTI file is also available upon request.

pHZ5

HIS3 2 μ *MAL62p* LexA NLS. *HIS3* 2 μ yeast vector for regulated expression of N-terminal LexA fusions using the *MAL62* promoter. Includes at the C-terminal end of LexA. This is the BD vector currently used by the Finley lab for most yeast two-hybrid studies. Finley et al., 2002, Gene 285, 49-57.

pHZ5.jpg pHZ5.gb pHZ5.pdf

pHZ5attR

HIS3 2 μ *MAL62p* LexA NLS attR. Destination vector version of pHZ5 for use with the GatewayTM (Invitrogen) in vitro cloning system. Finley et al., 2002, Gene 285, 49-57.

pHZ5attR.jpg pHZ5attR.gb pHZ5attR.pdf

pLexA(202+PL)

HIS3 2 μ *ADH1p* LexA. This is the granddaddy of all LexA expression vectors. *HIS3* 2 μ with the *ADH1* promoter driving expression of LexA. Most (possibly all) of the other LexA vectors shown here were derived from this common ancestor. Ruden et al., 1991, Nature 350, 250-252.

pLexA(202+PL).jpg pLexA(202+PL).gb pLexA(202+PL).pdf

Zhong et al., **Web supplement**

pEG202

*HIS3 2 μ ADHI*p LexA mcs. The most common plasmid used in the LexA two-hybrid system. Similar to pLexA(202+PL) but with a better multiple cloning site downstream of LexA. The sequence file was derived from the version marketed by Clontech and from Genbank Accession Number U89960. Estojak et al., 1995, Mol. Cell. Biol. 15, 5820-5829.

pEG202.jpg [U89960](#) pEG202.pdf

pNLex [also known as pNLex(NLS)]

*HIS3 2 μ ADHI*p LexA NLS. Derived from pLexA(202+PL). Contains an SV40 nuclear localization signal (NLS) coding sequence downstream of LexA and upstream of the mcs.

pNLexAattR

*HIS3 2 μ ADHI*p LexA NLS attR. Destination version of pNLex for use with the GatewayTM (Invitrogen) in vitro cloning system. Finley et al., 2002, Gene 285, 49-57.

pNLexAattR.jpg pNLexAattR.gb pNLexAattR.pdf

pJZ4

*TRP1 2 μ GALI*p NLS-B42AD-HAtag mcs *CYCI*t. For regulated expression of AD fusions from the *GALI* promoter. Similar to pJG4-5 except with an F1 origin and the *CYCI* terminator instead of *ADHI*t to reduce possible recombination with the BD vector. This is the AD vector currently used by the Finley lab for most yeast two-hybrid studies.

pJZ4.jpg pJZ4.gb pJZ4.pdf

pJG4-5

*TRP1 2 μ GALI*p NLS-B42AD-HAtag mcs *ADHI*t. This is the original AD vector for the Brent lab LexA two-hybrid system. The sequence file was derived from the version marketed by Clontech and from Genbank Accession Number U89961. Gyuris et al., 1993, Cell 75, 791-803.

pJZ4.jpg [U89961](#) pJZ4.pdf

Zhong et al., **Web supplement**

pHZ5attB-DmCycEI

Described in Zhong et al. pHZ5 derivative for expressing LexA fused to Drosophila cyclin E type I.
pHZ5attB-DmCycEI.jpg pHZ5attB-DmCycEI.gb pHZ5attB-DmCycEI.pdf

pNLexAattB-DmCycEI

Described in Zhong et al. pHZ5 derivative for expressing LexA fused to Drosophila cyclin E type I.
pNLexAattB-DmCycEI.jpg pNLexAattB-DmCycEI.gb pNLexAattB-DmCycEI.pdf

Destination vectors from Stanyon et al.

Yeast expression vectors for use with the Gateway™ (Invitrogen) *in vitro* cloning system. Stanyon et al., 2003, Biotechniques 35, 520-536.
StanyonVect.jpg