

	-CDEI-	-CDEII-	-CDEIII-	
AgCEN1	ATCACGTGAAA- TAAAT-		TCTTATA - TGTTTATATGTTCCGAAA- ATAAAAATAAGTTAT	208
AgCEN2	ATCATGTGACCG- TAAAC		TAATTTAT - TGATGGTATTTCCGAACTTATATTTAACTTAAAAAT	213
AgCEN3	TTCACGTGATAAATA- A		TCATTAT - TGTTTGTGTTTCCGAAA- ATATAAATGTTATTTTGG	211
AgCEN4	GGCACGTGACC- AATATC		TTAAATA - TGATGTGTGTTCCGAAATTATTTAAATTTTTAAT	209
AgCEN5	AGCACGTGACA- TAAAT-		ATAGATG - TGATACGATTTCCGAAACATAAAAAATTTT	204
AgCEN6	ATCACATGATCAATAAAA		TTATATA - TGATGTCTTTCCGAAAATATTTTTAAAAATACATA	211
AgCEN7	ATCACGTG- - - ATAAT	160bp AT-rich	TTTTGTA - TGTGTGTTTGTCCGAACTATAAAAAATGTTTAAA	212
EcymCEN1	ATCACATGATCATTATTT		T - TTATT - TGTGTAAGGTTCCGAACTAATTTTATTTATAAAT	209
EcymCEN2	ATCATGTGATAAAATATA		TAATACA - TGTTTAGATGTTCCGAAA- ATAAAAAT	199
EcymCEN3	TTCACGTGATATTTTTTA		TTATATAA - TGTTTTGATTTCCGAAAGGTTTTTATTTTTACTAT	209
EcymCEN4	ATCACATGATACAAAATA		TAGGT - - - TGTTTATGATTTCCGAAAATAAAAAAAT	203
EcymCEN5	ATCACGTGCTTATCTAAA		T - ATAAAT - GTTTTTGTGTTCCGAAAGATAAAATAAAT	203
EcymCEN6	AACACGTGATA- TATTTT		TTTTGTA - TGTTTTGGAGTCCGAAA- ATAAATAT	198
EcymCEN7	ATCACATGACTTCAAAAA		TAATATTT - TGTTTTGTTTCCGAACTAATTTATAAGTTAATAATGTATT	216
EcymCEN8	ATCACGTGTAATAAAA- A		TAAAAA - CGTATATTGTTCCGAACTATAAAAAATAGTTTATTAAT	213

Figure S1 Centromere DNA Element (CDE) conservation in *Emethocium*. CDEI is characterised by the palindrome CACGTG. CDEII is a 160bp AT-rich region, whereas CDEIII has a central conserved motif of TTCCGAA in a 25bp core sequence. These point-like centromeres differ from *S. cerevisiae* in the length of the CDEII element.

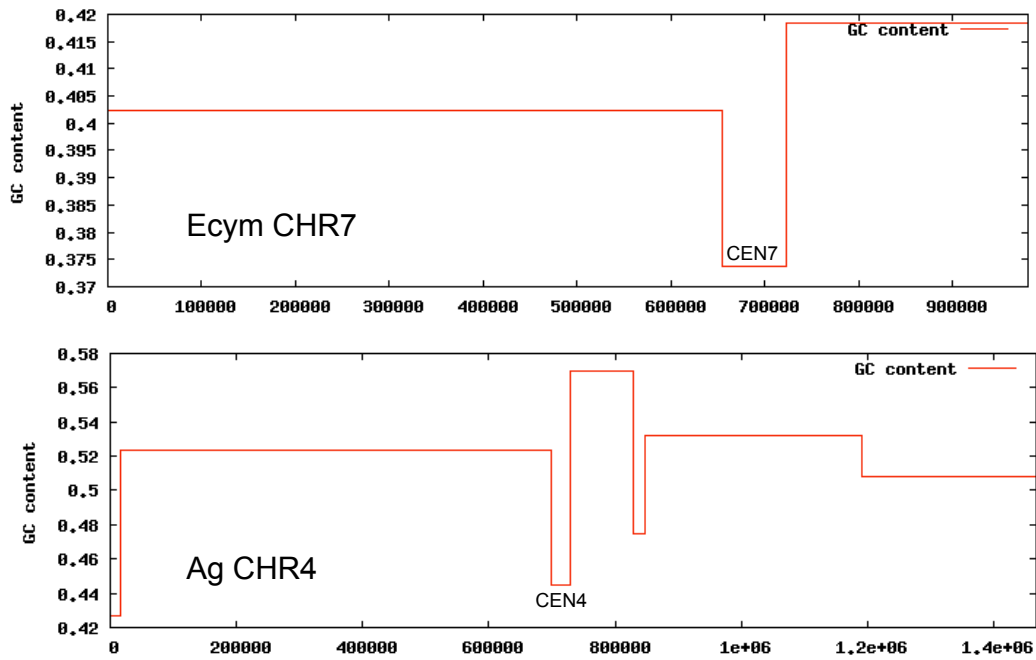


Figure S2 Analysis of GC-content on a chromosomal scale. GC-profiles were generated using the online tool at <http://tubic.tju.edu.cn/GC-Profile/>. (Gao and Zhang, 2006). Upper graph shows *E. cymbalariae* CHR 7 with its lower GC content around CEN7 (located at 695316-695514). Lower graph shows *A. gossypii* CHR4. CEN4 is located at 708288-708477. Two more regions with reduced GC content are found on this chromosome. One at the left telomere. The other between 827451-847198. For details on the genes in that region confer to Table S2.

The diagram shows a linear arrangement of rDNA repeat units. From left to right, the components are: 5S (indicated by a left-pointing arrow), NTS2, 5'-ETS, 18S, ITS1, 5.8S, ITS2, 28S, 3'-ETS, and NTS1 (indicated by a right-pointing arrow). Below this diagram is a table comparing the lengths of these regions and the total repeat unit size for three species: *Ag* (*A. gossypii*), *Ecym* (*E. cymbalariae*), and *Sc* (*S. cerevisiae*). The table also includes pairwise percentage identities for the 5S, 18S, 5.8S, and 28S rRNA genes.

Ag	121	734	618	1795	207	158	210	3390	964	8197bp
	100%			99%		97%		97%		
Ecym	121	696	625	1796	206	158	210	3391	858	8061bp
	100%			97%		92%		94%		
Sc	121	1243	701	1800	361	158	232	3396	1125	9137bp

Figure S3 Comparison of the rDNA repeat units of *A. gossypii* and *S. cerevisiae* (Wendland *et al.*, 1999) with the rDNA-repeat of *E. cymbalariae*. A single repeat unit (not to scale) is shown starting with the 5S rRNA gene that is divergently transcribed from the precursor 35S pre-rRNA (running from 5'-ETS to 3'-ETS). This precursor contains the 18S, 5.8S, and 25S rRNA genes separated by internal transcribed spacers (ITS1, ITS2) and flanked by external transcribed spacers (5'ETS, 3'ETS). The 5S rRNA gene and the 35S pre-rRNA are separated by non-transcribed spacers (NTS1, NTS2). Length of the individual mature rRNAs and spacer regions is indicated. Using pairwise comparisons % identity of rRNAs between *E. cymbalariae* and *A. gossypii* and between *E. cymbalariae* and *S. cerevisiae* is indicated.

Table S1 *E. cymbalariae* genome summary

CHR	lengths (bp)	coding ¹ (bp)	% encoding	Features	gene		tRNAs	LTRs	#genes with		total length of introns (bp)	telomeric repeats	Accession number
					density/kb	ORFs			introns ²	#of introns			
1	1.110.245	807.030	72.10	541	2.05	530	11	7	33	36	6525	0	CP002497
2	1.601.921	1.172.903	72.82	823	1.95	799	24	13	31	33	6329	1	CP002498
3	1.193.613	876.657	73.16	622	1.99	598	24	16	27	28	3364	1	CP002499
	1.541.034												
4	+rDNA repeat 1.385.851	1.152.108	74.34	792	1.95	767	25	7	38	39	6554	2	CP002500
5	+TY3	1.040.071	74.75	679	2.04	662	17	5	20	20	4127	2	CP002501
6	959.278	687.803	71.31	491	1.95	475	16	7	20	20	3713	1	CP002502
7	980.088	717.760	72.62	480	2.04	466	14	4	31	31	5988	0	CP002503
8	897.456	663.863	73.58	427	2.10	415	12	7	24	25	3471	2	CP002504
total	9.669.486	7.118.195	73.62	4855	2.05	4712	143	66	224	232	40.071	9/16	

1) The values in coding (bp) and % encoding refer to protein encoding genes, while gene density calculations also include tRNA genes.

2) The number of genes with introns refers to protein coding genes. There are also 44 tRNAs with introns.

Table S2 One *A. gossypii* GC-cold spot harbors many essential genes¹

<i>Ancestor</i>	<i>E. cymbalariae</i>	<i>A. gossypii</i>	<i>S. cerevisiae</i>	Function in <i>S. cerevisiae</i>
Anc_7.332	Ecym_8.082	ADR068W	YLR210W (CLB4)	B-type cyclin
Anc_7.331		absent	YDL154W (MSH5)	meiotic recombination
	Ecym_8.081			
Anc_7.330	Ecym_8.080	ADR069C	YDL153C (SAS10)	essential
Anc_7.329	Ecym_8.079	ADR070W	YDL150W (RPC53)	essential
Anc_7.328	Ecym_8.078	ADR071W	YDL149W (APG9)	Sporulation absent; viability decreased
Anc_7.327	Ecym_8.077	ADR072C	YDL148C (NOP14)	essential
Anc_7.326	Ecym_8.076	ADR073W	YDL147W (RPN5)	essential
Anc_7.325	Ecym_8.075	ADR074C	YLR211C	
Anc_7.324	Ecym_8.074	ADR075W	YDL146W	actin cytoskeleton defects; endocytosis decreased
Anc_7.323	Ecym_8.073	ADR076C	YLR212C (TUB4)	essential
Anc_7.322	Ecym_8.072	ADR077C	YDL145C (COP1)	essential

1. This is one example of a GC coldspot. A more detailed analysis of GC hotspots and GC coldspots will be presented elsewhere.

Table S3 Comparison of tandem duplicated genes of *A. gossypii* with *E. cymbalariae* homologs

Ag ^{1,2,3}	Ag	Ag	Ag	Sc	Ecym	Ecym	Ecym
AAL179W ³	AAL178			YJL079C (PRY1)	Ecym_6325	Ecym_6326	
	W			YKR013W (PRY2)			
				YJL078C (PRY3)			
ABL189W	ABL188			YDL237W	Ecym_6472	Ecym_6471	
	W						
ABR025C	ABR026C	ABR027C	ABR028C	YKL096W (CWP1)	Ecym_2423		
ABR182W	ABR183			YPR165W (RHO1)	Ecym_7211	Ecym_7212	
	W						
ABR246W	ABR247	ABR248W	ABR249	YIR035C/YIR036C	none		
³	W		W				
ACL202W³	ACL201	ACL200W		YMR238W (DFG5)	none		
	W						
ACR098C	ACR099C			YPL129W (ANC1)	Ecym_8318	Ecym_8319	
				YOR213C (SAS5)			
ACR143W	ACR144			YPL154C (PEP4)	Ecym_2396		
	W						
ACR272C³	ACR273			YKL096W (CWP1)	none		
	W						
ADL156C	ADL155C			YOL119C (MCH4)	Ecym_2054		
ADR081C	ADR082			YLR215C (CDC123)	Ecym_8069		
	C						
ADR336C	ADR337			YNR055C (HOL1)	Ecym_5082		
	C						
ADR403C	ADR404C	ADR405C		YAL051W (OAF1)	Ecym_5017	Ecym_5016	Ecym_5015

			YOR363C (PIP2)		
AER452C³	AER453C	AER454C	YJR107W	none	
AFL095W	AFL092C		YHR211W (FLO5)	Ecym_2200	Ecym_2201
AFR262C	AFR263C		YGL246C (RAI1)	Ecym_2600	
AGL352W ³	AGL351		YMR307W (GAS1)	Ecym_5666	Ecym_5665
	W				
AGL326W	AGL325		YJL172W (CPS1)	Ecym_7172	
	W				
AGR038C	AGR039		YDR046C (BAP3)	Ecym_2662	Ecym_2663
	C		YBR068C (BAP2)		
AGR188W	AGR189		YDR227W (SIR4)	Ecym_4126	Ecym_4125
	W				
AGR405C³	AGR406		YCL057W (PRD1)	Ecym_1011	
	C				

1) Ag = *Ashbya gossypii*; Sc = *Saccharomyces cerevisiae*; Ecym = *Eremothecium cymbalariae*

2) Genes printed in bold are absent or not duplicated in *E. cymbalariae* – no duplications in *E. cymbalariae* were detected that do not occur in *A. gossypii*.

3) Positioned at telomeric loci in *A. gossypii*

Table S3b Duplicated genes at telomeric loci in *Ashbya gossypii*

<i>Ashbya gossypii</i> gene	<i>Saccharomyces cerevisiae</i> gene	Location in <i>A. gossypii</i>
ACR082c	YAL018c	TEL3R
ACR083c		TEL3R
ADL027w		Internal
ADR122c		Internal
ADR403c	YAL051w	TEL4R
ADR404c	(OAF1)	TEL4R
ADR405c		TEL4R
AGR405c	YCL057w	TEL7R
AGR406c	(PRD1)	TEL7R
ABR228c	YFL041w	TEL2R
ADR239w	(FET5)	internal
AAR183c	YIL014w	TEL1R
ABR245c	(MNT3)	TEL2R
ADR020c		Internal
AEL345w		TEL5L
AFL235w		TEL6L
AFR301c	YIL159w	Internal
AGL364c	(BNR1)	TEL7L
ACL203c	YIL166c	TEL3L
AER444w		TEL5R
AFR229c		Internal
AGR235w		Internal
ABR246w	YIR035c	TEL2R
ABR247w		TEL2R
ABR248w		TEL2R
ABR249w		TEL2R
ACR171c		internal
AER445c	YJR076c	TEL5R
AFR436c	(CDC11)	Internal
ABR159c	YJR107w	Internal
ACL114w		Internal
AER452c		TEL5R
AER453c		TEL5R
AER454c		TEL5R

ABR025c	YKL096w	Internal
ABR026c	(CWP1)	Internal
ABR027c		Internal
ABR028c		Internal
ACR272c		TEL3R
ACR273w		TEL3R
ADL398c		TEL4L
AAR192c	YKL217w	TEL1R
ABL210c	(JEN1)	TEL2L
AFR333w		Internal
ACL200w	YMR238w	TEL3L
ACL201w	(DFG5)	TEL3L
ACL202w		TEL3L
AFR530w		Internal
AGL351w	YMR307w	TEL7L
AGL352w	(GAS1)	TEL7L
ABL123c	YPL154c	Internal
ACR143w	(PEP4)	Internal
ACR144w		Internal
AGR407c		TEL7R
ACL205c	YPR194c	TEL3L
ADL399c	(OPT2)	TEL4L
AGL027w		internal

Mating-type loci are excluded

Table S4 Genes found in *E. cymbalariae* but not in *A. gossypii*.

E. cymbalariae genes with homologs in *S. cerevisiae* that are absent from *A.**gossypii*

1	Ecym_1002	YCL067C HMLALPHA2
2	Ecym_1003	YCL066W HMLALPHA1
3	Ecym_1114	YCL067C MATALPHA1
4	Ecym_1115	YCL066W MATALPHA2
5	Ecym_1027	YIR030C DCG1
6	Ecym_1028	YDR524C-B
7	Ecym_1059	YDR506C
8	Ecym_1093	YMR318C ADH6
9	Ecym_1223	YBR067C TIP1
10	Ecym_1351	YCR019W MAK32
11	Ecym_1394	YDL209C CWC2
12	Ecym_1496	YCR021C HSP30
13	Ecym_2013	YNL327W EGT2
14	Ecym_2169	YPL223C GRE1
15	Ecym_2268	YGL108C
16	Ecym_2317	YDR387C
17	Ecym_2360	YOR264w DSE3
18	Ecym_2537	YPL039W
19	Ecym_2567	YBR161W CSH1
20	Ecym_2668	YBR071W
21	Ecym_2706	YML107C PML39
22	Ecym_2731	YGL159W
23	Ecym_2772	YLR376C PSY3
24	Ecym_3107	YLR286C CTS1
25	Ecym_3263	YLR394W CST9/ZIP3
26	Ecym_3264	YDL124W

27	Ecym_3364	YOL105C WSC3
28	Ecym_3399	YMR090W
29	Ecym_3408	YKL084W HOT13
30	Ecym_3569	YEL040W UTR2
31	Ecym_4001	YER185W PUG1
32	Ecym_4027	YOR084W LPX1
33	Ecym_4036	YKR087C OMA1
34	Ecym_4205	YDR171W HSP42
35	Ecym_4288	YLR097C HRT3
36	Ecym_4297	YDR119W-A
37	Ecym_4437	YPR134W
38	Ecym_4485	YIL001W
39	Ecym_4487	YIL002W-A
40	Ecym_4607	YML113W DAT1
41	Ecym_5140	YBR047W FMP23
42	Ecym_5363	YOR087W YVC1
43	Ecym_5427	YNL115C
44	Ecym_5553	YDR072C IPT1
45	Ecym_5557	YDR061W
46	Ecym_5577	YGR110W CLD1
47	Ecym_6004	YGL251C HFM1/MER3
48	Ecym_6006	YGL249W ZIP2
49	Ecym_6031	YOR390W
50	Ecym_6064	YLR040C
51	Ecym_6268	YLR363C NMD4
52	Ecym_7011	YJR152W DAL5
53	Ecym_7049	YBR188C NTC20
54	Ecym_7097	YHR149C SKG6
55	Ecym_7235	YBL005W PDR3

56	Ecym_7251	YMR073C IRC21
57	Ecym_7252	YMR072W ABF2
58	Ecym_7256	YMR069W NAT4
59	Ecym_7277	YPR010C-A
60	Ecym_7308	YFL003C MSH4
61	Ecym_7342	YGL041W-A
62	Ecym_7419	YOR320C GNT1
63	Ecym_8023	YIL073C SPO22/ZIP4
64	Ecym_8081	YDL154W MSH5
65	Ecym_8390	YDR032C PST2
66	Ecym_8420	YHL038C CBP2

***E. cymbalariae* genes absent from *S. cerevisiae* and *A. gossypii* but share**

homologs in other yeasts¹

67	Ecym_1007	KLLA0C00462g
68	Ecym_1012	KLLA0C00594g
69	Ecym_1082	KLLA0C02233g
70	Ecym_1420	KLTH0H09306g
71	Ecym_2062	KLLA0E15620g
72	Ecym_2283	KLLA0B00495g
73	Ecym_3096	KLLA0A04521g
74	Ecym_3372	KLLA0E14806g
75	Ecym_4528	KLLA0E03377g
76	Ecym_5045	KLLA0F23221g
77	Ecym_5162	KLLA0B04224g
78	Ecym_5211	KLLA0E15356g
79	Ecym_5262	KLLA0C04433g
80	Ecym_5281	KLTH0C08624g
81	Ecym_5338	KLLA0F21164g

82	Ecym_5415	KLLA0F23573g
83	Ecym_6130	KLLA0A02871g
84	Ecym_6199	KLLA0C11539g
85	Ecym_6395	KLLA0D11066p
86	Ecym_6433	KLLA0D08624p
87	Ecym_6440	KLLA0F20856g
88	Ecym_7076	KLLA0E24442g
89	Ecym_7169	KLLA0E05544g
90	Ecym_8149	KLLA0D10395g
91	Ecym_8160	KLLA0E17039g
92	Ecym_8221	KLLA0F22198g
93	Ecym_8354	KLLA0D14399g ScYAP5/YAP7
94	Ecym_1178	KLLA0E23079g
95	Ecym_2506	KLTH0A06908g
96	Ecym_2679	KLTH0A00440g
97	Ecym_5041	KLTH0D01408g
98	Ecym_7253	KLTH0D06886g
99	Ecym_7302	KLTH0A02728g
100	Ecym_4373	Kpol_1063p1
101	Ecym_3623	NFIA_005660
102	Ecym_3158	SCOCAAB17122
103	Ecym_3156	ZYRO0G01870g
104	Ecym_5064	ZYRO0D04224g

Hypothetical *E. cymbalariae* genes without homolog in the databases

105	Ecym_1213
106	Ecym_1460
107	Ecym_2057
108	Ecym_2064

109	Ecym_2234
110	Ecym_2655
111	Ecym_2740
112	Ecym_2756
113	Ecym_2780
114	Ecym_3228
115	Ecym_3234
116	Ecym_3288
117	Ecym_3377
118	Ecym_3379
119	Ecym_3380
120	Ecym_3401
121	Ecym_3441
122	Ecym_3536
123	Ecym_4267
124	Ecym_4268
125	Ecym_4495
126	Ecym_4582
127	Ecym_4583
128	Ecym_4774
129	Ecym_5223
130	Ecym_5230
131	Ecym_5238
132	Ecym_5449
133	Ecym_5602
134	Ecym_6046
135	Ecym_6063
136	Ecym_6119
137	Ecym_6120

138	Ecym_6121
139	Ecym_6172
140	Ecym_6237
141	Ecym_6297
142	Ecym_6371
143	Ecym_6450
144	Ecym_7298
145	Ecym_7394
146	Ecym_7433
147	Ecym_7444
148	Ecym_8269

1) KLLA: *Kluyveromyces lactis*; KLTH: *Lachancea thermotolerans*; Kpol: *Vanderwaltozyma polyspora*; NFIA: *Neosartorya fischeri*;
SCOC: *Schwanniomyces occidentalis*; ZYR: *Zygosaccharomyces rouxii*

Table S5 Genes found in *A. gossypii* but not in *E. cymbalariae*.

A. gossypii genes with homologs in *S. cerevisiae* that are absent from *E. cymbalariae*

1	AAL068C	YHR035W
2	AAL112C	YOR258W (HNT3)
3	AAL128C	YBR072W (HSP26)
4	AAR033W	YPR127W
5	AAR084W	YMR303C (ADH2)
6	AAR086W	YGR097W (ASK10)
7	AAR129C	YDR415C
8	AAR183C	YIL014W (MNT3)
9	ABL084C	YJR142W
10	ABL090W	YBR046C (ZTA1)
11	ABL210C	YKL217W (JEN1)
12	ABR050W	YMR012W (CLU1)
13	ABR053C	YMR015C (ERG5)
14	ABR113C	YLL055W
15	ABR185W	YCL057W (PRD1)
16	ABR229C	YJR159W (SOR1)
17	ABR245C	YIL014W (MNT3)
18	ACL085C	YNL092W
19	ACL203C	YIL166C
20	ACL205C	YPR194C (OPT2)
21	ACR211W	YDR380W (ARO10)
22	ADL015C	YNL104C (LEU4)
23	ADL194W	YOL141W (PPM2)
24	ADL199W	YML005W
25	ADL258W	YNL065W (AQR1)
26	ADL328C	YPR062W (FCY1)
27	ADL362C	YGR161C
28	ADL392W	YHR202W
29	ADL397C	YBR072W (HSP26)
30	ADR020C	YIL014W (MNT3)
31	ADR079C	YDL144C
32	ADR119W	YBR284W
33	ADR142W	YMR064W (AEP1)
34	ADR199C	YDR421W (ARO80)
35	ADR205C	YOR062C

36	ADR229C	YOR079C (ATX2)
37	ADR237C	YHL039W
38	ADR286C	YML004C (GLO1)
39	ADR365W	YOR337W (TEA1)
40	AEL057C	YCR023C
41	AEL099W	YDR338C
42	AEL114C	YJL163C
43	AEL143W	YCR028C (FEN2)
44	AEL151C	YJL190C (RPS22A)
45	AEL171C	YJL059W (YHC3)
46	AEL345W	YIL014W (MNT3)
47	AER313C	YDR452W (PPN1)
48	AER338C	YMR209C
49	AER401W	YDR368W (YPR1)
50	AER409C	YDR022C (CIS1)
51	AER426C	YIL137C
52	AER444W	YIL166C
53	AER445C	YJR076C (CDC11)
54	AER459w	YBR072W (HSP26)
55	AFL158C	YAL067C (SEO1)
56	AFL160C	YPL248C (GAL4)
57	AFL204C	YHR096C (HXT5)
58	AFL235W	YIL014W (MNT3)
59	AFR045W	YHR132C (ECM14)
60	AFR206C	YKL140W (TGL1)
61	AFR228W	YKR093W (PTR2)
62	AFR322C	YGR138C (TPO2)
63	AFR333W	YKL217W (JEN1)
64	AFR415C	YJR062C (NTA1)
65	AFR437W	YBR072W (HSP26)
66	AFR506C	YFL014W (HSP12)
67	AFR548C	YGL202W (ARO8)
68	AFR561W	YMR226C
69	AFR633W	YBR072W (HSP26)
70	AFR675W	YNL274C
71	AFR726W	YDR525W-A (SNA2)
72	AGL027W	YPR194C (OPT2)
73	AGL049C	YGR017W
74	AGL050C	YGR019W (UGA1)

75	AGL087C	YGL012W (ERG4)
76	AGL142C	YPL058C (PDR12)
77	AGL143C	YLR130C (ZRT2)
78	AGL322W	YBL039W-B
79	AGL359C	YER187W
80	AGR108C	YER176W (ECM32)
81	AGR110W	YER175C (TMT1)
82	AGR235W	YIL166C
83	AGR250C	YGR111W
84	AGR297C	YKR070W
85	AGR329C	YHR179W (OYE2)
86	AGR407C	YPL154C

A. gossypii* genes absent from *S. cerevisiae* and *E. cymbalariae

87	ABL004W
88	ABL060C
89	ABL062C
90	ABL067C
91	ABL157W
92	ABL167C
93	ABR042W
94	ACL007C
95	ACL049W
96	ACL115W
97	ACL133W
98	ACL156W
99	ACL204W
100	ACR002C
101	ACR141W
102	ACR169C
103	ADL011W
104	ADL070C
105	ADL112W
106	ADL148C
107	ADL359C
108	ADL393W
109	ADL400W
110	ADR057W
111	ADR100C

112	ADR245W
113	ADR246C
114	ADR347C
115	AER099C
116	AER101W
117	AER160C
118	AER382W
119	AER455C
120	AFL013C
121	AFL028W
122	AFL126W
123	AFL137W
124	AFL164C
125	AFL233C
126	AFL234C
127	AFR048W
128	AFR070W
129	AFR079C
130	AFR164W
131	AFR170C
132	AFR288C
133	AFR311C
134	AFR476C
135	AFR622W
136	AFR740W
137	AFR742W
138	AFR746C
139	AGL037W
140	AGL048C
141	AGL084C
142	AGL146W
143	AGL277W
144	AGL309W
145	AGL366C
146	AGL367C
147	AGL368W
148	AGL369W
149	AGR013C
150	AGR055C

151	AGR090C
152	AGR124C
153	AGR404W
