Supplemental material.

Generation of deletion constructs. Gene deletions were generated as described in Davidson et al. (2002) and and Gerik et al (2005). As an example, the *chs2* deletion construct was made by overlap PCR and fused together the 5' region of *CHS2* including 1017 bps of coding sequence (made by PCR using primers 1-CHS2 and 4-CHS2), the hygromycin cassette (Hua *et al*., 2000) utilizing primers 3-CHS2 and 6-CHS2, and the 3' region of *CHS2* including 1075 bps of coding sequence (made by PCR using primers 5-CHS2 and 2-CHS2). This fusion resulted in the deletion of 2769 bps of the *CHS2* gene. All other deletion constructs were made in a similar manner as *chs2*Δ. Primer sequences are listed in Table 1 of the supplemental material. These linear constructs were biolistically transformed into *C. neoformans* H99.

PCR analysis of transformants. A three primer PCR screen was used to prove homologous integration on both the 5' and 3' ends of all of the deletion constructs (Nelson *et al*., 2003). An example of this screen is the 5' end of the *chs2* deletion construct. Three PCR primers that corresponded to i) genomic sequence outside the deletion construct (7-CHS2), ii) genomic sequence from the deleted portion of the gene (9-CHS2), and iii) the HYG selectable marker cassette (Cn-actin7; TCCTCTCCTCCGACAACC) were used in a PCR reaction together. If the wild-type gene was present, a 1271 bp PCR product was made from the 7-CHS2 and 9-CHS2 primers, and if a homologous recombination event occurred, an approximately 200 bp larger PCR product from the 7-CHS2 and Cn-actin7 primers was synthesized. In this manner, homologous recombinants were distinguished from wild-type. Integration at the 3' end of the deletions was identified in the same manner using primers 8-CHS2, 10-CHS2 and Gal7-terminator50 (TGTCGGAATGGACGATCGACC). A PCR screen using primers 7-CHS2 and 8-CHS2, which were made to regions flanking recombination, amplified the entire region spanning the insertions. The wild-type construct was 5344 bp and the homologous recombinants were 5171 bp, demonstrating that a single copy of the transforming DNA had been inserted at the *CHS2* locus. A similar strategy was used to screen for all deletions that inserted the Hygromycin resistance cassette using primers 7-geneX, 9-geneX, and Cn-actin7 for the 5' screen, primers 8-geneX, 10-geneX and Gal7-

terminator50 for the 3' screen, and primers 7-geneX and 8-geneX for the full-length screen. For deletions containing the NAT resistance cassette, NAT-3 (AACGCCGTTGAATCCTCAGG) and Cn-actin7 primers were used in place of Cn-actin7 and Gal7-terminator50, respectively, in the triplex PCR screening for the 5' and 3' ends.

Supplemental Table 1.

Gene	primer #[1]	primer sequence
CHS1	1	AAGGTGAAGGTGCGGATGTACTCGCTTC
	2	GTACGATGCTCGACGCGAATGTTTCAGT
	3	TCAACCAACCACATCCCGCCCcaggaaacagctatgaccatg
	4	catggtcatagctgtttcctgGGGCGGGATGTGGTTGGTTGA
	5	cactggccgtcgttttacaacTCGGCGGGGTCAGTACTCCGTTT
	6	AAACGGAGTACTGACCCCGCCGAgttgtaaaacgacggccagtg
	7	GATGAATGGGAACGGGAAAAAGAAGCAG
	8	GCCTGAGACAAAACCTGACCCAACGAAT
	9	GCGACGTGTCGTTCAGGTCGAG
	10	TGGGCGAGGAGCGAGTATGACA
	11	TGGTAGGGTTTGTGACGATGG
	12	ATGAGTGGGGCATTGGGTAAG
CHS2	1	AGGACAGAGTGAGCGAATGAC
	2	CCAAAATAACCCGGTTAAGC
	3	GAAAGAATGGGGCGTCGGATCcaggaaacagctatgaccatg
	4	catggtcatagctgtttcctgGATCCGACGCCCCATTCTTTC
	5	cactggccgtcgttttacaacGTACTATGTTAAGCCACATCG
	6	CGATGTGGCTTAACATAGTACgttgtaaaacgacggccagtg
	7	AGACCATTTACGCTTGGTCC
	8	GACTCATACATGGTCATGCC
	9	ACAGGTGGTATCGTAGTAACC
	10	GCTCGACTTTCAATCCTTACC
	11	ATGTATGGCCGACCCAGTCCT
	12	TCACTCGCCTCTCGAGCAACT
CHS3	1	CATCACGTGCCCTCGGCGTCGGCTATCC
	2	AACAAACATTTAATACTGGGGAGGCGCC
	3	GGACCCAAGAATTGCTGGATcaggaaacagctatgaccatg
	4	catggtcatagctgtttcctgATCCAGCAATTCTTGGGTCC
	5	cactggccgtcgttttacaacTGATTCTTCCGGCATATTCC
	6	GGAATATGCCGGAAGAATCAgttgtaaaacgacggccagtg

	7	TCTCCACCCGACCGCTTTCCCATCAACC
	8	CCTAGATACTCTTATGTCCCTTGATCC
	9	CGATACCGATCTTTTCTCTCC
	10	GTGTATCTGATCTCACTTCCC
	11	GCGGTCGGTTTCATTACCTTC
	12	TATGGGCAGGAGGACCAAGTT
CHS4	1	GCATGGAGGAGGAGGTGCAGAGATGTAA
	2	TGTGTCAAAAATCCGTGGTGAGGAGACA
	3	GTTCGCAAGCACCGTTTCATCGCcaggaaacagctatgaccatg
	4	catggtcatagctgtttcctgGCGATGAAACGGTGCTTGCGAAC
	5	cactggccgtcgttttacaacCGCACCCCCACTCTATCCGTTCA
	6	TGAACGGATAGAGTGGGGGTGCGgttgtaaaacgacggccagtg
	7	TTGGTGAGTCCAAGTTTCTGACTTCCAG
	8	GGTGGCAAAAAGTCCTTTCCATATGAGG
	9	TGTCTGGCGCTGATCTCTGATCG
	10	TGGAATGCTTGGGAAGCAGCA
	11	AGCGGGACGTATTTGCTGTTC
	12	AACGGACCCGAAGTATGCACT
CHS5	1	TAGGCAGCAGTAACGAGCAGCGAGAAGA
	2	TCATACGTGGCAGCGAGACGGGATATAG
	3	AACACAGTGCAGCGGGCCAACcaggaaacagctatgaccatg
	4	catggtcatagctgtttcctgGTTGGCCCGCTGCACTGTGTT
	5	cactggccgtcgttttacaacTGAGAGGAGGGAGGCCATCAA
	6	TTGATGGCCTCCCTCCTCTCAgttgtaaaacgacggccagtg
	7	CACGGTTAAATCAGCAACTCCATCTGCAC
	8	GCACCCAGTACAGAAAGAACGGCTCACC
	9	AGGGTTGGCGAGGCTGACCTGA
	10	TGTGCGAATGCAGAGCTGGACA
	11	AATGCCCATACCCTGTCCAAC
	12	GCGATGTTTTTCATCCCGAAT
CHS6	1	GGGGGAGACGAACTATGGAGGGGAGATA
	2	CCCAAGCAGATCAAGGTACATGGTGGAA
	3	TATCACGCAAACCTCCTCCGCGcaggaaacagctatgaccatg
	4	catggtcatagctgtttcctgCGCGGAGGAGGTTTGCGTGATA
	5	cactggccgtcgttttacaacTTGACGAGGGTTCCGGCAGTT
	6	AACTGCCGGAACCCTCGTCAAgttgtaaaacgacggccagtg
	7	TCGCGGGAACTGATGAAATCAGCTGTAG
	8	GGTCAAGCTCCCCCTCCTCCTTATACCA
	9	CGGGGGTGATTGTATGGGAGCA
	10	CGCTGTCAAACGCCCTTCTCG
	11	GGCCCCCTCTTATGACTACCA

	12	ATCGAGGGTCGCTGATTGTTT
CHS7	1	GTCATCGGTCTACTAGCCCCACCTCTGC
	2	TGCCCCTGTCCTTCACTATCCTTCCTGT
	3	TGAGTGGGGGTCCTGACCTTCCcaggaaacagctatgaccatg
	4	catggtcatagctgtttcctgGGAAGGTCAGGACCCCCACTCA
	5	cactggccgtcgttttacaacGCATGCGTGCGTCTTCGCCTA
	6	TAGGCGAAGACGCACGCATGCgttgtaaaacgacggccagtg
	7	ATGACTTCCTTGGGCTGTAGTGGTAGGG
	8	AGCTGAGGGTCCAAACCTGACTTCTCAT
	9	CTGCGCTGGAAATTGAGGCAGA
	10	TGGGGCGTAAAACCATTGCAAGA
	11	GCCAAATGGCTCAATGACAAG
	12	GTCCAACAGCACGCAAATCTC
CHS8	1	CTTCCAGCCTCAGCTGCTTCCTTCTCTC
	2	CGACGACGATACAGGTTTTCCTTCGACA
	3	CCATCACATCTTCCACCCCAGCAcaggaaacagctatgaccatg
	4	catggtcatagctgtttcctgTGCTGGGGTGGAAGATGTGATGG
	5	cactggccgtcgttttacaacCGTTCGTCTGTTCGCCGGAGAGT
	6	ACTCTCCGGCGAACAGACGAACGgttgtaaaacgacggccagtg
	7	GTGGCCGCATCTGGTTCCATAATTGTT
	8	TCACTGTTGGGCTCTGCAGTCTCATGTC
	9	GGGGAGGTGAGACGGTCATCAAG
	10	CTGTGGGGCACAATGCTGACG
	11	CACATCTTCCACCCCAGCAAC
	12	TTTGCGTCCTATTTCGCCTGA
CSR1	1	CTGCCGAGCAACTCAAGCAGCCAGAACC
	2	ACGTAAGTGCCGATGTCTATCGTTTTCC
	3	CGCCTCTTCTCGATCAATGCCcaggaaacagctatgaccatg
	4	catggtcatagctgtttcctgGGCATTGATCGAGAAGAGGCG
	5	cactggccgtcgttttacaacCTGGACTTTCCTGATACGTCC
	6	GGACGTATCAGGAAAGTCCAGgttgtaaaacgacggccagtg
	7	CCCAGCGCCGCTCCCATACGTACGACAG
	8	CGATGACAGTAATTGACAACGGTGACTC
	9	CGCGTGGAAGAAGCGCCAACC
	10	TGCGCGCAGCAGGTATGTACC
CSR2	1	ACAGCATTTTCGCTGACCTGGAACTCCT
	2	AGCCCCCATCATTACCAAAACGACAGAA
	3	ATCAATTCGGCCCCAACTCCCGcaggaaacagctatgaccatg
	4	catggtcatagctgtttcctgCGGGAGTTGGGGCCGAATTGAT
	5	cactggccgtcgttttacaacAGCGGTGCAAGTGGGAAGGATG

	6	CATCCTTCCCACTTGCACCGCTgttgtaaaacgacggccagtg
	7	AACATACGTGGCACCTTGCACATCAACA
	8	GGTGAATACCGAACTTCTCCGGACACTG
	9	GCTGACGACACGGCTTCCCCTAT
	10	GATTAGGAGCCGCCAACCTTGC
CSR3	1	GAAATCCATCCGTACCCAACTGCCGAAT
	2	TTTATGCATTGGCCCCTCATTCACAACC
	3	ATCCCTCCAGCATCCCCACCCAcaggaaacagctatgaccatg
	4	catggtcatagctgtttcctgTGGGTGGGGATGCTGGAGGGAT
	5	cactggccgtcgttttacaacCCTTTCCCAATATCCACCAACGC
	6	GCGTTGGTGGATATTGGGAAAGGgttgtaaaacgacggccagtg
	7	AATTCCAAGGCCGAAGATTTTGCACCTG
	8	TTCAGCTGATCCGGAGTTGGTTGGAATC
	9	GGCGGCGCGTTCGATTGATTC
	10	TGCATGTGATTGCTGGTTGCTGA

1. Primer numbers correspond to the following designations.

Primers 1-10 were used to generate the gene deletion cassette and/or screen the potential deletion mutants as described above. Primers 11 and 12 were used to generate the unique gene-specific fragment that was used as a probe and in positive binding control ladder in the quantitative northern blots.