Electronic Supplementary Material (ESI) for Molecular BioSystems

Text S1

Supporting Materials and Methods

Strain Construction

ScLC427

Constructed by mating ScLC11 and ScLC152. Diploids were sporulated and G418 resistant meiotic progeny were verified for the proper deletion allele by PCR with primers oLC101 and oLC325 for *cpr1*Δ.

ScLC442, 443, 444

Constructed by mating ScLC427 and *bck1*Δ. Diploids were sporulated and G418 resistant meiotic progeny were verified for the proper deletion allele by PCR with primers oLC101 and oLC325 for *cpr1*Δ and oLC101 and oLC327 for *bck1*Δ.

ScLC445, 446, 447

Constructed by mating ScLC427 and *chs3*Δ. Diploids were sporulated and G418 resistant meiotic progeny were verified for the proper deletion allele by PCR with primers oLC101 and oLC325 for *cpr1*Δ and oLC101 and oLC328 for *chs3*Δ.

ScLC448, 449, 450

Constructed by mating ScLC427 and *skt5*Δ. Diploids were sporulated and G418 resistant meiotic progeny were verified for the proper deletion allele by PCR with primers oLC101 and oLC325 for *cpr1*Δ and oLC101 and oLC329 for *skt5*Δ.

ScLC451, 452, 453

Constructed by mating ScLC427 and *chs7*Δ. Diploids were sporulated and G418 resistant meiotic progeny were verified for the proper deletion allele by PCR with primers oLC101 and oLC325 for *cpr1*Δ and oLC101 and oLC330 for *chs7*Δ.

ScLC454, 455, 456

Constructed by mating ScLC428 and *bck1*Δ. Diploids were sporulated and G418 resistant meiotic progeny were verified for the proper deletion allele by PCR with primers oLC101 and oLC149 for *cnb1*Δ and oLC101 and oLC327 for *bck1*Δ.

ScLC457, 458, 459

Constructed by mating ScLC428 and *chs3*Δ. Diploids were sporulated and G418 resistant meiotic progeny were verified for the proper deletion alleles by PCR with primers oLC101 and oLC149 for *cnb1*Δ and oLC101 and oLC328 for *chs3*Δ.

ScLC460, 461, 462

Constructed by mating ScLC428 and *skt5*Δ. Diploids were sporulated and G418 resistant meiotic progeny were verified for the proper deletion alleles by PCR with primers oLC101 and oLC149 for *cnb1*Δ and oLC101 and oLC329 for *skt5*Δ.

ScLC1275

Constructed by mating ScLC489 and ScLC152 to obtain an alpha version of *chs3*Δ, which was then marker swapped with the NatMX cassette that was PCR amplified from pLC1 using oLC1138 and oLC1139 via transformation. NAT-resistant, G418-sensitive colonies were picked.

ScLC1278, 1279, 1280

Constructed by mating ScLC1275 and *cpr2*Δ. Diploids were sporulated and G418 resistant meiotic progeny were verified for the proper deletion alleles by PCR with primers oLC101 and oLC328 for *chs3*Δ and oLC101 and oLC424 for *cpr2*Δ.

ScLC1281, 1282, 1283

Constructed by mating ScLC1275 and *cpr3*Δ. Diploids were sporulated and G418 resistant meiotic progeny were verified for the proper deletion alleles by PCR with primers oLC101 and oLC328 for *chs3*Δ and oLC101 and oLC425 for *cpr3*Δ.

ScLC1284, 1285, 1286

Constructed by mating ScLC1275 to *cpr4*Δ. Diploids were sporulated and G418 resistant meiotic progeny were verified for the proper deletion alleles by PCR with primers oLC101 and oLC328 for *chs3*Δ and oLC101 and oLC426 for *cpr4*Δ.

ScLC1287, 1288, 1289

Constructed by mating ScLC1275 and *cpr5*Δ. Diploids were sporulated and G418 resistant meiotic progeny were verified for the proper deletion alleles by PCR with primers oLC101 and oLC328 for *chs3*Δ and oLC101 and oLC427 for *cpr5*Δ.

ScLC1290, 1291, 1292

Constructed by mating ScLC1275 and *cpr6*Δ. Diploids were sporulated and G418 resistant meiotic progeny were verified for the proper deletion alleles by PCR with primers oLC101 and oLC328 for *chs3*Δ and oLC101 and oLC428 for *cpr6*Δ.

ScLC1293, 1294, 1295

Constructed by mating ScLC1275 and *cpr7*Δ. Diploids were sporulated and G418 resistant meiotic progeny were verified for the proper deletion alleles by PCR with primers oLC101 and oLC328 for *chs3*Δ and oLC101 and oLC429 for *cpr7*Δ.

ScLC1296, 1297, 1298

Constructed by mating ScLC1275 to *cpr8*Δ. Diploids were sporulated and G418 resistant meiotic progeny were verified for the proper deletion alleles by PCR with primers oLC101 and oLC328 for *chs3*Δ and oLC101 and oLC430 for *cpr8*Δ.

ScLC1708

The *chs3::HygB* knock-out cassette was PCR amplified from pLC3 using oLC1519 and oLC1520, which have 45 bp of homology to *CHS3* on each end. 20 µl of PCR product was transformed into ScLC1516 using the standard LiOAC yeast transformation protocol. HygB

resistant transformants were PCR tested using primers oLC328 and oLC1388 to test for upstream integration of the HygB resistance cassette and oLC1389 and oLC1447 to test for downstream integration. Presence of the deletion was PCR tested using primers oLC328 and oLC1447, which would amplify a band ~2 kb for the deletion or 4170 bp for the wild-type allele.

ScLC1710

The *chs3::HygB* knock-out cassette was PCR amplified from pLC3 using primers oLC1519 and oLC1520, which have 45 bp of homology to *CHS3* on each end. 20 µl of PCR product was transformed into ScLC1533 using the standard LiOAC yeast transformation protocol. HygB resistant transformants were PCR tested with primers oLC328 and oLC1388 to test for upstream integration of the HygB resistance cassette and oLC1389 and oLC1447 to test for downstream integration. Presence of the deletion was PCR tested using primers oLC328 and oLC1447, which would amplify a band ~2 kb for the deletion or 4170 bp for the wild-type allele.

ScLC1711

The *chs3::HygB* knock-out cassette was PCR amplified from pLC3 using primers oLC1519 and oLC1520, which have 45 bp of homology to *CHS3* on each end. 20 µl of PCR product was transformed into ScLC1542 using the standard LiOAC yeast transformation protocol. HygB resistant transformants were PCR tested with primers oLC328 and oLC1388 to test for upstream integration of the HygB resistance cassette and oLC1389 and oLC1447 to test for downstream integration. Presence of the deletion was PCR tested using primers oLC328 and oLC1447, which would amplify a band ~2 kb for the deletion or 4170bp for the wild-type allele.

ScLC1712

The *chs3::HygB* knock-out cassette was PCR amplified from pLC3 using primers oLC1519 and oLC1520, which have 45 bp of homology to *CHS3* on each end. 20 µl of PCR product was transformed into ScLC1544 using the standard LiOAC yeast transformation protocol. HygB resistant transformants were PCR tested with primers oLC328 and oLC1388 to test for upstream integration of the HygB resistance cassette and oLC1389 and oLC1447 to test for downstream integration. Presence of the deletion was PCR tested using primers oLC328 and oLC1447, which would amplify a band ~2 kb for the deletion or 4170 bp for the wild-type allele.

ScLC1937, 1938

CNB1 was knocked out from ScLC1712 using pLC537, which was digested with NotI to liberate the NAT cassette. NAT resistant transformants were PCR tested with primers oLC1170 and oLC274 and oLC659 and oLC1173.

ScLC1939, 1940

CNB1 was knocked out from ScLC1710 using pLC537, which was digested with NotI to liberate the NAT cassette. NAT resistant transformants were PCR tested with primers oLC1170 and oLC274 and oLC659 and oLC1173.

Supporting References

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