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Large-scale genome reorganization in *Saccharomyces cerevisiae* through combinatorial loss of mini-chromosomes

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A highly efficient technique, termed PCR-mediated chromosome splitting (PCS), was used to create cells containing a variety of genomic constitutions in a haploid strain of *Saccharomyces cerevisiae*. Using PCS, we constructed two haploid strains, ZN92 and SH6484, that carry multiple mini-chromosomes. In strain ZN92, chromosomes IV and XI were split into 16 derivative chromosomes, seven of which had no known essential genes. Strain SH6484 was constructed to have 14 mini-chromosomes carrying only non-essential genes by splitting chromosomes I, II, III, VIII, XI, XIII, XIV, XV, and XVI. Both strains were cultured under defined nutrient conditions and analyzed for combinatorial loss of mini-chromosomes. During culture, cells with various combinations of mini-chromosomes arose, indicating that genomic reorganization could be achieved by splitting chromosomes to generate mini-chromosomes followed by their combinatorial loss. We found that although non-essential mini-chromosomes were lost in various combinations in ZN92, one mini-chromosome (18 kb) that harbored 12 genes was not lost. This finding suggests that the loss of some combination of these 12 non-essential genes might result in synthetic lethality. We also found examples of genome-wide amplifications induced by mini-chromosome loss. In SH6484, the mitochondrial genome, as well as the copy number of genomic regions not contained in the mini-chromosomes, was specifically amplified. We conclude that PCS allows for genomic reorganization, in terms of both combinations of mini-chromosomes and gene dosage, and we suggest that PCS could be useful for the efficient production of desired compounds by generating yeast strains with optimized genomic constitutions.

[**Key words:** Large scale genome reorganization; PCR-mediated chromosome splitting; *Saccharomyces cerevisiae*; DNA microarray; Mini chromosome]

Chromosome and genome engineering technologies offer powerful tools that can be utilized not only to breed commercially valuable strains but also to understand the function and the organization of the genome. Recently, we developed a method for splitting chromosomes called PCR-mediated chromosome splitting technology (PCS), a key technology for chromosome and genome engineering in budding yeast *Saccharomyces cerevisiae* (1–14). By a simple repeated transformation, this technology allows us to construct yeast cells containing multiple mini-chromosomes. As chromosomes of less than 50 kb are readily lost during mitotic cell divisions (15), yeast cells containing a particular set of mini-chromosomes can generate novel genomic constitutions through combinatorial loss of the mini-chromosomes. By culturing under different conditions, this technology, which we named genome reorganization technology (GReO technology), can be used to generate yeast cells containing different subsets of mini-chromosomes that are adapted to the particular cultural conditions employed. The objectives of this study were to validate the GReO technology and to investigate how much variety in genomic organization can be produced in yeast cells. To these ends, we constructed two model haploid strains of *S. cerevisiae*, one harboring seven non-essential mini-chromosomes and the other harboring fourteen non-essential mini-chromosomes; all were derived from natural chromosomes. Using a new DNA microarray technology called "3D-Gene" (which we recently developed and which has the advantageous features of an ultra-high sensitivity and an extremely low level of cross hybridization (16)), we investigated the extent of genomic reorganization in yeast cells following combinatorial loss of mini-chromosomes.

Our analyses showed that a wide degree of genomic reorganization could occur. Interestingly, and unexpectedly, we also found that the copy number of some mini-chromosomes increased despite the presence of a functional centromere that would be expected to limit the copy number of the mini-chromosome to one. Moreover, we

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TABLE 1. Yeast strains and plasmids used in this study.

Strain	Genotype	Remarks
FY833	*MAT*a *ura3-52 his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63*	Winston et al. (17)
FY834	*MATα ura3-52 his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63*	Winston et al. (17)
ZN1	*MATα ura3-52 his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 Ch4(TEL-932757.lys4) Ch4(hisG-URA3-hisG:lys4.931771-TEL)*	Derived from FY834
ZN3	*MATα ura3-52 his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 Ch4(TEL-511437.lys14:loxP-CgTRP1-loxP) Ch4(lys14.510818-932757.lys4) Ch4(hisG:lys4.931771-TEL)*	Derived from ZN1
ZN11	*MATα ura3-52 his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 Ch4(TEL-511437.lys14:loxP) Ch4(lys14.510818-932757.lys4) Ch4(hisG:lys4.931771-TEL)*	Derived from ZN3 by eliminating *CgTRP1* through transformation with pSH47
ZN12	*MATα ura3-52 his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 Ch4(TEL-511437.lys14:loxP) Ch4(lys14.510818-932757.lys4) Ch4(hisG:lys4.931771-1288498.ade8:loxP-CgTRP1-loxP) Ch4(ade8.1288499-TEL)*	Derived from ZN11
ZN13	*MATα ura3-52 his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 Ch4(TEL-160868) Ch4(loxP-CgLEU2-loxP:160869–511437.lys14:loxP) Ch4(lys14.510818-932757.lys4) Ch4(hisG:lys4.931771-1288498.ade8:loxP-CgTRP1-loxP) Ch4(ade8.1288499-TEL)*	Derived from ZN12
ZN14	*MATα ura3-52 his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 Ch4(TEL-160868) Ch4(loxP-CgLEU2-loxP:160869–258679) CH4(loxP-CgHIS3-loxP:258680–511437.lys14:loxP) Ch4(lys14.510818-932757.lys4) Ch4(hisG:lys4.931771-1288498.ade8:loxP-CgTRP1-loxP) Ch4(ade8.1288499-TEL)*	Derived from ZN13
ZN16	*MATα ura3-52 his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 Ch4(TEL-160868) Ch4(loxP:160869–258679) Ch4(loxP:258680–511437.lys14:loxP) Ch4(lys14.510818-932757.lys4) Ch4(hisG:lys4.931771-1288498.ade8:loxP) Ch4(ade8.1288499-TEL)*	Derived from ZN14 by eliminating *CgLEU2*, *CgTRP1* and *CgHIS3* through transformation with pSH47
ZN17	*MATα ura3-52 his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 Ch4(TEL-160868) Ch4(loxP:160869–239739) Ch4(loxP-CgLEU2-loxP:239740–258679) Ch4(loxP:258680–511437.lys14:loxP) Ch4(lys14.510818-932757.lys4) Ch4(hisG:lys4.931771-1288498.ade8:loxP) Ch4(ade8.1288499-TEL)*	Derived from ZN16
ZN22	*MATα ura3-52 his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 Ch4(TEL-130674) Ch4(loxP-CgHIS3-loxP:130675–160868) Ch4(loxP:160869–239739) Ch4(loxP-CgLEU2-loxP:239740–258679) Ch4(loxP:258680–511437.lys14:loxP) Ch4(lys14.510818-932757.lys4) Ch4(hisG:lys4.931771-1288498.ade8:loxP) Ch4(ade8.1288499-TEL)*	Derived from ZN17
ZN23	*MATα ura3-52 his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 Ch4(TEL-130674) Ch4(loxP-CgHIS3-loxP:130675–160868) Ch4(loxP:160869–239739) Ch4(loxP-CgLEU2-loxP:239740–258679) Ch4(loxP:258680–511437.lys14:loxP) Ch4(lys14.510818-932757.lys4) Ch4(hisG:lys4.931771-1288498.ade8:loxP) Ch4(ade8.1288499-1501017:loxP-CgTRP1-loxP) Ch4(1501018-TEL)*	Derived from ZN22
ZN26	*MATα ura3-52 his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 Ch4(TEL-130674) Ch4(loxP:130675–160868) Ch4(loxP:160869–239739) Ch4(loxP:239740–258679) Ch4(loxP:258680–511437.lys14:loxP) Ch4(lys14.510818-932757.lys4) Ch4(hisG:lys4.931771-1288498.ade8:loxP) Ch4(ade8.1288499-1501017:loxP) Ch4(1501018-TEL)*	Derived from ZN23 by eliminating *CgLEU2*, *CgTRP1* and *CgHIS3* through transformation with pSH47
ZN27	*MATα ura3-52 his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 Ch4(TEL-130674) Ch4(loxP:130675–160868) Ch4(loxP:160869–239739) Ch4(loxP:239740–258679) Ch4(loxP:258680–511437.lys14:loxP) Ch4(lys14.510818-679709:CgTRP1-loxP) Ch4(679710–932757.lys4) Ch4(hisG:lys4.931771-1288498.ade8:loxP) Ch4(ade8.1288499-1501017:loxP) Ch4(1501018-TEL)*	Derived from ZN26
ZN37	*MATα ura3-52 his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 Ch4(TEL-130674) Ch4(loxP:130675–160868) Ch4(loxP:160869–239739) Ch4(loxP:239740–258679) Ch4(loxP:258680–511437.lys14:loxP) Ch4(lys14.510818-630497:loxP-CgHIS3-loxP) Ch4(630498–679709:loxP-CgTRP1-loxP) Ch4(679710–932757.lys4) Ch4(hisG:lys4.931771-1288498.ade8:loxP) Ch4(ade8.1288499-1501017:loxP) Ch4(1501018-TEL)*	Derived from ZN27
ZN63	*MATα ura3-52 his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 Ch4(TEL-130674) Ch4(loxP:130675–160868) Ch4(loxP:160869–239739) Ch4(loxP:239740–258679) Ch4(loxP:258680–511437.lys14:loxP) Ch4(lys14.510818-630497:loxP-CgHIS3-loxP) Ch4(630498–679709:loxP-CgTRP1-loxP) Ch4(679710–932757.lys4) Ch4(hisG:lys4.931771-1288498.ade8:loxP) Ch4(ade8.1288499-1501017:loxP) Ch4(1501018-TEL) Ch11(TEL-521612:loxP-CgLEU2-loxP) Ch11(521613-TEL)*	Derived from ZN37
ZN71	*MATα ura3-52 his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 Ch4(TEL-130674) Ch4(loxP:130675–160868) Ch4(loxP:160869–239739) Ch4(loxP:239740–258679) Ch4(loxP:258680–511437.lys14:loxP) Ch4(lys14.510818-630497:loxP) Ch4(630498–679709:loxP) Ch4(679710–932757.lys4) Ch4(hisG:lys4.931771-1288498.ade8:loxP) Ch4(ade8.1288499-1501017:loxP) Ch4(1501018-TEL) Ch11(TEL-521612:loxP) Ch11(521613-TEL)*	Derived from ZN63 by eliminating *CgLEU2*, *CgTRP1* and *CgHIS3* through transformation with pSH47
ZN76	*MATα ura3-52 his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 Ch4(TEL-130674) Ch4(loxP:130675–160868) Ch4(loxP:160869–239739) Ch4(loxP:239740–258679) Ch4(loxP:258680–511437.lys14:loxP) Ch4(lys14.510818-630497:loxP) Ch4(630498–679709:loxP) Ch4(679710–932757.lys4) Ch4(hisG:lys4.931771-1288498.ade8:loxP) Ch4(ade8.1288499-1501017:loxP) Ch4(1501018-TEL) Ch11(TEL-521612:loxP) Ch11(521613–556764:loxP-CgTRP1-loxP) Ch11(556765-TEL)*	Derived from ZN71
ZN81	*MATα ura3-52 his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 Ch4(TEL-130674) Ch4(loxP:130675–160868) Ch4(loxP:160869–239739) Ch4(loxP:239740–258679) Ch4(loxP:258680–511437.lys14:loxP) Ch4(lys14.510818-630497:loxP) Ch4(630498–679709:loxP) Ch4(679710–932757.lys4) Ch4(hisG:lys4.931771-1288498.ade8:loxP) Ch4(ade8.1288499-1501017:loxP) Ch4(1501018-TEL) Ch11(TEL-521612:loxP) Ch11(521613–556764:loxP-CgTRP1-loxP) Ch11(556765–619189:CgLEU2-loxP) Ch11(619190-TEL)*	Derived from ZN76
ZN92	*MATα ura3-52 his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 Ch4(TEL-130674) Ch4(loxP:130675–160868) Ch4(loxP:160869–239739) Ch4(loxP:239740–258679) Ch4(loxP:258680–511437.lys14:loxP) Ch4(lys14.510818-630497:loxP) Ch4(630498–679709:loxP) Ch4(679710–932757.lys4) Ch4(hisG:lys4.931771-1288498.ade8:loxP) Ch4(ade8.1288499-1501017:loxP) Ch4(1501018-TEL) Ch11(TEL-21510) Ch11(loxP-CgHIS3-loxP:21511–521612:loxP) Ch11(521613–556764:CgTRP1-loxP) Ch11(556765–619189:loxP-CgLEU2-loxP) Ch11(619190-TEL)*	Derived from ZN81
SH6484	*MATa ura3-52 his3Δ200 leu2Δ1 lys2Δ202 trp1Δ63 Ch1(TEL-42447) Ch1(loxP:42448–109579) Ch1(loxP:109580–137801:loxP) Ch1(loxP:137802–181215:loxP) Ch1(181216-TEL) Ch2(TEL-743158:loxP) Ch2(743159-TEL) Ch3(TEL-143982:loxP) Ch3(143983–182046:loxP)*	Derived from FY833