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Research Article

SCRaMbLEing of a Synthetic Yeast Chromosome with Clustered Essential Genes Reveals Synthetic Lethal Interactions

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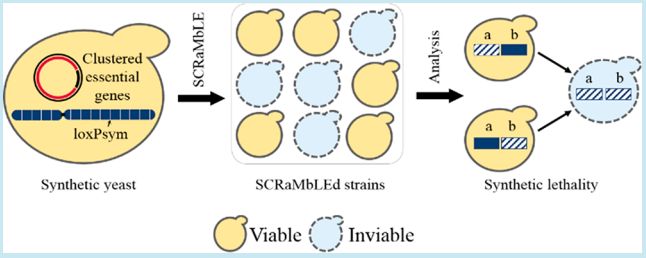
ABSTRACT: Genome-scale gene knockout is an important approach to the study of global genetic interactions. SCRaMbLEing of synthetic yeast chromosomes provides an efficient way to generate random deletion mutants. Here, we demonstrate the use of SCRaMbLE to explore synthetic lethal interactions. First, all essential genes of yeast chromosome III (chrIII) were clustered in a centromeric plasmid. We found that three types of reorganized clustered chrIII essential genes had similar transcriptional levels. Further, SCRaMbLEing of synthetic chromosome III (synIII) with supplementary clustered essential genes enables deletion of large chromosomal regions. Investigation of 141 SCRaMbLEd strains revealed varied deletion frequencies of synIII chromosomal regions. Among the no deletion detected regions, a hidden synthetic lethal interaction was revealed in the region of synIII 82−88 kb. This study shows that SCRaMbLE with clustered essential genes enhances streamlining of synthetic yeast chromosome and provides a novel strategy to uncover complex genetic interactions.



Systematic genetic screening is a powerful means to study genetic interaction networks in yeast. Single-, double- and triple-gene deletion mutants of yeast genome have been constructed to explore the global landscape of genetic interactions.[1−6] In *Saccharomyces cerevisiae*, a collection of single gene deletion mutants, covering 96% of annotated open reading frames, has been constructed by homologous recombination, which is a valuable resource for functional genomics. It demonstrated that ∼80% of genes in the genome of *Saccharomyces cerevisiae* are nonessential.[1] Further, millions of double deletion mutants were constructed by crossing between single mutants termed synthetic genetic array (SGA).[2] Genome-scale quantitative analysis revealed a comprehensive digenic interaction network, which enriched understanding of extensive functional cross-connections.[3−5] To further explore complex genetic interactions, ∼200 000 triple mutants were constructed by crossing between double mutants and single mutants.[6] The global trigenic interaction network is estimated to be ∼100 times as large as the digenic network, exploring the complexity of yeast genetic interactions.[6]

Although these large-scale analyses of genetic interactions were time-consuming and laborious, they paved the way to understanding the functional network.[7,8] However, it is still a great challenge to investigate complex interactions involving more genes using traditional gene knockout methods. PCR-mediated chromosomal deletion (PCD) was used to delete chromosomal segments harboring multiple nonessential genes.[9] The result showed that nonessential regions contain synthetic lethal combinations at a high frequency. This technique complements the study of yeast genetic interactions, but it is limited to individual nonessential chromosomal segments. With development of synthetic genomics, we are able to design and synthesize a yeast genome from the scratch.[10−19] Introduction of an inducible SCRaMbLE (Synthetic Chromosome Rearrangement and Modification by LoxPsym-mediated Evolution) system into the synthetic yeast genome make it an efficient method to generate random deletion mutants.[20−25] However, deletion using SCRaMbLE is limited by straggling locations of essential genes in the designer chromosomes.

Here, we perform SCRaMbLE of synthetic yeast chromosome III (synIII) with supplementation of clustered wild-type chromosome III (chrIII) essential genes. This strategy allows streamlining of the synthetic chromosome with deletion of large chromosomal segments. Further, varied deletion frequencies of synIII chromosomal regions were revealed. And a synthetic lethal interaction in the region of synIII 82−88 kb was verified among the no deletion detected regions. Overall, our findings highlight that SCRaMbLE is effective to study yeast genetic interactions.

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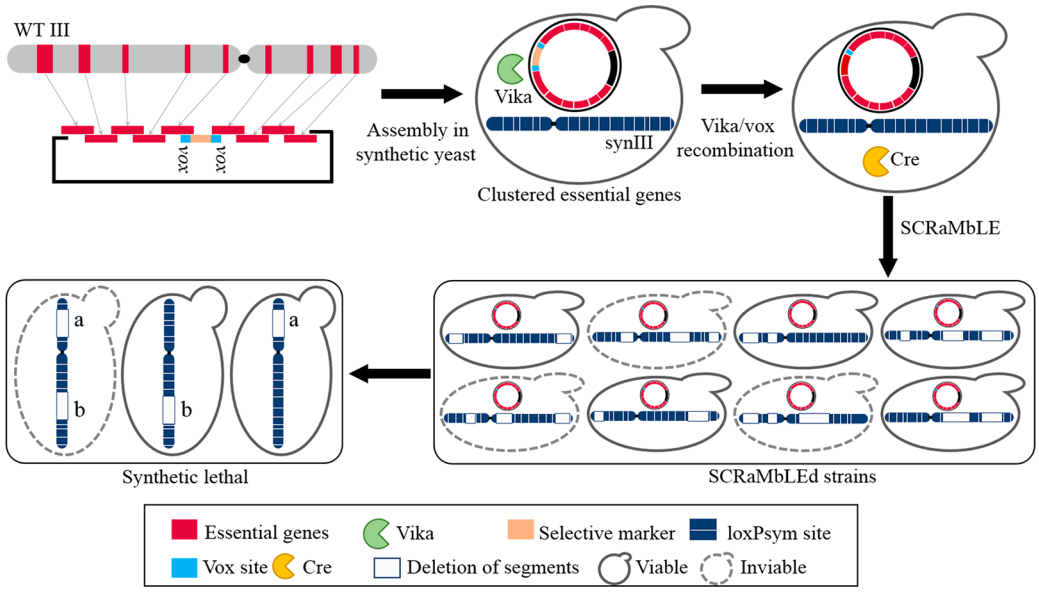

Figure 1. Strategy of SCRaMbLE with clustered essential genes to reveal genetic interactions. All essential genes of chrIII (red chunks) were clustered and assembled into a centromeric plasmid using homologous recombination in synIII strain, allowing SCRaMbLE with supplementation of the clustered essential genes once assembled. Using Vika/vox, a recombination system orthogonal to Cre/loxp, the selective marker in the middle of assembled fragments was deleted. Varied SCRaMbLEd strains were phenotypically tested and genotypically analyzed to explore synthetic lethal interactions. The white lines on the synthetic chromosome represent loxPsym sites. White blocks represent deletions of chromosomal regions in SCRaMbLEd strains. Yeast strains with normal, sick, and inviable growth fitness are indicated by oval with solid border, round dot border, and long dash border, respectively.

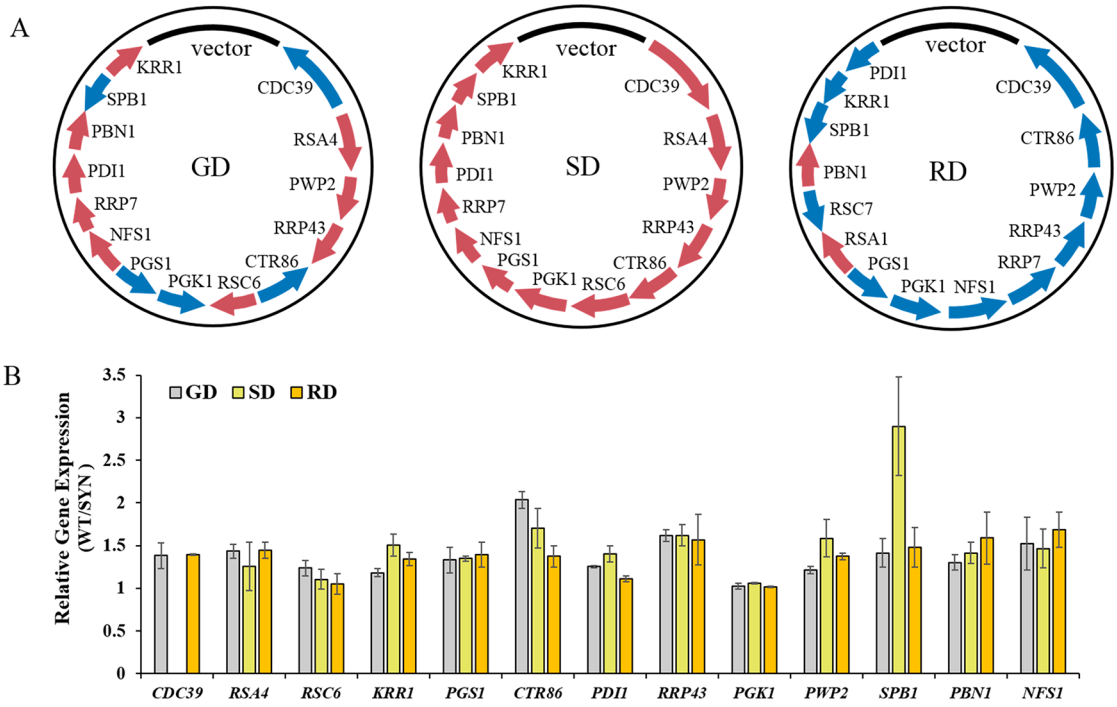

Figure 2. Reorganization of clustered essential genes of chrIII and transcription of the essential genes. (A) Detailed distributions of the clustered essential genes on three reorganized plasmids. In the genomic direction (GD) plasmid, the essential genes are arranged according to their natural chromosomal positions and orientations. In the same direction (SD) plasmid, the essential genes are arrayed in the same positions as GD plasmid but assembled in the same direction. In the random direction (RD) plasmid, the essential genes are arranged in random order according to the combined length of three individual genes. The plasmids harboring all 14 essential genes are up to 43 kb in length. The two directions of the arrows (red and blue) correspond to the directions of transcription and translation. (B) Relative gene transcription of wild-type essential genes on plasmids and synthetic essential genes on synIII. Sequencing reads of the transcriptome for wild-type and synthetic genes can be distinguished by PCRTags in their ORFs. Transcriptional levels of 13 essential genes (except *RRP7*) in three reorganized plasmids (GD, SD, RD) were analyzed. There is no transcription of *CDC39* in the SD plasmid because of the missing assembly of *CDC39* fragment. The transcription of *CDC39* in the right assembled SD plasmid was analyzed by qPCR (Supplementary Figure S8). Error bars represent standard deviation from three replicates.

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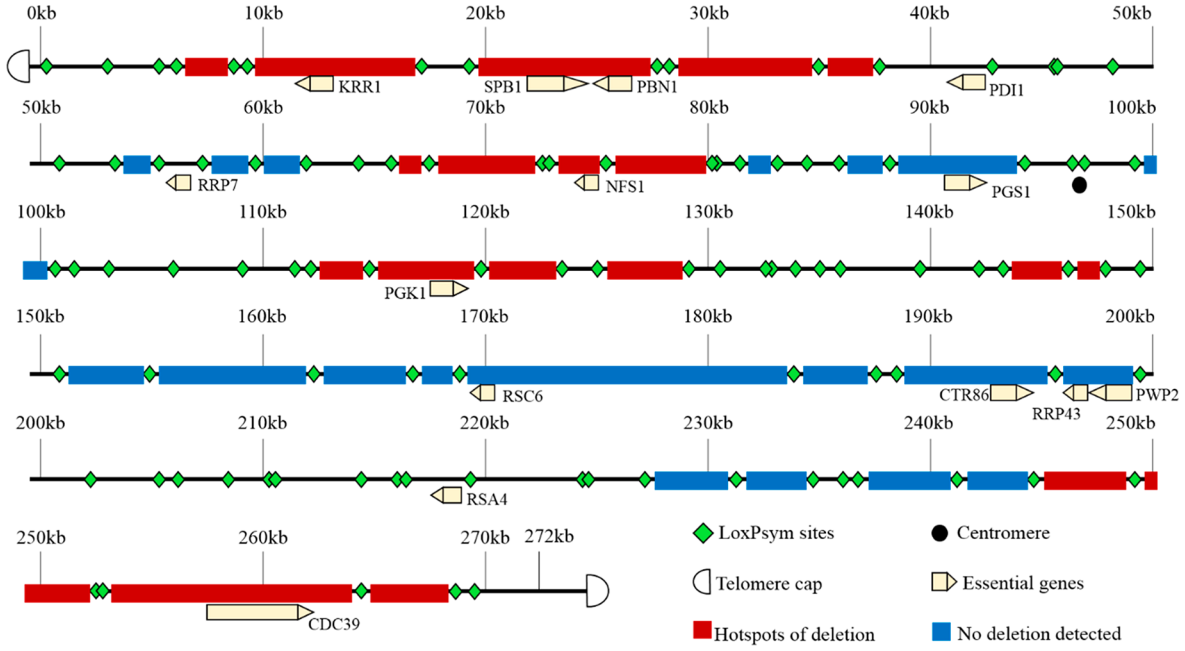

Figure 3. Varied deletion frequencies of synIII chromosomal regions triggered by SCRaMbLE. A total of 48 PCRTags on synIII were selected to detect the deletion frequencies. Hot spots and cold spots of deletion are indicated as red and blue horizontal filled bars, respectively. Hot spots (red horizontal filled bars) represent regions where the deletions were detected more than 10 times. Cold spots (blue horizontal filled bars) represent regions where no deletion were detected. Green diamonds represent loxPsym sites distributed on synIII. Yellow arrows represent open reading frames (ORFs) of essential genes.

RESULTS

SCRaMbLEing of Synthetic Yeast Chromosome III with Clustered Essential Genes Expands Deletable Regions. Previous studies have indicated that SCRaMbLE is an excellent strategy to induce yeast genome rearrangement and simplification, which relies on recombination reaction between loxPsym sites within synthetic chromosomes.[10,11] However, the loxPsym sites are inserted in the 3′-untranslated region (UTR) of nonessential genes, thus reducing the ability for deletions in a haploid synthetic yeast. As shown in Supplementary Figure S1, nonessential genes *MRC1*, *ADF1*, *FYV5*, *MOS1*, and *PRD1* cannot be deleted by Cre/loxPsym since essential gene *KRR1* exists in this segment. The synIII chromosome is separated by introduced loxPsym sites into ∼80 chromosomal segments that contain annotated open reading frames (ORFs).[11] Among them, 12 of the segments contain essential genes, which indicates that 15% of regions (accounting for 27.6% of synIII length) may not be deleted by SCRaMbLE.

To get rid of the limitation on chromosomal deletion caused by essential genes, we clustered all essential genes of chrIII into a centromeric plasmid as an additional supplement for the essential functions. All 14 clustered essential genes were amplified by PCR using wild-type BY4741 genome as a template; thus clustered essential genes on the plasmid can be distinguished from the essential genes on the synthetic chromosome by specific PCRTags in the ORFs.[10,11] The transcription units of essential genes were amplified from 500 base pairs before the start codon and 300 base pairs after the stop codon. All 14 fragments of essential genes were transformed in the synIII yeast and assembled into a centromeric plasmid with a total length of ∼43 kb in one step by homologous recombination. Using Vika/vox, a recombination system orthogonal to Cre/loxp, the auxotroph marker in the middle of assembled fragments can be deleted.[26,27] The synIII strain with additional clustered essential genes was then subjected to SCRaMbLE (Figure 1). PCRTag analysis and genome sequencing of SCRaMbLEd strains revealed deletions of essential genes in synIII (Supplementary Figure S2). For instance, essential genes *KRR1*, *SPB1*, *PBN1*, and *PGK1* in the synthetic chromosome III were deleted in a SCRaMbLEd strain yWPX023. The results from sequencing depth map of synIII also show deletion of essential genes *KRR1* and *PGK1* in strain yWPX025. Further, our result indicates that SCRaMbLE with clustered essential genes can facilitate deletion of large chromosomal segments. As shown in Supplementary Figure S3, a region with length of 42.6 kb in strain yWPX038 was deleted using this modified SCRaMbLE method. We also detected a SCRaMbLEd strain yWPX053 with a total of 97 kb deletion in synIII, which is 35.7% of synIII length.

Reorganization of Clustered Essential Genes of Chromosome III Has Little Transcriptional Effect. In the synthetic yeast genome project (Sc2.0), wild-type chromosomal segments were in situ replaced by counterpart synthetic DNA chunks.[28] In most cases, the synthetic yeast chromosomes result in normal growth fitness of host strains.[15−21] However, it is still a big question regarding the feasibility of clustering and reorganization of synthetic genome for future design. Here, 14 essential genes of chrIII were clustered and reordered, and transcription of the genes was tested. We designed and constructed three types of reorganized essential genes into three different plasmids (Figure 2A). For the genomic direction (GD) plasmid, the essential genes were arranged according to their natural chromosomal positions and orientations. For the same direction (SD) plasmid, the essential genes were arrayed in the same positions as GD plasmid, but all genes were assembled in the same direction

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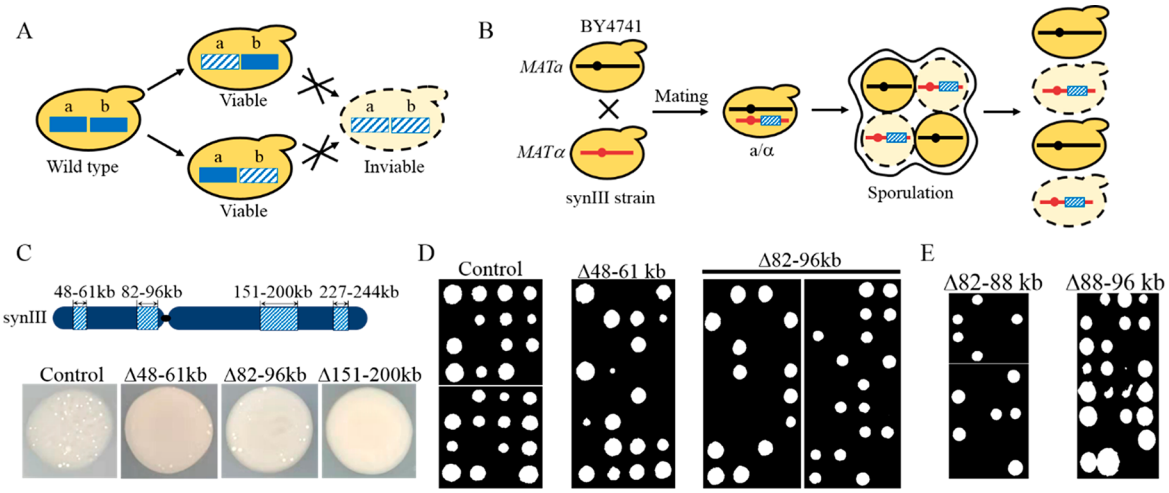

Figure 4. Synthetic lethal interactions were revealed in no deletion detected regions of SCRaMbLE. (A) Schematic representation of synthetic lethal interactions. Deletion of either gene a or gene b does not affect viability, whereas deletion of both at the same time is lethal. (B) The process of spore analysis for essential chromosomal region in the loss of heterozygosity strains (BY4741-synIII). (C) Random spore analysis of knockout diploid strains for the no deletion detected regions. Random spores were selected on SC-Arg with canavanine medium. (D) Tetrad analysis of synIII 48−61 kb and synIII 82−96 kb knockout diploid strains. The BY4741-synIII diploid was used as a control strain. (E) Tetrad analysis of synIII 82−88 kb and synIII 88−96 kb knockout diploid strain.

(SD). For the random direction (RD) plasmid, the essential genes were modularly assembled according to the combined length of three individual genes and thus were displayed disorderly. The synIII strains bearing the GD, SD, and RD plasmids were phenotypically normal despite the double expression of 14 essential genes.

To test functional effects of clustering and reorganization of the chrIII essential genes, three yeast strains harboring GD, SD, RD plasmids were analyzed by RNA sequencing individually. PCRTags, the synonymous mutation sequences in open reading frames (ORFs), can be used to distinguish the wild-type genes from synthetic genes for transcriptome sequencing.[10,11] Transcriptional level of all essential genes in the three types of clustered plasmids were investigated by alignment with specified PCRTags, except *RRP7*, which did not have PCRTags in the ORF. As shown in Figure 2B, transcription of the genes in GD, SD, and RD plasmids were similar to each other. It suggests that reorganization of the clustered 14 essential genes in yeast has little effect on gene expression. However, transcription of gene *SPB1* in the SD plasmid was slightly higher than that in GD and RD groups. Comparing the organization of *SPB1* in the three plasmids reveals that *SPB1* and *PBN1* are in the same direction in the SD plasmid. To test if the inversion of *SPB1* contributes to the increased transcription, the *SPB1* segment in the GD plasmid was inverted to the same direction as the SD plasmid, resulting a GD-2 plasmid (Supplementary Figure S4). Quantitative real-time PCR analysis of *SPB1* shows increased transcriptional level in the GD-2 plasmid compared with that in the GD plasmid. The results indicate that reorganization of clustered essential genes of chrIII has little transcriptional effect in yeast, although there may be effects in individual cases.

Varied Deletion Frequencies of synIII Chromosomal Regions Triggered by SCRaMbLE. In theory, SCRaMbLE can generate random deletions of chromosomal regions through Cre/loxPsym reactions between any two loxPsym sites on synthetic chromosomes.[10] However, deletion probability for different chromosomal regions is undetermined. Here, a synIII strain with clustered essential genes was SCRaMbLEd, and deletion frequencies of synIII chromosomal regions were statistically analyzed. From the total 186 pairs of PCRTags within 105 genes on synIII, 48 pairs of distributed PCRTags were selected to detect deletion events of the SCRaMbLEd strains (Supplementary Table S1). A total of 141 SCRaMbLEd strains with at least one deletion were detected, of which 107 strains were analyzed by 48 pairs of distributed PCRTags, and 34 strains were analyzed by whole genome sequencing (Supplementary Table S2). The detailed information about deletions of SCRaMbLEd strains are listed in Supplementary Figure S5. The result indicated that deletion events occurred in different loci of synIII. To further investigate varied deletion frequencies of different loci, 141 SCRaMbLEd strains were statistically analyzed. As shown in Figure 3, chromosomal regions that were deleted more than 10 times were considered as hot spots for deletion (marked in red), and chromosomal regions with no deletion detected were considered as cold spots for deletion (marked in blue).

Two hot spot deletion regions of synIII 6−38 kb and synIII 244−269 kb, both harboring essential genes, were located at two ends of the synthetic chromosome. This result is consistent with the higher prevalence of loss-of-heterozygosity (LOH) events near telomeres by genome analysis across 1011 *Saccharomyces cerevisiae* isolates.[29] Regions of synIII 66−80 kb, synIII 110−128 kb, and synIII 142−146 kb were also observed to be deleted at high frequency. This may be caused by nonessential functions within these regions.[1] In addition, no deletion event was detected in four regions larger than 10 kb in the synIII (synIII 48−61 kb, 82−96 kb, 151−200 kb, and 227−244 kb).

Synthetic Lethal Interactions Were Revealed in No Deletion Detected Regions of SCRaMbLE. Negative genetic interactions refers to an effect in which two mutations, when combined, result in a phenotype that is more severe than expected.[30] Synthetic lethality is a typical example of negative interaction in which two simultaneous mutations cause cell death, while each single mutation can produce living cells (Figure 4A). PCRTag analysis and whole genome sequencing revealed that 4 regions of synIII had never been deleted for the

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tested SCRaMbLEd strains. They are located at synIII 48−61 kb, 82−96 kb, 151−200 kb, and 227−244 kb, respectively. Three of the regions (synIII 48−61 kb, 82−96 kb, and 151−200 kb) contained known essential genes (*RRP7*, *PGS1*, *RRP43*, *RSC6*, *CTR86*, and *PWP2*), while one (synIII 227−244 kb) did not. We then investigated whether these regions are essential and contain synthetic lethal interactions. For the synIII 227−244 kb region, which contains only nonessential genes, it was reported that a region corresponding to synIII 227−244 kb was undeletable and contained synthetic lethal interactions, which was verified by PCR-mediated chromosome splitting (PCS).[9] For the remaining 3 no deletion detected chromosomal regions that contain essential genes, we first checked if this was caused by failed expressions of the supplementary essential genes in the clustered plasmids. As shown in Figure 2B, transcriptional level of the counterpart essential genes, *PGS1*, *RRP43*, *RSC6*, *CTR86*, and *PWP2*, was normal.

To determine whether the 3 no deletion detected regions are essential, we first try to knock out the 3 regions in haploid strains containing clustered essential genes individually. However, we failed to get the knockout strains by homologous recombination in the haploid synIII. We then knocked out the 3 regions in diploid strains individually, following by spore analysis of the loss of heterozygosity strains (Figure 4B). The diploid strains were derived from mating of synIII strain containing clustered essential genes with BY4741 strain. The result of random spore analysis shows that sporulation rate of the 3 knockout diploid strains are significantly reduced (Figure 4C). The diploid strain with deletion of synIII 151−200 kb failed to generate spores. This may be caused by deletion of functional genes involved in the process of sporulation. It was reported that null gene *BPH1*, *ELO2*, or *THR4* would decrease the sporulation efficiency.[31−33] Other methods are needed to determine the essential function of this region. Tetrad analysis of the producible knockout strains was used to uncover synthetic lethal interactions in the no deletion detected regions. For the diploid strain with synIII 48−61 kb deleted, even though the sporulation rate is relatively low, we were able to dissect a few tetrads with 3 or 4 viable spores. The result suggested that this region may not be essential under tested conditions. For the diploid strain with synIII 82−96 kb deleted, after dissection of 16 tetrads, the number of viable spores was always no more than two (Figure 4D). All viable spores were PCR verified for the existence of the synIII 82−96 kb segment (Supplementary Figure S6). The result suggests that deletion of this region leads to cellular death and there might be synthetic lethal interactions within this region. We further divided the undeletable region of synIII 82−96 kb into two parts, and two diploid strains with synIII 82−88 kb and 88−96 kb deleted were constructed individually. Tetrad analysis of the two strains reveals that the region of synIII 82−88 kb is essential (Figure 4E). There are 6 nonessential genes (*GBP2*, *SGF29*, *ILV6*, *STP22*, *VMA9*, and *YCL007C*) within this region, and individual deletion of the genes result in a viable cell.[1] From investigation of yeast genetic interactions on SGD (http://yeastgenome.org/), no synthetic lethal interactions have been reported in the region corresponding to synIII 82−88 kb.[4,5,34] This suggests that there is a hidden synthetic lethal interaction in this region. Further, we tried to explore the detailed relation of these genes by knocking out the individual ORFs and adjacent two ORFs in the BY4741 strains. As shown in Supplementary Figure S7, strains with the individual ORFs and adjacent two ORFs knocked out are viable on YPD medium, although the *STP22* and *VMA9* double knockout strain shows severe growth defect. We assumed that there may be a more complex interaction in this region.

DISCUSSION

Gene order plays an important role in the evolution of genome.[35,36] Gene order can be affected by various genomic rearrangement events, like inversions and translocations.[37] From "reading" a natural genome to "writing" a synthetic genome, we are exploring the boundary of how we can change the genome.[15−21] Clustering and reorganization of synthetic gene modules is one of the most fascinating aspects in the design of synthetic pathways or synthetic genomes. In our study, 14 essential genes of yeast chrIII were clustered into a plasmid. We found that although these genes were clustered in different orders, it had little effect on gene transcription. In prokaryotes, partial *M. mycoides* JCVI-syn3.0 genome was reorganized according to the functional category of genes, which also illustrated the feasibility of clustering and reorganization of synthetic genomes.[38] However, in one case, we found that inversion of *SPB1* fragment in the SD plasmid improved the transcriptional level of *SPB1*. Previous works have shown that an inversion of a synXII chromosomal region can cause abnormal expression of *ACE2* and leads to increased ethanol tolerance, and inversion of *crtI* in a β-carotene pathway by in vitro SCRaMbLE leads to higher production of β-carotene.[20,21] In conclusion, the results suggest that it is very promising for larger scale clustering and reorganization of yeast genome; however, it is worth noting that gene orders may have unexpected effect on expression of individual genes.

SCRaMbLE is capable of producing large amounts of deletions for genome streamlining in synthetic yeast.[12,22] Here, essential genes of chrIII were clustered in a plasmid to enable deletion of large chromosomal regions harboring essential genes. Our results indicate that SCRaMbLE with clustered essential genes is an effective method for streamlining of synthetic yeast genome. In the future, all essential genes of yeast genome can be clustered in a fully synthetic yeast to facilitate the study of simplified yeast genomes. However, many duplications of chromosomal segments were also detected in the SCRaMbLEd strains (Supplementary Figure S3). It is suspected that this may be caused by the supplementary essential genes, considering that more duplication events were possibly detected in SCRaMbLEd heterozygous diploid strains (with supplementation of another wild-type chromosome) than haploid strains.[23,24] In our study, synthetic lethal interactions were discovered in no deletion detected regions of SCRaMbLE. By means of SCRaMbLEing with more synthetic chromosomes for multiple rounds, synthetic yeast genome could be continuously simplified, providing a new strategy to study the complex negative interactions within large chromosomal regions. Besides deletions, SCRaMbLE can be used to discover the genetic interactions triggered by other structural variations, such as duplications, inversions, and translocations.

MATERIALS AND METHODS

Strains, Plasmids, and Media. The yeast strains used in this study are listed in Supplementary Table S3. Synthetic strain synIII (*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*) was used

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ACS Synth. Biol. 2020, 9, 1181−1189

as the strain for systematic chromosomal simplification. *S. cerevisiae* strain BY4741 (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) was used to mate with synIII to generate diploid strains. Plasmid pCLB2-Cre/EBD is available from Addgene, which is necessary for inducing SCRaMbLE. Yeast strains were grown at 30 °C in Yeast extract-Bacto peptone-Dextrose (YPD) medium containing 1% yeast extract, 2% peptone, and 2% glucose. The plasmid-bearing strains were cultured in synthetic complete (SC) medium. The drop-out mixture for making SC medium contained all possible supplements except leucine, histidine, and uracil. Two grams of drop-out mixture was needed for per liter of SC medium. SC medium containing 1 g/L 5-fluoroorotic (5-FOA) (Sigma-Aldrich) was used to select strains without *URA3* genes. SC-Arg medium with canavanine was used to select haploid strains.

Assembly of Clustered Essential Genes. Essential genes were individually amplified by PCR using genome of wild-type yeast as the template. During the progress of PCR, 59 bp homologous arms were introduced to neighboring genes by primers. At the same time, the first and the last genes that linked with the vector also carried the homologous arms of the vector fragment by PCR primers. Therefore, adjacent fragments have homologous fragments with each other. The vector pRS416 was linearized by *Not*I digestion. Then fragments of 14 essential genes and the linearized vector with homologous arms were transferred into strain synIII. All essential genes and vector were clustered into a complete plasmid by yeast-based homologous recombination in one step.[39−41] The correctness of assembly of GD, SD, and RD was checked by PCR verification of the junctions. The three reorganized plasmids (GD, SD, and RD) were constructed using the same method with different orders. In order to increase the efficiency of screening, we introduced a screening marker (*URA3*) in the middle of 14 fragments. We verified the transformants by testing the junctions between each gene. After verification, the marker *URA3* was knocked out by the Vika/vox system. The vox sequences were inserted into both ends of the marker, and the Vika gene was induced via the *GAL1* promoter.[26,27] SC medium containing 5-FOA was used to select strains with deletion of *URA3* marker.

RNA-Seq Analysis of Clustered Essential Genes. Yeast cells containing clustered essential genes in GD, SD, and RD plasmid were subjected to RNA sequencing to investigate the transcriptome. Three parallel samples were set for each yeast strain harboring the essential genes in a plasmid. The samples were tested using the BGISEQ-500 platform, and each sample produced an average of 21.42 Mb of data. The average alignment rate of the sample against the reference genome was 95.79%. The sequencing data is called raw reads or raw data, and then quality control (QC) of the raw reads is performed to determine whether the sequencing data is suitable for subsequent analysis. By trimming low quality reads, we quantified the transcriptional expression of clustered essential genes on plasmid relative to essential genes on chromosome. PCRTags were used to distinguish the transcription of wild-type genes from the transcription of synthetic genes.

Quantitative PCR (Q-PCR) Verification. Quantitative real-time PCR was used to verify the transcription of *SPB1* in different conditions. A single yeast colony was cultured in liquid medium until the OD_{600} was 0.8 to 1.2. RNA was extracted from collected cells through Column Fungal RNA Extraction Kit. The primers used in qPCR assay are listed in Supplementary Table S4. The relative gene expression data were quantified by comparing the Ct values of the target genes using the $2^{-\Delta\Delta Ct}$ method.[42] Unique Aptamer qPCR SYBR Green Master Mix was used for the qPCR reaction, and equipment was Quantagene q225 (Novogene). The reaction procedure was performed as follows: precycling, 50 °C/300 s; 40 cycles of 95 °C/10 s, 56 °C/20 s, and 72 °C/20 s; melt curve, which started from 60 to 95 °C. Three parallel samples were set up in the experiment.

SCRaMbLE. First, the Cre plasmid was transformed into the strain synIII with clustered essential genes. Second, the plasmid-bearing cells were cultured in SC-His-Ura medium to logarithmic growth phase. Third, cells were transferred to fresh medium to an OD_{600} of ∼0.5. β-Estradiol (Sigma-Aldrich) was added into the medium to a final concentration of 1 μM to induce SCRaMbLE for 8 h. Strains were collected by centrifugation and washed three times with sterile water. SCRaMbLEd strains were diluted 1000-fold and spread evenly on SC-His-Ura plates. The plates were incubated at 30 °C for 36 h.

Yeast Colony PCR for Detection of Chromosomal Deletions. The SCRaMbLEd colonies were resuspended in 50 μL of 20 mM NaOH. The cells were heated for 3 cycles of 95 °C/5 min and 4 °C/1 min prior to adding the PCR mix and performing the PCR program. The following PCR program was used: 95 °C/3 min; 30 cycles of 95 °C/15 s, 53 °C/15 s, and 72 °C/15 s; a final extension of 72 °C/5 min. PCR reaction was performed using 2× Rapid Taq Master Mix (Vazyme Biotech Co., Ltd.). Detection of PCRTags was performed by agarose gel electrophoresis. The absence of synthetic PCRTag amplicons (SYN) revealed the deletion of segments on the chromosome. The synthetic PCRTags used in this study are listed in Supplementary Table S1; 48 pairs of distributed PCRTags on synIII were selected to detect the deletion of different regions. SCRaMbLEd strains with deletions of these 48 genes are displayed in Supplementary Figure S5.

Whole Genome Sequencing of SCRaMbLEd Strains. The SCRaMbLEd strains were sequenced to confirm the integral genotype. Deep sequencing of all libraries was performed on the Illumina (BGISEQ-500) platform. The length of each read was 150 base pairs. The sequenced data were filtered, and the adapter sequence and low-quality data were removed, resulting in the clean data used for subsequent analysis. The variation information was obtained by aligning sample reads with reference genome. Through analysis of sequences linked by loxPsym sites, structural variations occurring throughout the chromosome can be identified.

Serial Dilution Assay. The candidate colonies were precultured overnight to keep strains in an activated state. Then, cells were transferred into sterile water in a 96-well plate with normalized concentration. Each suspension was diluted in a 10-fold gradient, and 3 μL of the diluted cells were spotted on the YPD medium. Plates were incubated at 30, 33, 35, and 37 °C. Cell phenotypic images were captured after 36 h of incubation.

Sporulation and Spore Analysis. The *S. cerevisiae* strain BY4741 was mated with the synIII strain to get diploid cells. For each query strain with putative synthetic lethal interactions, the target region was deleted from the diploid cell. The target segments were knocked out by yeast homologous recombination. Homologous fragments were synthesized by PCR. The primers used in knockout and verification are listed in Supplementary Table S5. Then

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ACS Synth. Biol. 2020, 9, 1181−1189

diploids were sporulated on nutrient-free medium and incubated at 25 °C for 3−10 days. The presence of tetrad was examined under a microscope. Following tetrad analysis of meiotic progeny derived from diploid cells, the tetrad spores were micromanipulated onto distinct positions on YPD agar medium. In addition to separating the spores, yeast was treated by zymolyase and vortex vibrated to conduct random spore analysis. The treated strains were cultured on haploid selection medium (SC-Arg with canavanine).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at the ACS Publications Web site. Supporting Information. Supplementary Figure S1−S8, Supplementary Table S1−S5. The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acssynbio.0c00059.

> Schematic diagram of distribution of sectional non-essential and essential genes on synIII, schematic diagram of deleting essential genes on synIII by SCRaMbLE with clustered essential genes, genotypes of SCRaMbLEd strains with deletion of large fragments, real-time qPCR analysis for the validation of gene expression, deletions of the 141 SCRaMbLEd strains in 48 pairs of PCRTags, PCRTag analysis of spores, phenotype of strains with the individual ORFs and adjacent two ORFs knocked out in the region of synIII 82−88 kb, validation of the assembly of SD plasmid, PCRTags of synthetic chromosome III used in this study, whole genome sequencing strains involved in this study, yeast strains and plasmids used in this study, primers used in this study for quantitative PCR (qPCR), primers used in segment knockout and validation (PDF)

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