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CRISPR-Cas9 Facilitated Multiple-Chromosome Fusion in *Saccharomyces cerevisiae*

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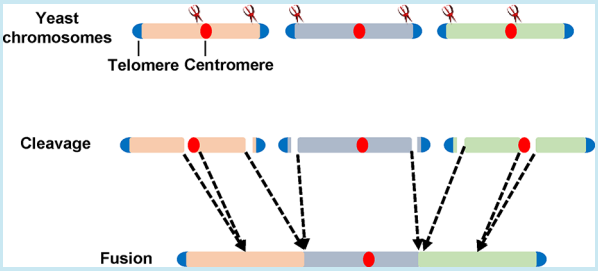
Supporting Information

ABSTRACT: Eukaryotic cells usually contain multiple linear chromosomes. Recently, we artificially created a functional single-chromosome yeast via sequential two-chromosome fusion utilizing the high performance of the CRISPR-Cas9 system and homologous recombination in *Saccharomyces cerevisiae*. In this paper, we adapted this method for the simultaneous fusion of multiple chromosomes. We demonstrated the fusion of two, two-chromosome sets with a 75% positive rate and three-chromosome fusions with a 50% positive rate. We also found that by using an additional selection marker, the positive rate of two-chromosome fusions reached 100%. Due to the simplicity, efficiency, and portability of this method, we expect that it can be easily adapted for multiple-chromosome fusions in other organisms.



Eukaryotes usually contain multiple linear chromosomes, and the number of chromosomes is varied among species. Chromosome fusion events incidentally occur during evolution. For example, there is evidence for a fusion event of human chromosome 2 from two ape chromosomes.[1] However, the impact of fusion events is not fully understood because naturally occurring fusion events are too rare to be readily studied. Recently, we utilized the highly efficient CRISPR-Cas9 system[2−4] and yeast homologous recombination to develop an efficient method for two-chromosome fusions.[5] However, the successful creation of a functional single-chromosome yeast via the fusion all of the 16 native chromosomes of a haploid *Saccharomyces cerevisiae* required 15 rounds of sequential two-chromosome fusions,[5] limiting the use of this method in other organisms with higher chromosome numbers. For simplicity of selection marker curation in sequential two-chromosome fusions,[5] the same marker, *URA3*, was used in two DNA targeting and selections for the ligation of chromosome segments, which could lead to large variations in the positive rate (20−100%) of two-chromosome fusions. In this study, we tested the utilization of this method for the simultaneous fusion of multiple chromosomes and further improved the efficiency of this method.

RESULTS AND DISCUSSION

To test for multiple-chromosome fusions, chromosomes VI (270 kb), I (230 kb), IX (440 kb), and II (813 kb) were chosen out of the 16 native chromosomes of *S. cerevisiae* BY4742 because the sizes of the disappearing natural chromosomes and the appearing artificially fused chromosomes were easily distinguishable. Detailed information on the deleted regions during the chromosome fusions is listed in Table S1. Moreover, to co-introduce the guide RNA expression plasmid and homologous recombination cassettes into the BY4742 (pCas9), both the lithium acetate (LiAc) transformation protocol[6] and the spheroplast transformation protocol[7] were tested.

For the simultaneous fusion of two, two-chromosome sets (VI and I, and IX and II, Figure 1a), two markers (*URA3* and *LYS2*) were used for the selection of each two-chromosome fusion set. We obtained dozens of transformants in two independent experiments using the spheroplast transformation, with a 75% positive rate. No colonies were obtained using the LiAc transformation experiment, which could be due to the lower efficiency of LiAc transformation. Southern hybridization (Figure 1b) confirmed the disappearance of the natural chromosomes I (230 kb), VI (270 kb), IX (440 kb), and II (813 kb) and the appearance of two new fused chromosomes VI+I (503 kb, with the *URA3* selection marker) and XI+II (1247 kb, with the *LYS2* selection marker).

The chromosome fusion method was also applied for the simultaneous fusion of three chromosomes (VI, I, and II) into one. Considering the linkage between centromere and telomere deletions in these three fused chromosomes, two selection markers were used. The *URA3* marker was used for the selection of the Chr. VI and Chr. I fusion, and the *LYS2* marker was used for the selection of the Chr. I and Chr. II fusion (Figure 1c). Dozens of transformants were obtained in two spheroplast transformation experiments, with a 50% (2/4) positive rate. Similarly, no colonies were obtained in the LiAc transformation

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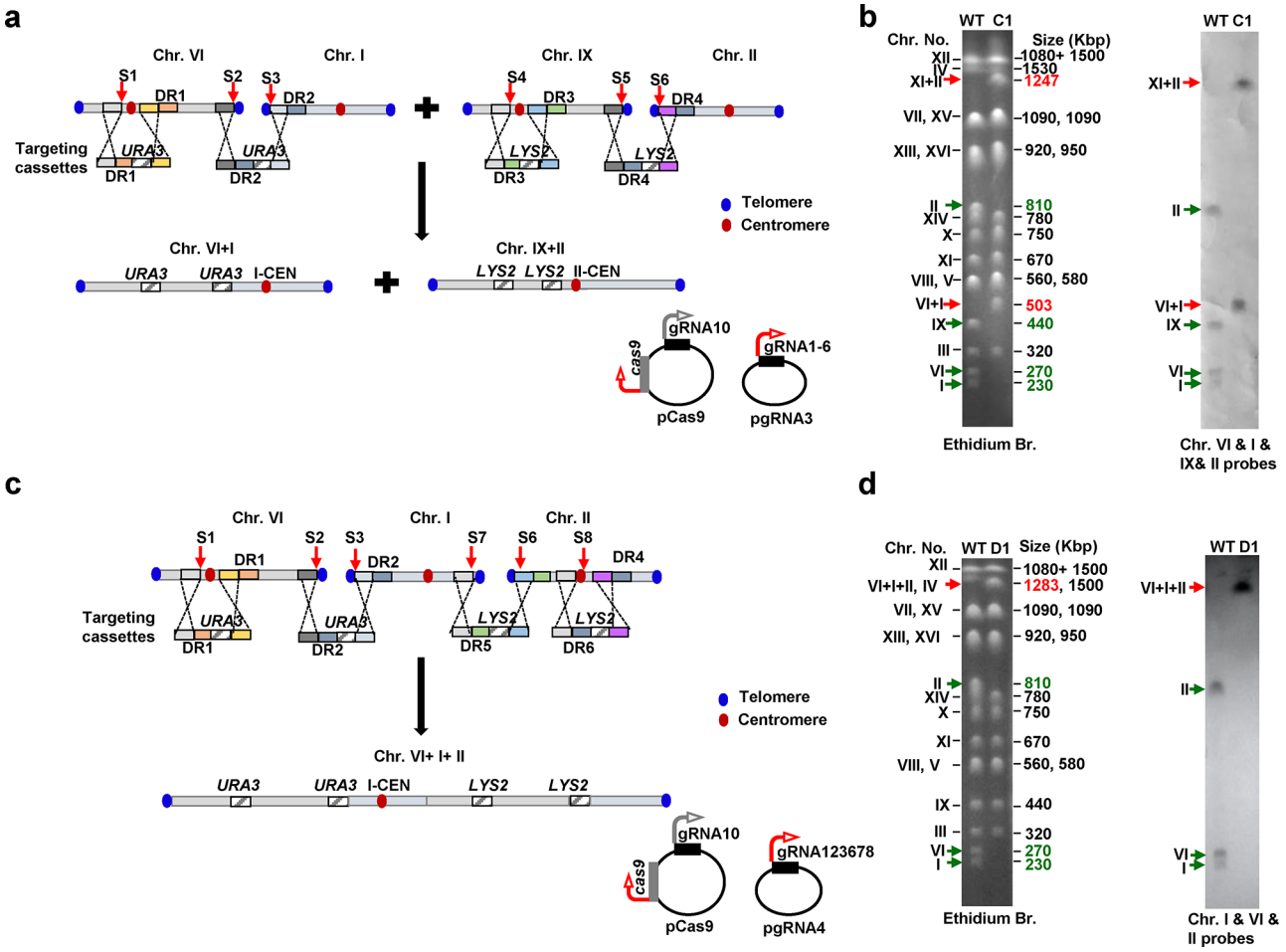

Figure 1. Simultaneous fusion of multiple chromosomes. (a) Schematic diagram of the simultaneous fusion of two, two-chromosome sets (VI and I, and IX and II). The to-be-deleted centromeres (sites S1 and S4) and telomeres (sites S2, S3, S5, and S6) were cut by the Cas9 nuclease under the guidance of gRNAs 1−6. (b) Confirmation of the simultaneous fusion of two, two-chromosome sets (VI and I, and IX and II) by pulsed-field gel electrophoresis (PFGE) and Southern blotting. The intact chromosomal DNA of the VI and I, and IX and II chromosome fusion clones C1 were separated by PFGE. Probes specific for Chr. VI, I, IX, and II were used in the Southern hybridization. (c) Schematic diagram of simultaneous fusion of three chromosomes (VI, I, and II). The to-be-deleted centromeres (sites S1 and S8) and telomeres (sites S2, S3, S7, and S6) were cut by the Cas9 nuclease under the guidance of gRNAs 1−3 and 6−8. (b) Confirmation of the simultaneous fusion of three chromosomes (VI, I, and II) by PFGE and Southern blotting. The intact chromosomal DNA of the VI, I, and II chromosome fusion clone D1 were separated by PFGE and stained with ethidium bromide. Probes specific for chromosomes VI, I, and II were used in the Southern hybridization. The green arrows indicate the to-be-fused chromosomes VI, I, and II, whereas the red arrows indicate the newly fused chromosome VI+I+II. WT: the wild-type strain BY4742. C1: positive colony containing the fused chromosomes VI+ I, IX+ II. D1: positive colony containing the fused chromosomes VI+ I+ II.

Table 1. Efficiency of the Chromosome Fusions

group	chromosomes joined in fusion: chromosome no.	chromosomes joined in fusion: chromosome length (kb)	lenth of fused chromosome (with markers) (kb)	chromosome fusion efficiency, no. of tranformants (positive rate): experiment 1[a] (LiAc)	experiment 2[b] (LiAc)	experiment 3[b] (protoplast)	experiment 4[b] (protoplast)
			two-chromosome fusion				
A	VI	270	503	60 (2/4)	56 (4/4)	150 (4/4)	634 (4/4)
	I	230					
B	IX	440	1247	36 (3/4)	124 (4/4)	672 (4/4)	832 (4/4)
	II	813					
			two sets of two-chromosome fusion				
C	VI	270	503		0	10 (3/4)	24 (3/4)
	I	230					
	IX	440	1247				
	II	813					
			three-chromosome fusion				
D	VI	270	1283		0	34 (2/4)	46 (2/4)
	I	230					
	II	813					

[a]Two-chromosome fusion with one selection marker. [b]Two-chromosome fusion with two selection markers.

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experiment. The successful fusion of the three chromosomes (VI, I, and II) was confirmed by Southern hybridization (Figure 1d).

To improve the efficiency of the chromosome fusions, we tested the two-chromosome fusions using two selection markers (Table 1). When using two selection markers (*URA3* and *LYS2*) for the centromere and telomere deletions, the positive rates of the group A and B two-chromosome fusions in three independent experiments were all 100% (4/4). In contrast, when the same selection marker (*URA3*) was used for the centromere and telomere deletions for the two-chromosome fusions, the positive rates were 50−75% (2/4 to 3/4).

In this study, we simultaneously fused two, two-chromosome sets, as well as three chromosomes using two selection markers using CRISPR-Cas9 facilitated homologous recombination in *S. cerevisiae*. We speculated that with more selection markers, more chromosomes could be simultaneously fused. This method could be a useful tool for creating multiple chromosome fusions in other organisms due to its simplicity and portability.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssynbio.8b00397.

Methods and additional tables (PDF)

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Notes

The authors declare no competing financial interest.

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