Front. Chem. Sci. Eng. 2018, 12(4): 806–814
https://doi.org/10.1007/s11705-018-1739-2

RESEARCH ARTICLE

Improving prodeoxyviolacein production via multiplex SCRaMbLE iterative cycles

Juan Wang[1,2*], Bin Jia[1,2*], Zexiong Xie[1,2], Yunxiang Li[1,2], Yingjin Yuan (✉)[1,2]

1 Key Laboratory of Systems Bioengineering (Ministry of Education), School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China
2 SynBio Research Platform, Collaborative Innovation Center of Chemical Science and Engineering (Tianjin), Tianjin University, Tianjin 300072, China

Abstract The synthetic chromosome rearrangement and modification by loxP-mediated evolution (SCRaMbLE) system has been used to improve prodeoxyviolacein (PDV) production in haploid yeast containing chromosome synV. To rapidly and continuously generate genome diversification with the desired phenotype, the multiplex SCRaMbLE iterative cycle strategy has been developed for the screening of high PDV production strains. Whole-genome sequencing analysis reveals large duplications, deletions, and even the whole genome duplications. The deletion of *YER151C* is proved to be responsible for the increase. This study demonstrates that artificial DNA rearrangement can be used to accelerate microbial evolution and the production of biobased chemicals.

Keywords synthetic biology, genome rearrangement, prodeoxyviolacein, SCRaMbLE, *Saccharomyces cerevisiae*

1 Introduction

Diversification in DNA and protein levels is the main cause of variety in phenotypes. Natural genome rearrangements have been observed in many species, including *Yersinia enterocolitica* [1], yeast [2], rice [3], mouse [4] and human [5]. However, natural genome rearrangements are a long-term and undirected process. Therefore, artificial methods are arising to address the problem. Genome shuffling has been developed to obtain genome diversity from the protoplast fusion of chemically mutated cells [6,7]. In this way, many genomic modifications are gathered in one cell and can be inherited permanently. However, the rearrangement scale of genome shuffling is limited to allelic sequences, which is inefficient. The synthetic chromosome recombination and modification by loxP-mediated evolution (SCRaMbLE) system, a key feature of the SC 2.0 project, has opened up a new way to generate mass structural variation (SV) and to explore the relationship between genotypes and phenotypes [8,9]. Because of the special structure of the 34 bp palindromic loxPsym site containing two 13 bp inverted repeats separated by an 8 bp symmetric spacer sequence, SCRaMbLE can generate genotypic diversity including genomic deletions, inversions, insertions (transpositions), or translocations within and between synthetic chromosomes as well as the corresponding phenotypic diversity.

Violacein is a blue-purple pigment usually produced by gram-negative bacteria, such as *Chromobacterium violaceum* [10]. Previous studies have shown antitumoral [11], antioxidant [12] and antimicrobial activities [13] in the constituents of *C. violaceum*. Recent research has reported the new use of violacein as an immunomodulatory, analgesic and antipyretic agent [14]. Lee et al. [15] expressed the violacein pathway in *Saccharomyces cerevisiae* for the first time. With a combinatorial method and several characterized promoters, they were able to screen for high violacein-producing strains and optimize the metabolic flux of all branches of this pathway.

Here, we choose prodeoxyviolacein, a key intermediate metabolite in the biosynthesis of violacein that relies on three genes, *vioA*, *vioB* and *vioE*, for optimization by SCRaMbLE. The synV haploid strain obtained from the work of Xie et al. [8] was used as the host to carry the heterogeneous pathway.

Received April 16, 2018; accepted April 22, 2018

E-mail: yjyuan@tju.edu.cn

*These authors contributed equally to this work

2 Materials and methods

2.1 Microbial strains and culture medium

The yWJR001 yeast strain integrating the prodeoxyviolacein pathway genes (*vioA*, *vioB*, *vioE*) was obtained from the yXZX846 (MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 LYS2 synV) *S. cerevisiae* strain in our laboratory. BY4741 (MATα his3Δ1 leu2Δ0 met15Δ0 ura3Δ0) was used as a transformation control in this study (See Table S1, Electronic Supplementary Material). YPD medium containing yeast extract (10 $g \cdot L^{-1}$), peptone (20 $g \cdot L^{-1}$), and glucose (20 $g \cdot L^{-1}$) was used to amplify and cultivate the strains before transformation. Synthetic complete medium lacking histidine (SC-His) and synthetic complete medium lacking leucine (SC-Leu) were used to screen colonies and select transformants. Synthetic complete galactose medium lacking histidine (SGal-His) was used to induce SCRaMbLE. YPD medium supplemented with 200 $mg \cdot L^{-1}$ geneticin (G418) was used to select the correct gene deletion strains.

Escherichia coli Trans5α (F-ψ80 lac ZΔM15 Δ (lacZYA-arg F) U169 endA1 recA1 hsdR17(rk−, mk +) supE44λ-thi-1 gyrA96 relA1 phoA) chemically competent cells were purchased from TransGen Biotech Company and used for bacterial transformation and propagation. These cells were grown at 37°C in Luria-Bertani (LB) broth containing 1% sodium chloride, 1% tryptone and 0.5% yeast extract. Selection was performed on solid medium (L B agar plate) containing 100 $\mu g \cdot mL^{-1}$ ampicillin.

2.2 Plasmid circuit construction and primers

The pRS416 plasmid was constructed using the yeast assembly method [16] and used as the vector for the prodeoxyviolacein genes. Specifically, the TEF1p, tTDH3p, tFBA1p and the TDH2t elements were PCR amplified from the genome of BY4741. The *vioA*, *vioB* and *vioE* genes were PCR amplified from the Registry of Standard Biological Parts. The pRS416 plasmid linearized by digestion with *Eco*RI was purified and transformed into BY4741 with all the other seven DNA fragments. Colonies with green color were picked to extract plasmids of pRS416-vio, which were then transformed into *E. coli* Trans5α. All the restriction endonucleases, T4 DNA Ligase and Phusion PCR Kits were purchased from New England BioLabs (NEB). PCR products were verified on a 1% agarose gel and purified by the TIANGEN Gel Midi Purification Kit (TIANGEN Biotech, DP209).

The pCRE4 (pGAL1-Cre-tCYC1) plasmid was obtained from previous work in our laboratory. In addition, pRS413 was transformed into pre-SCRaMbLEd strains to make a negative control (See Table S2). All the primers involved were synthesized by Genewiz and are listed in Table S3.

2.3 Yeast transformation

The standard protocol for yeast transformation using the LiAc/SS carrier has been described previously. To construct the pRS416-vio plasmid, 200 ng of each PCR product fragment was mixed with 300 ng of linearized vector. For plasmid transformation, 200 ng of plasmid was enough.

2.4 Integration of prodeoxyviolacein (PDV) pathway

Four plasmids bearing homologous arms of the CAN site in synV and the HO site in wtIV were obtained from our laboratory. The arms HO-A, HO-B-HIS3, CAN-A, and CAN-B-LEU2 were generated from *Hin*dIII and *Sac*I double digestion of the four plasmids. The PDV pathway was PCR amplified from pRS416-vio with a 40 bp homologous sequence on each side. For CAN site integration, CAN-A, CAN-B-LEU2 and the PCR product of the PDV pathway were cotransformed into yXZX846 and spread on SC-Leu agar to select the right transformant, which was then named yWJR001. Pathway duplication was conducted in yWJR001. The three fragments HO-A, HO-B-HIS3 and the PCR product of the PDV pathway were cotransformed into this strain, and SC-His agar was used to select double pathway transformants, which were named yWJV009.

2.5 Growth curve analysis

To verify the growth gap of the SCRaMbLEd strain yWJR026 and its ancestor yWJR001, we picked single colonies of each strain and inoculated them in 5 mL of YPD medium at 30°C overnight, and then cell culture was incubated at OD_{600} of 0.01 with 200 μL of fresh YPD medium in a 96-well plate. Data were measured in a Thermo MULTISKAN GO microplate reader at 600 $r \cdot min^{-1}$ and 30°C.

2.6 Deletion of target genes

The selective marker HIS3 was amplified from pRS413. The right and left homologous arms of the gene *YER151C* were amplified from the genome of yWJR001. The three parts were joined together by overlap PCR, and 200 ng of the gel-purified DNA fragment was directly transformed into yeast. The yWJV003 was generated by deleting *YER151C* in the yWJR001.

2.7 SCRaMbLE synV haploid yeast

First, we integrated the prodeoxyviolacein pathway into the CAN site of the synV chromosome to produce yWJR001, and then, the pCRE4 and pRS413 plasmids were transformed separately into this strain and plated onto SC-His agar medium. The plates were incubated at 30°C

for 72 h.

Second, single colonies were inoculated in 5 mL of SC-His at 30°C for 12 h, cells were centrifuged and washed twice with ddH_2O, and the OD_{600} was measured. Sample cultures were reinoculated into 5 mL of 2% galactose SGal-His medium with 1 μmol·L^{-1} estradiol (Sigma-Aldrich) at an OD_{600} of 1.0. SCRaMbLE was carried out at 30°C for 8 h.

Third, yeast cells were isolated from 1 mL of culture by spinning at 3000 r·min^{-1} in a centrifuge. The estradiol and SGal-His medium were removed completely by washing with ddH_2O. Then, the cells were resuspended in 1 mL of SC-His glucose medium.

Fourth, a series of dilutions of SCRaMbLEd cells were plated on SC-His glucose agar and cultivated at 30°C for 72 h. Strains with a darker color than the wild-type (wt) parent strain were picked to SCRaMbLE for the next cycle. We counted the darker-colored colonies and calculated their ratio in every SCRaMbLE cycle.

2.8 HPLC method for product quantification

Single colonies of SCRaMbLEd strains and the control strain were picked, inoculated into 5 mL of SC medium, and cultured at 220 r·min^{-1} and 30°C overnight. The cultures were then transplanted to 50 mL of fresh SC medium in a 200 mL conical flask with a final OD_{600} of 0.1, then grown for 72 h under the same conditions. We then took 10 mL of culture from each flask and dried it at 70°C to determine the dry cell weight. Another 2 mL of culture was centrifuged for 2 min at 6000 r·min^{-1} and washed with double-distilled water. Heat methanol extraction was adopted to extract the product. The cells were resuspended in 500 μL of methanol and heated at 75°C for at least 10 min until the cells changed to an off-white color. The mixture was vortexed twice during this time. The methanol extracts were centrifuged and filtered with a 0.22 μm filter for subsequent analysis. An HPLC system (Waters e2695) equipped with a BDS Hypersil C18 column (4.6 mm × 150 mm, 5 μm) and a UV/VIS detector (Waters 2489) was used to analyze the produced prodeoxyviolacein. We detected signals of prodeoxyviolacein at 610 nm, as described in a previous study [17]. In addition, the mobile phase consisted of methanol-water (7/3, v/v) with a flow rate of 0.3 mL·min^{-1} at 25°C. For the continuously improved strains, absolute quantification of the product was performed in a 250 mL round-bottom flask. The extraction process was the same as above.

2.9 Genome sequencing and verification

Yeast cultures were grown to saturation, and amplicon-free libraries were prepared. Paired-end sequencing of all libraries was performed on the Illumina HiSeq 2000 platform. The original figure data obtained by high-throughput sequencing were stored in FASTQ format, containing sequencing information and the corresponding sequencing quality information on the reads. The sequenced data were filtered, and the adapter sequence and low-quality data were removed, resulting in the clean data used for subsequent analysis. Structural variation (SV) refers to the insertion, deletion, inversion and translocation of the large segments in the genome level. The insertion, deletion, inversion, intrachromosomal translocation, and interchromosomal translocation between the reference and the sample were found by BreakDancer software. Whole-genome sequencing is available at the BIG Data Center under accession code CRA000772.

3 Results and discussion

3.1 Optimizing the SCRaMbLE to improve the production of PDV

In the yeast aromatic amino acid pathway, *ARO1, ARO2, ARO3* and *ARO4* catalyze the synthesis of erythrose 4-phosphate (E4P) and phosphoenolpyruvate to form chorismate [18]. L-Tryptophan (L-Trp) originates from the catalysis of chorismate by *TRP1, TRP2, TRP3, TRP4* and *TRP5* (Fig. 1(a)). PDV is synthesized from L-Trp by the genes *vioA*, *vioB*, and *vioE* [19]. To investigate whether SCRaMbLE of synV could be used *in vivo* to increase the production of prodeoxyviolacein, the heterologous PDV synthesis pathway along with a LEU2 marker was integrated into the YEL063C/CAN1 locus in synV to generate the yeast strain yWJR001 (Fig. 1(a)). As PDV is a dark green compound, green colonies were observed on the SC-Leu agar plate (Fig. 1(b)). As shown in Fig. 1(c), HPLC analysis under 610 nm showed a single peak for the cell extraction, which indicated that PDV is synthesized in the synV yeast without any other analog.

Previous research had demonstrated a controllable SCRaMbLE method by introducing the GAL1 promoter into the Cre/EBD expression cassette and constructing pCRE4 with a genetic switch [20]. The Cre/EBD fusion protein can act on loxPsym sites only when galactose and estradiol are present simultaneously, leading to many random interactions (Fig. 2(a)). To lower the leakage of SCRaMbLE and guarantee sustainable rearrangement, this genetic switch was used in our research (Fig. 2(a)). As there are 177 loxPsym segments in the synV chromosome, expressing Cre *in vivo* can result in large-scale genome rearrangements as well as corresponding phenotypic diversity (Fig. 2(b)). As loxPsym sites are positioned downstream of nonessential genes, expression of the activated Cre-EBD may delete large chromosome fragments containing essential genes in a synthetic chromosome, resulting in loss of viability. To assay the mortality caused by SCRaMbLE, the ancestral yeast yWJR001 containing pCRE4 was cultured in SGal-His medium with estradiol for 2, 4, 8, 12, 16 and 24 h, then plated on SC-His

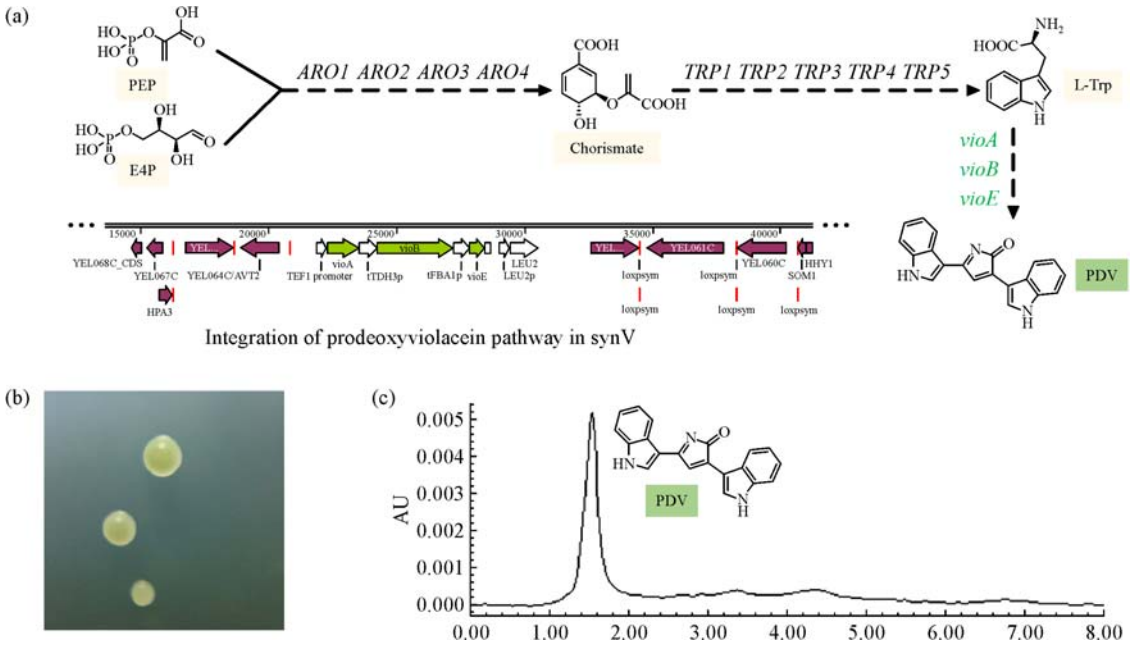

Fig. 1 Integration of heterogenous PDV pathway into synV yeast. (a) Tryptophan biosynthesis pathway and prodeoxyviolacein (PDV) pathway in yeast; (b) yeast containing the PDV pathway formed green colonies; (c) HPLC analysis of PDV extraction at 610 nm

agar for colony counting. The yeast yWJR001 containing pRS413 was used as a control. As shown in Fig. 2(c), the SCRaMbLE mortality was 58.21%, 92.29%, 99.47%, 99.89%, 99.98%, and 99.99% when cells were SCRaMbLEd for 2, 4, 8, 12, 16 and 24 h, respectively. This result indicated that the SCRaMbLE mortality is strongly associated with the induction time, as in traditional ultraviolet mutagenesis. To maintain a high rate of genome rearrangement in the whole yeast population, 8 h was chosen as the standard SCRaMbLE induction time.

3.2 Generating desired strain phenotypes by SCRaMbLE

To facilitate the screening, SCRaMbLEd cells were plated on SC-His (glucose) agar and incubated at 30°C for 48 h, followed by incubation at 4°C for 10–15 h until the colony color deepened enough for differentiation. After one round of SCRaMbLE from the initial strain yWJR001, many small colonies were observed among the deeper green colonies. As shown in Fig. 3(a), yWJR026 presents the deepest color, close to black-green, in contrast to the light green color of its ancestor yWJR001. The SCRaMbLEd strains yWJR026 showed an increase of 3.8-fold in the PDV yield compared with that of the non-SCRaMbLEd parent strain yWJR001 (Fig. 3(b)). The cell growth curves of yWJR001 and yWJR026 are shown in Fig. 3(c). A growth defect was observed in yWJR026. To determine the genomic SV in yWJR026 caused by SCRaMbLE, we deep sequenced yWJR026 and aligned the reads to chromosome synV. As shown in Fig. 3(d), three long fragments were duplicated to two copies, and the total length reached to 465.8 kb, covering approximately 80% of the whole synV chromosome. The PDV pathway was also observed to be duplicated, which was probably the main cause of the improved production. These results indicated that SCRaMbLE can generate large chromosome fragment duplication and can be used to improve biochemical production. Generally, the natural evolution of microorganisms is a long-term accumulation process [21]. We hypothesized that a stepwise SCRaMbLE strategy can be used to iteratively accumulate multiple rearrangements through multiple cycles of SCRaMbLE for enhanced PDV production. To rapidly and continuously generate genome diversification, MuSIC was used to iteratively accumulate desired phenotypes and genotypes through multiple cycles of SCRaMbLE. Cells with improved production yields were incubated for the subsequent cycle to perform stepwise screening accumulation (Fig. 2(c)).

To prove the concept of MuSIC, five cycles of SCRaMbLE were performed in the yWJR001 (Fig. 4(a)). Strains yWJR006, yWJR040, yWJR085, yWJR104 and yWJR111 were generated from SCRaMbLEd plates of yWJR001, yWJR006, yWJR040, yWJR085 and yWJR104, respectively. The color intensity of the cultures in each cycle increased with MuSIC. The PDV production levels of yWJR006, yWJR040, yWJR085, yWJR104 and yWJR111 increased 1.07-fold, 1.18-fold, 2.34-fold, 2.95-fold and 4.04-fold compared with yWJR001, respectively, representing a significant fold change from the production of yWJR001 (Fig. 4(b)). To analyze the frequency of various SCRaMbLEd phenotypes, we counted the ratio of

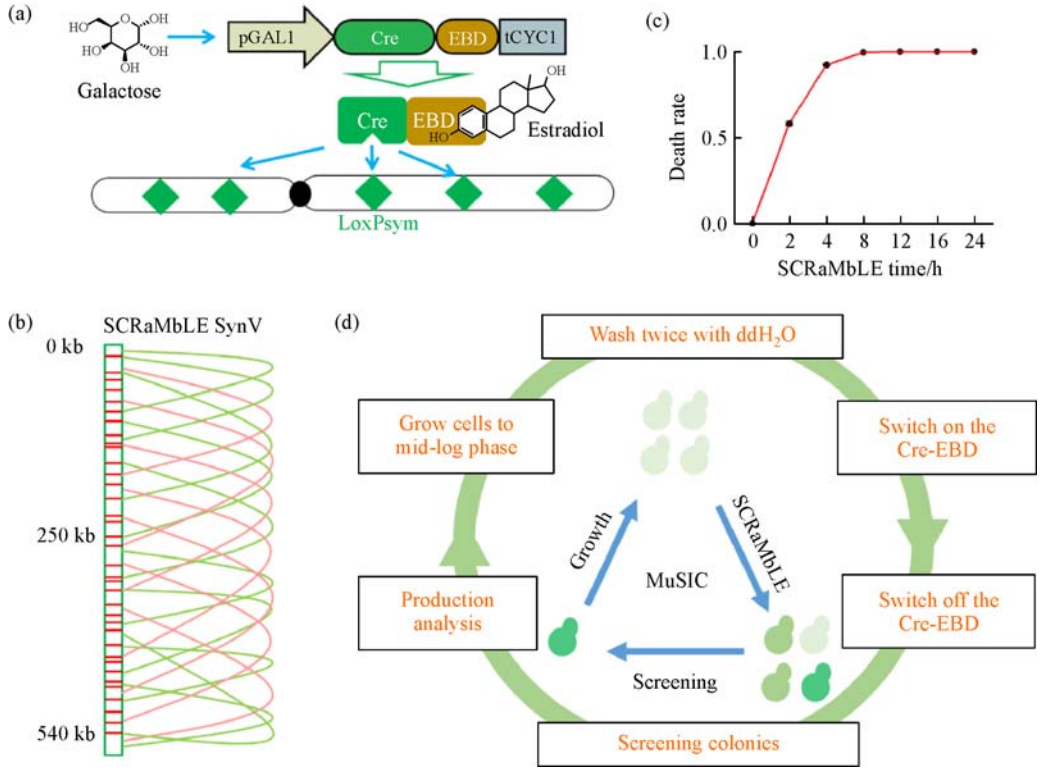

Fig. 2 Characterization of the SCRaMbLE system for improving the PDV production. (a) Cre expression for SCRaMbLE (The reaction between loxPsym sites and Cre-EBD fusion proteins was achieved when cells were induced with extra galactose and estradiol); (b) SCRaMbLE synV generated random recombination of 177 loxPsym segments in synV; (c) the death rate curve of the SCRaMbLE system; (d) Multiplex SCRaMbLE iterative cycles (MuSIC)

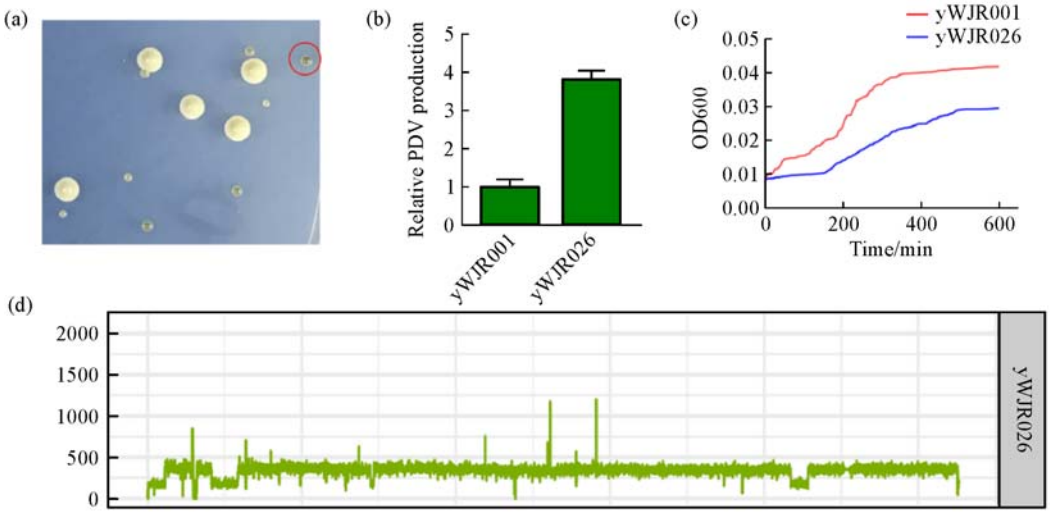

Fig. 3 Phenotypic verification and genome sequencing of the fitness-defective strain yWJR026. (a) Phenotypic diversity was observed after SCRaMbLE of yWJR001; (b) PDV production analysis of yWJR001 and yWJR026; (c) growth assay of yWJR026 and its ancestor yWJR001 in YPD medium; (d) deep sequencing analysis of yWJR026 (Long segment duplications were observed)

darker-colored strains in each cycle. As shown in Fig. 4(c), the darker color ratios from cycle1 to cycle5 were 6.99%, 2.87%, 2.46%, 1.22% and 1.36%, respectively. These results demonstrated the feasibility of generating optimized phenotypes with iterative SCRaMbLE. To assay the growth phenotype of the 5 SCRaMbLEd strains, 10-fold serial dilutions of yWJR001, yWJR006, yWJR040, yWJR085, yWJR104 and yWJR111 were spotted on

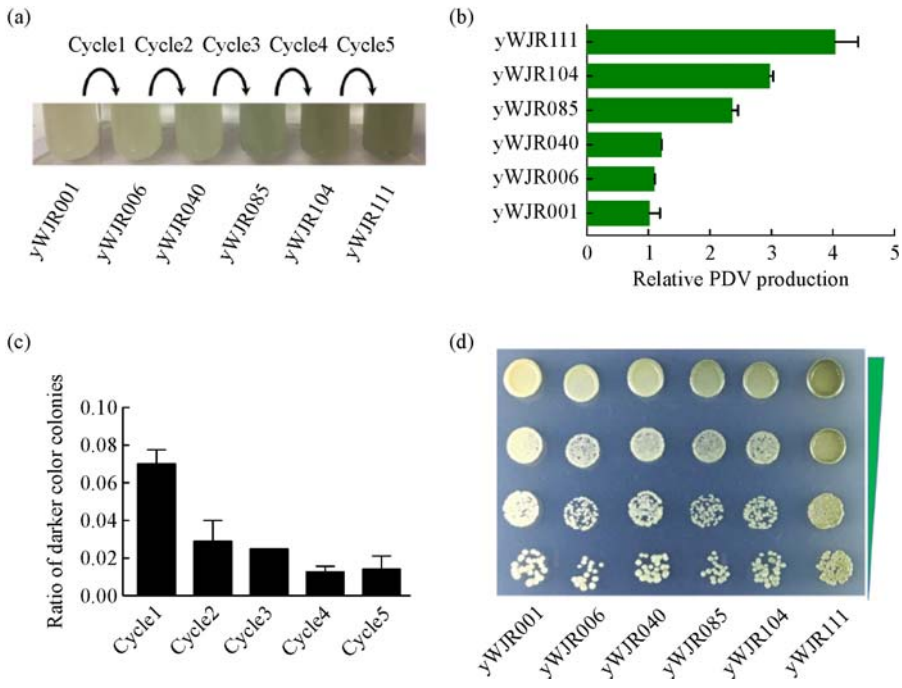

Fig. 4 MuSIC for continuously improving the PDV production. (a) Culture color corresponding to PDV yield from yWJR001, yWJR006, yWJR040, yWJR085, yWJR104 and yWJR111; (b) HPLC analysis of PDV from cultures of the 6 strains; (c) ratios of darker color colonies were calculated for each MuSIC cycle; (d) 10-fold dilution assays of the 6 strains

SC-His agar plates at 30°C for 48 h. As shown in Fig. 4(d), no significant growth defects were observed in these experiments.

3.3 Deep sequencing analysis of high-yield production strains

To determine the genomic SV caused by SCRaMbLE in high PDV production strains, we deep sequenced yWJR006, yWJR040, yWJR085 and yWJR104. As shown in Fig. 5(a), the deletion of *YER151C* was observed in yWJR006, yWJR040, yWJR085 and yWJR104. This result demonstrated that yWJR040, yWJR085 and yWJR104 inherited the deletion of *YER151C* from yWJR006. In addition, a decrease in copy number from YER158C to YER174C was observed in yWJR085 and yWJR104, and a decrease in copy number from YER025W to YER073W was observed in yWJR104 alone. A qPCR experiment demonstrated that the whole genome was duplicated into two copies, and deletions of YER158C to YER174C and YER025W to YER073W were observed on one copy of the synV chromosome (Fig. S1).

To assay whether *YER151C* deletion or pathway duplication influenced the heterogeneous prodeoxyviolacein yield, we performed a knockout of *YER151C* and the HO site (wild-type IV) integration of the second pathway genes in the ancestor yWJR001 by homologous recombination (Fig. 5(b)). As shown in Fig. 4(b), the selective marker HIS3 with homologous arms on the flank of *YER151C* was constructed by overlap-extension PCR, and three pathway genes were prepared with a HIS3 marker and homologous arms on each side. After transformation into the host, the transformants were spread on SC-His agar medium plates to screen for the correct one, followed by PCR verification. The PDV production was compared with that of yWJR001. The data showed a 1.21-fold improvement in PDV yield in the gene deletion strain yWJV003 and a 1.57-fold improvement in the pathway duplicated strain yWJV009 (Fig. 5(c)). *YER151C* is a ubiquitin-specific protease that is implicated in the regulation of a variety of cellular functions and plays a major role in stress response in eukaryotic cells by targeting misfolded and damaged proteins for degradation [22,23]. The previous literature also indicated that the deletion of genes related to autophagy can potentially modify global transcription in yeast [20], increasing the PDV production by improving the compatibility between the host cells and heterologous pathways. PDV pathway duplication can increase the expression of heterogeneous *vioA*, *vioB* and *vioE*, which improved the rate of conversion from L-Trp to PDV. These results were also consistent with those of previous works [15,17].

From each round of SCRaMbLE, we selected the top five strains with high production of prodeoxyviolacein for the next cycle of SCRaMbLE. However, some groups tended to generate strains with fitness-defective variations and few improvements. To increase the PDV production

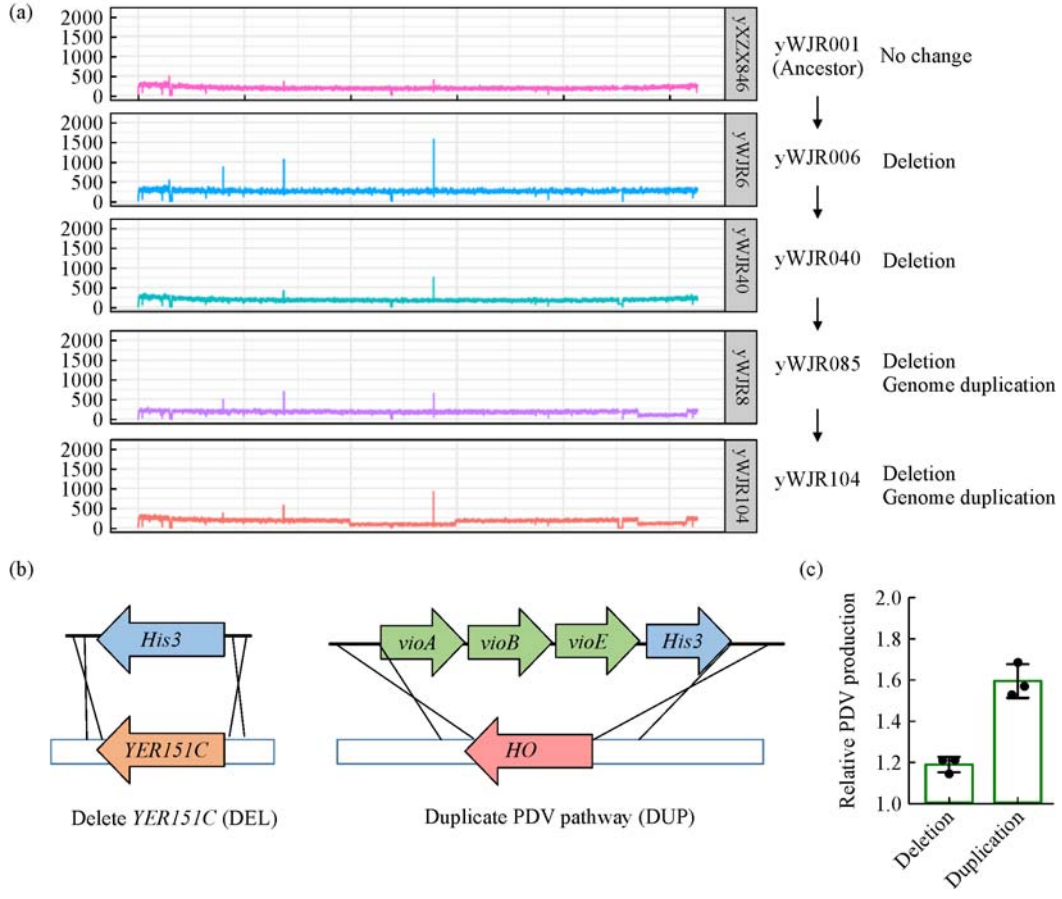

Fig. 5 Deep sequencing analysis of SCRaMbLEd variations and verification. (a) Deep sequencing of five related strains; (b) deletion of *YER151C* and duplication of PDV pathway were performed in yWJR001; (c) verification of the deletion of *YER151C* and duplication of the PDV pathway

iteratively, we selected the highest-performance groups for further cycles of SCRaMbLE. The strain yWJR111 was proved to have the best yield in our study. To prove the concept of our MuSIC strategy, five strains from the yWJR111 group were used for deep sequencing. Strains yWJR006 and yWJR026 both showed improved PDV production in the first SCRaMbLE cycle. Our results proved that *YER151C* deletion and PDV pathway duplication are responsible for the improvements in yWJR006 and yWJR026, respectively. Diversity in the genome structure variety was demonstrated to lead to changes in PDV production. With increasing PDV production, the strain color became darker throughout the 5 cycles.

Despite the duplication of genes in synthetic chromosome V, the wild-type chromosomes can also be duplicated to two copies, an unexpected case of genome rearrangement (Figs. S2–S6). This result demonstrated that SCRaMbLE can generate diploid yeasts from haploid strains. Moreover, SCRaMbLE in diploid yeasts can increase the diversity of genome structure variation, including the deletion of essential genes from one of the synthetic chromosomes. Our results indicated that different types of genome structure variations can improve PDV production. Further cycles of SCRaMbLE are predicted to continuously evolve the genome and improve the PDV production. In this work, PDV production was used for a proof-of-concept study. Combined with metabolic engineering methods, our MuSIC strategy may also be used to improve the production of other high-value chemicals, such as resveratrol [24] and astaxanthin [25].

Above all, the SCRaMbLE system contributes positively to the rapidly growing field of synthetic biology [26], and this work innovatively investigates a new strategy for the rapid evolution of the genome and the improvement of biochemical production.

4 Conclusions

In this study, we illustrate that SCRaMbLE can be used to generate genomic diversification and improve PDV production. SCRaMbLEing haploid yeast with a synthetic chromosome raised the lethality of the synthetic yeast by

the deletion of essential genes. A single SCRaMbLE can generate duplications and deletions, increasing PDV production. Multiple cycles of SCRaMbLE cannot only inherit genome structure variations but also continuously improve PDV production in yeast. Meanwhile, whole-genome sequencing (WGS) showed that the entire yeast genome can be duplicated during the MuSIC process. Verification of genome rearrangement events could be used to map the target genes and extend the limits of our biological knowledge. This parallelized design-SCRaMbLE-WGS-learn cycle could provide a standard operation program for optimizing heterogeneous pathways and improving the production of fuels, chemicals, pharmaceuticals, and other products.

Acknowledgements We thank the National Program on Key Basic Research Project of China (2014CB745100) and the National Natural Science Foundation of China (21750001 and 21621004) for funding.

Electronic Supplementary Material Supplementary material is available in the online version of this article at https://doi.org/10.1007/s11705–018-1739-2 and is accessible for authorized users.

References

1. Dymond J S, Richardson S M, Coombes C E, Babatz T, Muller H, Annaluru N, Blake W J, Schwerzmann J W, Dai J B, Lindstrom D L, et al. Synthetic chromosome arms function in yeast and generate phenotypic diversity by design. Nature, 2011, 477(7365): 471–476
2. Yue J X, Li J, Aigrain L, Hallin J, Persson K, Oliver K, Bergström A, Coupland P, Warringer J, Lagomarsino M C, et al. Contrasting evolutionary genome dynamics between domesticated and wild yeasts. Nature Genetics, 2017, 49(6): 913–924
3. Zhang Q J, Zhu T, Xia E H, Shi C, Liu Y L, Zhang Y, Liu Y, Jiang W K, Zhao Y J, Mao S Y, et al. Rapid diversification of five *Oryza* AA genomes associated with rice adaptation. Nucleic Acids Research, 2014, 111(46): e4954–e4962
4. Pevzner P, Tesler G. Genome rearrangements in mammalian evolution: Lessons from human and mouse genomes. Genome Research, 2003, 13(1): 37–45
5. Redon R, Ishikawa S, Fitch K R, Feuk L, George H, Andrews T D, Fiegler H, Shapero M H, Carson A R, Chen W W, et al. Global variation in copy number in the human genome. Nature, 2006, 444 (7118): 444–454
6. Zhang Y X, Perry K, Vinci V A, Powell K, Stemmer W P C, Del Cardayré S B. Genome shuffling leads to rapid phenotypic improvement in bacteria. Nature, 2002, 415(6872): 644–646
7. Biot-Pelletier D, Martin V J J. Evolutionary engineering by genome shuffling. Applied Microbiology and Biotechnology, 2014, 98(9): 3877–3887
8. Xie Z X, Li B Z, Mitchell L A, Wu Y, Qi X, Jin Z, Jia B, Wang X, Zeng B X, Liu H M, et al. "Perfect" designer chromosome V and behavior of a ring derivative. Science, 2017, 355(6329): 1046
9. Wu Y, Li B Z, Zhao M, Mitchell L A, Xie Z X, Lin Q H, Wang X, Xiao W H, Wang Y, Zhou X, et al. Bug mapping and fitness testing of chemically synthesized chromosome X. Science, 2017, 355 (6329): 1048
10. Durán N, Justo G Z, Durán M, Brocchi M, Cordi L, Tasic L, Castro G R, Nakazato G. Advances in chromobacterium violaceum and properties of violacein—its main secondary metabolite: A review. Biotechnology Advances, 2016, 34(5): 1030–1045
11. Melo P S, Maria S S, Vidal B C, Haun M, Durán N. Violacein cytotoxicity and induction of apoptosis in V79 cells. In Vitro Cellular & Developmental Biology, 2000, 36(8): 539–543
12. Konzen M, De Marco D, Cordova C A S, Vieira T O, Antônio R V, Creczynski-Pasa T B. Antioxidant properties of violacein: Possible relation on its biological function. Bioorganic & Medicinal Chemistry, 2006, 14(24): 8307–8313
13. Durán N, Antonio R V, Haun M, Pilli R A. Biosynthesis of a trypanocide by *Chromobacterium violaceum.* World Journal of Microbiology & Biotechnology, 1994, 10(6): 686–690
14. Antonisamy P, Ignacimuthu S. Immunomodulatory, analgesic and antipyretic effects of violacein isolated from *Chromobacterium violaceum.* Phytomedicine, 2010, 17(3–4): 300–304
15. Lee M E, Aswani A, Han A S, Tomlin C J, Dueber J E. Expression-level optimization of a multi-enzyme pathway in the absence of a high-throughput assay. Nucleic Acids Research, 2013, 41(22): 10668–10678
16. Lin Q, Jia B, Mitchell L A, Luo J C, Yang K, Zeller K I, Zhang W Q, Xu Z W, Stracquadanio G, Bader J S, Boeke J D, Yuan Y J. RADOM, an Efficient *in vivo* method for assembling designed DNA fragments up to 10 kb long in *Saccharomyces cerevisiae.* ACS Synthetic Biology, 2014, 4(3): 213–220
17. Liu D, Liu H, Li B Z, Qi H, Jia B, Zhou X, Du H X, Zhang W, Yuan Y J. Multigene pathway engineering with regulatory linkers (M-PERL). ACS Synthetic Biology, 2016, 5(12): 1535–1545
18. Knaggs A R. The biosynthesis of shikimate metabolites. Natural Product Reports, 2003, 20(1): 119–136
19. Zalatan J G, Lee M, Almeida E R, Gilbert L A, Whitehead E H, La Russa M, Tsai J C, Weissman J S, Dueber J E, Qi L S, Lim W A. Engineering complex synthetic transcriptional programs with CRISPR RNA scaffolds. Cell, 2015, 160(1–2): 339–350
20. Jia B, Wu Y, Li B Z, Mitchell L A, Liu H, Pan S, Wang J, Zhang H R, Liu H M, Chen Z X, et al. Precise control of SCRaMbLE in synthetic haploid and diploid yeast. Nature Communications, 2018, 9(1): 1933
21. Querol A, Fernández-Espinar M T, Del Olmo M, Barrio E. Adaptive evolution of wine yeast. International Journal of Food Microbiology, 2003, 86(1–2): 3–10
22. Gatti L, Hoe K L, Hayles J, Righetti S C, Carenini N B, Laura D, Kim D U, Park H O, Perego P. Ubiquitin-proteasome genes as targets for modulation of cisplatin sensitivity in fission yeast. BMC Genomics, 2011, 12(1): 44
23. Dodgson S E, Santaguida S, Kim S, Sheltzer J, Amon A. The pleiotropic deubiquitinase UBP3 confers aneuploidy tolerance. Genes & Development, 2016, 30(20): 2259–2271
24. Liu D, Li B Z, Liu H, Guo X J, Yuan Y J. Profiling influences of gene overexpression on heterologous resveratrol production in *Saccharomyces cerevisiae*. Frontiers of Chemical Science and

Engineering, 2017, 11(1): 117–125

25. Wang R Z, Gu X L, Yao M D, Pan C H, Liu H, Xiao W H, Wang Y, Yuan Y J. Engineering of β-carotene hydroxylase and ketolase for astaxanthin overproduction in *Saccharomyces cerevisiae*. Frontiers of Chemical Science and Engineering, 2017, 11(1): 89–99
26. Yuan Y J, Wu J C, Wang X. Collaborations of China with the world in Synbio. Frontiers of Chemical Science and Engineering, 2017, 11 (1): 1–2