Full Paper

A highly efficient targeted recombination system for engineering linear chromosomes of industrial bacteria *Streptomyces*

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Soil bacteria *Streptomyces* are the most important producers of secondary metabolites, including most known antibiotics. These bacteria and their close relatives are unique in possessing linear chromosomes, which typically harbor 20 to 30 biosynthetic gene clusters of tens to hundreds of kb in length. Many *Streptomyces* chromosomes are accompanied by linear plasmids with sizes ranging from several to several hundred kb. The large linear plasmids also often contain biosynthetic gene clusters. We have developed a targeted recombination procedure for arm exchanges between a linear plasmid and a linear chromosome. A chromosomal segment inserted in an artificially constructed plasmid allows homologous recombination between the two replicons at the homology. Depending on the design, the recombination may result in two recombinant replicons or a single recombinant chromosome with the loss of the recombinant plasmid that lacks a replication origin. The efficiency of such targeted recombination ranges from 9 to 83% depending on the locations of the homology (and thus the size of the chromosomal arm exchanged), essentially eliminating the necessity of selection. The targeted recombination is useful for the efficient engineering of the *Streptomyces* genome for large-scale deletion, addition, and shuffling.

Key Words: genome engineering; linear chromosome; linear plasmid; *Streptomyces*; targeted recombination

Introduction

Soil bacteria *Streptomyces* are important industrial microorganisms notable for being the richest source of secondary metabolites, including most known antibiotics, antifungal agents, food preservatives, anthelmintic agents, and antitumor agents.

These bacteria are unique in possessing linear chromosomes capped by terminal proteins (TP) covalently bound to the 5' ends of DNA (reviewed in Kirby and Chen (2011)). These replicons contain terminal inverted repeats (TIR) of various sizes. The telomere sequences are highly conserved for approximately 300 bp, which contain tightly packed palindromic sequences (Huang et al., 1998; Lin et al., 1993). The TPs are also highly conserved in sequence and size (185 aa), encoded by terminally located *tpg*. In most *Streptomyces*, *tpg* forms an operon with *tap*, which encodes an essential telomere-associated protein (Bao and Cohen, 2003). Both *Tpg* and *Tap* are crucial for completing replication of the telomeres (Yang et al., 2015).

TP-capped linear plasmids are present in many *Streptomyces* species. Some of them possess homologous telomeres and TPs as the chromosomes, while the others possess heterologous telomeres and TPs. For example, SCP1 of *Streptomyces coelicolor* A3(2) possess very different telomere sequences and 259-aa TP encoded by *tpc*, which forms an operon with *tac* (Huang et al., 2007). The latter encodes a 274-aa protein also essential for replication of SCP1. Artificially constructed linear plasmid carrying the SCP1 telomeres and *tac-tpc* operon was shown to co-exist and recombine with SCP1 at the *tac-tpc* homology to form recombinant plasmids in *Streptomyces lividans* (Huang et al., 2007). The recombination was observed in all the transformants examined.

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Fig. 1. Targeted recombination between a linear plasmid and a chromosome.

A. pLUS356 and derivatives. Chromosomal segments (1–4, 2r, and 4r) were inserted into pLUS356 at the *SacI* (S) and *HindIII* (H) sites in two orientations. Linear plasmids derived from pLUS356 (pLUS356L) and derivatives are shown to the right. *E. coli* and *Streptomyces* backbones are shown in open and red arcs, respectively. Red squares, *S. lividans* telomeres. ARS, autonomously replicating sequence. *tsr*, thiostrepton-resistance gene. 'A', *AseI* sites. B. Recombination between pHP1L and M145 chromosome. a. M145 chromosome (black line) recombines with pHP1L (red line) to form a recombinant chromosome and recombinant plasmid pHP1L-J'. The *AseI* sites, the fragment designations, the 'core region' (grey shade) (Bentley et al., 2002), the telomeres (black squares) of M145 chromosome are indicated. The size (in kb) of relevant sequences are shown in parentheses. The homologous segment (1) is marked by the red tick. b. PFGE (short pulse time) of uncut and *AseI*-digested M145[pHP1L] DNA. The results of the representative of 12 transformants is shown here. 'chr', chromosomal fragments. Selected *AseI* fragments with their size (in kb) are marked. c. PFGE-separated DNA was hybridized to *Segment 1* probe. *, unknown fragments sensitive to both Exonuclease III and λ Exonuclease (data not shown); presumably intermediates or products of aberrant replication. **, hybridization signal spilled over from the neighboring lane. C. Recombination between pHP2L and M145 chromosome. a. M145 chromosome recombines with pHP2L. b. PFGE (short pulse time) of uncut and *AseI* digested M145[pHP2L] DNA. c. PFGE-separated DNA was hybridized to *Segment 2* probe. The other labels are as in B. D. Recombination between pHP3L and M145 chromosome. a. M145 chromosome recombines with pHP3L. b. PFGE (short pulse time) of uncut and *AseI* digested M145[pHP2L] DNA. c. PFGE-separated DNA was hybridized to *Segment 3* probe. The other labels are as in B. For the transformants, only the results of a representative one are shown. **, hybridization signal spilled over from the neighboring lane.

Recombination between a linear plasmid and chromosome was observed in the formation of SCP1'-*cysD* plasmid in *S. coelicolor* (Yamasaki and Kinashi, 2004) and in arm replacement in *Streptomyces rimosus* (Gravius et al., 1994; Pandza et al., 1998). Recombination between different arms of sister chromosomes resulted in recombinant chromosomes with extra long TIR in *Streptomyces ambofaciens* (Fischer et al., 1998). The 15.4-kb identity between the right arm of linear plasmid SLP2 and both arms of the *Streptomyces lividans* chromosome is assumed to have resulted from past recombination between the two replicons (Huang et al., 2003). Exchanges of arm sequences, which are diverse in sequence, are very important in shaping diverse genomes in *Streptomyces* during evolution (Chen et al., 2002).

In this study, we devise a targeted recombination procedure, in which a specific sequence of the chromosome is placed on the plasmid to provide a substrate for homologous recombination between the plasmid and the chromosome. Three kb of homology was sufficient to achieve recombination efficiencies ranging from 9% to 83%. Curing of some recombinant plasmids resulted in cultures with a truncated chromosome. At least 424 kb of the left arm of the chromosome might be deleted from the chromosome without affecting viability. The targeted recombination strategy is useful for engineering *Streptomyces* chromosomes for deletions of specific arm sequences and/or insertions of long heterologous sequences (such as antibiotic biosynthesis gene clusters) into the chromosomes.

Material and Methods

Bacteria and media. Microbiological and genetic manipulations of *Streptomyces* were according to Kieser et al. (2000). *S. coelicolor* A3(2) M145 (Hopwood et al., 1983) was used as the host for the plasmid-chromosome recombination studies. Liquid cultures of *Streptomyces* were grown in TSB containing 0.4% glycine or YEME containing 0.5% glycine. For solid cultures of *Streptomyces*, R5, YM, or Minimum Medium was used. *E. coli* ET12567 (MacNeil et al., 1992) were cultured in LB medium.

Construction of linear plasmids. Linear plasmids were constructed according to Qin and Cohen (1998). The starting material, plasmid pLUS356 (Fig. 1A) (Huang et al., 2013), was an *E. coli-Streptomyces* shuttle plasmid harboring a linear plasmid sequence consisting of a pair of *S. lividans* telomeres (Huang et al., 2003) flanking a thiostrepton-resistance gene (*tsr*), and an autonomously replicating sequence (ARS) of linear plasmid pSLA2 (Qin and Cohen, 1998).

pLUS356 and its derivatives were propagated in *E. coli* ET12567. Plasmid DNA linearized with *AseI* was used to transform M145. Thiostrepton-resistant (Thio^r) transformants were isolated, in which the expected TP-capped linear plasmids were generally found. For example, transformation of *AseI*-linearized pLUS356 produced 7.9-kb linear plasmid pLUS356L (Figs. 1A and B-b).

The unique *HindIII* and *SacI* sites on pLUS356 were used for insertion of chromosomal segments. The inserted segments and hybridization probes were amplified by PCR using the primers listed in Supplementary Table S1. The

Fig. 2. Lack of recombination between pHP3L and the chromosome of *recA* mutant T145-15.

The procedures and labels are as in Fig. 1. A. PFGE (short pulse time) of uncut T145-15[pHP3L] DNA. B. PFGE-separated DNA hybridized with *Segment 3* DNA probe. Shown are the results of four independent isolates of T145-15[pHP3L]. The other three transformants were included for comparison. 'M', molecular weight markers. The other labels are as in Fig. 1.

coding information of the inserted segments is given in Supplementary Fig. S1.

DNA manipulations. Basic recombinant DNA procedures were according to Green and Sambrook (2012). Pulsed-field gel electrophoresis (PFGE) procedure was according to Lin et al. (1993) with minor modifications. For separation of restriction fragments, a relative short pulse time from 5 to 200 s (ramped) was used in CHEF-DR III Systems (Bio-Rad) for 19–30 h. For separation of intact chromosomes and plasmids, a long pulse time from 30–60 m (ramped) in Rotaphor System 6.0 (Biometra) for 4 d.

Due to a wide dynamic range (over 1,000:1) of the fluorescence signals of the ethidium bromide-stained DNA, visualization of the small plasmids (7–11 kb) demanded increased brightness adjustment that resulted in unavoidable overexposure of the largest chromosomal DNA fragments.

For hybridization, Digoxigenin (DIG) DNA Labeling Mix (Roche) was used to prepare the probes, and Anti-DIG-AP, Fab fragments (Roche) for Dig antibody, and CSPD (Roche) were used for detection. The hybridization signals were captured on ImageQuant LAS 4000 Mini, and quantified using Multi Gauge software (GE Healthcare Life Sciences).

Curing of linear plasmids. Spores of the plasmid-harboring cultures were spread on YM agar to form single colonies. The colonies were picked and plated on YM and YM-Thio agars to screen for Thio sensitive (Thio^s) derivatives. PCR was performed on the Thio^s colonies for the presence of a 100 to 101-kb DNA sequence from the left end of the chromosome to detect arm deletions.

Results and Discussion

Highly-efficient targeted recombination between a linear plasmid and a chromosome

To provide the substrate for recombination, we ligated a 3.1-kb *HindIII*-*SacI* fragment at position 197 to 200-kb from the left end of an M145 chromosome (designated 'Segment 1') to an *HindIII*-*SacI* digested pLUS356 to produce pHP1 (Fig. 1A). Transformation of M145 with *AseI*-linearized pHP1 produced an 11-kb linear plasmid pHP1L in the Thio^r transformants (Fig. 1B-b).

Recombination between pHP1L and the chromosome produced a 208-kb recombinant linear plasmid carrying the 197-kb left arm of the chromosome (Fig. 1B-a), as detected by fluorescence stain in PFGE gel (Fig. 1B-b) and hybridization to a *Segment 1* probe (Fig. 1B-c). The 8,470-kb recombinant chromosome expected from recombination could not be mobilized under the short pulse time used for PFGE in Fig. 1B (Lin et al., 1993). Only some degraded fragments appeared in the upper 'compression zone'.

AseI cut M145 chromosomal DNA into 16 fragments (Kieser et al., 1992) (Fig. 1B-b). In M145, *Segment 1* resides in 240-kb *AseI*-J in M145[pHP1L]. Recombination separated *AseI*-J into 208-kb *AseI*-J' and 43-kb *AseI*-J (Fig. 1B-a). The former was relocated to the recombinant plasmid, which was designated pHP1L-J'.

pHP1L-J' contained no *AseI* site, and could not be detected by fluorescence stain due to its low abundance and co-migration with *AseI*-K (Fig. 1B-b), but was detected by hybridization (Fig. 1B-c). *AseI*-J was revealed by fluorescence stain (Fig. 1B-b) and hybridization using *Segment 1* as the probe (Fig. 1B-c).

AseI-J remained in M145[pHP1L], indicating that intact M145 chromosomes were present in M145[pHP1L] hyphae. To quantify the extent of recombination, we defined 'recombination efficiency' as the fraction of recombinant chromosomes/total chromosomes, i.e., $AseI\text{-}J'/(AseI\text{-}J + AseI\text{-}J')$ in this case. For six transformant samples, the recombination efficiencies ranged from 0.67 to 0.81 with a mean of 0.73.

The formal term 'recombination frequency' in classical genetics usually involves two long homologous sequences in 1-to-1 pairs on which multiple crossovers are likely to occur. Recombination frequencies in this situation never exceed 0.5. The targeted recombination, on the other hand, is not only unequal in sequences exchanged but also in numbers of participating partners. The >0.5 'recombination efficiency' observed may also reflect the shorter replication cycles for the shortened recombinant chromosomes or faster growth rates of the cultures that harbored them.

A second 3.1-kb *HindIII*-*SacI* fragment at position 424 to 427 kb (designated 'Segment 2') was inserted in the same orientation as *Segment 1* into pUS356 to produce pHP2 (Fig. 1A). M145 transformed with *AseI*-linearized pHP2 DNA produced 11-kb pHP2L (Fig. 1C-b). Recombination between pHP2L and the chromosome gave rise to an expected 435-kb recombinant plasmid as revealed by fluorescence stain (Fig. 1C-b) and hybridization (Fig. 1C-c).

Segment 2 resides in the 632-kb *AseI*-F in M145. Recombination separated *AseI*-F into 195-kb *AseI*-F' and 449-kb *AseI*-F (Fig. 1C-a). The former was relocated to the recombinant plasmid, which was designated pHP2L-F'. *AseI*-F' could not be detected by fluorescence stain due to co-migration with 180-kb *AseI*-L (Fig. 1C-b), but hybridized to *Segment 2* as expected (Fig. 1C-c). *AseI*-F in M145[pHP2L] was detected in fluorescence stain (Fig. 1C-b) and hybridization (Fig. 1C-c).

AseI-F, which represented the intact chromosome, was still present in M145[pHP2L]. The recombination efficiency, as calculated by $AseI\text{-}F'/(AseI\text{-}F + AseI\text{-}F')$, for six random transformants examined ranged from 0.80 to 0.88 with a mean of 0.83.

A third (2.8-kb) *HindIII*-*SacI* fragment (designated *Segment 3*) at position 880 to 883 kb was inserted in the same orientation as *Segment 1* into pLUS356 to give pHP3 (Fig. 1A). Transformation of M145 using linearized pHP3 DNA produced 11-kb linear plasmid pHP3L (Fig. 1D-b).

Recombination between pHP3L and the chromosome generated an 891-kb recombinant plasmid and a 7,788-kb recombinant chromosome (Fig. 1D-a). The recombinant plasmid was detected by fluorescence stain (Fig. 1D-b) and hybridization (Fig. 1D-c). M145[pHP3L] DNA was analyzed by *SspI* restriction.

Segment 3 resides in 596-kb *SspI*-C (Kieser et al., 1992). Recombination separated *SspI*-C into 343-kb *SspI*-C' into 264-kb *SspI*-C (Fig. 1D-a). The former was relocated to the recombinant plasmid, which was designated pHP3L-C'. *SspI*-C' was revealed by hybridization (Fig. 1D-c), but not by fluorescence stain due to co-migration with *SspI*-N (Fig. 1D-b). *SspI*-C was also revealed by hybridization (Fig. 1D-c), but not by fluorescence stain due to co-migration with *SspI*-P (Fig. 1D-b). The recombination efficiency between pHP3L and the chromosome, as calculated by the fraction $SspI\text{-}C'/(SspI\text{-}C + SspI\text{-}C')$, ranged from 0.05 to 0.16 with a mean of 0.09, about one order lower than those in M145[pHP1L] and M145[pHP2L].

Target recombination mediated by *RecA* pathway

To check whether the targeted recombination was mediated by the *RecA* pathway of homologous recombination, linearized pHP3 DNA was used to transform *recA* null mutant of M145, T145-15 (Huang and Chen, 2006). The results showed that none of the transformants possessed the recombinant plasmid pHP3L-C' (Fig. 2), indicating that the targeted recombination was mediated by *recA*.

Chimeric chromosomes generated by targeted recombination

A fourth (3.9-kb) *SacI* fragment (designated *Segment 4*) at position 4,177 to 4,181 kb from the left end of the chromosome was inserted in the same orientation as *Segment 1* into the *SacI* site of pLUS356 to give pHP4 (Fig. 1A). Transformation of M145 using linearized pHP4 DNA produced 11-kb linear plasmid pHP4L (Fig. 3B-a).

Segment 4 was near the center of the chromosome, left of *oriC*. Recombination between pHP4L and the chromosome would generate a 4.2-Mb and 4.5-Mb recombinant chromosomes, possessing pSLA2 ARS, and *oriC*, respec-

Fig. 3. Chimeric chromosomes generated by targeted recombination.

A. M145 chromosome recombines with pHP4L (top) or pHP4rL (bottom) to form two chimeric chromosomes. Open circle, *oriC*. Dashed line, chimeric chromosome without a replication origin. The other labels are as in Fig. 1B. **B.** DNA of three transformants each of M145[pHP4L] and M145[pHP4rL] were subjected to PFGE (long pulse time) with or without prior protease E ('PE') digestion. **a.**, and hybridized to cosmid 7H2 (**b**) and 5B8 (**c**) probes. The size (in Mb) of the *Streptomyces* DNA and *Schizosaccharomyces pombe* chromosomes are indicated.

tively, as replication origins (Fig. 3A). PFGE with a long pulse time revealed intact chromosomal DNA ('8.7'), DNA of 4.0–5.0 Mb ('4–5'), and smaller fragments with a wide size distribution ('frag', Fig. 3B-b). The latter was also observed in M145 and M145[pLUS356L], and was presumably the degradation products of the chromosomal DNA.

The DNA in the 4.0–5.0 Mb region hybridized to cosmids 7H2 and 5B8 (Redenbach et al., 1996) from the left and right arm of the chromosome, respectively (Figs. 3B-b and B-c), suggesting that both arms were present. TP-capped DNA is immobile in PFGE without proteolytic treatment (Lin et al., 1993). Omission of protease E treatment of the samples resulted in the disappearance of the DNA in the 8.7 and 4.0–5.0 Mb region, indicating the pres-

ence of TP on these molecules. These results indicate that, while intact M145 chromosome dominated in the transformants, both chimeric chromosomes were present albeit in low abundance.

Segment 4 resides on a 13-kb *KpnI* fragment of M145 chromosome, and would reside on an 18-kb *KpnI* fragment on the 4.2-Mb recombinant chromosome. Colonies grown from individual spores were checked for the presence of these two fragments by hybridization to a *Segment 4* probe. Of 100 colonies checked, all contained the 13-kb fragment, 59 contained both fragments, and none contained the 18-kb fragment only. These, and above-mentioned, results suggested that an intact chromosome was essential for the viability of M145[pHP4L] cultures.

Segment 4 was inserted in the reverse orientation into

pLUS356 to give pHP4r (Fig. 1A). Transformation of M145 using linearized pHP4r DNA produced 11-kb linear plasmid pHP4rL (Fig. 3B). Recombination of pHP4rL and the chromosome would also generate 4.2-Mb and 4.5-Mb recombinant chromosomes (Fig. 3A). The former would possess no replication origin, and the latter would possess *oriC* and the plasmid ARS. Fluorescence stain revealed the presence of DNA of 4.0–5.0 Mb in the PFGE gel (Fig. 3B-a). Hybridization with both 7H2 and 5B8 probes and the dependence on protease E treatment for appearance suggested that both chimeric chromosomes were present (Figs. 3B-b and B-c). The 4.2-Mb DNA, lacking a replication origin, probably represented a non-replicating recombination product. As in M145[pHP4L], native intact chromosomes dominated.

Curing of the recombinant plasmids

Attempts were also made to isolate truncated chromosomes that had lost the recombinant plasmids in M145[pHP2L], M145[pHP1L], and M145[pHP3L]. In polyploid hyphae, all the native and recombinant replicons co-existed, and it was necessary to screen single haploid spores. Colonies grown from single spores of these strains were screened for Thio^s segregants, which had lost both the pLUS356 derivatives and recombinant plasmids. These plasmidless isolates would possess either a truncated or intact chromosome. PCR was performed on these colonies to check the existence of a chromosomal sequence at position 100–101 kb from the left end of the chromosome, which should be absent in the truncated chromosomes.

Two of 50 M145[pHP1L] colonies screened was Thio^s and PCR negative. One of them, designated D197, was chosen for further studies. *AseI*-digested D197 DNA was subjected to PFGE (Fig. 4A-a) and hybridization using *S. lividans* telomere (Fig. 4A-b) and *S. coelicolor* telomere (Fig. 4A-c) as the probes. The results showed the absence of pHP1L plasmid as expected. *AseI*-J (containing an *S. coelicolor* telomere) in M145 was also absent. In its place was *AseI*-J' (containing an *S. lividans* telomere). Hybridization using two 1.5-kb sequences at 50 and 150 kb from the left end (both residing in *AseI*-J') of M145 chromosome confirmed the absence of *AseI*-J' in D197 (Fig. 4B-a). These results indicated that D197 was plasmidless and harbored a truncated chromosome lacking the 197-kb left arm. pHP1L-J' and the F' fragment of pHP2L-F' were not detected, presumably due to the relatively short (350 bp) probe and relatively low copy numbers of these fragments.

Of M145[pHP2L], 18 out of 50 colonies screened were Thio^s and PCR negative. One of them, designated D423, was selected for further studies. *AseI* restriction and hybridization analyses indicated the absence of pHP2L and *AseI*-F and the presence of *AseI*-F' (Fig. 4A). Hybridization using four 1.5-kb sequences at 50, 150, 250, and 350 kb (the latter two residing in *AseI*-F') from the left end of M145 revealed no hybridizing fragment in D423 (Fig. 4B-b). These results confirmed the lack of any plasmid and the presence of a truncated chromosome lacking the 424-kb left arm in D423.

In contrast, 18 Thio^s variants of 92 M145[pHP3L] colonies screened were all PCR positive, indicating that these plasmidless strains still harbored intact chromosomes.

Fig. 4. Truncated chromosomes in D197 and D423.

A. *AseI*-digested D197 and D423 DNA were separated by PFGE (short pulse time) (a), and hybridized to *S. lividans* telomere DNA (b), and *S. coelicolor* telomere DNA (c). B. *AseI*-digested D197 and D423 DNA were separated by PFGE (short pulse time), and hybridized to Mix 1 probes containing two 1.5-kb PCR products at positions 50 and 150 kb of the chromosome (a), and to Mix 2 probes containing four 1.5-kb PCR products at positions 50, 150, 250, and 350 kb (b).

Both D197 and D423 grew on minimum medium and sporulated normally, indicating that the 424-kb left arm of the M145 chromosome contained no genes that are essential for viability and for biosynthesis of amino acids or other factors necessary for growth and sporulation in minimum medium. The inability to isolate variants with a truncated chromosome in M145[pHP3L] suggested that the deletion of 427–880 kb sequences resulted in either lethality or handicapped growth. In support for this notion, the recombinant plasmid pHP3L-C' persisted in a relatively higher abundance than pHP1L-J' and pHP2L-F' in their respective transformants (compare Figs. 1B-b, C-b, and D-b).

Yamasaki and Kinashi (2004) identified two chimeric chromosomes of 7.2 and 1.8 Mb in *S. coelicolor* 2106 as the result of non-homologous recombination between SCP1 and the right arm of the chromosome. The rightmost 1.6-Mb of the chromosome, including the *tap*-*tpg* operon, methylenomycin biosynthesis gene cluster, and *cysD* gene, joined with the left arm of SCP1 could not be cured as expected (Yamasaki and Kinashi, 2004).

Application for identification of essential regions and genome engineering

The targeted recombination described here provides a convenient method for engineering *Streptomyces* chromosomes with specific deletions or additions. It is particularly useful for moving biosynthetic gene clusters for secondary metabolites, which are typically hundreds of kb in length. Shuffling of these clusters among *Streptomyces* genomes offers the potential to increase productivities and/or efficient efflux, and to discover cryptic or hybrid metabolites (Katz and Baltz, 2016). The high efficiency of the chromosome-plasmid recombination makes special selection unnecessary. The relative ease of curing the linear plasmid also facilitates the stabilization of the engi-

neered chromosomes.

In this study, we demonstrated this technology at the left arm of *S. coelicolor* chromosome. The same strategy applied to the right arm, however, would stop short at about 100 kb from the end, where the *tap-tpg* operon resides. The operon is essential for replication of the M145 chromosome in linear form, and may be deleted only when the chromosome is circularized (Lin et al., 1993). This is true for most other *Streptomyces* chromosomes.

Currently, several site-specific recombinases, transposons, reporter genes, and I-SceI endonuclease systems have been utilized for large-scale genome manipulations in *Streptomyces* and related bacteria (Siegl and Luzhetskyy, 2012). PCR-targeted gene replacement procedure of Gust et al. (2003) is also very useful for generating large deletions of chromosomal segments, including ten secondary metabolite biosynthetic gene clusters from *S. coelicolor* chromosome (Zhou et al., 2012). The targeted recombination strategy adds an alternative approach for genome shuffling in *Streptomyces* genomes. A homology of approximately 3 kb was sufficient to achieve an efficiency of 9 to 81% depending on the locations.

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Supplementary Materials

Supplementary materials are available in our J-STAGE site (<http://www.jstage.jst.go.jp/browse/jgam>).

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