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A versatile PCR-based tandem epitope tagging system for *Streptomyces coelicolor* genome

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ABSTRACT

Epitope tagging approaches have been widely used for the analysis of functions, interactions and subcellular distributions of proteins. However, incorporating epitope sequence into protein loci in *Streptomyces* is time-consuming procedure due to the absence of the versatile tagging methods. Here, we developed a versatile PCR-based tandem epitope tagging tool for the *Streptomyces* genome engineering. We constructed a series of template plasmids that carry repeated sequence of c-myc epitope, Flp recombinase target (FRT) sites, and apramycin resistance marker to insert epitope tags into any desired spot of the chromosomal loci. A DNA module which includes the tandem epitope-encoding sequence and a selectable marker was amplified by PCR with primers that carry homologous extensions to the last portion and downstream region of the targeted gene. We fused the epitope tags at the 3′ region of global transcription factors of *Streptomyces coelicolor* to test the validity of this system. The proper insertion of the epitope tag was confirmed by PCR and western blot analysis. The recombinants showed the identical phenotype to the wild-type that proved the conservation of *in vivo* function of the tagged proteins. Finally, the direct binding targets were successfully detected by chromatin immunoprecipitation with the increase in the signal-to-noise ratio. The epitope tagging system describes here would provide wide applications to study the protein functions in *S. coelicolor*.

1. Introduction

Streptomycetes are soil-dwelling bacteria, which produce a diverse range of secondary metabolites such as natural antibiotics. Members of this genus have complex regulatory systems at transcription and translation levels for sensing and signal transduction to adapt a wide range of nutritional and environmental conditions. In particular, there are regulatory networks for the fine control of morphological differentiation depending on the growth patterns [1]. Thus, characterizing *in vivo* functions of hundreds of regulators in *Streptomyces* has become important issue to understand the complex regulatory systems and to engineer their antibiotics production.

In general, gene overexpression or disruption methods have been widely used to determine the functions of the genes of interest and their link to regulatory mechanisms. Among those, one-step gene inactivation based upon PCR-based homologous recombination has been developed in several bacterial species including *Escherichia coli* and yeast [2,3]. This system has led to a versatile genetic strategy enabling rapid and effective gene inactivation and been also engineered to generate epitope tagged proteins [4]. This rapid immune-activity generation method has become a simple and efficient molecular genetic method for rapid characterization, purification, and *in vivo* localization of the protein of interest [5]. To improve the detection sensitivity of the tagging system, tandem epitope tagging systems were also developed in yeast, *E. coli* and other systems [6]. The system includes a series of template plasmid containing tandem myc sequence, Flp recombinase target (FRT) site, and antibiotic resistance gene was established [7].

The tagging system combined with chromatin immunoprecipitation (ChIP) is being widely used to pursue a systematic and high-throughput cataloging of the genome-wide binding sites of regulatory proteins of interest [8]. Typical ChIP experiments require antibodies highly specific against the target proteins. More importantly, the affinity and specificity between the antibody and the target proteins should be high enough to maintain their interaction under highly stringent experimental conditions to discard non-specific interaction between the antibody and other proteins. It has been reported that antibodies specific to the

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target proteins are designed for ChIP applications in *Streptomyces coelicolor* [9,10]. However, use of well-known and inexpensive commercial antibodies will provide a systematic platform which leads to more diverse studies for the regulatory proteins in *Streptomyces*. Here, we design a series of template plasmid for tandem epitope tagging of *S. coelicolor* proteins and show their versatility to readily fuse the tandem epitope into desired location in *S. coelicolor* genome.

2. Materials and methods

2.1. Strains and growth conditions

All strains used are *S. coelicolor* A3(2) M145, and *E. coli* K-12 MG1655 and its derivatives. *S. coelicolor* A3(2) M145 was grown in R5 complex medium composed of 103 g sucrose, 0.25 g K_2SO_4, 10.12 g $MgCl_2{\cdot}6H_2O$, 10 g glucose, 0.1 g Difco casamino acids, 2 mL trace element solution composed of $ZnCl_2$ 40 mg, $FeCl_3{\cdot}6H_2O$ 200 mg, $CuCl_2{\cdot}2H_2O$ 10 mg, $MnCl_2{\cdot}4H_2O$ 10 mg, $Na_2B_4O_7{\cdot}10H_2O$ 10 mg, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 10 mg in 1 L of deionized water, 5 g yeast extract, 5.73 g TES buffer, and 7 mL 1 N NaOH in 1 L of distilled water. *E. coli* BW25113 was used to propagate the recombination plasmid pIJ790 and *S. coelicolor* cosmids. *E. coli* BT340 carrying pCP20 was used for FLP-mediated site-specific recombination. *E. coli* ET12567/pUZ8002 was the nonmethylating plasmid donor strain for intergeneric conjugation with *S. coelicolor* strain M145 [11]. Apramycin (50 μg/mL), chloramphenicol (25 μg/mL) or kanamycin (50 μg/mL) was added to growth media when required.

2.2. Construction of template plasmids

Oligonucleotide encoding tandem myc sequences were chemically synthesized. In accordance with codon usage, the myc sequence (Glu-Gln-Lys-Leu-Ile-Ser-Glu-Glu-Asp-Leu) was optimized for the gene expression in *S. coelicolor* (GAG CAG AAG CTG ATC AGC GAG GAG GAC CTG). PCR was performed using primers carrying EcoRI and BamHI restriction sites in a final volume of 100 μL containing 2.5 U LA Taq DNA polymerase (TAKARA), 50 μL GC buffer I, 35 μL DDW, 8 μL (50 μM) dNTP, 5 μL DMSO and 1 μL (50 ng) plasmid containing the tandem myc tag sequence as a template. PCR amplification conditions were 30 cycles with 30 s denaturation at 94 °C, 30 s annealing at 60 °C, and 30 s extension at 72 °C. The PCR product was then cloned into pUC18 and confirmed by DNA sequencing. A gene cassette, which contained the flanking FRT sites and apramycin resistance gene, was amplified from pIJ773 [11] using primers carrying oligonucleotide extensions with BamHI and HindIII restriction sites. The gene cassette was then ligated into the pUC18:(*n*)-myc to obtain the pJN1 plasmid series.

2.3. Tandem epitope tagging to S. coelicolor transcription factors

Linear DNA fragments were amplified using pairs of primers which were 59-bp in length with 39-bp homology extensions overlapping upstream and downstream from stop codon of target genes and 20-bp priming sequences from pJN1 template plasmid series. Each PCR product was purified, digested with DpnI, repurified, and then electroporated into *E. coli* strain harboring pIJ790 (which expresses the λ Red recombination system under the control of an inducible promoter) and *S. coelicolor* cosmid (which contains a genomic region of interest). The cells were then incubated at 37 °C for 1 h in 1 mL of LB and spread onto LB-agar medium supplemented with apramycin. The myc-inserted cosmid was transported into the methylation-deficient *E. coli* strain ET12567 containing the RP4 derivative pUZ8002, and then transferred to *S. coelicolor M145* by conjugation [12]. Single-crossover exconjugants were selected on MS containing kanamycin and nalidixic acid, to obtain transconjugants. The genomic DNA was then isolated and plasmid integration was confirmed by PCR with the primers of 300 bp upstream and downstream from stop codon.

2.4. Chromatin immunoprecipitation

Fifty-milliliter cultures of cells harboring 6× myc-fused transcription factors were grown in R5 complex media at 37 °C. The cells were cross-linked by 1% formaldehyde at room temperature for 30 min. Following the quenching of unused formaldehyde with 125 mM glycine at room temperature for 5 min, the cross-linked cells were harvested by centrifugation and washed three times with 50 mL ice-cold Tris-buffered saline (Sigma, St. Louis, MO, USA). The washed cells were resuspended in 1.5 mL lysis buffer composed of 10 mM Tris–HCl (pH 7.5), 100 mM NaCl, 1 mM EDTA, protease inhibitor cocktail (Sigma), and 1 kU lysozyme (EPICENTRE, Madison, WI, USA). The cells were incubated at room temperature for 30 min and then treated with 2 mL 2× IP buffer (100 mM Tris–HCl, pH 7.5, 200 mM NaCl, 1 mM EDTA, 2% Triton® X-100). The lysate was then sonicated eight times for 20 s each in an ice bath to fragment the chromatin complexes. The range of the DNA size resulting from the sonication procedure was 300–1000 bp, and the average DNA size was 500 bp. Cell debris was removed by centrifugation at 37,000×*g* for 10 min at 4 °C, and the resulting supernatant was used as cell extract for the immunoprecipitation. To immunoprecipitate the protein–DNA complex, 3 μg of anti-c-myc antibody (9E10, Santa Cruz Biotech) were then added into the cell extract, respectively. For the nonspecific control (mock-IP), 2 μg of normal mouse IgG (Upstate) was added into the cell extract. They were then incubated overnight at 4 °C, and 50 μL of the Dynabeads Pan Mouse IgG beads (Invitrogen) was added into the mixture. After 5 h of incubation at 4 °C, the beads were washed twice with the IP buffer (50 mM Tris–HCl at pH 7.5, 140 mM NaCl, 1 mM EDTA, and 1% (v/v) Triton X-100), once with the wash buffer I (50 mM Tris–HCl at pH 7.5, 500 mM NaCl, 1% (v/v) Triton X-100, and 1 mM EDTA), once with wash buffer II (10 mM Tris–HCl buffer at pH 8.0, 250 mM LiCl, 1% (v/v) Triton X-100, and 1 mM EDTA), and once with TE buffer (10 mM Tris–HCl at pH 8.0, 1 mM EDTA) in order. After removing the TE buffer, the beads were resuspended in 200 μL of elution buffer (50 mM Tris–HCl at pH 8.0, 10 mM EDTA, and 1% SDS) and incubated overnight at 65 °C for reverse cross-linking. After reversal of the cross-links, RNAs were removed by incubation with 200 μL of TE buffer with 1 μL of RNaseA (QIAGEN) for 2 h at 37 °C. Proteins in the DNA sample were then removed by incubation with 4 μL of proteinase K solution (Invitrogen) for 2 h at 55 °C. The sample was then purified with a PCR purification kit (MACHEREY-NAGEL). To measure the enrichment of the protein-binding targets in the DNA samples, 1 μL of IP or mock-IP DNA was used to carry out gene specific real-time qPCR with the specific primers to the promoter regions. The *hrdB*, housekeeping sigma factor, promoter sequence was used as a negative control in all cases. All real-time qPCR reactions were done in triplicate. The samples were cycled at 94 °C for 15 s, 60 °C for 30 s and 72 °C for 30 s (total 40 cycles) on a iQ5 (Bio-Rad). The threshold cycle values were calculated automatically by the iCycler iQ optical system software (Bio-Rad Laboratories). Primer sequences used in this study are available on request.

2.5. Western blot analysis

Each sample was subjected to electrophoresis in a SDS–10% polyacrylamide gel and the resolved proteins were electrotransferred to a Hybond™-ECL membrane (Amersham Biosciences, Piscataway, NJ, USA). The ECL™ Western detection kit (Amersham

Biosciences), mouse monoclonal 9E10 antibody, and horseradish peroxidase (HRP)-conjugated sheep anti-mouse IgG (Amersham Biosciences) were used to detect the tandem-myc tagged proteins. Fifty milliliters BCA protein assay kit (Pierce, Rockford, IL, USA) were used to quantify the amount of proteins.

3. Results and discussion

The PCR-based tagging strategy applied here starts with amplifying a DNA segment, which begins with the tandem epitope sequence followed by a drug-resistance gene flanked by FRT sites. In addition, the amplifiable segment has homologous sequences to the last portion and to a downstream region of the targeted gene. The precise insertion of the DNA segment into the cosmid containing the target gene is achieved by electroporating the PCR-amplified DNA segment into *E. coli* BW25113/pIJ790 containing the cosmid followed by λ-Red-mediated recombination. The epitope-inserted cosmid is then transported into the methylation-deficient *E. coli* (ET12567/pUZ8002) and transferred to *S. coelicolor M145* by conjugation [13]. To provide versatile PCR amplification of the DNA segment, we constructed a series of template plasmids encoding 2×, 4×, and 6× myc epitope. Each template plasmid encodes two priming sites, the repeated epitope sequence with a stop codon, and the apramycin resistance gene flanked by directly repeated FRT sites (Fig. 1).

To demonstrate the functionality of the tandem epitope tagging system, we fused the tandem myc tag to several transcription factors of *S. coelicolor*. One of the targets was a quorum receptor protein, ScbR (SCO6265), whose regulatory function has been previously studied [14]. Although ScbR has been revealed to have global effects on transcription regulation, little is known about its direct binding targets except *cpkO* [15]. Another target was NdgR (SCO5552), an IclR-like regulator which is involved in amino-acid-dependent growth, quorum sensing, and antibiotic production. NdgR showed the binding to intergenic region of *ndgR-leuC in vitro* [16], however none of *in vivo* measurement has been made to explore regulatory interaction between NdgR and such *cis*-acting elements. The correct insertion of the tandem epitope into the desired genomic loci was first validated by PCR and subsequently by western blot analysis. To this end, genomic DNA was extracted from each tagged strain as a PCR template. PCR was then carried out by using the primer pair of both sides between the start codon and the end of apramycin cassette (Fig. 1). The PCR products which have several restriction sites capable of one-step confirmation were digested to three DNA fragments after XhoI/BamHI treatment (Fig. 2A). While the apramycin resistance gene and open reading frame (ORF) region indicate the same size (the upper and middle bands, respectively), the lowest bands contained DNA sequences encoding the tandem myc epitope and varied in size depending on the number of tag repeats. Western blot analysis revealed that the intensity and the size of tandem epitope-fused transcription factors were proportional to the number of tandem myc copies as expected (Fig. 2B).

With confirming the correct integration of the tandem epitope-encoding sequence into the desired locus, the most important aspect of tandem epitope tagging is to maintain the proper function of tandem epitope-fused proteins [7]. The major uncertainty in an epitope-tagging strategy is that the fused proteins potentially lose their *in vivo* functions. To address the potential drawback, we compared the mRNA expression level of the target genes and the phenotype of tagged strains with those of the wild-type. First, we compared the regulatory function of 6× myc fused ScbR with that of wild-type ScbR. As a quorum receptor protein, ScbR plays an important regulatory role in the onset of antibiotic production in *S. coelicolor* with a signaling molecule, a γ-butyrolactone SCB1 [15]. In the absence of signaling molecules, ScbR represses transcription of the cryptic type I polyketide synthase gene cluster (*cpk*) by directly binding to promoter region of *cpkO* (SCO6280), the activator of the *cpk* gene cluster [15]. On the other hand, SCB1 at high concentration binds to ScbR to form a SCB1–ScbR complex, thereby relieving the repression. Thus *cpkO* is constitutively expressed in a *scbR* null mutant irrespective of the presence of SCB1. While the negative regulation of the *cpkO* gene was abolished in the *scbR* null mutant strain, the JN116 strain harboring the 6× myc fused ScbR maintained the negative regulatory effect at the same magnitude as in the wild-type under early exponential growth phase (Fig. 3A). Phenotypically, when liquid cultured cell was transferred to and spread out on a solid R5-medium, the wild-type strain forms spores (Fig. 3C). As in the case with the wild-type strain, the formation of spores was clearly observed from the JN116 strain. However, the deletion of the *scbR* gene abolishes the formation of spores. This observation, along with the expression data shown earlier, demonstrates that the ScbR maintained its regulatory functions.

In a previous study, deletion of the *ndgR* gene causes slow cell growth and various phenotypes depending upon which amino acids were used in the minimal media [16]. In particular, growth of the *ndgR* null mutant in the minimal medium supplemented with *N*-acetylglucosamine and asparagine increased the production of actinorhodin (ACT) compared to that of the wild-type. How-

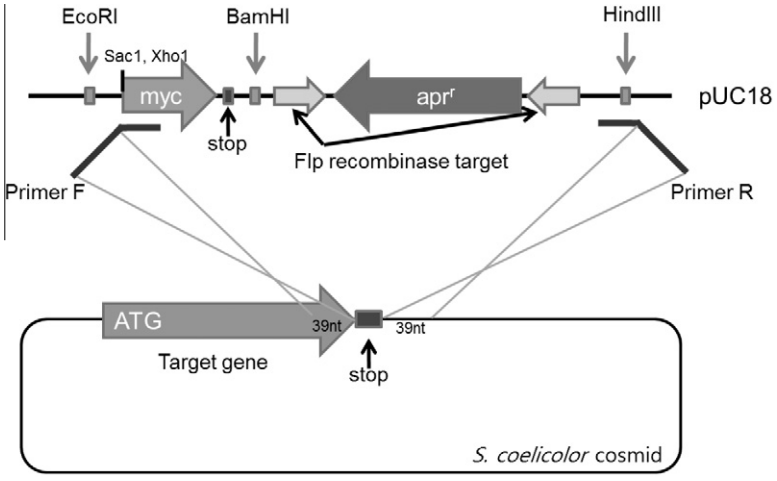

Fig. 1. PCR-based tandem epitope tagging system for *Streptomyces coelicolor*. A DNA module that involves tandem myc sequence and the antibiotic resistance marker (apr^r) is amplified with primers carrying extensions homologous to the upstream and downstream of the translation stop codon of target gene.

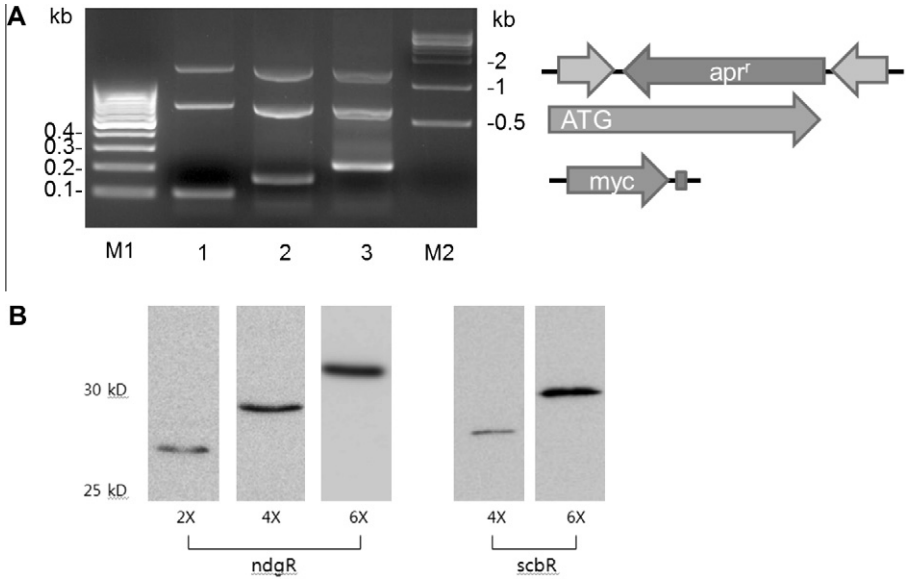

Fig. 2. Confirmation of correct insertion of epitope tags into the chromosomal genes in *Streptomyces coelicolor*. (A) PCR verification of tandem myc-tagged transcription factor, ScbR. M1, M2 lanes contain 100-bp, 1 kb DNA ladder, respectively. Lanes 2, 3, 4 contain two, four, and six myc tags, respectively. (B) Western blot analysis verification of tandem myc-tagged transcription factor, NdgR and ScbR. It showed that the intensity of spot was proportional to the number of myc copies.

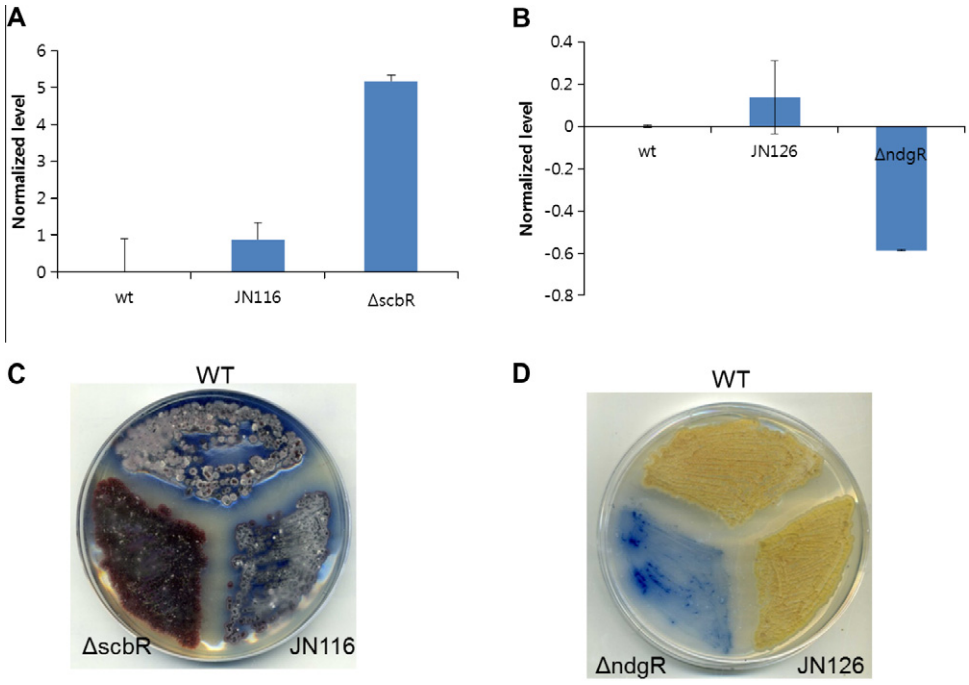

Fig. 3. Conservation of *in vivo* function of scbR-6X myc and ndgR-6X myc (A) CpkO expression in wild-type (M145), *S. coelicolor* JN116, and ScbR null mutant. (B) LeuC expression in wild-type (M145), *S. coelicolor* JN126, and NdgR null mutant. Normalized level means the relative DNA quantity in log2 scale. (C) Phenotype on the R5-solid media plate. JN116 produced spores like WT but *scbR* knockout mutant did not have. (D) Phenotype on the GlcNAc/ASN minimal media plate. NdgR knockout mutant showed slow growth rate but produced actinorhodin more than wild-type and JN126. Wild-type and JN126 had similar phenotypes.

ever, JN126 strain harboring 6× myc fused NdgR exhibited similar level of ACT production with the wild-type (Fig. 3D). Judging from the ACT production levels, the 6× myc tag does not significantly affect the regulatory role of the NdgR. Based upon the enhancement of ACT production level of the *ndgR* null mutant grown in minimal media containing certain amino acids such as leucine, the regulatory interaction was revealed by electrophoretic mobility shift assay between NdgR and *ndgR-leuC* intergenic region [16]. Moreover, expression of the *leuC* gene (SCO5553) in JN126 strain was similar to the wild-type, whereas transcript level of the *leuC* gene was slightly decreased in the *ndgR* null mutant (Fig. 3B). Consequently, it was concluded that both tandem myc-tagged ScbR and NdgR retained their regulatory function of transcription over *cpkO* and *leuC*, respectively.

The tandem epitope fused ScbR and NdgR did not affect *in vivo* functions of the transcription factors, thus recombinants could be directly used for chromatin immunoprecipitation (ChIP) experiments. This ChIP technique is useful to demonstrate cellular DNA–protein interactions under a diverse set of physiological conditions [8]. In ChIP procedure, the cross-linked DNA–protein com-

plex is enriched through immunoprecipitation by the specific antibody against the protein of interest. Also, highly stringent salt and detergent conditions are used to remove nonspecific interactions between cellular proteins and the antibody being used. As a result, affinity and specificity between the protein of interest and the specific antibody is critical to increase the enrichment yield. As shown in Fig. 4, interactions between 6× myc fused protein and the target promoter regions were determined by the quantitative PCR. First, the enrichment level of *cpkO* promoter, known as a target of ScbR, was 100-fold greater than that of nonspecific ChIP sample (Fig. 4A). Similarly, the normalized ChIP DNA value of ndgR promoter region came out to be eight times greater compared to that of nonspecific sample (Fig. 4B). As a negative control experiment, no differences were observed between specific and nonspecific DNA using a promoter region of a housekeeping sigma factor, *hrdB*. Judging from the enrichment of the known ScbR and NdgR binding sites, it meant that tandem myc tagged proteins maintained their correct interactions with their target regulatory sites.

Branched-chain amino acid (BCAA) degradation pathway is the important Pathway for supplying CoA related compounds which are the major precursors for polyketide antibiotics in *Streptomyces* [17,18]. Especially, leucine is the best source of precursors for actinorhodin biosynthesis in minimal medium. Biosynthesis of leucine in most bacteria involves three steps starting from α-ketoisovalerate, which is the intermediate of valine biosynthetic pathway from pyruvate. Each step is taken charge of α-isopropylmalate synthase (*leuA*), β-isopropylmalate dehydratase (*leuC*, *leuD*) and β-isopropylmalate dehydrogenase (*leuB*), respectively. In final step, BCAA aminotransferase transfers amine group from glutamate to α-ketoisocaproate. In enteric bacteria, the genes for the corresponding enzymes are clustered in a single operon [19]. But in *Streptomyces*, the genes for the leucine biosynthesis are scattered across the genome. And two homologous enzymes, SCO2528 and SCO5529, are annotated as an α-isopropylmalate synthase, LeuA.

In the previous report, AreB, an orthologous of NdgR in *Streptomyces clavuligerus*, has been found to control *leuCD* and secondary metabolite [20]. Another orthologous, LtbR in *Corynebacterium glutamicum*, regulates tryptophan biosynthesis, ribose uptake and leucine biosynthetic pathway by binding to the promoter region of *leuB* and *leuCD* [21]. As we found out new targets of NdgR in *S. coelicolor*, we measured the enrichment yield of the promoter regions of enzymes involved in the leucine biosynthetic pathway, *leuA* and *leuB*. The promoter region of one of the two genes annotated as *leuA*, SCO5529, showed threefold increased level compared to that of nonspecific ChIP sample but not in SCO2528 (Fig. 4B). The enrichment level of *leuB*, SCO5522, was fivefold greater than that of nonspecific sample (Fig. 4B). Taken together above data, NdgR regulates entire pathway for leucine biosynthesis in spite of scattered location of corresponding enzymes. In final step, two homologous enzymes, SCO1546 and SCO5523 (IlvE), are annotated as a BCAA aminotransferase. But they did not seem to be regulated by NdgR in this condition by measuring enrichment of promoter region of them although SCO5523 was down-regulated in *ndgR* deletion mutant detected by two-dimensional electrophoresis. Even though it is located at the downstream of *leuB* gene, SCO5522, the mRNA is differentially expressed between SCO5522 and SCO5523 (data not shown). Disruption of *leuCD* gene makes leucine auxotroph [22] but mRNA expression level of *leuCD* was slightly decreased in NdgR mutant in *S. coelicolor* (Fig. 3b). Despite NdgR regulates entire pathway for leucine biosynthesis directly, it seems not to be an essential regulator for leucine biosynthesis.

We demonstrated that this PCR-based tandem epitope tagging system is a versatile tool for investigating the regulatory roles

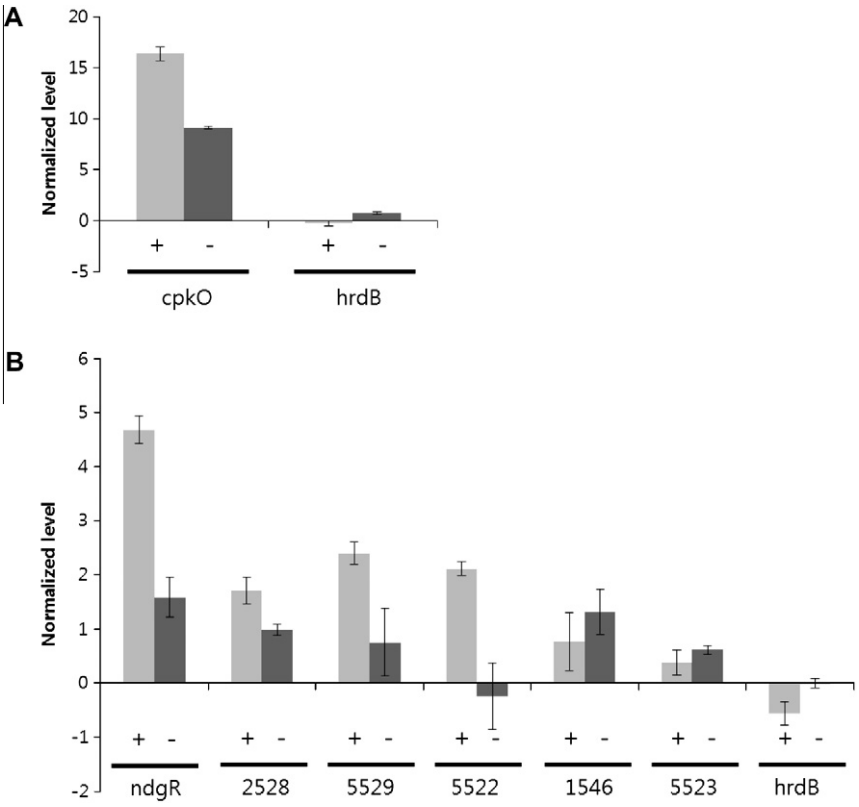

Fig. 4. Verification of tagging system using chromatin immunoprecipitation (ChIP). Enrichment of promoter regions in specific DNA sample and nonspecific DNA sample was monitored by quantitative real-time PCR (qRT-PCR). Normalized level means the relative DNA quantity in log2 scale. (A) Normalized DNA quantity in specific DNA ChIP sample (+) and nonspecific DNA ChIP sample (−) of *scbR*. (B) Normalized DNA quantity in specific DNA ChIP sample (+) and nonspecific DNA ChIP sample (−) of *ndgR*.

of transcription factors in *S. coelicolor*. With a series of template plasmid which has tandem myc epitopes and apramycin resistance cassette, we can fuse the epitope tag into any desired chromosomal loci. Expression levels of target genes and phenotype showed that endogenous tagging did not change *in vivo* activity of the transcription factor of interest. In addition, the conservation of DNA binding activity of tagged protein was confirmed by ChIP-qPCR, so that this tool can be used for identifying direct binding sites at the genome-scale by combining with microarray or massively parallel sequencing, ChIP-chip or ChIP-seq, respectively [23]. Since the myc tag is a commonly used epitope, inexpensive antibodies are commercially available without making specific antibodies against the proteins of interest. Enhancement of detection sensitivity by the repeated epitope sequence allowed stringent conditions for washing to remove the nonspecific binding and to increase signal-to-noise ratio.

Acknowledgments

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References

[1] S.D. Bentley, K.F. Chater, A.M. Cerdeno-Tarraga, et al., Complete genome sequence of the model actinomycete *Streptomyces coelicolor* A3(2), Nature 417 (2002) 141–147.
[2] A. Baudin, O. Ozier-Kalogeropoulos, A. Denouel, et al., A simple and efficient method for direct gene deletion in *Saccharomyces cerevisiae*, Nucleic Acids Res. 21 (1993) 3329–3330.
[3] K.A. Datsenko, B.L. Wanner, One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products, Proc. Natl. Acad. Sci. USA 97 (2000) 6640–6645.
[4] S. Uzzau, N. Figueroa-Bossi, S. Rubino, et al., Epitope tagging of chromosomal genes in *Salmonella*, Proc. Natl. Acad. Sci. USA 98 (2001) 15264–15269.
[5] J.W. Jarvik, C.A. Telmer, Epitope tagging, Ann. Rev. Genet. 32 (1998) 601–618.
[6] M.S. Longtine, A. McKenzie 3rd, D.J. Demarini, et al., Additional modules for versatile and economical PCR-based gene deletion and modification in *Saccharomyces cerevisiae*, Yeast 14 (1998) 953–961.
[7] B.K. Cho, E.M. Knight, B.O. Palsson, PCR-based tandem epitope tagging system for *Escherichia coli* genome engineering, Biotechniques 40 (2006) 67–72.
[8] V.A. Spencer, J.M. Sun, L. Li, et al., Chromatin immunoprecipitation: a tool for studying histone acetylation and transcription factor binding, Methods 31 (2003) 67–75.
[9] G. Bucca, E. Laing, V. Mersinias, et al., Development and application of versatile high density microarrays for genome-wide analysis of *Streptomyces coelicolor*: characterization of the HspR regulon, Genome Biol. 10 (2009) R5.
[10] C.D. den Hengst, N.T. Tran, M.J. Bibb, et al., Genes essential for morphological development and antibiotic production in *Streptomyces coelicolor* are targets of BldD during vegetative growth, Mol. Microbiol. 78 (2010) 361–379.
[11] B. Gust, G. Chandra, D. Jakimowicz, et al., Lambda red-mediated genetic manipulation of antibiotic-producing Streptomyces, Adv. Appl. Microbiol. 54 (2004) 107–128.
[12] F. Flett, V. Mersinias, C.P. Smith, High efficiency intergeneric conjugal transfer of plasmid DNA from *Escherichia coli* to methyl DNA-restricting streptomycetes, FEMS Microbiol. Lett. 155 (1997) 223–229.
[13] B. Gust, G.L. Challis, K. Fowler, et al., PCR-targeted *Streptomyces* gene replacement identifies a protein domain needed for biosynthesis of the sesquiterpene soil odor geosmin, Proc. Natl. Acad. Sci. USA 100 (2003) 1541–1546.
[14] A. Chatterjee, L. Drews, S. Mehra, et al., Convergent transcription in the butyrolactone regulon in *Streptomyces coelicolor* confers a bistable genetic switch for antibiotic biosynthesis, PLoS ONE 6 (2011) e21974.
[15] E. Takano, H. Kinoshita, V. Mersinias, et al., A bacterial hormone (the SCB1) directly controls the expression of a pathway-specific regulatory gene in the cryptic type I polyketide biosynthetic gene cluster of *Streptomyces coelicolor*, Mol. Microbiol. 56 (2005) 465–479.
[16] Y.H. Yang, E. Song, E.J. Kim, et al., NdgR, an IclR-like regulator involved in amino-acid-dependent growth, quorum sensing, and antibiotic production in *Streptomyces coelicolor*, Appl. Microbiol. Biotechnol. 82 (2009) 501–511.
[17] C.D. Denoya, R.W. Fedechko, E.W. Hafner, et al., A second branched-chain alpha-keto acid dehydrogenase gene cluster (*bkdFGH*) from *Streptomyces avermitilis*: its relationship to avermectin biosynthesis and the construction of a *bkdF* mutant suitable for the production of novel antiparasitic avermectins, J. Bacteriol. 177 (1995) 3504–3511.
[18] K. Stirrett, C. Denoya, J. Westpheling, Branched-chain amino acid catabolism provides precursors for the Type II polyketide antibiotic, actinorhodin, via pathways that are nutrient dependent, J. Ind. Microbiol. Biotechnol. 36 (2009) 129–137.
[19] J. Hercomb, G. Thierbach, S. Baumberg, et al., Cloning, characterization and expression in *Escherichia coli* of a leucine biosynthetic gene from *Streptomyces rochei*, J. Gen. Microbiol. 133 (1987) 317–322.
[20] I. Santamarta, M.T. Lopez-Garcia, R. Perez-Redondo, et al., Connecting primary and secondary metabolism: AreB, an IclR-like protein, binds the ARE(ccaR) sequence of *S. clavuligerus* and modulates leucine biosynthesis and cephamycin C and clavulanic acid production, Mol. Microbiol. 66 (2007) 511–524.
[21] I. Brune, N. Jochmann, K. Brinkrolf, et al., The IclR-type transcriptional repressor LtbR regulates the expression of leucine and tryptophan biosynthesis genes in the amino acid producer *Corynebacterium glutamicum*, J. Bacteriol. 189 (2007) 2720–2733.
[22] P. Castelli, S. Donadio, F. Marinelli, et al., Complementation of a *Streptomyces lividans* Leu-mutant by the *Actinoplanes teichomyceticus leuC* gene, Gene 158 (1995) 97–100.
[23] D.A. Gilchrist, D.C. Fargo, K. Adelman, Using ChIP-chip and ChIP-seq to study the regulation of gene expression: genome-wide localization studies reveal widespread regulation of transcription elongation, Methods 48 (2009) 398–408.