Table S1. Bacterial strains used in this work

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| **Strain** | **Relevant genotype or description** | **Source or reference** |
| ***Escherichia coli*** | | |
| DH5α | F- endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG Φ80dlacZΔM15 Δ(lacZYA-argF)U169, hsdR17(rK- mK+), λ– | Promega |
| BL21(DE3)pLysS | F-, *ompT*, *hsdSB* (rB-, mB-), *gal*, *dcm* (DE3), pLysS (CamR) | Promega |
| BW25113/pIJ790 | *lacI*+*rrnB*T14 Δ*lacZ*WJ16 *hsdR*514 Δ*araBAD*AH33 Δ*rhaBAD*LD78 *rph-1* Δ*(araB–D)567* Δ*(rhaD–B)568* Δ*lacZ4787*(::*rrnB-3*) *hsdR514* *rph-1* pIJ790  Recombineering strain harbouring arabinose-inducible RED genes on the plasmid pIJ790. | (Gust et al., 2003) |
| ET12567/pUZ8002 | strain for conjugal transfer of DNA from *E. coli* to *Streptomyces* (*dam* *dcm* *hsdS* CamR TetR on the bacterial chromosome; *tra* KanR RP4 23 on pUZ8002) | (Kieser et al., 2000) |
| ***Streptomyces coelicolor* A3(2)** | | |
| M145 | wild type strain, *S. coelicolor* A3(2) (SCP1- SCP2-) | (Bentley et al., 2002) |
| *∆cpkO* (P193) | SCP1- SCP2- *cpkO::aac3(IV)* | This work |
| *∆cpkO-φ* (P194) | SCP1- SCP2- *cpkO::aac3(IV)* pIJ10257 | This work |
| *cpkOCO* (P195) | SCP1- SCP2- *cpkO::aac3(IV)* pIJ10257-cpkOCO | This work |
| *∆cpkN* (P196) | SCP1- SCP2- *cpkN*::Tn5062 | This work |
| *∆cpkN-φ* (P197) | SCP1- SCP2- *cpkN*::Tn5062 pIJ10257 | This work |
| *cpkNCO* (P198) | SCP1- SCP2- *cpkN*::Tn5062 pIJ10257-cpkNCO | This work |
| *∆cpkN-scoTOE* (P199) | SCP1- SCP2- *cpkN*::Tn5062 pIJ10257-scoTOE | This work |
| M145, P193 and P196-derivatives for luciferase reporter assay | The strains listed above harbouring pFLUXH derivatives containing sequences of different *cpk* promoters: pcpkA, pcpkD, pcpkO, pscoT, pcpkN, pscF, pscbA, pscbR and pscbR2 | This work |

Table S2. Plasmids/constructs used in this work. PCR fragments were routinely cloned into either pGEM-T Easy or pTZ57R/T, sequenced, and recloned into appropriate restriction sites of final vectors.

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| **Name** | **Relevant genotype or description** | **Source or reference** |
| pGEM-T Easy | T-vector for direct cloning of PCR products (AmpR) | Promega |
| pTZ57R/T | T-vector from InstT/A Cloning kit for direct cloning of PCR products (AmpR) | Fermentas (Thermo Scientific) |
| pTZ-MYCO | pTZ57R/T containing *Mycobacterium smegmatis* (NC\_008596.1) intergenic region between MSMEG\_6643 and MSMEG\_6645 amplified with primer pair MF (CGTAGGCGAGGGCCTATGAA) and MR (CGCGATCTTCCACGCGAGAT) | gift from J. Hołówka (unpublished) |
| pET28a(+) | Novagen pET system overexpression plasmid | Novagen |
| pET28a(+)-scbR2 | pET28a(+) containing *scbR2* gene sequence amplified with primer pair R2\_HE\_F, R2\_HE\_Rv and cloned into NdeI, XhoI sites. | This work |
| pD01-HN-scbR | pET28a(+) containing *scbR* gene sequence amplified with primer pair ScbREXFW, ScbREXRV and cloned into NdeI, EcoRI sites | This work |
| St1G7 | SuperCos1 cosmid carrying fragment of  *S. coelicolor* A3(2) chromosome encompassing part of *cpk* gene cluster (bp 6905834 to 6947687) | http://www.strepdb.streptomyces.org.uk |
| St1G7-cpkODM | St1G7 cosmid, in which *cpkO* gene sequence was replaced (by means of PCR-targeting) with an apramycin resistance gene *aac(3)IV* amplified using primers CpkODM-Fw, CpkODM-Rv. Recombineering was performed by PCR-targeting in *E.coli* BW25113/pIJ790 | This work |
| 11B05.G04 | Transposon-mutagenised cosmid in which *cpkN* sequence was disrupted with Tn5062 transposon containing *aac(3)IV* casette. | (Fernandez-Martinez et al., 2011) |
| pIJ10257 | ΦBT1 integrating overexpression plasmid containing strong constitutive promter *ermEp\*.* | (Hong et al., 2005) |
| pIJ10257XermEp | pIJ10257 derivative lacking *ermEp\** promoter. pIJ10257 was cut with KpnI, HindIII, its ends were blunted with polymerase T4 and autoligated. | This work |
| pIJ10257-cpkOCO | pIJ10257XermEp containing sequence of *cpkO* gene with its native promoter (amplified with primers CpkO\_c257\_RED\_Eco105I\_F, CpkO\_c257\_RED\_Eco105I\_R) digested with Eco105I and cloned into PvuII site of the plasmid. | This work |
| pIJ10257-cpkNCO | pIJ10257 containing sequence of *cpkN* gene with its native promoter (amplified with primers CPKN\_KHp, CPKN\_KHR) cloned into KpnI, XhoI sites. | This work |
| pIJ10257-scoTOE | pIJ10257 containing sequence of *scoT* gene (amplified with primers TE-K-Hind, TE-P-Nde) cloned under the strong constitutive promoter *ermEp\** - sites NdeI, HindIII | This work |
| pFLUXH | ΦBT1 integrating reporter plasmid with a promoterless luciferase operon *luxCDAEB* and hygromycin resistance cassette | (Szafran et al., 2016) |
| pFLUXH-pcpkA  pFLUXH-pcpkD | pFLUXH-derivatives containing promoter sequence  pcpkA/pcpkD (amplified with primers p6275\_Nde, p6276\_Nde) cloned into NdeI site and selected for desired insert direction using luxout primer. | This work |
| pFLUXH-pcpkO | pFLUXH-derivative containing *cpkO* promoter sequence  pcpkO (amplified with primers p6280\_Nde, KSO-FW) cloned into NdeI, BamHI\* sites. | This work |
| pFLUXH-pcpkN | pFLUXH-derivative containing *cpkN* promoter sequence  pcpkN (amplified with primers p6288\_Nde, CPKN\_KHp) cloned into NdeI, BamHI\* sites. | This work |
| pFLUXH-pscF | pFLUXH-derivative containing *scF* promoter sequence pscF (amplified with primers accA1-Rv, p6272\_Nde) cloned into NdeI, BamHI\* sites. | This work |
| pFLUXH-pscoT  pFLUXH-pscbR2 | pFLUXH-derivatives containing promoter sequence  pscoT/pscbR2 (amplified with primers p6287\_Nde, p6286\_Nde) cloned into NdeI site and selected for desired insert direction using luxout primer. | This work |
| pFLUXH-pscbA | pFLUXH-derivative containing *scbA* promoter sequence  pscbA (amplified with primers scbA\_lux, p6266\_Nde) cloned into NdeI, BamHI\* sites. | This work |
| pFLUXH-pscbR | pFLUXH-derivative containing *scbR* promoter sequence  pscbR (amplified with primers SCBA-FW, p6265\_Nde) cloned into NdeI, BamHI\* sites. | This work |
| pET28-scbR2 | pET28a(+)-derivative containing *scbR2* gene sequence amplified with primers R2\_HE\_F, R2\_HE\_Rv, digested with PagI, XhoI and cloned into NdeI, XhoI sites of the plasmid. | This work |
| pD01-HN-scbR | pET28a(+)-derivative containing *scbR* gene sequence amplified with primers ScbREXFW, ScbREXRV, digested with NdeI, EcoRI and cloned into NdeI, EcoRI sites of the plasmid. | This work |

\*BamHI site flanking the insert in pTZ57R/T was used to cut out the insert for further cloning into pFLUXH

Table S3. Primers used in this work. Restriction sites are in bold.

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| **Name** | **Sequence 5’-3’** | **Restriction sites** | **Application/amplified fragment** |
| CPKN\_KHp | **AGATCTGGTACC**GTGGCGCGAGCACACCAC | BglII, KpnI | pKH3prom |
| CPKN\_KHR | **CTCGAGAAGCTTCTAGA**CCGGCCGGGTCGAGATCG | XhoI, HindIII, XbaI |
| CpkO\_c257\_RED\_Eco105I\_F | GATAATTTATCACCGCAGATGGTTACCTCGCCTCTGACC**TACGTA**CCGTCCCGGCGGTCGCCGGA | Eco105I | pIJ10257XermEp-cpkOCO |
| CpkO\_c257\_RED\_Eco105I\_R | ACTCTAGTTAATTAATCACTCGAGATCTCATATGGGGCC**TACGTA**TCAGATCGCCCCGCCTCCG | Eco105I |
| TE-K-Hind | TTTTTTT**AAGCTT**GTCGTACGTACACGGA | HindIII | pIJ10257-scoTOE |
| TE-P-Nde | TTTTTTTTT**CATATG**GGAAGTGACTGGTT | NdeI |
| CpkODM-Fw | TTTCGGATGCTCGGTCCACTCGAGGTGTTGTCCGGCGAGATTCCGGGGATCCGTCGACC | - | St1G7-cpkODM |
| CpkODM-Rv | GACGGCGGACCGCGGGCGGGCTCGGAGCAGCGGGGGTCATGTAGGCTGGAGCTGCTTC | - |
| p6275\_Nde | **CATATG**CGGCTGCCCTTTCCTGGCTGT | NdeI | pFLUXH-pcpkA  pFLUXH-pcpkD |
| p6276\_Nde | **CATATG**GATTTACTCTCCTTCGACAAG | NdeI |
| p6280\_Nde | **CATATG**TCCCCCAGTCCTGCACGCTGT | NdeI | pFLUXH-pcpkO |
| KSO-FW | ATCATCCGGGACACCGACGGA | - |
| p6288\_Nde | **CATATG**CTCACACTCCTGTCCCGGCAC | NdeI | pFLUXH-pcpkN |
| CPKN\_KHp | **AGATCTGGTACC**GTGGCGCGAGCACACCAC | BglII, KpnI |
| accA1-Rv | GGCGATGAGCACCTTGCGCA | - | pFLUXH-pscF |
| p6272\_Nde | **CATATG**CGAACCTCCGTGAGAACAAGA | NdeI |
| p6287\_Nde | **CATATG**CTTTTCCCCTTACCGTTCGAC | NdeI | pFLUXH-pscoT  pFLUXH-pscbR2 |
| p6286\_Nde | **CATATG**GTGCTCCGTGGTCGCGATCGT | NdeI |
| scbA\_Lux | CAAGCGGTGACAGAACAACA | - | pFLUXH-pscbA |
| p6266\_Nde | **CATATG**TCCCCCCCAGGAATCATGTGA | NdeI |
| SCBA-FW | TATCCAGCTGACCGGGAACGC | - | pFLUXH-pscbR |
| p6265\_Nde | **CATATG**TGCCTCCTTGTTCATGTCTCC | NdeI |
| luxout | GCTCTCGGGGAAGATCTCGAC | - | verification of insert orientation in pFLUXH |
| R2\_HE\_F | AAAAAA**TCATGA**CCAAGCAGGAG | PagI | pET28-scbR2 |
| R2\_HE\_Rv | AAAAAA**CTCGAG**GTGCGGCGC | XhoI |
| ScbREXFW | **GGATCCCATATG**GCCAAGCAGGACC | BamHI, NdeI | pD01-HN-scbR |
| ScbREXRV | **GAATTC**TCA**AAGCTT**GTCCTTCCCGGTCGGTGC | EcoRI, HindIII |
| NF | GTGAGTCCCCAGTGGGTACT | - | pcpkN, pcpkN-a and pcpkN-up fragments as shown in Fig. 5. |
| NR | GCCGTCGTCGCCGACGATCT | - |
| UP1 | GAACGCGGGACTCACCG | - |
| UP1C | CGGTGAGTCCCGCGTTC | - |
| UP2 | **GGATCC**GACCTGAGGGTGTT | BamHI |
| pTZBAM700  (pTZBAM800) | IRDye-ATGCAGGCCTCTGCA | - | amplification and IRDye\* labeling of fragments for EMSA cloned in pTZ57R/T |
| pTZXBA700  (pTZXBA800) | IRDye-TCGGTACCTCGCGAA | - |

\* IRDye 700 (ex. 685 nm, em.: 700 nm); IRDye 800 (ex. 785 nm, em. 800 nm)