1. Sequence of artificial operon PHC and TA

As for PHC (phaA-hbd-crt), the sequence of phaA, hbd and crt was marked in red, blue and green respectively, the short section highlighted in yellow is the designed SD sequence.

catatgactgacgttgcatcatgatatcggccgcccggcaccggcgttgccggaagtggggtgccgtgctggcaagaat
cgacctgggctctgtctcgagacaagcttcagttttggttgagtttttgaatgtaaatagtggtgctgcagactaatttttgcag
agggagtttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttgaattttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttttt
The sequence of TA operon is shown below (ter and adhEl was highlighted in red or blue, respectively)

ATG AGATCAA ACATGG TAGAAG GGAAGTTAGG AAATTTTTGACAATTTGCTCATCACCAGGACGGATGCAAAAAAGGCCTT GCAGCATG GAAGTTAGG AAATTTTTGACAATTTGCTCATCACCAGGACGGATGCAAAAAAGGCCTT GCAGCATG GAAGTTAGG AAATTTTTGACAATTTGCTCATCACCAGGACGGATGCAAAAAAGGCCTT GCAGCATG GAAGTTAGG AAATTTTTGACAATTTGCTCATCACCAGGACGGATGCAAAAAAGGCCTT GCAGCATG GAAGTTAGG AAATTTTTGACAATTTGCTCATCACCAGGACGGATGCAAAAAAGGCCTT GCAGCATG GAAGTTAGG AAATTTTTGACAATTTGCTCATCACCAGGACGGATGCAAAAAAGGCCTT GCAGCATG GAAGTTAGG AAATTTTTGACAATTTGCTCATCACCAGGACGGATGCAAAAAAGGCCTT GCAGCATG GAAGTTAGG AAATTTTTGACAATTTGCTCATCACCAGGACGGATGCAAAAAAGGCCTT GCAGCATG GAAGTTAGG AAATTTTTGACAATTTGCTCATCACCAGGACGGATGCAAAAAAGGCCTT GCAGCATG GAAGTTAGG AAATTTTTT
ACAAGAATATTAATAGGAGAAGTTACCTCCTTAGGTGAAGAAGAACCTTTTGCCCACGAAAAACTATCTCCTGT
TTGGCTATGTATGAGGCTGACAATTTTGATGATGCTTTAAAAAAAGCAGTAACTCTAATAAATCTTAGGAGGCC
TCGGCCATACCTCAGGAATATATGCAGATGAAATAAAACGCAAGAGATAAATATAGTAGATTTAGTAGTGGCATG
AAAACGCTAAGACCTTTGTAATATATCCCAACCTCAACAGGTGCAAGTGGAGATCTATATAATTTAGAATACC
ACCTTCTTTACGCTTTGCGGATTTTTGAGGAGAAATTCGTTTCCAGAATGTTGTGTCCAAAAACATCTCTT
TGAATATTTAACCCTAGCTGAAAGCCTTTGTAATATCCCAACCTCACAAGGTGCAAGTGGAGATCTATATAATTTAGAATACC
TTAATAAGGTTGGAGAGAAGCTGATCTTAAAAACCCATAAAAAAGCAACTGAAGAAATGCCCTCCTCTCCTTTATGCA
GACACTATAATAGCTTATTGAGGACTTACCCTGAAATGAGCTCTGGAATAGTTAATATTATAGAAGTAGC
AGAATCTTATGTAATATGAGCTTTTGCACATTTTATGACACTATAAAATTTGAGATCTTAAAACCTGTTT
AGAGTTCCACATAAAGTATATTTTAAG

2. Construction of plasmid pENA, pCNA, pENA-TA and pCNA-PHC

As seen in Figure below, DNA fragment of Phya was amplified using genomic DNA of E. coli strain Bw25113, with primer pair Phya-F and Phya-R. The PCR product and plasmid DNAs of pACYCduet-1/pETduet-1 were all double digested with EcoRI and HindIII, purified fragments were ligated by T4 DNA ligase to produce pCNA and pENA.

(DNA sequence of Phya-F is aaccgaattccttctcttcattcttctagcaacgg
DNA sequence of Phya-R is: ttggtaatcttcatgtggtactggttcggactatggtcgctcttcctcttg)

As shown below as well, synthesized DNA of TA was treated with NdeI and XhoI, and ligated into same enzymes treated pENA to get pENA-TA; artificial operon PHC was treated with NdeI and KpnI, ligation of digested fragment with same enzymes treated pCNA generated pCNA-PHC.
3. Illustration of Construction of pCNA-PHCF by the aid of helper plasmid pKD46

① PCR preparation of a fragment containing \textit{kan} cassette flanked by frt sites and homologous sequences used for recombination with plasmid pCNA-PHC. 

② Co-transformation of pCNA-PHC and the PCR product.
Separation of plasmid pCNA-PHCF from pCNA-PHC.

4. The Sequence of the fragment frt-sm-frt inserted in vector pENA-TAF
   frt sites is highlighted in blue, and the DNA encoding streptomycin
   resistant enzyme (sm) is labelled in red.

   gaattccggagatggcttacaagcaaaatccgccaacaaaaactctccgtatgtgattgagcaagcaactg
   ggtttttgtacccgggcccctaatgatccctattcctcaagttctatttctctgaaagtgataggaacttcgagt
cgcgtacattttgtcagctccgagtccgaccagtgactagctatgtgatgttttattttttctaatctcattcataatgtat
cgcgtcatgacaataaaccctgtataatgtatagtggatatgtaattgaaaaaggagagatattgaggaagcctggga
atcgccgaagtacgactcaactatcagaggtagttgggccagctcagctcagcaccagctgcgtgagtttctggtgtgc
ccgtacattttgtacgctccgagtccgaccagtgactagctatgtgatgttttattttttctaatctcattcataatgtat
cgcgtcatgacaataaaccctgtataatgtatagtggatatgtaattgaaaaaggagagatattgaggaagcctggga
5. Genetic confirmation of BuS2

Strain BuS2 was genetically confirmed by PCR: genomic DNAs from wild type BW25113 and mutant BuS2 were isolated and used as the templates; Primers were designed from both ends located about 300 bps each upstream or downstream of individual gene ldhA or adhE on the genome. The length of PCR product of in-frame deletion of ldhA was expected to be about 600 bps; and the PCR product from adhE mutant should be about 700 bps; as seen in the figure below, both adhE and ldhA in BuS2 were confirmed to have been deleted from the genome.