**Improving the plasmid stability by a *hok*/*sok* system for** **L-homoserine production in *Escherichia coli***

Bing-Yao Sun#, Xin-Yi Tao#, Hua-Lu Sui, Feng-Qing Wang, Qing-Hai Liu, Bei Gao, Jian, Zhao, Yu-Shu Ma\*, Min Liu \*, and Dong-Zhi Wei

State Key Lab of Bioreactor Engineering, Newworld Institute of Biotechnology, East China University of Science and Technology, Shanghai 200237, China.

# Bing-Yao Sun and Xin-Yi Tao contribute equally for this work.

\*Address correspondence to

Min Liu: lmin@ecust.edu.cn

Yu-Shu Ma: myushu@ecust.edu.cn

E-mail addresses for other authors:

Bing-Yao Sun: sby18818278548@163.com

Xin-Yi Tao: xytao@ecust.edu.cn

Hua-Lu Sui: shl18616321432@163.com

Feng-Qing Wang: fqwang@ecust.edu.cn

Qing-Hai Liu: liuqinghai@ecust.edu.cn

Bei Gao: gaobei@ecust.edu.cn

Jian Zhao: zhaojian@ecust.edu.cn

Dong-Zhi Wei: dzhwei@ecust.edu.cn

Declarations of interest: none

**Figure captions**

**Table S1.** Primers used in the work.

**Fig. S1** The growth clones of SHL12 (cultured without antibiotics in test tube) on solid plate with or without antibiotics.

**Table S1**

|  |  |
| --- | --- |
| Name | Sequences (5’ to 3’) |
| pKK-*metL*-F | GAAGGAGATATACCGGTACCATGAGTGTGATTGCGCAGGC |
| pKK-*metL*-R | AGCTTGGCTGCAGGAATTCTTACAACAACTGTGCCAGCCG |
| hok/sok-1-F | GGCTGGCACAGTTGTTGTAAGTGGATGGTGCCGAACAAACT |
| hok/sok-1-R | TCTCATCCGCCAAAACAGCCCCTGGCAGTCTGGTTGTTCAT |
| hok/sok-2-F | CACTGATTAAGCATTGGTAAGTGGATGGTGCCGAACAAACT |
| hok/sok-2-R | GAGTAAACTTGGTCTGACAGCCTGGCAGTCTGGTTGTTCAT |
| pTargetF-*ppc* (trc) | ACTAGTATTATACCTAGGACTGAG |
| pTargetR-*ppc* (trc) | CAATGCGACGTGAAGGATACGTTTTAGAGCTAGAAATAGCAAGTT |
| *ppc*-1 | CGCGAACTGATAACCCAGG |
| *ppc*-2 | CACACATTATACGAGCCGGATGATTAATTGTCGCAGGAATTTACGTCAT |
| *ppc*-3 | TCGTATAATGTGTGGTCACAAAGGAGATATACATGAACGAACAATATTC |
| *ppc*-4 | CGACGGGTAATTTCGGTTGG |
| pTargetF-*thrA* (trc) | ACTAGTATTATACCTAGGACTGAG |
| pTargetR-*thrA* (trc) | AAAGCCCGCACTGTCAGGTGGTTTTAGAGCTAGAAATAGCAAGTT |
| *thrA*-1 | TGGTGCCGCTGTTAGAGGAAA |
| *thrA*-2 | CACACATTATACGAGCCGGATGATTAATTGTCAGGTAACCAGTTCAGAA |
| *thrA*-3 | TCGTATAATGTGTGGTCACAAAGGAGATATACATGAAACGCATTAGCAC |
| *thrA*-4 | CAGCGCAGCGTTGATGCTATCCG |
| pTargetF-*asd* (trc) | ACTAGTATTATACCTAGGACTGAG |
| pTargetR-*asd* (trc) | TAAATGTGCCGGTCTCCTCTGTTTTAGAGCTAGAAATAGCAAGTT |
| *asd*-1 | AGACAACAACATCAGCGTGGCG |
| *asd*-2 | CACACATTATACGAGCCGGATGATTAATTGTCATAGCGAAGCCATCAGT |
| *asd*-3 | TCGTATAATGTGTGGTCACAAAGGAGATATACATGAAAAATGTTGGTTT |
| *asd*-4 | AAACCACCCAACGACATCAACA |
| pTargetF-*pntAB* (trc) | ACTAGTATTATACCTAGGACTGAG |
| pTargetR-*pntAB* (trc) | GCTTGTGTGGCTCCTGACACGTTTTAGAGCTAGAAATAGCAAGTT |
| *pntAB*-1 | GGAGAATGCCATAGCAGAAA |
| *pntAB*-2 | CACACATTATACGAGCCGGATGATTAATTGTCTATGCCTGTGATCTAGC |
| *pntAB*-3 | TCGTATAATGTGTGGTCACAAAGGAGATATACATGCGAATTGGCATACC |
| *pntAB*-4pTargetF-*rhtA* (trc)pTargetF-*rhtA* (trc) | CAACACCCGCACCAATCACCACTAGTATTATACCTAGGACTGAGGTACTGGATATACCCAATGCGTTTTAGAGCTAGAAATAGCAAGTT |
| *rhtA*-1 | AACCGTAGTCGCTGGTGTCG |
| *rhtA*-2 | CACACATTATACGAGCCGGATGATTAATTGTCAACGTTCTGTTACATGA |
| *rhtA*-3 | TCGTATAATGTGTGGTCACAAAGGAGATATACATGCCTGGTTCATTACG |
| *rhtA*-4 | TAGCAGGAACCACAGACCAA |
| aspA-1 | GTAGACAGCGGACGGCAAGG |
| aspA-2 | GCTTGAAAAAGAAGGTTCACTCGTACAGGGTAGTACAAAT |
| aspA-3 | ATTTGTACTACCCTGTACGAGTGAACCTTCTTTTTCAAGC |
| aspA-4 | GTGACATACCGATAACTGAC |
| aspC-1 | GCTGAACGAAGGCGATACCA |
| aspC-2 | AACCATAATGGAACCTCGTCGCATTAAAAACAATGAAGCC |
| aspC-3 | GGCTTCATTGTTTTTAATGCGACGAGGTTCCATTATGGTT |
| aspC-4 | GCAGCGAACTACGGTGAAAC |
| aspAC-1 | ATTGTAAGAAGGGATATACCATGTCAAACAACATTCGTAT |
| aspAC-2 | GGTATATCCCTTCTTACAATTCCACATTACTGTTCGCTTTCATCAG |
| aspAC-3 | TGTGGAATTGTAAGAAGGGATATACCATGTTTGAGAACATTACCGC |
| aspAC-4 | ACAGCCAAGCTTGGCTGCAGTTACAGCACTGCCACAATCG |
| MAC-F | TGATTAATTGTCAACAGCTCTTACAGCACTGCCACAATCG |
| MAC-R | CACGATGCGTCCGGCGTAGATTGACAATTAATCATCGGCT |
| aspAC-1(pri) | CACGATGCGTCCGGCGTAGAACCGACACTTAAAGTGATCC |
| aspAC-2(pri) | TACTGATGAAAACGCAGGCTTTACTGTTCGCTTTCATCAG |
| aspAC-3(pri) | CTGATGAAAGCGAACAGTAAAGCCTGCGTTTTCATCAGTA |
| aspAC-4(pri) | ACAGCCAAGCTTGGCTGCAGTTACAGCACTGCCACAATCG |

a Restriction sites are underlined.

**Fig. S1**

