

Continuous Secretion of Human Epidermal Growth Factor Based on *Escherichia coli* Biofilm

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Table S1. Primers used in this study

Primer	Sequence Number	Sequence
<i>arpA</i> -sg-F	NO.1	gtcctaggtataataactagtagttttatcattagttacattgttttagag ctagaaatag
<i>yfbL</i> -sg-F	NO.2	gtcctaggtataataactagttctctgaccacctgaattatgttttaga gctagaaatag
<i>yddE</i> -sg-F	NO.3	gtcctaggtataataactagtagagcgctgatccacttcgtttag agctagaaatag
<i>yjcF</i> -sg-F	NO.4	gtcctaggtataataactagtgaaatggcaattgcttaaatgttttag agctagaaatag
<i>yjjM</i> -sg-F	NO.5	gtcctaggtataataactagttagccgatgacgttcgagcagtttta gagctagaaatag
sg-R	NO.6	actagtattatacctaggactgagctagctgtcaaggatc
<i>arpA</i> -up-F	NO.7	taaatccaaacaggcgtgcatggtag
<i>arpA</i> -up-R	NO.8	gccttcaaccagtcagctccttcgggtgggcggaatatta aacactattctctgatgg
<i>yfbL</i> -up-F	NO.9	aacactcataactcattataaataatg
<i>yfbL</i> -up-R	NO.10	gccttcaaccagtcagctccttcgggtgggcgccatggg gcattcatagaataaacag
<i>yddE</i> -up-F	NO.11	taccgtggatgagttagcgtggtg
<i>yddE</i> -up-R	NO.12	gccttcaaccagtcagctccttcgggtgggcgccgctat cagttaacagattggctg
<i>yjcF</i> -up-F	NO.13	tgctgaactttaccacctgcgcgccacg
<i>yjcF</i> -up-R	NO.14	gccttcaaccagtcagctccttcgggtgggcggatgttta atatccgtctctatcgtg
<i>yjjM</i> -up-F	NO.15	aattgtttacgtaattgtgcattacg
<i>yjjM</i> -up-R	NO.16	caaccagtcagctccttcgggtgggcgctggcaatagct ttcgatgttctcg

<i>hEGF-arpA-F</i>	NO.17	ccatcagagaatagtgttaattccgcccaccggaagga gctgactgggtgaaggc
<i>hEGF-arpA-R</i>	NO.18	ggctaagaataaagaacacaactgaaggatgtacggcg cgtccattcgccaatccgg
<i>hEGF-yfbL-F</i>	NO.19	ctgtttattctatgaatgccccatggcgcccaccggaagga gctgactgggtgaaggc
<i>hEGF-yfbL-R</i>	NO.20	cgataaaagcgggtgcagtaatcattacggggcgctccatt cgccaatccgg
<i>hEGF-yddE-F</i>	NO.21	cagccaatctgttaactgatagcggcgcccaccggaaggagct gactgggtgaaggc
<i>hEGF-yddE-R</i>	NO.22	cgatattctgccgggttgccgatccaggtcggggcacgtccc attcgccaatccgg
<i>hEGF-yjcF-F</i>	NO.23	cacgatagagacggatattaacatccgcccaccggaaggagc tgactgggtgaaggc
<i>hEGF-yjcF-R</i>	NO.24	cgaaatggttatatgtaccggctctaatatggggcgcgctc ccattcgccaatccgg
<i>hEGF-yjjM-F</i>	NO.25	cgagaacatgcgaaagctattgccgacgccaccgga aggagctgactgggtg
<i>hEGF-yjjM-R</i>	NO.26	gcactttctaacctgcctgatcatgatcgggcgcgctccc attcgccaatccgg
<i>arpA-dn-F</i>	NO.27	ccggattggcgaatgggacgcgcccgtacatcctcagt tgtgttctttattcttagcc
<i>arpA-dn-R</i>	NO.28	acacttactcaaaccattgccccaatatttg
<i>yfbL-dn-F</i>	NO.29	ccggattggcgaatgggacgcgccccgtaatgattact gacaccgttttatcg
<i>yfbL-dn-R</i>	NO.30	ttccaggatcaatcccaggaagccatc
<i>yddE-dn-F</i>	NO.31	ccggattggcgaatgggacgcgcccgcgacctggatc

		ggcaaaccggcagaatatcg
<i>yddE</i> -dn-R	NO.32	tgatagttctccagtcacaggagaacg
<i>yjcF</i> -dn-F	NO.33	ccggattggcgaatgggacgcgcccgcattagagc cgtacatataaccatttcg
<i>yjcF</i> -dn-R	NO.34	ttatccaattcgaattttgcaggttc
<i>yjiM</i> -dn-F	NO.35	ccggattggcgaatgggacgcgcccgcattatcagg caggttgagaaagtgc
<i>yjiM</i> -dn-R	NO.36	ttgatgatatggcgcggggtcatatcc
pET28a-F	NO.37	gcagcagccatcatcatcatc
pET28a-R	NO.38	tctccttctaaagttaaacaaa
pBbE1a-F	NO.39	ccaggcatcaaataaacgaaag
pBbE1a-R	NO.40	atgtatatctccttctaaagatctttgaattc
pET28a- <i>dgcC</i> -F	NO.41	ttgtttaactttaagaaggagaatgtccaaaaataatga atgatgaaaact
pET28a- <i>dgcC</i> -R	NO.42	gatgatgatgatggctgctgctcaggccgccacttcggtg
pET28a- <i>csgD</i> -F	NO.43	ttgtttaactttaagaaggagaatgtttaatgaagtccatag tattcatggtc
pET28a- <i>csgD</i> -R	NO.44	gatgatgatgatggctgctgcttatcgctgaggttatcg
pET28a- <i>bcsA</i> -F	NO.45	ttgtttaactttaagaaggagaatgagtatcctgacccggt
pET28a- <i>bcsA</i> -R	NO.46	gatgatgatgatggctgctgctcattgtgagccaaagcc
pET28a- <i>bcsB</i> -F	NO.47	ttgtttaactttaagaaggagaatgaaaagaaactattct gga
pET28a- <i>bcsB</i> -R	NO.48	atgatgatgatggctgctgcttactcgttatccgggttaa

pBbE1a- <i>bcsB</i> -F	NO.49	agatctttaagaaggagatatatatgaaaagaaaacta ttctggattgtgc
pBbE1a- <i>bcsB</i> -R	NO.50	cttcgttttatttgatgcctgggtactcgttatccgggtaag ac

For genome-integrated expression of *hEGF*, the N20 sequence of sgRNA in pTarget was redesigned to target specific gene locus using primers NO.1 to 6 in Table S1. Primers NO.7 to 16 in Table S1 were used to amplify the upstream homologues of the target locus using *E. coli* BL21(DE3) genome as template, and primers NO.17 to 26 in Table S1 were used to amplify the target gene *hEGF* using plasmid pET30a-PelB-*hEGF* as template, and primers NO.27 to 36 in Table S1 were used to amplify the downstream homologues of the target locus using *E. coli* BL21(DE3) genome as template. These fragments were ligated by overlap PCR. For expression of biofilm-related genes, plasmid fragments were amplified using primers NO.37 to 40 listed in Table S1, and biofilm genes *dgcC*, *csgD*, *bcsA* and *bcsB* were amplified from the genomic DNA of *E. coli* MG1655 using primers NO.41 to 50 listed in Table S1, which were linked by overlap PCR.

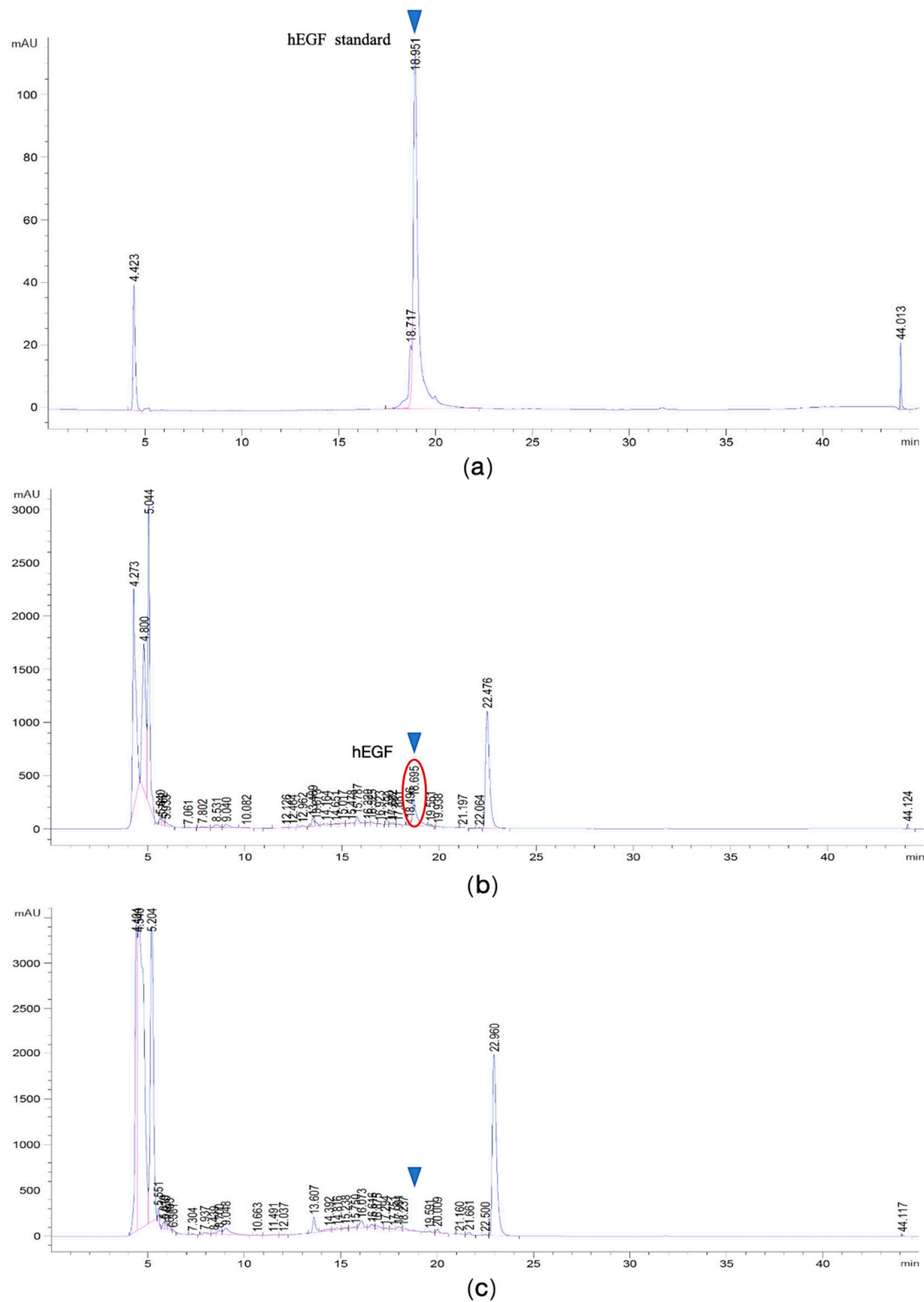


Figure S1 Elution profile. (a) hEGF standard (25 mg/L); (b) BL21-*hEGF*-C5 (37 mg/L); (c) BL21 (DE3).