

## Supplementary Information

### Optimizing Cell-Free Protein Synthesis for Increased Yield and Activity of Colicins

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**Supplementary Table S1.** Primers used in this study. “\*” indicates phosphorothioated bases.

Primer	Purpose	Sequence (5' → 3')
pJL1-F	Amplification of pJL1 backbone for Gibson Assembly	CAAAGCCCCGAAAGGAAGCTGAGTTG
pJL1-R		CTTAAAGTTAAACAAAATTATTTCTAGAGGG
Gacol-F	Amplification of <i>cm</i> a gene encoding colicin M	TTTGTTTAACTTTAAGAAGGAGATATACATATG
Gacol-R		TTCCTTTCGGGCTTTGTTAGCAGCCGGTCGACTTA
ColE1-F	Amplification of <i>cea</i> gene encoding colicin E1	ATTTTGTTTAACTTTAAGAAGGAGATATACATATGGAAACCGCGGTAGCGTAC
ColE1-R		CGGGCTTTGTTAGCAGCCGGTCGACTTATTTTTCGAACTGCGGATGGCTCCAAA TCCCTAACACCTCATTATAG
ColE3-F	Amplification of <i>ceaC</i> gene encoding colicin E3	ATTTTGTTTAACTTTAAGAAGGAGATATACATATGAGCGGTGGCGATGGACG
ColE3-R		CGGGCTTTGTTAGCAGCCGGTCGACTCATTTTTCGAACTGCGGATGGCTCCAAA GATATTTCTTGATATTTTC
E3Imm-F	Amplification of <i>imm</i> gene encoding E3 immunity	ATTTTGTTTAACTTTAAGAAGGAGATATACATATGGGACTTAAATTGG
E3Imm-R		CGGGCTTTGTTAGCAGCCGGTCGACTCATTTTTCGAACTGCGGATGGCTCCACC AATCACCATC
T7Mega-F	Addition of T7 promoter	A*G*ATCTCGATCCC CGCAAATTAATACGACTCACTATAGGGAGACCACAACG GTTTCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGG
TAA-R	Addition of overlap region with T7 terminator	TATTGCTCAGCGGTGGCAGCAGCCAACTCAGCTTCCTTTCGGGCTTTGTTAGCA GCCGG
T7Mega-R	T7 terminator addition	T*A*ATCAGAATTGGCTTTCAGCAAAAAACCCCTCAAGACCCGTTTAGAGGCC CAAGGGGTTATGCTAGTTATTGCTCAGCGGTGGCAGC
T7-Pro	Sequence checking for colicin amplification	TAATACGACTCACTATAGGG
T7-Ter		GCTAGTTATTGCCTCAGCGG

**Supplementary Table S2.** DNA sequences of all colicin genes. T7 promoter, terminator, colicin genes, and E3 immunity gene are highlighted as blue, red, turquoise, and green, respectively. Bolded lowercase characters indicate Strep-tag sequence.

Name	Size (bp)	Sequence 5' → 3'
<i>cma</i> encoding M	1073	<p>AGATCTCGATCCCGCGAAAT<b>TAATACGACTCACTATAGGGAG</b>ACCACAACGGTTCCCTCTAGAAATA            ATTTTGTTAACTTTAAGAAGGAGATATACATATGGAAACCTTAACTGTTTCATGCACCATCACCATCA            ACTAACTTACCAAGTTATGGCAATGGTGCATTTTCTCTTTCAGCACCGCATGTTCTGGTGGTGGACCT            CTTTGTAGTCCAGTTGTTTATAGTTTTTCCAGAGTCCAAACATGTGTCTTCAGGCTTTAACTCAACTT            GAGGATTACATCAAAAAACATGGGGCTAGCAACCCTCTCACATTGCAGATCATATCGACAAATATTG            GTTACTTCTGTAACGCCGACCGAAATCTGGTCTTCACCCTGGAATAAGCGTTTATGACGTTACCACT            TCTCAAACCAGCGCCAAGTCAATATGACTATCGCTCAATGAATATGAAACAAATGAGCGGTAATGT            CACTACACCAATTGTGGCGCTTGTCTACTATTTATGGGGTAATGGCGCTGAAAGGAGCGTTAATATCG            CCAACATTGGTCTTAAAATTTCCCTATGAAAATTAATCAGATAAAAGACATTATAAAATCTGGTGTGA            GTAGGTACATTCCCTGTTTCTACAAAGTTCACACATGCCACTGGTGATTATAATGTTATTACCGGTGCA            TATCTTGGTAATATCACACTGAAAACAGAAGGTACTTTAACTATCTCTGCCAATGGCTCCTGGACTTA            CAATGGCGTTGTTTCGTTTCATATGATGATAAATACGATTTAACGCCAGCACTCACCGTGGCGTCATCG            GAGAGTCGCTACAAGGCTCGGGGCGATGTTTTCTGGTAAAGAGTACCAGATACTGTTCTTGGTGAA            ATTCACATTAAGAAAGTGGTAAGCGAt<b>ggagccatccgcagttcgaaaa</b>TAAGTCGACCGGCTGCTAACAAAG            CCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCAT<b>AACCCCTTGGGGCCTC</b>  <b>TAAACGGGTCTTGAGGGGTTTTT</b>GCTGAAAGCCAATTCTGATTA</p>
<i>cea</i> encoding E1	1826	<p>AGATCTCGATCCCGCGAAAT<b>TAATACGACTCACTATAGGGAG</b>ACCACAACGGTTCCCTCTAGAAATA            ATTTTGTTAACTTTAAGAAGGAGATATACATATGGAAACCGCGGTAGCGTACTATAAAGATGGTGGT            CTTATGATGATAAGGGACAGGTAATTACTCTTTTGAATGGTACTCCTGACGGGAGTGGCTCTGG            CGGCGGAGGTGGAAAAGGAGGCAGTAAAAGTGAAGTTCTGCAGCTATTCATGCAACTGCTAAATGG            TCTACTGCTCAATTAAGAAAACACAGGCAGAGCAGGCTGCCCGGGCAAAGCTGCAGCGGAAGCA            CAGGCGAAAGCAAAGGCAAACAGGGATGCGCTGACTCAGCGCCTGAAGGATATCGTGAATGAGGCT            CTTTCGTCACAATGCCTCACGTACGCCTTACGCAACAGAGCTTGCTCATGCTAATAATGCAGCTATGCA            GCGGAAGACGAGCGTTTGCGCCTTGCGAAAGCAGAAGAAAAGCCCGTAAAGAAGCGGAAGCAGC            AGAAAAGGCTTTTCAGGAAGCAGAACAACGACGTAAGAGATTGAACGGGAGAAGGCTGAAACAGA            ACGCCAGTTGAAACTGGCTGAAGCTGAAGAGAAACGACTGGCTGCATTGAGTGAAGAAGCTAAAGCT            GTTGAGATCGCCAAAAAAACTTTCTGCTGCACAATCTGAAGTGGTGAATAATGGATGGAGAGATTA            AGACTCTCAATTCTCGTTTAAAGCTCCAGTATCCATGCCCGTGATGCAGAAATGAAAACGCTCGCTGGA            AAACGAAATGAACTGGCTCAGGCATCCGCTAAATATAAAGAACTGGATGAGCTGGTCAAAAAACTAT            CACCAAGAGCCAATGATCCGCTCAGAACCCTTTTTTTTGAAGCAACCAGACGACGGGTTGGGGCC            GGTAAGATTAGAGAAGAAAACAAAAACAGGTAACAGCATCAGAAACACGTATTAACCGGATAAAT</p>

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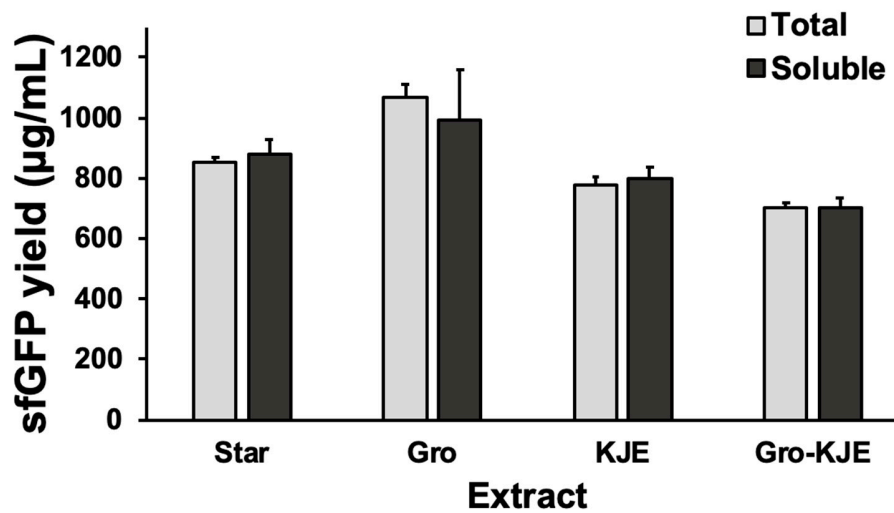
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E3

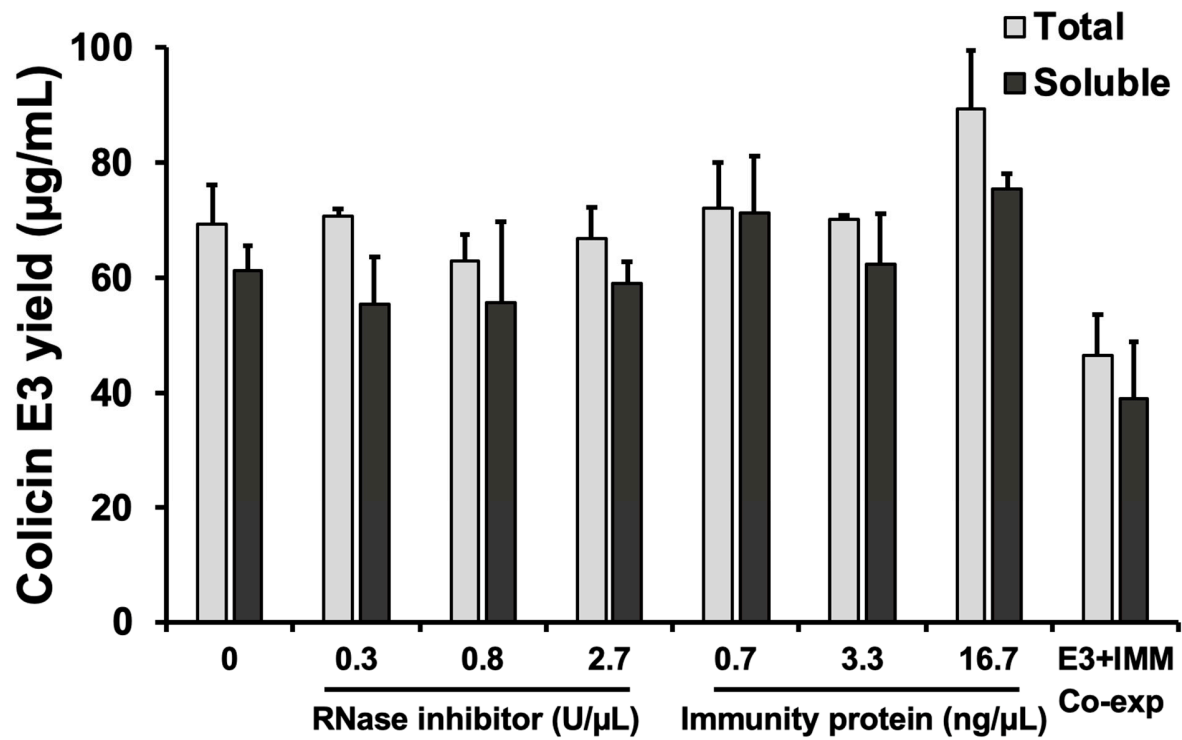
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E3+Imm operon	2156	AGATCTCGATCCCGCGAAAT <b>TAATACGACTCACTATAGGGAG</b> ACCACAACGGTTTCCCTCTAGAAATA ATTTTGTTTAACTTTAAGAAGGAGATATACATATGAGCGGTGGCGATGGACGCGGCCATAACACGGG CGCGCATAGCACAAGTGGTAACATTAATGGTGGCCGACCGGGCTTGGTGTAGGTGGTGGTCTTCTG ATGGCTCCGGATGGAGTTCGGAAAATAACCCGTGGGGTGGTGGTTCCGGTAGCGGCATTCACTGGGG TGGTGGTTCCGGTCATGGTAATGGCGGGGGGAATGGTAATCCGGTGGTGGTTCGGGAACAGGCGGT AATCTGTCAGCAGTAGCTGCGCCAGTGGCATTGGTTTTCCGGCACTTCCACTCCAGGAGCTGGCGG TCTGGCGGTCAGTATTCAGCGGGAGCATTATCGGCAGCTATTGCTGATATTATGGCTGCCCTGAAAG GACCGTTTAAATTTGGTCTTTGGGGGTGGCTTTATATGGTGTATTGCCATCACAAATAGCGAAAGAT GACCCAATATGATGTCAAAGATTGTGACGTCATTACCCGCAGATGATATTACTGAATCACCTGTCAG TTCATTACCTCTCGATAAGGCAACAGTAAACGTAATGTTCTGTGTTGTTGATGATGTAAAAGACGAGC GACAGAATATTTGGTGTTCAGGTGTTCCGATGAGTGTCCGGTGGTTGATGCAAAACCTACCGAA CGTCCGGGTGTTTTACGGCATCAATCCAGGTGCACCTGTTCTGAATATTTCAAGTTAATAACAGTACG CCAGCAGTACAGACATTAAGCCAGGTGTTACAAATAATACTGATAAGGATGTTCCGCCGGCAGGAT TACTCAGGGTGGTAATACCAGGGATGCAGTTATTCGATTCCCGAAGGACAGCGGTCATAATGCCGTA TATGTTTCAGTGAGTGATGTTCTTAGCCCTGACCAGGTAAAACAACGTCAAGATGAAGAAAATCGCC GTCAGCAGGAATGGGATGCTACGCATCCGGTTGAAGCGGTGAGCGAAATTATGAACGCGCGCGTGC AGAGCTGAATCAGGCAAATGAAGATGTTGCCAGAAATCAGGAGCGACAGGCTAAAGCTGTTCAAGTT TATAATTCGCGTAAAAGCGAACTTGATGCAGCGAATAAACTCTTGCTGATGCAATAGCTGAAATAA AACAATTTAATCGATTTGCCCATGACCCAATGGCTGGCGGTACAGAAATGTGGCAAATGGCCGGGCTT AAAGCCCAGCGGGCGCAGACGGATGTAATAATAAGCAGGCTGCATTTGATGCTGCTGCAAAAGAGA AGTCAGATGCTGATGCTGCATTGAGTTCTGCTATGGAAAGCAGGAAGAAGAAGAAGATAAGAAAA GGAGTGCTGAAAATAATTTAAACGATGAAAAGAATAAGCCAGAAAAGGTTTTAAAGATTACGGGCA TGATTATCATCCAGCTCCGAAAACCTGAGAATATTAAGGGCTTGGTGATCTTAAGCCTGGGATACCAA AAACACCAAAGCAGAATGGTGGTGGAAAACGCAAGCGCTGGACTGGAGATAAAGGGCGTAAGATTT ATGAGTGGGATTCTCAGCATGGTGAGCTTGAGGGGTATCGTGCCAGTGATGGTCAGCATCTTGCTCA TTTGACCCTAAAACAGGCAATCAGTTGAAAGGTCCAGATCCGAAACGAAATATCAAGAAATATCTTT GAGAGGAAGTT <b>ATGGGACTTAAATTGGATTTAACTTGGTTTTGATAAAAAGTACAGAAGATTTTAAAGG</b> <b>TGAGGAGTATTCAAAGATTTTGGAGATGACGGTTCAGTTATGGAAAAGTCTAGGTGTGCCTTTTAAAG</b> <b>ATAATGTTAATAACGGTTGCTTTGATGTTATAGCTGAATGGGTACCTTTGCTACAACCATACTTTAATC</b> <b>ATCAAATTGATATTTCCGATAATGAGTATTTTGTTCGTTTGATTATCGTGATGGTGAATTGGTGA</b> GTCG ACCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCA T <b>AACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTT</b> GCTGAAAGCCAATTCTGATTA



**Supplementary Figure S1.** Overall CFPS capacity of chaperone-enriched extracts. Total and soluble protein yields of sfGFP produced in chaperone-enriched extracts determined by  $^{14}\text{C}$ -Leu scintillation counting at 30°C for 20 h.



**Supplementary Figure S2.** Effect of additives in colicin E3 yield. Yield of cell-free synthesized colicin E3 with different concentrations of RNase inhibitor and E3 immunity protein and with co-expression (co-exp) of immunity protein. Error bars indicate standard deviation from three independent CFPS reactions.