

Supplementary file 1. Oligonucleotides and plasmids used in this study.

Oligonucleotides	Source	Lab Stock #
ePEC non-template DNA (consensus) GGTCAGTACGTCCGGCATAGTTGCGCCCGTAAATTAGATCTTCC AGTGG	IDT	#9563
ePEC non-template DNA (bubble scaffold) GGTCAGTACGTCCGGTCGATCTTCGCCCGTAAATTAGATCTTCC AGTGG	IDT	#8333
ePEC template DNA (consensus) CCACTGGAAGATCTGAATTACGGGCGCAACTATGCCGGACGTA CTGACC	IDT	#8334
ePEC RNA -11G (consensus) UUUUUUGGCAUAGUU	IDT	#8342
ePEC RNA -11U UUUUUUUGCAUAGUU	IDT	#10797
ePEC non-template DNA (Bochkareva <i>et al.</i> , 2012) ACTTACAGTAATCCGAAGTACACACGGCGAATAGCCA	IDT	#8195
ePEC template DNA (Bochkareva <i>et al.</i> , 2012) TGGCTATTGCCGTGTACTTCGGATTACTGTAAGT	IDT	#8196
ePEC non-template long DNA (Bochkareva <i>et al.</i> , 2012) ACTTACAGTAATCCGAAGTACACACGGCGAATAGCCATTCCAGTG G	IDT	#8289
ePEC template long DNA (Bochkareva <i>et al.</i> , 2012) CCACTGGAATGGCTATTGCCGTGTACTTCGGATTACTGTAAG T	IDT	#8290
ePEC RNA (Bochkareva <i>et al.</i> , 2012) AAUAAUCCGAAGU	IDT	#7974
ePEC tandem scaffold non-template DNA GGTACCGGCATAGTTGCGCCCGGCAAAGATGCGCCCGTAAATT CAGATCTTCCAGTGG	IDT	#12623
ePEC tandem scaffold template DNA CCACTGGAAGATCTGAATTACGGGCGCATCTTGCCGGCGCA ACTATGCCGGTACC	IDT	#12624
EC non-template DNA for DS-6MI control GGTGATCTAAGGTTCCAGAGGGAGCGXTCATGTCAGTAGAGTAGC GAGTGG X=6-MI	Fidelity Systems	#11159
EC template DNA for DS-6MI control CCACTCGCTACTCTACTGACATGACCGCTCCTCTGGAACCTTAGA TCACC	IDT	#11242
EC RNA for DS-6MI control CUCACAACCAGAGGGAG	IDT	#8106
ePEC non-template DNA for DS-6MI pause GGTCAGTACGTCCGGCATAGTTGCGXTCGTAATTAGATCTTCC AGTGG	Fidelity Systems	#11156

<u>ePEC template DNA for DS-6MI pause</u> CCACTGGAAGATCTGAATTACGACCGCAACTATGCCGGACGTAC TGACC	IDT	#11241
<u>ePEC RNA for DS-6MI pause</u> UUUUUUGGCAUAGUUG	IDT	#8401
<u>ePEC non-template DNA for US-6MI control</u> ACTTGTAGCGATCTAAGGTTCCAGAGGAGCGGGCATGTCAGTAGA GTAGC	IDT	#8102
<u>ePEC template DNA for US-6MI control</u> GCTACTCTACTGACATGACCGCTCCTCTXGAACCTTAGATCGCTA CAAGT	Fidelity Systems	#8110
<u>ePEC non-template DNA for US-6MI pause</u> GGTCAGTACGTCCGCCATAGTTGCGCCCCGTAAATTAGATCTTCC AGTGG	IDT	#8399
<u>ePEC template DNA for US-6MI pause</u> CCACTGGAAGATCTGAATTACGGGCGCAACTATXGC GGACGTA CTGACC	Fidelity Systems	#8385
<u>ePEC RNA for US-6MI pause</u> UUUUUUGGCAUAGUUG	IDT	#8402
<u>ePEC template DNA (usFJ variant)</u> CCACTGGAAGATCTGAATTACGGGCGCAACTATGGCGGACGTA CTGACC	IDT	#9762
<u>ePEC RNA (usFJ variant)</u> UUUUUUGGCAUAGUU	IDT	#8763
<u>ePEC template DNA (Hyb variant)</u> CCACTGGAAGATCTGAATTACGGGCGCGAGTATGCCGGACGTA CTGACC	IDT	#11639
<u>ePEC RNA (Hyb variant)</u> UUUUUUGGCAUACUC	IDT	#11640
<u>ePEC non-template DNA (dsFJ variant)</u> GGTCAGTACGTCCGGCATAGTTGCGGGCGTAAATTAGATCTTCC AGTGG	IDT	#10806
<u>ePEC non-template DNA (DSDNA variant)</u> GGTCAGTACGTCCGGCATAGTTGCGGGCGTAAATTAGATCTTCC AGTGG	IDT	#11178
<u>ePEC template DNA (DSDNA variant)</u> CCACTGGAAGATCTGAATTACGACCGCAACTATGCCGGACGTAC TGACC	IDT	#11241
<u>ePEC template DNA (usFJ+Hyb variant)</u> CCACTGGAAGATCTGAATTACGGGCGCGAGTATGGCGGACGTA CTGACC	IDT	#11951
<u>ePEC RNA (usFJ+Hyb variant)</u> UUUUUUGGCAUACUC	IDT	#11952
<u>ePEC non-template DNA (usFJ+dsFJ variant)</u> GGTCAGTACGTCCGCCATAGTTGCGCCCCGTAAATTAGATCTTCC AGTGG	IDT	#11533

<u>ePEC non-template DNA (usFJ+DSDNA variant)</u> GGTCAGTACGTCCGCCATAGTTGCGGGCGTAAATTAGATCTTCC AGTGG	IDT	#11534
<u>ePEC template DNA (usFJ+DSDNA variant)</u> CCACTGGAAGATCTGAATTACGACCGCAACTATGGCGGACGTAC TGACC	IDT	#11535
<u>ePEC non-template DNA (Hyb+DSDNA variant)</u> GGTCAGTACGTCCGGCATAGTTGCGGGCGTAAATTAGATCTTCC AGTGG	IDT	#11178
<u>ePEC non-template DNA (dsFJ+DSDNA variant)</u> GGTCAGTACGTCCGGCATAGTTGCGGGCGTAAATTAGATCTTCC AGTGG	IDT	#11537
<u>ePEC non-template DNA (Double usFJ variant)</u> GGTCAGTACGTCCCGTCATAGTTGCGCCCCGTAAATTAGATCTTCC AGTGG	IDT	#10800
<u>ePEC template DNA (Double usFJ variant)</u> CCACTGGAAGATCTGAATTACGCGCGCAACTATGACGGACGTA CTGACC	IDT	#10801
<u>ePEC RNA (Double usFJ variant)</u> UUUUUUUUUCAUAGUU	IDT	#10798
<u>hisPEC1 non-template DNA</u> GCGTCCTATCGATCTTCGGAAGAGATTAGAG	IDT	#10924
<u>hisPEC1 template DNA</u> CTCTGAATCTCTTCAGCACACATCAGGACGC	IDT	#10919
<u>hisPEC1 RNA (pause -2 RNA)</u> UCAUCCGGCGAUGUGUG	IDT	#6593
<u>hisPEC1 exit-channel duplex RNA</u> CCGGAUGA	IDT	#6594
<u>Control EC2, hisPEC2 non-template DNA</u> GGTCAGTACGTCCCTGTCGATCTTCGGAAGAGAATTAGATCTTCC AGTGG	IDT	#8847
<u>Control EC2 template DNA</u> CCACTGGAAGATCTGAATTCTCTTCCCCTAGCTCAGGACGTAC TGACC	IDT	#8848
<u>Control EC2 RNA</u> UUUUUUUGAGCUAGAGG	IDT	#8855
<u>hisPEC2 template DNA</u> CCACTGGAAGATCTGAATTCTCTTCCAGCACACATCAGGACGTAC TGACC	IDT	#8851
<u>hisPEC2 RNA (pause RNA)</u> CCUGACUAGUCUUUCAGGCGAUGUGUGCU	IDT	#4865

Plasmids	Source	Lab Stock #
<u>pRM756</u> RNAP($\alpha_2\beta\beta'\omega$) overexpression plasmid. His ₁₀ -ppx tag at <i>rpoC</i> -C-terminus	Windgassen <i>et al.</i> , 2014	#2956
<u>pRM843</u> RNAP($\alpha_2\beta\beta'\omega$) overexpression plasmid. HMK-Strep tag at <i>rpoC</i> -C-terminus, <i>rpoB</i> -His ₁₀ -ppx.	Hein <i>et al.</i> , 2014	#5143
<u>pRM1151</u> Derivative of pRM843. RNAP($\alpha_2\beta\beta'\omega$) overexpression plasmid containing <i>rpoB</i> substitution R542A.	This work	#5451
<u>pRM 1147</u> Derivative of pRM756. RNAP($\alpha_2\beta\beta'\omega$) overexpression plasmid containing <i>rpoC</i> substitution K334A	This work	#5447
<u>pDJ115</u> RNAP($\alpha_2\beta\beta'\omega$) overexpression plasmid containing β' substitutions I937C- P1139C with His ₁₀ and HMK tags at β' C-terminus	Nayak <i>et al.</i> , 2013	#4954
<u>pDJ116</u> RNAP($\alpha_2\beta\beta'\omega$) overexpression plasmid containing β' substitutions I937C- T1135C with His ₁₀ and HMK tags at β' C-terminus	Nayak <i>et al.</i> , 2013	#4955
<u>pDJ124</u> RNAP($\alpha_2\beta\beta'\omega$) overexpression plasmid containing β' substitution I937C- Q736C with His ₁₀ and HMK tags on the β' C-terminus	Nayak <i>et al.</i> , 2013	#4963
<u>pDJ129</u> RNAP($\alpha_2\beta\beta'\omega$) overexpression plasmid containing β' substitution I937C- G687C with His ₁₀ and HMK tags on the β' C-terminus	Nayak <i>et al.</i> , 2013	#4968
<u>pRM950</u> RNAP($\alpha_2\beta\beta'\omega$) overexpression plasmid containing substitutions $\beta'258iC$, $\beta1045iC$, and $\beta 843C$. HMK-Strep tag at β' C-terminus, His ₁₀ -precision protease tag at β N-terminus.	Kang <i>et al.</i> , 2018a	#5250
<u>pRM918</u> RNAP($\alpha_2\beta\beta'\omega$) overexpression plasmid containing substitutions $\beta'258iC$, $\beta1044C$. HMK-Strep tag at β' C-terminus, His ₁₀ -precision protease tag at β N terminus	Hein <i>et al.</i> , 2014	#5218

X , 6-Methylisoxanthopterin.