

## SUPPLEMENTAL INFORMATION FOR

# Genomic biases in the anaerobic fungal mevalonate pathway lead to heterologous toxicity underpredicted by codon adaptation indices

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Table S1 – Codon usage table for anaerobic fungus *Piromyces sp. UH3-1*. %Usage represents overall usage of the codon relative to the total of all codons used for all genes (coding sequences, CDS). Ratio = relative usage based on the frequency that a codon is used for the related amino acid.

	CODON	AMINO ACID	% USAGE	RATIO	CODON	AMINO ACID	% USAGE	RATIO	CODON	AMINO ACID	% USAGE	RATIO	CODON	AMINO ACID	% USAGE	RATIO	
U	UUU	Phe (F)	3.0%	0.71	UCU	Ser (S)	2.3%	0.28	UAU	Tyr (Y)	3.8%	0.82	UGU	Cys (C)	1.4%	0.86	U
	UUC	Phe (F)	1.1%	0.29	UCC	Ser (S)	0.7%	0.08	UAC	Tyr (Y)	0.8%	0.18	UGC	Cys (C)	0.2%	0.14	C
	UUA	Leu (L)	4.9%	0.60	UCA	Ser (S)	2.6%	0.30	UAA	STOP	0.1%	0.80	UGA	STOP	0.0%	0.09	A
	UUG	Leu (L)	0.7%	0.09	UCG	Ser (S)	0.2%	0.03	UAG	STOP	0.0%	0.11	UGG	Trp (W)	0.8%	1.00	G
C	CUU	Leu (L)	1.7%	0.22	CCU	Pro (P)	0.9%	0.27	CAU	His (H)	1.4%	0.86	CGU	Arg (R)	0.8%	0.26	U
	CUC	Leu (L)	0.2%	0.03	CCC	Pro (P)	0.1%	0.05	CAC	His (H)	0.2%	0.14	CGC	Arg (R)	0.0%	0.01	C
	CUA	Leu (L)	0.4%	0.05	CCA	Pro (P)	2.3%	0.65	CAA	Gln (Q)	3.0%	0.91	CGA	Arg (R)	0.2%	0.06	A
	CUG	Leu (L)	0.1%	0.01	CCG	Pro (P)	0.1%	0.03	CAG	Gln (Q)	0.3%	0.09	CGG	Arg (R)	0.0%	0.01	G
A	AUU	Ile (I)	5.4%	0.62	ACU	Thr (T)	2.6%	0.48	AAU	Asn (N)	9.0%	0.88	AGU	Ser (S)	2.3%	0.27	U
	AUC	Ile (I)	0.7%	0.09	ACC	Thr (T)	0.8%	0.15	AAC	Asn (N)	1.3%	0.12	AGC	Ser (S)	0.3%	0.03	C
	AUA	Ile (I)	2.7%	0.29	ACA	Thr (T)	1.8%	0.32	AAA	Lys (K)	7.0%	0.75	AGA	Arg (R)	1.9%	0.60	A
	AUG	Met (M)	2.0%	1.00	ACG	Thr (T)	0.2%	0.05	AAG	Lys (K)	2.0%	0.25	AGG	Arg (R)	0.2%	0.06	G
G	GUU	Val (V)	2.6%	0.57	GCU	Ala (A)	2.1%	0.55	GAU	Asp (D)	5.3%	0.90	GGU	Gly (G)	2.4%	0.58	U
	GUC	Val (V)	0.4%	0.09	GCC	Ala (A)	0.5%	0.14	GAC	Asp (D)	0.6%	0.10	GGC	Gly (G)	0.2%	0.05	C
	GUA	Val (V)	1.3%	0.28	GCA	Ala (A)	1.1%	0.28	GAA	Glu (E)	6.7%	0.91	GGA	Gly (G)	1.4%	0.34	A
	GUG	Val (V)	0.2%	0.06	GCG	Ala (A)	0.1%	0.03	GAG	Glu (E)	0.5%	0.09	GGG	Gly (G)	0.1%	0.03	G
	U				C				A				G				

Table S2 – Codon usage table for *E. coli*. %Usage represents overall usage of the codon relative to the total of all codons used for all genes (coding sequences, CDS). Ratio = relative usage based on the frequency that a codon is used for the related amino acid.

	CODON	AMINO ACID	% USAGE	RATIO	CODON	AMINO ACID	% USAGE	RATIO	CODON	AMINO ACID	% USAGE	RATIO	CODON	AMINO ACID	% USAGE	RATIO	
U	UUU	Phe (F)	1.9%	0.51	UCU	Ser (S)	1.1%	0.19	UAU	Tyr (Y)	1.6%	0.53	UGU	Cys (C)	0.4%	0.43	U
	UUC	Phe (F)	1.8%	0.49	UCC	Ser (S)	1.0%	0.17	UAC	Tyr (Y)	1.4%	0.47	UGC	Cys (C)	0.6%	0.57	C
	UUA	Leu (L)	1.0%	0.11	UCA	Ser (S)	0.7%	0.12	UAA	STOP	0.2%	0.62	UGA	STOP	0.1%	0.30	A
	UUG	Leu (L)	1.1%	0.11	UCG	Ser (S)	0.8%	0.13	UAG	STOP	0.03%	0.09	UGG	Trp (W)	1.4%	1.00	G
C	CUU	Leu (L)	1.0%	0.10	CCU	Pro (P)	0.7%	0.16	CAU	His (H)	1.2%	0.52	CGU	Arg (R)	2.4%	0.42	U
	CUC	Leu (L)	0.9%	0.10	CCC	Pro (P)	0.4%	0.10	CAC	His (H)	1.1%	0.48	CGC	Arg (R)	2.2%	0.37	C
	CUA	Leu (L)	0.3%	0.03	CCA	Pro (P)	0.8%	0.20	CAA	Gln (Q)	1.3%	0.31	CGA	Arg (R)	0.3%	0.05	A
	CUG	Leu (L)	5.2%	0.55	CCG	Pro (P)	2.4%	0.55	CAG	Gln (Q)	2.9%	0.69	CGG	Arg (R)	0.5%	0.08	G
A	AUU	Ile (I)	2.7%	0.47	ACU	Thr (T)	1.2%	0.21	AAU	Asn (N)	1.6%	0.39	AGU	Ser (S)	0.7%	0.13	U
	AUC	Ile (I)	2.7%	0.46	ACC	Thr (T)	1.6%	0.43	AAC	Asn (N)	2.6%	0.61	AGC	Ser (S)	1.5%	0.27	C
	AUA	Ile (I)	0.4%	0.07	ACA	Thr (T)	1.4%	0.30	AAA	Lys (K)	3.8%	0.76	AGA	Arg (R)	0.2%	0.04	A
	AUG	Met (M)	2.6%	1.00	ACG	Thr (T)	1.3%	0.23	AAG	Lys (K)	1.2%	0.24	AGG	Arg (R)	0.2%	0.03	G
G	GUU	Val (V)	2.0%	0.29	GCU	Ala (A)	1.8%	0.19	GAU	Asp (D)	3.3%	0.59	GGU	Gly (G)	2.8%	0.38	U
	GUC	Val (V)	1.4%	0.20	GCC	Ala (A)	2.3%	0.25	GAC	Asp (D)	2.3%	0.41	GGC	Gly (G)	3.0%	0.40	C
	GUA	Val (V)	1.2%	0.17	GCA	Ala (A)	2.1%	0.22	GAA	Glu (E)	4.4%	0.70	GGA	Gly (G)	0.7%	0.09	A
	GUG	Val (V)	2.4%	0.34	GCG	Ala (A)	3.2%	0.34	GAG	Glu (E)	1.9%	0.30	GGG	Gly (G)	0.9%	0.13	G
	U				C				A				G				

Table S3 – Codon usage table for *S. cerevisiae*. %Usage represents overall usage of the codon relative to the total of all codons used for all genes (coding sequences, CDS). Ratio = relative usage based on the frequency that a codon is used for the related amino acid.

	CODON	AMINO ACID	% USAGE	RATIO	CODON	AMINO ACID	% USAGE	RATIO	CODON	AMINO ACID	% USAGE	RATIO	CODON	AMINO ACID	% USAGE	RATIO	
U	UUU	Phe (F)	2.6%	0.59	UCU	Ser (S)	2.3%	0.26	UAU	Tyr (Y)	1.9%	0.56	UGU	Cys (C)	0.8%	0.63	U
	UUC	Phe (F)	1.8%	0.41	UCC	Ser (S)	1.4%	0.16	UAC	Tyr (Y)	1.5%	0.44	UGC	Cys (C)	0.5%	0.37	C
	UUA	Leu (L)	2.6%	0.28	UCA	Ser (S)	1.9%	0.21	UAA	STOP	0.1%	0.47	UGA	STOP	0.1%	0.30	A
	UUG	Leu (L)	2.7%	0.29	UCG	Ser (S)	0.9%	0.10	UAG	STOP	0.05%	0.23	UGG	Trp (W)	1.0%	1.00	G
C	CUU	Leu (L)	1.2%	0.13	CCU	Pro (P)	1.4%	0.31	CAU	His (H)	1.4%	0.64	CGU	Arg (R)	0.6%	0.14	U
	CUC	Leu (L)	0.5%	0.06	CCC	Pro (P)	0.7%	0.15	CAC	His (H)	0.8%	0.36	CGC	Arg (R)	0.3%	0.06	C
	CUA	Leu (L)	1.3%	0.14	CCA	Pro (P)	1.8%	0.42	CAA	Gln (Q)	2.7%	0.69	CGA	Arg (R)	0.3%	0.07	A
	CUG	Leu (L)	1.0%	0.11	CCG	Pro (P)	0.5%	0.12	CAG	Gln (Q)	1.2%	0.31	CGG	Arg (R)	0.2%	0.04	G
A	AUU	Ile (I)	3.0%	0.46	ACU	Thr (T)	2.0%	0.35	AAU	Asn (N)	3.6%	0.59	AGU	Ser (S)	1.4%	0.16	U
	AUC	Ile (I)	1.7%	0.26	ACC	Thr (T)	1.3%	0.22	AAC	Asn (N)	2.5%	0.41	AGC	Ser (S)	1.0%	0.11	C
	AUA	Ile (I)	1.8%	0.27	ACA	Thr (T)	1.8%	0.30	AAA	Lys (K)	4.2%	0.58	AGA	Arg (R)	2.1%	0.48	A
	AUG	Met (M)	2.1%	1.00	ACG	Thr (T)	0.8%	0.14	AAG	Lys (K)	3.1%	0.42	AGG	Arg (R)	0.9%	0.21	G
G	GUU	Val (V)	2.2%	0.39	GCU	Ala (A)	2.1%	0.38	GAU	Asp (D)	3.8%	0.65	GGU	Gly (G)	2.4%	0.47	U
	GUC	Val (V)	1.2%	0.21	GCC	Ala (A)	1.3%	0.22	GAC	Asp (D)	2.0%	0.16	GGC	Gly (G)	1.0%	0.19	C
	GUA	Val (V)	1.2%	0.21	GCA	Ala (A)	1.6%	0.29	GAA	Glu (E)	4.6%	0.70	GGA	Gly (G)	1.1%	0.22	A
	GUG	Val (V)	1.1%	0.19	GCG	Ala (A)	0.6%	0.11	GAG	Glu (E)	1.9%	0.30	GGG	Gly (G)	0.6%	0.12	G
	U				C				A				G				

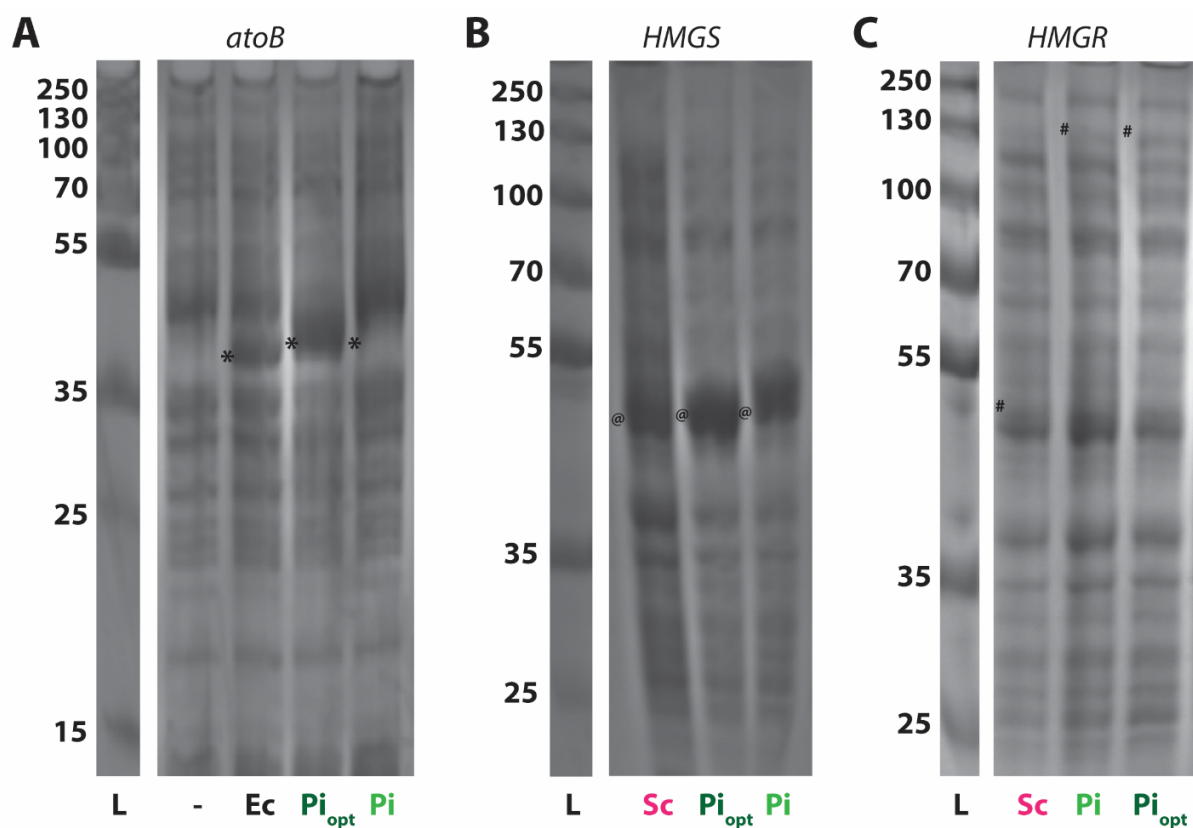


Figure S1 – SDS-PAGE analysis of mevalonate homologs. A) *atoB*, B) *HMGS*, and C) *HMGR* homologs from *E. coli* (Ec), *S. cerevisiae* (Sc), *P. indiana* (Pi) and the *E. coli*-optimized *P. indiana* (Pi<sub>opt</sub>) gene; \* shows the expected sizes/location of the Ec and Pi *atoB* homologs (40.3 and 43.9 kDa, respectively), @ shows the expected sizes/location of the Sc and Pi *HMGS* homologs (51.5 and 52.3 kDa, respectively), and # show the expected sizes/location of the Sc and Pi *HMGR* homologs (53.1 and 112.1 kDa, respectively). L indicates the Page Ruler PLUS ladder and “-” indicates the *E. coli* host control with an uninduced vector. Whole cell lysates of actively growing cultures were analyzed ~ 2 hours after induction and loading was relativized to OD<sub>600</sub>.

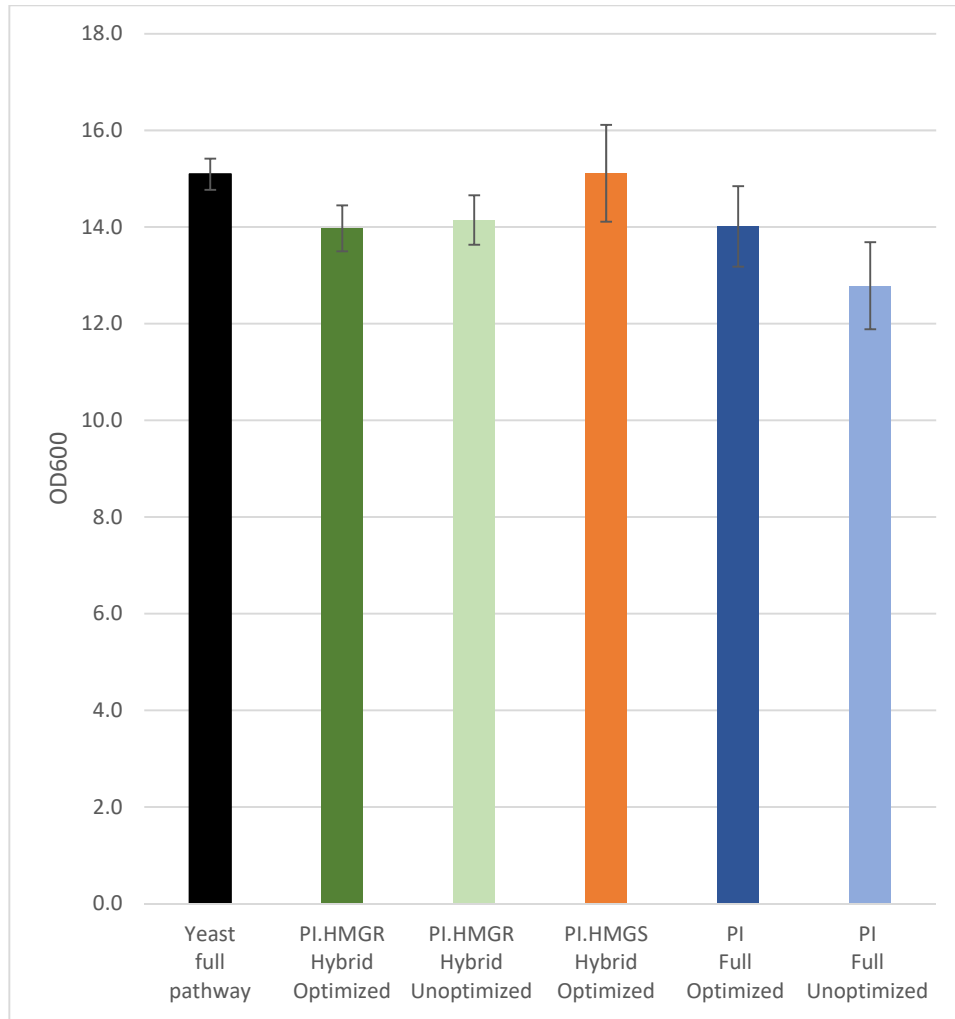


Figure S2: OD of 50 ml cultures containing various mevalonate pathways after 20 hours of growth. Yeast pathway = the *S. cerevisiae* from Martin et al [236]. Full PI pathways = genes from *P. indiana* (Figure 3B) either native or optimized for *E. coli*. Hybrid pathways use the yeast construct and swap the indicated gene for the original yeast homolog. All pathways evaluated with *atoB<sub>H9</sub>-HMGS<sub>C4</sub>-HMGR<sub>H9</sub>* promoter organization. Error bars = standard deviation.

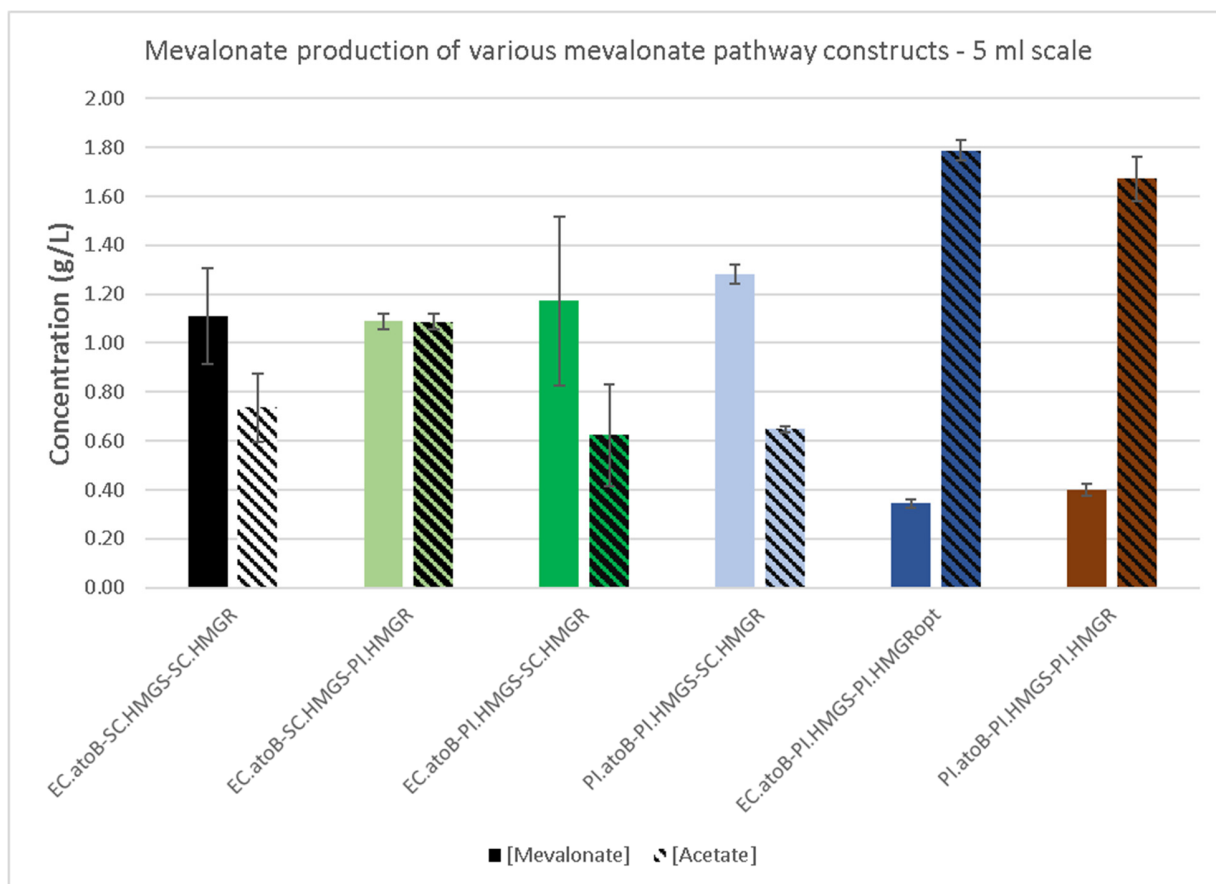


Figure S3: Mevalonate and acetate titers of original and hybrid mevalonate pathways at 5 ml scale. Mevalonate production (solid) and acetate accumulation (striped) from various mevalonate pathway hybrids containing Martin et al (black) or *E. coli*-codon-optimized *P. indiana* genes (various colors, see Figure 6-7) after 20 hrs of culture. All pathways are configured in the high-producing h9-promoter configuration. Errors bars represent standard deviation.

Table S4 – Oligos used in this study

<b>Name</b>	<b>Sequence 5'→3'</b>	<b>Purpose</b>
2.3.1.9_FWD_XhoI	TGGT <u>ctcgag</u> <b>ATG</b> ACTCTTCAAAAAGATGTTTACATTGTTTCCGC	pETM6-Pi.atoB construction
2.3.1.9_Rev_BcuI	TGGT <u>actagt</u> <u>cccggg</u> yTAAAGAACTTCAATAACGACAGATGAGGC	
2.3.3.10_fwd_XmaI	TGGT <u>ctcgag</u> <u>cccggg</u> <b>ATGA</b> ArGCTAATAAAyrTTGGTATTGTT	pETM6-Pi.HMGS construction
2.3.3.10_REV_NotI	TGGT <u>actagt</u> <u>gcccgc</u> cTTATTGrTATmTTTCATArGTTCTrCG	
1.1.1.88_fwd-BglII	TGGT <u>agatctc</u> <b>ATG</b> ATGACGACAGTACCwATAGAAG	pETM6-Pi.HMGR construction
1.1.1.88_rev-XhoI	TGGT <u>ctcgag</u> TTAAGTATTTTCGAGAACGTCCTTTGAG	
M13F	TGTAAAACGACGGCCAGT	pETM6-Pi.atob, HMGS, & HMGR opt gBLOCK amplification for construction
M13R	CAGGAAACAGCTATGAC	
T7_pro_seq	<u>gtgatgtcggcgatatagg</u>	Promoter Sequencing primer

Start sites bolded, Restrictions sites underlined

Table S5 – *E. coli* strains used in this study

<b>Name</b>	<b>Relevant genotype</b>	<b>Source</b>
DH5a	F– endA1 glnV44 thi-1 recA1 relA1 gyrA96 deoR nupG purB20 φ80dlacZΔM15 Δ(lacZYA-argF)U169, hsdR17(rK–mK+), λ–	ThermoFisher
BL21	F– ompT gal dcm lon hsdSB(rB–mB–) λ(DE3 [lacI lacUV5- T7p07 ind1 sam7 nin5]) [malB+]K-12(λS)	Invitrogen



BL21 CodonPlus RIPL	F- ompT hsdS(rB – mB – ) dcm+ Tetr gal $\lambda$ (DE3) endA Hte [argU proL Camr ] [argU ileY leuW Strep/Spectr	Agilent Technologies
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Table S6 – Gene sequences used in this study.

<b>Sc.atoB* Silent mutation of NdeI for pETM6 subcloning</b>
ATGAAAAATTGTGTCATCGTCAGTGCGGTACGTACTGCTATCGGTAGTTTTAACGGTT CACTCGCTTCCACCAGCGCCATCGACCTGGGGGCGACAGTAATTAAGCCGCCATTG AACGTGCAAAAATCGATTCACAACACGTTGATGAAGTGATTATGGGTAACTGTTAC AAGCCGGGCTGGGGCAAAATCCGGCGCGTCAGGCACTGTTAAAAAGCGGGCTGGCA GAAACGGTGTGCGGATTCACGGTCAATAAAGTATGTGGTTCGGGTCTTAAAAGTGTG GCGCTTGCCGCCAGGCCATTCAGGCAGGTCAGGCGCAGAGCATTGTGGCGGGGGGT ATGGAAAATATGAGTTTAGCCCCCTACTTACTCGATGCAAAAGCACGCTCTGGTTATC GTCTTGAGACGGACAGGTTTATGACGTAATCCTGCGCGATGGCCTGATGTGCGCCA CCCATGGTTATCACATGGGGATTACCGCCGAAAACGTGGCTAAAGAGTACGGAATTA CCCGTGAAATGCAGGATGAACTGGCGCTACATTCACAGCGTAAAGCGGCAGCCGCA ATTGAGTCCGGTGCTTTTACAGCCGAAATCGTCCCGGTAAATGTTGTCACTCGAAAGA AAACCTTCGTCTTCAGTCAAGACGAATTCCCGAAAGCGAATTCAACGGCTGAAGCGT TAGGTGCATTGCGCCCGGCCTTCGATAAAGCAGGAACAGTCACCGCTGGGAACGCGT CTGGTATTAACGACGGTGCTGCCGCTCTGGTGATTATGGAAGAATCTGCGGCGCTGGC AGCAGGCCTTACCCCCCTGGCTCGCATTAAAAGTTATGCCAGCGGTGGCGTGCCCCC CGCATTGATGGGTATGGGGCCAGTACCTGCCACGCAAAAAGCGTTACAACCTGGCGGG GCTGCAACTGGCGGATATTGATCTCATTGAGGCTAATGAAGCATTGCTGCACAGTTC CTTGCCGTGGGAAAAACCTGGGCTTTGATTCTGAGAAAGTGAATGTCAACGGCGGG GCCATCGCGCTCGGGCATCCTATCGGTGCCAGTGGTGCTCGTATTCTGGTCACACTAT TACATGCCATGCAGGCACGCGATAAAACGCTGGGGCTGGCAACACTGTGCATTGGCG GCGGTCAGGGAATTGCGATGGTGATTGAACGGTTGAATTAA
<b>Sc.HMGS</b>
ATGACTGAACTAAAAAAACAAAAGACCGCTGAACAAAAAACAGACCTCAAAATGT CGGTATTAAGGTATCCAAATTTACATCCCAACTCAATGTGTCAACCAATCTGAGCTA GAGAAATTTGATGGCGTTTCTCAAGGTAAATACACAATTGGTCTGGGCCAAACCAAC ATGTCTTTTGTCAATGACAGAGAAGATATCTACTCGATGTCCCTAACTGTTTTGTCTAA GTTGATCAAGAGTTACAACATCGACACCAACAAAATTGGTAGATTAGAAGTCGGTAC TGAAACTCTGATTGACAAGTCCAAGTCTGTCAAGTCTGTCTTGATGCAATTGTTTGGT GAAAACACTGACGTCGAAGGTATTGACACGCTTAATGCCTGTTACGGTGGTACCAAC GCGTTGTTCAACTCTTTGAACTGGATTGAATCTAACGCATGGGATGGTAGAGACGCCA TTGTAGTTTGCGGTGATATTGCCATCTACGATAAGGGTGCCGCAAGACCAACCGGTG GTGCCGGTACTGTTGCTATGTGGATCGGTCCTGATGCTCCAATTGTATTTGACTCTGTA AGAGCTTCTTACATGGAACACGCCTACGATTTTTACAAGCCAGATTTACCAGCGAAT ATCCTTACGTCGATGGTCATTTTTTATTAACTTGTTACGTCAAGGCTCTTGATCAAGTT TACAAGAGTTATTCCAAGAAGGCTATTTCTAAAGGGTTGGTTAGCGATCCCGCTGGTT CGGATGCTTTGAACGTTTTGAAATATTTGACTACAACGTTTTCCATGTTCCAACCTGT AAATTGGTCACAAAATCATACGGTAGATTACTATATAACGATTTTACAGAGCCAATCCT CAATTGTTCCAGAAAGTTGACGCCGAATTAGCTACTCGCGATTATGACGAATCTTTAA CCGATAAGAACATTGAAAAAACTTTTGTTAATGTTGCTAAGCCATTCCACAAAGAGA GAGTTGCCCAATCTTTGATTGTTCCAACAAACACAGGTAACATGTACACCGCATCTGT

TTATGCCGCCTTTGCATCTCTATTAAACTATGTTGGATCTGACGACTTACAAGGCAAG  
CGTGTTGGTTTATTTTCTTACGGTTCCGGTTTAGCTGCATCTCTATATTCTTGCAAAATT  
GTTGGTGACGTCCAACATATTATCAAGGAATTAGATATTACTAACAAATTAGCCAAG  
AGAATCACCGAAACTCCAAAGGATTACGAAGCTGCCATCGAATTGAGAGAAAATGC  
CCATTTGAAGAAGAAGCTTCAAACCTCAAGGTTCCATTGAGCATTTGCAAAGTGGTGTT  
TACTACTTGACCAACATCGATGACAAATTTAGAAGATCTTACGATGTTAAAAAATAA

**Sc.HMGR\* - Silent mutation of NdeI for pETM6 subcloning**

ATGGTTTTAACCAATAAAACAGTCATTTCTGGATCGAAAGTCAAAAGTTTATCATCTG  
CGCAATCGAGCTCATCAGGACCTTCATCATCTAGTGAGGAAGATGATTCCCGCGATA  
TTGAAAGCTTGGATAAGAAAATACGTCCTTTAGAAGAATTAGAAGCATTATTAAGTA  
GTGGAAATACAAAACAATTGAAGAACAAAGAGGTGCTGCCTTGGTTATTCACGGTA  
AGTTACCTTTGTACGCTTTGGAGAAAAAATTAGGTGATACTACGAGAGCGGTTGCGGT  
ACGTAGGAAGGCTCTTTCAATTTTGGCAGAAGCTCCTGTATTAGCATCTGATCGTTTA  
CCATATAAAAATTATGACTACGACCGCGTATTTGGCGCTTGTGTGAAAATGTTATAG  
GTTACATGCCTTTGCCCCGTTGGTGTTATAGGCCCTTGGTTATCGATGGTACATCTTAT  
CATATACCAATGGCAACTACAGAGGGTTGTTTGGTAGCTTCTGCCATGCGTGGCTGTA  
AGGCAATCAATGCTGGCGGTGGTGCAACAACCTGTTTTAACTAAGGATGGTATGACAA  
GAGGCCCAGTAGTCCGTTTCCCAACTTTGAAAAGATCTGGTGCCTGTAAGATATGGTT  
AGACTCAGAAGAGGGGACAAAACGCAATTAAAAAAGCTTTTAACTCTACATCAAGAT  
TTGCACGTCTGCAACATATTCAAACCTGTCTAGCAGGAGATTTACTCTTCATGAGATTT  
AGAACAACACTACTGGTGACGCAATGGGTATGAATATGATTTCTAAAGGTGTCGAATAC  
TCATTAAAGCAAATGGTAGAAGAGTATGGCTGGGAAGATATGGAGGTTGTCTCCGTT  
TCTGGTAACTACTGTACCGACAAAAAACCAGCTGCCATCAACTGGATCGAAGGTCGT  
GGTAAGAGTGTGCTCGCAGAAGCTACTATTCCTGGTGATGTTGTCAGAAAAGTGTTAA  
AAAGTGATGTTTCCGCATTGGTTGAGTTGAACATTGCTAAGAATTTGGTTGGATCTGC  
AATGGCTGGGTCTGTTGGTGGATTAAACGCACATGCAGCTAATTTAGTGACAGCTGTT  
TTCTTGGCATTAGGACAAGATCCTGCACAAAATGTTGAAAGTTCCAACCTGTATAACAT  
TGATGAAAGAAGTGGACGGTGATTTGAGAATTTCCGTATCCATGCCATCCATCGAAG  
TAGGTACCATCGGTGGTGGTACTGTTCTAGAACCACAAGGTGCCATGTTGGACTTATT  
AGGTGTAAGAGGCCCCGCATGCTACCGCTCCTGGTACCAACGCACGTCAATTAGCAAG  
AATAGTTGCCTGTGCCGTCTTGGCAGGTGAATTATCCTTATGTGCTGCCCTAGCAGCC  
GGCCATTTGGTTCAAAGTCACATGACCCACAACAGGAAACCTGCTGAACCAACAAA  
ACCTAACAATTTGGACGCCACTGATATAAATCGTTTGAAAGATGGGTCCGTCACCTGC  
ATTAAATCCTAA

**Pi.atoB**

ATGACTCTTCAAAAAGATGTTTACATTGTTTCCGCTGTTTCGTA CTCCAATTGGTGGTTT  
AAGTGGTTCTTTAGCTCAATTTAGTGCTACTCAACTTGGTAGCATTGCTATCAAAGGT  
GCTTTAGAAAAGGCCAATGTTAAGCCAGAAGAAGTTCAAGAAGTATTCTTCGGAAAT  
GTTTTAACTGCTAACCTTGGTCAAAACCCAGCTCGTCAAGCTGCTTTAGGTGCTGGTA  
TTCCAAACACTGTTGTTTGTACCACTGTTAACAAGGTTTGTGCTTCCGCTATGAAGGCC  
ACTATTCTTGGTGCTCAAACCTATTATTTTAGGTGAAAATGATTTAGTTGTTGTAGGTGG  
ACAAGAATCTATGACTAACAACCTCCATACTACATTCCAAAAGCTAGAGCTGGTTGTCTG  
TTACGGTAACCAACAAATTGTTGATGGTATCATTCAAGATGGTCTTTACGATGTTTAC

AACCAATATCAAATGGGTGTTGCTGCTGATGCTACTGCTGCTGAATACAATATCACTA  
GAAATGATCAAGATGATTTTCGCTATTAGAAGTTACAAACTTTCCCAAGAATCCAATG  
CTAACGGTTATGTTGTTCGATGAAATTATTCCAGTTGAAATTCCAGCCACTAAGAGAAC  
CCCAGCTTCTACTGTTACTACTGATGATGAAATTAACCATCTTAATGAAGCCAAGTTA  
AGAGCTGTTAGACCAGCTTTCGTCACTGATGGTACTGTTACTGCTCCAAATGCTTCTA  
CTATTAGTGATGGTGCTGCTGCTCTTGTCTTAGCTTCTAAGGAAAAGGTTGAAGCTTTA  
GGTTTAAAGCCAATTGCTAAGATTATCGGTTGGGGTGATGCTGCTCATGATCCAGCTC  
ACTTCACTACTGCTCCATCTTTAGCTATTCCAAAGGCTTTAAAGCATGCTAACAAAAC  
TCAAGATGAAATTGATTTCTTTGAAATTAATGAAGCTTTCGCTGTTGTTTCCCTTGTTA  
ACAGTAAGATTTTAAATATTCCAATTGAAAAATTAAATGTCTTTGGTGGTGCTTGTAG  
TATGGGTCACCCACTTGGTTGTTCCGGTGCTAGAATTATTGTTACTTTAATCAATGTCT  
TAAAGAAGAAGGGTGGTAAGCTTGGTTGTGCTGCTATCTGTAATGGTGGTGGTGGTGC  
CTCATCTGTCGTTATTGAAGTTCTTAACCCGGACTAG

#### **Pi.HMGS**

ATGAAGGCTAATAATGTTGGTATTGTTGCTATGGATATTTACTTCCCAAAACAATACG  
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TTTAGGTCAAACCAGAATGGCTTTCTGTGATGATAGAGAAGATATTAAGTCTGTTTGT  
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GATTAGAAGTCGGTACTGAAACTATTATTGATAAGTCTAAATCAGTCAAGTCTACTTT  
AATGCAACTTTTCGCTGAATCTGGAAATACTGATATTGAAGGTATTGATACCACTAAC  
GCTTGTTATGGTGGTACTAACGCTTTATTCAACACTGTAACTGGATGGAATCATCTGC  
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ATGCTAGACCAACCGGTGGTTGTGGTGTGTTGTGCTTTATTAATTGGTCCAAACGCTCC  
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TGGTGAAAAGATTGATATTGAAACCGTTCCATACTTTGTCTTCCACACTCCATACTCTA  
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AGCAAGTTATTCCATCATTATACATTCCAAAGAACTGTGGTAACATGTACTGTGGTTC  
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GTCATGAATTCTACTGCTGATATTGCTAAGACCTTAAATATTAAGGAAAGATTAGAAA  
GCAGAAATGAAGTCAAACCAGAAGAATTCGAAAAGATCATGTCTTTAAGAGAAAAG  
ACTTACCAACTTAAGGATTACACTCCAGTTAGTAAGACTGAAATGTTCCCAGGTACTT  
ACTATCTTAAGCACGTCGATGAAAAATTCCGCAGAACCTATGAAAGATATCAATAA

#### **Pi.HMGR**

ATGATGACGACAGTACCTATAGAAGTTAAAACACAAACAGAACAAAAAGTAAATAT  
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ATTTCCAAATCTTTTCCACCTACTTCACCACTTATATCTAAAGCTTCTTTTCTTCATTC  
ACCATCCTCAGGATCTGTATCAAATTTAACTGAATCAGCTTCAGATGTTTCAATTCAC  
CGTAATGAATCTACATCAGATTTAAGCAGTTTAGGTGTTTATGAAGAAAAGTTTCCAA

TTACTTCATTGGATGAACATATTGCTGATAATATGATTGAAAATTGTGTTGGTACTCTT  
GGATTGCCAGTTGGTGTGCTTTAAACTTCAATATTGATAGTAAACCAATTATAGTTCC  
AATGGCTATCGAAGAACCATCAGTAGTAGCAGCTGTTTCTGGTGCTGCCAAAACAGT  
AGCACAATTCGGTAAAGGAAAAACATTTTATACCAAACTTCTGAAAGAAATATTAC  
TTTTGCCCAAGTGGTTATTTTAGATATTCCGGATAATCGCTTAGATGAAAATGAGAAG  
AAGTTAAACGATATGAAAAATGAAATTATTACTTACGCTAATCAATATTGTCAAAAT  
ATGTATTCAAGGGGTGGTGGTGCTGTAATTTAGTAGTTCGTAGAGTTAAGAAGAAC  
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TATGTCATTTTCATATTGATGTTTGTGATGCTATGGGTGCAAACGTGTGCTAGTACTGTT  
GCAGAAGGTGTCGCACCATTCCTTGCTGATTTAACAAATGGTCGTATTGGATTAAGAA  
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AATGGTATCGATGCTGTTGCCTTAGCAACTGGTCAAGACTGGAGAGCTATTGAAGCT  
GCTGCACATGCTTATGCTGCTTCTGGTTCTAGTGTAAGTGTAAATGAACCAGTTAAAG  
GTCATTATAAATCTTTAACTAGTTATTGGATGGAAGAAGTTGAAGAAGAAGTAGATA  
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TACTAAGGGTGGTGTATTAAAAACAAATCCAGTTTATCATTACACTTTAGGTCTTATG  
GGTCATCCAGATTCTAAGGCTTTGTCAGCTATTTTTGCTTGTGTTGGTCTTGCTCAAAA  
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GCTCACGAATTACATAGTACTTTGAGAAAGCATTTAGAAGGTTCTGATTCCCAATCAT  
CAAAACCACCAAGTATGTTCTATTTTGAAGAATATGTTCCAGAAGGTGAAGATAAAA  
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TGAATATCCAACCTGGATCAAATGATGATCCAATATCTCAACTTTTATTTGGTAATAAA  
GATTACACATGGATTTTCATCAATGCTTAATGTATTAGATAAATTCCAATTTAGTACAG  
CTTCTCAAGGACGTGCTAACTTTGTTTTAGCAAAAAAATTAAGTCTTATCAATGCT  
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ATTGAAAGAATCTTTAGACATTCAAAGAGACCAAAGTCAAGTACTTCATCATCCTTA  
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TCTGCTAATCAACAACCTTCAAACCAAAATATTCACTTACCTGGTCCAATTTCTGCTC  
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AAGAATATTTTAGATATTGCAGATGATGTTAAAAATTTAAGTGATCCAACCTTAATTC  
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TATAATTAATAATACTGCAAAGGTTCAAGAACCACCTTATCTTAGTTCCTTCAACCGT  
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ATCTTTTGATCCAACCTCTTATTAAGTCCCGTAGAATTAATTTCTTATTAACATTAGGTA  
GTTACTTAGAATGGGAATTAGCTAAGGATCATGATCTTGGTCGTCTAGGACGTGATCT  
TATGCTTGTTCAAAACCAAAATCAAAAAATTAGTCACGATGGTAGTATTGGTAATGGT  
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AATATGTTCCACTTTGAAGATGATGATGATACTGATGATTCTGCTAGTACCCCATCTA  
TATCTAATATAGTTGAATCTGTAGATAATGAACCATACTATCAACGTTATAATGATGA  
AATTAAACAATATTTATTAGAACTGATCATTCACAAATAAAATCACAATTATTTAAT  
ACTGAATCATGTCTTTTATTCTCAAAGGACGTTCTCGAAAATACTTAA

**Pi.atoB-opt**

ATGACTCTCCAGAAAGATGTGTATATTGTTTCCGCGGTGCGTACCCCAATCGGCGGAT  
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GGCTCTGGAAGAGGCCAATGTTAAGCCGGAGGAAGTTCAGGAAGTATTTTTCGGCAA  
TGTGCTGACGGCGAACCTGGGTCAAACCCGCGCGCTCAGGCAGCCCTGGGCGCGG  
GTATTCCAAACACTGTGGTTTGTACCACCGTGAACAAAGTTTTCGCTTCCGCAATGAA  
AGCCACAATCCTGGGCGCGCAGACGATTATCTTGGGTGAGAACGATCTGGTGGTAGT  
AGGGGGACAAGAATCTATGACTAACACACCGTACTATATCCCGAAAGCCCGCGCGG  
GCTGCCGTTACGGTAACCAGCAGATTGTGGACGGCATCATTACAGGATGGCCTTTACG  
ATGTGTACAACCAGTATCAAATGGGGGTTGCAGCTGACGCGACCGCCGCAGAATACA  
ATATCACGCGTAATGACCAGGATGATTTTCGCGATTTCGCAGCTATAAACTGTCCCAGG  
AATCCAATGCTAACGGTTATGTGGTTCGATGAAATCATTCCAGTAGAAATCCCGGCCA  
CTAAACGCACCCAGCGTCTACAGTGACGACCGATGACGAAATTAACCATCTTAACG  
AAGCCAAATTACGTGCAGTTCGCCCCGGCCTTTGTCACTGATGGCACAGTGACGGCGC  
CGAATGCTTCTACCATCAGCGATGGTGCAGCGGCCCTGGTCCTGGCGTCTAAAGAAA  
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CGGTGGTTTCCCTGGTGAACAGCAAAATTTTAAATATCCCGATTGAAAAATTGAATGT  
CTTTGGCGGTGCTTGCAGCATGGGCCACCCGCTGGGGTGTTCCGGTGCACGCATCATT  
GTTACACTGATCAACGTCTTGAAAAAAAAGGGCGGTAAACTGGGCTGCGCGGCCATC  
TGTAATGGTGGCGGCGGTGCCTCATCTGTCGTGATTGAAGTACTTAACCCGGATTAG

**Pi.HMGS-opt**

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CAACAGTGTGTGTCTGACGGTGGTGAAGAGTCTGATGGAAAAGTACAACATCGACTA  
TAACCAAGTTGGCCGTCTGGAAGTGGGCACCGAGACGATCATCGACAAGAGCAAGA  
GCGTGAAGAGCACGCTGATGCAGCTGTTTTCGGAAAGCGGTAACACCGACATCGAA  
GGCATCGACACCACCAACGCGTGCTACGGTGGCACGAACGCGCTGTTCAACACGGTG  
AACTGGATGGAAAGCAGTGCCTGGGATGGTCGCTTCGGTCTGGTGGTTGCGGGCGAT  
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CTGCTGATCGGCCCCGAATGCGCCGATCGTTATGGAACAAGGCGTGCGCGCGACCCAC  
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GACGGCCATCTGAGCAATGTGTGCTATCTGCGCAGCGTGGACATCTGCTACAATCGCT  
ACATCGAGAAAGTGCAGAAGCGTACCGGCGAGAAGATCGATATCGAGACGGTGCCG  
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CCTTTAACGACTTCATCAAGGATCAAGAAAACCCAAAATACGCGGGTCTGGAAGAA

CACAAAGGCAAGACCCTCAGCGAGACCTACTACGATAAGCCGCTGGAGAAAGCCTT  
CATGGCGTACACCAAGGAGAAGTTCCAGAAACAAGTTATTCCGAGTCTCTACATCCC  
GAAGAATTGCGGCAACATGTACTGCGGCAGCGTTTACAGCGCCCTCATCAGTCTGGT  
GAGCCAGATCCCGAGTGAAGAGCTGCTCAACAAGCGCATCGTGCTCTTCAGCTATGG  
TAGCGGTCTGGCGGCCAGCATGTACAGCTTCAAGGTGATGAACAGCACCGCCGACAT  
CGCGAAGACGCTCAACATCAAGGAACGTCTGGAGAGTCGCAACGAAGTTAAGCCGG  
AGGAGTTCGAGAAGATCATGAGTCTCCGCGAGAAGACCTACCAGCTCAAAGACTAC  
ACGCCGGTTAGCAAGACCGAGATGTTTCCGGGCACCTACTATCTGAAGCACGTTGAT  
GAGAAGTTCCGCGCACCTACGAACGTTACCAGTAA

**Pi.HMGR-opt**

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CCCGGATACCGGGATCTTCCGTAAGAATATCCTGGACATTGCGGATGATGTTAAAAA  
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GACCTCGGTGCTGCTGGGACGTGACCTCATGTTGATCCAGAATTACAACAAAAAGGT  
AGCCATGATGGCTCTATTGGTAATGGGATCACAAACAGCTTTATTATCTGGAAAGAG  
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GACAATGAGTCGTACTATCAACGTTATAATGACGAAATCAAACAGTATCTGCTGGAA  
ACTGATCATTCTGAGATTAAATCGCAGCTGTTTAACACGGAGAGCTGCCTTCTGTTCT  
CCAAGGACGTGCTCGAAAACACTATTGAAGAGTACCAGAAGTATTACAATGTGAAA  
AACCTGCTGAACAACCTGAATACGAACTAA



Table S7 – Plasmids used in this study.

<b><u>Name</u></b>	<b><u>Relevant Phenotype</u></b>	<b><u>Plasmid origin of replication</u></b>	<b><u>Source</u></b>
pETM6-T7-mCherry	AmpR, lacO/I, P <sub>T7</sub> mCherry	ColE1 (pBR322)	Xu et al., 2012
pETM6-H9-mCherry	AmpR, lacO/I, P <sub>H9</sub> mCherry	ColE1 (pBR322)	Jones et al., 2015b
pETM6-C4-mCherry	AmpR, lacO/I, P <sub>CA</sub> mCherry	ColE1 (pBR322)	Jones et al., 2015b
pMevT	AmpR, lacO/I, P <sub>lac</sub> -Ec.atoB-Sc.HMGS-Sc.HMGR	p15a	Martin et al, 2003
pETM6-T7-Ec.atoB	AmpR, lacO/I, P <sub>T7</sub> Ec.atoB	ColE1 (pBR322)	This Study
pETM6-H9-Ec.atoB	AmpR, lacO/I, P <sub>H9</sub> Ec.atoB	ColE1 (pBR322)	This Study
pETM6-T7-Sc.HMGS	AmpR, lacO/I, P <sub>T7</sub> Sc.HMGS	ColE1 (pBR322)	This Study
pETM6-C4-Sc.HMGS	AmpR, lacO/I, P <sub>C4</sub> Sc.HMGS	ColE1 (pBR322)	This Study
pETM6-T7-Sc.HMGR	AmpR, lacO/I, P <sub>T7</sub> Sc.HMGR	ColE1 (pBR322)	This Study
pETM6-H9-Sc.HMGR	AmpR, lacO/I, P <sub>H9</sub> Sc.HMGR	ColE1 (pBR322)	This Study
pETM6-H9-Ec.atoB_C4-Sc.HMGS	AmpR, lacO/I, P <sub>H9</sub> Ec.atoB, P <sub>C4</sub> Sc.HMGS	ColE1 (pBR322)	This Study
pETM6-H9-Ec.atoB_C4-Sc.HMGS_H9-Sc.HMGR	AmpR, lacO/I, P <sub>H9</sub> Ec.atoB, P <sub>C4</sub> Sc.HMGS, P <sub>H9</sub> Sc.HMGR	ColE1 (pBR322)	This Study
pETM6-H9-Ec.atoB_C4-Sc.HMGS_H9-Pi.HMGR	AmpR, lacO/I, P <sub>H9</sub> Ec.atoB, P <sub>C4</sub> Sc.HMGS, P <sub>H9</sub> Pi.HMGR	ColE1 (pBR322)	This Study
pETM6-T7-Pi.atoB	AmpR, lacO/I, P <sub>T7</sub> Pi.atoB	ColE1 (pBR322)	This Study
pETM6-H9-Pi.atoB	AmpR, lacO/I, P <sub>H9</sub> Pi.atoB	ColE1 (pBR322)	This Study
pETM6-T7-Pi.HMGS	AmpR, lacO/I, P <sub>T7</sub> Pi.HMGS	ColE1 (pBR322)	This Study
pETM6-C4-Pi.HMGS	AmpR, lacO/I, P <sub>C4</sub> Pi.HMGS	ColE1 (pBR322)	This Study

pETM6-T7-Pi.HMGR	AmpR, lacO/I, P <sub>T7</sub> Pi.HMGR	ColE1 (pBR322)	This Study
pETM6-H9-Pi.HMGR	AmpR, lacO/I, P <sub>H9</sub> Pi.HMGR	ColE1 (pBR322)	This Study
pETM6-T7-9-AGF_T7-10-AGF	AmpR, lacO/I, P <sub>T7</sub> Pi.atoB, P <sub>T7</sub> Pi.HMGS	ColE1 (pBR322)	This Study
pETM6-H9-9-AGF_T7-10-AGF	AmpR, lacO/I, P <sub>H9</sub> Pi.atoB, P <sub>T7</sub> Pi.HMGS	ColE1 (pBR322)	This Study
pETM6-T7-9-AGF_T7-10-AGF_T7-88-AGF	AmpR, lacO/I, P <sub>T7</sub> Pi.atoB, P <sub>T7</sub> Pi.HMGS, P <sub>T7</sub> Pi.HMGR	ColE1 (pBR322)	This Study
pETM6-H9-9-AGF_T7-10-AGF_H9-88-AGF	AmpR, lacO/I, P <sub>H9</sub> Pi.atoB, P <sub>T7</sub> Pi.HMGS, P <sub>H9</sub> Pi.HMGR	ColE1 (pBR322)	This Study
pETM6-T7-Pi.atoB-opt	AmpR, lacO/I, P <sub>T7</sub> Pi.atoB-opt	ColE1 (pBR322)	This Study
pETM6-H9-Pi.atoB-opt	AmpR, lacO/I, P <sub>H9</sub> Pi.atoB-opt	ColE1 (pBR322)	This Study
pETM6-T7-Pi.HMGS-opt	AmpR, lacO/I, P <sub>T7</sub> Pi.HMGS-opt	ColE1 (pBR322)	This Study
pETM6-C4-Pi.HMGS-opt	AmpR, lacO/I, P <sub>C4</sub> Pi.HMGS-opt	ColE1 (pBR322)	This Study
pETM6-T7-Pi.HMGR-opt	AmpR, lacO/I, P <sub>T7</sub> Pi.HMGR-opt	ColE1 (pBR322)	This Study
pETM6-H9-Pi.HMGR-opt	AmpR, lacO/I, P <sub>H9</sub> Pi.HMGR-opt	ColE1 (pBR322)	This Study
pETM6-H9-Ec.atoB_C4-Sc.HMGS_H9-Pi.HMGR-opt	AmpR, lacO/I, P <sub>H9</sub> Ec.atoB, P <sub>C4</sub> Sc.HMGS, P <sub>H9</sub> Pi.HMGR-opt	ColE1 (pBR322)	This Study
pETM6-H9-Pi.atoB-opt_C4-Pi.HMGS-opt_H9-Sc.HMGR	AmpR, lacO/I, P <sub>H9</sub> Pi.atoB, P <sub>C4</sub> Pi.HMGS, P <sub>H9</sub> Sc.HMGR-opt	ColE1 (pBR322)	This Study
pETM6-H9-Pi.atoB-opt_C4-Pi.HMGS-opt_H9-Pi.HMGR-opt	AmpR, lacO/I, P <sub>H9</sub> Pi.atoB, P <sub>C4</sub> Pi.HMGS, P <sub>H9</sub> Pi.HMGR-opt	ColE1 (pBR322)	This Study
pETM6-H9-Ec.atoB_C4-Pi.HMGS-opt_H9-Sc.HMGR	AmpR, lacO/I, P <sub>H9</sub> Ec.atoB, P <sub>C4</sub> Pi.HMGS-opt, P <sub>H9</sub> Sc.HMGR	ColE1 (pBR322)	This Study
pETM6-H9-Ec.atoB_C4-Pi.HMGS-opt_H9-Pi.HMGR-opt	AmpR, lacO/I, P <sub>H9</sub> Ec.atoB, P <sub>C4</sub> Pi.HMGS-opt, P <sub>H9</sub> Pi.HMGR-opt	ColE1 (pBR322)	This Study
pETM6-H9-Pi.atoB-opt_C4-Sc.HMGS_H9-Sc.HMGR	AmpR, lacO/I, P <sub>H9</sub> Pi.atoB-opt, P <sub>C4</sub> Sc.HMGS, P <sub>H9</sub> Sc.HMGR	ColE1 (pBR322)	This Study
pETM6-H9-Pi.atoB-opt_C4-Sc.HMGS_H9-Pi.HMGR-opt	AmpR, lacO/I, P <sub>H9</sub> Pi.atoB-opt, P <sub>C4</sub> Sc.HMGS, P <sub>H9</sub> Pi.HMGR-opt	ColE1 (pBR322)	This Study