Two dynamic, N-terminal regions are required for function in Ribosomal RNA Adenine Dimethylase family members

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Running title: Two N-terminal regions required for RRAD function

Keywords: rRNA, methyltransferase, RNA modification, ribosome biogenesis, antibiotic resistance

Supporting Information

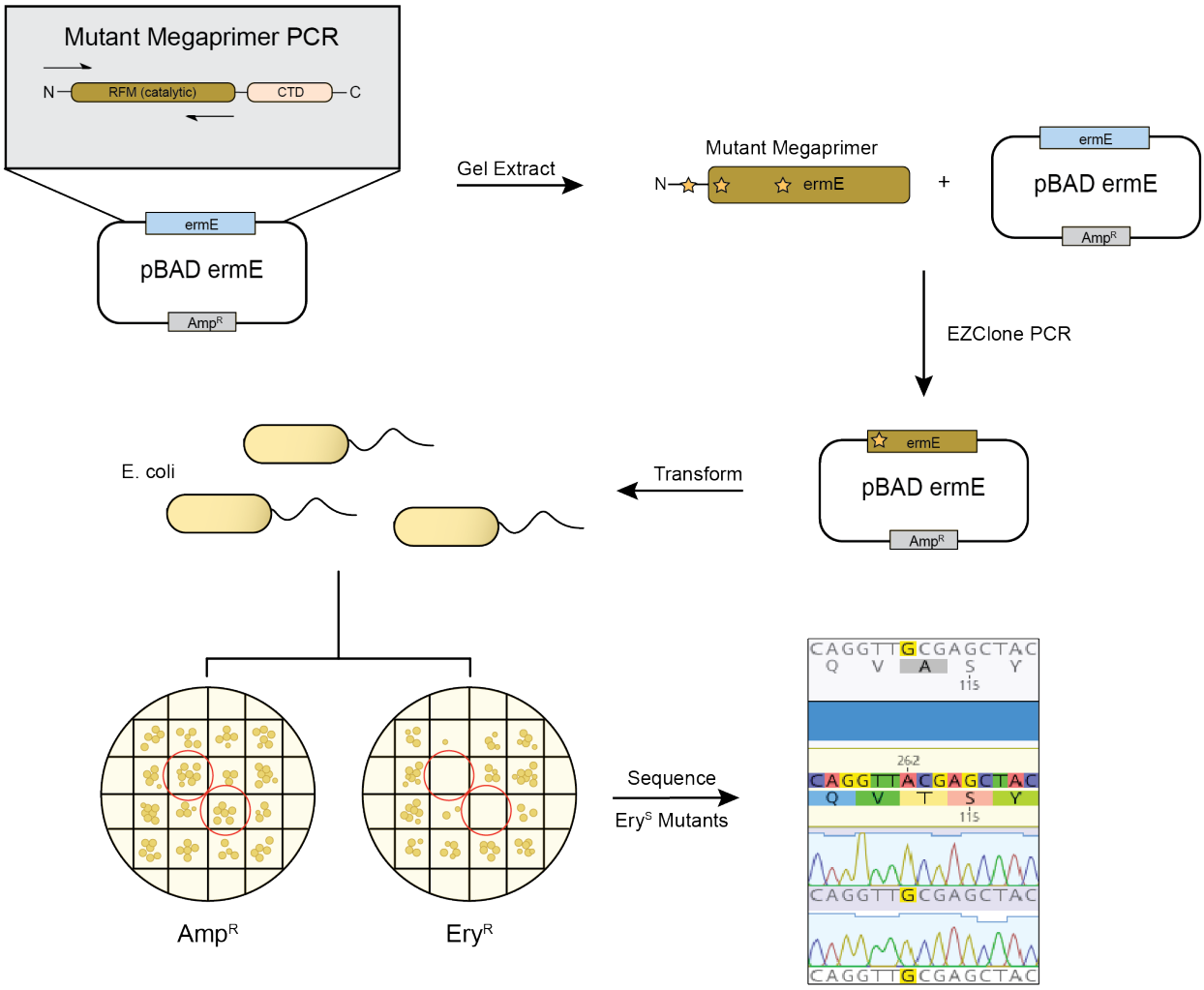

Figure S1. A description of the random mutagenesis workflow.

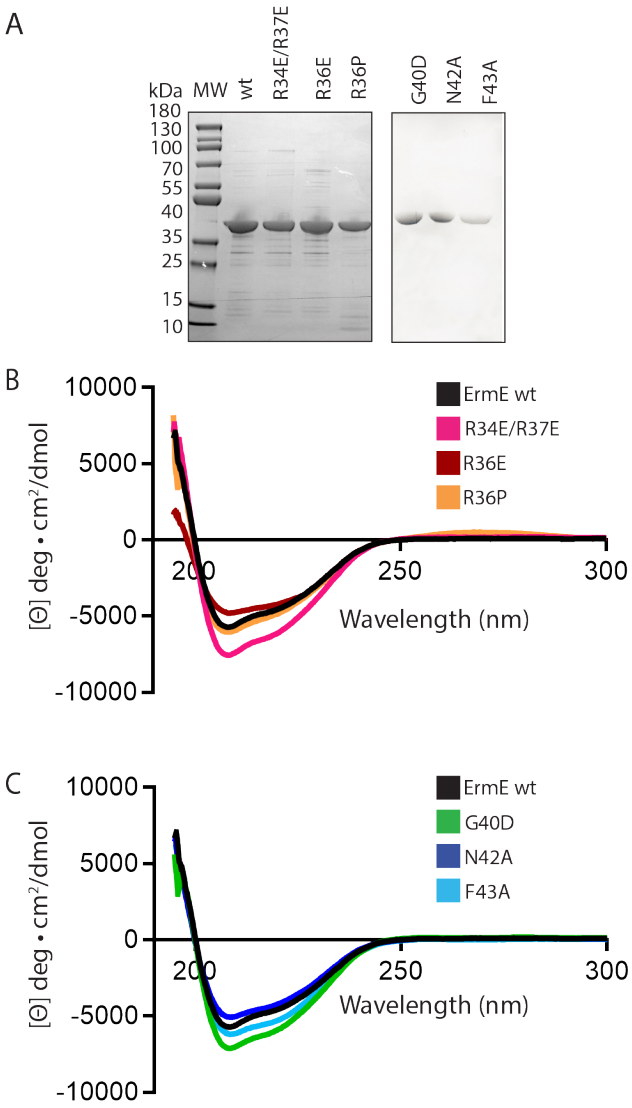

Figure S2. Selected site-directed mutants of ErmE maintain native structure. (A) SDS-PAGE analysis of wt ErmE and site-directed mutants is shown. (B) Circular dichroism spectroscopy of site-directed mutants within the N-terminal basic patch. (C) Circular dichroism spectroscopy of site-directed mutants within motif X.

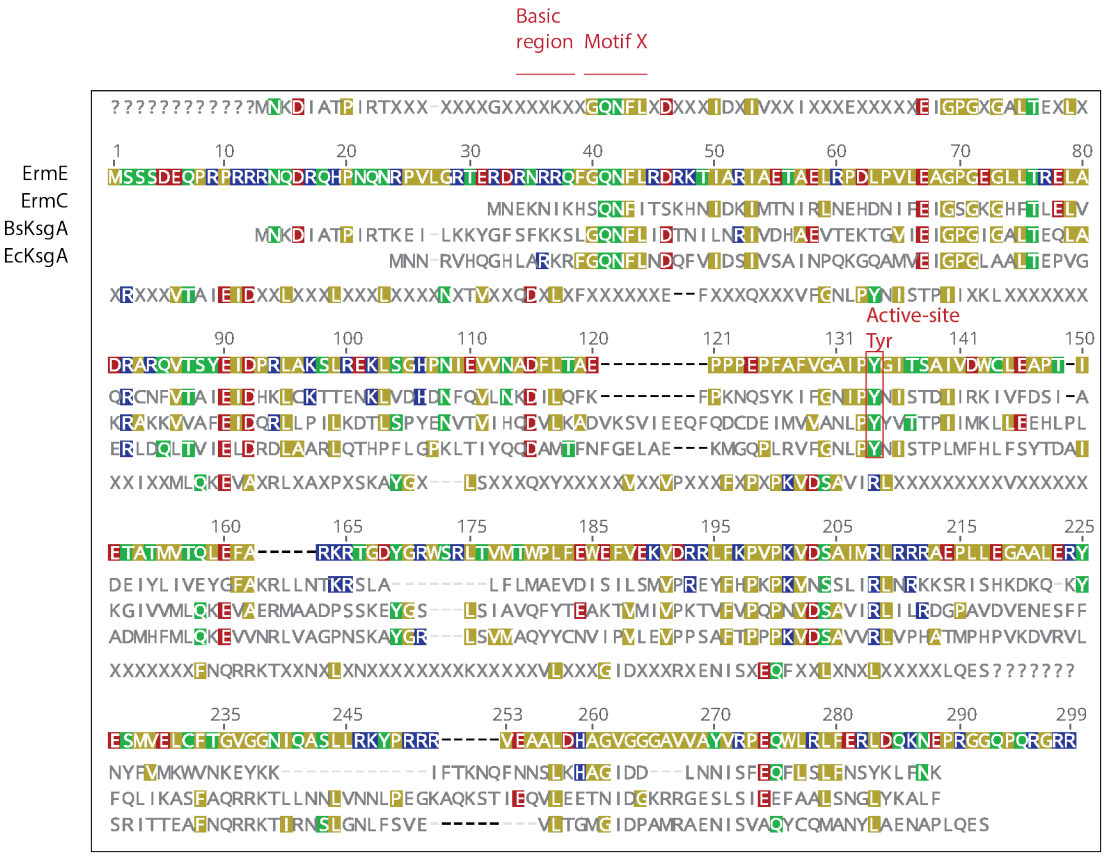

Figure S3. Multiple sequence alignment. A Clustal Omega alignment of *S. erythraea* ErmE, *B. subtilis* ErmC, *B. subtilis* KsgA and *E. coli* KsgA. The ErmE construct has a low sequence-complexity C-terminal region removed. Numbering is according to the ErmE sequence.

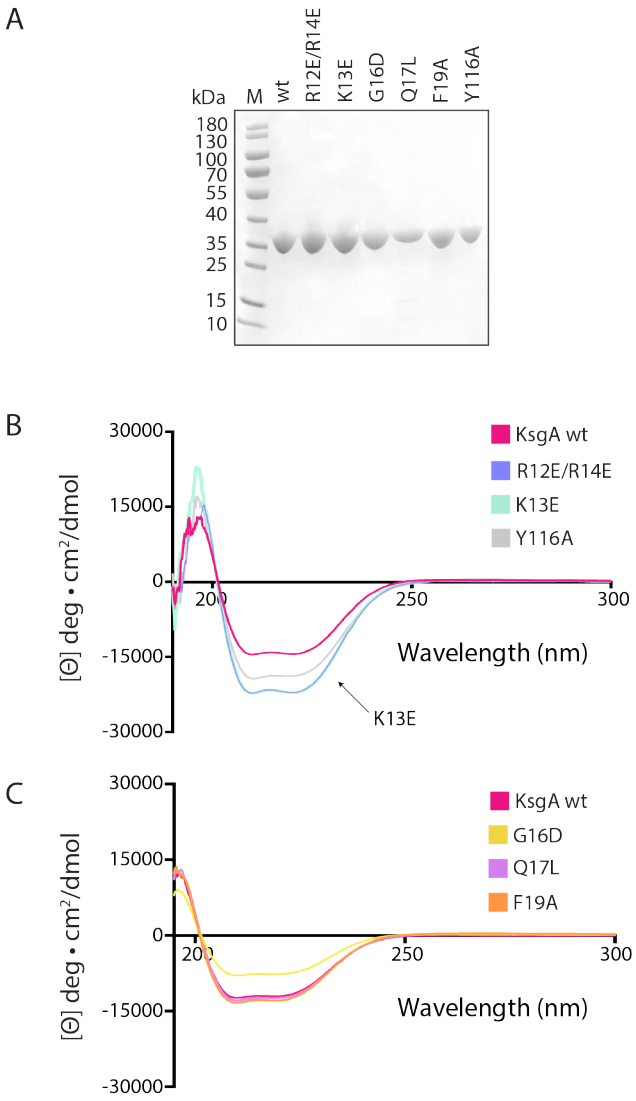

Figure S4. Selected site-directed mutants of KsgA maintain native structure. (A) SDS-PAGE analysis of wt KsgA and site-directed mutants is shown. (B) Circular dichroism spectroscopy of site-directed mutants within the N-terminal basic patch along with Y116A. (C) Circular dichroism spectroscopy of site-directed mutants within motif X.

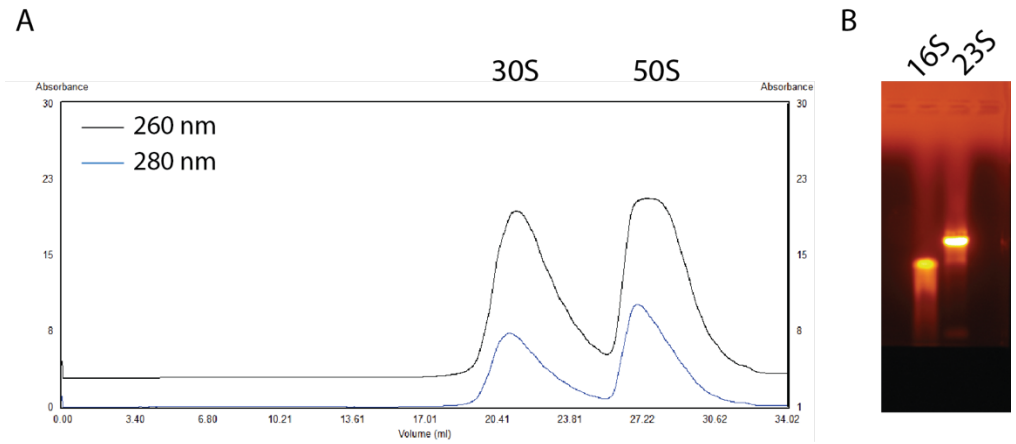

Figure S5. Purification of 30S ribosomal subunits. (A) Elution profile from ultracentrifugation of 10%-40% w/v sucrose gradient loaded with pelleted ribosomal subunits. (B) A 0.8% w/v agarose gel of the 30S fraction and 50S fraction from the sucrose gradient displays bands consistent with the 16S rRNA and 23S rRNA. The fractions containing 30S subunits were used as substrates for in vitro methylation by KsgA.

Table S1. Oligos used in this study.

Name	Use	F/R	Sequence
prDAM001	Genemorph pBAD *ermE*	F	GTTTTTTGGGCTAACAGGAGGAATTAAC
prDAM002	Genemorph pBAD *ermE*	R	GCCCCTTCCAGCAGTGG
prDAM041	*ermC* K4E Q5	F	CATGAACGAGGAGAATATAAAACACAG
prDAM042	*ermC* K4E Q5	R	GTTAATTCCTCCTGTTAGC
prDAM025	*ermC* K4E/K7E Q5	F	ATAGAGCACAGTCAAAACTTTATTACTTC
prDAM026	*ermC* K4E/K7E Q5	R	ATTCTCCTCGTTCATGGTTAATTCC
prDAM015	*ermC* S9D Q5	F	TATAAAACACGATCAAAACTTTATTACTTCAAAACATAATATAG
prDAM016	*ermC* S9D Q5	R	TTTTTCTCGTTCATGGTTAATTC
prDAM017	*ermC* Q10L Q5	F	AAAACACAGTCTAAACTTTATTACTTC
prDAM018	*ermC* Q10L Q5	R	ATATTTTTCTCGTTCATGGTTAATTC
prDAM019	*ermC* N11A Q5	F	ACACAGTCAAGCCTTTATTACTTCAAAACATAATATAG
prDAM020	*ermC* N11A Q5	R	TTTATATTTTTCTCGTTCATGG
prDAM021	*ermC* F12A Q5	F	CAGTCAAAACGCTATTACTTCAAAACATAATATAG
prDAM022	*ermC* F12A Q5	R	TGTTTTATATTTTTCTCGTTCATG
prDAM023	*ermC* I13A Q5	F	TCAAAACTTTGCTACTTCAAAACATAATATAGATAAAATAATG
prDAM024	*ermC* I13A Q5	R	CTGTGTTTTATATTTTTCTCGTTC
prLOA001	*ermC* S9D/Q10L Q5	F	TATAAAACACGATCTAAACTTTATTACTTCAAAACATAATATAG
prLOA002	*ermC* S9D/Q10L Q5	R	TTTTTCTCGTTCATGGTTAATTC
prLOA003	*ermC* S9D/N11A Q5	F	AGCCTTTATTACTTCAAAACATAATATAGATAAAATAATG
prLOA004	*ermC* S9D/N11A Q5	R	TGATCGTGTTTTATATTTTTCTCGTTC
prLOA005	*ermC* Q10L/N11A Q5	F	AAAACACAGTCTTGCCTTTATTACTTCAAAACATAATATAG
prLOA006	*ermC* Q10L/N11A Q5	R	ATATTTTTCTCGTTCATGGTTAATTC
prASC011	*ermC* Q10L/F12A Q5	F	CGCTATTACTTCAAAACATAATATAGATAAAATAATG
prASC012	*ermC* Q10L/F12A Q5	R	TTTAGACTGTGTTTTATATTTTTCTCG
prLOA007	*ermC* N11A/F12A Q5	F	ACACAGTCAAGCCGCTATTACTTCAAAACATAATATAGATAAAATAATG
prLOA008	*ermC* N11A/F12A Q5	R	TTTATATTTTTCTCGTTCATGG
prDAM035	*ermE* R34E QC	F	AGAACGTGATGAGAATCGCCGCCAATTTG
prDAM036	*ermE* R34E QC	R	GTACGGCCCAACACAGGG
prDAM037	*ermE* R36E QC	F	TGATCGTAATGAGCGCCAATTTGGCCAG
prDAM038	*ermE* R36E QC	R	CGTTCTGTACGGCCCAAC
prRMP115	*ermE* R36P QC	F	CAGAACGTGATCGTAATCCCCGCCAATTTGGCC
prRMP114	*ermE* R36P QC	R	GGCCAAATTGGCGGGGATTACGATCACGTTCTG
prDAM039	*ermE* R37E QC	F	TCGTAATCGCGAGCAATTTGGCCAGAACTTTCTG
prDAM040	*ermE* R37E QC	R	TCACGTTCTGTACGGCCC
prDAM027	*ermE* R34E/R37E Q5	F	CGCGAGCAATTTGGCCAGAACTTTCTG
prDAM028	*ermE* R34E/R37E Q5	R	ATTCTCATCACGTTCTGTACGGCC
prRMP121	*ermE* R47S QC	F	CCAGAACTTTCTGCGGGATAGCAAAACCATTGCT
prRMP120	*ermE* R47S QC	R	AGCAATGGTTTTGCTATCCCGCAGAAAGTTCTGG
prRMP117	*ermE* G40D QC	F	AATCGCCGCCAATTTGACCAGAACTTTCTGCGG
prRMP116	*ermE* G40D QC	R	CCGCAGAAAGTTCTGGTCAAATTGGCGGCGATT
prASC002	*ermE* N42A QC	F	CCGCCAATTTGGCCAGGCCTTTCTGCGGGATCGC
prASC001	*ermE* N42A QC	R	GCGATCCCGCAGAAAGGCCTGGCCAAATTGGCGG
prACS004	*ermE* F43A QC	F	CGCCAATTTGGCCAGAACGCTCTGCGGGATCGCAAAAC
prACS003	*ermE* F43A QC	R	GTTTTGCGATCCCGCAGAGCGTTCTGGCCAAATTGGCG
prASC006	*ermE* L44A QC	F	CGCCAATTTGGCCAGAACTTTGCGCGGGATCGCA
prASC005	*ermE* L44A QC	R	TGCGATCCCGCGCAAAGTTCTGGCCAAATTGGCG

prDAM067	*ksgA* R12E/R14E Q5	F	AGAGTTCGGGCAAAACTTTCTCAACG
prDAM068	*ksgA* R12E/R14E Q5	R	TTCTCGGCTAAGTGGCCCTGGTG
prDAM079	*ksgA* K13E QC	F	GCCCGAAGCGCTCACGGGCTAAGTGGCCCTG
prDAM080	*ksgA* K13E QC	R	CAGGGCCACTTAGCCCGTGAGCGCTTCGGGC
prDAM081	*ksgA* G16D QC	F	CTGATCGTTGAGAAAGTTTTGATCGAAGCGTTTACGGGCTAAG
prDAM082	*ksgA* G16D QC	R	CTTAGCCCGTAAACGCTTCGATCAAAACTTTCTCAACGATCAG
prDAM083	*ksgA* Q17L Q5	F	ACGCTTCGGGCTGAACTTTCTCAAC
prDAM084	*ksgA* Q17L Q5	R	TTACGGGCTAAGTGGCCC
prDAM083	*ksgA* N18A QC	F	GAACTGATCGTTGAGAAAGGCTTGCCCGAAGCGTTTACGG
prDAM084	*ksgA* N18A QC	R	CCGTAAACGCTTCGGGCAAGCCTTTCTCAACGATCAGTTC
prDAM077	*ksgA* F19A Q5	F	CGGGCAAAACGCGCTCAACGATCAG
prDAM078	*ksgA* F19A Q5	R	AAGCGTTTACGGGCTAAG
prDAM085	*ksgA* Y116A Q5	F	CAACCTGCCTGCTAACATCTCCACGCCG
prDAM086	*ksgA* Y116A Q5	R	GCCGCTGCGTGTTTTCGG
prDAM061	Excise *ksgA* from Top10 E. coli	F	ATGAATAATCGAGTCCACCAGGGCC
prDAM062	Excise *ksgA* from Top10 E. coli	R	ACTCTCCTGCAAAGGCGCGTTCT
prDAM063	Linearize pBAD IP	F	GGGCCCGAACAAAAACTC
prDAM064	Linearize pBAD IP	R	GGTTAATTCCTCCTGTTAGC
prDAM065	*ksgA* Homology Ends to pBAD	F	GCTAACAGGAGGAATTAACCATGAATAATCGAGTCCACCAGG
prDAM066	*ksgA* Homology Ends to pBAD	R	ATGAGTTTTTGTTCGGGCCCACTCTCCTGCAAAGGCGC

QC, QuikChange. IP, inverse PCR. F/R forward or reverse primer.

Table S2. Gene sequences used in the study.

Name	Sequence
S. erythraea ermE (codon optimized, truncated low complexity C-terminal region)	ATGAGTAGCTCTGACGAGCAACCACGTCCACGCCGTCGCAATCAGGATC GTCAACACCCGAACCAGAATCGCCCTGTGTTGGGCCGTACAGAACGTGA TCGTAATCGCCGCCAATTTGGCCAGAACTTTCTGCGGGATCGCAAAACCA TTGCTCGGATTGCAGAAACCGCGGAATTACGCCCGGATTTACCGGTACTG GAAGCCGGACCAGGTGAAGGCCTGCTGACTCGCGAACTCGCTGATCGT GCGCGTCAGGTTACGAGCTACGAGATCGATCCTCGTTTAGCCAAATCCTT GCGCGAGAAACTGTCAGGCCATCCGAACATCGAGGTGGTGAATGCCGAT TTCCTGACTGCCGAACCGCCGCCTGAACCGTTCGCATTCGTGGGTGCGAT TCCCTACGGGATTACCAGCGCGATTGTGGACTGGTGTTTGGAGGCGCCT ACCATCGAAACCGCTACCATGGTGACGCAGCTGGAGTTTGCTCGCAAAC GCACGGGTGACTATGGTCGGTGGAGTCGCCTTACGGTCATGACCTGGCC GCTTTTCGAATGGGAGTTCGTGGAGAAAGTGGATCGGCGCCTCTTTAAA CCCGTCCCGAAAGTCGATTCGGCCATTATGCGCCTGCGTCGCCGCGCAGA ACCACTGCTGGAAGGGGCAGCGCTGGAACGTTACGAATCGATGGTAGA ACTGTGCTTTACAGGCGTTGGCGGCAACATCCAGGCGTCCTTACTCCGCA AGTATCCCCGTCGCCGTGTTGAAGCGGCACTGGACCATGCGGGTGTTGG CGGAGGGGCCGTAGTCGCCTATGTTCGCCCGGAACAGTGGCTTCGCCTG TTTGAACGCCTGGACCAGAAGAACGAACCGCGTGGCGGTCAACCGCAA CGTGGTCGTCGCCTCGAGCTTGGGCCC**GAACAAAAACTCATCTCAGAA GAGGATCTGAATAGCGCCGTCGACCATCATCATCATCATCAT**TGA
B. subtilis ermC'	ATGAACGAGAAAAATATAAAACACAGTCAAAACTTTATTACTTCAAAACAT AATATAGATAAAATAATGACAAATATAAGATTAAATGAACATGATAATATCT TTGAAATCGGCTCAGGAAAAGGGCATTTTACCCTTGAATTAGTACAGAG GTGTAATTTCGTAACTGCCATTGAAATAGACCATAAATTATGCAAAACTAC AGAAAATAAACTTGTTGATCACGATAATTTCCAAGTTTTAAACAAGGATAT ATTGCAGTTTAAATTTCCTAAAAACCAATCCTATAAAATATTTGGTAATATA CCTTATAACATAAGTACGGATATAATACGCAAAATTGTTTTTGATAGTATAG CTGATGAGATTTATTTAATCGTGGAATACGGGTTTGCTAAAAGATTATTAA ATACAAAACGCTCATTGGCATTATTTTTAATGGCAGAAGTTGATATTTCTAT ATTAAGTATGGTTCCAAGAGAATATTTTCATCCTAAACCTAAAGTGAATAG CTCACTTATCAGATTAAATAGAAAAAAATCAAGAATATCACACAAAGATAA ACAGAAGTATAATTATTTCGTTATGAAATGGGTTAACAAAGAATACAAGA AAATATTTACAAAAAATCAATTTAACAATTCCTTAAAACATGCAGGAATTG ACGATTTAAACAATATTAGCTTTGAACAATTCTTATCTCTTTTCAATAGCTAT AAATTATTTAATAAGCTTGGGCCC**GAACAAAAACTCATCTCAGAAGAGG ATCTGAATAGCGCCGTCGACCATCATCATCATCATCAT**TGA
E. coli ksgA	ACGATTTATCAGCAGGATGCGATGACCTTTAACTTTGGTGAACTGGCCGA GAAAATGGGTCAGCCGCTGCGTGTTTTCGGCAACCTGCCTTATAACATCT CCACGCCGTTGATGTTCCATCTGTTTAGCTATACTGATGCCATTGCCGACAT GCACTTTATGTTGCAAAAAGAGGTGGTGAATCGTCTGGTTGCAGGACCG AACAGCAAAGCGTATGGTCGATTAAGCGTCATGGCGCAATACTATTGCAA TGTGATCCCGGTACTGGAAGTACCGCCGTCAGCCTTTACACCACCACCCA AAGTGGATTCCGCCGTCGTGCGCCTGGTTCCTCATGCAACGATGCCTCAC CCGGTTAAAGATGTTCGTGTGTTGAGCCGCATCACCACCGAAGCCTTTAA

	CCAGCGTCGTAAAACCATTCGTAACAGCCTCGGCAACCTGTTTAGCGTCG AGGTGTTAACGGGAATGGGGATCGACCCGGCGATGCGAGCGGAAAATA TCTCTGTCGCGCAATATTGCCAGATGGCGAACTATCTGGCGGAGAACGCG CCTTTGCAGGAGAGTGGGCCC**GAACAAAAACTCATCTCAGAAGAGGAT CTGAATAGCGCCGTCGACCATCATCATCATCATCAT**TGA

MYC epitope and hexahistidine tag are shown in bold.