

Legends and Supplementary Figures S1 and S2.

***Thermococcus kodakarensis* genetics: TK1827-encoded  $\beta$ -glycosidase,  
new positive selections, targeted and repetitive deletion technology**

**Thomas J. Santangelo, Ľubomíra Čuboňová and John N. Reeve<sup>\*</sup>.**

Fig. S1. Structure and sequence of pTS535. (A). Plasmid pTS535 was constructed from the pUC118 backbone (4) and retains the pUC118 replication machineries,  $\beta$ -lactamase encoding Amp<sup>R</sup> gene and multiple cloning site, designated MCS2 in this figure. The (trp-6MP<sup>S</sup>) cassette [P<sub>TK2279</sub>-trpE (TK0254) + P<sub>hmtB</sub>-TK0664] was cloned adjacent to MCS2, and a synthetic DNA sequence was cloned downstream of TK0664 to provide MCS1. (B) The 5,463 bp sequence of pTS535. The Amp<sup>R</sup>, TK0664 and trpE coding sequences are shown in upper case, brackets delineate the MCS1 and MCS2 sequences, and the TATA-box elements of the P<sub>hmtB</sub> and P<sub>2279</sub> promoters (1-3) are identified.

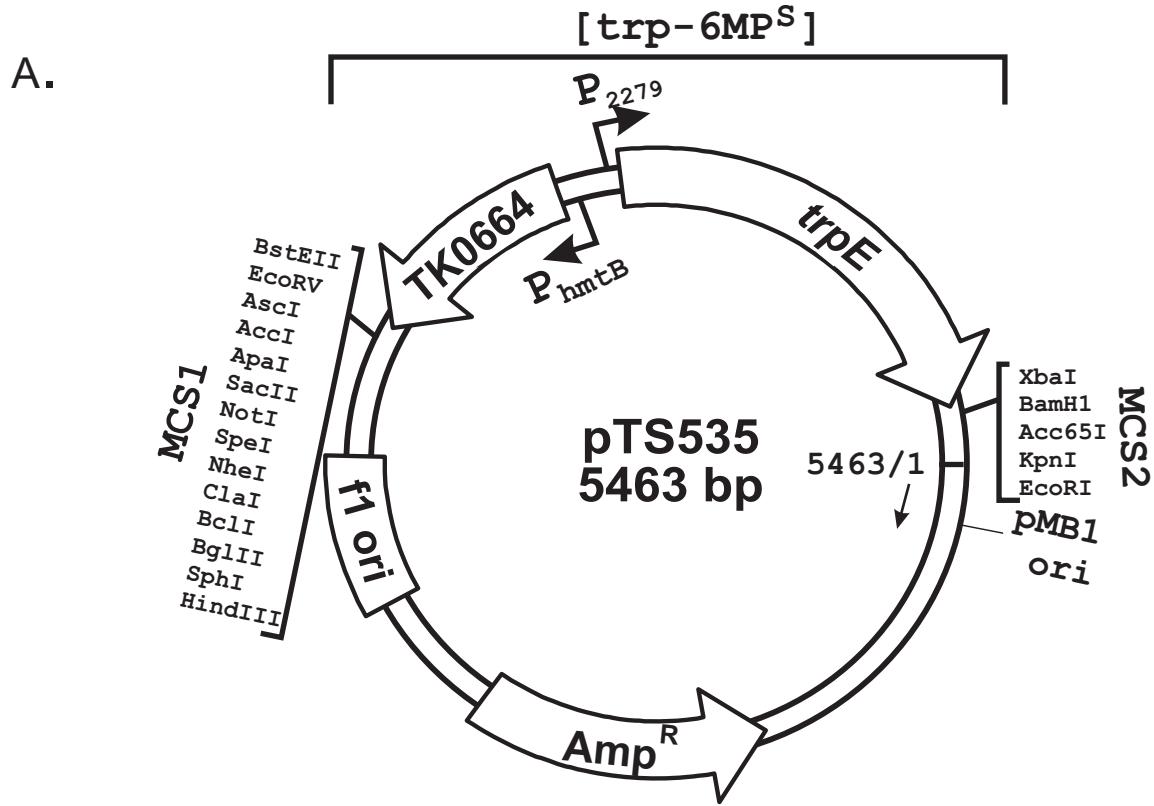
FIG. S2. Construction of *T. kodakarensis* TS538 and TS541. (A) Plasmid pTS538 was constructed from pTS535 (Fig. S1) by cloning the genes, as shown, on either side of the (trp-6MP<sup>S</sup>) cassette and used to transform *T. kodakarensis* TS517 (Table 1). A transformant, designated *T. kodakarensis* TS538i, selected by growth on plates lacking tryptophan, had the genome structure shown. Dilutions of a culture of *T. kodakarensis*

TS538i were spread on plates containing 6MP, and a 6MP<sup>R</sup>, tryptophan auxotroph, designated *T. kodakarensis* TS538, had the genome shown. (B) Plasmid pTS541 was constructed by cloning the genes, as shown, into pTS535 (Fig. S1) and used to transform *T. kodakarensis* TS517. A transformant, designated *T. kodakarensis* TS541i, selected by growth on plates lacking tryptophan, had the genome structure shown. Dilutions of a culture of *T. kodakarensis* TS541i were spread on plates containing 6MP, and a 6MP<sup>R</sup>, tryptophan auxotroph, designated *T. kodakarensis* TS541, had the genome shown.

## REFERENCES

1. **Santangelo, T.J., L. Čuboňová, R. Matsumi, H. Atomi, T. Imanaka, and J.N. Reeve.** 2008. Polarity in archaeal operon transcription in *Thermococcus kodakaraensis*. *J. Bacteriol.* **190**:2244-2248.
2. **Santangelo, T.J., L. Čuboňová, and J.N. Reeve.** 2008. Shuttle vector expression in *Thermococcus kodakaraensis*: contributions of *cis* elements to protein synthesis in a hyperthermophilic *Archaeon*. *Appl. Environ. Microbiol.* **74**:3099-3104.
3. **Santangelo, T.J., L. Čuboňová, K.M. Skinner, and J.N. Reeve.** 2009. Archaeal intrinsic transcription termination in vivo. *J. Bacteriol.* **191**: 7102-7108.
4. **Vieira, J., and J. Messing.** 1987. Production of single-stranded plasmid DNA. *Meth. Enzymol.* **153**:3-11.

FIG. S1.



B.

1 cttccgttctcgctactgactcgctcggtcggtcggtcggtatcagtcactcaaaggcg  
 taatacggttatccacagaatcaaggataacgcggaaagaacatgtgagcaaaaggccagaaaaggccaggacc  
 gtaaaaaggccgcgttgcgtttccataggctcccccgtacgagcatcacaaaatcgacgctcaagtc  
 agaggtggcgaaacccgacaggactataaagataaccaggcgcccccttggaaagctccctcgctcccttgc  
 cgaccctggcgttaccggataacctgtccgccttcctcccttggaaagctggcgcttcatacgctcgtga  
 ggtatctcagttcggttaggtcgctccaaagctggctgtgcacgaaccccccttgcggccaccgctgc  
 cttatccggttaactatcgcttgcgttggacttgcactggcactggcagcagccactggtaaca  
 ggattagcagagcgaggatgttaggcgtgtacagagtcttgcgttgcactacaggctacactagaagga  
 cagtatttggatctgcgtctgtgaagccagttacccggaaaaagagttggtagcttgcgttgcggcaaaaa  
 ccaccgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgtt  
 tgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgtt  
 tgatctttctacgggtctgcgtctgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgttgcgtt  
 ggatcttcaccttagatcccttaattttaaatggatcttgcgttgcgttgcgttgcgttgcgttgcgtt  
 acagTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTGTTCATCCATAGTTGCCTGACTCC  
 CCGTCGTAGATAACTACGATAACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATAACCGCGAGACCCACGCT  
 CACCGGCTCCAGATTATCAGCAATAAACCCAGCCAGCCGGAAAGGGCCAGCGCAGAAGTGGTCTGCAACTTTATCCG  
 CCTCCATCCAGTCTATTAAATTGTGCCGGGAAGCTAGAGTAAGTAGTCCGGAGTTAATAGTTGCGAACGTTGTTG  
 CCATTGCTACAGGCATCGTGGTGTACGCTCGTGTGTTGGTATGGCTTCAATTAGCTCCGGTCCCAACGATCAAGGC  
 GAGTTACATGATCCCCATGTTGCAAAAAGCGGTTAGCTCCTCGGTCCGATGTTGTCAAGAAGTAAGTGG  
 CCGCAGTGTATCACTCATGGTATGGCAGCAGTCATAATTCTCTTACTGTATGCCATCCGTAAAGATGCTTTCTG  
 TGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGATGCGGCAGCGAGTTGCTCTGCCGGCGTCAATAC  
 GGGATAATACCGGCCACATAGCAGAACTTTAAAAGGCTCATCATTGGAAAACGTTCTCGGGCGAAAACCTCTCAA  
 GGATCTTACCGCTGTTGAGATCCAGTCAGTGTAAACCCACTCGTGCACCCAACGTATCTCAGCATCTTCCCTTCA  
 CCAGCGTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGAAAAAGGGAATAAGGGCGACACGGAAATGTTGAA  
 TACTCATactttcaatattttcaatattttcaatattttcaatattttcaatattttcaatattttcaatattttcaat

R  
Amp<sup>R</sup>

**FIG. S1 (cont.)**

MCS1

TK0664

trpE

MCS2

gtatttagaaaaataacaaatagggttcgcacattccccgaaaagtgccacctgacgtctaagaaaccatta  
 ttatcatgacattaacctataaaaataggcgatcacaaggcccttcgtctcgccgttccggatgacgggtaaa  
 acctctgacacatgcagctcccgagacggcacagctgtctgttaagcggtatgcggggagcagacaagccgtcagg  
 gcgctcagcggtgttggcggtgtcgggcttaactatgcggcatcagagcagattgtactgagagtgacc  
 ataaaattgtaaacgttaatatttgttaattcgcttaatattttgttaatcagctcatttttaaccaatagg  
 ccgaaatcgccaaaatccctataaatcaaaaagaatagcccagatagggttagtgttccagtttgaacaaga  
 gtccactattaaagaacgtggactccaacgtcaaaggcgaaaaaccgtctatcagggcgtggccactacgtgaac  
 catcacccaaatacaagttttgggtcgaggtgcgtaaagcactaaatcggaaccctaaaggagccccgattta  
 gagcttgcacggggaaagccggcgaacgtggcgagaaaggaaagaaacgcggagcggcgctagggcgctgg  
 caagtgtagcggtcacgcgtcgtaccacccacaccgcgcgttaatgcgcgtacaggcgctactatggtt  
 gcttgcgtatgcgggtgtgaaataccgcacagatgcgttaaggagaaaataccgcattcaggcgccattcgc  
 gctgcaactgttggaaaggcgatcggtcggcccttcgttattacccagctggcgaaaggggatgtctgc  
 aaggcgattaaagtggtaacgccagggtttccagtcacgcgttgcataacgcggccagtcgaagcttgcattg

[HindIII, SphI, BglIII, BclI, ClaI, NheI, SpeI, NotI, SacII, ApaI, AccI, AscI, EcoRV, BstEII]

cagatctgatcatcgatgtcgactgtcgccgcggccgtcgacggcgccgatatcgtaaaccgtcgatgc  
 ctctcgctgtcatttcgttatctcgccggagtagacgcgtgtgttcttgcattctcctcaaccccttccctgtcc  
 aagtcacctccCTAGGCTTTGAGCCACTTCCCTTCCTGAACTTAAAACCTTCTCATTCAGGCCATCCTGAGG  
 GCCTCTCGAGCTTGCCCTCGGGACTTCGAGACCCTGGAGCTCCTGAAGAGCTCGATTATCTCGTGGTGTAC  
 GCTTCTTTCCCTCAAGAATGTTGCTCACGAGGTTATCATATCCTCGACGAAGTCCACGGGAAGACTATCCAGGCC  
 CAGTCTATCTCCTCCGTAGTACTCGGGCTTGAAGCGGGAGCCTCTGATCGTCAGGAGTGTGCAGTCCTGACTTCC  
 GCAGGGCTTGTCTCGACGTAGTTTGCCAGCGTCAGGCTCTCACCAAGTATCGCTGATGTCGTCAGCGATGAGA  
 ACCTTTTCCCCTGAGGCTGTAGTTGCTGCCGTACTTGAGCTGGCTTGCCGAGTAGCGTTACTCCCCAG  
 TGCTCCACCTTGAGGCTTACAAGGTCTTGATTCCAGGTAGTCGAGTAGCTCGCCGCGACCCAGCCGCCCC  
 GCGAGACCAACCACCATCTGGCTTCCAGCCTTCTCCAGAAATCTCCAGGCGCTTCTCGCCCACCTTCAATG  
 TCTTCCAAGAACGAGCAGGCTTGCAGGAAACTTCTTCATatgcacccatccgtatattatattactatcccta  
 tataaatatatcgctctgcagggtccagctggcacaacgcgtatccgcattttgtcaccggaaaatttttaatactaagggtt

TATA-box

← P<sub>hmbt</sub>

TATA-box

→ P<sub>2279</sub>

aatthaatctcgagcggtttagtccttgcggcttggagaggccgttaaaaagggtatgcataATGCCTCTCAA  
 AAAGCTGAAGCCGTTGACCCTTGAAAGCTCTACAGCGCCCTAGAGACTTGGGATGCCATTCTATGCTCCGCTCTGC  
 CGAGAAGGACTCCAGGAAGGCCAGATTCACCTACATATCGGCCAGCCGGAGTCGTGAGGTGCGAGGGGAC  
 TGAGATCGACGGGGAGCGAGTTCCGACGAGAGAACCCCTCAGAGCTCTAAAGGGCTCATGGGGAGAGGGTCGA  
 GGGCAGGAGGTTCATGGGTGGCTTCGTTGAGTACCTCCATCGGTTACACTCCATCATCGGGGGAGATCGA  
 AGAGCCCTCGGTCTCGGCTACTACCCCTGGACCTTCATCTACGACCACTCTACCGCGCTTTCTTCTTACCT  
 CAGAGAGGCTCTTCGACCCCGAGGCCTTAGTTGAAAGGGCAGGAGGGAAAGAGTCACGGCTTAAGACGGCGGTT  
 GGAGGTCATATCCACCGACGGCATGGAAGAGTTGTTGAAATCGTCAGGCTGGAGGAGTACATCTACTCGGG  
 GGACGTCTCCAGGTGGTCTGTCGCGAGTACAGGGTTAGAACGGATCTCGATGCCCTCGAAATCTACAAGCGGCT  
 CGTGGAGCTCAACCCCTCCCCGTACACCTTCATCCTGGAGTTGAGAAGACCGCTGAGGGGCTCACCGAAACCAT  
 GGGTCCGTCAGGGGGAGAACCTCAAGATAAAACCCATAGCAGGAACAGCGCCAGGGGGAGGACGGGGAGGAAGA  
 CCGGGAGCTGAAAAGGCCCTACTCTCGACGAGAAAGAGCGAGCTGAGCACGTCATGCTCGTTGACCTGCTAGAAA  
 CGACGTCAAGGAGGGTTCAAAGCCGGAGCGTTAGGCTAACCGCTTCTTCGACGTCCTGAAGTACAGCCACGTCCA  
 GCACATAGAGAGCGAGGTGGCTGGTAACTCGATGAGGGAAAAACGCGTTGACGCCATGGAGGCGCTTCCGGC  
 GGGAACACTAACCGAGCCCCGAAGATAAGGGCGATGGAGATCATAGACGAGCTGGAGAGGAGCAGGAGAAAGGTCTA  
 CGGGGGAGCAGTGGGTTACTTCTCCCTCACCGGGGACGCCGACATGGCGATAGCGATAAGGATGGCCAGATCGAGGG  
 CAGGAAAGCGAGCGTTAGGGCGGGGCAGGAATAGTGGCGGATTCAGTTCCAGAGAAGGAGTTCTCGAGACCGAGAA  
 CAAAATGAGAGCAGTTCTGAAGGCCTGGGGGTGAGGGAAATGAgctggactctagaggatccccgggtaccgagct

[XbaI, BamH1, XmaI, Acc65I, KpnI, EcoRI]

cgaattttaatcatggcatagctgtttccgtgtgaaattgttatccgtcacaattccacacaacatcagggcg  
 gaagcataaagtgtaaagcctgggtgcctaattgcgtgagctactcacattaattgcgttgcgtactgcccgtt  
 tccagtcggaaaacctgtcgccagctgcattatgcataatcgccacaacgcgcggggagaggcggttgcgtattggc  
 gct 5463

FIG. S2.

