

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, Lubomíra Čuboňová and John N. Reeve \***

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

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FIG. S1 (cont.)

gtatttagaaaaataaacaataggggttccgcgcacatttccccgaaaagtgccacctgacgtctaagaaaccatta  
 ttatcatgacattaacctataaaaaatagggcgtatcacgagggccttctcgtctcgcggttcgggtgatgacggtgaaa  
 acctctgacacatgcagctcccggagacggtcacagcttgtctgtaagcggatgccgggagcagacaagcccgtcagg  
 gcgctcagcgggtgttgccgggtgtcggggctggcttaactatgcggcatcagagcagattgtactgagagtgacc  
 ataaaattgtaaacgttaataatgtttaaattcgcgttaaattttgttaaatacagctcatttttaaccaatagg  
 ccgaaatcggcaaaatcccttataaatcaaaagaaatagcccagataggggttgagtgtgttccagtttggaacaaga  
 gtccactattaagaacgtggactccaacgtcaaagggcgaaaaaccgtctatcagggcgaaggccactacgtgaac  
 catcacccaaatcaagtttttggggctcagagtgccgtaaagcactaaatcgggaaccctaaagggagccccgattta  
 gagcttgacgggaaagccggcgaacgtggcgagaaaggaaggggaagaaagcgaaggagcggggcgttagggcgtgg  
 caagtgtagcggctacgctgcgcgtaaccaccacaccgcgcgcttaatgcgcgctacagggcgcgtaactatgggtt  
 gctttgacgtagcgggtgtgaaataccgcacagatgcgtaaggagaaaaataccgcacagggccatttcgcaattcag  
 gctgcgcaactgttgggaagggcgaatcgggtgcgggctcttcgctattacgccagctggcgaaagggggatgtgctgc  
 aagggcattaagttgggtaacgccaggggtttccagtcacgacgttgtaaaacgacggccagtgcaagcttgcagtg

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

cagatctgatcatcgatgctagcactagtgcggccgcccggctcgacggcgcgcccgataatcggtaaaccgctcagtg  
 ctctcgtctgtgcatcttctgataatctcgcgggagtagacgctgtggttcttgatctcctcaaccttcttctccctgtcc  
 aagctacctctcccTcAGGCTTTGAGCCACTTCCCTTCCCTGAACTTAAAAACCTTCCCTCATTTCAGCCATCTGAGG  
 GCCTCTTCGAGCTTGCCTTTCGGGACTTCGAGACCGTGGAGCTCCTTGAAGAGCTCGATTATCTCGTCGGTGCCTTACC  
 GCTTCTTTTCCCTCAAGAATGTTGCTCACGAGGTTTATCATATCTTCGACGAAGTTCACGGGAAGACTATCCAGGCC  
 CAGTCTATCTCCTCTCCGTAGTACTCGGGCTTGAAGCGGGAGCCTCTGATCGTCAGGAGTGTTCAGTCTGACTTCC  
 GCAGGGCTCTTGCTCTCGACGTAGTTTTTGGCCAGCGTCAGGCTCTCACCAGTATCGCTGATGTCTGTCGACGATGAGA  
 ACCTTTTTTCCCGCTGAGGTCGTAGTTCGTGCCGTACTTGAAGCTTGGCCTTGGCGTCCGGAGTAGCGGTTACTCCCCAG  
 TGCTCCACCTTGAAGGCTTACAAGGCTTTTGAATTTCCAGGTAGTCGCAGTAGAGTCTCGCCGCGACCCAGCCGCCCTG  
 GCGAGACCAACCACCATCTGGCTTCCAGCCTTCTTCCAGAATCTTCCAGGCGCCTTCTTCCGCCACCTTTCAATG  
 TCTTCCCAAGAAGCGAGCCTTGCAGGAACTTCTTCATatgcatcacctccgtgatattatctataactataatcccta  
 tataaataatcgctctgcaggtccagctgccgcaacgcgcattttgctcacccgaaaattttaaataactaagggtt

TATA-box

← P<sub>hmtB</sub>

TATA-box

P<sub>2279</sub> →

aatttaatctcgcgctttgagtccttctgacggctccttgagagggccgttaaaaagggtgatgcatATGCCCTCTCAA  
 AAAGCTGAAGCCCCTTGACCTTTGAAGCTCTACAGCGCCCTTAGAGACTTTGGGATGCCATTATGCTCCGCTCTGC  
 CGAGAAGGACTCCAGGAAGGCCAGATTACCTACATATCGGCCGAGCCGGAGTTCGTCTGGAGGTTCGGCGAGGGGAC  
 TGAGATCGACGGGGAGCGAGTTTCCGACGAGAGGAACCCCTCAGAGCTCTTAAAGGGCTCATGGGGGAGAGGTTCGA  
 GGGCAGGAGTTTATGGGTGGCTTCGTTGGCTACGTCTCCTACGATTCGGTACACTCCATCATCGGGGGGAAGATCGA  
 AGAGCCCTCGGTCTTCGGCTACTACCCCTGGACCTTCTACGACCCTTACCAGGCGCTCTTCTCTTTTACCT  
 CAGAGAGGCTCCTTTTCGACCCGAGGCCCTTAGTTGAAAGGGCCAGGAGGAAGAGTACGGCTTGAAGACGGCGGTTT  
 GGAGGTCAATCCACCGACGCGGGCATGGAAGAGTTCGTTGAAATCGTCAGGGCTGGGAAGGAGTACATCTACTCGGG  
 GGACGTCTTCCAGGTGGTTCTGTCTGCGCGAGTACAGGGTTAGAACGGATCTCGATGCCCTCGAAATCTACAAGCGGCT  
 CGTGGAGCTCAACCCCTCCCGTACACCTTCTTGGAGTTCGAGAAGACCGTTCGTAGGGGCTCACCCGAAACCAT  
 GGGTTCCGTCGAGGGGAGAACCTTCAAGATAAACCCCATAGCAGGAACAGCGCCGAGGGGAAGGACGGGGGAGGAAGA  
 CCGGGAGCTGGAAAAGGCCCTACTCTCCGACGAGAAAGAGCGAGCTGAGCACGTCTGCTCGTTGACCTTGCTAGAAA  
 CGACGTCAAGAGGGTTTCAAAGCCCGGAGCGTTAGGCTAACCCGCTTCTTTCGACGTCTGAAGTACAGCCACGTCCA  
 GCACATAGAGAGCGAGGTGGTTCGGTGAACCTCGATGAGGGGAAAAACGCGTTCGACGCCATGGAGGCGGCTTTTCCGGC  
 GGGAACTAACCAGGAGCCCCGAAGATAAGGGCGATGGAGATCATAGACGAGCTGGAGAGGAGCAGGAGAAAGGTCTA  
 CGGGGGAGCAGTGGGTTACTTCTCCCTCACCGGGGACGCCGACATGGCGATAGCGATAAGGATGGCCGAGATCGAGGG  
 CAGGAAAGCGAGCGTTAGGGCCGGGGCAGGAATAGTGGCGGATTAGTTCAGAGAAAGGAGTCTTTCGAGACCGAGAA  
 CAAAATGAGAGCAGTTCTGAAGGCGCTGGGGGTGAGGGAATGAgctggactctagaggatccccgggtaccgagct

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

cgaattgttaatcatgggtcatagctgtttcctgtgtgaaattgttatccgctcacaattccacacaacatacagagccg  
 gaagcataaagtgtaaagcctgggggtgcctaatgagtgagctaacacattaattgcgttgccgctcactgcccgtt  
 tccagtcgggaaacctgtcgtgccagctgcaatgaatcggccaacgcgccccggggagagggcgggttgcgtaattgggc  
 gct 5463

MCS1

TK0664

t<sub>1</sub>rP<sub>E</sub>

MCS2

FIG. S2.

