

1 *Supplementary Materials*

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3 **A Genetic System for *Methanocaldococcus jannaschii*, an**
4 **Evolutionary Deeply Rooted Hyperthermophilic Methanarchaeon**
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36 ***Methanocaldococcus jannaschii*, gene knock out, protein overexpression**
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38 **1 Supplementary Figure and Tables**

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40 **1.1 Supplementary Figure**

P_{Sta}

TAATATTCACCAAAATTAATATTTATTTTTGGTCTTTTTTAACATTATTCCTCATAGGTTTTATAAATTTCTACTA
AATATTTGGATATATCAAAATTCGTTTTTAACCATTAAAAAAGTTGCAAATGTTTGAACTTTTTTATTTTAG
AATTTGTCAAAAATTATATGATAAATTTGAAATATCAAAGATATAATAGAAATTCATATCGTAAAACTATTGTTA
TAACCGTTTCAT**ATCGTAAGCTTTATATA**GTAGTTTCGTCGAAGGTATATACC**G**TCAATCAAATAACAACATA
AAAACTT**AGGTGA**TAAGGT**ATC**
+1

P_{flaB1B2}

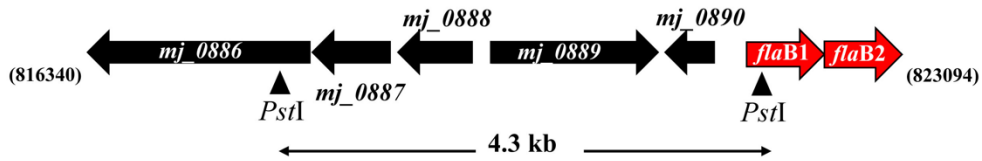
GTTTATCCATTAGTTTATATAAATTTGGATTTGTTTAAATTTATTTGGCATAATCATTACGTTAATTTGCTATTT
AAGATTGACTATCGTTTTTACCTACACAAACCTCTACATAAACACCGATAAAATTGACTACGCAATAAATTA
CGCAAAAAGAATATATTTTAAGAAAATTACAGTTTATAGT**G**AGGTGGATTGAGGAAAAGTGTGGGGGAAT
TGTGTGGGGGAGGAAAATACTACAAGCAATTGAGGGATTAATATACTC**AGGTGA**TATGAGATGAAG**ATC**
+1

RBS **BRE/TATA Box** **Start Codon**

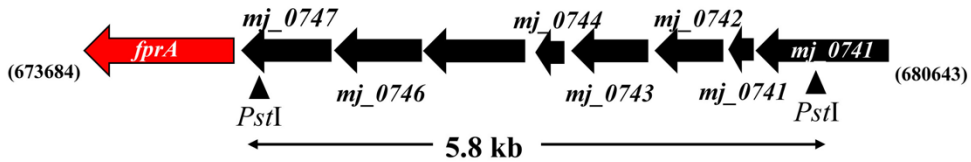
41 **Figure S1. DNA sequences of promoters used in the study.** Promoter name, respective gene
42 or operon: P_{sta}, S-layer protein gene of *Methanocaldococcus* sp. FS406-22 (locus tag number,
43 *mfs40622_1341*); P_{flaB1B2}, flagellin B1B2 operon of *Methanocaldococcus jannaschii* (locus tag
44 number, *mj_0891-2*). Indicated BRE/TATA box regions and the transcription start sites are either
45 directly based on or inferred from published work with *M. jannaschii* (4, 5). +1, transcription start
46 site.
47

Locus

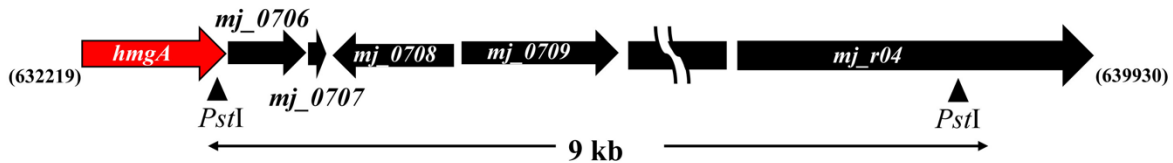
flaB1B2



fprA



hmgA



48 **Figure S2.** *PstI* restriction map for the select regions of *M. jannaschii* genome. This
49 information was used to assign identities to the bands a-c in the Southern blot (Fig. 3C). The
50 expected sizes of the fragments resulting from *PstI* digestion are shown. These fragments lack
51 *SacI* restriction sites.

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1 MDYKDHDGDY KDHD IDYKDD DDKGSAAWS HPQFEKGGGS GGSGGGWS
 51 HPQFEKSGMK KYESRRSKIA DG~~VY~~WVGVLD WDIRMYHG~~YT~~ LKGT~~TY~~NAYL
 101 VFGDEKVALI DNTYPG~~TSAQ~~ MWGR~~IKDA~~FE KEGREFKIDV IVQNHVEKDH
 151 SGALPEI~~HKK~~ FPDAP~~IYCTE~~ VAVEGLK~~KHY~~ PSLKDAQ~~FKV~~ VHTGDTVDLG
 201 GKT~~LT~~FLEAP LLHWPDS~~MFT~~ FYNEGGILFS NDAFGQ~~HLCF~~ PAHKRF~~DKDI~~
 251 PEYVLMDANQ KFYANLITPL SKLVLK~~KFEE~~ VIQLGLLEKI KMIAPSHGQI
 301 WTDPMKVIKA YQDFATG~~KAA~~ KDKAVIVYDT MHYSTQ~~KMAH~~ AFAEGLMSEG
 351 IDVVMYFLHY DERSEIVKDI L~~DAKAV~~LFGI PTIYDEP~~PYS~~ IGDIIYYLRG
 401 LKFNRTGF~~KR~~ LAVTFGSMGG EGGAVAKIAE DLAKCGFEVI NQYELYVPT
 451 EDEL~~TNC~~YNM GKELAKRIKE MKIE

54

55 **Figure S3. Regions of FprA represented by the thermolysine peptides identified in MALDI**
 56 **mass spectrometric analysis.** Red, areas covered in the analysis, respectively; underlined,
 57 3xFLAG tag; underlined and italics, strep-tag. The sequence coverage in the analysis, 261/474 or
 58 55%.

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60 **1.2 Supplementary Tables**

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62 **Table S1. Primers and synthetic DNA**

Name	DNA sequence (5' -> 3')
Primer 1	CTCAAAGGAATAATCCTCTG
Primer 2	GGAACATTCAGACCAGAAAG
Primer 3	GTGGTTTTAGCATACTATCTGG
Primer 4	GCATAAACTTCTGGTTTGC
3xFLAG- 2xSTREP	ATGGACTATAAGGATCACGATGGAGATTATAAAGATCATGATATTGATTATAA GGATGACGATGATAAAGGATCAGCTGCTTCATGGTCACACCCACAATTTGAAA AAGGAGGTGGATCAGGAGGTGGTTCTGGTGGAGGATCTTGGTCTCATCCACAA TTCGAGAAGTCAGGA

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65 **Table S2. DNA sequences of coding regions, promoters, and a terminator**

Sequence identity (locus tag number) ^a	DNA sequence (5' -> 3')
<i>hmgA</i> (<i>mj_0705</i>)	ATGGAAAATTATAATGACATCCTTGAAAAAATGTTAAATGGAGAAAATAAAGCCATATCAATTAG ATAAAATGTTGGTTCAAAAATAGCAACTGAAATTAGAAGAAAATTTATGAAAAAAGGTGG GATTGAATTTAAGCATATATGCAATTACTCAATAGATGAAGAAATGGCTATGAAGAAGATATA GAAAATATGATTGGAGCTATACAGATTCATTGGGTTTTGCTGGGCCCTTAAAGATAAATGGGG AATATGCAAGGGGAGAGTTTTACATCCCATTTGGCAACAACCTGAAGGAGCTTTGGTGGCATCGGT TAATAGGGGTTGCTCAATAATAACAAAAATGTGGGGGGCAACTGTTAGGGTTATAGATGATAAG ATGACAAGAGCCCCTTGCTTAAAAACAAGAGTGTGTAGATGCAATAAAAGTCAGAGATTGGA TTAGAGAAAACCTTTGAGAGGATAAAGGAAGTTGCTGAATCAACAACAGGCATGGAAAGCTAAT AAAGATAGAGCCAATTTAATCGTTGGAAAGAAATCTATATCCAAGATTTGTATTTAAAACCTGGA GATGCCATGGGCATGAATATGGTTACAATTGCAACAGAGAGGCATGTAATTTTATAGAGGGGG AATTAAAAAAAGAAGGCATATTTGTTAAAACAGTTGCTGTGAGTGGAAATGCATGTGTAGATAA AAAGCCTAGTGGAAATGAACCTAATTAATGGTAGAGGAAATCTATTGTAGCAGAGGTATTTTA ACTGAGAAGGAAGTTAATAAATACCTAAAACCCTTCCCAAGCTATAGCTGAAGTAAATAGGT TAAAGAATTATATAGGTTTCAGCAATAAGCAATTCATGGGATTCAATGCCCATTTATGCAATAT CATTGGGGCAATATTTCTTAGCTACTGGGCAAGATGAGGCACATATAGTTGAGGGTAGTTTAGGA ATTACAATGGCTGAAGTTGAAGATGATGGATTATTTTTTCAGTAACTCTCCCAGATGTTCCCTA TTGGGACTGTTGGAGGAGGAACAAGGGTTGAAACAAAAAGAGTGTCTAGAGATGCTTGGTTG CTACGGAGATAAATAAGCTTTAAAATTTGCTGAAATTTGTTGAGCTGCAGTTTTAGCTGGGGAG TTATCTTTATTAGGAGCTTTAGCAGCTGGACATTTAGGAAAAGCTCATCAAGAAGCTCGGTAGAT AA
<i>fprA</i> (<i>mj_0748</i>)	ATGAAAAATATGAAAGCAGACGCAGCAAAATAGCTGATGGTGTATATTTGGGTGGGGTTTTAG ACTGGGACATAAGAATGTATCAGGCTACACATTAAGGAACAACATACAATGCCTATTTAGT CTTTGGAGATGAAAAGTTGCTTTAATAGACAACATACCAGGAACCTCCGCTCAAATGTGG GGGAGGATAAAAGATGCATTTGAAAAAGAGGGGAGGGAATTTAAAATGATGTAATCGTTCAAA ACCACGTAGAAAAGACCACAGTGGAGCTCTCCCTGAAATACACAAAAAATTTCCAGATGCACC AATATACTGTACTGAGGTAGCTGTTGAGGGACTTAAAAGCACTATCCATCATTAAAAGACGCT CAATTTAAGGTTGTTTACATACAGGAGATACAGTTGATTTAGGAGGAAAGACATTAACATTTCTAG AAGCTCCTCTATTACACTGGCCAGATAGTATGTTACCTTCTACAACGAAGGGGGAATTTTATT CTCAAACGATGCATTTGGACAGCATCTCTGCTTCCCAGCACACAAGAGATTTGATAAAGATATT CCAGAGTATGTGTTAATGGATGCAAACCAGAAGTTTATGCTAATTTAATTAATTCACCTCCATCA AGCTTGTATTAAGAAAATTTGAGGAAGTTATTTCAGTTGGGATTATTTAGAAAAGATAAAAAATGAT TGCCCCATCACACGGGCAGATATGGACAGACCCAATGAAAGTTATTAAGGCATATCAAGACTTT GCTACTGGTAAAGCAGCTAAGGATAAAGCAGTTATCGTTTTATGATACATGCACTACTCAACAC AAAAGATGGCTCATGCATTTGAGAGGGTTAATGAGTGAAGGAAATGATGTTGTAATGTATTT CTTACACTACGATGAGAGAAGTGAATGTTAAAGACATCTTAGATGCTAAGGCAGTTCTCTTT GGAATTTCCAACAATCTATGATGACCATATCCATCAATTTGGAGATATCATATACTACTTGAAG GATTGAAATTTAACAGAACAGGATTTAAGAGATTGGCGGTTACTTTTGGTTCAATGGGGGAGAG AGGTGGAGCAGTTGCTAAGATTGCTGAAGACTTGGCGAAATGTGGATTTGAAGTTATTAATCAA TATGAAGCTACTATGTCCTAACAGAGGATGAATTAACAACCTGCTACAATATGGGTAAAGAAAT TGGCTAAGAGAATTAAGAGATGAAGATTGAATGA
<i>P_{sla}</i> (<i>MFS40622_1341</i> for <i>sla</i>)	AAATATTCACAAAATTAATATTTATTTTTGGTCTTTTTTAAACATTATTCCTCATAGGTTTTAT AAATTTCTACTAAATATTTGGATATATCAAAATTCGTTTTTAAACCATTTAAAAAAGTTGCAAAAT GTTTGTAATTTTTATTTTAGAATTTGTCAAAAATTTATATGATAAATTTGAAATATCAAAGAT ATAATAGAAATTCATATCGTAAAACCTATTTGTTATAACCGTTTCATATCGTAAGCTTTATATAGT AGTTTCGTCGAAGGTATATACCGTCAATCAAATAACAACATAA
<i>P_{flaB1}</i> (<i>mj_0891</i> for <i>flaB1</i>)	GTTTATCCATTAGTTTATATAAATTTGGATTTGTTTAAATTTATTTGGCATAATCATTACGTTA ATTTGCTATTTAAGATTGACTATCGTTTTTACCTACACAACCTCTACATAAACACCGATAAAA TTGACTACGCAATAAATACGCAAAAAGAATATATTTTAAAGAAAATTACAGTTTATAGTGAGGT GGATTGAGGAAAGTGTGGGGGAATTTGTTGGGGGAGGAAAACTACTACAAGCAATTTGAGGGATTA ATATACTCAGGTGATATGAGATGAAG
<i>T_{sla}</i> (<i>MFS40622_1341</i> for <i>sla</i>)	ATCCTCTAAATCTTTTTTTATTTTTTTAGATGAC

66 ^a P, promoter; T, terminator; *hmgA*, *fprA*, *sla*, and *flaB1*, genes for HMG CoA reductase, F₄₂₀H₂ oxidase,
67 S-layer protein, and flagellin, respectively.

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