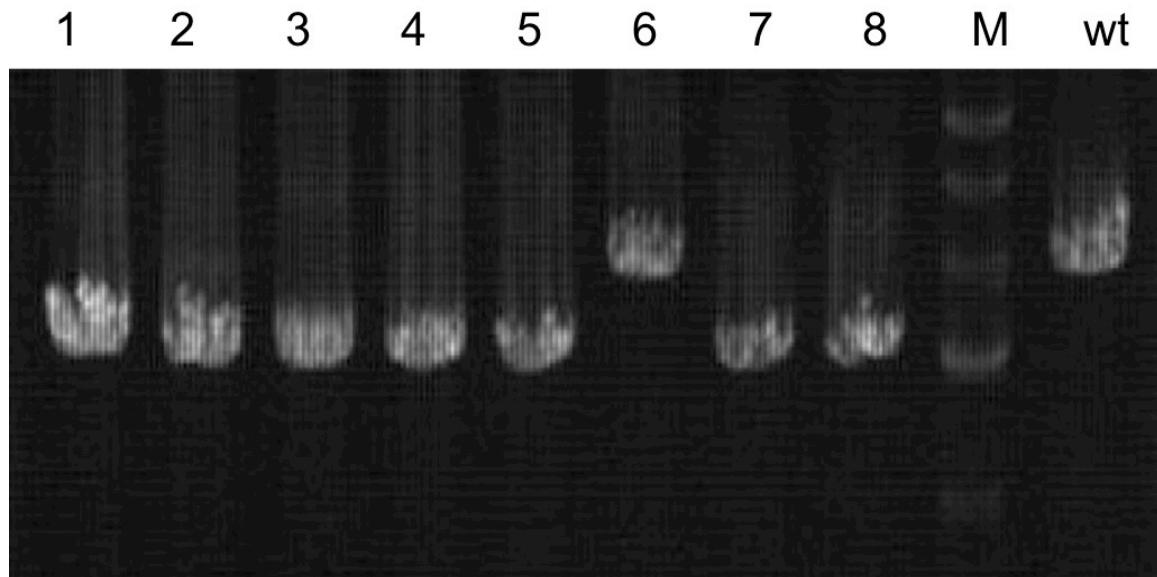


484

485 **Figure S1**

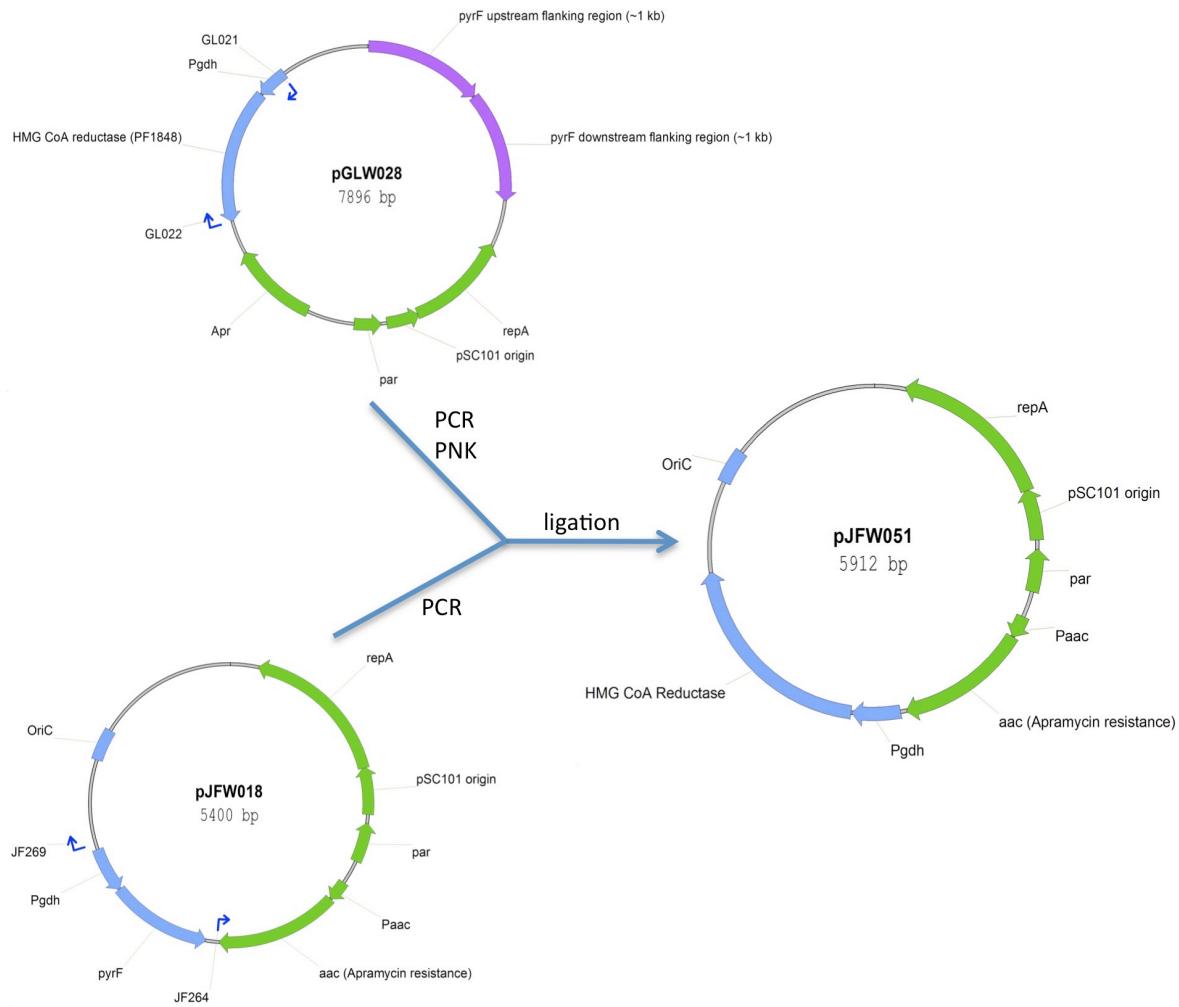
486



487

488 **Figure S1.** Screening of putative *trpAB* marker replacement mutants. A 3.9kb
489 product is produced from the wild type, while a 3.1kb band is produced by the
490 targeted marker replacement.

491 **Figure S2**



492

493 **Figure S2.** Construction of pJFW051. A 4.4kb fragment was amplified by PCR
 494 from pJFW018 using primers JF264 and JF269. The *P_{gdh}-hmg* cassette (22) was
 495 amplified from pGLW28 (21) using primers GL021 and GL022, treated with T4
 496 polynucleotide kinase and ligated into the 4.4kb fragment to produce pJFW051.

497

498

499 **Table S1. Primer Sequences**

Primer	Sequence (5' to 3')
pyrF500bpF	AAAACAGATATCCGAAATACTCGA

pyrF500bpF GGTTATCTCCCAATCTTATCCCT
pyrF250bpF ACGAGGCAATAAAGTCGACGCAA
pyrF250bpF TAAGATCTCCTTATCAGAGAGCT
pyrF150bpF GAGATTGTCAAGAGACTCTATGAT
pyrF150bpF TCTGTGGTCTTATCGAACTCCGCA
pyrF100bpF TACTTGGGCAGATAAAATTGGAAG
pyrF100bpF AGCATCTGCTTGCAATTCCCTCT
pyrF50bpF AGTCGTCTTAAAGGGAGACCAACT
pyrF50bpR ATCTCTTAACATCCTTCTTGCTT
pyrF40bpF AGGGAGACCAACTCCCCAGGGAAATATT
pyrF40bpR TTGATTATCTCTTAACATCCTTCTTGCTT
pyrF30bpF ACTCCCCAGGGAAATATTGGAGGT
pyrF30bpR CTTAACATCCTTCTTGCTTAGGG
pyrF20bpF GAAATATGGAGGTGGCTGAATGATTGTAC
pyrF20bpR CTTCTGCTTAGGGGGTGCTTATCTTGAG
pyrF10bpF AGGTGGCTGAATGATTGTACTAGCGTTGGA
pyrF10bpR AGGGGGTGCTTATCTTGAGCTCCATTCTT
pyrF0bpF ATGATTGTACTAGCGTTGGACGTGTATGAG
pyrF0bpR TTATCTTGAGCTCCATTCTTCACCTCCTC
GL021 GATTGAAAATGGAGTGAGCTGAG
GL022 TCATCTCCAAGCATTATGAG
GL055 AGAGAGAGGCATGCCACCTACCTCCTATATTGTTCCATG
GL058 GAGAGAGGGCGCGCCGTCAAGAGGATGATTAGGTAGAGC
GL158 TCAAATGCTCATCATTAGTTTATG
JF264 CTCCAACGTCATCTCGTTCTC
JF269 TCCATTGAAATTGTGCTCTAG
JF307 AATGAGCATGCGTTATAAACACATTCTC
JF311 AAGCTCTAGAAAAGATATTGGAGGTTCC
JF357 TTAATATGCCCAAGGCCTACTCCTTGCCGTACACCATATGATCGTGAG
JF358 AGGAGTAGGCCTGGGCATATTACGTGCTGACTGATAGAGGATTAGCTAA
T
TGAAC
JF355.1 AGGAGTAGGCCTGGGCATATTACGTGCTGACTGATTGAAAATGGAGTGA
TGAG
JF356.1 TTAATATGCCCAAGGCCTACTCCTTGCCGTGATTATCTTGAGCTCCATT
TCAC
JF322 ACTGCCAGCATGCTCGTAG
JF326 AGATGCTAGCGAAGGGGATGAAGTTG
JF359 TTCGTGTTATGATGCCACTAACCGTCTGCTCGACCTCAGATTCAAAAC
TCATAG
JF360 TTAGTGGGCATCATAACACGAAAGAGAGTGATTAGAGCCTGTCGTAGACC
JF355.2 ACGCTTAGTGGGCATCATAACACGAAAGAGAGTGATTGAAAATGGAGTGA
TGAG
JF356.2 TGTTATGATGCCACTAACCGTCTGCTCGGATTATCTTGAGCTCCATT
TCACC
JF392 AAGAGAAGTCGGCAATTCAC

JF393	ACGAAACCAGGGTCTCGCGTAACACGCTCACTCACCAAACCACATTTTG C
JF394	TGTTACGCCGAGACCCGGTTCGTCTCATTTAGGGATCTAAAATTTGT/ AAC
JF395	TAGGGAGTATAAGAGAAGAGC
JF355.3	TGTTACGCCGAGACCCGGTTCGTCTCATGATTGAAAATGGAGTGAGCT AG
JF356.3	AAACCGGGTCTCGCGTAACACGCTCACTGATTATCTTGAGCTCCATT TTCACC
WN008	CCAACCACATTCAGTTCACCTCCGCCTT
WN009	GTGAAGTGAATGTGGTTGGTAGTTGG
WN010	CTAAAAAAAGATTTAGATCCCTAAAAGTTCCCTCTA

500

500

REFERENCES

- 501 1. **Alani, E., L. Cao, and N. Kleckner.** 1987. A method for gene disruption that
502 allows repeated use of URA3 selection in the construction of multiply
503 disrupted yeast strains. *Genetics* **116**:541-5.
- 504 2. **Allers, T.** 2011. Personal Communication.
- 505 3. **Allers, T., H. P. Ngo, M. Mevarech, and R. G. Lloyd.** 2004. Development
506 of additional selectable markers for the halophilic archaeon *Haloferax*
507 *volcanii* based on the *leuB* and *trpA* genes. *Appl Environ Microbiol* **70**:943-
508 53.
- 509 4. **Bitan-Banin, G., R. Ortenberg, and M. Mevarech.** 2003. Development of a
510 gene knockout system for the halophilic archaeon *Haloferax volcanii* by use
511 of the *pyrE* gene. *J Bacteriol* **185**:772-8.
- 512 5. **Blumer-Schuette, S. E., I. Kataeva, J. Westpheling, M. W. W. Adams,**
513 **and R. M. Kelly.** 2008. Extremely thermophilic microorganisms for biomass
514 conversion: status and prospects. *Current Opinion in Biotechnology* **19**:210-
515 217.
- 516 6. **Bridger, S. L., S. M. Clarkson, K. Stirrett, M. B. Debarry, G. L. Lipscomb,**
517 **G. J. Schut, J. Westpheling, R. A. Scott, and M. W. Adams.** 2011.
518 Deletion Strains Reveal Metabolic Roles for Key Elemental Sulfur-
519 Responsive Proteins in *Pyrococcus furiosus*. *J Bacteriol* **193**:6498-504.
- 520 7. **Chandrayan, S. K., P. M. McTernan, R. C. Hopkins, J. Sun, F. E. Jenney,**
521 **Jr., and M. W. Adams.** 2012. Engineering hyperthermophilic archaeon

- 522 *Pyrococcus furiosus* to overproduce its cytoplasmic [NiFe]-hydrogenase. J
523 Biol Chem **287**:3257-64.
- 524 8. **Chung, D., J. R. Huddleston, J. Farkas, and J. Westpheling.** 2011.
525 Identification and characterization of Cbel, a novel thermostable restriction
526 enzyme from *Caldicellulosiruptor bescii* DSM 6725 and a member of a new
527 subfamily of HaeIII-like enzymes Journal of Industrial Microbiology &
528 Biotechnology.
- 529 9. **Deng, L., H. Zhu, Z. Chen, Y. X. Liang, and Q. She.** 2009. Unmarked gene
530 deletion and host-vector system for the hyperthermophilic crenarchaeon
531 *Sulfolobus islandicus*. Extremophiles **13**:735-46.
- 532 10. **Farkas, J., D. Chung, M. Debarry, M. W. Adams, and J. Westpheling.**
533 2011. Defining Components of the Chromosomal Origin of Replication of the
534 Hyperthermophilic Archaeon, *Pyrococcus furiosus*, Needed for Construction
535 of a Stable Replicating Shuttle Vector. Appl Environ Microbiol **77**:6343-6349.
- 536 11. **Fiala, G., and K. O. Stetter.** 1986. *Pyrococcus furiosus* sp. nov. represents
537 a novel genus of marine heterotrophic archaebacteria growing optimally at
538 100°C. Archives of Microbiology **145**:56-61.
- 539 12. **Fukui, T., H. Atomi, T. Kanai, R. Matsumi, S. Fujiwara, and T. Imanaka.**
540 2005. Complete genome sequence of the hyperthermophilic archaeon
541 *Thermococcus kodakaraensis* KOD1 and comparison with *Pyrococcus*
542 genomes. Genome Res **15**:352-63.
- 543 13. **Grogan, D. W., and K. R. Stengel.** 2008. Recombination of synthetic
544 oligonucleotides with prokaryotic chromosomes: substrate requirements of

- 545 the *Escherichia coli*/λRed and *Sulfolobus acidocaldarius* recombination
546 systems. Molecular Microbiology **69**:1255-1265.
- 547 14. **Haldenby, S.** 2007. Genetic Analysis of RadB, a Paralogue of the Archaeal
548 Rad51/RecA Homologue, RadA. University of Nottingham.
- 549 15. **Haldenby, S., M. F. White, and T. Allers.** 2009. RecA family proteins in
550 archaea: RadA and its cousins. Biochemical Society Transactions **37**:102-
551 107.
- 552 16. **Hopkins, R. C., J. Sun, F. E. Jenney, Jr., S. K. Chandrayan, P. M.**
553 **McTernan, and M. W. Adams.** 2011. Homologous Expression of a
554 Subcomplex of *Pyrococcus furiosus* Hydrogenase that Interacts with
555 Pyruvate Ferredoxin Oxidoreductase. PLoS ONE **6**:e26569.
- 556 17. **Horton, R. M., Z. L. Cai, S. N. Ho, and L. R. Pease.** 1990. Gene splicing by
557 overlap extension: tailor-made genes using the polymerase chain reaction.
558 Biotechniques **8**:528-535.
- 559 18. **Ishikawa, K., M. Watanabe, T. Kuroita, I. Uchiyama, J. M. Bujnicki, B.**
560 **Kawakami, M. Tanokura, and I. Kobayashi.** 2005. Discovery of a novel
561 restriction endonuclease by genome comparison and application of a wheat-
562 germ-based cell-free translation assay: *Pabl* (5'-GTA/C) from the
563 hyperthermophilic archaeon *Pyrococcus abyssi*. Nucleic Acids Research
564 **33**:e112.
- 565 19. **Komori, K., T. Miyata, J. DiRuggiero, R. Holley-Shanks, I. Hayashi, I. K.**
566 **O. Cann, K. Mayanagi, H. Shinagawa, and Y. Ishino.** 2000. Both RadA

- 567 and RadB Are Involved in Homologous Recombination in *Pyrococcus*
568 *furirosus*. The Journal of Biological Chemistry **275**:33782-33790.
- 569 20. **Kurosawa, N., and D. W. Grogan.** 2005. Homologous recombination of
570 exogenous DNA with the *Sulfolobus acidocaldarius* genome: properties and
571 uses. FEMS Microbiol Lett **253**:141-9.
- 572 21. **Lipscomb, G. L., K. Stirrett, G. J. Schut, F. Yang, F. E. Jenney, Jr., R. A.**
573 **Scott, M. W. Adams, and J. Westpheling.** 2011. Natural competence in
574 the hyperthermophilic archaeon *Pyrococcus furirosus* facilitates genetic
575 manipulation: construction of markerless deletions of genes encoding the
576 two cytoplasmic hydrogenases. Appl Environ Microbiol **77**:2232-8.
- 577 22. **Matsumi, R., K. Manabe, T. Fukui, H. Atomi, and T. Imanaka.** 2007.
578 Disruption of a sugar transporter gene cluster in a hyperthermophilic
579 archaeon using a host-marker system based on antibiotic resistance. J
580 Bacteriol **189**:2683-91.
- 581 23. **Miozzari, G., P. Niederberger, and R. Hutter.** 1977. Action of tryptophan
582 analogues in *Saccharomyces cerevisiae*. Arch Microbiol **115**:307-16.
- 583 24. **Peck, R. F., S. DasSarma, and M. P. Krebs.** 2000. Homologous gene
584 knockout in the archaeon *Halobacterium salinarum* with *ura3* as a
585 counterselectable marker. Mol Microbiol **35**:667-76.
- 586 25. **Pingoud, V., C. Conzelmann, S. Kinzebach, A. Sudina, V. Metelev, E.**
587 **Kubareva, J. M. Bujnicki, R. Lurz, G. Luder, S. Y. Xu, and A. Pingoud.**
588 2003. PspGI, a type II restriction endonuclease from the extreme
589 thermophile *Pyrococcus* sp. structural and functional studies to investigate

- 590 an evolutionary relationship with several mesophilic restriction enzymes. J
591 Mol Biol **329**:913-29.
- 592 26. **Pritchett, M. A., J. K. Zhang, and W. W. Metcalf.** 2004. Development of a
593 markerless genetic exchange method for *Methanosarcina acetivorans* C2A
594 and its use in construction of new genetic tools for methanogenic archaea.
595 Appl Environ Microbiol **70**:1425-33.
- 596 27. **Sandler, S. J., P. Hugenholtz, C. Schleper, E. F. DeLong, N. R. Pace,**
597 **and A. J. Clark.** 1999. Diversity of *radA* genes from cultured and uncultured
598 Archaea: Comparative analysis of putative RadA proteins and their use as a
599 phylogenetic marker. Journal of Bacteriology **181**:907-915.
- 600 28. **Sandler, S. J., L. H. Satin, H. S. Samra, and A. J. Clark.** 1996. *recA*-like
601 genes from three archaean species with putative protein products similar to
602 Rad51 and Dmc1 proteins of the yeast *Saccharomyces cerevisiae*. Nucleic
603 Acids Res **24**:2125-32.
- 604 29. **Sato, T., T. Fukui, H. Atomi, and T. Imanaka.** 2005. Improved and
605 Versatile Transformation System Allowing Multiple Genetic Manipulations of
606 the Hyperthermophilic Archaeon *Thermococcus kodakaraensis*. Applied and
607 Environmental Microbiology **71**:3889-3899.
- 608 30. **Sato, T., T. Fukui, H. Atomi, and T. Imanaka.** 2003. Targeted Gene
609 Disruption by Homologous Recombination in the Hyperthermophilic
610 Archaeon *Thermococcus kodakaraensis* KOD1. Journal of Bacteriology
611 **185**:210-220.

- 612 31. **Spitalny, P., and M. Thomm.** 2008. A polymerase III-like reinitiation
613 mechanism is operating in regulation of histone expression in archaea. Mol
614 Microbiol **67**:958-70.
- 615 32. **Stetter, K. O.** 1996. Hyperthermophilic prokaryotes. FEMS Microbiology
616 Reviews **18**:149-158.
- 617 33. **Stothard, P.** 2000. The sequence manipulation suite: JavaScript programs
618 for analyzing and formatting protein and DNA sequences. Biotechniques
619 **28**:1102, 1104.
- 620 34. **Strand, K. R., C. Sun, T. Li, F. E. Jenney, Jr., G. J. Schut, and M. W.**
621 **Adams.** 2010. Oxidative stress protection and the repair response to
622 hydrogen peroxide in the hyperthermophilic archaeon *Pyrococcus furiosus*
623 and in related species. Arch Microbiol **192**:447-59.
- 624 35. **Tang, X., S. Ezaki, S. Fujiwara, M. Takagi, H. Atomi, and T. Imanaka.**
625 1999. The tryptophan biosynthesis gene cluster trpCDEGFBA from
626 *Pyrococcus kodakaraensis* KOD1 is regulated at the transcriptional level
627 and expressed as a single mRNA. Mol Gen Genet **262**:815-21.
- 628 36. **Toyn, J. H., P. L. Gunyuzlu, W. H. White, L. A. Thompson, and G. F.**
629 **Hollis.** 2000. A counterselection for the tryptophan pathway in yeast: 5-
630 fluoroanthranilic acid resistance. Yeast **16**:553-60.
- 631 37. **Wang, G., S. P. Kennedy, S. Fasiludeen, C. Rensing, and S. DasSarma.**
632 2004. Arsenic resistance in *Halobacterium* sp. strain NRC-1 examined by
633 using an improved gene knockout system. J Bacteriol **186**:3187-94.

634 38. **Woods, W. G., and M. L. Dyall-Smith.** 1997. Construction and analysis of a
635 recombination-deficient (*radA*) mutant of *Haloferax volcanii*. Molecular
636 Microbiology **23**:791-797.

637

638