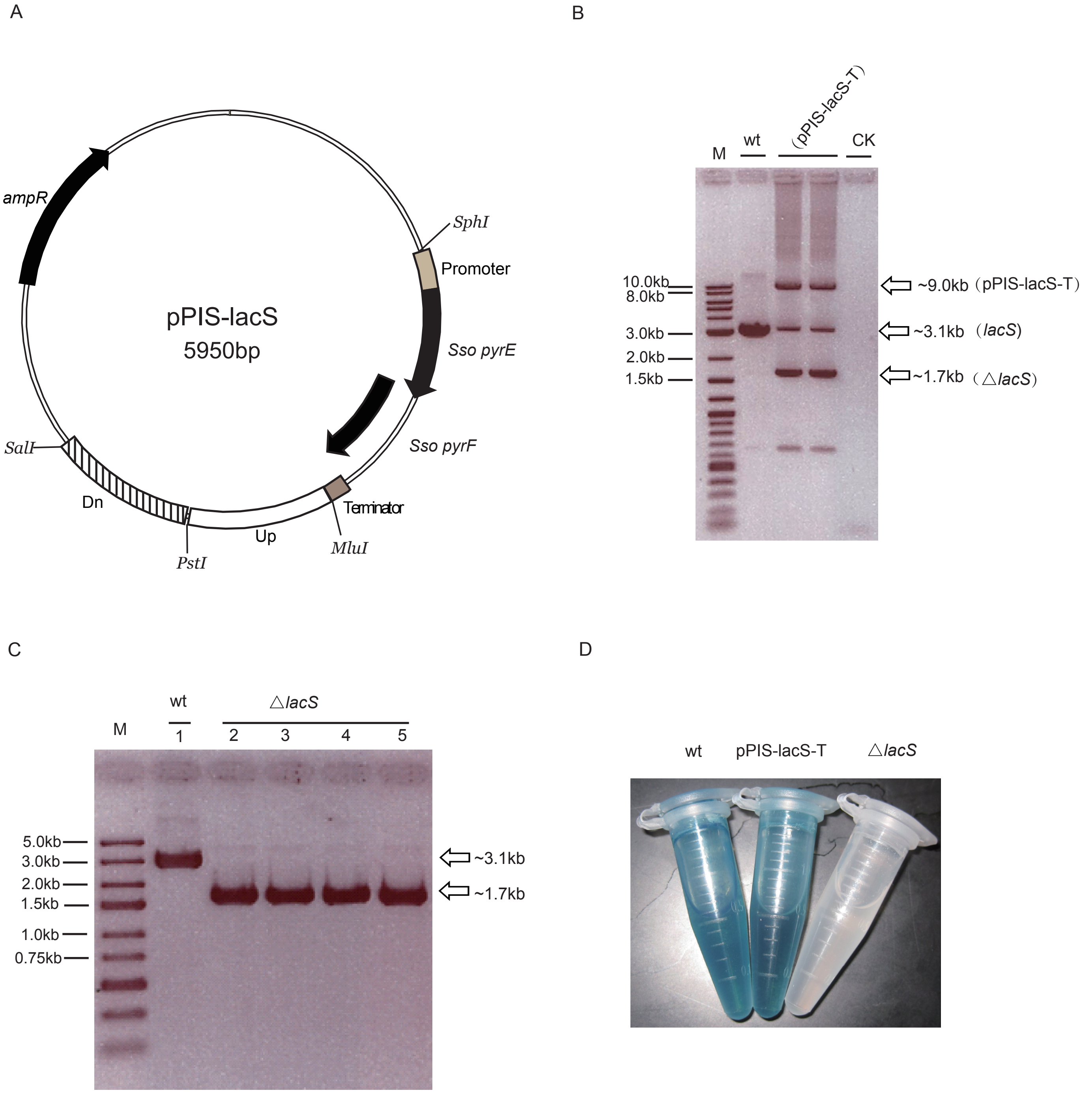
A
pPIS-lacS
5950bp
ampR
SphI
Promoter
Sso pyrE
Sso pyrF
Terminator
MluI
Up
PstI
Dn
SalI
B
M
wt
（pPIS-lacS-T）
CK
10.0kb
8.0kb
3.0kb
2.0kb
1.5kb
~9.0kb（pPIS-lacS-T）
~3.1kb （lacS)
~1.7kb（△lacS）
C
M
wt
△lacS
1
2
3
4
5
5.0kb
3.0kb
2.0kb
1.5kb
1.0kb
0.75kb
~3.1kb
~1.7kb
D
wt
pPIS-lacS-T
△lacS

Fig. S1. Construction of a *lacS* deletion on M.16.04 RJW002 (Δ*pyrEF*) chromosome via conventional Plasmid Integration and Segregation (PIS) method. (A) Cloning of *lacS* knockout plasmid (pPIS-lacS). Diagram shows major features of pPIS-lacS, containing *pyrEF* marker from *S. solfataricus* P2, upstream (Up-arm) and downstream (Dn-arm) flanking region of *lacS*. For flow chart of constructing *lacS* deletion mutants via PIS strategy, see reference (1) (B) Identification of pPIS-lacS transformants (pPIS-lacS-T) by PCR. *lacS* alleles from wild-type and two transformants were analyzed using primer pairs *lacS* flankP-F/R. The expected size range of PCR products are as follows: wild-type gene, ~3.1kb; transformants allele: ~9.0kb; mutants allele: ~1.7kb. 2-Log DNA Ladders (0.1-10.0kb, NEB) were loaded in lane M. (C) Identification of *lacS* deletion mutants using *lacS* flankP-F/R. Lane M: GeneRuler Express DNA Ladder. Lane 1 and 2-5 showed amplification of *lacS* alleles from wild-type strain and *lacS* deletion mutants respectively. (D) X-gal staining of wild-type strain, pPIS-lacS-T and *lacS* deletion mutant.

Table. S1 Primers used in this study

Primers	Sequence (5’---3’)[a]
SsopyrEF-F	5'CATGCCATGGCATCAAACTTATGTCTCATTACAAT3'
SsopyrEF-R	5'ACTAACGCGTTACTGGCGTAGTTTAACATAAGG3'
lacS-Up-F	5'AAAACGCGTCCTTATCTCTGGATTGATGC3'
lacS-Up-R	5'AGAACTGCAGAGACCAACCGAACCTAAAGT3'
lacS-Dn-F	5'AGAACTGCAGATAAGACCATTAAGGCACTAGAC3'
lacS-Dn-R	5'AAGCGTCGACTTACTAAACAGAGGAGT3'
lacS-flankP-F	5'TACGGGAAGTAACACGGAGC3'

lacS-flankP-R	5'GTGGCACTTATTTGAGGGTATTA3'
SsolacS-F	5’AAGCGTCGACCTCCTCTTATTATTAGAATTGTACGC3’
SsolacS-R	5’AAAACGCGTCCTAGTGTTGCAAGGCAGAT3’
argD-Up-F	5' CGCGGATCCCCTTGACCTTGTAAAATCACTGT3'
argD-Up-R	5'CGGGGTACCCTGTTCTGACATCTTTCTTTCTTAT3'
argD-Dn -F	5'CGGGGTACCAGTTCACAATAGTTTTTTATATTAATCTC3'
argD-Dn -R	5'AAGCGTCGACTGGAAACTTACCATTAGGCTG3'
argD-flankP-F	5'ATCCCAAGGTTACCGTGAA 3’
argD-flankP-R	5'TATCCTTTCACCAGGCTTTG3’
pyrEF-flankP-F	5'CGCTACTCTATAGCCTTCACTTC3'
pyrEF-flankP-R	5'AGATGATGAGGTGGACAGGTT3'
SsoargD-F	5'CATGCCATGGATTCTCCAATATATGGGGTTT3'
SsoargD-R	5'ACATGCATGCGAAACGGCCGTACTTTCTTACTGCTTTGATCAA3'
SsoargD-R1	5'ACTAACGCGTTACTTTCTTACTGCTTTGATCAA3'
SsoargD-F2	5'AAGCGTCGACATTCTCCAATATATGGGGTTT3'
SsoargD-R2	5'TCACCCCGGGTACTTTCTTACTGCTTTGATCAA3'
upsEF-Up-F	5'TCGCGTCGACGGTAATCCAGACAAGAACGC3'
upsEF-Up-R	5'CGGGGTACCAACAGAATACTCTTGAAGAATATTCA3'
upsEF-Dn-F	5'CGGGGTACCTAAAAAGGTAAATTATTTTCGATG3'
upsEF-Dn-R	5'ACGCGGATCCTTCCTTAACAGGTATGTTGCTAT3'
upsEF-Tg-F	5'ACATGCATGCAACATATACAAGTGGCGAGTC3'
upsEF-Tg-R	5'GAAACGGCCGTTAGATTAAGTTAAAGATAACGTTTC3'

upsEF-flankP-F	5'ATTCCCTCGAAATCTCTGC3'
upsEF-flankP-R	5'CGCTGTTCCACATTTTAGAG3'

[a] Added restriction sites are underlined.

Reference

1. **Deng L, Zhu H, Chen Z, Liang YX, She Q.** 2009. Unmarked gene deletion and host-vector system for the hyperthermophilic crenarchaeon Sulfolobus islandicus. Extremophiles **13:**735-746.