Supplementary data

Table S1

Methanococcus maripaludis strains used in this study.

Strains	Genotype or description	Source or reference
S2	Wild-type *M. maripaludis*	*(Whitman et al., 1986)*
Mm900	S2 Δ*hpt*	(Moore and Leigh, 2005)
FSD6	Mm900 Δ*sel*D::*pac*	This study
FSD10	Mm900 Δ*sel*D	This study
FSD102	Mm900 Δ*sel*D Δ*pstk*::*pac*	This study
FSD103	Mm900 Δ*sel*D Δ*spcS*::*pac*	This study
FHM1	Mm900 Δ*hrsM*::*pac*	This study
FHM11	Mm900 Δ*hrsM*::*pac* Δ*pstk*::*neo*	This study

Table S2
Plasmids used in this study.

Plasmids	Description	Source or reference
pCR2.1TOPO	Amp^r Kan^r cloning vector	Invitrogen
pCRPrtNeo	*hmv* promoter–*hpt* fusion + Neo^r cassette in pCR2.1TOPO	(Moore and Leigh, 2005)
pIJA03	Pur^r methanogen integration vector	(Stathopoulos et al., 2001)
pIJA03hpt	the 745 bp BglII/XhoI fragment containing the hmv promoter–hpt fusion was excised from pCRPrtNeo and ligated into BglII/XhoI digested pIJA03	This study
pIJA04	from pIJA03hpt the *pac* gene was removed by XbaI/SacII- digestion and religation after blunting overhangs	This study
pJK301	Amp^R cloning vector used to construct markerless deletions in *Methanosarcina barkeri*; contains a *pac–hpt* operon flanked by two *flp* recombinase recognition (*FRT*) sites	(Welander and Metcalf, 2008)
pJK301Δhpt	the *Methanosarcina acetivorans hpt* gene was removed from pJK301 by NdeI/MfeI-digestion and religation after blunting overhangs; the KpnI site adjacent to the *mcr*	This study
	terminator was removed by site-directed mutagenesis	
pJKM12	the *hmv* promoter–*hpt–mcr* terminator cassette encoded on pIJA04 was excised with XhoI/KpnI and ligated into XhoI/KpnI digested pJK301Δhpt	This study
pJKM12N	AatII-cut neo^R PCR product (using primers 12NEOF and12NEOR and pCRPrtNeo as template) cloned into AatII-cut pJKM12; correct orientation confirmed by sequencing; replaces Pur^R with Neo^R	This study
pJKM12ΔselD	deletion construct of *selD* in pJKM12. PCR products using primers SELDNF-SELDNR and SELDCF-SELDCR cloned into NotI/BamHI and HindIII/XhoI sites of pJKM12	This study
pJKM12ΔpstK	deletion construct of *pstK* in pJKM12. PCR products using primers PSTKNF-PSTKNR and PSTKCF-PSTKCR cloned into NotI/BamHI and HindIII/XhoI sites of pJKM12	This study
pJKM12NΔpstK	deletion construct of *pstK* in pJKM12N. PCR products using primers PSTKNF-PSTKNR and PSTKCF-PSTKCR cloned into NotI/BamHI and HindIII/XhoI sites of pJKMN12	This study
pJKM12ΔhrsM	deletion construct of *hrsM* in pJKM12. PCR products using primers HRSMNF-HRSMNR and HRSMCF-HRSMCR cloned into NotI/BamHI and HindIII/XhoI sites of pJKM12	This study
pJKM12ΔspcS	deletion construct of *spcS* in pJKM12. PCR products using primers SPCSNF-SPCSNR and SPCSCF-SPCSCR cloned into NotI/BamHI and HindIII/XhoI sites of pJKM12	This study
pMEV2	Neo^r shuttle vector	(Lin and Whitman, 2004)
pECSD	PstI/XbaI-cut *E. coli sel*D PCR product (using primers ECSDF and ECSDR) cloned into NsiI/XbaI-cut pMEV2	This study
pECSA	NsiI/XbaI-cut *E. coli selA* PCR product (using primers ECSAF and ECSAR) was ligated to the *hpt*-containing XbaI/NarI restriction fragment from pCRPrtNeo; this construct was then ligated into NsiI/NarI-digested pMEV2	This study
pFLPH	*S. cerevisiae* Flp recombinase expression vector. PstI/XbaI- cut *flp* PCR product(using primers FLPF and FLPR) was ligated into NsiI/XbaI-digested pMEV2	This study

Table S3

Oligonucleotides used in this study. Added recognition sites for restriction endonucleases are underlined.

Primer	Sequence	added restriction site
FLPF	AACTGCAGATGCCACAATTTGGTATATT	PstI
FLPR	GCTCTAGATTATATGCGTCTATTTATGT	XbaI
12NEOF	GGAATTCGACGTCATGATTGAACAAGATGGATTGCA	AatII
12NEOR	GGAATTCGACGTCTCAGAAGAACTCGTC	AatII
SELDNF	ATAAGAATGCGGCCGCGAGGGGCAACAGTATTTAGCG	NotI
SELDNR	CGGGATCCCTGGTAGAATTACATGCCGC	BamHI
SELDCF	CCCAAGCTTCCAGAATACAAGGATAAATTAA	HindIII
SELDCR	CCGCTCGAGGTTATACATGCCGACAGTAC	XhoI
PSTKNF	ATAAGAATGCGGCCGCGTCTGCTCCGATTTTGACGACTTCGTTAG	NotI
PSTKNR	CGGGATCCCAAAGGCGCTTTGAGATATA	BamHI
PSTKCF	CCCAAGCTTTCTGAGAAGTTAGATTTTGA	HindIII
PSTKCR	CCGCTCGAGCTTGCATCCGGAGTTTCTCC	XhoI
HRSMNF	ATAAGAATGCGGCCGCTCCAGAAACATCTTCTATAAACAAAC	NotI
HRSMNR	CGGGATCCGGGTCCATGGTATCACTATTATTATACGTTTATTCTT	BamHI
HRSMCF	CCCAAGCTTAGGGTAAAAAACCAAGAAACC	HindIII
HRSMCR	CCGCTCGAGAATTACTTGATGAAACATCA	XhoI
SPCSNF	ATAAGAATGCGGCCGCATTATCTCGAATAAGTTCTC	NotI
SPCSNR	CGGGATCCCCTTCAATATTAAAATCGAGCATTATATCACG	BamHI
SPCSCF	CCCAAGCTTTGGAAACTGTTATTTGGGAACTTATACC	HindIII
SPCSCR	CCGCTCGAGAATTTTAATTGTACTACCAT	XhoI
YBBBNF	ATAAGAATGCGGCCGCCCCCTACCTGTAACGTATGC	NotI
YBBBNR	CGGGATCCCACCAGAGATACAGTAGTAAAA	BamHI
YBBBCF	CCCAAGCTTGGATTTAAAGTAAAAAGACTTGTAGGTG	HindIII
YBBBCR	CCGCTCGAGCCAAATGTTGGAAAATCAAC	XhoI
ECSDF	AACTGCAGATGAGCGAGAACTCGATTCGTTTGACCC	PstI
ECSDR	GCTCTAGATTAACGAATCTCAACCATGG	XbaI
ECSAF	CCAATGCATATGACAACCGAAACGCGTTCCCTCTAT	NsiI
ECSAR	GCTCTAGAGTCATTTCAACAACATCTCCA	XbaI
FRCA-F	AAGACACGAAGGGCATACCAAACTT	
FRCA-R	GGCAAATTCCACAGAATCTCGAAA	
VHCD-F	GTAGTGGCAATCTCCAAGGTGGC	
VHCD-R	GGTGCAGATACAGCAGGAGTTGGTA	
VHU-F	ATGTTACCAGTGAGGATACGGTGCA	
VHU-R	TGCAACGAATACAGCATCAGCG	

Figure S1
Generation of marked and markerless deletion mutants of *selD* (MMP0904) in *M. maripaludis* Mm900. (A) Disruption of the gene encoding SelD in *M. maripaludis* Mm900 by the *FRT-pac-FRT* cassette as a result of two homologous recombinations upon transformation with suicide plasmid pJKM12ΔselD gave rise to six clones (named FSD1-FSD6). From the FSD6 strain the *pac*-cassette was removed by *flp*-recombinase mediated recombination to yield strain FDS10. (B) Replacement of wild-type *selD* in strain Mm900 (lane 7) with the *FRT-pac-FRT* cassette was confirmed by Southern hybridization. Lanes 1-6: FSD1-FSD6. Genomic DNAs were digested with ClaI and MscI and hybridized with a DIG-labeled probe complementary to a 640bp fragment overlapping the upstream flanking region and first 230 bp of MMP0904. (C) Southern hybridization confirming the markerless deletion of *selD* in strain FSD10. Genomic DNA of strains Mm900 (lane 1), FSD6 (lane 2) and FSD10 (lane 3) was digested with ClaI and MscI and hybridized with the same probe as in (B).

Figure S2
Generation of a marked deletion mutant of *pstK* (MMP1490) in *M. maripaludis* strain FSD10. (A) The gene encoding PSTK in the *M. maripaludis* FSD10 strain was disrupted by the *FRT-pac-FRT* cassette as a result of two homologous recombinations upon transformation with suicide plasmid pJKM12ΔpstK. After verification of the genotype, one randomly selected *pstK* mutant was designated FSD102. (B) Disruption of *pstK* was confirmed by Southern hybridization. Genomic DNA of 3 randomly selected puromycin resistant clones (lane 1-3) and of FSD10 (lane 4) was digested with PacI and NsiI and hybridized with a DIG-labeled probe complementary to a 530 bp fragment overlapping the last70 bp of MMP1490 and the adjacent downstream flanking region.

Figure S3
Generation of a marked deletion mutant of *spcS* (MMP0595) in *M. maripaludis* strain FSD10. (A) The gene encoding SepSecS in the *M. maripaludis* FSD10 strain was disrupted by the *FRT-pac-FRT* cassette as a result of two homologous recombinations upon transformation with suicide plasmid pJKM12ΔspcS. After verification of the genotype, one randomly selected *spcS* mutant was designated FSD103. (B) Disruption of *spcS* was confirmed by Southern hybridization. Genomic DNA of FSD103 (lane 1) and of FSD10 (lane 2) was digested with BamHI and hybridized with a DIG-labeled probe complementary to a 480 bp fragment overlapping the upstream flanking region and first 23 bp of MMP0595.

Figure S4
Generation of a marked deletion mutant of *pstK* (MMP1490) in *M. maripaludis* strain FHM1. (A) The gene encoding PSTK in the *M. maripaludis* FHM1 strain was disrupted by the *FRT-neo-FRT* cassette as a result of two homologous recombinations upon transformation with suicide plasmid pJKM12NΔpstK. After verification of the genotype, one randomly selected *pstK* mutant was designated FHM11. (B) Disruption of *pstK* was confirmed by Southern hybridization. Genomic DNA of FHM11 (lane 1) and of FHM1 (lane 2) was digested with HindIII and hybridized with a DIG-labeled probe complementary to a 530 bp fragment overlapping the upstream flanking region and first 320 bp of MMP1490.

Figure S1

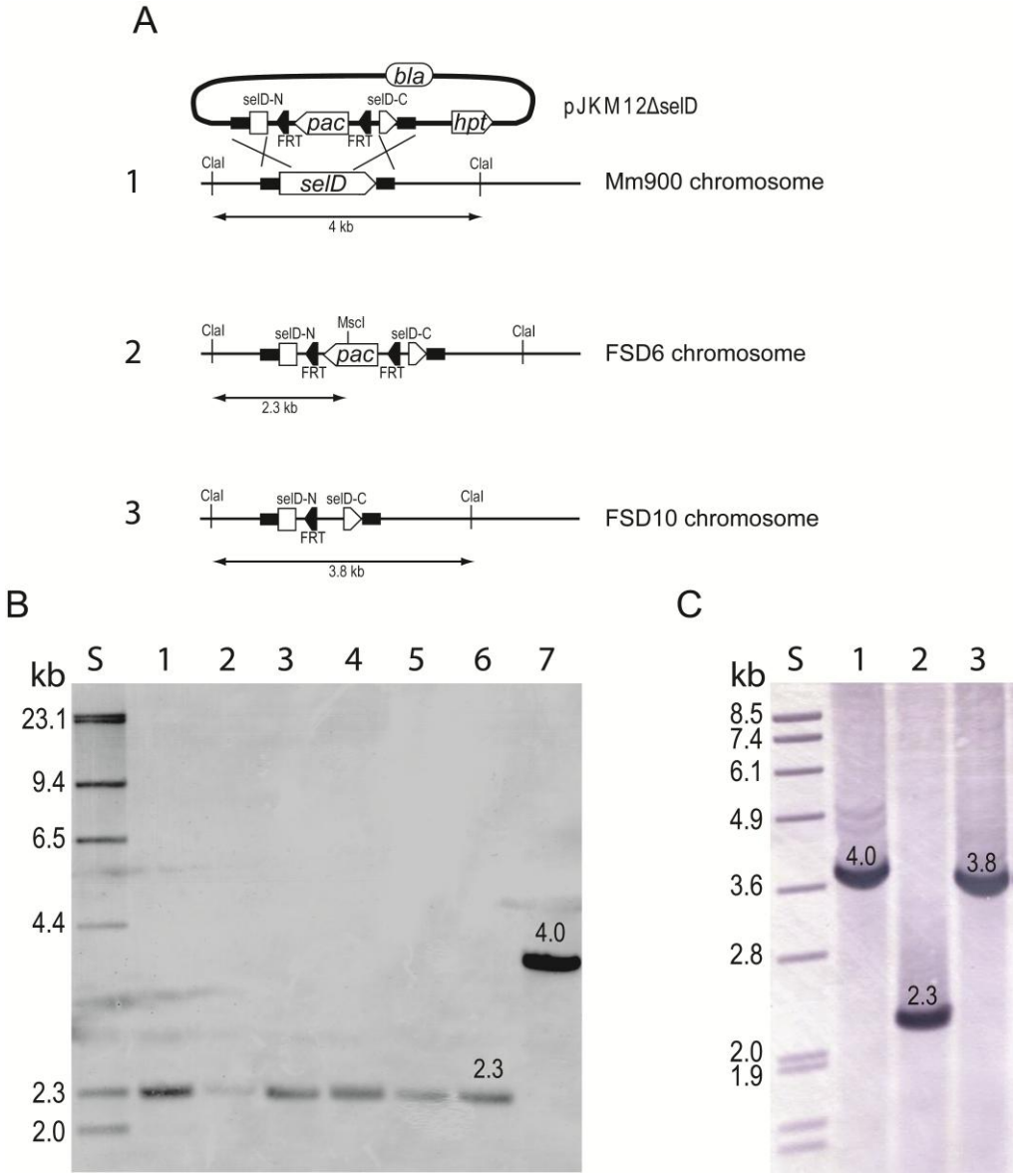
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Figure S2

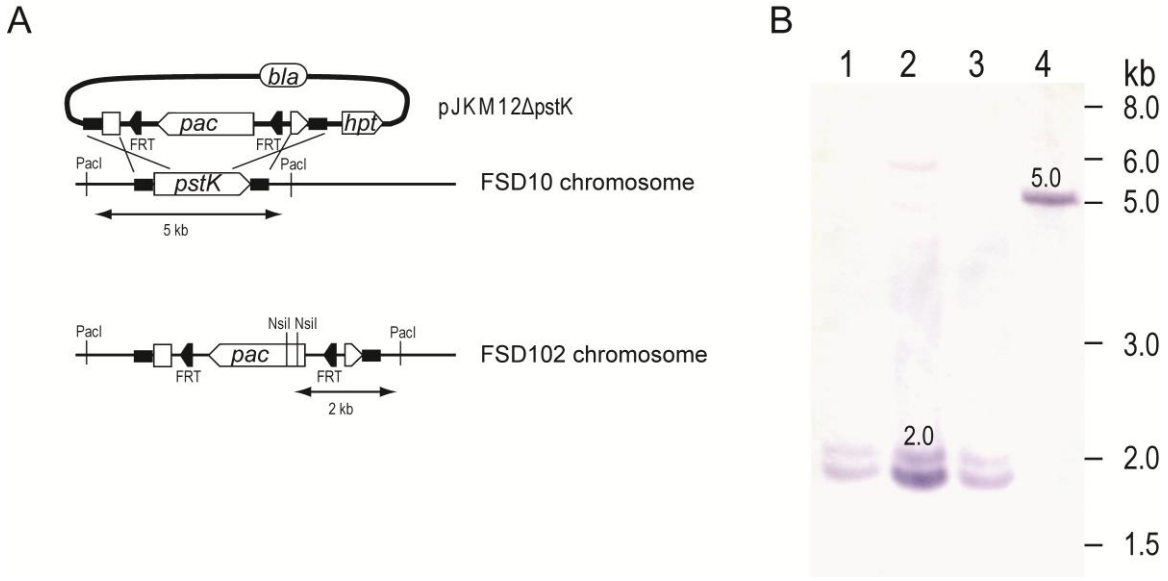


Figure S3

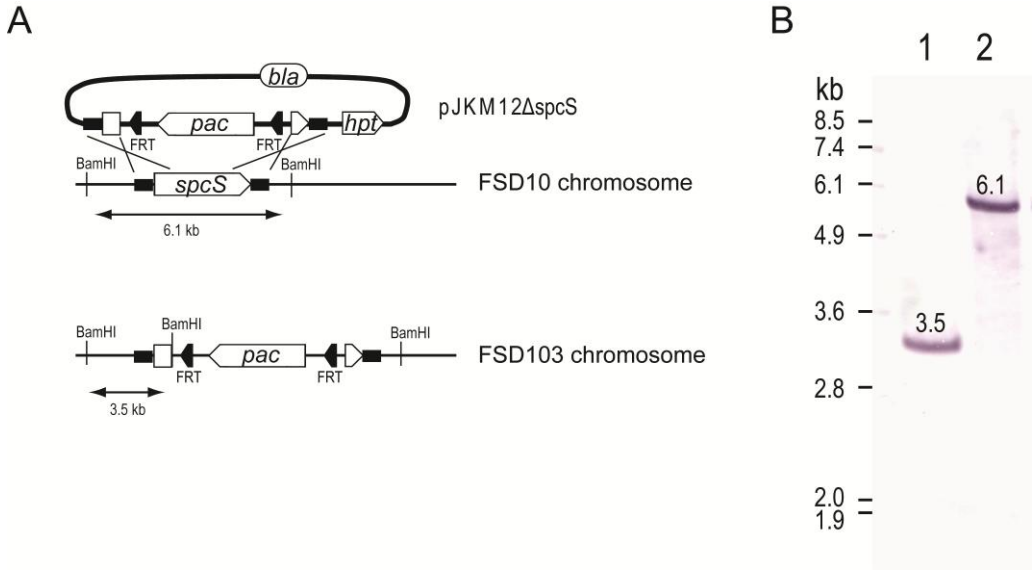
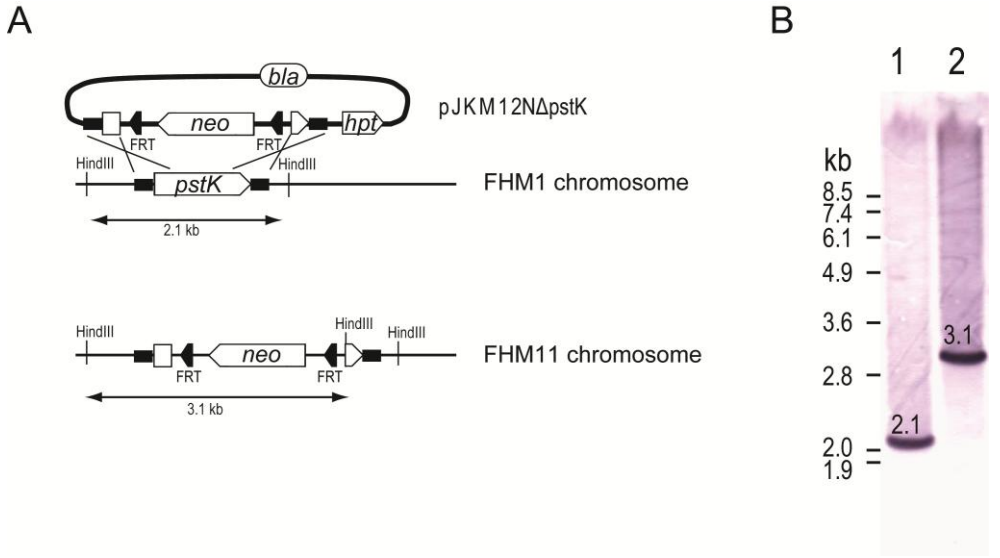


Figure S4



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