

Supplementary information S1 (table) | Commonly used plasmid vectors for archaeal genetics

Vector	Host species	Archaeal replicon	Archaeal marker(s)	Size (kb)	References	Notes
pWL102, pWL104	Har, Hbt, Hvo	pHV2	<i>Mev^R</i>	10.5, 8.7	1–4	pHV2 is an indigenous plasmid of <i>Haloferax volcanii</i> . Laboratory strains WFD11 and DS70 are cured of pHV2, allowing the use of this replicon for shuttle vectors
pWL204	Hvo	pHV2	<i>Mev^R</i>	10.4	5	An expression vector based on pWL102 with a tRNA ^{Lys} promoter
pMDS99	Hvo	pHV2	<i>Mev^R</i>	7.3	6	A <i>Mev^R</i> marker from <i>Haloarcula hispanica</i>
pUBP2	Har, Hbt, Hvo	pHH9 (pHH1)	<i>Mev^R</i>	12.3	2,3	pHH9 is derived from <i>Halobacterium halobium</i> plasmid pHH1
pNG168	Hbt	pNRC100	<i>Mev^R</i>	8.9	4	Contains a polylinker in <i>lacZ'</i> for blue/white screening in <i>Escherichia coli</i>
pMPK54, 56	Hbt	pGRB1	<i>Mev^R</i>	8.6, 5.4	7,8	pMPK56 is more stable than pMPK54, but does not have an <i>E. coli</i> replicon or marker
pNOB102	Har, Hbt	pNOB1	<i>Mev^R</i>	9.1	9,10	pNOB1 is from <i>Natronobacterium</i> spp strain AS7091; see also pNOB103
pMLH3	Hvo	pHK2	<i>Mev^R; Nov^R</i>	11.3	11	A dual-marker plasmid
pMDS20	Hvo	pHK2	<i>Nov^R</i>	10	11–13	Derived from pMDS10; see also pMDS1, 2, 8, 9 and 11
pWL-Nov	Hvo	pHV2	<i>Nov^R</i>	9.4	14	Derived from pWL102
pBR-Nov, pUC-Nov	Hvo	–	<i>Nov^R</i>	5.7, 5.1	14	These are integrative vectors based on pBR322 and pUC18
pHRZ	Hbt	ND	<i>Ani^R; Ths^R</i>	12.6	15	Derived from pHRZ
pHRZH	Hbt	–	<i>Ani^R; Ths^R</i>	14.4	15	
pMPK408	Hbt	–	<i>ura3</i>	4.1	16,17	
pGB70	Hvo	–	<i>pyrE2</i>	3.3	18	pGB70 is a vector for gene knockout using 5-FOA counterselection; requires a <i>H. volcanii</i> Δ <i>pyrE2</i> host

pTA131, 132, 133, 192	Hvo	–	<i>pyrE2; trpA; leuB; hdrB</i>	3.6, 3.9, 4.1, 3.7	19	Contain a polylinker in <i>lacZ'</i> for blue/white screening in <i>E. coli</i> . Require <i>H. volcanii</i> $\Delta pyrE2$, $\Delta trpA$, $\Delta leuB$ or $\Delta hdrB$ hosts, respectively; pTA131 is a vector for gene knockout using 5-FOA counterselection
pTA230, 231, 232, 233	Hvo	pHV2	<i>pyrE2; trpA; leuB; hdrB</i>	7.4, 7.7, 7.8, 7.4	19	Shuttle vectors that are derived from pTA131, 132, 133, 192, with similar host requirements
pMIP1	Mvo	–	<i>Pur^R</i>	7.4	20	Also contains <i>hisA</i> sequences for integration into the <i>Methanococcus voltae</i> genome
pMEB.2	Mma	–	<i>Pur^R</i>	5.0	20,21	pUC18 with <i>pac</i> cassette for <i>Pur^R</i>
pKAS102	Mma	–	<i>Pur^R</i>	12.1	22	Derived from pMIP1, with a 4.7 kb insert of the <i>Methanococcus maripaludis</i> <i>argH</i> gene, for integration into the genome
pWAY1, 2	Mmz	–	<i>Pur^R</i>	5.5	23	
pUC:: <i>pac</i>	Mvo	–	<i>Pur^R</i>	5.0	24,25	pUC BM21 with <i>pac</i> cassette for <i>Pur^R</i>
pJK29, 41	Mac Mba	–	<i>Pur^R</i>	3.2, 3.5	26	Contain a polylinker in <i>lacZ'</i> for blue/white screening in <i>E. coli</i> ; require <i>E. coli</i> <i>pir</i> host for replication using <i>oriR6K</i> origin. See also pJK31, 33, 35, 37 and 39
pWLG13, 18	Mma	–	<i>Pur^R</i>	4.4, 7.5	27	Contain the <i>P_{hmvA}</i> promoter for gene expression in <i>M. maripaludis</i> . pWL18 also contains a polylinker in <i>lacZ'</i> for blue/white screening in <i>E. coli</i> . See also pWLG11, 12A, 14
pWM307, 311, 321	Mac Mba	pC2A	<i>Pur^R</i>	8.2, 8.7, 8.9	28,29	pWM311 contains a polylinker in <i>lacZ'</i> for blue/white screening in <i>E. coli</i> . See also pWM309, 313, 315, and 317, 319
pDLT44	Mma	pURB500	<i>Pur^R</i>	12.7	30	Not very stable in <i>E. coli</i>
pWLG30	Mma	pURB500	<i>Pur^R</i>	12.7	27	Derived from pWLG14. Contains the <i>P_{hmvA}</i> promoter for gene expression in <i>M. maripaludis</i> . See also pWL30+ <i>lacZ</i> (which contains <i>lacZ'</i> for blue/white screening in <i>E. coli</i>)
PRCZ113	Mma	–	<i>Pur^R</i>	16	31	Transcriptional reporter construct using <i>lacZ</i> ; includes the <i>M. maripaludis</i> <i>argH</i> gene for integration into the genome
Miptre	Mvo	–	<i>Pur^R</i>	8.7	32	Transcriptional reporter construct using <i>treA</i> ; includes the <i>M. voltae</i> <i>hisA</i> gene for integration into the genome

pMudpur	Mma	–	<i>Pur^R</i>	7.3	33	Contains a Mud transposon for insertional mutagenesis
pWM381	Mac	–	<i>Pur^R</i>	7.5	34	Mini-mariner delivery plasmid for <i>in vivo</i> transposon mutagenesis in <i>Methanosarcina acetivorans</i> . See also pWM379, 383, 385
pPAC60	Mvo	–	<i>Pur^R</i>	4.7	35	Vector for insertional mutagenesis; includes the <i>hmvA</i> promoter to drive transcription of genes downstream from insertion
pMP44	Mac	–	<i>hpt; Pur^R</i>	4.2	36	Counter-selectable plasmid for markerless gene deletion in <i>M. acetivorans</i> . Requires an <i>E. coli pir</i> host for replication using the <i>oriR6K</i> origin
pCRPrNeo	Mma	–	<i>hpt; Neo^R</i>	6.5	37	Counter-selectable plasmid for markerless gene deletion in <i>M. maripaludis</i>
pJLA5, 6	Mma	–	<i>Neo^R</i>	5.6, 5.8	38	
pHisuid	Mvo	–	<i>hisA</i>	6.6	39	Integration of plasmid reconstitutes the <i>hisA</i> gene; requires a <i>M. voltae hisA</i> host. Also contains the <i>uidA</i> glucuronidase gene of <i>E. coli</i> under control of the <i>hmvA</i> promoter. See also pHisuid- <i>vhc</i> and - <i>frc</i>
pPB31–35	Mac	pC2A	<i>PA^R</i>	11.3	29,40	Contain apolylinker in <i>lacZ'</i> for blue/white screening in <i>E. coli</i> . Require an <i>E. coli pir</i> host for replication using <i>oriR6K</i> origin
pJK89	Mac	pC2A	<i>proC</i>	9.1	36	Requires <i>M. acetivorans ΔproC</i> host
pKMSD48	Sso	SSV1	–	18.5	41	Derived from the SSV1 virus with insertion of pBluescript II SK+. No selectable marker; transformants form viral plaques on a lawn of sensitive cells
pEXSs	Sso	SSV1	<i>hph</i>	6.4	42	Replicon that is based on the SSV1 virus autonomously replicating sequence; the copy number in <i>Sulfolobus solfataricus</i> can be increased by exposure of cells to mitomycin C or UV. Not widely used
pEXADH	Sso	SSV1	<i>hph adh-hT</i>	7.7	43	Dual-marker plasmid derived from pEXSs
pEXlacOP	Sso	SSV1	<i>hph lacS–lacTr</i>	11.7	44	Derived from pEXSs. Contains <i>lacTr–lacS</i> operon; requires an <i>S. solfataricus</i> GθW <i>ΔlacS–ΔlacTr</i> host
pSSV64	Sso	SSV1	<i>pyrEF</i>	20.1	45	Derived from pKMSD48; requires an <i>S. solfataricus pyrE</i> or <i>pyrF</i> mutant host
pJM03	Sso	SSV1	<i>pyrEF; lacS</i>	21.8	45	Derived from pSSV64; requires an <i>S. solfataricus lacS pyrE/F</i> host

pNOB8:: <i>lacS</i>	Sso	pNOB8	<i>lacS</i>	43	46	pNOB8 is a self-spreading conjugative plasmid; a selectable marker is not necessary. <i>lacS</i> is used as a phenotypic marker. pNOB8 imposes a severe growth defect on the host strain
pRN1-pUC18	Sso	pRN1	–	8.0	47	Used to develop transformation protocols for <i>S. solfataricus</i>
pRN1:: <i>lacS</i>	Sso	pRN1	<i>lacS</i>	7.1	48	<i>lacS</i> is used as a phenotypic marker. pRN1-based plasmids are not widely used
pDMI1	Sac	23S rRNA intron	–	3.7	49	Replicon that uses self-spreading mobile intron from the 23S rRNA gene of <i>Desulfurococcus mobilis</i> . Selectable marker is not necessary
pCSV1	Pfu, Sac	pGT5	–	6.1	49	pGT5 plasmid from <i>Pyrococcus furiosus</i> ; replicates in both Euryarchaeota and Crenarchaeota
pAG21	Pfu, Sac	pGT5	<i>adh</i>	6.5	50	See also pAG1 and 2; less stable in <i>E. coli</i> than pAG21 owing to high copy number
pYS2	Pab	pGT5	<i>pyrE</i>	6.4	51	<i>pyrE</i> marker from <i>Sulfolobus acidocaldarius</i> . Requires a <i>pyrE</i> mutant of the <i>Pyrococcus abyssi</i> GE9 strain (devoid of pGT5)
pUDT2	Tko	–	<i>pyrF</i>	6.0	52	Plasmid for the deletion of the <i>trpE</i> gene from the genome by double crossover. Used to develop a gene-knockout system for <i>Thermococcus kodakaraensis</i>

ND, replicon not determined; –, integrative vector. Har, *Haloarcula marismortui*; Hbt, *Halobacterium* species; Hvo, *Haloferax volcanii*; Mac, *Methanosarcina acetivorans*; Mba, *Methanosarcina burkeri*; Mma, *Methanococcus maripaludis*; Mmz, *Methanosarcina mazei*; Mth, *Methanothermobacter thermautotrophicus*; Mvo, *Methanococcus voltae*; Pab, *Pyrococcus abyssi*; Pfu, *Pyrococcus furiosus*; Sso, *Sulfolobus solfataricus*; Tko, *Thermococcus kodakaraensis*. For more information on markers, see also TABLE 2.

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