Design and control of extrachromosomal elements in *Methylorubrum extorquens* AM1

Martina Carrillo[1], Marcel Wagner[2], Florian Petit[1], Amelie Dransfeld[1,2], Anke Becker[2], Tobias J. Erb[1,2]*

[1]Max Planck Institute for Terrestrial Microbiology, Department of Biochemistry & Synthetic Metabolism, Karl-von-Frisch-Str. 10, 35043 Marburg, Germany; [2]LOEWE Center for Synthetic Microbiology, 35043 Marburg, Germany

*Correspondence: toerb@mpi-marburg.mpg.de

SUPPORTING INFORMATION

Contents of the material supplied as Supporting Information:

Table S1 Strains used in this study.

Strains	Description	Reference
CM2720	*Methylorubrum extorquens* AM1 strain deficient in cellulose production	Delaney *et al.*, 2013[1]
SmCreΔhsdR	*Sinorhizobium meliloti* Rm1021 *cre* expression strain with *hsdR* deletion, *tauX*:: *cre-tetRA* (Tc^R)	Döhlemann *et al*., 2016[2]
Escherichia coli DH5α	F^- Φ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F) U169 *rec*A1 *end*A1 *hsd*R17(r_k^-, m_k^+) *pho*A *sup*E44 *thi*-1 *gyr*A96 *rel*A1 λ^-	Thermo Fischer Scientific
E. coli TOP10	F- *mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 ΔlacX74 recA1 araD139 Δ(ara leu) 7697 galU galK rpsL (StrR) endA1 nupG*	Thermo Fischer Scientific
E. coli TOP10 Δ*dapA*	F- *mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 ΔlacX74 recA1 araD139 Δ(ara leu) 7697 galU galK rpsL (StrR) endA1 nupG dapA::FRT-cat-FRT*; DAP deficient	This study

DAP – diaminopimelic acid

Table S2 Plasmids used in this study.

Plasmids	Description	Cloning strategy	Reference
pRK2013	Mobilization helper plasmid, Km^R	n. a.	Figurski and Helinski, 1979[3]
pK18mob2	Suicide vector, Km^R	n. a.	Tauch *et al.*, 1998[4]
pKD3	FRT-flanked chloramphenicol resistance cassette, Amp^R Cm^R	n. a.	Datsenko *et al.*, 2000[5]
pACYC184	Carrying p15A replication origin, Cm^R, Tc^R	n. a.	Chang and Cohen, 1978[6]
pXG-10	Carrying pSC101* replication origin, Cm^R	n. a.	Urban and Vogel, 2007[7]
pIND4	pMG160 origin, $P_{A1/O4/O3}$ promoter, empty vector, Km^R	n. a.	Ind *et al.*, 2009[8]
pTE1830	pMG160 origin, $P_{A1/O4/O3}$ promoter, empty vector, Gm^R	PCR products of prMC276+MC278 from pIND4, prMC228+MC277 from pIND4, and prMC274+MC275 from pLAR-Gm (*aacC1*) were assembled via Gibson assembly.	This study
pTE1841	pMG160 origin, $P_{A1/O4/O3}$ promoter, empty vector, Tc^R	NheI, NotI 5.283 bp fragment from pIND4, PCR products of pr276+295 from pIND4, prMC311+MC277 from pIND4, and prMC296+MC297 from pTE100 (*tetA*) were assembled via Gibson assembly.	This study
pTE100	Promoter-less, oriV-traJ' origin, Tc^R	n. a.	Schada v. B. *et al*, 2015[9]
pTE101	Promoter-less, oriV-traJ' origin, Km^R	n. a.	Schada v. B. *et al*, 2015[9]
pTE102	P_{mxaF} promoter, oriV-traJ' origin, Tc^R	n. a.	Schada v. B. *et al*, 2015[9]
pTE102-mChe	P_{mxaF} promoter, mCherry, oriV-traJ' origin, Tc^R	n. a.	Schada v. B. *et al*, 2015[9]
pTE104-mChe	P_{coxB} promoter, mCherry, oriV-traJ' origin, Tc^R	n. a.	Schada v. B. *et al*, 2015[9]
pLoriVSm	Unloaded library vector for *repABC* regions, pK18mob2-derivative, Km^R	n. a.	Döhlemann *et al.*, 2017[10]
pLsynTer-1	Library plasmid providing *synTer1*-MCS, Km^R	n. a.	Döhlemann *et al.*, 2017[10]
pLsynTer-2	Library plasmid providing *synTer2*-MCS, Km^R	n. a.	Döhlemann *et al.*, 2017[10]
pLsynTer-3	Library plasmid providing *synTer3*-MCS, Km^R	n. a.	Döhlemann *et al.*, 2017[10]
pLAR-Km	Library plasmid providing a kanamycin resistance cassette, Km^R	n. a.	Döhlemann *et al.*, 2017[10]

pLAR-Gm	Library plasmid providing a gentamicin resistance cassette Km^R, Gm^R	n. a.	Döhlemann *et al.*, 2017[10]
pLAR-Tc	Library plasmid providing a tetracyclin resistance cassette Km^R, Tc^R	n. a.	Döhlemann *et al.*, 2017[10]
pLoriT	Unloaded library vector for oriT parts, pK18mob2-derivative, Km^R	n. a.	Döhlemann *et al.*, 2017[10]
pLoriT-1	Library plasmid providing a *mob* site	n. a.	Döhlemann *et al.*, 2017[10]
pAD1	pLoriVSm with *repABC*_Mex-DM4, Km^R	Backbone: ScaI linearized and dephosphorylated pLoriVSm; Insert: T4 PNK phosphorylated PCR product of prAD7+8 from *Methylorubrum extorquens* DM4 gDNA.	This study
pAD2	pLoriVSm with *repABC*_Ocar-2a, Km^R	Backbone: ScaI linearized and dephosphorylated pLoriVSm; Insert: T4 PNK phosphorylated PCR product of prAD13+14 from *Oligotropha carboxidovorans* OM5 gDNA.	This study
pAD3	pLoriVSm with *repABC*_Mrad-JCM, Km^R	Backbone: ScaI linearized and dephosphorylated pLoriVSm; Insert: T4 PNK phosphorylated PCR product of prAD9+10 from *Methylobacterium radiotolerans* JCM2831 gDNA.	This study
pAD4	pLoriVSm with *repABC*_Ocar-1, Km^R	Backbone: ScaI linearized and dephosphorylated pLoriVSm; Insert: T4 PNK phosphorylated PCR product of prAD11+12 from *Oligotropha carboxidovorans* OM5 gDNA.	This study
pAD5	pLoriVSm with *repABC*_Nham-2a, Km^R	Backbone: ScaI linearized and dephosphorylated pLoriVSm; Insert: T4 PNK phosphorylated PCR product of prAD3+4 from *Nitrobacter hamburgensis* X14 gDNA.	This study
pAD6	pLoriVSm with *repABC*_Nham-3, Km^R	Backbone: ScaI linearized and dephosphorylated pLoriVSm; Insert: T4 PNK phosphorylated PCR product of prAD5+6 from *Nitrobacter hamburgensis* X14 gDNA.	This study
pAD7	pLoriVSm with *repABC*_Ocar-2b, Km^R	Backbone: ScaI linearized and dephosphorylated pLoriVSm; Insert: T4 PNK phosphorylated PCR product of prAD13+15 from *Oligotropha carboxidovorans* OM5 gDNA.	This study
pAD8	pLoriVSm with *repABC*_Mex-CM4, Km^R	Backbone: ScaI linearized and dephosphorylated pLoriVSm; Insert: T4 PNK phosphorylated PCR product of prAD24+25 from *Methylorubrum extorquens* CM4 gDNA.	This study
pAD9	pLoriVSm with *repABC*_Mnod-1, Km^R	Backbone: ScaI linearized and dephosphorylated pLoriVSm; Insert: T4 PNK phosphorylated PCR product of prAD26+27 from *Methylobacterium nodulans* ORS 2060 gDNA.	This study
pAD10	pLoriVSm with *repABC*_Mnod-2, Km^R	Backbone: ScaI linearized and dephosphorylated pLoriVSm; Insert: T4 PNK phosphorylated PCR product of prAD28+29 from *Methylobacterium nodulans* ORS 2060 gDNA.	This study
pAD11	pLoriVSm with *repABC*_Nham-2b, Km^R	Backbone: ScaI linearized and dephosphorylated pLoriVSm; Insert: T4 PNK phosphorylated PCR product of prAD1+2 from *Nitrobacter hamburgensis* X14 gDNA.	This study
pMW216	Km^R pMB1 origin; *repABC*_Mex-DM4; MCS 1	Phosphorylated PCR products of primers AR_for + AR_rev from pLAR-Km (P_{min2}-*nptII*), pMB1_for + pMB1_rev from pK18mob2 (pMB1 origin), oriVSm_for + oriVSm_rev from pAD1 (*repABC*_MexDM4), synTer-MCS_for + synTer-MCS_rev from pLsynTer-1 (MCS1) were *in vitro* assembled via ligase cycling reaction using bridging oligonucleotides BO1, BO2, BO3 and BO4.	This study
pMW217	Km^R pMB1 origin; *repABC*_Mrad-JCM; MCS 1	Phosphorylated PCR products of primers AR_for + AR_rev from pLAR-Km (P_{min2}-*nptII*), pMB1_for + pMB1_rev from pK18mob2 (pMB1 origin), oriVSm_for + oriVSm_rev from pAD3 (*repABC*_Mrad-JCM), synTer-MCS_for + synTer-MCS_rev from pLsynTer-1 (MCS1) were *in vitro* assembled via ligase cycling reaction using bridging oligonucleotides BO1, BO2, BO3 and BO4.	This study

pMW218	Km^R pMB1 origin; *repABC*_Nham-3; MCS 1	Phosphorylated PCR products of primers AR_for + AR_rev from pLAR-Km (P_{min2}-*nptII*), pMB1_for + pMB1_rev from pK18mob2 (pMB1 origin), oriVSm_for + oriVSm_rev from pAD6 (*repABC*_Nham-3), synTer-MCS_for + synTer-MCS_rev from pLsynTer-1 (MCS1) were *in vitro* assembled via ligase cycling reaction using bridging oligonucleotides BO1, BO2, BO3 and BO4.	This study
pMW219	Km^R pMB1 origin; *repABC*_Mex-CM4; MCS 1	Phosphorylated PCR products of primers AR_for + AR_rev from pLAR-Km (P_{min2}-*nptII*), pMB1_for + pMB1_rev from pK18mob2 (pMB1 origin), oriVSm_for + oriVSm_rev from pAD8 (*repABC*_Mex-CM4), synTer-MCS_for + synTer-MCS_rev from pLsynTer-1 (MCS1) were *in vitro* assembled via ligase cycling reaction using bridging oligonucleotides BO1, BO2, BO3 and BO4.	This study
pMW220	Gm^R p15A origin; *repABC*_Mex-DM4 ; MCS 2	Phosphorylated PCR products of primers AR_for + AR_rev from pLAR-Gm (P_{min2}-*aacC1*), p15A_for + p15A_rev from pACYC184 (p15A origin), oriVSm_for + oriVSm_rev from pAD1 (*repABC*_Mex-DM4), synTer-MCS_for + synTer-MCS_rev from pLsynTer-2 (MCS2) were *in vitro* assembled via ligase cycling reaction using bridging oligonucleotides BO1, BO2, BO3 and BO4.	This study
pMW221	Gm^R p15A origin; *repABC*_Nham-3; MCS 1	Phosphorylated PCR products of primers AR_for + AR_rev from pLAR-Gm (P_{min2}-*aacC1*), p15A_for + p15A_rev from pACYC184 (p15A origin), oriVSm_for + oriVSm_rev from pAD6 (*repABC*_Nham-3), synTer-MCS_for + synTer-MCS_rev from pLsynTer-1 (MCS1) were *in vitro* assembled via ligase cycling reaction using bridging oligonucleotides BO1, BO2, BO3 and BO4.	This study
pMW223	Tc^R p15A origin; *repABC*_Mex-DM4; MCS 2	Phosphorylated PCR products of primers AR_for + AR_rev from pLAR-Tc (P_{min2}- *tetR*), p15A_for + p15A_rev from pACYC184 (p15A origin), oriVSm_for + oriVSm_rev from pAD1 (*repABC*_Mex-DM4), synTer-MCS_for + synTer-MCS_rev from pLsynTer-2 (MCS2) were *in vitro* assembled via ligase cycling reaction using bridging oligonucleotides BO1, BO2, BO3 and BO4.	This study
pMW224	Tc^R pSC101* origin; *repABC*_Nham-3; MCS 3	Phosphorylated PCR products of primers AR_for + AR_rev from pLAR-Tc (P_{min2}- *tetR*), pSC101*_for + pSC101*_rev from pXG-10 (pSC101* origin), oriVSm_for + oriVSm_rev from pAD6 (*repABC*_Nham-3), synTer-MCS_for + synTer-MCS_rev from pLsynTer-3 (MCS3) were *in vitro* assembled via ligase cycling reaction using bridging oligonucleotides BO1, BO2, BO3 and BO4.	This study
pMW231	Library plasmid providing the gene for the Cre recombinase under the control of $P_{L/O4/A1}$	Backbone: ScaI linearized and dephosphorylated pLoriT; Insert: T4 PNK phosphorylated PCR products of prMW1041+1042 from pTE1887 and prMW1028+1029 from SmCreΔhsdR gDNA. Three- component ligation with T4 DNA ligase.	This study
pMW232	Library plasmid providing the gene for the Cre recombinase under the control of P_{coxB}	Backbone: ScaI linearized and dephosphorylated pLoriT; Insert: T4 PNK phosphorylated PCR products of prMW1043+1044 from pTE104-mChe and prMW1028+1029 from SmCreΔhsdR gDNA. Three- component ligation with T4 DNA ligase.	This study
pMW233	Gm^R; pMB1 origin; *repABC*_Mex-CM4; MCS 1; oriT	Phosphorylated PCR products of primers AR_for + AR_rev from pLAR-Gm (P_{min2}-*aacC1*), pMB1_for + pMB1_rev from pK18mob2 (pMB1 origin), oriVSm_for + oriVSm_rev from pAD8 (*repABC*_Mex-CM4), synTer-MCS_for + synTer-MCS_rev from pLsynTer-1 (MCS1), mob_for	This study

		+ mob_rev from pLoriT-1 (*mob* site) were *in vitro* assembled via ligase cycling reaction using bridging oligonucleotides BO1, BO2, BO3a, BO3b and BO4.	
pMW234	Gm^R; pMB1 origin; *repABC*_Nham-3; MCS 2; oriT	Phosphorylated PCR products of primers AR_for + AR_rev from pLAR-Gm (P_{min2}-*aacC1*), pMB1_for + pMB1_rev from pK18mob2 (pMB1 origin), oriVSm_for + oriVSm_rev from pAD6 (*repABC*_Nham-3), synTer-MCS_for + synTer-MCS_rev from pLsynTer-2 (MCS2), mob_for + mob_rev from pLoriT-1 (*mob* site) were *in vitro* assembled via ligase cycling reaction using bridging oligonucleotides BO1, BO2, BO3a, BO3b and BO4.	This study
pMW235	Tc^R; pMB1 origin; *repABC*_Mex-DM4; MCS 3; oriT	Phosphorylated PCR products of primers AR_for + AR_rev from pLAR-Tc (P_{min2}- *tetR*), pMB1_for + pMB1_rev from pK18mob2 (pMB1 origin), oriVSm_for + oriVSm_rev from pAD1 (*repABC*_Mex-DM4), synTer-MCS_for + synTer-MCS_rev from pLsynTer-3 (MCS3), mob_for + mob_rev from pLoriT-1 (*mob* site) were *in vitro* assembled via ligase cycling reaction using bridging oligonucleotides BO1, BO2, BO3a, BO3b and BO4.	This study
pMW236	Gm^R; pMB1 origin; *repABC*_Mrad-JCM; MCS 1; oriT	Phosphorylated PCR products of primers AR_for + AR_rev from pLAR-Gm (P_{min2}-*aacC1*), pMB1_for + pMB1_rev from pK18mob2 (pMB1 origin), oriVSm_for + oriVSm_rev from pAD3 (*repABC*_Mrad-JCM), synTer-MCS_for + synTer-MCS_rev from pLsynTer-1 (MCS1), mob_for + mob_rev from pLoriT-1 (*mob* site) were *in vitro* assembled via ligase cycling reaction using bridging oligonucleotides BO1, BO2, BO3a, BO3b and BO4.	This study
pMW237	Km^R; pMB1 origin; *repABC*_Mex-CM4; MCS 1; P_{coxB}-*cre*	Phosphorylated PCR products of primers AR_for + AR_rev from pLAR-Km (P_{min2}-*nptII*), pMB1_for + pMB1_rev from pK18mob2 (pMB1 origin), oriVSm_for + oriVSm_rev from pAD8 (*repABC*_Mex-CM4), synTer-MCS_for + synTer-MCS_rev from pLsynTer-1 (MCS1), mob_for + mob_rev from pMW232 (P_{coxB}-*cre*) were *in vitro* assembled via ligase cycling reaction using bridging oligonucleotides BO1, BO2, BO3a, BO3b and BO4.	This study
pMW238	Km^R; pMB1 origin; *repABC*_Mex-CM4; MCS 1; $P_{L/O4/A1}$-*cre*	Phosphorylated PCR products of primers AR_for + AR_rev from pLAR-Km (P_{min2}-*nptII*), pMB1_for + pMB1_rev from pK18mob2 (pMB1 origin), oriVSm_for + oriVSm_rev from pAD8 (*repABC*_Mex-CM4), synTer-MCS_for + synTer-MCS_rev from pLsynTer-1 (MCS1), mob_for + mob_rev from pMW231 ($P_{L/O4/A1}$ -*cre*) were *in vitro* assembled via ligase cycling reaction using bridging oligonucleotides BO1, BO2, BO3a, BO3b and BO4.	This study
pTE1179	pK18mob2 with homologous region of *katA* (META1p3421), Km^R	Backbone: XbaI, KpnI linearized pK18mob2; Insert: XbaI, KpnI digest of PCR product from *M. extorquens* AM1 gDNA prMC157+158. Ligation with T4 DNA ligase.	This study
pTE1870	$P_{A1/O4/O3}$-mCherry_AAV protein degradation tag, Km^R	Backbone: BsrGI, HindIII digested pTE1853; Insert: primer hybridization product prMC398+399. Ligation with T4 DNA ligase.	This study
pTE1875	P_{mxaF}-mCherry_AAV protein degradation tag, Tc^R	Backbone: SpeI, KpnI lin pTE102; Insert: XbaI, KpnI 786bp fragment from pTE1870. Ligation with T4 DNA ligase.	This study
pTE1899	pTE1179 with P_{mxaF}-mCherry_AAV tag expression cassette, Km^R	Backbone: XbaI linearized pTE1179; Insert: XbaI, SpeI 1.3 kb fragment of pTE1875. Ligation with T4 DNA ligase. Product 1.	This study
pTE1825	pIND4-derivative with $P_{A1/O4/O3}$-mCherry	Backbone: HindIII, XbaI linearized pIND4; Insert: HindIII, XbaI 740 bp fragment of pTE102-mChe. Ligation with T4 DNA ligase.	This study
pTE1852	KpnI site at bp 1292 removed by SNP mutation, Km^R	QuikChange mutagenesis of pTE1825 with prMC348+349.	This study
pTE1853	$P_{A1/O4/O3}$-mCherry; KpnI sites removed, Km^R	QuikChange mutagenesis of pTE1852 with prMC353+354.	This study

pTE1855	$P_{L/O4}$-mCherry, Km^R	Backbone: XbaI, AatII linearized pTE1853; Insert: primer hybridization product prMC361-8. Ligation with T4 DNA ligase.	This study
pTE1856	$P_{L/O4/O3}$-mCherry, Km^R	Backbone: XbaI, AatII linearized pTE1853; Insert: primer hybridization product prMC361-367+369+370. Ligation with T4 DNA ligase.	This study
pTE1877	$P_{L/O4/A1}$-mCherry, Km^R	Backbone: XbaI, AatII linearized pTE1853; Insert: primer hybridization product prMC361-366+422+421. Ligation with T4 DNA ligase.	This study
pTE1878	$P_{A1/O5/O4}$-mCherry, Km^R	Backbone: XbaI, AatII linearized pTE1853; Insert: primer hybridization product prMC361-363+381+383+378+419+420. Ligation with T4 DNA ligase.	This study
pTE1879	$P_{A1con/O5/O4}$-mCherry, Km^R	Backbone: XbaI, AatII linearized pTE1853; Insert: primer hybridization product prMC361-363+377+381+383+419+423. Ligation with T4 DNA ligase	This study
pTE1880	$P_{A1/O4}$-mCherry, Km^R	Backbone: XbaI, AatII linearized pTE1853; Insert: primer hybridization product prMC361-363+376+380+382+419+420. Ligation with T4 DNA ligase.	This study
pTE1881	$P_{A1/O4s}$-mCherry, Km^R	Backbone: XbaI, AatII linearized pTE1853; Insert: primer hybridization product prMC361-363+379+380+382+419+424. Ligation with T4 DNA ligase.	This study
pTE1882	$P_{A1/O4s\_GA}$-mCherry, Km^R	Backbone: XbaI, AatII linearized pTE1853; Insert: primer hybridization product prMC361-363+380+419+424+425+426. Ligation with T4 DNA ligase.	This study
pTE1863	$P_{T5s/A1URS/O3}$-mCherry, Km^R	Backbone: XbaI, AatII linearized pTE1853; Insert: primer hybridization product prMC361-363+369+373-375+380. Ligation with T4 DNA ligase.	This study
pTE2714	$P_{A1/O4/O3}$ promoter; empty multiple cloning site with Strep-II tag, Km^R	Backbone: XbaI, HindIII cut pTE1853; Insert: primer hybridization product prMC404-408. Ligation with T4 DNA ligase.	This study
pTE1885	$P_{L/O4}$ promoter; empty multiple cloning site with Strep-II tag, Km^R	Backbone: XbaI, HindIII cut pTE1855; Insert: primer hybridization product prMC404-408. Ligation with T4 DNA ligase.	This study
pTE1886	$P_{L/O4/O3}$ promoter; empty multiple cloning site with Strep-II tag, Km^R	Backbone: XbaI, HindIII cut pTE1856; Insert: primer hybridization product prMC404-408. Ligation with T4 DNA ligase.	This study
pTE1887	$P_{L/O4/A1}$ promoter; empty multiple cloning site with Strep-II tag, Km^R	Backbone: XbaI, HindIII cut pTE1877; Insert: primer hybridization product prMC404-408. Ligation with T4 DNA ligase.	This study
pTE1888	$P_{A1/O5/O4}$ promoter; empty multiple cloning site with Strep-II tag, Km^R	Backbone: XbaI, HindIII cut pTE1878; Insert: primer hybridization product prMC404-408. Ligation with T4 DNA ligase.	This study
pTE1889	$P_{A1con/O5/O4}$ promoter; empty multiple cloning site with Strep-II tag, Km^R	Backbone: XbaI, HindIII cut pTE1879; Insert: primer hybridization product prMC404-408. Ligation with T4 DNA ligase.	This study
pTE1890	$P_{A1/O4}$ promoter; empty multiple cloning site with Strep-II tag, Km^R	Backbone: XbaI, HindIII cut pTE1880; Insert: primer hybridization product prMC404-408. Ligation with T4 DNA ligase.	This study
pTE1891	$P_{A1/O4s}$ promoter; empty multiple cloning site with Strep-II tag, Km^R	Backbone: XbaI, HindIII cut pTE1881; Insert: primer hybridization product prMC404-408. Ligation with T4 DNA ligase.	This study
pTE1892	$P_{A1/O4s\_GA}$ promoter; empty multiple cloning site with Strep-II tag, Km^R	Backbone: XbaI, HindIII cut pTE1882; Insert: primer hybridization product prMC404-408. Ligation with T4 DNA ligase.	This study
pTE1893	$P_{T5s/A1}$ promoter; empty multiple cloning site with Strep-II tag, Km^R	Backbone: XbaI, HindIII cut pTE1863; Insert: primer hybridization product prMC404-408. Ligation with T4 DNA ligase.	This study
pTE2704	P_{mxaF}-mCherry_AAV; *repABC*_Mex-DM4, Km^R	Backbone: ScaI, PacI linearized pMW216; Insert: ScaI, PacI digest of PCR product prMC437+438 of pTE1875. Ligation with T4 DNA ligase.	This study

pTE2705	P_{mxaF}-mCherry_AAV; *repABC*_Mrad-JCM, Km^R	Backbone: ScaI, PacI linearized pMW217; Insert: ScaI, PacI digest of PCR product prMC437+438 of pTE1875. Ligation with T4 DNA ligase.	This study
pTE2706	P_{mxaF}-mCherry_AAV; *repABC*_Nham-3, Km^R	Backbone: ScaI, PacI linearized pMW218; Insert: ScaI, PacI digest of PCR product prMC437+438 of pTE1875. Ligation with T4 DNA ligase.	This study
pTE2707	P_{mxaF}-mCherry_AAV; *repABC*_Mex-CM4, Km^R	Backbone: ScaI, PacI linearized pMW219; Insert: ScaI, PacI digest of PCR product prMC437+438 of pTE1875. Ligation with T4 DNA ligase.	This study

n. a. – not applicable, gDNA – genomic DNA, Amp^R – ampicillin resistance, Cm^R – chloramphenicol resistance, Gm^R – gentamicin resistance, Km^R – kanamycin resistance, Tc^R – tetracycline resistance, pr – primers

Table S3 Primers used in this study.

Primer #	Sequence
MC147	ATTCAACTTCCCGCACGAGT
MC148	GGCACCTTGTTGATCATCGC
MC157	TGATCTAGAGATTCTGACGACCCGTCAGG
MC158	TATGGTACCTCGCGCTGGTTCGAATAGAC
MC228	GAAACGCCTGGTATCTTTATAGTC
MC261	TGCCATACCAAACGTACCATTGAGACACTTGTTTGCACAGAGGATGGCCCTGGGAATTAGCCATGGTC
MC262	AGACCCAGTTCCTTACATGCCCATTTCACCGGGATTGGATTGGGTTCGACTCAGTCTTGAGCGATTGTG TAGG
MC274	ACAGTAATACAAGGGGTGTTATGTTACGCAGCAGCAACG
MC275	CAACCAATTAACCAATTCTGTTAGGTGGCGGTACTTGGG
MC276	AACACCCCTTGTATTACTG
MC277	CAGAATTGGTTAATTGGTTG
MC278	ATAAAGATACCAGGCGTTTC
MC295	TCGACGCGGGCCGAGCTTTG
MC296	CAACCAATTAACCAATTCTGATCAGCGATCGGCTCGTTGC
MC297	ACAGTAATACAAGGGGTGTTTCATGCTTGACACTTTATCAC
MC311	TTATTGGTGAGAATCCAAGC
MC348	CGCGCGAATTGCAGTTACCATTTATCAGGG
MC349	CCCTGATAAATGGTAACTGCAATTCGCGCG
MC352	CGAGCTCGGTACTGACGTAGCCCAGC
MC353	GCTGGGCTACGTCAGTACCGAGCTCG
MC361	CTAAGAAACCATTATTATCATGAC
MC362	ATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTC
MC363	CCTATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGT
MC364	TTCACCTCGAGAAAAGATAAATTATCTCTGGCGGTGTTGACATGTG
MC365	ATCTTTTCTCGAGGTGAAGACGAAAGGGCCTCGTGATACG
MC366	AGTATCATTGTTATCCGCTCACATGTCAACACCGCCAGAGATAATTT
MC367	CTAGAGTCAGTGCGTCCTGCTGATGTGCTC
MC368	AGCGGATAACAATGATACTGAGCACATCAGCAGGACGCACTGACT
MC369	CTAGACTGTGTGAAATTGTTATCCGCTCACAATTGAATCTA
MC370	AGCGGATAACAATGATACTTAGATTCAATTGTGAGCGGATAACAATTTCACACAGT
MC373	AGCGCTCACAATTTATAATTAGATTCAATTGTGAGCGGATAACAATTTCACACAGT
MC375	ATTATAAATTGTGAGCGCTCACAAAGCAACACTCTTTTTG
MC376	AGTATCATTGTTATCCGCTCACAAGTCAACACTCTTTTTG
MC377	ATTATAATTGTTATCCGCTCACAAGTCAACTAAATTGTTACCGCTCA
MC378	AGTATCATTGTTATCCGCTCACAAGTCAACTAAATTGTTACCGCTCA
MC379	AGTATCAATTGTGAGCGCTCACAAGTCAACACTCTTTTTG
MC380	ATAAATTTTCTCGAGGTGAAGACGAAAGGGCCTCGTGATACG
MC381	CAATTTTTTCTCGAGGTGAAGACGAAAGGGCCTCGTGATACG
MC382	TTCACCTCGAGAAAATTTATCAAAAAGAGTGTTGACTTGTGAGCG
MC383	TTCACCTCGAGAAAAAATTGTGAGCGGTAACAATTTAGTTGACTTGTGAGCG
MC398	GTACAAGGCCGCGAACGACGAAAACTACGCCGCGGCCGTCTGAACTAGTCCTGCAGGTACCTTAAGA
MC399	AGCTTCTTAAGGTACCTGCAGGACTAGTTCAGACGGCCGCGGCGTAGTTTTCGTCGTTCGCGGCCTT
MC404	CTAGAATTAAAGAGGAGAAATTAACCATGGCGAGCTGGAGCCATCCGCAGTTCGAA
MC405	AGCTCGCCATGGTTAATTTCTCCTCTTTAATT
MC406	AAGATCGAAGGCCGCCATATGTCGGGCGGATCCTGAACTAGTCCTGCAGGTACCGTA
MC407	TCCGCCCGACATATGGCGGCCTTCGATCTTTTCGAACTGCGGATGGCTCC
MC408	AGCTTACGGTACCTGCAGGACTAGTTCAGGA
MC419	CTAGAGTCGCCGTGTCCCTCTCGATGAATCTA
MC420	GATAACAATGATACTTAGATTCATCGAGAGGGACACGGCGACT
MC421	CTAGAGTCGCCGTGTCCCTCTCGATGTGCTC
MC422	AGCGGATAACAATGATACTGAGCACATCGAGAGGGACACGGCGACT
MC423	GATAACAATTATAATTAGATTCATCGAGAGGGACACGGCGACT
MC424	GATAACAATTATAATTAGATTCATCGAGAGGGACACGGCGACT
MC425	AGTATCAATTGTGAGTGCTCACAAGTCAACACTCTTTTTG
MC426	TTCACCTCGAGAAAATTTATCAAAAAGAGTGTTGACTTGTGAGCA
MC437	CTGACAGTACTCAAGCTAGCTTCCCGCTTGGTC
MC438	CGCTTAATTAACGACGGCCAGTGAATTAGG
AD1	ACCCGATCGCGAAAACTTCC
AD2	CTCGAGGGTTGGGCGAAAG
AD3	ATGGATCCGAAATCGCCCAG
AD4	AACTTCCTTCGTAGCGGTC

AD5	AACGAAGTTCGGCCATGACC
AD6	GGCGAACGCGAGGAGAAATT
AD7	ATGCTGCGGATCGCTAAAAAGA
AD8	TTCGGCTTGGTCTCGATCA
AD9	AGGTCTCGGAAAGACATCCG
AD10	CGGATCCAGGTGTTCAGGAA
AD11	ATCATTTGCGGCTCGCCTAG
AD12	GTCCAGACCATCGGGCTCTA
AD13	GGAAAGCCTGGTGCTGAAAT
AD14	TTTGTCGTCCTTGAAGGGATAGTT
AD15	CGGATCATCGATCGCATTCTG
AD24	ACCATCAAGAACTGCCCGTT
AD25	GTGACGGAATGACGCCGTAA
AD26	ATCTCCATTCACACCGCTGCTT
AD27	TTTGTCCGCGAGGTCTTCGTT
AD28	GTGCTTCGTCATGGCTCCAT
AD29	AATGACCTCTGACCGGCGTTA
MW125	GCGGTCGACGTTATGGAGCAGCAACGATGTT
MW126	GCGCTGCAGAGTACTTTAGGTGGCGGTACTTGGGTCG
MW1028	GCGCATATGTCCAATTTACTGACCGTACACCAAAAT
MW1029	GCTAGCGAATTCTAGTCGCCATC
MW1041	CGCCATATGTAATTTCTCCTCTTTAATTCTAGAGTC
MW1042	TCGCGCTAACTTACATTAATTG
MW1043	CGTATCCCCAGAGGCAGCCAAATTGC
MW1044	GCGCATATGCCCGCTTGGCTCCCCTGGTG
MW1046	ATAACTTCGTATATGGTATTATATACGAACGGTAG
MW1047	TCGACTACCGTTCGTATATAATACCATATACGAAGTTAT
MW1048	GTACCGTTCGTATAGCATACATTATACGAAGTTAT
MW1049	ATAACTTCGTATAATGTATGCTATACGAACGGTACTGCA
JD253	CTCGCAGAGCAGGATTCCCGTT
JD254	GGCAGGATAGGTGAAGTAGGCCCA
AR_for	GACCTTTTCTCCGACGAATAGA
AR_rev	GTCTTATCTGAAAGTTGTGCCTG
oriVSm_for	GAAACTGTCACTGGTCCGT
oriVSm_rev	TTCAGTTACGATAGAGTTCCACG
synTer-MCS_for	CTATTGAAGGAACACTGTATCTCG
synTer-MCS_rev	GTCAACCCGCTTACACTC
pMB1_for	TACTACTGTTTCAGACTGGCGTAATCACTCAGTAGATCAAAGGATCTTCTTGAGATCCTTTTTTTC
pMB1_rev	TGGACAGAATAGTCTTACTCAGTGATTGCCAGTCGCGTTGCTGGCGTTTTTC
p15A_for	TACTACTGTTTCAGACTGGCGTAATCACTCAGTCTACATTTGAAGAGATAAATTGCACTG
p15A_rev	TGGACAGAATAGTCTTACTCAGTGATTGCCAGTTAGCGGAGTGTATACTGGC
pSC101*_for	TACTACTGTTTCAGACTGGCGTAATCACTCAGTCCTAGGGTACGGGTTTTGC
pSC101*_rev	TGGACAGAATAGTCTTACTCAGTGATTGCCAGTGACAGTAAGACGGGTAAGCCT
mob_for	TGTGACGATAAGTTCCCTACTG
mob_rev	CCTCTATTGATAACGGGTGACA
BO1	AACTTCTCGTGGAACTCTATCGTAACTGAAGTCTATTGAAGGAACACTGTATCTCGGTCA
BO2	GTTATCAGAAGAGTGTAAGCGGGTTGACTATACTACTGTTTCAGACTGGCGTAATCACTC
BO3	GGCAATCACTGAGTAAGACTATTCTGTCCAGACCTTTTCTCCGACGAATAGAGTAACAGA
BO3a	GGCAATCACTGAGTAAGACTATTCTGTCCATGTGACGATAAGTTCCCTACTGACAGAATC
BO3b	GGTTACAGTGTCACCCGTTATCAATAGAGGGACCTTTTCTCCGACGAATAGAGTAACAGA
BO4	GATTGGTCAGGCACAACTTTCAGATAAGACGAAACTGTCACTGGTCCGTTGATACAATCC

Table S4 Fold induction of IPTG-inducible promoters in *M. extorquens* AM1. Mean ± SD.

Promoter	Fluorescence/OD_{600}		Fold induction	Maximum strength relative to the P_{mxaF} promoter (%)*
	0 mM IPTG	1 mM IPTG		
background	339 ± 14	355 ± 13	n. a.	n. a.
P_{mxaF}	2152 ± 16	n. a.	n. a.	100
$P_{A1/O4/O3}$	376 ± 18	1355 ± 11	27	55.6
$P_{L/O4}$	526 ± 8	3172 ± 147	15	156.8
$P_{L/O4/O3}$	361 ± 4	1132 ± 66	36	43.2
$P_{L/O4/A1}$	471 ± 1	3332 ± 43	23	165.7
$P_{A1/O5/O4}$	416 ±8	1297 ± 14	12	52.4
$P_{A1con/O5/O4}$	384 ± 6	1286 ± 91	21	51.8
$P_{A1/O4}$	441 ± 4	2531 ± 131	21	121.1
$P_{A1/O4s}$	420 ± 14	855 ± 17	6	27.8
$P_{A1/O4s\_GA}$	678 ± 5	2235 ± 224	6	104.6
$P_{T5s/A1}$	353 ± 6	510 ± 4	11	8.6

n. a. – not applicable. *Values after induction with 1 mM IPTG.

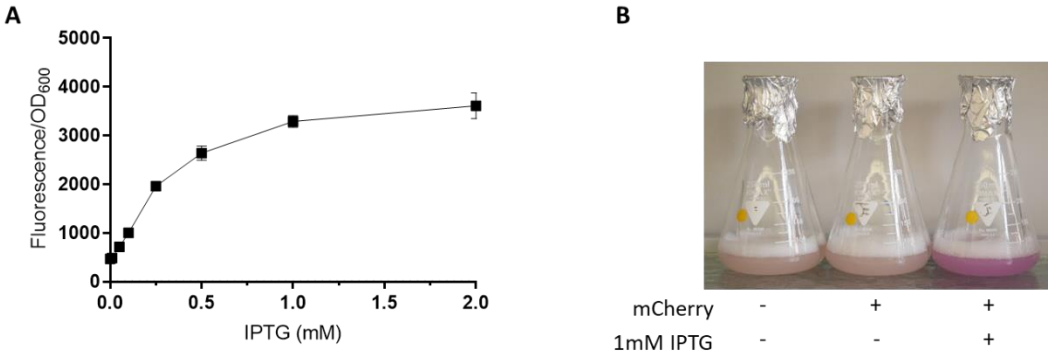

Fig. S1 $P_{L/O4/A1}$ promoter in *M. extorquens* AM1. Dynamic range of expression with 0-2 mM IPTG (**A**). Cultures expressing ± mCherry before and after induction with 1 mM IPTG; culture of an empty vector is shown as a reference (**B**).

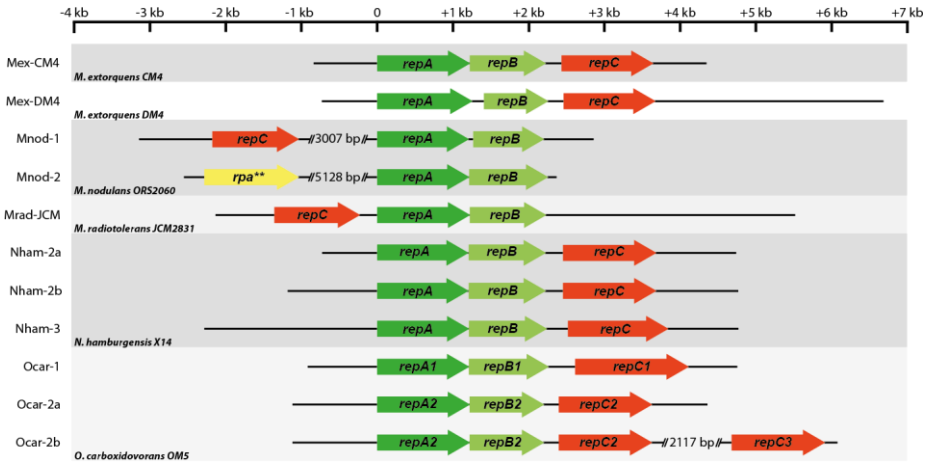

Fig. S2 Overview of the individual *repABC* regions tested in this study. All *repABC* regions are shown with the start codon of *repA* aligned at the zero mark. The black lines depict the regions amplified outside of the *repABC* coding sequences to include necessary *parS* sites. A scale bar is given as a reference.

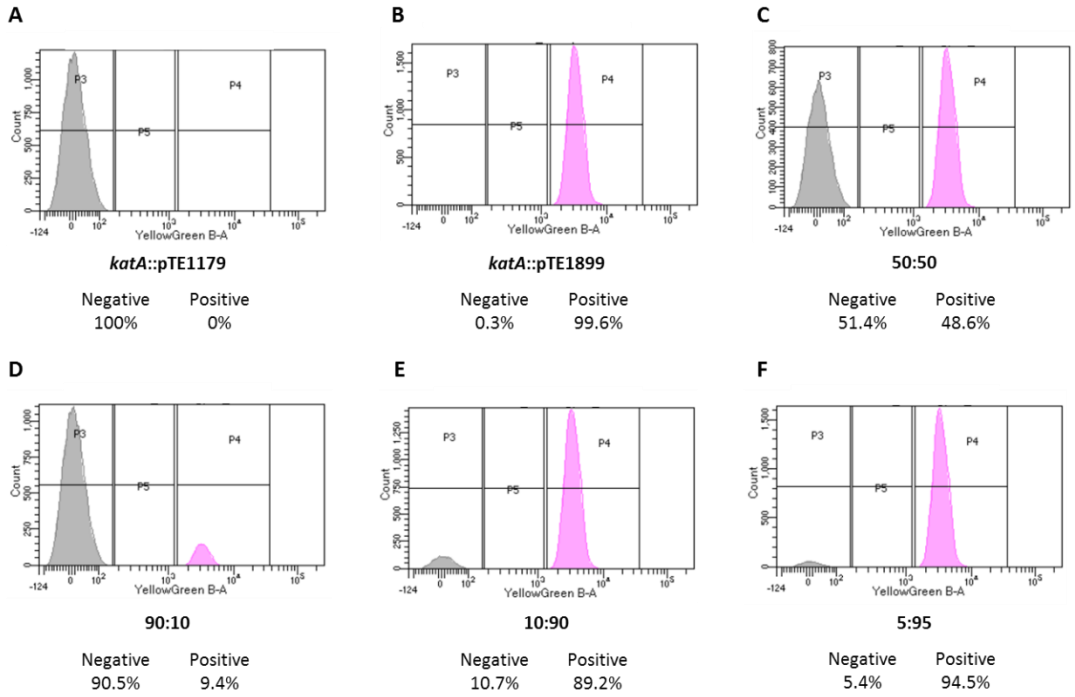

Fig. S3 Verification of flow cytometry sensitivity. Control strains were created by integrating the suicide vector pK18mob2 with (*katA*::pTE1899) and without (*katA*::pTE1179) a mCherry expression cassette into the chromosome of *M. extorquens*. Gates for were adjusted to distinguish between fluorescent (shown in pink) and non-fluorescent (shown in grey) cells using *katA*::pTE1179 (A), *katA*::pTE1899 (B), and mixtures thereof (C-F). 30,000 events were recorded per sample.

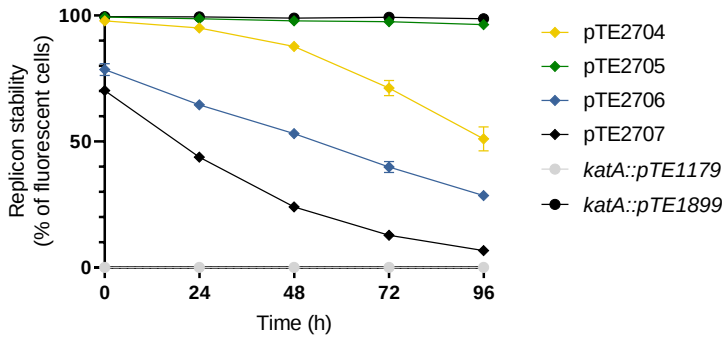

Fig. S4 Replicon stability measured by flow cytometry of mini-chromosomes with Mex-DM4, Mrad-JCM, Nham-3, and Mex-CM4 *repABC* regions (pTE2704-7, respectively) and an mCherry expression cassette. Control strains were created by integrating the suicide vector pK18mob2 with (*katA*::pTE1899) and without (*katA*::pTE1179) an mCherry expression cassette into the chromosome of *M. extorquens* AM1. 30,000 events were recorded per sample.

Table S5 DNA sequences of inducible promoters characterized in this study[a].

Promoter	Sequence
$P_{A1/O4/O3}$	GACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTTC ACCTCGAGAAAA**TTTATCAAAAAGAGTGTTGACTTGTGAGCGGATAACAATGATACTTAGATTCAATTGTG AGCGGATAACAATTTCACACA**TCTAGA
$P_{L/O4}$	GACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTTC ACCTCGAGAAAA**GATAAATTATCTCTGGCGGTGTTGACATGTGAGCGGATAACAATGATACTGAGCACATC AGCAGGACGCACTGAC**TCTAGA
$P_{L/O4/O3}$	GACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTTC ACCTCGAGAAAA**GATAAATTATCTCTGGCGGTGTTGACATGTGAGCGGATAACAATGATACTTAGATTCAA TTGTGAGCGGATAACAATTTCACACAG**TCTAGA
$P_{L/O4/A1}$	GACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTTC ACCTCGAGAAAA**GATAAATTATCTCTGGCGGTGTTGACATGTGAGCGGATAACAATGATACTGAGCACATC GAGAGGGACACGGCGA**CTCTAGA
$P_{A1/O5/O4}$	GACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTTC ACCTCGAGAAAA**AATTGTGAGCGGTAACAATTTAGTTGACTTGTGAGCGGATAACAATGATACTTAGATTC ATCGAGAGGGACACGGCGA**CTCTAGA
$P_{A1con/O5/O4}$	GACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTTC ACCTCGAGAAAA**AATTGTGAGCGGTAACAATTTAGTTGACTTGTGAGCGGATAACAATTATAATTAGATTC ATCGAGAGGGACACGGCGA**CTCTAGA
$P_{A1/O4}$	GACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTTC ACCTCGAGAAAA**TTTATCAAAAAGAGTGTTGACTTGTGAGCGGATAACAATGATACTTAGATTCATCGAGA GGGACACGGCGA**CTCTAGA
$P_{A1/O4s}$	GACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTTC ACCTCGAGAAAA**TTTATCAAAAAGAGTGTTGACTTGTGAGCGCTCACAATTGATACTTAGATTCATCGAGA GGGACACGGCGA**CTCTAGA
$P_{A1/O4s\_GA}$	GACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTTC ACCTCGAGAAAA**TTTATCAAAAAGAGTGTTGACTTGTGAGCACTCACAATTGATACTTAGATTCATCGAGA GGGACACGGCGA**CTCTAGA
$P_{T5s/A1}$	GACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTTC ACCTCGAGAAAA**TTTATCAAAAAGAGTGTTGCTTTGTGAGCGCTCACAATTTATAATTAGATTCAATTGTGA GCGGATAACAATTTCACACAG**TCTAGA

[a]The promoter region is shown in **bold**, AatII and XbaI restriction sites are underlined. The surrounding sequences were included only to facilitate primer hybridization.

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