Electronic Supporting Information

Challenges and advances in genetic manipulation of filamentous actinomycetes – the remarkable producers of specialized metabolites

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Table S1: List of plasmids according to the *Streptomyces*/*E. coli* replicon, transposon elements and functions. Selected examples are reported with their properties

1. Integrating shuttle plasmids

E. coli **replicon**	**Plasmid**	**Features**	**Comments**	**References**
pUC18	pIJ8600	8.1 kb, *oriT* RK2, *int* ΦC31, *attP* ΦC31, P_{tipA}, *t0* (terminator from phage λ), *tsr, acc(3)*IV	expression vector (gene expression under the control of the thiostrepton-inducible P_{tipA})	[1]
pUC18	pIJ8630 and pIJ8660	7.7 kb, *int* ΦC31, *acc(3)*IV, *oriT* RK2, *egfp*	pIJ8600-derivatives, in which the P_{tipA} promoter was deleted and *egfp* was inserted to be used as indicator	[1; 2, 3]
pIC20R	pKT02	6.078 kb, *int* VWB, *attP* (VWB), *tsr, bla*	- integrating vector derived from phage VWB from *S. venezuelae* - integration into the $tRNA^{Arg}$	[4; 5]
pUC18	pMT3226	8.4 kb, *oriT* RK2, *attP* ΦC31, *int* ΦC31, *gylR, xylE*, *fdt*, *bla*	- integrating expression vector, *gylR*-cassette for glycerol - inducible gene expression	[6]
pUC18	pSET152	5.5 kb, *oriT* RK2, *attP* (ΦC31), *int* ΦC31, *lacZα*, MCS, *oriT,*	derived from ΦC31 phage	[7; 8]
pBR322	pOJ436	11.1 kb, *oriT* RK2, *acc(3)*IV, *attP* (ΦC31), *int* ΦC31,P_ T7 /T3 (promoters), *cos* sites,	derived from ΦC31 phage	[7; 9; 10; 11]
pBR327	pIJ4201 pIJ4210	8.1 kb, *IS*117 derivatives, *tsr, aphII*, *orf1*, *orf2*,*3* only in pIJ4210, *attM*, *bla*	- integrate into specific sites - transposable elements (derived from *IS*117 from *S. coelicolor*) - pIJ4201 and pIJ4210 differ in the orientation of the resistance genes - the *aphII* gene from Tn*5* lacks its own promoter	[12;13]
pBR322	pIJ4696, pIJ4698 pIJ4700	pIJ4696: 7.5 kb; pIJ4698: 10.7 kb and pIJ4700: 7.8 kb, *IS*117 derivatives, *bla*, *orf1*, *orf2,3*, *attM, hph^R* in pIJ4696, *aadA*, *tsr* and *aphII*, only in pIJ4698	- for gene expression - pIJ4698 can be used as positive selection replacement vector	[14; 15; 16; 17; 18]
pUC18	pSET151	6.2 kb, *tsr, xylE, oriT, lacZα,* MCS, *aac(3)IV*	- non-replicating vector, integration of DNA fragments into specific sites in the chromosome, can be used for reversible integration - derived from pHJL401 by deleting the SCP2* replicon on a Bell fragment and inserting a cassette containing *oriT* and the *xylE* structural gene driven	[7; 19; 20]

			by P_{ermE}	
2. **Plasmids grouped according to *Streptomyces* replicon**				
pIJ101		8.8 kb, *rep, ori* (ds origin for RCR (rolling circle replication), *rep*, *sti*, *tra, clt*, *korA*, *kilA*, *spdAB, kilB, korB*, *orf56* (unknown function)	- replicative plasmid (RCP), approx. 300 copies per chromosome - pock formation in *S. lividans* and *S. coelicolor*	21; 22
	pIJ699	9.5 kb, *ori* pA15 (*E. coli* replicon), P*tet* (Promoter), *vph, aphII, ter* (terminator sequence), ter, *tsr*	- positive selection vector - replicative plasmid, which can be used for gene expression - the pA15 replicon is removed during the cloning procedure and the obtained hybrid plasmids replicate only in actinomycetes (mainly *Streptomyces*) - selection on neomycin/kanamycin is only possible in *E. coli* not in actinomycetes - Self-religation after removing the „stuffer“ fragment leads to the generation of a fragment with a perfect palindrome from the inverted *fd* ter sequence, rendering the plasmid “invisible”	23
	pIJ702	5.65 kb, Δ*traB*	- cloning vector	21; 24
	pIJ486 pIJ487	6.2 kb, Δ*traB*	- cloning vector (the two vectors differ for the MCS orientation) - is lost spontaneously	25; 26
	pIJ303	Δ*traB* deleted	- ts, (lost at 37°C)	27; 28;
	pMT660	5.6 kb, Δ*traB*	- ts, derivative of pIJ702	29
	pWHM3	7.2 kb, Δ*traB*, *ori* pU18	- vector for knockouts - unstable replicon	30; 31
	pHZ1351 and pHZ1358	8.0 kb, Δ*traB* 10 kb, deletion of the stability region	- vector for knockouts - pIJ101 with deleted region between *sti* and *rep*	6; 32
	pANT1200-1202	pANT1200: 8 kp, pANT1201: 6.6 kb, pANT1202: 6.3 kb *ori* (pUC), *rep* (pIJ101), resistance gene(s), *ter* sequences, *snpA* promoter, *snpR* gene	- expression vectors - pANT1200: *bla* and *tsr* - pANT1201: *aphII* (kanamycin resistance gene under the control of the *acc(3)*IV promoter) - pANT1202: *acc(3)*IV	6; 33
pSG5		12.2 kb replicative, conjugative plasmid, naturally temperature sensitive (ts) plasmid (replication stops above 34°C)	- expression vectors - multicopy plasmid (copy number 20-50) - wide range within *Streptomyces*	34; 35; 36
	pSG5 derivatives pCZA185, pKC1139, pGM series	contain only *rep* gene and ds *ori* of pSG5	- pSG5 derivatives are extremely useful for transposon delivery, for gene description and gene replacement by including homologues regions - in most cases replicative vectors	- pGM series[34; 35; 36] - pKC1139[7; 37] - pCZA185 1994[38]

	pHZ132	10.1 kb cosmid, *ori* (pAT153), *vph, cos* site, *oriT*), *tsr*, *bla*	- bifunctional pSG5 derivative (shuttle plasmid) - for gene expression	
SCP2*		31.4 kb transposase-like protein, regions for replication (*rep*), stability and transfer	- low copy number plasmid	39; 40; 41; 42; 43; 44
	pSCP103	24.5 kb SCP2*-derivative with deleted fragment of approx. 4 kb (PstI-A + B fragments circularised in their native configuration)	- lack of partition function (instable, do not contain stability region)	45; 41; 42
	pIJ922	24.0 kb, *tsr, traA*	- pSCP103 derivative	46; 42; 47
	pIJ698	29.9 kb, pSCP103 derivative *aphII*, *vph*, pA15, *ter*, *tsr/hph*^{R} *cassette*	- shuttle vector (replication in *E. coli,* p15A) - derived from pSCP103	42; 23
	pIJ80	pSCP103 derivative, *aphI*		6
	pKC505	18.7 kb pSCP103 derivative, SCP2* replication and fertility function, *ori* (*E. coli*), *acc(3)*IV	- shuttle cosmid vector - can be conjugally transferred between different streptomycetes	48
	pMT603	18.7 kb pSCP103 derivative *tsr*, *mel* gene (melanin production)	- cloning vector (pMT603) based on the low copy number plasmid SCP2* - pMT6O3 is unstable because it lacks the SCP2* stability region	49
	pRM5	pSCP103 derivative, transfer and stability region were deleted, includes pBR327 parts, *tsr*	- designed for cloning (and expression) polyketide biosynthetic gene clusters (combinatorial biosynthesis)	50
	pHJL401	5.9 kb, higher copy number pSCP103-derivative *E. coli* replicon (pUC19)	- shuttle vectors - the region around the replication origin of pSCP103 was used for construction of pHJL401, which resulted in an increased copy number vector (10 copies)	20
	pKC1064	10 kb, *ori* (SPC2*), *lacZα* (MCS), P_{tipA}, t*fd* (ter), *ori* (pUC vector), *bla, oriT* (RK4)	- cloning and expression vector - gene expression under the control of the inducible *tipA* promoter	51
	pKC1218	5.8 kb, *ori* (SPC2*), *lacZα* (MCS), *ori* (pUC18), *acc(3)*IV, *oriT* (RK2)	- replicates autonomously in actinomycetes (*Streptomyces*) - for gene expression	7
	pIJ4231	pSCP103 derivative (see below, pSCP103), IS*117* integration site, *vph*, *hph*^{R}	- transfer-proficient SCP2* derivative containing a cloned IS*117 attB* site - used for gene expression	52
SLP1		17.2 kb	- SLP1 plasmids found in *Streptomyces lividans* after mating with *S. coelicolor* strain A3(2) originate as deletion mutants of a 17 kb segment of the *S. coelicolor* chromosome - integrates into specific, highly conserved chromosomal tRNA gene (40 bp copy of the 3´end of the respective tRNA^{Tyr} gene) - deletion mutants of SLP1 resulted in formation of autonomously replicating, conjugative, pock-forming CCC plasmids	53

	pCAO106	21.1 kb, SLP1 derivative, *bla*, transfer and replication function, *attP*, Kan^R, *ori* (p15A)	-integrate into *S. lividans* chromosome via *attP* site, is transfer-proficient and forms pocks	6
	pCAO170	SLP1/ derivative, pCAO106 containing *tsr*	-integrate into *S. lividans* chromosome via *attP* site, is transfer proficient and forms pocks	6
3.	**Plasmids containing transposable elements**			
	pIJ4201, pIJ4210, pIJ4696, pIJ4698, pIJ4700	see 1. Integrating shuttle plasmids		
	pCZA163	5.8 kb, *ori* (pUC19), *bla* *orfA* upstream and *orfB* downstream of the *acc(3)*IV, P_{lac} upstream of the Tn*5096*	- transposon mutagenesis - shuttle vector	54
	pOJ446	10.4 kb cosmid, *ori* (SCP2*), (*cos)3*$^{\lambda}$, *rep* (pUC), *oriT* RK2, *acc(3)*IV	- cloning vectors (derived from pKC505) for the conjugal transfer of DNA from *E. coli* to actinomycetes - autonomous replication, or site-specific integration at the bacteriophage ΦC31 attachment site	7; 55
	pCZA258	6.9 kb, *ori* (ColEI), P*lac*, MCS, hph^R, Tn*5099-10*, *orfA* upstream and *orfB* downstream-PstI and SphI sites, *oriT* (RK2), *bla*	- transposon integration - shuttle vector	38
	pCZA276	7 kb, *ori* (pUC19), P_{lac}, MCS, hph^R, Tn*5100-4*, *orfA* upstream and *orfB* downstream-*PstI* and *SphI* sites (*orfA*-*orfB* are in the opposite direction compared to pCZA258), *oriT* (RK2), *bla*	- shuttle vector - the ORFA-ORFB is in the opposite direction compared to pCZA258	38
4.	**λ cosmid vectors**			
	pOJ446	10.4 kb cosmid, *ori* (SCP2*), (*cos)3*$^{\lambda}$, *rep* (pUC), *oriT* RK2, *acc(3)*IV		
	SuperCos1 or pSuperCos1	- 8 kb cosmid, *ori* (SV40), (*cos)2* sites, MCS, *bla*, *ori* (ColEI), *aphII*	- selection on kanamycin - size limitation: 36-53 kb insert (allowing packaging into λ head)	56; 57
	pOJ436	see 1. Integrating shuttle plasmids		
	pHZ1358	see, 2. pIJ101 derivatives		
	pKC505	see 2. SCP2* derivatives		
5.	**Phage-derived integrating plasmids**			
pSAM2		11-kb, *attP, int*, *ds* origin of replication, *rep* operon (replication), *xis*, *tra*, *spd, kor*, *pra* and *orf131* (regulatory genes)	- phage-derived integrative, conjugative vector - integrates into specific, highly conserved chromosomal tRNA gene (40 bp copy of the 3´end of the respective $tRNA_{Pro}$ gene) - the plasmid can reintegrate from the genome	58; 59
	pPM927	integrative vectors (integration in non-tRNA sequence	- mostly conjugative and integrative plasmids, which can be used for gene	60; 6; 61,62

		possible)	expression - wide host range	
ΦC31 phage	ΦC31-derived vectors	phage-derived integrating vectors	- mostly conjugative and integrative plasmids, which can be used for gene expression - integrate more efficiently compared to pSAM2 vectors	63; 64; 65; 66; 67; 68; 69; 70; 71; 72; 73; 74; 75; 76; 77; 78, 7
	pIJ8600	see 1. Integrating shuttle plasmids		
	pIJ8630 and pIJ8660	see 1. Integrating shuttle plasmids		
	pOJ446	see 3. Plasmids containing transposable elements		
	KC515	-38.3 kb, Δ*attP*, phage ΦC31-based cloning vectors - *vph* gene (promoterless viomycin phosphotransferase gene conferring resistance), *tsr*	- phage KC515-derived systems are used for gene disruption and replacement - contains single sites for the enzymes *BamHI, BglII, PstI, PvuII, SstI* (two sites) and *XhoI,* available for the insertion of DNA of up to 4 kb	79; 80
	KC516	38.3 kb, Δ*attP*, phage ΦC31-based cloning vectors *vph* gene, *tsr*	allows DNA insert of up to 4-4.5 kb	79
	KC857	40.1 kb, Δ*attP*, phage ΦC31-based cloning vectors *vph* gene, *tsr, xylE* reporter gene, *ter*(fd) (ter)	- allows DNA insert of up to 2.2 kb - useful for *in situ* fusion to *xylE*	6
	PM8	39.0 kb, Δ*attP*, phage ΦC31-based cloning vectors *hph*^{R}, *tsr, xylE* reporter gene, *ter*(fd) (ter)	- allows insertion of up to 3.3 kb	6
6.	**Expression vectors**			
	pIJ8600, pMT3226	see 1. Integrating shuttle plasmids		
	pANT1200-1202	see 2. pIJ101 derivatives		
	pCZA185	see 2. pSG5 derivatives		
	pKC1064	see 2. SCP2* derivatives		
	pRM5	see 2. SCP2* derivatives		
	pANT849-851	5.3-5.8 kb, *ori* (pIJ101), *tsr,* P*snpA* (small neutral protease SnpA promoter) upstream of the MCS, *snpR* (SnpA-regulator gene), *rep* (pIJ101) in pANT850-851, *aphI* gene	- for gene expression - reporter protein levels up to 50-fold higher compared to *melC1* promoter	81; 82; 83; 84, 85
	pIJ4123 and pIJ6021	9.2 kb (pIJ4123) and 7.8 kb (pIJ6021) pIJ4123 and pIJ6021 differ in MCS pIJ4123 contains a N-terminal His-Tag *ori* (pIJ101), *tsr,* Kan^R gene under the control of P_{tipA} and *t0*, *ter*, *ori* (pIJ101)	- thiostrepton inducibile P_{tipA} - *E. coli-Streptomyce*s shuttle vectors for gene expression	86
	pHZ1271-1272	10.2 kb (pHZ1272) and 11.7 kb (pHZ1271) pHZ1271 and pHZ1272 differ in the MCS pHZ1271 contains a N-terminal His-Tag	- high copy number - structurally stable in *E. coli* and *Streptomyces lividans* - pIJ4123 and pIJ6021 derivatives	87

		ori (pIJ101), *tsr,* Kan^R gene under the control of P_{tipA} and *t0*, *ter*, *ori* (pBR322), *bla*	- *E. coli*-Streptomyces shuttle vectors for gene expression	
	pCJR24	4.6 kb, *ori* (pUC19)*, tsr*, *actII-ORF4* with downstream actinorhodin promoters (P*actI* and P*actIII*), *bla*	- *E. coli*-Streptomyces shuttle vectors for regulated gene expression of antibiotic biosynthesis genes	88
pJV1		11.1 kb, transfer region similar to pSN22	- wide host range - several cloning vectors were derived from pJV1 (e.g. pB series or the cosmid vector pFD666) - multicopy plasmid (replicative plasmid)	89; 90; 30
7. **Unstable and temperature sensitive plasmids**				
	pHZ1351, pHZ1358, and pMT660	see 2.2 pIJ101 derivatives		
	pCZA185	see 2. pSG5 derivatives		
	pCZA185 and pKC1139	see 2. pSG5 derivatives		
	pIJ80 and pIJ4680	see 2. SCP2* derivatives		
	pHZ132	see 2. pSG5 derivatives		
	pGM9	6.2 kb, *rep* (pSG5), *ori* (pSG5), *ts*, *tsr,* Ble^R, *aphII*	- naturally *ts* for replication - for gene expression	34; 91; 35; 36
	pGM11	5.3 kb, *rep* (pSG5), *ts, ori* (pSG5), *aphII*, *ter*	- naturally *ts* for replication - for gene expression - *aacC1*not expressed (lacks promoter)	34; 91; 35; 36
	pGM16-17	pGM16 (5.1 kb) and pGM17 (6.8 kb) contain different selection markers, *rep* (pSG5), *ori* (pSG5), *ts* pGM16: *tsr, Ble^R* with an upstream promoter of *aphII* from Tn*5* and MCS pGM17: *tsr* was replaced by *cat*, *ble^R* and *aphII*	- naturally *ts* for replication - for gene expression	34; 92
	pGM160	- approx. 7.8 kb, *ori* (pSG5), *rep* (pSG5), *ts, aacC1*, *Ble^R,*	- *E. coli-Streptomyces* shuttle vector (bifunctional pSG5-derived vector) - naturally *ts* for replication - can be used for gene expression (at 28°C-30°C) and for integration into the genome (at 37°C-39°C and without selection)	34; 92; 93
8. **Examples of CRISPR-vectors**				
pSG5-derivative, pJVD52.1	pCRISP omyces	11.4 kb, *oriT, rpsL* promoter, *cas9*, *gapdh* promoter, *lacZα* cloning site, $P_{T7/T3}$ promoter, gRNA site, *ori*, *bla*, *rep* (pSG5)	- multiplex genome editing of *Streptomyces* species - *Streptomyces* codon-optimized *cas9* gene	94; 95

pSG5-derivative, pGM1190	pCRISPR-Cas9	11.2 kb, the *S. coelicolor*-codon optimized *cas9* gene was cloned into the vector pGM1190 pGM1190: *ts*, *tsr*, *aac(3)IV*, *oriT*, *t0* ter, RBS, P_{tipA}, *fd* ter	- multiplex genome editing of *Streptomyces* species - *S. coelicolor*-codon optimized *cas9* gene	34; 92; 93; 96
9. **Other plasmids/vectors**				
SLP1		see, **1.5 SLP1 derivatives** for SLP1 plasmid		
	pIJ61	15.7 kb, *attP* was replaced by *aphII*, *tsr*	- bifunctional plasmid (*E. coli-Streptomyces* shuttle vector) - non-integrating, autonomous conjugative, self-transmissible, pock-forming CCC plasmid, with a copy number of 4-5	97; 98;99
pBR327	pBR327 derivatives	3.2 kb, derived from pBR322 *ori*, *bla*, Tet^R	parts of the vector were used for the construction of hybrid vectors (e.g. *E. coli-Streptomyces* shuttle vector)	100; 101; 102; 103; 104; 105, 106;
	pGUS derivatives based on pKCLP2, pKC1139	*gusA* as reporter system	mostly used for knock-in/knock-outs	107; 108; 109, 110

Abbreviations: aacC1= gentamicin resistance gene, *aadA*= aminoglycoside resistance gene, *acc(3)*IV= apramycin resistance gene, *aphI*= kanamycin resistance gene, *aphII*= neomycin resistance, *att*= attachment site, *bla*= ampicillin resistance gene, Ble^R= bleomycin resistance, cat= chloramphenicol resistance gene, *clt*= cis-acting transfer function (*oriT*-related), *egfp*= gene encoding recombinant Green Fluorescence Protein, *fd*= transcriptional terminator, *gylR*= cassette for glycerol inducible gene expression, *gusA*= GusA directs conversion of X-Gluc into coloured 5,5′-dibromo-4,4′-dichloroindigo, hph^R= hygromycin resistance, *int*= integrase, Kan^R= kanamycin resistance, *kilA*= promoter and amino-terminal region of *tra* (lethality in the absence of the KorA repressor), *kilB*= lethality in the absence of KorB, KorA=kill override (repressor for *tra* and the *korA* expression), KorB= regulator for *kilB*, LacZα= β-galactosidase (first 59 residues), the α-peptide, MCS= Multiple Cloning Sites, *orf*= open reading frame, *ori*= origin of replication, P_{tipA}= thiostrepton inducibile promoter, $P_{T7\ /T3}$= T7/T3 promoters, *rep*= replicon region, RBS= Ribosome Binding Site, *snpR*= SnpA-regulator gene, snpAp= protease promoter, *spd*= spreading pock-formation, spdAB= spread genes, *sti*= strong incompatibility, Tet^R= tetracycline resistance, ter= terminator, *ts*= temperature sensitive plasmid, *tra*= transfer gene, *tsr*= thiostrepton resistance gene, *vph*= viomycin resistance gene, *xylE*= reporter gene encoding catechol 2,3-dioxygenase,

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