

## Plasmids page:

To request any of these plasmids please contact [jic.strepstrains@jic.ac.uk](mailto:jic.strepstrains@jic.ac.uk). Note that all materials are shared subject to MTA and a small charge will be made for strain handling and shipping. The charges are £50 per strain/plasmid for up to three items and then £50 per item for >3 items up to a maximum of 10 (£400). To request exemption from these charges please state your case when you email [jic.strepstrains@jic.ac.uk](mailto:jic.strepstrains@jic.ac.uk).

Visit the *Streptomyces* genome database StrepDB for annotations and other tools (linked from <http://streptomyces.org.uk>)

For protocols and more information on organisms and plasmids visit <http://actinobase.org> which also has a link to a free download of the Practical *Streptomyces* Genetics manual.

Plasmid	Description	Resistance	Replicative status and copy number in <i>Streptomyces</i>	Comments / (Reference)]
<b>Expression vectors</b>				
plJ86	Expression vector ( <i>ermE</i> *p)	AprR (ES)	Conjugative, multi-copy	plJ101 origin, constitutive expression vector (1)
plJ6021	Expression vector ( <i>tipAp</i> )	KanR (S), TsrR (S)	Non-conjugative, multi-copy	Tsr-inducible ( <i>tipAp</i> ) expression vector (2)
plJ6902	Expression vector ( <i>tipAp</i> )	AprR (ES), TsrR (S)	Conjugative, integrative (phiC31)	Tsr-inducible ( <i>tipAp</i> ) expression vector (3)
plJ8671	Expression vector ( <i>tipAp</i> )	AprR (ES), TsrR (S)	Conjugative, requires insert for homologous integration	Allows Tsr-inducible expression of chromosomal genes after homologous recombination
plJ10257	Expression vector ( <i>ermE</i> *p)	HygR (ES)	Conjugative, integrative (phiBT1)	Constitutive ( <i>ermE</i> *p) expression vector (4)
plJ10727	Expression vector (aphp)	AprR (ES)	Conjugative, multi-copy	plJ101 origin, derived from plJ86, constitutive (aphp) expression vector

plJ12477	Expression vector ( <i>ermE</i> *p)	AprR (ES), KanR (ES)	Conjugative, multi-copy	plJ101 origin, derived from plJ86 by addition of neo from SuperCos1, constitutive ( <i>ermE</i> *p) expression vector (5)
plJ12551	Expression vector ( <i>ermE</i> *p)	AprR (ES)	Conjugative, integrative (phiC31)	Constitutive ( <i>ermE</i> *p) expression vector (6)
plJ12552	Expression vector ( <i>ermE</i> *p)	AprR (ES)	Conjugative, integrative (phiC31)	<i>ermE</i> *p in opposite orientation to plJ12551, constitutive expression vector
plJ12901	Expression vector ( <i>tipAp</i> )	KanR (ES), TsrR (S)	Conjugative, multi-copy, unstable	pSG5 origin, temperature-sensitive, Tsr-inducible ( <i>tipAp</i> ) expression vector
plJ13042	Expression vector ( <i>ermE</i> *p)	AprR (ES), TsrR (S)	Conjugative, multi-copy	plJ101 origin, derived from plJ86 by addition of <i>tsr</i> , constitutive ( <i>ermE</i> *p) expression vector
plJ12739	Expression vector ( <i>tipAp</i> )	AprR (ES), TsrR (S)	Conjugative, multi-copy, unstable	pSG5 origin, temperature-sensitive, Tsr-inducible ( <i>tipAp</i> ) Meganuclease gene (7)
plJ12742	Expression vector ( <i>ermE</i> *p)	AprR (ES), TsrR (S)	Conjugative, multi-copy, unstable	pSG5 origin, temperature-sensitive, <i>ermE</i> *p constitutive Meganuclease gene (7)

#### Mutational plasmids

plJ12738	Insertional vector with Scel site	AprR (ES)	Conjugative, requires insert for homologous integration	Vector for integration by homologous recombination before Meganuclease cleavage (7)
plJ12739	Meganuclease delivery plasmid	AprR (ES), TsrR (S)	Conjugative, multi-copy, unstable	pSG5 origin, temperature-sensitive, Tsr-inducible ( <i>tipAp</i> ) Meganuclease gene
plJ12742	Meganuclease delivery plasmid	AprR (ES), TsrR (S)	Conjugative, multi-copy, unstable	pSG5 origin, temperature-sensitive, <i>ermE</i> *p constitutive Meganuclease gene (7)
plJ12900	Deletion/replacement vector	KanR (ES), TsrR (S)	Non-conjugative, multi-copy, unstable	pSG5 origin, temperature-sensitive, useful for deletion after introduction of homologous flanking regions and AprR/oriT

plJ12901	Deletion/replacement vector	KanR (ES), TsrR (S)	Conjugative, multi-copy, unstable	plJ12900 with oriT, useful for generation of unmarked deletions
<b>Reporter vectors</b>				
plJ486/7	KanR reporter plasmid	TsrR (S)	Non-conjugative, multi-copy	Kan reporter plasmids (8)
plJ8630	EGFP reporter plasmid	AprR (ES)	Conjugative, integrative (phiC31)	Cloning sites: BgIII, KpnI, XbaI, NdeI (9)
plJ8660	EGFP reporter plasmid	AprR (ES)	Conjugative, integrative (phiC31)	Cloning sites: BgIII, EcoRV, BamHI, XbaI, NsiI, KpnI, NdeI (9)
plJ10500	Triple flag-tag plasmid	HygR (ES)	Conjugative, integrative (phiBT1)	Flag (3x) cassette in EcoRV site of pMS82 (10)
plJ10740	Positive control for pGUS assays	AprR (ES)	Conjugative, integrative (phiC31)	pGUS with <i>ermE</i> *p (M. Feeney, unpublished)
plJ10741	Positive control for plJ10742 assays	HygR (ES), SpcR (E)	Conjugative, integrative (phiBT1)	plJ10742 with <i>ermE</i> *p (11)
plJ10742	Beta-glucuronidase reporter plasmid	HygR (ES), SpcR (E)	Conjugative, integrative (phiBT1)	pMS82 with <i>gusA</i> (beta-glucuronidase assays) (11)
pSS150	<i>ermE</i> *p-sfGFP reporter plasmid	HygR (ES)	Conjugative, integrative (phiBT1)	plJ10257-based plasmid for the constitutive expression of cytoplasmic sfGFP (12)
plJ10772 (pSS172)	Cloning vector with MCS and mCherry gene	HygR (ES)	Conjugative, integrative (phiBT1)	plJ10770-based (13)
plJ10773 (pSS173)	Cloning vector with MCS and YPet gene	HygR (ES)	Conjugative, integrative (phiBT1)	plJ10770-based (12)
<b>Cloning vectors</b>				
plJ10706	Cloning vector with MCS	HygR (ES)	Conjugative, integrative (phiC31)	pSET152 derivative with AprR replaced with HygR (14)
plJ10750 (pSS10)	Cloning vector with MCS	HygR (ES)	Conjugative, integrative (phiBT1)	pMS82 derivative with additional cloning sites (AprR gene promoter reads into MCS)

				(S.Schlümpert, unpublished)
pIJ10770 (pSS170)	Cloning vector with MCS	HygR (ES)	Conjugative, integrative (phiBT1)	pIJ10750 derivative with AprR gene promoter removed (13)
pIJ12330	Cloning vector with MCS	HygR (ES), AprR (ES)	Conjugative, integrative (phiC31)	pIJ10706 derivative with additional resistance marker - useful for establishing conjugation/selection conditions
pIJ12331	Cloning vector with MCS	HygR (ES), SpcR/StrR (S)	Conjugative, integrative (phiC31)	pIJ10706 derivative with additional resistance marker - useful for establishing conjugation/selection conditions
pIJ12332	Cloning vector with MCS	HygR (ES), VioR (S)	Conjugative, integrative (phiC31)	pIJ10706 derivative with additional resistance marker - useful for establishing conjugation/selection conditions
pIJ12333	Cloning vector with MCS	HygR (ES), TsrR (S)	Conjugative, integrative (phiC31)	pIJ10706 derivative with additional resistance marker - useful for establishing conjugation/selection conditions (6)
<b>Cosmid vectors</b>				
pIJ10702	Conjugative, integrative cosmid vector	AprR (ES), Amp (E )	Conjugative, integrative (phiC31)	Derived from SuperCos1 (15)
<b>PCR targeting</b>				
pIJ773	PCR-targeting - AprR, oriT cassette	AprR (ES), Ampr (E )	Non-conjugative, multi-copy in <i>E. coli</i>	With FRT sites (16)
pIJ773_del_oriT	PCR-targeting - AprR cassette	AprR (ES), Ampr (E )	Non-conjugative, multi-copy in <i>E. coli</i>	With FRT sites
pIJ778	PCR-targeting - SpcR/StrR, oriT cassette	SpcR/StrR (S), AmpR/SpcR (E)	Non-conjugative, multi-copy in <i>E. coli</i>	With FRT sites (17)

plJ799	PCR-targeting - AprR, oriT cassette	AprR (ES), AmpR (E)	Non-conjugative, multi-copy in <i>E. coli</i>	No FRT sites, flanked by bla sequences (to replace bla by AprR-oriT to construct conjugative SuperCos (including flipped) derivatives (18)
plJ10700	PCR-targeting - HygR, oriT cassette	HygR (ES), AmpR (E)	Non-conjugative, multi-copy in <i>E. coli</i>	With FRT sites (16)
plJ10701	PCR-targeting - HygR, oriT cassette	HygR (ES), AmpR (E)	Non-conjugative, multi-copy in <i>E. coli</i>	No FRT sites, flanked by bla sequences (to replace bla by HygR-oriT to construct conjugative SuperCos (including flipped) derivatives (16)

#### Other material

pSET152	Cloning vector	AprR (ES)	Conjugative, integrative (phiC31)	(19)
pKC1132	Suicide vector with oriT	AprR (ES)	Conjugative, non-integrative	Blue/white screening in <i>E. coli</i> for insertion of deletion/disruption cassette (20)

#### Abbreviations

##### Antibiotics:

Amp, ampicillin	Hyg, hygromycin	Str, streptomycin
Apr, apramycin	Kan, kanamycin	Tsr, thiostrepton
Cml, chloramphenicol	Spc, spectinomycin	Vio, viomycin

##### Strains:

E = *E. coli*

S = *Streptomyces*

## References

1. Thanapipatsiri A, Claesen J, Gomez-Escribano JP, Bibb M, Thamchaipenet A. A *Streptomyces coelicolor* host for the heterologous expression of Type III polyketide synthase genes. *Microb Cell Factories*. 2015 Sep 16;14(1):145.
2. Takano E, White J, Thompson CJ, Bibb MJ. Construction of thiostrepton-inducible, high-copy-number expression vectors for use in *Streptomyces* spp. *Gene*. 1995 Dec 1;166(1):133–7.
3. Huang J, Shi J, Molle V, Sohlberg B, Weaver D, Bibb MJ, et al. Cross-regulation among disparate antibiotic biosynthetic pathways of *Streptomyces coelicolor*. *Mol Microbiol*. 2005;58(5):1276–87.
4. Hong HJ, Hutchings MI, Hill LM, Buttner MJ. The role of the novel Fem protein VanK in vancomycin resistance in *Streptomyces coelicolor*. *J Biol Chem*. 2005 Apr 1;280(13):13055–61.
5. Claesen J. Cloning and analysis of the cypemycin biosynthetic gene cluster. *Univ East Angl* 2010.
6. Sherwood EJ, Hesketh AR, Bibb MJ. Cloning and Analysis of the Planosporicin Lantibiotic Biosynthetic Gene Cluster of *Planomonospora alba*. *J Bacteriol*. 2013 May;195(10):2309–21.
7. Fernández-Martínez LT, Bibb MJ. Use of the meganuclease I-SceI of *Saccharomyces cerevisiae* to select for gene deletions in actinomycetes. *Sci Rep*. 2014 Nov 18;4:7100.
8. Ward JM, Janssen GR, Kieser T, Bibb MJ, Buttner MJ, Bibb MJ. Construction and characterisation of a series of multi-copy promoter-probe plasmid vectors for *Streptomyces* using the aminoglycoside phosphotransferase gene from *Tn5* as indicator. *Mol Gen Genet MGG*. 1986 Jun;203(3):468–78.
9. Sun J, Kelemen GH, Fernández-Abalos JM, Bibb MJ. Green fluorescent protein as a reporter for spatial and temporal gene expression in *Streptomyces coelicolor* A3(2). *Microbiol Read Engl*. 1999 Sep;145 ( Pt 9):2221–7.
10. Pullan ST, Chandra G, Bibb MJ, Merrick M. Genome-wide analysis of the role of GlnR in *Streptomyces venezuelae* provides new insights into global nitrogen regulation in actinomycetes. *BMC Genomics*. 2011 Apr 4;12:175.
11. Feeney MA, Chandra G, Findlay KC, Paget MSB, Buttner MJ. Translational Control of the SigR-Directed Oxidative Stress Response in *Streptomyces* via IF3-Mediated Repression of a Noncanonical GTC Start Codon. *mBio*. 2017 Jun 13;8(3):e00815-17.

12. Casu B, Sallmen JW, Schlimpert S, Pilhofer M. Cytoplasmic contractile injection systems mediate cell death in *Streptomyces*. *Nat Microbiol.* 2023;8(4):711–26.
13. Schlimpert S, Wasserstrom S, Chandra G, Bibb MJ, Findlay KC, Flärdh K, et al. Two dynamin-like proteins stabilize FtsZ rings during *Streptomyces* sporulation. *Proc Natl Acad Sci.* 2017 Jul 25;114(30):E6176–83.
14. Foulston LC, Bibb MJ. Microbisporicin gene cluster reveals unusual features of lantibiotic biosynthesis in actinomycetes. *Proc Natl Acad Sci.* 2010 Jul 27;107(30):13461–6.
15. Yanai K, Murakami T, Bibb M. Amplification of the entire kanamycin biosynthetic gene cluster during empirical strain improvement of *Streptomyces kanamyceticus*. *Proc Natl Acad Sci.* 2006 Jun 20;103(25):9661–6.
16. Gust B, Chandra G, Jakimowicz D, Yuqing T, Bruton CJ, Chater KF.  $\lambda$  Red-Mediated Genetic Manipulation of Antibiotic-Producing *Streptomyces* - ScienceDirect. *Adv Appl Microbiol.* 2004;54:107–28.
17. Gust B, Challis GL, Fowler K, Kieser T, Chater KF. PCR-targeted *Streptomyces* gene replacement identifies a protein domain needed for biosynthesis of the sesquiterpene soil odor geosmin. *Proc Natl Acad Sci.* 2003 Feb 18;100(4):1541–6.
18. Gust B, O'Rourke S, Bird N, Kieser T, Chater K. Recombineering in *Streptomyces coelicolor*.  
<https://streptomyces.org.uk/redirect/RecombineeringFEMSMP-2006-5.pdf>.
19. Kieser T, Bibb MJ, Buttner MJ, Chater KF, Hopwood DA. Practical *Streptomyces* Genetics. John Innes Foundation; 2000. 613 p.
20. Bierman M, Logan R, O'Brien K, Seno ET, Rao RN, Schoner BE. Plasmid cloning vectors for the conjugal transfer of DNA from *Escherichia coli* to *Streptomyces* spp. *Gene.* 1992 Jul 1;116(1):43–9.