Mini-Tn7 transposons for site-specific tagging of bacteria with fluorescent proteins

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Mini-Tn*7* transposons – the delivery plasmids

See also the documents: "The Tn*7* transposon " and "How to use the mini-Tn*7* transposons".
Note ! The delivery plasmids presented here are all pUC19 derivatives and can replicate in *E. coli* and other enterics, but for example not in *Pseudomonas.* Only characters inserted as part of the mini-Tn*7* transposon are described in the table below.

Note also: Sequences of the plasmids are not available as the system is based on plasmids, which were constructed long time ago when sequencing was not common. For available information about the base plasmids see McKown *et al*. 1988; Bao *et al*., 1991; Højberg *et al*., 1999 and Koch *et al*., 2001.

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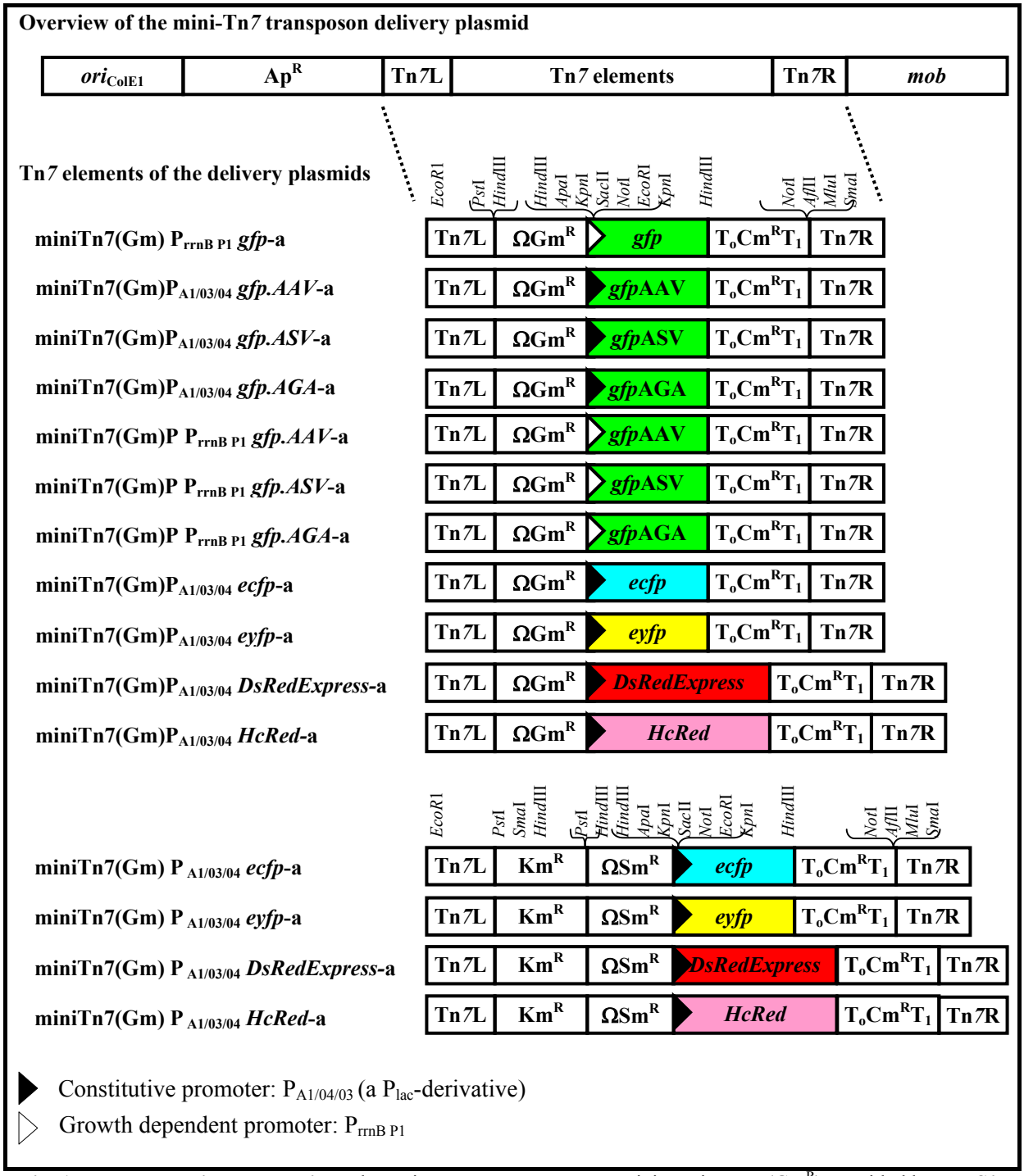

Fig. 1. The Tn*7* delivery plasmids. The resistance genes are: gentamicin resistance (Gm^R) provided by *aacC1* encoding acetyltransferase-3-1, from plasmid pAComegaGm, chloramphenicol resistance (Cm^R) provided by *cat* encoding chloramphenicol-acetyl-transferase, kanamycin resistance (Km^R) provided by neomycin phosphotransferase (nptII), original from Tn903 (Oka *et al*., 1981) and streptomycin resistance (Sm^R) provided by *aadA* from pUCBM20 and original from Tn9 (Boehringer Mannheim). All plasmids are based on pUC19 and carry resistance to ampicillin (Koch *et al*., 2001). Ω shows that the resistance gene is flanked by transcription and translation terminators (Fellay *et al*., 1987) and the fluorescent proteins are from Clontech Laboratories (Palo Alto, CA). All constructs contain the ribosomal binding site, RBSII, in front of the fluorescent gene and terminator T_0 and T_1 flanking *cat* after, as indicated and described by Andersen *et al*., 1998. *GfpAAV*, *gfpAGA* and *gfpASV* encode unstable versions of Gfp protein (Andersen *et al*., 1998; Ramos *et al* 2000). Most constructs are also available with the fluorescent protein transcribed in the direction from the right to left, though we have not indications that the orientation should have an effect on expression (data not shown). Useful known restriction sites are indicated, the figure is not drawn to scale.

Table 2. Mini-Tn7 transposons – the delivery plasmids (see also Fig. 1)

Name of plasmid	Antibiotic[1] resistance to	Promoter (driving the fluorescent protein)[2]	Fluorescent protein or other marker [2,3]	Comments For details on the constructions, see the section "construction of delivery plasmids"	Strain harbouring the plasmid *E. coli*	Reference	Cloned by [4]	Our strain number
pBK-miniTn7-ΩGm	Gm →	-	-	see reference	XL1-Blue	Koch *et al*., 2001	BK	AKN62
pBK-miniTn7-ΩSm1	Sm	-	-	see reference	XL1-Blue	Koch *et al*., 2001	BK	AKN63
pBK-miniTn7-KmΩSm1	Km →	-	-	see reference	XL1-Blue	Koch *et al*., 2001	BK	AKN64
pBK-miniTn7-*gfp*1	Km→,Cm→	$P_{A1/04/03}$ [5]	GFP→	see reference	XL1-Blue	Koch *et al*., 2001	BK	AKN65
pBK-miniTn7-*gfp*2	Gm→,Cm→	$P_{A1/04/03}$	GFP→	see reference	XL1-Blue	Koch *et al*., 2001	BK	AKN66
pBK-miniTn7-*gfp*3	Km→,Sm, Cm→	$P_{A1/04/03}$	GFP→	see reference	XL1-Blue	Koch *et al*., 2001	BK	AKN67
miniTn7(Gm)$P_{A1/04/03}$–*ecfp-a*	Gm→,Cm→	$P_{A1/04/03}$	ECFP→	$P_{A1/04/03}$–*ecfp* cloned into *Not*I site of pBK-miniTn7-ΩGm	JM105	Klausen *et al*., 2003	AKN	AKN34
miniTn7(Gm)$P_{A1/04/03}$–*ecfp*-b	Gm→,Cm←	$P_{A1/04/03}$	ECFP←	$P_{A1/04/03}$–*ecfp* cloned into *Not*I site of pBK-miniTn7-ΩGm	JM105	Lambertsen *et al*., 2003	AKN	AKN35
miniTn7(Gm)$P_{A1/04/03}$–*eyfp*-b	Gm→,Cm←	$P_{A1/04/03}$	EYFP←	$P_{A1/04/03}$–*eyfp* cloned into *Not*I site of pBK-miniTn7-ΩGm	JM105	Lambertsen *et al*., 2003	AKN	AKN36
miniTn7(Gm)$P_{A1/04/03}$–*eyfp*-a	Gm→,Cm→	$P_{A1/04/03}$	EYFP→	$P_{A1/04/03}$–*eyfp* cloned into *Not*I site of pBK-miniTn7-ΩGm	JM105	Klausen *et al*., 2003	AKN	AKN69

[1] An arrow after the resistance marker indicates the direction of transcription of the gene compared to the left, Tn7L, and right, Tn7R, side of the transposon. Gm: gentamicin resistance is provided by *aacC1* encoding acetyltransferase-3-1, from pAComegaGm. Cm: chloramphenicol resistance provided by *cat* encoding chloramphenicol-acetyl-transferase. Km: kanamycin resistance provided by neomycin phosphotransferase (nptII), original from Tn903 (Oka *et al*., 1981), Sm: streptomycin resistance provided by from pUCBM20, original from Tn9 (Boehringer Mannheim). All plasmids are based on pUC19 and carry resistance to ampicillin.

[2] All constructs contain the ribosomal binding site: RBSII and terminator T_0 and T_1 flanking *cat* (Cm resistance) after the fluorescent gene as described by Andersen *et al*., 1998.

[3] An arrow after the encoded protein indicates the direction of transcription of the gene compared to the left Tn7L, and right, Tn7R, side of the transposon. The *gfp* gene used is the derivative *gfp*mut3*.

[4] AKN: Anne Kirstine Nielsen, TJ: Tove Johansen, BK: Birgit Koch, LL: Lotte Lambertsen

[5] Lac derived promoter (Lanzer and Bujard, 1988).

Name of plasmid	Antibiotic[1] resistance to	Promoter (driving the fluorescent protein)[2]	Fluorescent protein or other marker [2,3]	Comments For details on the constructions, see the section "construction of delivery plasmids"	Strain harbouring the plasmid *E. coli*	Reference	Cloned by [4]	Our strain number
miniTn7(Gm)$P_{A1/04/03}$–*dsred*-a	Gm→,Cm→	$P_{A1/04/03}$	DsRed→	$P_{A1/04/03}$–*dsred* cloned into *Not*I site of pBK-miniTn7-ΩGm	JM105	Lambertsen *et al*., 2003	AKN	AKN38
miniTn7(Gm)P_{rrnB1}–*gfp*-a	Gm→,Cm→	P_{rrnB1} [6]	GFP→	P_{rr}-*gfp* cloned into *Not*I site of pBK-miniTn7-ΩGm	MT102	Lambertsen *et al*., 2003	TJ	SM1973 or AKN137
miniTn7(Gm)P_{rrnB1}–*gfp*AAV-a	Gm→,Cm→	P_{rrnB1}	GFP-AAV→	P_{rr}-*gfp*-AAV cloned into *Not*I site of pBK-miniTn7-ΩGm	MT102	Lambertsen *et al*., 2003	TJ	SM1974 or AKN138
miniTn7(Gm)P_{rrnB1} –*gfp*ASV-a	Gm→,Cm→	P_{rrnB1}	GFP-ASV→	P_{rr}-*gfp*-ASV cloned into *Not*I site of pBK-miniTn7-ΩGm	MT102	Lambertsen *et al*., 2003	TJ	SM1975 or AKN139
miniTn7(Gm)P_{rrnB1}–*gfp*AGA-a	Gm→,Cm→	P_{rrnB1}	GFP-AGA→	P_{rr}-*gfp*-AGA cloned into *Not*I site of pBK-miniTn7-ΩGm	MT102	Lambertsen *et al*., 2003	TJ	SM1976 or AKN140
miniTn7(Km, Sm)$P_{A1/04/03}$–*ecfp*-a	Km→,Sm→ Cm→	$P_{A1/04/03}$	ECFP→	$P_{A1/04/03}$–*ecfp* cloned into *Not*I site of pBK-miniTn7-KmΩSm1	MT102	Lambertsen *et al*., 2003	AKN	AKN84
miniTn7(Km, Sm)$P_{A1/04/03}$–*ecfp*-b	Km→ Sm→ Cm←	$P_{A1/04/03}$	ECFP←	$P_{A1/04/03}$–*ecfp* cloned into *Not*I site of pBK-miniTn7-KmΩSm1	MT102	Lambertsen *et al*., 2003	AKN	AKN99
miniTn7(Km,Sm)$P_{A1/04/03}$–*eyfp*-a	Km→,Sm→ Cm→	$P_{A1/04/03}$	EYFP→	$P_{A1/04/03}$–*eyfp* cloned into *Not*I site of pBK-miniTn7-KmΩSm1	MT102	Lambertsen *et al*., 2003	AKN	AKN86
miniTn7(Km, Sm)$P_{A1/04/03}$–*eyfp*-b	Km→,Sm→ Cm←	$P_{A1/04/03}$	EYFP←	$P_{A1/04/03}$–*eyfp* cloned into *Not*I site of pBK-miniTn7-KmΩSm1	MT102	Lambertsen *et al*., 2003	AKN	AKN85
miniTn7(Gm)$P_{A1/04/03}$–*gfp*AAV-a	Gm→,Cm→	$P_{A1/04/03}$	GFP-AAV→	P_{rr}-*gfp*-AAV cloned into *Not*I site of pBK-miniTn7-ΩGm	JM105	Lambertsen *et al*., 2003	AKN	AKN104
miniTn7(Gm) $P_{A1/04/03}$–*gfp*ASV-a	Gm→,Cm→	$P_{A1/04/03}$	GFP-ASV→	$P_{A1/04/03}$-*gfp*-ASV cloned into *Not*I site of pBK-miniTn7-ΩGm	JM105	Lambertsen *et al*., 2003	AKN	AKN102
miniTn7(Gm) $P_{A1/04/03}$–*gfp*AGA-a	Gm→,Cm→	$P_{A1/04/03}$	GFP-AGA→	$P_{A1/04/03}$-*gfp*-AGA cloned into *Not*I site of pBK-miniTn7-ΩGm	JM105	Lambertsen *et al*., 2003	AKN	AKN100
miniTn7(Gm) $P_{A1/04/03}$–*gfp*AAV-b	Gm→,Cm←	$P_{A1/04/03}$	GFP-AAV←	$P_{A1/04/03}$-*gfp*-AAV cloned into *Not*I site of pBK-miniTn7-ΩGm	JM105	Lambertsen *et al*., 2003	AKN	AKN105
miniTn7(Gm)$P_{A1/04/03}$–*gfp*ASV-b	Gm→,Cm←	$P_{A1/04/03}$	GFP-ASV←	$P_{A1/04/03}$-*gfp*-ASV cloned into *Not*I site of pBK-miniTn7-ΩGm	JM105	Lambertsen *et al*., 2003	AKN	AKN103
miniTn7(Gm)$P_{A1/04/03}$–*gfp*AGA-b	Gm→,Cm←	$P_{A1/04/03}$	GFP-AGA←	$P_{A1/04/03}$-*gfp*-AGA cloned into *Not*I site of pBK-miniTn7-ΩGm	JM105	Lambertsen *et al*., 2003	AKN	AKN101

[6] A ribosomal promoter. Growth rate regulated (Bartlett and Gourse, 1994).

Name of plasmid	**Antibiotic[1] resistance to**	**Promoter** (driving the fluorescent protein)[2]	**Fluorescent protein or other marker** [2,3]	**Comments For details on the constructions, see the section "construction of delivery plasmids"**	**Strain harbouring the plasmid *E. coli***	**Reference**	**Cloned by** [4]	**Our strain number**
miniTn7(Gm)$P_{A1/04/03}$− DsRedExpress-a	Gm→,Cm→	$P_{A1/04/03}$	DsRedExpress →	$P_{A1/04/03}$- DsRedExpress (AKN122) cloned into *Not*I site of pBK-miniTn7-ΩGm	JM105	Lambertsen *et al*., 2003	LL	AKN132
miniTn7(Gm)$P_{A1/04/03}$− DsRedExpress-b	Gm→,Cm←	$P_{A1/04/03}$	DsRedExpress ←	$P_{A1/04/03}$- DsRedExpress (AKN122) cloned into *Not*I site of pBK-miniTn7-ΩGm	JM105	Lambertsen *et al*., 2003	LL	AKN131
miniTn7(Km, Sm)$P_{A1/04/03}$− DsRedExpress-a	Km→, Sm, Cm→	$P_{A1/04/03}$	DsRedExpress →	$P_{A1/04/03}$- DsRedExpress (AKN122) cloned into *Not*I site of pBK-miniTn7-KmΩSm1	JM105	Lambertsen *et al*., 2003	LL	AKN133
miniTn7(Km, Sm)$P_{A1/04/03}$− DsRedExpress-b	Km→, Sm, Cm←	$P_{A1/04/03}$	DsRedExpress ←	$P_{A1/04/03}$- DsRedExpress (AKN122) cloned into *Not*I site of pBK-miniTn7-KmΩSm1	JM105	Lambertsen *et al*., 2003	LL	AKN134
miniTn7(Gm)$P_{A1/04/03}$–*HcRed*-a	Gm→,Cm→	$P_{A1/04/03}$	HcRed1→	$P_{A1/04/03}$- HcRed1 (AKN118) cloned into *Not*I site of pBK-miniTn7-ΩGm	JM105	Lambertsen *et al*., 2003	LL	AKN123
miniTn7(Gm)$P_{A1/04/03}$–*HcRed*-b	Gm→,Cm←	$P_{A1/04/03}$	HcRed1←	$P_{A1/04/03}$- HcRed1 (AKN118) cloned into *Not*I site of pBK-miniTn7-ΩGm	JM105	Lambertsen *et al*., 2003	LL	AKN124
miniTn7(Km, Sm)$P_{A1/04/03}$–*HcRed*-a	Km→,Sm, Cm→	$P_{A1/04/03}$	HcRed1→	$P_{A1/04/03}$- HcRed1 (AKN118) cloned into *Not*I site of pBK-miniTn7-KmΩSm1	JM105	Lambertsen *et al*., 2003	LL	AKN125
miniTn7(Km, Sm)$P_{A1/04/03}$–*HcRed*-b	Km→,Sm, Cm←	$P_{A1/04/03}$	HcRed1←	$P_{A1/04/03}$- HcRed1 (AKN118) cloned into *Not*I site of pBK-miniTn7-KmΩSm1	JM105	Lambertsen *et al*., 2003	LL	AKN126
pUX-BF13				Helper plasmid, providing the Tn*7* transposase proteins	*E. coli* SM10::λpir	Bao *et al*., 1991		AKN68 or SM1958

Construction of mini-Tn*7* transposon delivery plasmids for fluorescent tagging

All delivery plasmids (Fig. 1) are based on the mini-Tn*7* delivery plasmids published by Koch *et al*., 2001. They are all pUC-plasmid derivatives containing ampicillin resistance. They also carry the mobilisation genes (*mob*) from RP4, which provide these plasmids with the ability to be mobilised into a host by the RP4/ RK2 plasmid. Apart from this they carry the DNA fragment that will be inserted by transposition, containing genes encoding an antibiotic resistance marker and a fluorescent protein, flanked by the left Tn7L and right Tn7R end of the Tn7 transposon. All constructs contain the ribosomal binding site: RBSII in front of the fluorescent gene and terminator T_0 and T_1 flanking *cat* (Cm resistance) after the fluorescent gene as described by Andersen *et al*., 1998. Below are the details about the constructed plasmids.

All DNA manipulations were performed essentially as described by Sambrook *et al*., 1989, except for DNA plasmid purification which was performed using the Plasmid DNA purification QIAprep Spin Miniprep Kit (Qiagen GmbH) and DNA fragments and PCR products, were purified using GFX^{TM} PCR DNA and Gel Band Purification Kit (Amersham Phamacia Biotech Europe). Enzymes were purchased from GibcoBRL, Life Technologies.

All delivery plasmids have been tested by transposition into *Pseudomonas putida* KT2440 using either electroporation or mobilitation (by four-parental mating with *P. putida* KT2440, *E. coli* HB101/RK600 (mobilises the other plasmids), *E. coli* SM10::λpir/ pUX-BF13 (helper contains the transposase genes) and *E. coli* containing the delivery-plasmid being tested. Inserts were tested as described by Lambertsen *et al*., (2003) and in the document "How to use the Mini-Tn7 transposon".

miniTn*7*(Gm)P$_{A1/04/03}$–*ecfp*-a and **miniTn*7*(Gm)P$_{A1/04/03}$–*ecfp*-b** were constructed by:
a) PCR amplifying the *ecfp* gene as a 740 bp fragment from the templates pECFP (Clontech) using the primer pair 5'-atatagcatgctgagcaagggcgaggagctg-3' and 5'-ctctcaagcttattacttgtacagctcgtccatgcc-3', which also introduce a *Sph*I and *Hind*III restriction site. (b) Cloning the *SphI-Hind*III digested PCR fragments into the *SphI-Hind*III site of pTTN50 (a pUC18Not::P$_{A1/04/03}$ *dsRed*; Tolker-Nielsen *et al*., 2000), resulting in pUC18Not::P$_{A1/04/03}$ *ecfp*. (c) Cloning the app. 2,000 bp *Not*I fragment from this plasmid into the *Not*I site of pBK-miniTn7-ΩGm (Koch *et al*., 2001). d) Orientation of the inserted *Not*I-fragment was tested by restriction with *Knp*I and the construction in which *ecfp* is transcribed in the direction from Tn7L to Tn7R was named -a, the other -b.

miniTn7(Gm)*P$_{A1/04/03}$*–*eyfp*-a and **miniTn7(Gm)P$_{A1/04/03}$–*eyfp*-b** were constructed by:
a) PCR amplifying the *eyfp* gene as a 740 bp fragment from the templates pEYFP (Clontech) using the primer pair 5'-atatagcatgctgagcaagggcgaggagctg-3' and 5'-ctctcaagcttattacttgtacagctcgtccatgcc-3', which also introduce a *Sph*I and *Hind*III restriction site. (b) Cloning the *SphI-Hind*III digested PCR fragments into the *SphI-Hind*III site of pTTN50 (a pUC18Not::P$_{A1/04/03}$ *dsRed*; Tolker-Nielsen *et al*., 2000), resulting in pUC18Not::P$_{A1/04/03}$ *eyfp*. (c) Cloning the app. 2,000 bp NotI fragment from this plasmid into the *Not*I site of pBK-miniTn7-ΩGm (Koch *et al*., 2001). d) Orientation of the inserted *Not*I-fragment was tested by restriction with *Knp*I and the construction in which *eyfp* is transcribed in the direction from Tn7L to Tn7R was named -a, the other -b.

miniTn7(Gm)*P$_{A1/04/03}$*–*dsred*-a was constructed by:
a) Cloning an app. 2,000 bp *Not*I fragment from pTTN50 (a pUC18Not::P$_{A1/04/03}$ *dsRed*; dsRed from Clontech, Tolker-Nielsen *et al*., 2000) into the *Not*I site of pBK-miniTn7-ΩGm (Koch *et al*., 2001). b) Orientation of the inserted *Not*I-fragment was tested by restriction

with *Knp*I and the construction in which *dsRed* is transcribed in the direction from Tn7L to Tn7R was named -a.
Note this mini-Tn*7* insert does only give a visible colour after several days, when used in *Pseudomonas putida* KT2440.

miniTn7(Gm)*P_{rrnB1}*–*gfp*-a was constructed by:
a) Cloning an app. 2,000 bp *Not*I fragment from pSM1690 (pLOW2Not-*rrnB*P1-*gfp*mut3b*; Sternberg *et al*., 1999) into the *Not*I site of pBK-miniTn7-ΩGm (Koch *et al*., 2001). b) Orientation of the inserted *Not*I-fragment was tested by restriction with *Knp*I and the construction in which *gfp* is transcribed in the direction from Tn7L to Tn7R was named -a.

miniTn7(Gm)*P_{rrnB1}*–*gfp*AAV-a was constructed by:
a) Cloning an app. 2,000 bp *Not*I fragment from pSM1606 (pLOW2Not-*rrnB*P1-*gfp*(AAV); Sternberg *et al*., 1999) into the *Not*I site of pBK-miniTn7-ΩGm (Koch *et al*., 2001). b) Orientation of the inserted *Not*I-fragment was tested by restriction with *Knp*I and the construction in which *gfp* is transcribed in the direction from Tn7L to Tn7R was named -a.

miniTn7(Gm)*P_{rrnB1}* –*gfp*ASV-a was constructed by:
a) Cloning an app. 2,000 bp *Not*I fragment from pTTN129 (pLOW2Not-*rrnB*P1-*gfp*(ASV), constructed as described in Sternberg *et al*., 1999 into the *Not*I site of pBK-miniTn7-ΩGm (Koch *et al*., 2001). b) Orientation of the inserted *Not*I-fragment was tested by restriction with *Knp*I and the construction in which *gfp* is transcribed in the direction from Tn7L to Tn7R was named –a.

miniTn7(Gm)*P_{rrnB1}*–*gfp*AGA-a was constructed by: a) Cloning an app. 2,000 bp *Not*I fragment from pSM1692 (pLOW2Not-*rrnB*P1-*gfp*(AGA); Ramos *et al*., 2000; Sternberg *et al*., 1999 into the *Not*I site of pBK-miniTn7-ΩGm (Koch *et al*., 2001). b) Orientation of the inserted *Not*I-fragment was tested by restriction with *Knp*I and the construction in which *gfp* is transcribed in the direction from Tn7L to Tn7R was named –a.

miniTn7(Km, Sm)*$P_{A1/04/03}$*–*ecfp*-a and **miniTn7(Km, Sm)*$P_{A1/04/03}$*–*ecfp*-b** were constructed by cloning the app. 2,000 bp *Not*I fragment from pUC18Not::$P_{A1/04/03}$ *ecfp* into the *Not*I site of pBK-miniTn7-KmΩSm1 (Koch *et al*., 2001). b) Orientation of the inserted *Not*I-fragment was tested by restriction with *Knp*I and the construction in which *ecfp* is transcribed in the direction from Tn7L to Tn7R was named -a, the other -b.

miniTn7(Km,Sm)*$P_{A1/04/03}$*–*eyfp*-a and **miniTn7(Km, Sm)*$P_{A1/04/03}$*–*eyfp*-b** were constructed by: a) cloning the app. 2,000 bp *Not*I fragment from pUC18Not::$P_{A1/04/03}$ *eyfp* into the *Not*I site of pBK-miniTn7-KmΩSm1 (Koch *et al*., 2001). b) Orientation of the inserted *Not*I-fragment was tested by restriction with *Knp*I and the construction in which *ecfp* is transcribed in the direction from Tn7L to Tn7R was named -a, the other -b.

miniTn7(Gm)*$P_{A1/04/03}$*–*gfp*AAV-a and **miniTn7(Gm) *$P_{A1/04/03}$* –*gfp*AAV-b** were constructed by: a) Cloning an app. 2,000 bp *Not*I fragment from JBA112 (Andersen *et al*., 1998)into the *Not*I site of pBK-miniTn7-ΩGm (Koch *et al*., 2001). b) Orientation of the inserted *Not*I-fragment was tested by restriction with *Knp*I and the construction in which *gfp* is transcribed in the direction from Tn7L to Tn7R was named –a, the other b.

miniTn7(Gm) *$P_{A1/04/03}$* –*gfp*ASV-a and **miniTn7(Gm)*$P_{A1/04/03}$* –*gfp*ASV-b** were constructed by: a) Cloning an app. 2,000 bp *Not*I fragment from JBA113 (Andersen *et al*., 1998)into the

*Not*I site of pBK-miniTn7-ΩGm (Koch *et al*., 2001). b) Orientation of the inserted *Not*I-fragment was tested by restriction with *Knp*I and the construction in which *gfp* is transcribed in the direction from Tn7L to Tn7R was named –a, the other b.

miniTn7(Gm) $P_{A1/04/03}$–*gfp*AGA-a and **miniTn7(Gm)$P_{A1/04/03}$–*gfp*AGA-b** were constructed by: a) Cloning an app. 2,000 bp *Not*I fragment from JBA47 (Andersen *et al*., 1998; Ramos *et al*., 2000) into the *Not*I site of pBK-miniTn7-ΩGm (Koch *et al*., 2001). b) Orientation of the inserted *Not*I-fragment was tested by restriction with *Knp*I and the construction in which *gfp* is transcribed in the direction from Tn7L to Tn7R was named –a, the other b.

miniTn7(Gm)$P_{A1/04/03}$–DsRedExpress-a and **miniTn7(Gm)$P_{A1/04/03}$–DsRedExpress-b** were constructed by a) cloning the *Nco*I-*Not*I fragment (klenow treated) of pDsRedExpress (Clontech) into pJBA27 (Andersen *et al*., 1998) restricted by *Hind*III and klenow treated, then restricted by *Sph*I and T4-DNA polymerase treated to form the plasmid p18-$P_{A1/04/03}$ DsRedExpress. The app. 2000 bp *Not*I-fragment from this plasmid was inserted into the *Not*I site of pBK-miniTn7-ΩGm (Koch *et al*., 2001). b) Orientation of the inserted *Not*I-fragment was tested by restriction with *Pst*I and the construction in which *DsRedExpress* is transcribed in the direction from Tn7L to Tn7R was named –a, the other b.
Note this mini-Tn*7* insert does only give a visible colour after 1-2 days, when used in *Pseudomonas putida* KT2440.

miniTn7(Km, Sm)$P_{A1/04/03}$–DsRedExpress-a and **miniTn7(Km, Sm)$P_{A1/04/03}$–DsRedExpress-b** were constructed by a) cloning the *Nco*I-*Not*I fragment (klenow treated) of pDsRedExpress (Clontech) into pJBA27 (Andersen *et al*., 1998) restricted by *Hind*III and klenow treated, then restricted by *Sph*I and T4-DNA polymerase treated to form the plasmid p18-$P_{A1/04/03}$ DsRedExpress. The app. 2000 bp *Not*I-fragment from this plasmid was inserted into the *Not*I site of pBK-miniTn7-KmΩSm1 (Koch *et al*., 2001). b) Orientation of the inserted *Not*I-fragment was tested by restriction with *Pst*I and the construction in which *DsRedExpress* is transcribed in the direction from Tn7L to Tn7R was named –a, the other b.
Note this mini-Tn*7* insert does only give a visible colour after 1-2 days, when used in *Pseudomonas putida* KT2440.

miniTn7(Gm)$P_{A1/04/03}$–*HcRed*-a and **miniTn7(Gm)$P_{A1/04/03}$–*HcRed*-b** were constructed by a) cloning the *Nco*I-*Not*I fragment (klenow treated) of pHcRed (Clontech) into pJBA27 (Andersen *et al*., 1998) restricted by *Hind*III and klenow treated, then restricted by *Sph*I and T4-DNA polymerase treated to form the plasmid p18-$P_{A1/04/03}$ HcRed. The app. 2000 bp *Not*I-fragment from this plasmid was inserted into the *Not*I site of pBK-miniTn7-ΩGm (Koch *et al*., 2001). b) Orientation of the inserted *Not*I-fragment was tested by restriction with *Nar*I and the construction in which *HcRed* is transcribed in the direction from Tn7L to Tn7R was named –a, the other b.
Note this mini-Tn*7* insert does only give a visible colour after 1 day, when used in *Pseudomonas putida* KT2440.

miniTn7(Km, Sm)$P_{A1/04/03}$–*HcRed*-a and **miniTn7(Km, Sm)$P_{A1/04/03}$–*HcRed*-b** were constructed by a) cloning the *Nco*I-*Not*I fragment (klenow treated) of pHcRed (Clontech) into pJBA27 (Andersen *et al*., 1998) restricted by *Hind*III and klenow treated, then restricted by *Sph*I and T4-DNA polymerase treated to form the plasmid p18-$P_{A1/04/03}$ HcRed. The app. 2000 bp *Not*I-fragment from this plasmid was inserted into the *Not*I site of pBK-miniTn7-KmΩSm1 (Koch *et al*., 2001). b) Orientation of the inserted *Not*I-fragment

was tested by restriction with *Nar*I and the construction in which *HcRed* is transcribed in the direction from Tn7L to Tn7R was named –a, the other b.
Note this mini-Tn*7* insert does only give a visible colour after 1 day, when used in *Pseudomonas putida* KT2440.

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