

A pair of mobilizable shuttle vectors conferring resistance to spectinomycin for molecular cloning in *Escherichia coli* and in Gram-positive bacteria

Patrick Trieu-Cuot*, Cécile Carlier, Claire Poyart-Salmeron and Patrice Courvalin
Unité des Agents Antibactériens, CNRS UA 271, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris Cedex 15, France

Submitted May 4, 1990

We previously designed a vector strategy that allows transfer by conjugation of recombinant plasmids from *Escherichia coli* to various Gram-positive bacilli and cocci (1). We report here the construction of plasmid vectors which exploit this broad host range transfer system. These vectors, pAT28 and pAT29, are composed of 1) the origins of replication of pUC (2) and of the broad host range enterococcal plasmid pAMβ1 (3); 2) a spectinomycin resistance gene expressed in Gram-negative and in Gram-positive bacteria (4); 3) the origin of transfer of the IncP plasmid RK2; and 4) the multiple cloning site and the *lacZα* reporter gene of pUC18 (pAT28) and pUC19 (pAT29). These plasmids can be efficiently mobilized by any self-transferable IncP plasmid co-resident in *E. coli* donors (1). They are 6.7 kilobases in length and contain nine unique cloning sites (Figure 1) which allow easy screening of derivatives containing DNA inserts by α-complementation in *E. coli* carrying the M15 deletion of *lacZα* (2). Plasmids pAT28, pAT29 and recombinant derivatives were transferred by filter mating (1) from *E. coli* to *Enterococcus faecalis, Lactococcus lactis, Listeria innocua* and *Listeria monocytogenes* at frequencies ranging from 10^{-6} to 10^{-7}. This powerful transfer system offers an alternative to transformation and electroporation for introducing plasmid DNA in a large range of Gram-positive organisms.

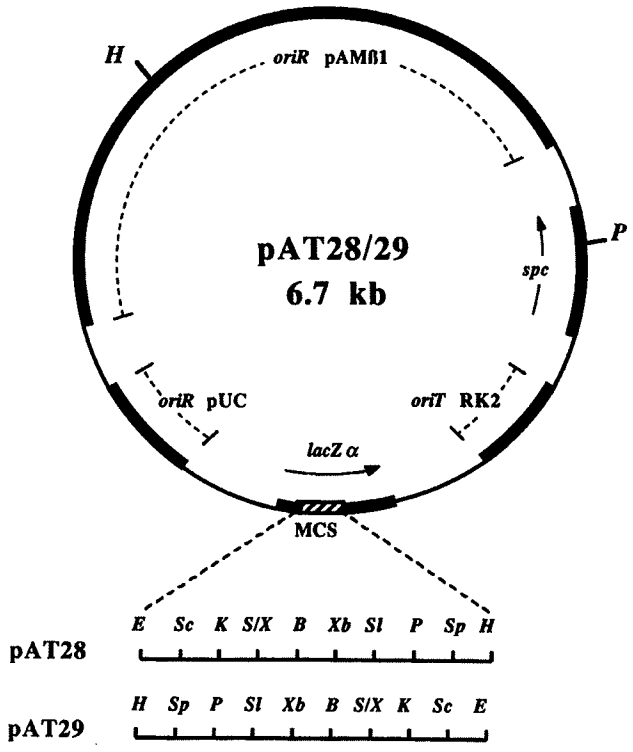

Figure 1. Structure of the mobilizable shuttle vectors pAT28 and pAT29. *oriR*, origin of replication; *oriT*, origin of transfer; MCS, multiple cloning site; *spc*, spectinomycin resistance gene. Arrows indicate the direction and extent of transcription. *B, Bam*HI; *E, Eco*RI; *H, Hin*dIII; *K, Kpn*I; *P, Pst*I; *Sl; Sal*I; *Sc, Sac*I, *S, Sma*I; *Sp, Sph*I; *Xb, Xba*I; *X, Xma*I. Note the presence of two *Hin*dIII and *Pst*I restriction sites.

ACKNOWLEDGEMENTS

This work was supported by a grant (ATP Bactéries Lactiques) from the Centre National de la Recherche Scientifique. C.P.-S. was the recipient of a fellowship from the Fondation pour la Recherche Médicale.

REFERENCES

1. Trieu-Cuot,P., Carlier,C., Martin,P. and Courvalin,P. (1987) *FEMS Microbiol. Lett.* **48**, 289–294.
2. Yanisch-Perron,C., Vieira,J. and Messing,J. (1985) *Gene* **33**, 103–119.
3. Leblanc,D.J. and Lee,L.N. (1984) *J. Bacteriol.* **157**, 445–453.
4. Murphy,E. (1985) *Mol. Gen. Genet.* **200**, 33–39.

* To whom correspondence should be addressed