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Two new multi-purpose multicopy *Schizosaccharomyces pombe* shuttle vectors, pSP1 and pSP2

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Abstract. Plasmids pSP1 and pSP2 are two new *Schizosaccharomyces pombe ars1* multicopy vectors with the *Saccharomyces cerevisiae LEU2* and *URA3* genes as selectable markers. They are derivatives of *S. cerevisiae* integrative plasmids. These plasmids allow classical molecular genetic techniques, such as mutagenesis, nested deletions and sequencing, to be performed directly.

Key words: Autonomously replicating sequence – Recombinant DNA – Nested deletions – Mutagenesis – *Schizosaccharomyces pombe*

Although *S. pombe* molecular genetics has been possible for more than a decade (Beach and Nurse 1981), little has been done to improve the cloning and manipulations of its DNA. Initially, genes of *S. pombe* have been cloned using *S. cerevisiae-Escherichia coli* shuttle vectors bearing the 2-μm autonomously replicating sequence (ars) for replication: pDB248 (Beach and Nurse 1981), pDB262 (Beach et al. 1982a), pDB248′ (Beach et al. 1982b), YEp13 (Broach et al. 1979), and pWH5 (Wright et al. 1986). In contrast to common belief these 2-μm-based vectors do not behave as real high-copy plasmids in *S. pombe* (Heyer et al. 1986). The region that functions as an ars in *S. pombe* does not include the complete replication origin that functions in *S. cerevisiae* (Gaillardin et al. 1983; G. C. unpublished data) and not all 2-μm-based plasmids transform *S. pombe* at high frequency.

The isolation and characterisation of ars sequences that promote high transformation efficiency (Losson and Lacroute 1983; Maundrell et al. 1988) was crucial for the design of multicopy plasmids such as pFL20 (Losson and Lacroute 1983), pIRT2 (Hindley et al. 1987), pON163 and pDW232 (Weilguny et al. 1991).

We designed two versatile *S. pombe* vectors by introducing the *S. pombe ars1* element into the integrative *S. cerevisiae* plasmids pRS305 and pRS306 (Sikorski and Hieter 1989). These two constructs carry the *LEU2* (pRS305) or *URA3* (pRS306) gene within the *Nde*I site of PRSS56 (Sikorski and Hieter 1989), a chimeric plasmid constructed from pBluescribe and pBluescript (Stratagene; Sikorski and Hieter 1989). These plasmids contain the f1 phage origin of DNA replication for the production of single-stranded DNA in vivo, the T3/T7 phage promoters for the in-vitro synthesis of RNA, and a large polylinker region in the alpha fragment of the *LacZ* gene allowing the blue/white color screen of recombinants. Nested deletions of inserts can be performed (Henikoff 1984, 1990) and single-stranded DNA can be prepared for sequencing (Sanger et al. 1977). The *S. cerevisiae LEU2* and *URA3* genes complement *S. pombe* mutants carrying the *leu1*$^-$ and *ura4*$^-$ alleles respectively. The 1.2-kbp *Eco*RI *ars1* DNA fragment from *S. pombe* (Losson and Lacroute 1983) was filled in with T4 DNA polymerase in the presence of dATP and dTTP and introduced into the *Aat*II sites of pRS305 and pRS306 which were blunted with the exonuclease activity of the T4 DNA polymerase in the presence of dCTP (Sambrook et al. 1989). The derivatives were named pSP1 and pSP2 respectively (plasmid *S. pombe*) (Fig. 1). Although the ligation of a blunt *Aat*II site to a blunt *Eco*RI site should recreate an *Eco*RI site, these sites are not present in pSP2 probably due to a fortuitus nucleotide misincorporation during the blunt-ending reaction. Therefore, *Eco*RI is unique and in the cloning area of pSP2, while pSP1 carries three sites. The natural *Nde*I site in *S. pombe ars1* was removed by a fill-in reaction (D.B., unpublished data) prior its use in the construction of these vectors. As a consequence *Nde*I is not present in pSP1 and is unique in the *URA3* fragment of pSP2. The structure of the plasmids is available through compilation of the components from computer database.

Both vectors transform *S. pombe* at high efficiency and have been used successfully to subclone various genes (Matsumoto and Beach 1991; G. C. unpublished

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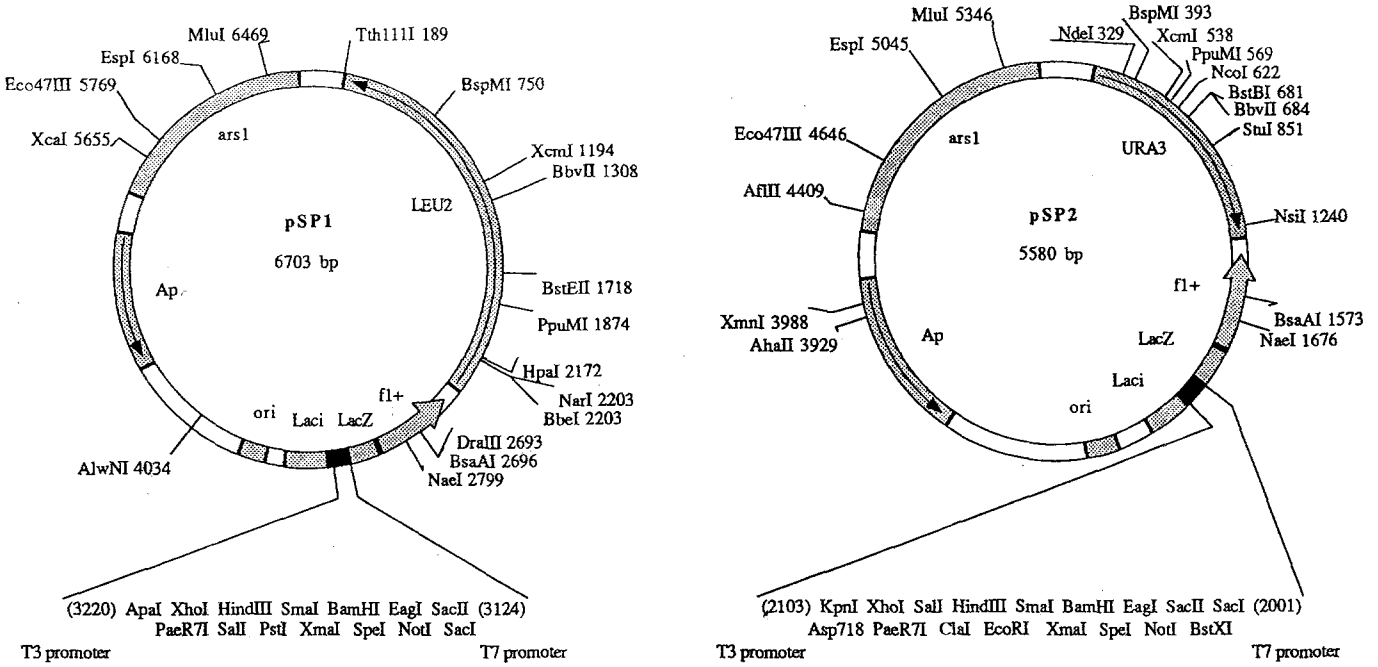

Fig. 1. Structure of the pSP1 and pSP2 vectors. The unique restriction sites are shown. The T3 promoter resides upstream of the *Kpn*I (not shown in pSP1) and transcription proceeds toward the *Sac*I site; the T7 promoter resides upstream of the *Sac*I site and initiates transcription in the opposite direction. *Ap*, ampicillin resistance β-lactamase gene; *LEU2*, *S. cerevisiae* β-IPM dehydrogenase gene; *URA3*, *S. cerevisiae* orotidine-5'P decarboxylase gene; *LacZ*, β-galactosidase gene; *Lac*I, Lac repressor gene; *ori*, ColE1 origin of replication; *f1*$^+$, f1 origin of replication; *ars1*, *S. pombe* autonomously replicating sequence previously deleted of its *Nde*I site

data), generate nested deletions in DNA fragments as large as 7.5 kbp (Henikoff 1984) for identification of the complementing region by direct transformation of yeast, and for DNA sequencing (G. C., unpublished results).

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