A series of vectors to construct *lacZ* fusions for the study of gene expression in *Schizosaccharomyces pombe*

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Abstract We have constructed a series of plasmids to facilitate the fusion of promoters with or without coding regions of genes of *Schizosaccharomyces pombe* to the *lacZ* gene of *Escherichia coli*. These vectors carry a multiple cloning region in which fission yeast DNA may be inserted in three different reading frames with respect to the coding region of *lacZ*. The plasmids were constructed with the *ura4*$^+$ or the *his3*$^+$ marker of *S. pombe*. Functionality of the plasmids was tested measuring in parallel the expression of fructose 1,6-bisphosphatase and β-galactosidase under the control of the *fbp1*$^+$ promoter in different conditions.

Key words: Expression vector; *lacZ* fusion; *Schizosaccharomyces pombe*

1. Introduction

Analysis of factors that influence gene expression is one important question in different fields of current biology. Ideally, quantitation of changes in gene expression between different situations needs careful analysis of specific mRNAs. Since the available techniques to perform these measurements are laborious they are not practical when a large number of samples needs to be processed as happens during the characterization by deletion analysis of regulatory regions in the promoter of a gene. An alternative method that is widely used is the fusion of promoter regions of the gene under study to the coding sequence of a reporter gene. The changes in the amount or activity of the reporter protein are accepted as an estimate of those in the expression of the gene itself. Although this method is indirect and several factors different from gene expression may influence the amount of protein measured, it has become very popular due to its simplicity and versatility. Among the several reporter genes available (see for example [1]) the most commonly used is still the *lacZ* gene from *Escherichia coli* that encodes β-galactosidase.

To facilitate the construction of *lacZ* fusions in *Saccharomyces cerevisiae*, Myers et al. [2] designed plasmids in which the sites of the multiple cloning region of pUC8 and pUC18 could be used to insert yeast DNA fragments in three different reading frames with respect to the coding region of *lacZ*. Due to the increasing interest in the study of the regulation of gene expression in the fission yeast *Schizosaccharomyces pombe* we have constructed a series of integrative and episomal vectors carrying *ura4*$^+$ [3] marker from *S. pombe* that allow the fusion of *S. pombe* promoters to *lacZ*. We also constructed similar episomal plasmids with the *his3*$^+$ [4] marker from *S. pombe*. We evaluated the functionality of these vectors by assaying in different growth conditions the expression of the *lacZ* gene under the control of the promoter of the *fbp1*$^+$ gene [5]. The vectors presented can, in principle, be used to study the regulation of other genes of the fission yeast. (The collection of vectors constructed has been deposited in the Spanish Type Culture Collection[2].)

2. Materials and methods

2.1. Yeast and bacterial strains and culture conditions

S. pombe strains CJM094 *h*$^-$ *ade6-204 ura4-294* (originally provided by S. Moreno, Salamanca, Spain) and TPY39 *h*$^-$ *ade6-M210 ura4-D18 leu1-32 his3-D2* (provided by K.L. Gould, Nashville, TN, USA) were used throughout this work. They were grown at 30°C in a synthetic medium [6] with 2% glucose or 3% glycerol+0.05% glucose as carbon sources and the adequate auxotrophic requirements at a final concentration of 100 μg/ml. Transformation of *S. pombe* was done with lithium chloride as in [6].

Escherichia coli DH5α was used for plasmid multiplication. It was grown on LB [7] supplemented with 50 μg/ml ampicillin.

2.2. Plasmids and DNA manipulations

The following plasmids were used: pUC18 [8], pBluescript®KS$^+$ (Stratagene), YEp353-358 and YEp356R-358R [2], obtained from the ATCC (Rockville, MD, USA), pREP4 [9], pAF1[10] (sent by K.L. Gould, Nashville, TN, USA) and pAOV6 [5] (sent by M. Boutry, Louvain la Neuve, Belgium).

2.3. Construction of promoter fusions

The plasmids that contain the *fbp1*$^+$ promoter fused in frame with the *lacZ* coding sequence were constructed as follows. The promoter region of *fbp1*$^+$ was amplified by PCR using DNA from plasmid pAV06 using the following oligonucleotides as primers: upstream 5′-CGTTGCTGAATTCCAGCACAC-3′; downstream 5′-GCATCC-GTTCGACTTCATCGC-3′. The *Eco*RI site (underlined) was added to facilitate subcloning. The 1.4-kbp PCR product was digested with *Eco*RI and *Bgl*II and the resulting 1.1-kbp fragment was ligated respectively into plasmids pSPI-353 and pSPE-353 (see Section 3), previously cut with *Eco*RI and *Bam*HI. The cloned fragment contains 3 amino acids from the coding region of *S. pombe* fructose 1,6-bisphosphatase.

2.4. Extracts and enzymatic assays

Yeast extracts were prepared by shaking with glass beads in 20 mM imidazole, pH 7 as described [11]. For β-galactosidase assay the centrifugation step was omitted. β-Galactosidase was assayed and units defined as in [12] with a centrifugation step before reading optical density. Fructose 1,6-bisphosphatase was assayed as in [13]. Protein was determined by the method of Lowry et al. [14] using the Pierce Protein Assay Reagent.

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3. Results and discussion

3.1. Construction of episomal and integrative lacZ fusion plasmids for expression of β-galactosidase in the fission yeast carrying the ura4+ or his3+ markers

Vectors to allow fusion of DNA fragments in three different reading frames to the *E. coli lacZ* gene were first constructed by Minton [15]. Myers et al. [2] made use of these vectors to construct a series of *S. cerevisiae-E. coli* shuttle plasmids. We have modified these last vectors to introduce in them the genes *ura4*$^+$ or *his3*$^+$ and the *ARS201* fragment from *S. pombe*. Plasmids YEp353-358 and YEp356R-358R were digested with *Nsi*I (*Ava*III) and the 1.6-kbp band containing the *S. cerevisiae URA3* gene and a part of the 2 μ sequence was eliminated. We introduced in these treated plasmids different cassettes with *ura4*$^+$, *ura4*$^+$*-ARS201* or *his3*$^+$*-ARS201* as *Pst*I fragments (see below).

3.2. Preparation of the S. pombe cassettes ura4+, ura4+-ARS-201 and his3+-ARS-201 for constructions

The *ura4*$^+$ gene was prepared to be inserted in the *Nsi*I-digested plasmids as follows. The 1.85-kbp *Hin*dIII-*Hin*dIII fragment from pREP4 carrying the *S. pombe ura4*$^+$ gene was introduced into pBluescript®KS$^+$ digested with *Hin*dIII;

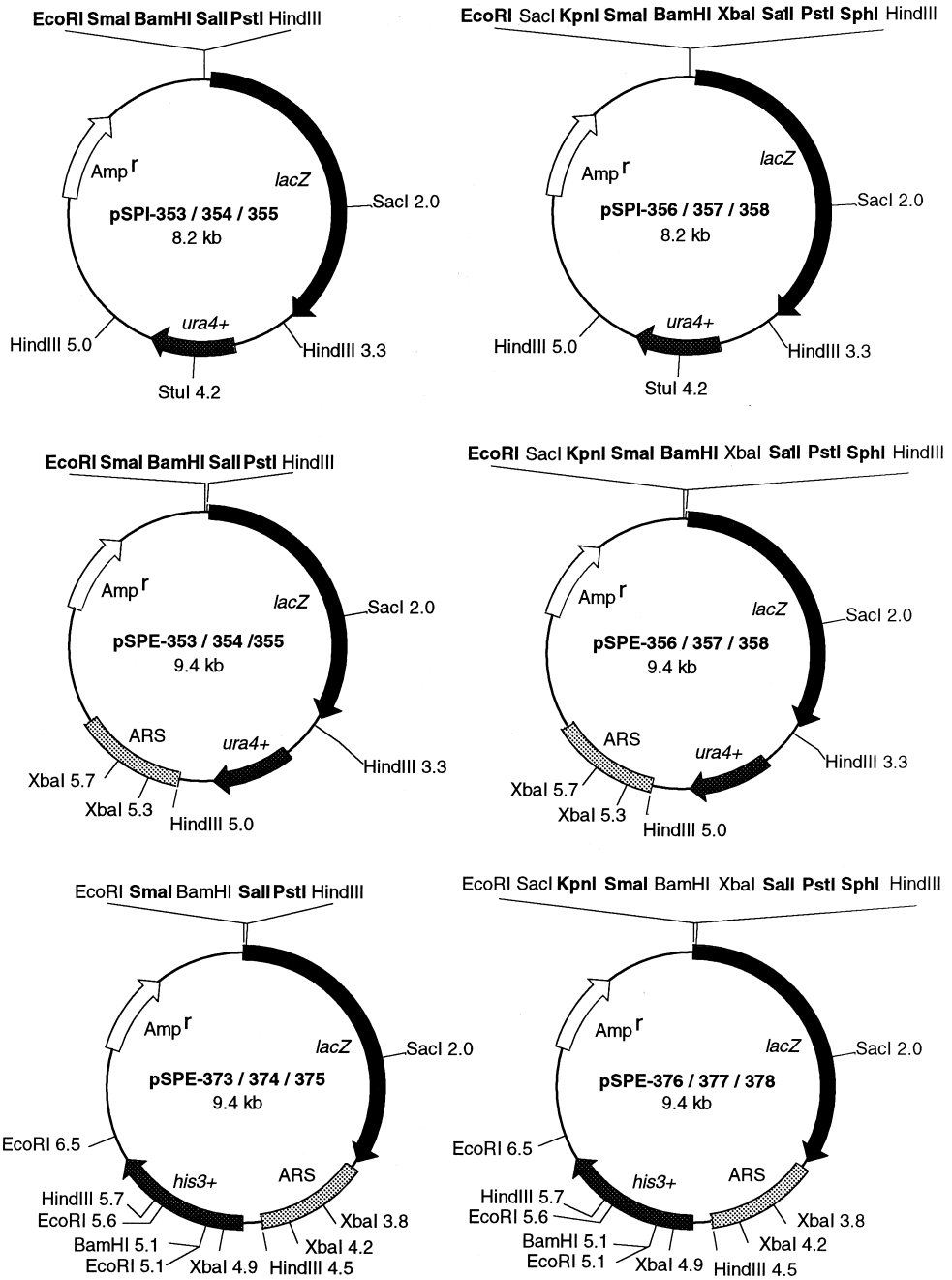

Fig. 1. Basic structure of the plasmids to construct *lacZ* fusions for expression in *S. pombe*. Plasmids with the *ura4*$^+$ marker are available as episomal or integrative while those with the *his3*$^+$ marker exist only as episomal. Sites in bold are unique in the corresponding plasmid. Plasmids pSPI356R to 358R, pSPE356R to 358R and pSPE376R to 378R have the polylinker in the opposite orientation. For details of the constructions see text.

Table 1
Reading frame and restriction sites of the multiple cloning region of the pSPI and pSPE vectors

Plasmid	Reading frame and restriction sites
pSPI-353/pSPE-353/pSPE-373 4938 4939 4940	SmaI SalI HindIII ga att ccc ggg gat ccg tcg acc tgc agc caa gct tgc gat ccc EcoRI BamHI PstI
pSPI-354/pSPE-354/pSPE-374 4941 4942 4943	SmaI SalI HindIII gaa ttc ccg ggg atc cgt cga cct gca gcc aag ctt gct ccc EcoRI BamHI PstI
pSPI-355/pSPE-355/pSPE-375 4944 4945 4946	SmaI SalI HindIII g aat tcc cgg gga tcc gtc gac ctg cag cca agc ttc gat ccc EcoRI BamHI PstI
pSPI-356/pSPE-356/pSPE-376 4947 4948 4950	SacI SmaI XbaI PstI HindIII ga att cga gct cgg tac ccg ggg atc ctc tag agt cga cct gca ggc atg caa gct tgc gat ccc EcoRI KpnI BamHI SalI SphI
pSPI-357/pSPE-357/pSPE-377 4954 4955 4956	SacI SmaI XbaI PstI HindIII gaa ttc gac ctc ggt acc cgg gga tcc tct aga gtc gac ctg cag gca tgc aag ctt gct ccc EcoRI KpnI BamHI SalI SphI
pSPI-358/pSPE-358/pSPE-378 4960 4961 4962	SacI SmaI XbaI PstI HindIII g aat tcg agc tcg gta ccc ggg gat cct cta gag tcg acc tgc agg cat gca agc ttc gat ccc EcoRI KpnI BamHI SalI SphI
pSPI-356R/pSPE-356R/pSPE-376R 4951 4952 4953	SphI SalI BamHI KpnI EcoRI aag ctt gca tgc ctg cag gtc gac tct aga gga tcc ccg ggt acc gag ctc gaa ttc cca gct tgc gat ccc HindIII PstI XbaI SmaI SacI
pSPI-357R/pSPE-357R/pSPE-377R 4957 4958 4959	SphI SalI BamHI KpnI EcoRI a agc ttg cat gcc tgc agg tcg act cta gag gat ccc cgg gta ccg agc tcg aat tcc cag ctt gct ccc HindIII PstI XbaI SmaI SacI
pSPI-358R/pSPE-358R/pSPE-378R 4963 4964 4965	SphI SalI BamHI KpnI EcoRI aa gct tgc atg cct gca ggt cga ctc tag agg atc ccc ggg tac cga gct cga att ccc agc ttc gat ccc HindIII PstI XbaI SmaI SacI

The recognition sites of the different restriction enzymes are underlined. For unique restriction sites in each vector see Fig. 1. The reading frame is according to [2]. The number under each plasmid is the one given in the Spanish Type Culture Collection to *E. coli* DH5α transformed with the corresponding plasmid.

the resulting plasmid was digested with *Sal*I and *Bam*HI and the 1.85-kbp fragment was cloned into the *Sal*I and *Bam*HI digested plasmid pUC18 previously cut with *Sma*I and *Eco*RI, blunt ended and religated. The restriction sites *Sal*I and *Cla*I flanking *ura4*$^+$ were eliminated by cutting and filling-in to yield plasmid pMJ1. The *Eco*RI site also flanking *ura4*$^+$ was eliminated from this plasmid by cutting and filling-in to produce plasmid pMJ2.

The *ARS201* fragment was obtained as a 1.2-kbp *Eco*RI-*Eco*RI fragment from pREP4. It was blunt ended and cloned into pMJ1 digested with *Eco*RI and blunt ended producing plasmid pMJ3.

The *his3*$^+$*-ARS-201* cassette was prepared to be inserted in the *Nsi*I digested plasmids as follows: The 1.9-kbp *Sma*I-*Sal*I blunt ended fragment from pAF1 with the gene *his3*$^+$, was introduced into a 3.9-kbp fragment of pMJ3 obtained after blunt ending the product of a *Hin*dIII digestion to yield plasmid pMJ4.

3.3. Construction of a set of integrative plasmids

For the construction of pSPI plasmids (*Schizosaccharomyces Pombe* Integrative) the 1.85-kbp *Pst*I-*Pst*I fragment from plasmid pMJ2, carrying the *ura4*$^+$ gene was cloned into all plasmids digested with *Nsi*I as described above. Plasmids pSPI-353 through pSPI-358 and pSPI356R to 358R were obtained (Fig. 1). Plasmids pSPI-353 to pSPI-355 carry the multiple cloning region of pUC8 while the rest carry that of pUC18 [16].

3.4. Construction of a set of episomal plasmids

For the construction of the pSPE plasmids (*Schizosaccharomyces Pombe* Episomal) the 3-kbp *Pst*I-*Pst*I fragment from plasmid pMJ3, carrying the *ura 4*$^+$*-ARS201* cassette was cloned into all plasmids digested with *Nsi*I as above yielding plasmids pSPE353 to pSPE358 and pSPE356R to pSPE358R (Fig. 1). Plasmids pSPE353 to pSPE355 carry the multiple cloning region of pUC8 while the rest carry that of pUC18 [16]. To allow an easy reference to the corresponding plasmids of *S. cerevisiae* and avoid proliferation of designations, the *ura4*$^+$ containing plasmids carry the same numbers as those of Myers et al. [2] with the letters SP to indicate *S. pombe*.

To increase the versatility of these plasmids and allow their use in strains with a marker different from *ura4*$^+$ we generated another series of episomal plasmids carrying the *his3*$^+$ marker. The 3.1-kbp *Pst*I-*Pst*I fragment from the pMJ4 plasmid carrying the *his3*$^+$*-ARS201* cassette was cloned into all the plas-

Table 2
Fructose 1,6-bisphosphatase (FbPase) and β-galactosidase (β-Gal) activities (mU/mg protein) of *S. pombe* strains transformed with episomal (pSPE353-fbp1) or integrative (pSPI353-fbp1) plasmids carrying *fbp1*$^+$*-lacZ* fusions

	pSPE353-fbp1		pSPI353-fbp1	
	FbPase	β-Gal	FbPase	β-Gal
Glucose (X)	< 1	140	< 1	70
Glucose (S)	30	5500	40	2100
Glycerol (X)	90	4600	80	1450

The transformed yeasts were grown as described in Section 2 and harvested during the exponential (X) or the stationary (S) phase of growth. Enzymatic activities were assayed as described in Section 2.

mids digested with *Nsi*I, yielding pSPE373 to pSPE378 and pSPE376R to pSPE378R (Fig. 1).

The structures of the multiple cloning sites of these vectors based on the sequences provided by Myers et al. [2] are shown in Table 1.

3.5. Expression of β-galactosidase from the vectors

To test the functionality of the plasmids constructed we measured the expression of β-galactosidase driven from the *fbp1*$^+$ promoter in different growth conditions using the episomal and integrative plasmids pSPE353 and pSPI353. This last construct was integrated in the chromosomal *ura4*$^+$ locus after linearization with *Stu*I that is internal to the *ura4*$^+$ gene and is not present in the rest of the construct sequence. The vectors did not express β-galactosidase in *S. pombe* in the absence of sequences of a yeast promoter (results not shown). As may be seen in Table 2 the values of β-galactosidase expressed under the control of the *fbp1*$^+$ promoter varied in the same direction as those of the fructose 1,6-bisphosphatase measured in the same cultures. Parallel assays with plasmids carrying the *his3*$^+$ marker (results not shown) demonstrated the adequacy of the other constructs.

Constructs with the plasmids described may also be useful to localize gene products intracellularly as antibodies against β-galactosidase are commercially available.

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