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Research Article

Construction and characterization of a series of vectors for *Schizosaccharomyces pombe*

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Abstract

A set of vectors was created to allow cloning and expression studies in *Schizosaccharomyces pombe*. These vectors had a uniform backbone with an efficient *Sz. pombe* ARS, ARS3002, but different selectable markers — *his3*$^+$, *leu1*$^+$, *ade6*$^+$ and *ura4*$^+$. The vectors functioned efficiently as autonomously replicating plasmids that could also be converted into integrating vectors. The *ura4*$^+$-containing vector was used to construct a *Sz. pombe* genomic library. Copyright © 2005 John Wiley & Sons, Ltd.

Keywords: ***Schizosaccharomyces pombe*; vectors; genomic library**

Introduction

Vectors that can easily be used between different organisms as well as for different functions within the same organism are fundamental tools in molecular biology and genetics. Such vectors can be used for mutagenesis of genes as well as overexpression studies. The versatile pRS vectors in *Saccharomyces cerevisiae* (Sikorski and Hieter, 1989) have greatly aided in the genetic and molecular studies of this organism and we have constructed a similar set of vectors for studies in *Schizosaccharomyces pombe*.

Sz. pombe has been extensively utilized in studies in cell biology, genetics and molecular biology and numerous plasmid vectors are in use today (Siam *et al*., 2004). However, a uniform set of vectors that allows the use of multiple markers that can be rapidly converted from an episome to an integrating vector does not exist for this organism.

In an effort to redress this, we have constructed a set of *Sz. pombe* integrating and autonomously replicating vectors, based on the bluescript backbone (Keeney and Boeke, 1994). These vectors are relatively small, contain multiple cloning sites, replicate in bacteria and allow for selection of four *Sz. pombe* markers, *his3*$^+$, *ade6*$^+$, *leu1*$^+$ and *ura4*$^+$. Coupled with *Sz. pombe* strains with specific auxotrophic mutations; these vectors will be useful as basic tools for genetic and molecular manipulation.

Materials and methods

Genomic DNA isolation

Samples (25 ml) of an overnight culture of *Sz. pombe* strain ROP9 was used to inoculate 1 l YES media and the cells were allowed to grow overnight at 30 °C. The cells were centrifuged at 5000 r.p.m. for 7 min in a GS3 rotor, then washed in 15 ml 50 mM citrate/phosphate, 40 mM EDTA and 1.2 M sorbitol. The cells were resuspended in the buffer and transferred to a 50 ml Falcon tube; 43.7 µl β-mercaptoethanol and 375–600 µl 10 mg/ml zymolyase 20T were added and the cells were incubated at 37 °C with shaking for 1 h. Completion of spheroplasting was monitored microscopically. To 10 µl cells, 1 µl 10% SDS was added and the cells were checked for lysis. When the cells turned dark and ghost-like, the incubation was complete. The spheroplasts were centrifuged at 2200 r.p.m. for 5 min in a benchtop centrifuge. The cells

were resuspended in 15 ml 5× TE and 1.5 ml 10% SDS was added to lyse the cells. The lysate was gently mixed and incubated at 65 °C for 5 min. The lysate was transferred to screw-cap tubes and 5 ml 5 M potassium acetate was added, the suspension was incubated on ice for 30 min and then centrifuged at 5000 r.p.m. for 15 min in a SS34 rotor. The supernatant was passed through four layers of muslin and 20 ml isopropanol added to the DNA solution. After incubation at −20 °C for 5 min, the DNA was centrifuged at 10 000 r.p.m. for 15 min. The DNA pellet was resuspended in 3 ml 5× TE and 6 µl 10 mg/ml RNaseA was added and incubated at 37 °C for 2 h. The DNA was extracted with an equal volume of phenol : chloroform and reprecipitated with sodium acetate and ethanol.

To 4 ml DNA, 4.2 g CsCl was added. In a second tube, 125 µl Hoeschst dye was diluted in 4 ml 100 mM Tris–HCl, 100 mM EDTA and 200 mM NaCl with 4.2 g CsCl. The CsCl containing DNA was mixed with the Hoeschst dye solution in equal volume. The material was placed in tubes, sealed and centrifuged at 55 000 r.p.m. at 20 °C for 20 h in a Vti65.2 rotor. The DNA in the tubes was visualized with long-range UV light. The material was removed with a syringe and needle, and extracted with a 5 : 1 isopropanol : water mixture. The lower phase contained DNA and was extracted three times with isopropanol. Finally, three volumes of cold 70% ethanol was added slowly along the side of the tube and the phases were mixed in a single quick motion. DNA precipitated at the phase junction as a fibrous network. The DNA was removed by touching it with a glass Pasteur pipette and resuspended in 300 µl TE overnight at 4 °C.

Library generation

Plasmid pRO314 was isolated from 1 l *Escherichia coli* grown in 2x Ty medium to an A600 of 0.7. The plasmid DNA was purified on a CsCl gradient and resuspended in 500 ul TE, dialysed against water and reprecipitated with ethanol. The DNA was finally resuspended in 1 ml water and stored.

Separating the digested DNA on an agarose gel monitored the extent of digestion of the genomic DNA. A time point was chosen at which the bulk DNA had an average size of 4 kb. 250 µg bulk DNA was then digested for 2 h with 12.5 units Sau3A enzyme, loaded onto 5–25% sucrose gradient and centrifuged in a SW41 rotor for 18 h at 25 000 r.p.m. 500 µl fractions were collected and analysed on an agarose gel. The DNA in each fraction was initially diluted 1 : 1, phenol : chloroform-extracted and precipitated with sodium acetate and alcohol. The precipitated DNA was resuspended in 200 µl water and stored.

The vector was digested with *Bam*HI, phosphatased, run on a agarose gel, and the cut DNA was eluted from the gel, quantitated and used for ligations with the *Sz. pombe* genomic DNA. The proportion of insert to vector was determined empirically, maximizing for insert ligation with low background of vector alone.

Results and discussion

Generation of the vectors

The bluescript backbone has been extensively used in the generation of numerous vectors. Plasmid pJK142 (Keeney and Boeke, 1994) is a modified version of pBSII (SK+) vector that contains unique *Aat*II and *Nde*I restriction enzyme recognition sites between the bacterial *β-lactamase* and *lacZ* genes. The bluescript multiple cloning site, present in the *lacZ* gene, aids in the transferability of the cloned fragments of DNA, while the presence of the $f1^+$ origin allows recovery of the sense strand of the *lacZ* gene when the *E. coli* host strain is co-transfected with helper phage. For these reasons we decided to generate *Sz. pombe* vectors in this backbone.

Stable propagation of episomes in *Sz. pombe* requires a functional *Sz. pombe* origin of replication, and different *Sz. pombe* vectors have utilized different origins of replication. There is a natural cluster of three replication origins near the $ura4^+$ gene on chromosome III of *Sz. pombe* (Dubey *et al*., 1994; Zhu *et al*., 1992, 1994). The *cis*-acting sequences that determine these three origins are also capable of specifying origins in plasmids. However, the three origins fire with different efficiencies (ARS3002 > ARS3003 > ARS3004) and we chose the most efficient origin, ARS3002, for our plasmid constructs. While the ~800 bp *Eco*RV–*Eco*RI fragment, called the core ARS3002, is sufficient for plasmid origin function, the adjacent ~400 bp *Eco*RI–*Hin*cII fragment, called the replication enhancer, increases plasmid origin activity (Kim and Huberman, 1999; Kim *et al*., 2001), and we

therefore decided to use the entire 1.2 kb fragment in our vector construction. Primers (Roog 124 and Roog 147) were designed such that the *Eco*RV and *Hin*cII fragment was flanked by *Asc*I and *Aat*II sites instead, and this PCR-amplified ARS3002 fragment was cloned into the *Aat*II site of pJK142 to give the resulting plasmid, pCA1, containing ARS3002 flanked by both *Aat*II and *Asc*I restriction sites. This configuration of restriction sites allowed the removal of the ARS element as required by digestion with either of these two enzymes. The *Eco*RI site in the 1.2 kb ARS3002 fragment was deleted by partially digesting plasmid pCA1 with *Eco*RI, followed by end-filling and religation to obtain plasmid pRO322. This change did not affect the function of the ARS element and generated a plasmid with a unique *Eco*RI site present in the polylinker.

To insert the *Sz. pombe* selectable markers into this backbone, plasmid pRO322 was digested with *Nde*I. The *ura4*$^+$ gene of *Sz. pombe* was PCR-amplified from plasmid pJK4 (Keeney and Boeke, 1994) with oligonucleotides Roog 137 and Roog 138, such that the gene was flanked by *Fse*I and *Ase*I sites. The PCR-amplified DNA was digested with *Ase*I and ligated into the *Nde*I-digested pRO322 vector backbone. Two plasmids were generated, pRO314 and pRO318, with *Fse*I restriction sites flanking the *ura4*$^+$ gene, and the two plasmids were identical except for the orientation of the *ura4*$^+$ gene (Figure 1).

pRO314 was next digested with *Fse*I to drop out the *ura4*$^+$ gene and the vector was religated to generate plasmid pRO323. This plasmid contained a unique *Fse*I restriction site in place of the *Nde*I site of the parent plasmid (pRO322).

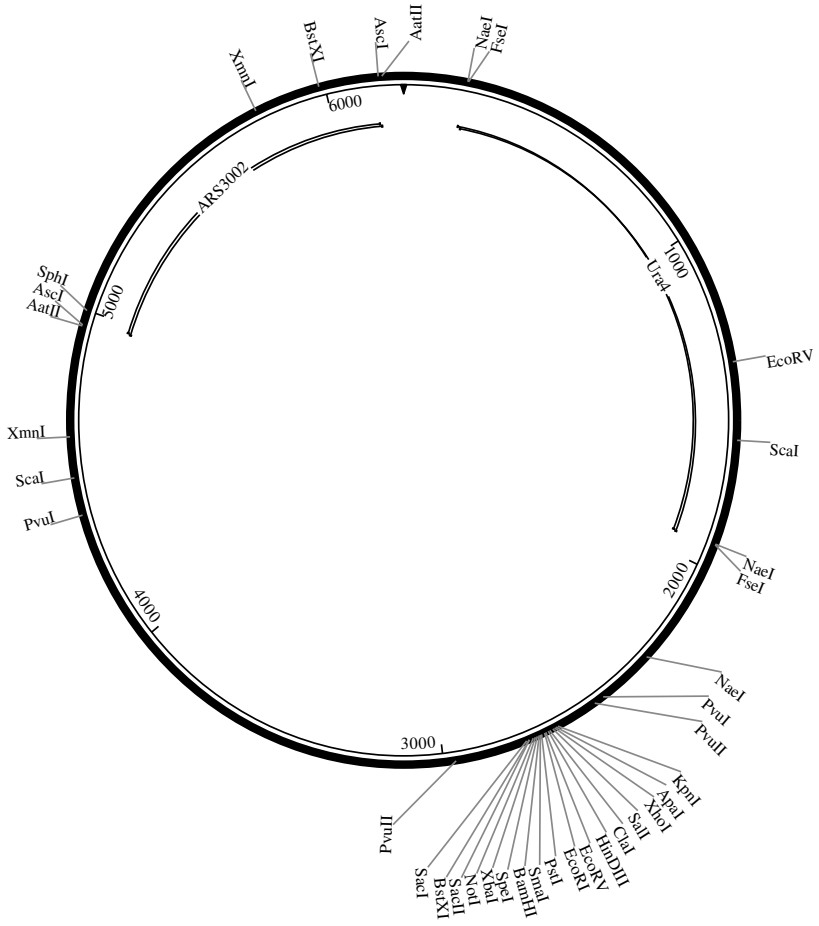

Figure 1. Maps of *Sz. pombe* ARS-containing vectors. Restriction map of vectors with *ura4*$^+$ is shown. Only a few selected restriction sites are shown. The unique and absent restriction sites are listed in Table 1

To clone the remaining selectable markers, *his3*$^+$, *leu1*$^+$ and *ade6*$^+$, the genes for these markers were PCR-amplified with oligonucleotides containing *Fse*I sites flanking the genes. Following amplification, the PCR products and plasmid pRO323 were digested with *Fse*I and ligated together.

Plasmids pRO315 and pRO319 contained the *his3*$^+$ gene [PCR-amplified from pAF1 (Ohi *et al.*, 1996) with oligonucleotides Roog139 and 140],

Table 1. Unique and absent restriction sites for the four plasmids

URA4		***HIS3***		***LEU1***		***ADE6***	
Absent	**Unique**	**Absent**	**Unique**	**Absent**	**Unique**	**Absent**	**Unique**
*Aar*I	*Acc*65I	*Afe*I	*Acc*65I	*Aar*I	*Acc*65I	*Aar*I	*Acc*65I
*Afe*I	*Ale*I	*Afl*II	*Ale*I	*All*II	*Ale*I	*Afe*I	*Ale*I
*Afl*II	*Alo*I	*Age*I	*Alo*I	*Age*I	*Alo*I	*Afl*II	*Alo*I
*Age*I	*Alw*NI	*Avr*II	*Alw*NI	*Avr*II	*Alw*NI	*Age*I	*Alw*NI
*Bae*I	*Apa*I	*Bae*I	*Apa*I	*Bae*I	*Apa*I	*Avr*II	*Apa*I
*Bbe*I	*Asi*SI	*Bbe*I	*Asi*SI	*Bbe*I	*Asi*SI	*Bbe*I	*Asi*SI
*Bcl*I	*Avr*II	*Rbv*CI	*Bpu*10I	*Bbv*CI	*Bam*HI	*Bbv*CI	*Baa*AI
*Bgl*II	*Bam*HI	*Bcl*I	*Bsa*AI	*Bcl*I	*Bpu*10I	*Bcl*I	*Bsm*I
*Blp*I	*Bbv*CI	*Bgl*II	*Bsm*I	*Bgl*II	*Bsm*I	*Bgl*II	*Cla*I
*Bmt*I	*Bpu*10I	*Blp*I	*Eag*I	*Blp*I	*Bsu*36I	*Bmt*I	*Eag*I
*Bpl*I	*Bea*AI	*Bmt*I	*Eco*ICRI	*Bmt*I	*Btg*I	*Bpl*I	*Eco*ICRI
*Bse*RI	*Bsg*I	*Bpl*I	*Hin*cII	*Bpl*I	*Cla*I	*Bpu*10I	*Kpn*I
*Bsi*WI	*Bsm*I	*Bse*RI	*Kpn*I	*Bse*RI	*Eag*I	*Bsi*WI	*Not*I
*Bsp*EI	*Bsu*36I	*Bsg*I	*Mfe*I	*Bsg*I	*Eco*ICRI	*Bsp*EI	*Pac*I
*Bsp*MI	*Btg*I	*Bsl*FI	*Not*I	*Bsp*EI	*Eco*RI	*Bsp*MI	*Pfo*I
*Bst*API	*Cla*I	*Bsm*FI	*Psp*OMI	*Bsp*MI	*Hin*eII	*Bsr*GI	*Psp*OMI
*Bst*BI	*Eag*I	*Bsr*GI	*Pst*I	*Bsr*GI	*Kpn*I	*Bst*BI	*Pst*I
*Bst*EII	*Eco*ICRI	*Bst*API	*Sac*I	*Bst*API	*Not*I	*Bst*EII	*Sac*I
*Csp*CI	*Eco*NI	*Bst*EII	*Sac*II	*Bst*BI	*Pac*I	*Bsu*36I	*Sac*II
*Hpa*I	*Eco*RI	*Bsu*36I	*Sal*I	*Bst*EII	*Pfo*I	*Cap*CI	*Sal*I
*Kas*I	*Hin*cII	*Csp*CI	*Sap*I	*Bst*Z17I	*Psp*OMI	*Fal*I	*Sap*I
*Mlu*I	*Hin*dIII	*Ful*I	*Sma*I	*Csp*CI	*Pst*I	*Hpa*I	*Sma*I
*Mac*I	*Kpn*I	*Hpa*I	*Spe*I	*Fal*I	*Sac*I	*Kas*I	*Sph*I
*Nar*I	*Mfe*I	*Kas*I	*Sph*I	*Hpa*I	*Sac*II	*Mlu*I	*Xma*I
*Nco*I	*Not*I	*Mlu*I	*Xma*I	*Kas*I	*Sal*I	*Msc*I	
*Nde*I	*Pac*I	*Nar*I		*Mlu*I	*Sap*I	*Nar*I	
*Nhe*I	*Pfl*MI	*Nco*I		*Msc*I	*Sma*I	*Nhe*I	
*Nru*I	*Pfo*I	*Nhe*I		*Nar*I	*Spe*I	*Nru*I	
*Pas*I	*Psp*OMI	*Nru*I		*Nco*I	*Sph*I	*Pfl*MI	
*Pme*I	*Psp*XI	*Pas*I		*Nhe*I	*Xba*I	*Pme*I	
*Pml*I	*Pst*I	*Pfl*MI		*Pas*I	*Xho*I	*Pml*I	
*Ppu*MI	*Sac*I	*Pme*I		*Pfl*MI	*Xma*I	*Ppu*MI	
*Psh*AI	*Sac*II	*Pml*I		*Pme*I		*Psh*AI	
*Rsr*II	*Sal*I	*Ppu*MI		*Pml*I		*Rsr*II	
*San*DI	*Sap*I	*Psh*AI		*Ppu*MI		*San*DI	
*Sbf*I	*Sma*I	*Rsr*II		*Psh*AI		*Sbf*I	
*Sex*AI	*Spe*I	*San*DI		*Rsr*II		*Sex*AI	
*Sfi*I	*Sph*I	*Sbf*I		*San*DI		*Sfi*I	
*Sfo*I	*Stu*I	*Sex*AI		*Sbf*I		*Sfo*I	
*Sgr*AI	*Xba*I	*Sfi*I		*Sex*AI		*Sgr*AI	
*Sna*BI	*Xho*I	*Sfo*I		*Sfi*I		*Sna*BI	
*Srf*I	*Xma*I	*Sgr*AI		*Sfo*I		*Srf*I	
*Swa*I		*Sna*BI		*Sgr*AI		*Stu*I	
		*Srf*I		*Srf*I		*Swa*I	
		*Sty*I		*Stu*I		*Tth*111I	
		*Swa*I		*Swa*I		*Xcm*I	
		*Tth*111I		*Xcm*I			

while pRO316 and pRO320 contained the *leu1*$^+$ gene [PCR-amplified from pJK13 (Keeney and Boeke, 1994) with oligonucleotides Roog135 and 136] and pRO317 and pRO321 contained the *ade6*$^+$ gene [PCR-amplified from pEN81 (Nimmo *et al*., 1994) with oligonucleotides Roog133 and 134]. The two plasmids for each of the selectable markers were identical except for the orientation of the marker gene, and the sequences for these vectors are available upon request. Other markers could be inserted in place of the current markers.

Integrating plasmids can be constructed from the ARS-containing plasmid by digesting the appropriate vectors with *Asc*I or *Aat*II and religation. The presence of these restriction sites flanking the ARS element should be useful in converting these vectors from autonomously replicating plasmids to integrating ones.

To test these plasmids, we transformed strain ROP192 (*h*$^+$ *ade6-M216 arg3-D4 his3-D1 leu1-32 ura4-D18*) with plasmids pRO318 and pRO316 and selected for the plasmids on EMM plates lacking uracil or leucine, respectively. Both ARS-containing plasmids gave transformants, although with equivalent amounts of plasmid there were a greater number of transformants with pRO318 than with pRO316.

Analyses of the plasmid generated

We next characterized the plasmids we had generated. The *ura4*$^+$ containing vector was taken as a representative of these plasmids and characterized. The autonomously replicating plasmids were tested for copy number by transforming *Sz. pombe* strain ROP19 (*h*$^-$ *ade6-704 leu1-32 ura4-294*) with pRO314. The strains were allowed to grow overnight in selective EMM media and total genomic DNA isolated from these strains (Hoffman and Winston, 1987) was digested with *Eco*RV and probed with an *ura4*$^+$ probe. This probe hybridized to a ~7 kb fragment of the genomic *ura4*$^+$ locus. The copy number of the plasmid was determined by comparing the radioactive signal of the plasmid band to the genomic *ura4*$^+$ band. Six independent *ura4* transformants were checked and the average plasmid copy number was determined to be 7.8 ± 1.9 copies/cell.

We next determined the plasmid loss rate for two of these plasmids, pRO314 and pRO320, in wild-type *Sz. pombe*. Three independent transformants were grown selectively in EMM media for 10 generations and the cultures were diluted and plated onto YES plates. The colonies after growth on YES plates were replica-plated onto EMM selective plates and the number of colonies able to grow on these plates was ascertained. For pRO314, an average of $17 \pm 3.5\%$ of the colonies had lost the plasmid, while for pRO320, approximately $19 \pm 5.1\%$ had lost the plasmid.

We were also interested in determining whether the extended ARS3002 plasmids that we had constructed had a higher transformation efficiency compared to other ARS plasmids. For example, the transformation frequency of core ARS3002-containing plasmids is only about 40% of the efficiency obtained with plasmids carrying the extended ARS3002 (Kim and Huberman, 1999; Kim *et al*., 2001). We chose to analyse pRep4, a plasmid with a different *Sz. pombe* ARS element, ARS1 (Maundrell, 1990). We transformed equal amounts of pRep4 and pRO318 plasmid DNA into strain ROP53 (*h*$^-$ *ura4-294 leu1-32*) and allowed colonies to develop. Quantitation of our results indicated that the extended ARS3002-containing plasmid (pRO318) gave rise to ~25% more transformants than with pRep4 (Figure 2A).

Under selective conditions, colony growth is limited by the replication efficiency of the plasmid (Kim and Huberman, 1998; Huberman, 1999) and plasmids with weak ARS elements form colonies of heterogeneous size, while plasmids with strong ARS elements give rise to large, uniform-sized transformants (Maundrell *et al*., 1988; Brun *et al*., 1995). Thus, measurement of colony size provides an indication of ARS activity. Analysis of our transformants with pRep4 and pRO318 did not demonstrate any significant differences in colony size between these two plasmids (Figure 2A), suggesting that, while the number of transformants/μg plasmid was higher with pRO318, the replication efficiency and segregation of the plasmid was similar in the two plasmids.

Integration of a tagged *hst4*$^+$ gene

To test the utility of these plasmids, a genomic fragment containing the *hst4*$^+$ gene and 500 bp flanking DNA was cloned into pRO314. The resulting autonomously replicating plasmid (pRO741) expressed the *hst4*$^+$ gene and was able to rescue

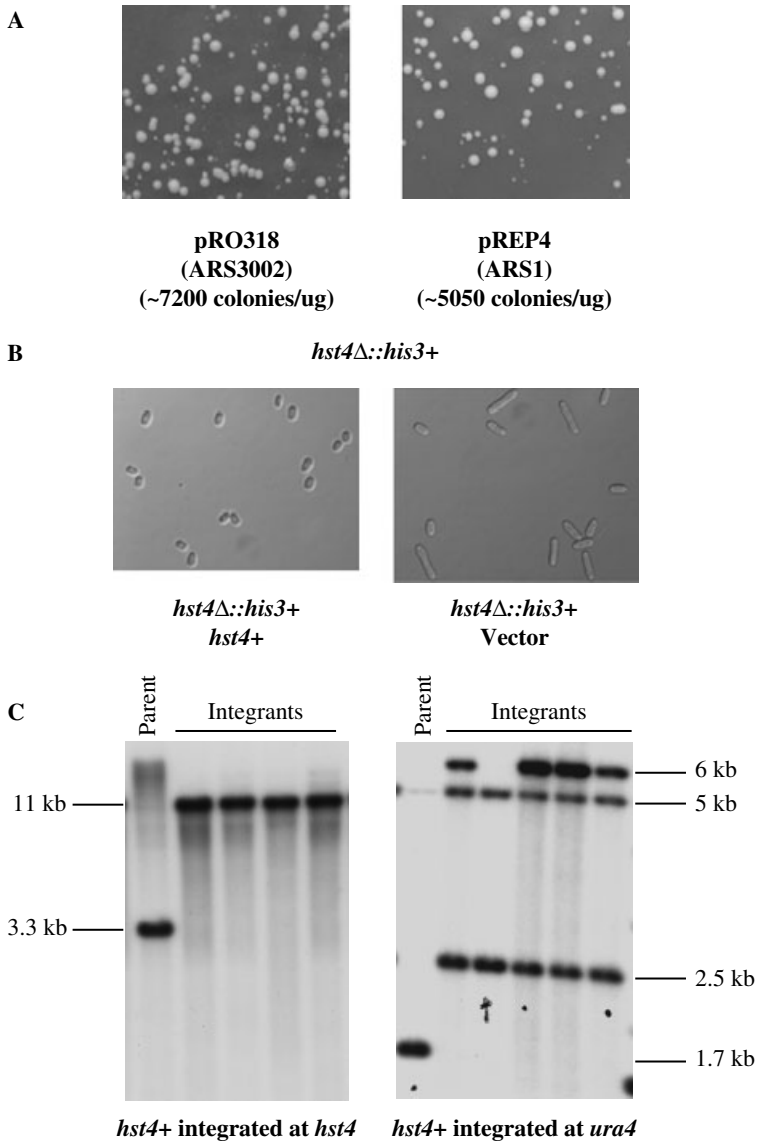

Figure 2. Analyses of the *ura4*$^+$-containing vector. (A) Transformation efficiency of pRO318. Equal amounts of plasmid pRO318 and pREP4 were transformed into strain ROP53. Transformants were plated on EMM plates lacking uracil and colonies were allowed to develop over 3 days prior to photography. (B) Complementation analyses of *hst4*Δ cells with a plasmid containing wild-type *hst4*$^+$. An *hst4*Δ strain was transformed with vector (pRO314) or a vector containing full-length *hst4*$^+$ (pRO741). After growth in EMM media, the cells were visualized under the microscope and photographed. (C) DNA blot analyses of integrants. Integrating plasmid pRO665 containing a wild-type *hst4*$^+$ gene was digested within the *ura4*$^+$ or *hst4*$^+$ gene and the linearized DNA was transformed into strain ROP 53. Integrants were grown in liquid medium and DNA was isolated. For integrants at the *ura4*$^+$ locus, DNA was digested with *Hin*dIII and the DNA blots were probed with *ura4* probe. For integrants at the *hst4*$^+$ locus, the DNA was digested with *Sph*I and the blots were probed with *hst4*

the long cell phenotype associated with a *hst4*Δ strain (Figure 2B; Freeman-Cook *et al*., 1999).

We also generated an integrating version of this plasmid in which the ARS3002 element was deleted (pRO665), and tested the ability of this plasmid to integrate the cloned *hst4*$^+$ gene into *Sz. pombe* at either the *ura4*$^+$ or the *hst4*$^+$ locus (Figure 2C). The plasmid was linearized in the *ura4*$^+$ gene with *Stu*I or within the *hst4*$^+$ gene by digestion with *Bsi*WI, and Rop53 was

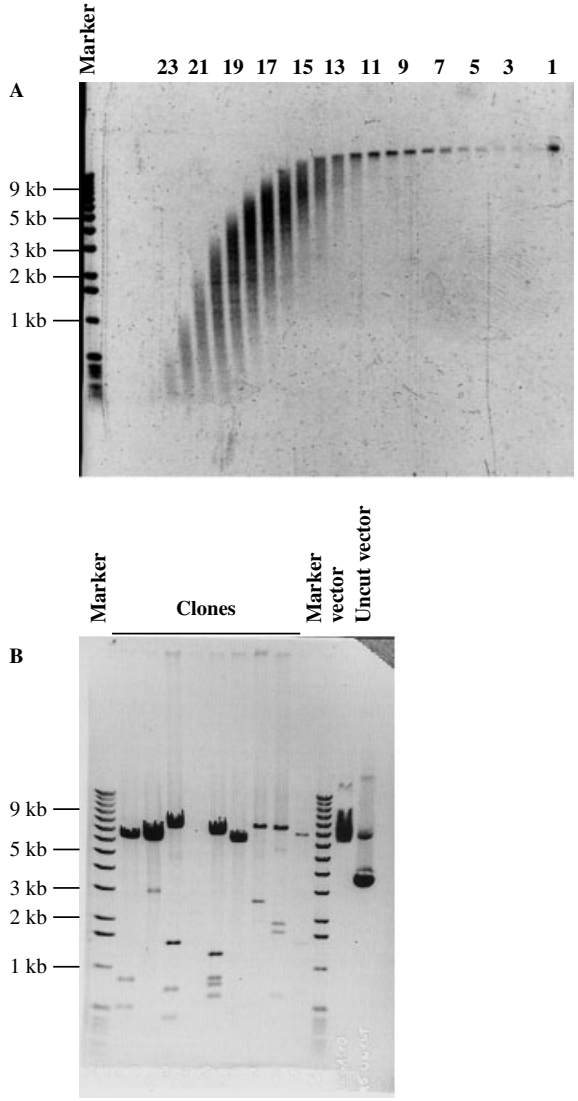

Figure 3. Generation of genomic library. (A) Sucrose gradient purification of *Sz. pombe* DNA. *Sz. pombe* genomic DNA (from strain ROP9) was partially digested with *Sau*3A and fractionated on a 5–25% sucrose gradient. The gradient was fractionated from the bottom and ca. 1 μl each fraction was loaded on an agarose gel and the DNA size analysed. (B) Size analysis of the genomic clones. Nine bacterial clones containing *Sz. pombe* genomic DNA were picked at random and the plasmids were isolated and digested with *Bam*HI to determine the presence and size of the inserts

transformed with the DNA and ura$^+$ transformants were selected on EMM plates. The transformants were analysed for integration by DNA blot analysis. Integration of the plasmid at the *ura4* locus was expected to convert a 1.7 kb fragment into two fragments, 2.5 and 5 kb in size. In addition to the two expected fragments, we also observed an additional 6 kb fragment that most likely reflects multiple integrations at the *ura4* locus. Interestingly, we did not observe multiple integrations when the plasmid was integrated at the *hst4* locus and the 3.3 kb wild-type *hst4*$^+$ band was converted to a 11 kb band, as expected.

Generation of a *Sz. pombe* genomic library

We next generated a *Sz. pombe* genomic DNA library in pRO314. *Sz. pombe* genomic DNA isolated from strain ROP9 (*h*$^+$ *his5-303 lys1-131*) was purified on a caesium chloride gradient and a time course of digestion with Sau3A was performed to standardize the extent of digestion. A time point was chosen at which the bulk DNA had an average size range of 4 kb and the digested DNA was fractionated on a sucrose gradient. The fractionation profile is shown in Figure 3A and fractions 18 and 19 were used for the generation of the *Sz. pombe* library. Plasmid pRO314 was digested to completion with *Bam*HI, phosphatased and ligated with the *Sau*3A-digested genomic DNA. The proportion of insert to vector was empirically determined to maximize for insert ligation. Approximately 70% of clones contained inserts of average size 2 kb (Figure 3B).

The library is proficient for use in multicopy suppressor screens as well as for complementation analyses and should be a useful resource for the general *Sz. pombe* community.

References

Dubey DD, Zhu J, Carlson DL, Sharma K, Huberman JA. 1994. Three ARS elements contribute to the *ura4* replication origin region in the fission yeast, *Schizosaccharomyces pombe*. *EMBO J* **13**: 3638–3647.

Freeman-Cook LL, Sherman JM, Brachmann CB, *et al*. 1999. The *Schizosaccharomyces pombe hst4*$^+$ gene is a *SIR2* homologue with silencing and centromeric functions. *Mol Biol Cell* **10**: 3171–3186.

Hoffman CS, Winston F. 1987. A ten minute DNA preparation from yeast efficiently releases autonomous plasmids for transformation of *Escherichia coli*. *Gene* **57**: 267–272.

Keeney JB, Boeke JD. 1994. Efficient targeted integration at *leu1–32* and *ura4–294* in *Schizosaccharomyces pombe*. *Genetics* **136**: 849–856.

Kim SM, Huberman JA. 1999. Influence of a replication enhancer on the hierarchy of origin efficiencies within a cluster of DNA replication origins. *J Mol Biol* **288**: 867–882.

Kim SM, Zhang DY, Huberman JA. 2001. Multiple redundant sequence elements within the fission yeast *ura4* replication origin enhancer. *BMC Mol Biol* **2**: 1.

Maundrell K. 1990. *nmt1* of fission yeast. A highly transcribed gene completely repressed by thiamine. *J Biol Chem* **265**: 10 857–10 864.

Nimmo ER, Cranston G, Allshire RC. 1994. Telomere-associated chromosome breakage in fission yeast results in variegated expression of adjacent genes. *EMBO J* **13**: 3801–3811.

Ohi R, Feoktistova A, Gould KL. 1996. Construction of vectors and a genomic library for use with *his3*-deficient strains of *Schizosaccharomyces pombe*. *Gene* **174**: 315–318.

Siam R, Dolan WP, Forsburg SL. 2004. Choosing and using *Schizosaccharomyces pombe* plasmids. *Methods* **33**: 189–198.

Sikorski RS, Hieter P. 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* **122**: 19–27.

Zhu J, Brun C, Kurooka H, Yanagida M, Huberman JA. 1992. Identification and characterization of a complex chromosomal replication origin in *Schizosaccharomyces pombe*. *Chromosoma* **102**: S7–16.

Zhu J, Carlson DL, Dubey DD, Sharma K, Huberman JA. 1994. Comparison of the two major ARS elements of the *ura4* replication origin region with other ARS elements in the fission yeast, *Schizosaccharomyces pombe*. *Chromosoma* **103**: 414–422.

