The Journal of Microbiology, December 2009, p. 789-795
DOI 10.1007/s12275-009-0010-4

Vol. 47, No. 6

Generation of Expression Vectors for High-Throughput Functional Analysis of Target Genes in *Schizosaccharomyces pombe*

Jiwon Ahn[1,3], Chung-Hae Choi[1], Chang-Mo Kang[2], Chun-Ho Kim[2], Hee-Moon Park[3], Kyung-Bin Song[4], Kwang-Lae Hoe[1], Misun Won[1*], and Kyung-Sook Chung[1*]

[1]Medical Genomics Research Center, KRIBB, Daejeon 305-806, Republic of Korea
[2]Laboratory of Cytogenetics and Tissue Regeneration, KIRAMS, Seoul 139-706, Republic of Korea
[3]Department of Microbiology and [4]Department of Food Science and Technology, Chungnam National University, Daejeon 305-764, Republic of Korea

(Received January 7, 2009 / Accepted July 2, 2009)

An immediate challenge in the post-genomic era is to assign a biological functions to proteins unraveled by genome analysis. This report is based on studies conducted using *Schizosaccharomyces pombe*, a simple model organism, and presents various vector systems as tools for high-throughput functional analysis of human genes. We constructed *S. pombe* expression vectors for efficient cloning of genes via the Gateway system. We modified the pREP and pSLF series vectors, which are widely used for gene expression in *S. pombe*. The vectors constructed have a uniform backbone of *S. pombe* autonomously replicating sequence (ARS) elements with different selective markers, namely, *ura4*$^+$ and *Saccharomyces cerevisiae* LEU2 complementing *leu1*. These vectors contain 3 different strengths of the inducible promoter *nmt1*, which affect the expression levels of the cloned open reading frames (ORFs). Further, target proteins can be fused with an N-terminal or C-terminal tag such as triple hemagglutinin (3× HA), enhanced green fluorescent protein (EGFP), or *Discosoma* red fluorescent protein (DsRed). We tested the feasibility of the constructed vectors by using 3 human genes, namely, *RAB18*, *SCC-112*, and *PTEN*. Proper expression of tagged *RAB18* was confirmed by western blot analysis. Further, localization of RAB18, SCC112, and PTEN was demonstrated. The constructed vectors can be utilized for high-throughput functional analysis of heterologous genes.

***Keywords*:** expression vector, EGFP, DsRed, HA, gateway system, fission yeast

With the identification of more than 35,000 genes in the human genome (Lander *et al.*, 2001; Venter *et al.*, 2001), there is an increasing demand for high-throughput expression of human genes. Researchers have identified thousands of candidate genes involved in various cellular processes directly relevant to human diseases, and some of these genes could prove to be novel drug targets. *Schizosacchromyces pombe* has been extensively utilized in studies of cross-species gene functions involved in basic cellular processes in cell biology, genetics, and molecular biology. Manipulation of genes in *S. pombe* can produce observable phenotypes. For example, deletion or overexpression of genes involved in cell division or signal transduction causes growth defects and/or morphological changes (Chung *et al.*, 2007; Moon *et al.*, 2008). This characteristic makes *S. pombe* a potential model organism for the investigation of disease-related genes (Bischoff *et al.*, 1992; Ink *et al.*, 1997; Jang *et al.*, 1997). Therefore, studies involving deletion and overexpression of genes are pivotal to the functional analysis of genes in *S. pombe*. In addition, fluorescent protein fusions have been particularly useful for examining subcellular localization of proteins and detecting interactions between 2 proteins in living cells (Prasher, 1995; Sheff and Thorn, 2004; Alting-Mees *et al.*, 2006).

Numerous vectors are used in molecular biology studies in *S. pombe* (Siam *et al.*, 2004; Adams *et al.*, 2005; Van Driessche *et al.*, 2005). Auxotrophic markers such as *ura4*, *his3*, *arg6*, and *ade6* have commonly been used in *S. pombe* episomal vectors. *Saccharomyces cerevisiae* LEU2 is also a useful marker that is known to complement *leu1* (Grimm and Kohli, 1988; Apolinario *et al.*, 1993; Toh-e, 1995; Waddell and Jenkins, 1995; Adams *et al.*, 2005) in *S. pombe*. pREP series vectors are general-purpose episomal vectors that contain the replication origin, *ura4*$^+$ or LEU2 as the selective marker, and the inducible promoter *nmt1* (Forsburg, 1993). pSLF series vectors contain a triple hemagglutinin (3× HA) epitope tag, the replication origin, *ura4*$^+$ as the selective marker, and the inducible promoter *nmt1* (Forsburg and Sherman, 1997). However, currently available vectors are still inadequate for relevant studies. Transfer of open reading frames (ORFs) from one vector to another requires restriction enzyme cloning, which is a very arduous and time-consuming procedure. To overcome this problem, 288 *S. cerevisiae* expression vectors compatible with the Gateway recombination-based cloning system have been constructed (Alberti *et al.*, 2007). However, a few Gateway vectors with promoters that can be constitutively expressed with the

* To whom correspondence should be addressed.
(Tel) 82-42-860-4179; (Fax) 82-42-860-4597
(E-mail) K.S. Chung: kschung@kribb.re.kr
M. Won: mwon@kribb.re.kr

cloned gene have been reported in *S. pombe* (Matsuyama *et al.*, 2008).

In this report, we describe the construction of Gateway-compatible destination vectors by insertion of Gateway conversion cassettes in existing *S. pombe* expression vectors. We modified the pREP and pSLF series shuttle vectors to express proteins fused with N-terminal or C-ternimal tag sequences such as 3×HA, enhanced green fluorescent pro-

Table 1. Summary of plasmids generated in this study

Name	Tag	Marker	Promoter strength	Comments	Reference
General cloning Vectors					
pREP3X			high		
pREP41X	none	LEU2	medium		Forsburg (1993) (at ATCC)
pREP81X			low		
pSLF173			high		
pSLF273	3xHA	*ura4*$^+$	medium		Forsburg and Sherman (1997) (at ATCC)
pSLF373			low		
pSLF175			high		This study
pSLF275	GFP	LEU2	medium	derived from pREP3X, 41X, 81X	This study
pSLF375			low		This study
pSLF177			high		This study
pSLF277	GFP	*ura4*$^+$	medium	derived from pSLF173, 273, 373	This study
pSLF377			low		This study
pREP5X			high		This study
pREP45X	RFP	LEU2	medium	derived from 3X, 41X, 81X	This study
pREP85X			low		This study
pSLF179			high		This study
pSLF279	RFP	*ura4*$^+$	medium	derived from pSLF173, 273, 373	This study
pSLF379			low		This study
Destination vectors					
pDES3X	none		high	derived from pREP3X, 41X, 81X/Cassette C[a]	This study
pDES41X	none	LEU2	medium		This study
pDES81X	none		low		This study
N-terminal tagging vectors					
pDES173N	3xHA		high	derived from pSLF173, 273, 373 /Cassette B[a]	Chung *et al.* (2007)
pDES273N	3xHA	*ura4*$^+$	medium		This study
pDES373N	3xHA		low		This study
pDES175N	GFP		high	derived from pSLF175, 275, 375 /Cassette B[a]	This study
pDES275N	GFP	LEU2	medium		This study
pDES375N	GFP		low		This study
pDES177N	GFP		high	derived from pSLF177, 277, 377 /Cassette B[a]	This study
pDES277N	GFP	*ura4*$^+$	medium		This study
pDES377N	GFP		low		This study
pDES5XN	RFP		high	derived from pSLF5X, 45X, 85X /Cassette C[a]	This study
pDES45XN	RFP	LEU2	medium		This study
pDES85XN	RFP		low		This study
pDES179N	RFP		high	derived from pSLF179, 279, 379 /Cassette C[a]	This study
pDES279N	RFP	*ura4*$^+$	medium		This study
pDES379N	RFP		low		This study
C-terminal tagging vectors					
pDES173C	3xHA		high	derived from pSLF173, 273, 373 /Cassette C[a]	This study
pDES273C	3xHA	*ura4*$^+$	medium		This study
pDES373C	3xHA		low		This study
pDES175C	GFP		high	derived from pSLF175, 275, 375 /Cassette C[a]	This study
pDES275C	GFP	LEU2	medium		This study
pDES375C	GFP		low		This study
pDES179C	RFP		high	derived from pSLF179, 279, 379 /Cassette C[a]	This study
pDES279C	RFP	*ura4*$^+$	medium		This study
pDES379C	RFP		low		This study

[a] These cassettes make the vectors Gateway-compatible by insertion to general purpose vectors.

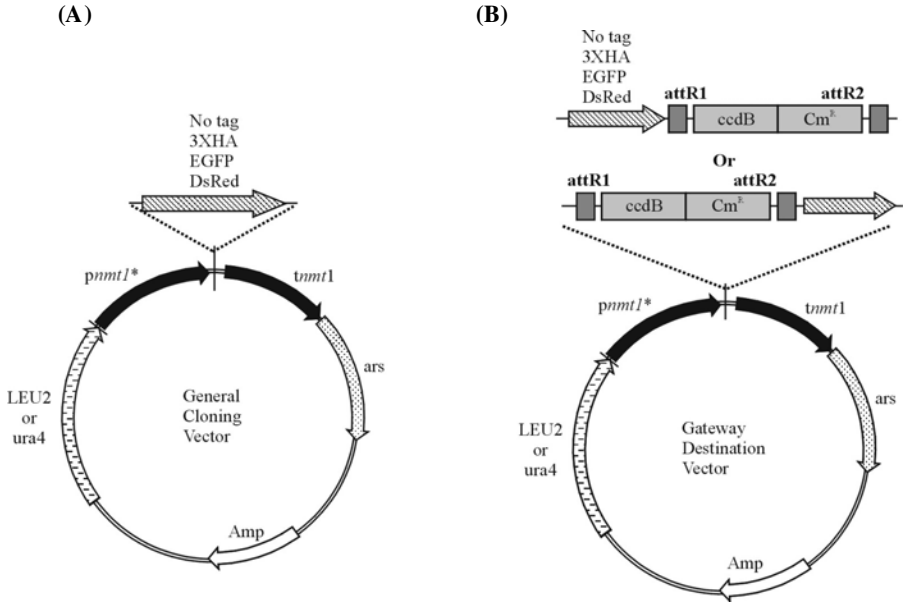

Fig. 1. Overview of the new vector constructions in *S. pombe*. (A) Generation of expression vectors with a GFP or RFP tag suitable for the intracellular localization of proteins. The vectors contain *ura4*$^+$ or LEU2 as the selection marker and 3 different strengths (*nmt1**) of the inducible promoter *nmt1*. The TATA box of the *nmt1* promoter has been mutated to modify its overall strength. (B) Generation of destination vectors that are untagged, N-terminal or C-terminal tags like HA, EGFP, and DsRed. p*nmt1* and t*nmt1* represent the promoter and terminator of the *nmt1* gene. p*nmt1* is regulated by concentration of thiamine in the media.

tein (EGFP), and *Discosoma* red fluorescent protein (DsRed) (Forsburg, 1993; Forsburg and Sherman, 1997). These vectors will be useful in genetic and molecular manipulations for high-throughput functional analysis of heterologous genes in *S. pombe*.

Materials and Methods

Strains and media

The fission yeast *S. pombe* strain ED665 (*h*$^-$, *ade6-M210*, *leu1-32*, *ura4-D18*) was used for the validation of vectors. Yeast cells were grown in YES medium (0.5% yeast extract, 3% glucose, and supplements) or Edinburgh minimal medium (EMM) supplemented with adenine, uracil, and/or leucine. Media preparation and basic manipulation for *S. pombe* were carried out as described by Moreno *et al.* (1991).

Escherichia coli strains DH5α and DB3.1 were used for the subcloning of polymerase chain reaction (PCR) products and destination cassettes and the amplification of constructed plasmids.

Materials

The Gateway conversion cassette system and LR Clonase enzyme were purchased from Invitrogen (USA). Klenow fragment of DNA polymerase I and restriction endonucleases were purchased from Roche (Germany) and NEB (England). Anti-RAB18, anti-HA (12CA5), anti-green fluorescent protein (GFP) (G6539), and anti-red fluorescent protein (RFP) (sc-33353) antibodies were purchased from Roche (England), Sigma-Aldrich (USA), and Santa Cruz Biotech (USA), respectively.

Generation of expression vectors with a GFP or RFP tag as parental plasmids

The *S. pombe* expression vector pREP3X, pSLF172, and pSLF173 series were obtained from recombinant DNA vectors in the American Type Culture Collection (ATCC) (Table 1).

To construct fluorescent protein-tagged vectors, the coding sequences of the fluorescent proteins were cloned into the *S. pombe* autonomously replicating sequence (ARS) element-based vectors. GFP and monomeric DsRed were amplified by PCR, using the following primers: for GFP; 5'-GCTCTCGA GATGGTGAGCAAGGGCGAGGAG-3' and 5'-TCAGGATC CCTTGTACAGCTCGTCCATGCC-3' derived from pEGFP-1 (Clontech, USA); for DsRed; 5'-GATCTCGAGATGGCCT CCTCCGAGGACGTC-3' and 5'-ATCGTCGACGGCGCCG GTGGAGTGGCGGCC-3' derived from pDsRed-N1 (Clontech). The PCR-generated EGFP fragments were inserted into the *Xho*I/*Bam*HI sites of the pSLF173, pSLF273, and pSLF373 vectors (*ura4*$^+$), resulting into the pSLF177 series (EGFP/*ura4*$^+$). The *Xho*I/*Bam*HI fragments of the pSLF177 series were then inserted into the pREP3X, pREP41X, and pREP81X vectors (No tag/LEU2), resulting into the pSLF175 series (EGFP/LEU2). The PCR products of monomeric DsRed were inserted into the *Xho*I/*Sal*I site of the pREP3X vector (No tag/LEU2) to produce the pREP5X vector (RFP/LEU2). The *Xho*I/*Sma*I fragments of pREP5X were inserted into the pREP41X and pREP81X vectors (No tag/LEU2) to produce the pREP45X and pREP85X vectors (RFP/LEU2), respectively, and into the pSLF173, pSLF273, and pSLF373 vectors (*ura4*$^+$) to produce the pSLF179 series (RFP/*ura4*$^+$).

Generation of destination vectors for *S. pombe*

We inserted the Gateway cassette B or C possessing the chloramphenicol or *ccdB* resistance gene in frame with the HA, GFP, or RFP tag (Fig. 1). Insertion of the cassette into general-purpose vectors makes them Gateway compatible. To generate the destination vectors for N-terminal or C-terminal fusion proteins, the reading frame in the *att*R1 recombination site should be the same as that in the fused region (Fig. 1B). Thus, we used 2 conversion cassettes with different reading frames for correct translation of the expression clones. To construct the N-terminal tagging vectors, the conversion cassette **'B'** was inserted into the *Sma*I restriction site of the pSLF173 series, the *Bam*HI/*Sma*I restriction site of the pSLF175 series, and the *Sma*I restriction site of the pSLF177 series, resulting into the pDES173N series (3×HA/*ura4*$^+$), the pDES175N series (EGFP/LEU2) and the pDES177N series (EGFP/*ura4*$^+$), respectively. The conversion cassette **'C'** was ligated into the *Sma*I-digested pREP3X series, pREP5X series, and pSLF179 series to produce the destination vector pDES3X series (No tag/LEU2), pDES5XN series (RFP/LEU2), and pDES179N series (RFP/*ura4*$^+$), respectively. To construct the destination vectors for C-terminal fusion proteins, the pSLF173, the pSLF175, and pSLF179 series vectors were digested with *Xho*I and the ends of vectors were filled with the Klenow fragment. The conversion cassette **'C'** was inserted into the digested fragments of the pSLF173 series, pSLF175 series, and pSLF179 series with blunt ends, resulting into the pDES173C series (3×HA/*ura4*$^+$), pDES175C series (EGFP/LEU2), and pDES 177C series (EGFP/*ura4*$^+$), respectively. Proper integration and orientation of the Gateway cassette was confirmed by DNA sequencing.

Cloning of ectopic genes into destination vectors

Full-length cDNAs of the human genes *RAB18*, *SCC-112*, and *PTEN* were amplified by PCR and cloned into the entry vector pENTR3C. To generate the recombination expression plasmids, 200 ng each of the entry vector and destination vector was mixed with 2 μl of the LR Clonase enzyme (Invitrogen, USA). The mixture was incubated at room temperature for 3 h. After adding 1 μl of protease K, the mixture was incubated at 37°C for 10 min to terminate the clonase reaction.

Ectopic expression of genes in *S. pombe*

The recombination vectors were introduced into the ED665 h^- auxotroph haploid strain of *S. pombe* by the lithium acetate transformation method (Moreno *et al.*, 1991). The colonies carrying the recombination vectors were first grown in EMM containing thiamine, which suppressed the expression of the ectopic genes, and were then washed 3 times with distilled water. The cultured cells were transferred to thiamine-free EMM and grown for additional 12 h to remove the residual thiamine from the cells. For ectopic gene expression, the cells were retransferred to fresh thiamine-free EMM and cultured for 12 h to confirm the cellular localization of the encoded protein and for 18 h to detect the protein by western blot analysis (Forsburg and Sherman, 1997; Chung *et al.*, 2001). Overexpression of the cloned genes was confirmed by western blot analysis performed using HA, GFP, and DsRed antibodies.

Western blot analysis

Proteins were prepared in a lysis buffer as previously described (Jang *et al.*, 1997). Cells were lysed by the glass bead method in a lysis buffer [20 mM Tris-HCl; pH 7.5, 10 mM EGTA, 2 mM EDTA, 0.25 M Sucrose, 1% Nonidet P-40 (NP-40)] containing a protease inhibitor cocktail (Complete™ mini; Roche, Germany) and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Immunodetections were performed using monoclonal antibodies raised against the HA, GFP, and DsRed tags; a horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotech, USA); and enhanced chemiluminescence (ECL) detection kit (Millipore, USA).

Microscopy

Cell morphology and fluorescence were examined using a fluorescence microscope and differential interference contrast microscope (Carl Zeiss Co., Germany). The cells were pelleted and resuspended in 50 mM citrate/phosphate buffer and immobilized on coverslips for imaging.

Results and Discussion

Generation of expression vectors with a GFP or RFP tag as parental plasmids

An important approach to study gene function is to examine the subcellular localization of the encoded protein. Protein tagging can allow for simple and efficient biological and biochemical analyses of the gene products. The location of a reporter protein in a subcellular compartment, as directed by the unknown fused protein, often provides additional supporting evidence for the function of the gene of interest. Fluorescent protein fusions can be used to detect interactions between 2 proteins as well as protein localization (Yan and Marriott, 2003; Park and Raines, 2004). GFP and monomeric DsRed (RFP) have become popular protein tags used for determining protein localization and imaging living-cell fluorescence (Rodrigues *et al.*, 2001; Campbell *et al.*, 2002; Sheff and Thorn, 2004). GFP and DsRed were originally isolated from the luminescent jellyfish *Aequorea victoria* and *Discosoma* sp., respectively

To generate the parent plasmids for tagged expression vectors, the coding sequences of fluorescent proteins such as GFP and monomeric DsRed (RFP) were amplified by PCR. The amplified DNA fragments were then cloned into the pREP3X and pSLF173 series vectors, which contain 3 different strengths of the inducible promoter *nmt1* (Fig. 1). p*nmt1* is a strong promoter in *S. pombe* and is transcriptionally repressed by thiamine. The weaker versions of p*nmt1* contain mutations in the TATA box, which attenuate induction of expression (Basi *et al.*, 1993). PCR-generated EGFP fragments were inserted into the pSLF173, pSLF273, and pSLF373 vectors (*ura4*$^+$), resulting into the pSLF177 series (EGFP/*ura4*$^+$). The EGFP fragments of the pSLF177 series were then inserted into the pREP3X, pREP41X, and pREP81X vectors (No Tag/LEU2), resulting into the pSLF175 series (EGFP/LEU2). The PCR products of monomeric DsRed were inserted into the pREP3X vector (LEU2) to

produce the pREP5X vector (RFP/LEU2). The *Xho*I/*Sma*I fragments (carrying DsRed) of pREP5X were inserted into the pREP41X and pREP81X vectors (No Tag/LEU2) to produce the pREP45X and pREP85X vectors (RFP/LEU2),

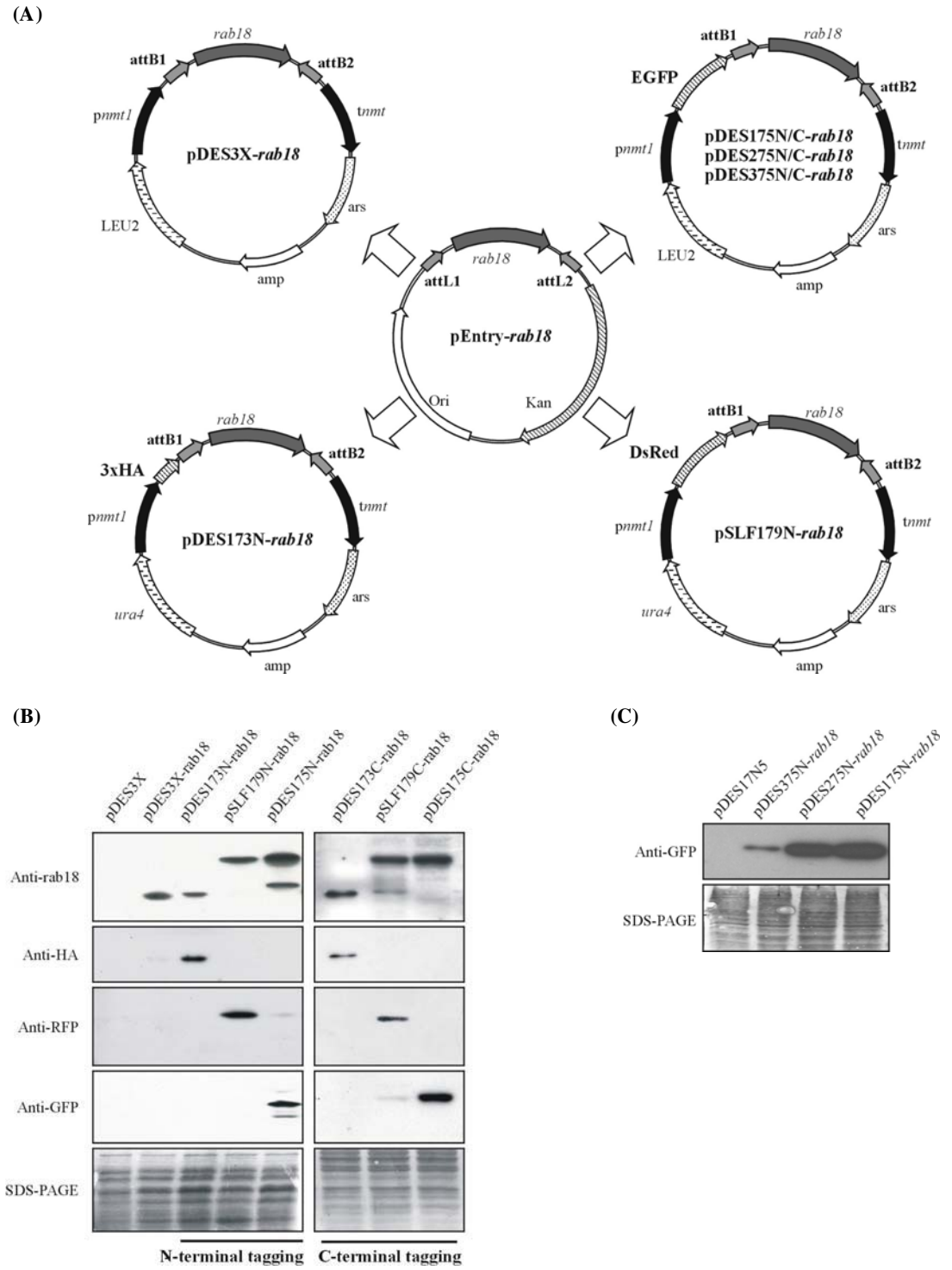

Fig. 2. Validation of the vectors generated in *S. pombe*. (A) Practical application of the Gateway vectors to test correct fusion. An entry clone containing human *RAB18* was used to generate untagged, HA-, EGFP-, and DsRed-tagged expression constructs. (B) Protein tagging was confirmed by western blot analysis. A total of 30 μg of proteins were used in SDS-PAGE. Western blot analysis detected the appropriately sized fusion proteins, using anti-RAB18, anti-HA, anti-RFP, and anti-GFP antibodies. (C) Verification of promoter strength. pDES 175N, pDES275N, and pDES375N vectors were used for full-, medium-, and low-strength promoters to test the 3 versions of the *nmt1* promoter. Western blot analysis detected the GFP fusion proteins with anti-GFP antibody.

respectively, and into the pSLF173, pSLF273, and pSLF373 vectors (*ura4*$^+$) to produce the pSLF179 series (RFP/*ura4*$^+$).

Proper integration and amplification of these vectors was confirmed by DNA sequencing of the cloned gene. The tags are important for studying the functions and localization of heterologous genes. The resultant plasmids are listed in Table 1.

Generation of destination vectors for *S. pombe*

The current challenge in the post-genomic era is to assign a function(s) to the increasing number of genes being discovered. To accomplish this, comprehensive analysis including ectopic expression, subcellular localization, and functional complementation will be performed. *S. pombe* is a potential model organism for investigating foreign gene functions. However, conventional cloning strategies that involve restriction digestion and ligation are arduous and time consuming, and their implementation is often restricted by inappropriate restriction enzyme sites. To address this problem and achieve high-throughput screening of candidate genes in *S. pombe*, we constructed destination vectors compatible with the entry vector of the Gateway recombination-based cloning system. In the Gateway system, the clonase enzyme removes ectopic genes by cutting from the *att*L sites of the entry vector (Fig. 2A) and inserts these genes by ligation at the *att*R sites of the destination vector. Finally, *att*B sites are generated by recombination between the *att*L and *att*R sites (Fig. 2A), and expression clones carrying the ectopic genes are constructed.

In this study, the destination vectors were constructed by inserting the Gateway cassette possessing the chloramphenicol or ccdB resistance gene into the expression vectors generated for tagging of the target protein. The conversion cassette **'B'** was introduced into the pSLF173, pSLF175, and pSLF177 series, generating the N-terminal tagging vectors pDES173N (3×HA/*ura4*$^+$) (Chung *et al.*, 2007), pDES175N (EGFP/LEU2), and pDES177N (EGFP/*ura4*$^+$) series, respectively. The conversion cassette **'C'** was also ligated into the pREP5X, pSLF179, and pREP3X series to produce the N-terminal tagging vectors pDES5XN (RFP/LEU2) and pDES179N (RFP/*ura4*$^+$), and the no tagging vectors pDES3X (No tag/LEU2) series. To construct the C-terminal tagging vectors, the conversion cassette **'C'** was introduced into the pSLF173, pSLF175, and pSLF179 series, resulting into the pDES173C (3×HA/*ura4*$^+$), pDES175C (EGFP/LEU2), and pDES179C (RFP/*ura4*$^+$) series, respectively. The resultant Gateway destination vectors are listed in Table 1.

Validation of the generated plasmids

We tested the feasibility of the constructed vectors by using human genes that are of interest to our laboratory. Recombination between the recognition site of the entry clone containing the gene of interest and that of the destination plasmid is brought about by the LR Clonase enzyme, and an expression clone containing the gene of interest is generated. First, nine recombination plasmids with no tags or N-terminal fusion tags were produced using an entry vector carrying the human gene *RAB18* encoding RAS-like small G-protein, nine different destination vectors (pDES3X, pDES173N, pDES175N, pDES275N, pDES375N, pDES179N, pDES173C, pDES175C, and pDES179C) and the Gateway LR Clonase enzyme (Fig. 2A). This reaction mixture was used to transform *E. coli* cells, and clones containing the human genes in the destination vectors were selected on LB agar plates containing ampicillin. The expression clones were introduced into *S. pombe* strain ED665 and induced by the *nmt1* promoter. After 18 h of induction, production of RAB18, and N-terminal and C-terminal tagged RAB18 was assayed by western immunoblotting. Blotting experiments were performed using antibodies raised against RAB18, HA, GFP, and RFP. As expected, the fusion proteins in all the constructs were significantly expressed (Fig. 2B), and the strain carrying pDES3X, pDES173N, pDES175N, pDES179N, pDES173C, pDES175C, and pDES179C, all of which express the RAB18 fusion protein under full-strength p*nmt1*, substantially expressed RAB18 after 18 h of induction. Less protein was expressed under the medium- and low-strength promoters (pDES275N and pDES375N) (Fig. 2C). Second, entry clones containing the cDNA clone of human *SCC-112* (encoding a nuclear cell cycle regulatory protein) and that of human *PTEN* (encoding a tumor suppressor protein that negatively regulates the AKT/PKB signaling pathway) were used in the Gateway LR reaction to generate pDES175N-*SCC-112* and pDES175N-*PTEN*, respectively. We selected these genes because the subcellular localization of SCC-112 (Zheng *et al.*, 2008), PTEN (Das *et al.*, 2003), and RAB18 (Ozeki *et al.*, 2005) has been well characterized; SCC-112 localizes in the nucleus, PTEN in the cytoplasm, and RAB18 in the cytoplasm and plasma membrane. After 12 h of induction, cells were processed for fluorescence microscopy (Fig. 3). We observed the same localization patterns of the fusion proteins as previously described.

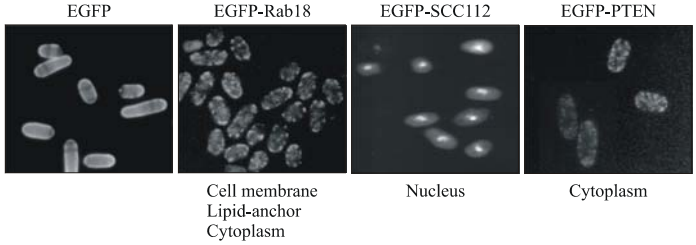

Fig. 3. Practical application of Gateway vectors for correct localization of fusion protein. The Gateway-generated GFP-tagged proteins localized at the same sites as reported previously. EGFP-RAB18 localized in the plasma membrane and cytoplasm, EGFP-SCC-112 in the nucleus, and EGFP-PTEN in the cytoplasm. EGFP alone was distributed throughout the cytoplasm as the control.

In this report, we have described the construction of expression vectors containing a destination cassette suitable for high-throughput cloning of target genes via the Gateway system. These plasmids can be used to express N-terminal or C-terminal tagged target proteins for affinity binding and/or functional analysis of target genes. The availability of these vectors makes *S. pombe* a robust system for a genome-wide investigation of the mechanisms underlying many cellular processes and for the discovery of novel therapeutic targets for human diseases.

Acknowledgements

This work was supported, in part, by a grant from the 21st Century Frontier Program for Functional Analysis of the Human Genome, a Nuclear R&D Program from the Ministry of Science and Technology, and a grant from the Korea Research Council of Fundamental Science and Technology.

References

Adams, C., D. Haldar, and R.T. Kamakaka. 2005. Construction and characterization of a series of vectors for *Schizosaccharomyces pombe*. *Yeast* 22, 1307-1314.

Alberti, S., A.D. Gitler, and S. Lindquist. 2007. A suite of Gateway® cloning vectors for high-throughput genetic analysis in *Saccharomyces cerevisiae*. *Yeast* 24, 913-919.

Alting-Mees, M.A., E.P. Risseeuw, E. Liu, M. Desautels, W.A. Crosby, and S.M. Hemmingsen. 2006. Intracellular expression of recombinant antibody fluorescent protein fusions for localization of target antigens in *Schizosaccharomyces pombe*. *Methods Mol. Biol.* 313, 97-105.

Apolinario, E., M. Nocero, M. Jin, and C.S. Hoffman. 1993. Cloning and manipulation of the *Schizosaccharomyces pombe his7*$^+$ gene as a new selectable marker for molecular genetic studies. *Curr. Genet.* 24, 491-495.

Basi, G., E. Schmid, and K. Maundrell. 1993. TATA box mutations in the *Schizosaccharomyces pombe nmt1* promoter affect transcription efficiency but not the transcription start point or thiamine repressibility. *Gene* 123, 131-136.

Bischoff, J.R., D. Casso, and D. Beach. 1992. Human p53 inhibits growth in *Schizosaccharomyces pombe*. *Mol. Cell. Biol.* 12, 1405-1411.

Campbell, R.E., O. Tour, A.E. Palmer, P.A. Steinbach, G.S. Baird, D.A. Zacharias, and R.Y. Tsien. 2002. A monomeric red fluorescent protein. *Proc. Natl. Acad. Sci. USA* 99, 7877-7882.

Chung, K.S., Y.J. Jang, N.S. Kim, S.Y. Park, S.J. Choi, J.Y. Kim, J.H. Ahn, H.J. Lee, J.H. Lim, J.H. Song, J.H. Ji, J.H. Oh, K.B. Song, H.S. Yoo, and M. Won. 2007. Rapid screen of human genes for relevance to cancer using fission yeast. *J. Biomol. Screen* 12, 568-577.

Chung, K.S., M. Won, S.B. Lee, Y.J. Jang, K.L. Hoe, D.U. Kim, J.W. Lee, K.W. Kim, and H.S. Yoo. 2001. Isolation of a novel gene from *Schizosaccharomyces pombe*: *stm1*$^+$ encoding a seven-transmembrane loop protein that may couple with the heterotrimeric Gα2 protein, Gpa2. *J. Biol. Chem.* 276, 40190-40201.

Das, S., J.E. Dixon, and W. Cho. 2003. Membrane-binding and activation mechanism of PTEN. *Proc. Natl. Acad. Sci. USA* 100, 7491-7496.

Forsburg, S.L. 1993. Comparison of *Schizosaccharomyces pombe* expression systems. *Nucleic Acids Res.* 21, 2955-2956.

Forsburg, S.L. and D.A. Sherman. 1997. General purpose tagging vectors for fission yeast. *Gene* 191, 191-195.

Grimm, C. and J. Kohli. 1988. Observations on integrative transformation in *Schizosaccharomyces pombe*. *Mol. Gen. Genet.* 215, 87-93.

Ink, B., M. Zornig, B. Baum, N. Hajibagheri, C. James, T. Chittenden, and G. Evan. 1997. Human Bak induces cell death in *Schizosaccharomyces pombe* with morphological changes similar to those with apoptosis in mammalian cells. *Mol. Cell. Biol.* 17, 2468-2474.

Jang, Y.J., M. Won, K.S. Chung, D.U. Kim, K.L. Hoe, C. Park, and H.S. Yoo. 1997. A novel protein, Psp1, essential for cell cycle progression of *Schizosaccharomyces pombe* is phosphorylated by Cdc2-Cdc13 upon entry into G_0-like stationary phase of cell growth. *J. Biol. Chem.* 272, 19993-20002.

Lander, E.S., L.M. Linton, B. Birren, C. Nusbaum, M.C. Zody, J. Baldwin, K. Devon, K. Dewar, M. Doyle, W. FitzHugh, R. Funke, D. Gage, K. Harris, A. Heaford, J. Howland, L. Kann, J. Lehoczky, R. LeVine, P. McEwan, K. McKernan, *et al.* 2001. Initial sequencing and analysis of the human genome. *Nature* 409, 860-921.

Matsuyama, A., A. Shirai, and M. Yoshida. 2008. A series of promoters for constitutive expression of heterologous genes in fission yeast. *Yeast* 25, 371-376.

Moon, D., J.A. Bae, H.J. Cho, and J.H. Yoon. 2008. The fission yeast homologue of Gle1 is essential for growth and involved in mRNA export. *J. Microbiol.* 46, 422-428.

Moreno, S., A. Klar, and P. Nurse. 1991. Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*. *Methods Enzymol.* 194, 795-823.

Ozeki, S., J. Cheng, K. Tauchi-Sato, N. Hatano, H. Taniguchi, and T. Fujimoto. 2005. Rab18 localizes to lipid droplets and induces their close apposition to the endoplasmic reticulum-derived membrane. *J. Cell Sci.* 118, 2601-2611.

Park, S.H. and R.T. Raines. 2004. Fluorescence polarization assay to quantify protein-protein interactions. *Methods Mol. Biol.* 261, 161-166.

Prasher, D.C. 1995. Using GFP to see the light. *Trends Genet.* 11, 320-323.

Rodrigues, F., M. van Hemert, H.Y. Steensma, M. Corte-Real, and C. Leao. 2001. Red fluorescent protein (DsRed) as a reporter in *Saccharomyces cerevisiae*. *J. Bacteriol.* 183, 3791-3794.

Sheff, M.A. and K.S. Thorn. 2004. Optimized cassettes for fluorescent protein tagging in *Saccharomyces cerevisiae*. *Yeast* 21, 661-670.

Siam, R., W.P. Dolan, and S.L. Forsburg. 2004. Choosing and using *Schizosaccharomyces pombe* plasmids. *Methods* 33, 189-198.

Toh-e, A. 1995. Construction of a marker gene cassette which is repeatedly usable for gene disruption in yeast. *Curr. Genet.* 27, 293-297.

Van Driessche, B., L. Tafforeau, P. Hentges, A.M. Carr, and J. Vandenhaute. 2005. Additional vectors for PCR-based gene tagging in *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* using nourseothricin resistance. *Yeast* 22, 1061-1068.

Venter, J.C., M.D. Adams, E.W. Myers, P.W. Li, R.J. Mural, G.G. Sutton, H.O. Smith, M. Yandell, C.A. Evans, R.A. Holt, J.D. Gocayne, P. Amanatides, R.M. Ballew, D.H. Huson, J.R. Wortman, Q. Zhang, C.D. Kodira, X.H. Zheng, L. Chen, M. Skupski, *et al.* 2001. The sequence of the human genome. *Science* 291, 1304-1351.

Waddell, S. and J.R. Jenkins. 1995. *arg3*$^+$, a new selection marker system for *Schizosaccharomyces pombe*: Application of *ura4*$^+$ as a removable integration marker. *Nucleic Acids Res.* 23, 1836-1837.

Yan, Y. and G. Marriott. 2003. Analysis of protein interactions using fluorescence technologies. *Curr. Opin. Chem. Biol.* 7, 635-640.

Zheng, M.Z., L.M. Zheng, and Y.X. Zeng. 2008. *SCC-112* gene is involved in tumor progression and promotes the cell proliferation in G_2/M phase. *J. Cancer Res. Clin. Oncol.* 134, 453-462.