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A Simple and Immediate Method for Simultaneously Evaluating Expression Level and Plasmid Maintenance in Yeast

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To allow the comprehensive assessments of yeast expression systems, a simple and immediate method for simultaneously evaluating the expression level and plasmid maintenance in yeast was demonstrated. This method uses green fluorescent protein (GFP) and flow cytometry (FCM) and is characterized by a dual analysis of the average intensity of GFP fluorescence and the population of GFP-expressing cells. The FCM analysis of GFP fluorescence intensity rapidly quantifies the expression level without complex manipulations, such as the enzymatic reaction of a *lacZ* reporter assay. Moreover, the single-cell analysis revealed that the proportion of cells expressing GFP in the cell cluster reflects the plasmid retention rate; therefore, the FCM analysis of the GFP-expressing population allows the immediate estimation of the plasmid retention rate without the 2- or 3-day incubation required for colony counting. We show that the FCM analysis with GFP reporter is a suitable method to explore the hopeful expression vector and host strain or establish the several expression systems exhibiting the characteristic properties in yeast.

Key words: yeast, expression system, plasmid maintenance, green fluorescent protein, flow cytometry.

Abbreviations: *CEN/ARS*, centromere-autonomously replicating sequence; *EGFP*, enhanced green fluorescent protein; FCM, flow cytometry; GFP, green fluorescent protein; IR, inverted repeat; *MCS*, multiple cloning site; *PGK5′*, *PGK1* promoter; *PGK3′*; *PGK1* terminator; SD, synthetic dextrose; YPDA, yeast extract-peptone-dextrose-adenine.

Yeasts such as *Saccharomyces cerevisiae, Kluyveromyces lactis, Yarrowia lipolytica* and *Pichia pastoris* are attractive host strains for the production of recombinant proteins (*1*, *2*). Expression systems have been developed for this purpose, and the transformation efficiencies, vector stabilities, copy numbers and expression levels have been investigated. However, few reports objectively provide a comprehensive comparison of the expression systems. The lack of exhaustive assessments makes it difficult to select the expression vector and host strain from the diversity of options including promoters, selectable markers, origins and genotypes.

For the practical use of expression vectors and host strains, the expression level and stability of gene maintenance are especially important. The investigation of plasmid maintenance by colony counting is the classical method for evaluating the origins and selectable markers, and the examination of expression levels by the *lacZ* reporter assay is the classical method for selecting promoters and host strains (*3*, *4*). Although these methods are reliable, they are time consuming and labor intensive; therefore, it is beneficial to develop a more convenient method.

Flow cytometry (FCM) can rapidly and quantitatively measure the fluorescence of cells without any complex procedures; FCM requires only the harvest, resuspension and measurement of cultivated cells. Moreover, FCM measures the fluorescence intensity at the single-cell level, and therefore it distinguishes the cell clusters displaying distinctive fluorescence levels and measures the population of cell clusters (*5*). Green fluorescent protein (GFP) and FCM are a useful combination for the evaluation of expression systems. However, GFP and FCM are not commonly used for the evaluation of expression systems, and their utility for this purpose has not been completely explored.

In this report, we evaluated a set of expression vectors carrying several selectable markers and replicative origins in three typical *Saccharomyces cerevisiae* strains as a comprehensive selection model to survey the advantages of GFP and FCM for valuating yeast expression systems. The histogram plots obtained from the FCM analyses of wide range of candidates directed the evidence that the proportion of GFP-expressing cells in the yeast cell cluster correlates with the plasmid retention rate and a simple and immediate method to simultaneously estimate the expression level and plasmid maintenance in yeast by using GFP and FCM was demonstrated.

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Table 1. **Yeast strains and plasmids.**

Strain or plasmid[a]	Description
BY4741	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*
YPH499	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1*
W303-1A	*MAT***a** *leu2-3,112 his3-11,15 trp1-1 can1-100 ade2-1 ura3-1*
pGK402	Integration; *ADE2* marker; 6458 bp; created from pRS402
pGK403	Integration; *HIS3* marker; 5386 bp; created from pRS403
pGK404	Integration; *TRP1* marker; 5204 bp; created from pRS404
pGK405	Integration; *LEU2* marker; 6437 bp; created from pRS405
pGK406	Integration; *URA3* marker; 5314 bp; created from pRS406
pGK412	*CEN/ARS ori; ADE2* marker; 6972 bp; created from pRS412
pGK413	*CEN/ARS ori; HIS3* marker; 5900 bp; created from pRS413
pGK414	*CEN/ARS ori; TRP1* marker; 5718 bp; created from pRS414
pGK415	*CEN/ARS ori; LEU2* marker; 6951 bp; created from pRS415
pGK416	*CEN/ARS ori; URA3* marker; 5828 bp; created from pRS416
pGKRS422	2 μ *ori* (short form, pRS original); *ADE2* marker; 7798 bp; created from pRS422
pGKRS423	2 μ *ori* (short form, pRS original); *HIS3* marker; 6727 bp; created from pRS423
pGKRS424	2 μ *ori* (short form, pRS original); *TRP1* marker; 6546 bp; created from pRS424
pGKRS425	2 μ *ori* (short form, pRS original); *LEU2* marker; 7779 bp; created from pRS425
pGKRS426	2 μ *ori* (short form, pRS original); *URA3* marker; 6656 bp; created from pRS426
pGK422	2 μ *ori* (long form, from pWI3); *ADE2* marker; 8698 bp; created from pRS402
pGK423	2 μ *ori* (long form, from pWI3); *HIS3* marker; 7626 bp; created from pRS403
pGK424	2 μ *ori* (long form, from pWI3); *TRP1* marker; 7444 bp; created from pRS404
pGK425	2 μ *ori* (long form, from pWI3); *LEU2* marker; 8677 bp; created from pRS405
pGK426	2 μ *ori* (long form, from pWI3); *URA3* marker; 7554 bp; created from pRS406

[a]All plasmids include *PGK1* promoters. The *EGFP* gene was inserted into each plasmid and designated as pGK(RS)4XX-EGFP.

MATERIALS AND METHODS

Yeast Strains and Media—Saccharomyces cerevisiae BY4741 (*6*), YPH499 (*7*) and W303-1A (*8*) were selected as host strains, and their genotypes are listed in Table 1. YPDA media contained 10 g/l yeast extract, 20 g/l peptone, 20 g/l dextrose and 150 mg/l adenine. Synthetic dextrose (SD) media containing 6.7 g/l yeast nitrogen base without amino acids and 20 g/l dextrose were supplemented with appropriate amino acids and nucleotides depending on the desired selectable markers (150 mg/l adenine, 20 mg/l histidine, 60 mg/l leucine, 30 mg/l lysine, 20 mg/l methionine, 40 mg/l tryptophan and 20 mg/l uracil). For solid media, the concentrations of adenine were changed to 55 mg/l and 20 g/l agar were added.

Plasmid Constructions—All GFP-expressing plasmids were derived from the pRS vector series (*3*, *7*) and were controlled by *PGK1* promoters (Table 1 and Fig. 1). The different lengths of 2 μ origins were prepared as a short form (1345 bp) and a long form (2234 bp). The short 2 μ was originally included in pRS42X (X; 2–6). The long 2 μ was amplified from pWI3 (*9*) with oligonucleotides (5′cccGACGTCcacggactatagactatacta and 5′cctGACGTCt gaaccagtcctaaaacgagt) and inserted into pRS40X (X; 2–6) at the *Aat*II site with the same direction. The resulting plasmids were designated as pRS40X+2 μm (X; 2–6).

The fragment containing the *PGK1* promoter (*PGK5′*), multiple cloning cite (*MCS*) and *PGK1* terminator (*PGK3'*) was prepared by digestion of pTA2-PGK (*5*) with *Xho*I and *Not*I sites and then ligated with the same sites into the pRS vector series (pRS40X, integration types; pRS41X, *CEN/ARS* types; pRS42X, short 2 μ types; and pRS40X+2 μm, long 2 μ types) to create pGK40X, pGK41X, pGKRS42X and pGK42X (X; 2–6), respectively.

The enhanced green fluorescent protein (*EGFP*) genes attached with the *Sal*I site and *Bam*HI site were amplified from pEGFP (Takara Bio, Shiga, Japan) with oligonucleotides (5′ttttGTCGACatggtgagcaagggcgagga and 5′ccccGGATCCttacttgtacagctcgtcca) and ligated into pGK40X, pGK41X, pGKRS42X and pGK42X (X; 3 and 5), producing pGK40X-EGFP, pGK41X-EGFP, pGKRS42X-EGFP and pGK42X-EGFP (X; 3 and 5). Similarly, pGK40X-EGFP, pGK41X-EGFP, pGKRS42X-EGFP and pGK42X-EGFP (X; 2, 4 and 6) were created with oligonucleotides including *Nhe*I site or *Eco*RI site (5′ttttGCTAGCatggtgagcaagggcg and 5′ggggGAATTCtta cttgtacagctcgtcca).

Transformation and Cultivation—The transformation procedure followed the lithium acetate method (*10*). The integration plasmids pGK40X-EGFP were linearized with a single restriction site in the selectable markers (X = 2, *Eco*RV; X = 3, *Nde*I; X = 4, *Eco*RV; X = 5, *Eco*RV and X = 6, *Eco*RV), and W303-1A was transformed with the linear DNA fragments. Similarly, pGK40X-EGFP was linearized with *Eco*RV (X; 2, 5, 6) or *Bsp*EI (X; 4), and YPH499 was transformed with the linear fragment. All episomal plasmids pGK41X-EGFP, pGKRS42X-EGFP and pGK42X-EGFP (X; 2–6) transformed BY4741, YPH499, and W303-1A, depending on the auxotrophies without the limited enzymatic digestion.

All transformants were grown in the appropriate SD media overnight and were then inoculated into 10 ml of fresh SD media or YPDA media to give an initial optical density of 0.1 at 600 nm. Yeast cells were grown at 30°C

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on a rotatory shaker set at 150 r.p.m. for 24 h in SD media or for up to 48 h in YPDA media. Cultured cells were harvested for use in the experiments.

FCM Analysis—The GFP expression levels were analysed by a flow cytometer (FACSCanto II; Becton Dickinson and Co., Franklin Lakes, NJ, USA). A total of 10,000 cells were analysed for each transformant. The green fluorescence signal was collected through a 530/30-nm band-pass filter, and the GFP fluorescence intensity was defined as the GFP-A (green fluorescence signal) mean of 10,000 cells. P1 and P2 regions were determined for separating the lower fluorescence cluster (P1) and the higher fluorescence cluster (P2). P1 cell clusters were defined as GFP-non-expressing cells, and P2 cell clusters were defined as GFP-expressing cells.

Measurement of Plasmid Retention Rate—Cells were prepared to give an initial optical density of 1.0 at 600 nm and were diluted by 100,000-fold with distilled water. Diluted cell suspensions (800 μl) were respectively spread on three sets of YPDA plates and appropriate auxotrophic SD plates and then incubated 30°C for 2–3 days. Plasmid retention rates were determined as the number of colonies on SD plates divided by the number on YPDA plates.

RESULTS AND DISCUSSION

A Set of pGK Vectors and S. cerevisiae Strains as a Model for Evaluating Yeast Expression Systems—Yeast pRS vector series were categorized into genomic integration types (pRS40X, single-copy series), episomal plasmid types with *CEN/ARS* origins (pRS41X, single-copy series) and episomal plasmid types with 2 μ origins (pRS42X, multi-copy series). These vectors possess five types of selectable markers (X = 2, *ADE2*; X = 3, *HIS3*; X = 4, *TRP1*; X = 5, *LEU2*; X = 6, *URA3*) (*3*, *7*). To demonstrate the usefulness of our method with GFP and FCM, we constructed a set of yeast expression vectors controlled under the *PGK1* promoters (pGK vector series) from the pRS vector series (Table 1 and Fig. 1). The single-copy pGK vectors were designated as pGK40X (integration types) and pGK41X (*CEN/ARS* types), and two types of multi-copy pGK vectors (2 μ types) were prepared. In addition to pGKRS42X based on the original pRS42X vectors, which possess 1345 bp of polynucleotides containing 2 μ origins, the comparable vectors carrying long forms of 2 μ sequences (2234 bp of polynucleotides) were constructed by inserting the 2234 bp of polynucleotides amplified from pWI3 (*9*) into the pRS40X vectors (pGK42X). Both lengths of 2 μ sequences were derived from YEp24 (*11*) and include inverted repeat (IR) sequences (*12*); but, the entire lengths of the genes coding for Flp1, Rep1, Raf1 and Rep2 proteins were not included (*Saccharomyces* Genome Database; http://www.yeastgenome.org/). *EGFP* genes were inserted into all pGK vectors, and these pGK vectors for GFP expression were introduced into three typical *S. cerevisiae* laboratory strains (BY4741, YPH499 and W303-1A) (Table 1 and Fig. 1). Four auxotrophic alleles (*his3Δ1*, *leu2Δ0*, *met15Δ0* and *ura3Δ0*) of BY4741 and *his3-Δ200* alleles of YPH499 eliminate the homologies to selectable markers of pRS vectors and

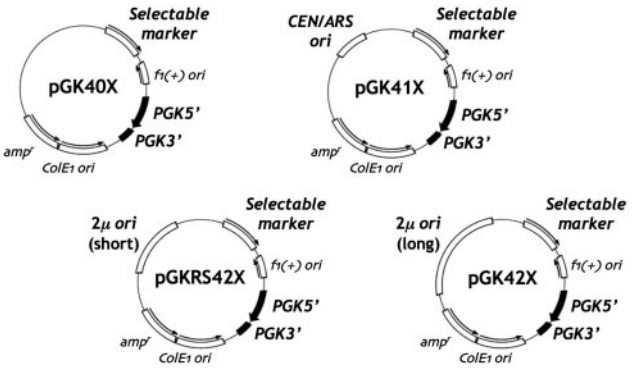

Fig. 1. **Illustration of a series of pGK expression vectors.** Each vector possesses the *ADE2* marker (X = 2), *HIS3* marker (X = 3), *TRP1* marker (X = 4), *LEU2* marker (X = 5) or *URA3* marker (X = 6). The short form of 2 μ *ori* (1345 bp) originates from pRS42X, and the long form of 2 μ *ori* (2234 bp) is derived from pWI3. *PGK5′* indicates the *PGK1* promoter, and *PGK3′* indicates the *PGK1* terminator. The *EGFP* gene was inserted into each vector and designated as pGK(RS)4XX-EGFP.

are unavailable for genomic integrations (Table 1). All transformants were grown in the SD selectable media for 24 h, and the 10,000 cells were analysed on FCM.

Simple Evaluations of Expression Levels by Analysing GFP Fluorescence Intensities with FCM—To evaluate the expression levels of pGK vectors carrying various origins or selectable markers, we determined the mean intensity of GFP fluorescence per cell in BY4741 (Fig. 2A). The expression levels of multi-copy vectors were ~5–7-fold higher than those of single-copy vectors. There were no differences in expression levels caused by the lengths of 2 μ *ori*. The marker-dependent expression levels were: *URA3* > *LEU2* > *HIS3*. To compare the expression levels of host strains, we evaluated YPH499 and W303-1A (Fig. 2B and C). In the case of YPH499, the expression levels of multi-copy vectors were about 5–25-fold higher than those of single-copy vectors as also observed for BY4741, suggesting that the number of gene replications profoundly influences the expression levels (Fig. 2A and B). However, comparing the lengths of 2 μ sequences in YPH499, the short 2 μ vectors showed somewhat higher expression levels (Fig. 2B). The marker-dependent expression levels were moderately accommodated as follows: *URA3* (approximately equal to *ADE2* and *TRP1*) > *LEU2* > *HIS3*. In contrast, the expression levels of W303-1A were dissimilar to those of BY4741 and YPH499 (Fig. 2C). Both the long and short forms of 2 μ vectors in W303-1A had expression levels that were similar to single-copy vectors. The relatively low expression levels of 2 μ vectors in W303-1A correspond to the previous report of the expression of the ZZ domain derived from *Staphylococcus aureus* protein A by the *GAL1* promoter (*13*). As the result of analysing all 51 types of transformants containing GFP expression plasmids carrying the *PGK1* promoters and several varieties of selectable markers and replicative origins (empty yeasts, 10 types of BY4741 transformants; 20 types of YPH499 transformants; 21 types of W303-1A transformants, Fig. 2), BY4741 harbouring pGK426-EGFP (long 2 μ *ori* and *URA3* marker) exhibited the

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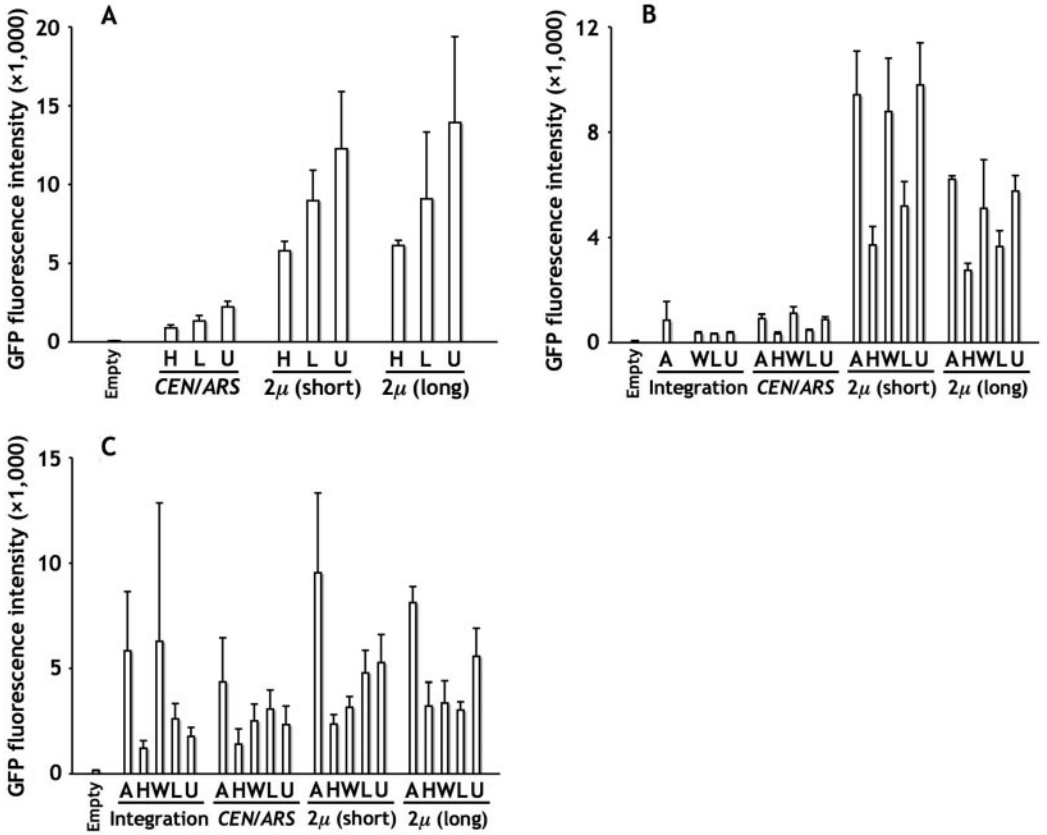

Fig. 2. **GFP fluorescence intensities.** All transformants were grown in SD selectable media for 24 h. The mean value of the green fluorescence signal of 10,000 cells is displayed. Error bars show the standard deviation of three different transformants. Each auxotrophic selectable marker is indicated by a single character code: A, *ADE2*; H, *HIS3*; W, *TRP1*; L, *LEU2*; U, *URA3*. (A) BY4741 (B) YPH499 (C) W303-1A.

highest expression level. Previous reports have identified BY4741 or BY4742 as the host strain displaying the highest expression level in the expression of the ZZ domain or the Fc part of human immunoglobulin G (*13*, *14*); these results support the validity of our method that uses GFP and FCM to quantitatively evaluate and compare the expression systems in yeast.

The evaluations of expression levels in yeasts are usually performed by *lacZ* or *luc* reporter assays. However, these enzymatic reactions require complex procedures, such as the extraction of cell contents (containing enzyme), addition of agents (buffer and substrate) and accurate measurement of reaction time. FCM can rapidly, quantitatively and individually measure the fluorescence of a given number of cells and requires only the harvesting, re-suspension and measurement of the cultivated cells. Thus, our method that uses GFP and FCM allows for the relatively simple and quick quantitative evaluation of the expression level per cell.

Immediate Estimations of Plasmid Retention Rates by Analysing GFP-Expressing Populations with FCM—Next, we analysed the populations of cells expressing GFP by utilizing the ability of FCM, which measures the fluorescence intensity at the single-cell level. As shown in the histogram plots, the 10,000 cells were divided into two cell clusters (as typically seen in cells carrying 2μ vectors, Fig. 3 and Supplementary Fig. 1). We defined the cell cluster displaying higher fluorescence as the GFP-expressing cells (Fig. 3 and Supplementary Fig. 1, P2 regions), and we examined the populations of GFP-expressing cells included in the 10,000 cells (Fig. 4). In *GFP*-integrated cells (YPH499/pGK40X, X = 2, 4, 5 and 6; W303-1A/pGK40X, X = 2–6), the populations of GFP-expressing cells were nearly 10,000 cells (almost 100% of the cells) (Fig. 4B and C). For cells carrying the *CEN/ARS* vectors, there were approximately 8,000–9,000 GFP-expressing cells (80–90% of cells) in all strains (Fig. 4A–C). In BY4741 and YPH499, although the long forms of 2μ vectors showed similar GFP-expressing populations to the *CEN/ARS* vectors, the GFP-expressing populations of short 2μ vectors were slightly lower than those of long 2μ vectors (Fig. 4A and B). However, the populations of GFP-expressing cells of W303-1A carrying 2μ vectors were extremely low. The small population of GFP-expressing cells corresponded to their low expression levels, suggesting that the 2μ vectors are unfavourable for W303-1A (Figs 2C and 4C). This obvious decay in W303-1A may be caused by the natural 2μ circular yeast plasmids, which are present in nearly all strains of *S. cerevisiae* including laboratory strains (*12*), although BY4741 and YPH499 would have been cognate with the strains lacking natural 2μ circular plasmids for the utilization of artificial

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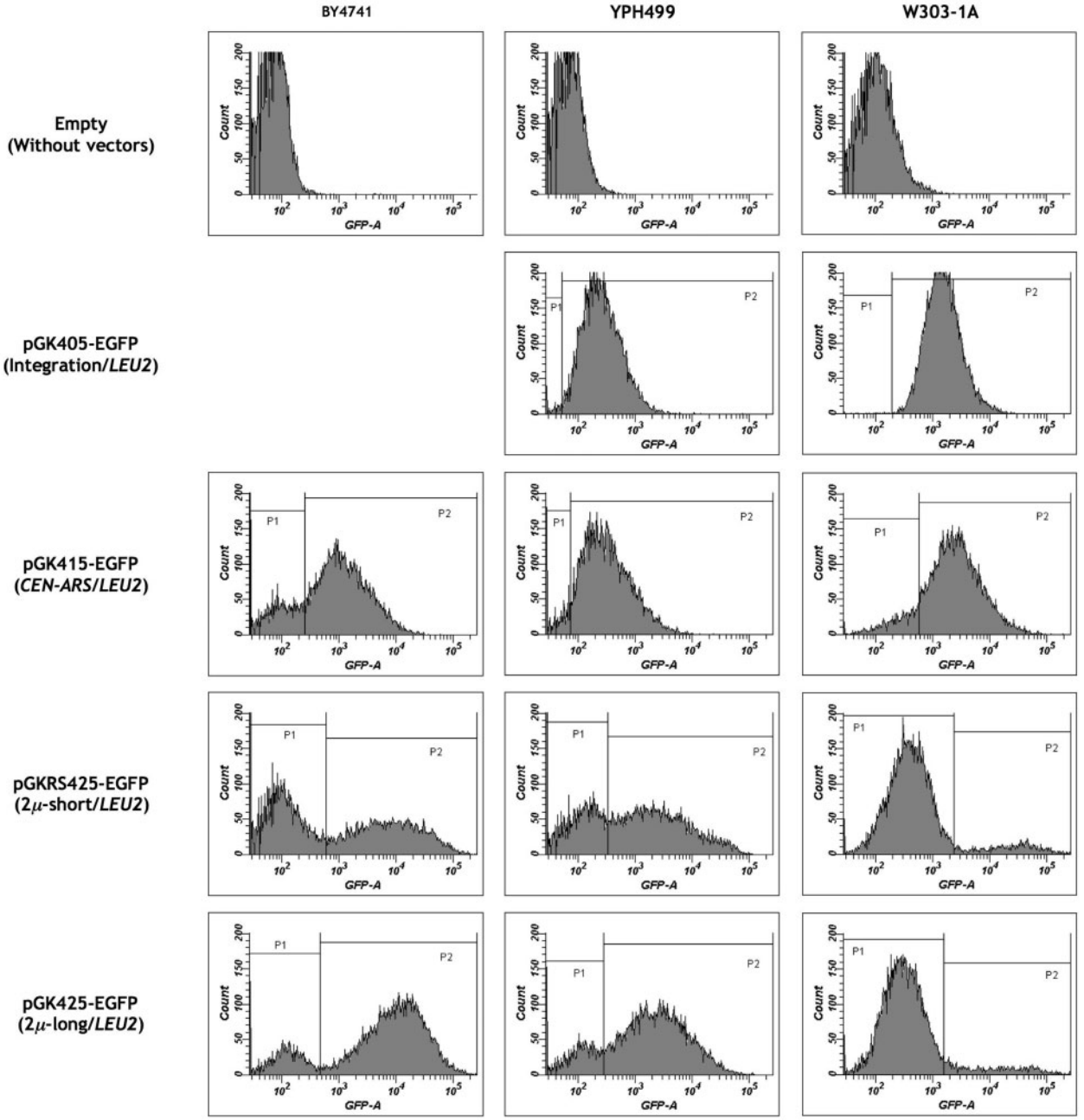

Fig. 3. **Histogram plots of FCM analyses measuring GFP expression.** All transformants were grown in SD selectable media for 24 h. Each histogram contains data from 10,000 cells. The vertical axis indicates the number of cells, and the horizontal axis indicates the green fluorescence signal (GFP-A). The P1 and P2 regions were determined for separating the lower fluorescence cluster (P1) and the higher fluorescence cluster (P2). P2 cell clusters were defined as GFP-expressing cells. BY4741, YPH499 and W303-1A were used as the host strains to evaluate a set of vectors expressing GFP under the control of the *PGK1* promoter. For instance, the histograms of empty yeasts and *LEU2* selectable transformants are exhibited (all data are shown in Supplementary Fig. 1). Three different transformants were analysed, and one typical histogram is displayed.

high-copy 2 μ plasmids (*6*, *7*). In response to the dramatically different results of GFP-expressing populations in W303-1A carrying 2 μ vectors, we assumed that the proportions of GFP-expressing cells were linked to the plasmid maintenance.

To verify our assumption, we examined the plasmid maintenance by colony counting on YPDA plates and SD selectable plates. Figure 5 shows the plasmid retention rates of all transformants. In *GFP*-integrated cells, plasmid retention rates of YPH499 and W303-1A were almost 100% (Fig. 5B and C). The *CEN*/*ARS* vectors retained ~80% of plasmids in all strains (Fig. 5A–C). While the plasmid retention rates of long 2 μ vectors were similar to those of *CEN*/*ARS* vectors, short 2 μ vectors displayed slightly lower plasmid retention rates than *CEN*/*ARS* and long 2 μ vectors in BY4741 and

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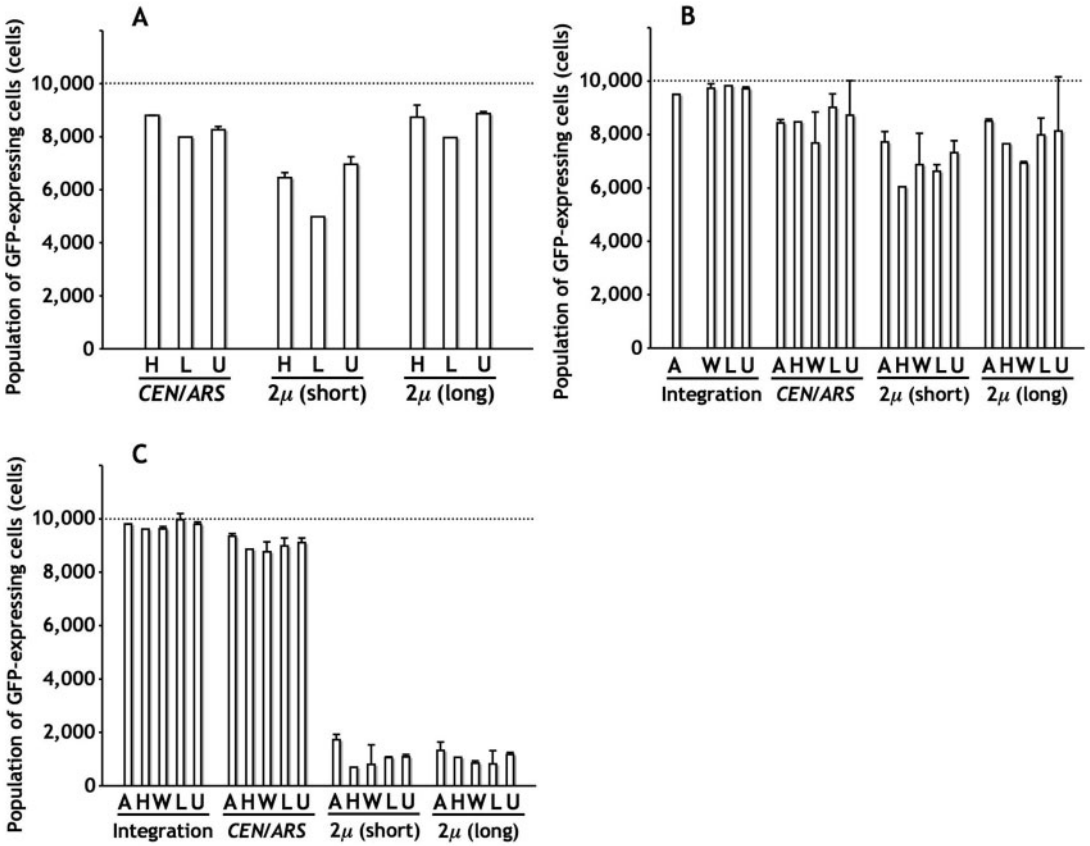

Fig. 4. **Populations of GFP-expressing cells.** All transformants were grown in SD selectable media for 24 h. The number of cells in the P2 regions (as shown in Fig. 3 and Supplementary Fig. 1) are displayed. Error bars indicate the standard deviation of three different transformants. Each auxotrophic selectable marker is indicated by single character code: A, *ADE2*; H, *HIS3*; W, *TRP1*; L, *LEU2*; U, *URA3*. (A) BY4741 (B) YPH499 (C) W303-1A.

YPH499 (Fig. 5A and B). In contrast, the plasmid retention rates of W303-1A carrying 2 μ vectors were extremely low (Fig. 5C). As a result, the level of plasmid maintenance was: integration vectors (pGK40X) > *CEN/ARS* vectors (pGK41X) or 2 μ long vectors (pGK42X) > 2 μ short vectors (pGKRS42X). However, the expression levels of single-copy vectors were lower than those of multi-copy vectors (Fig. 2), and multi-copy 2 μ vectors were therefore suitable for the transiently large amount of protein production. These plasmid retention rates grown in the SD selectable media were remarkably consistent with the patterns of GFP-expressing populations by FCM analyses (Figs 4 and 5), and they correspond reasonably well to the previous studies of the stabilities of pRS vectors (*3*). For additional validation, BY4741 harbouring 2 μ short vector (pGKRS425-EGFP) and 2 μ long vector (pGK425-EGFP) were cultivated in YPDA media and they were monitored by FCM for up to 48 h. Compared to those grown in SD selectable media, the expression levels were low (data not shown) and the GFP-expressing populations were decreased by about 20% (Supplementary Fig. 2). The behaviours were almost similar between the cases of 24 h and 48 h, and the populations were also corresponding to the measurements by colony counting (Supplementary Fig. 2). These results suggest that our method with GFP and FCM permits the immediate and effortless estimation of the plasmid retention rate without requiring the dilution and plating of cultivated cells followed by a 2- or 3-day incubation.

In summary, we demonstrated that the proportion of GFP-expressing cells in the yeast cell cluster reflects the plasmid retention rate, and a simple and immediate method for the simultaneous evaluation of the expression level and plasmid maintenance was established. This method, which uses GFP and FCM, can rapidly quantify the expression level by analysing GFP fluorescence intensity without complex manipulations such as the enzymatic reaction of the *lacZ* reporter assay and can immediately estimate the plasmid retention rate by analysing the population of GFP-expressing cells without 2 or 3 days of incubation (as required for colony counting). Our method allows comprehensive assessments of yeast expression systems and is valuable to establish several expression systems exhibiting characteristic properties in yeasts, although it may not make changes to assure the expressions for all proteins. We are sure that our system is a practical tool for the evaluation of promoters, host strains, selectable markers and replicative origins and can be used to explore the hopeful expression vector and host strain in various yeasts.

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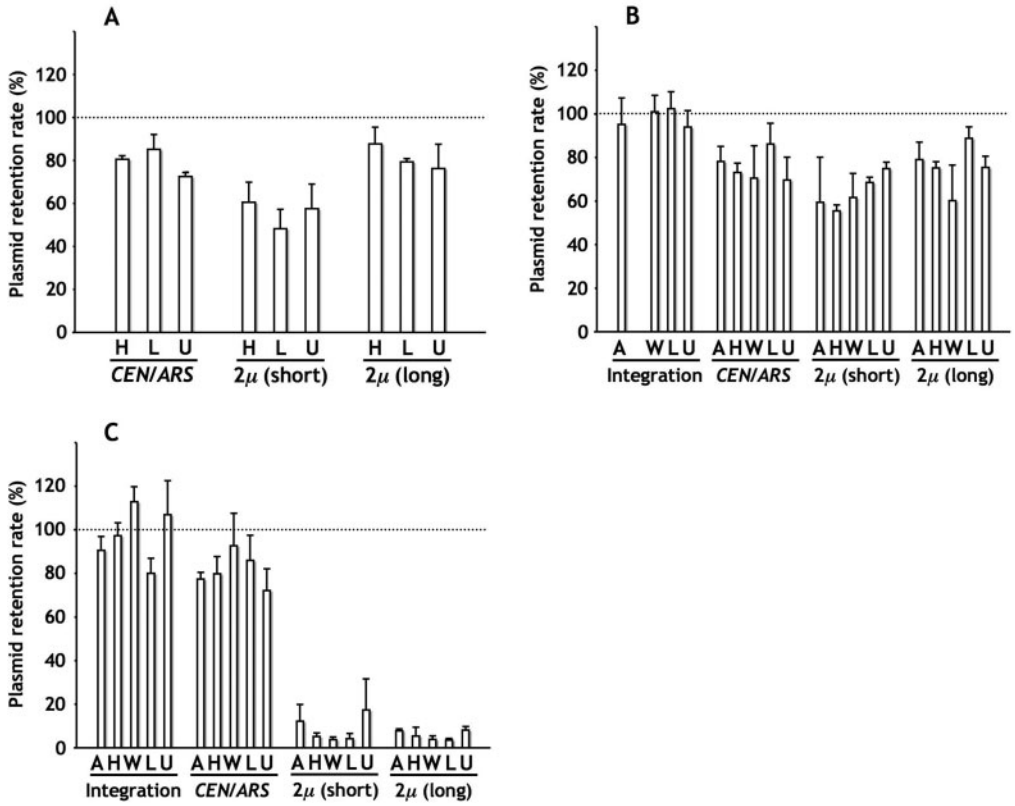

Fig. 5. **Plasmid retention rates measured by colony counting.** All transformants were grown in SD selectable media for 24 h. Error bars indicate the standard deviation of three different transformants. Each auxotrophic selectable marker is indicated by a single character code: A, *ADE2*; H, *HIS3*; W, *TRP1*; L, *LEU2*; U, *URA3*. (A) BY4741 (B) YPH499 (C) W303-1A.

SUPPLEMENTARY DATA

Supplementary data are available at *JB* online.

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CONFLICT OF INTEREST

None declared.

REFERENCES

1. Buckholz, R.G. and Gleeson, M.A. (1991) Yeast systems for the commercial production of heterologous proteins. *Biotechnology (N.Y.)* **9**, 1067–1072
2. Porro, D., Sauer, M., Branduardi, P., and Mattanovich, D. (2005) Recombinant protein production in yeasts. *Mol. Biotechnol.* **31**, 245–259
3. Christianson, T.W., Sikorski, R.S., Dante, M., Shero, J.H., and Hieter, P. (1992) Multifunctional yeast high-copy-number shuttle vectors. *Gene* **110**, 119–122
4. Mumberg, D., Müller, R., and Funk, M. (1995) Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. *Gene* **156**, 119–122
5. Ishii, J., Tanaka, T., Matsumura, S., Tatematsu, K., Kuroda, S., Ogino, C., Fukuda, H., and Kondo, A. (2008) Yeast-based fluorescence reporter assay of G protein-coupled receptor signalling for flow cytometric screening: *FAR1*-disruption recovers loss of episomal plasmid caused by signalling in yeast. *J. Biochem.* **143**, 667–674
6. Brachmann, C.B., Davies, A., Cost, G.J., Caputo, E., Li, J., Hieter, P., and Boeke, J.D. (1998) Designer deletion strains derived from *Saccharomyces cerevisiae* S288C: a useful set of strains and plasmids for PCR-mediated gene disruption and other applications. *Yeast* **14**, 115–132
7. Sikorski, R.S. and 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
8. Thomas, B.J. and Rothstein, R. (1989) Elevated recombination rates in transcriptionally active DNA. *Cell* **56**, 619–630
9. Kanai, T., Atomi, H., Umemura, K., Ueno, H., Teranishi, Y., Ueda, M., and Tanaka, A. (1996) A novel heterologous gene expression system in *Saccharomyces cerevisiae* using the

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isocitrate lyase gene promoter from *Candida tropicalis*. *Appl. Microbiol. Biotechnol.* **44**, 759–765
10. Gietz, D., St. Jean, A., Woods, R.A., and Schiestl, R.H. (1992) Improved method for high efficiency transformation of intact yeast cells. *Nucleic Acids Res.* **20**, 1425
11. Botstein, D., Falco, S.C., Stewart, S.E., Brennan, M., Scherer, S., Stinchcomb, D.T., Struhl, K., and Davis, R.W. (1979) Sterile host yeasts (SHY): a eukaryotic system of biological containment for recombinant DNA experiments. *Gene* **8**, 17–24
12. Hartley, J.L. and Donelson, J.E. (1980) Nucleotide sequence of the yeast plasmid. *Nature* **286**, 860–865
13. Shibasaki, S., Kawabata, A., Ishii, J., Yagi, S., Kadonosono, T., Kato, M., Fukuda, N., Kondo, A., and Ueda, M. (2007) Construction of a novel synergistic system for production and recovery of secreted recombinant proteins by the cell surface engineering. *Appl. Microbiol. Biotechnol.* **75**, 821–828
14. Fukuda, N., Ishii, J., Shibasaki, S., Ueda, M., Fukuda, H., and Kondo, A. (2007) High-efficiency recovery of target cells using improved yeast display system for detection of protein-protein interactions. *Appl. Microbiol. Biotechnol.* **76**, 151–158

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