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Construction of plasmids

e design of the reporter plasmid was based on the pSL6Z vector that integrates into the yeast chromosome at a position upstream of the leu1 gene. e major fragment, including the origin of replication, selectable marker, and lipocortin I terminator (TLPI), was ampli ed from pSL6Z using the primers pSL6Z_F and pSL6Z_R and digested with PstI and SalI. Fragments containing P p1 were ampli ed from the genomic DNA of S. pombe using the primers P p1_F and P p1_R and digested with PstI and NcoI. e open reading frame (ORF) of GFP was ampli ed using GFP_F and GFP_R primers from the Monster Green uorescent protein phMGFP vector (Promega Japan) and digested with NcoI and wen r

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factor dimer Atf1¬–Pcr1. Atf1–Pcr1 binds to UAS1 and de-represses p1 transcription. In glucose-rich conditions, Pka1 activation in the glucose signaling pathway inhibits binding of Atf1–Pcr1 to UAS1. At

at 100 µM glucose was approximately half that at 1 µM glucose, and uorescence was absent at 20 mM glucose. However, the uorescence decrease at 1 µM was not signi cantly di erent. erefore, this signi cant decrease in uorescence intensity at 10 µM glucose indicated that the reporter system can measure a glucose concentration of 10 µM as in Figure 4(A). In shorter time of incubation, 100 µM and 5 mM glucose were detected at 9 hours (B) and 3 hours (C), respectively.

Conclusions

To develop a glucose biosensor, we constructed a hybrid p1–GFP gene fusion reporter for monitoring the expression level of GFP in response to glucose. e method can measure glucose concentrations of about 10 µM, which is su cient to determine a range of glucose levels in human blood, saliva, and tears. e yeast strains developed in this study as a glucose biosensor may also be used as a platform for analyzing the activity of heterologous GPCRs; particularly orphan GPCRs, in future studies.

Con ict of Interest

e authors declared that there is no con ict of interest.

Author Contribution

Kanako Sasai: Methodology, Validation, Formal analysis, Investigation, Writing- Original dra preparation. Sho Hidaka: Methodology. Toshiya Osada: Writing- Review & Editing, Supervision.

References

1. Gether U, Kobilka BK (1998) G protein-coupled receptors. J Biol Chem 273: 17979-17982.
2. Iiri T, Farfel Z, Bourne HR (1998) G-protein diseases furnish a model for the turn-on switch. Nature 394: 35-38.
3. Sprang SR (1997) G protein mechanisms: insights from structural analysis. Annu Rev Biochem 6 5-32.

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