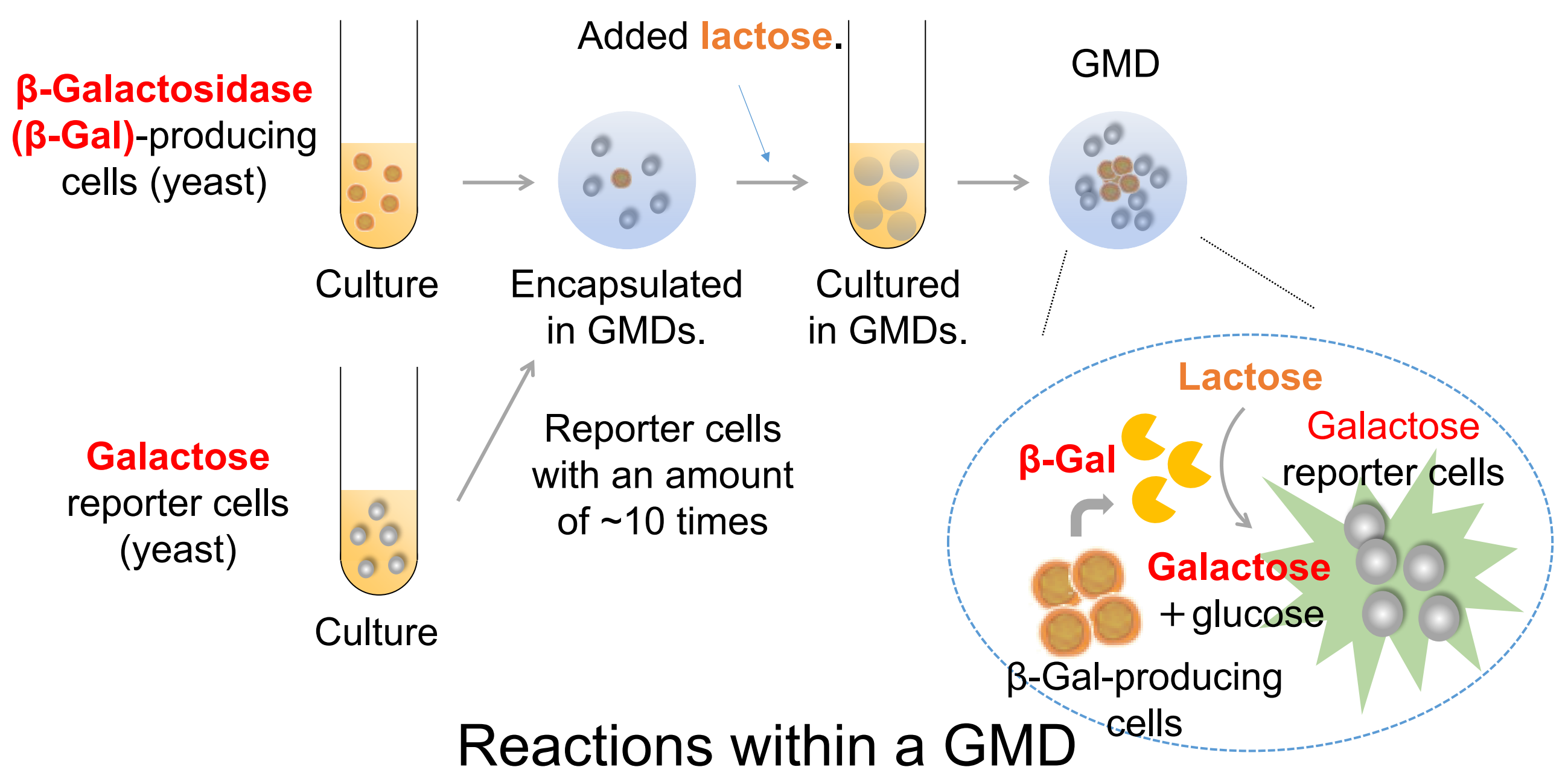
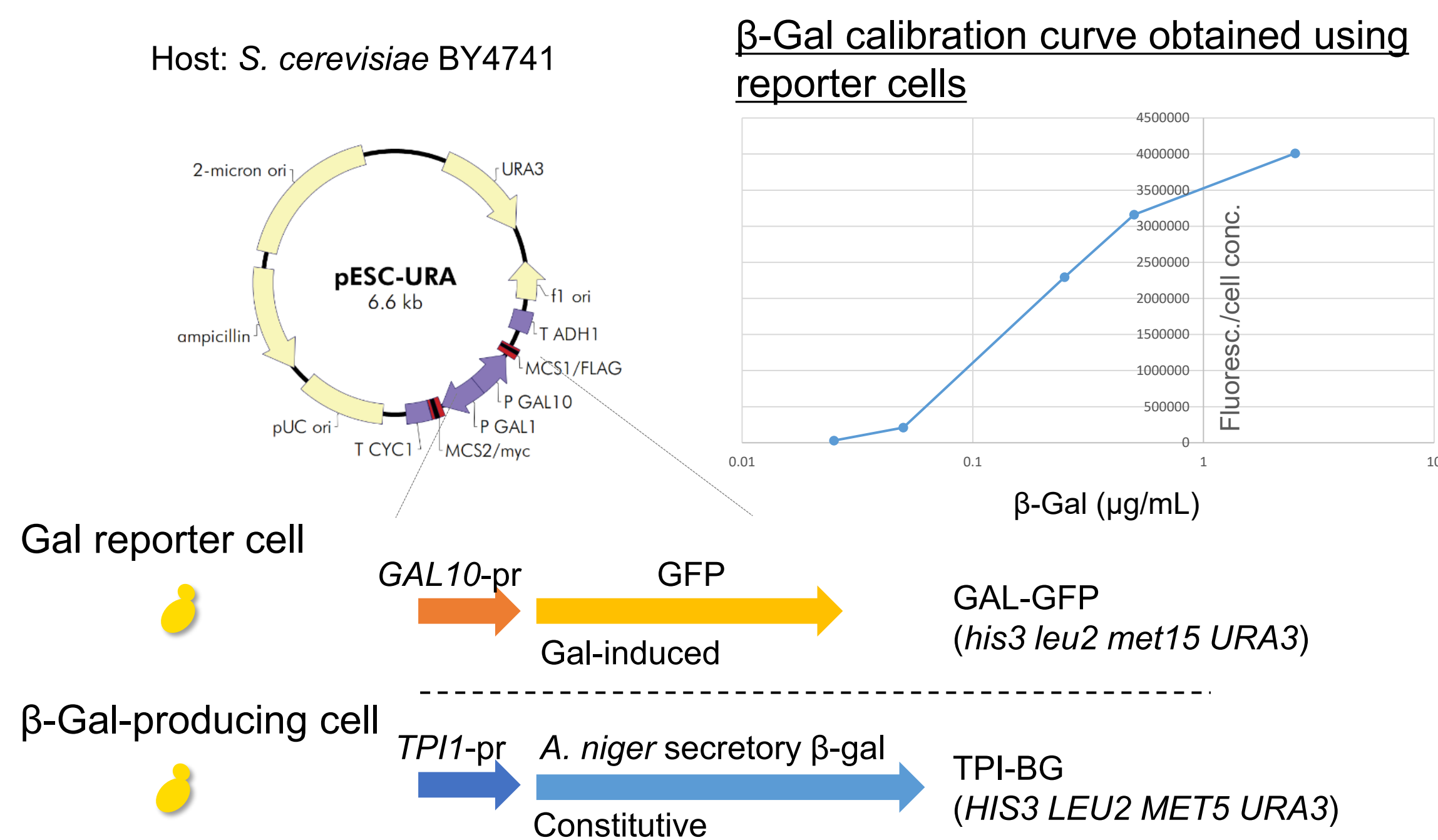


Rapid Screening of Mutant Strains with Improved Productivity Using GMDs

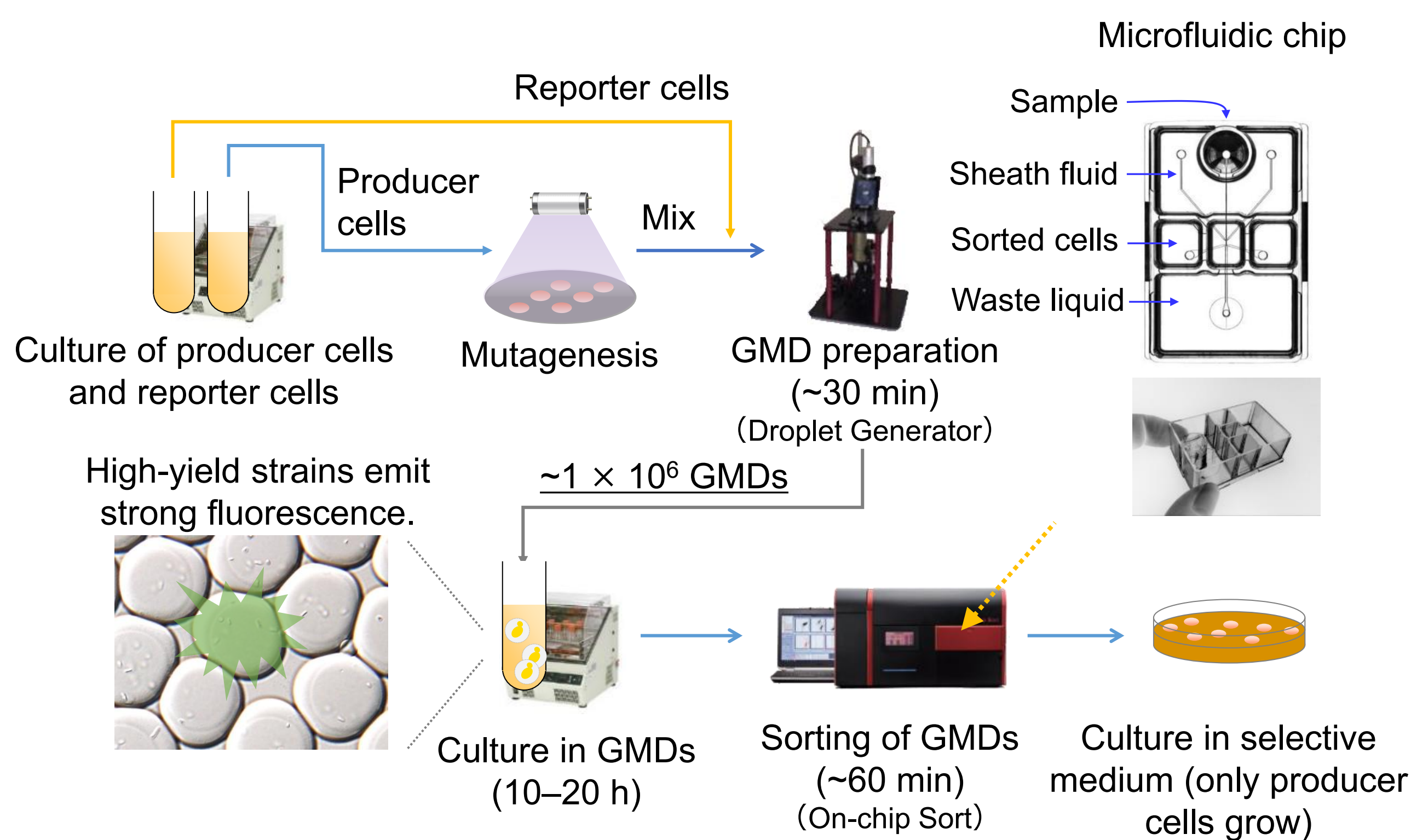
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Abstract: We developed a screening system for *E. coli* and lactic acid bacteria strains with enhanced production of compounds such as tryptophan and GABA. The system uses reporter cells expressing EGFP under the control of compound-inducible promoters to detect the target compounds produced in gel microdrops (GMDs). We also developed a screening system for high-yield strains of β -galactosidase (β -Gal) based on the detection of compounds generated by enzymatic reactions. Random mutations were introduced into the producer strains, which were then mixed with reporter cells and encapsulated the mixture in GMDs. After culture, approximately 3×10^5 GMDs were sorted. Hundreds of candidate strains were obtained and cultured individually. Strains with approximately 2–4-fold increases in yield were obtained with a probability of about 50%.

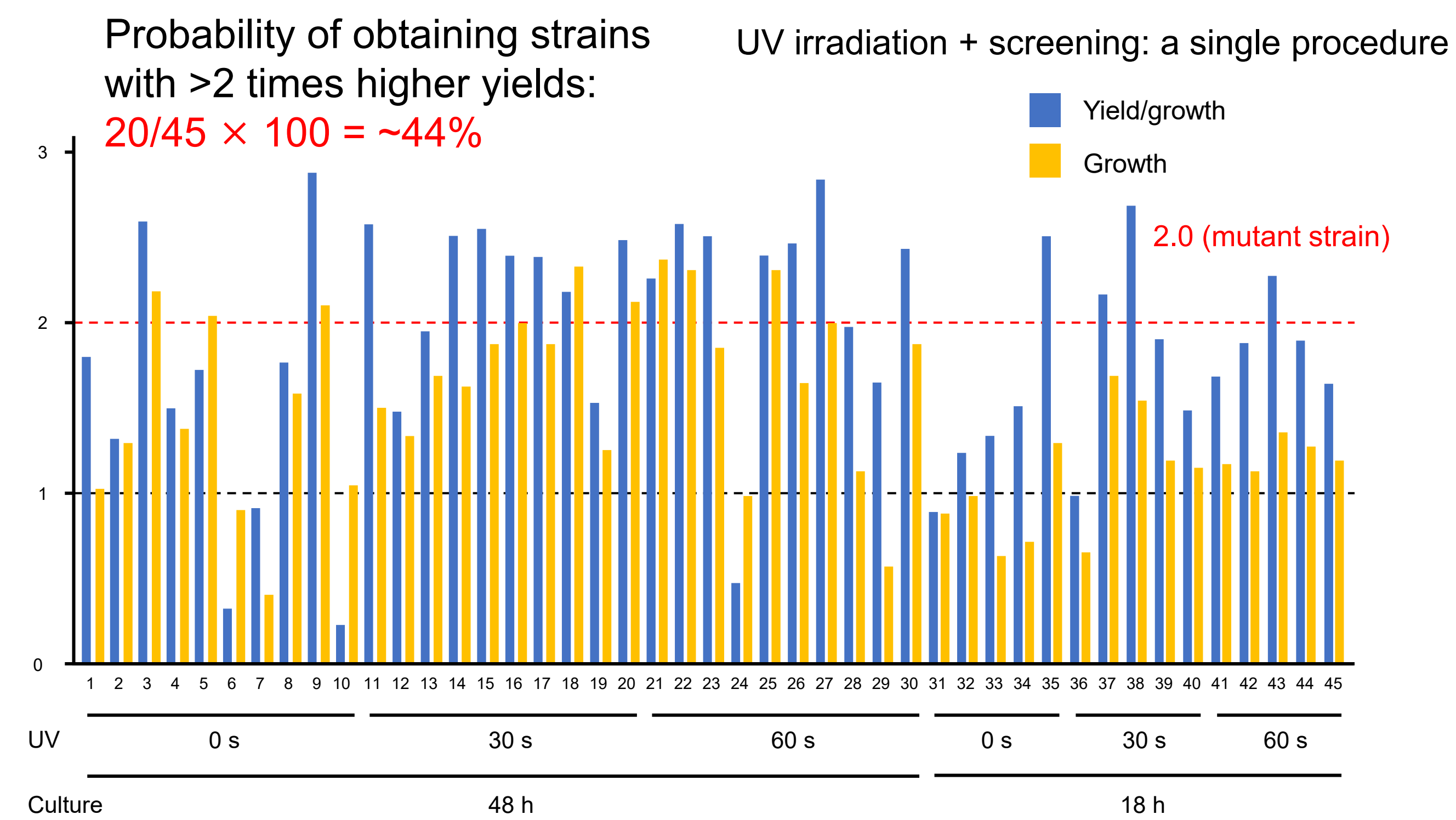
1. Construction of β -Gal-producing cells and Gal reporter cells 2. Detection of β -Gal in GMDs using reporter cells



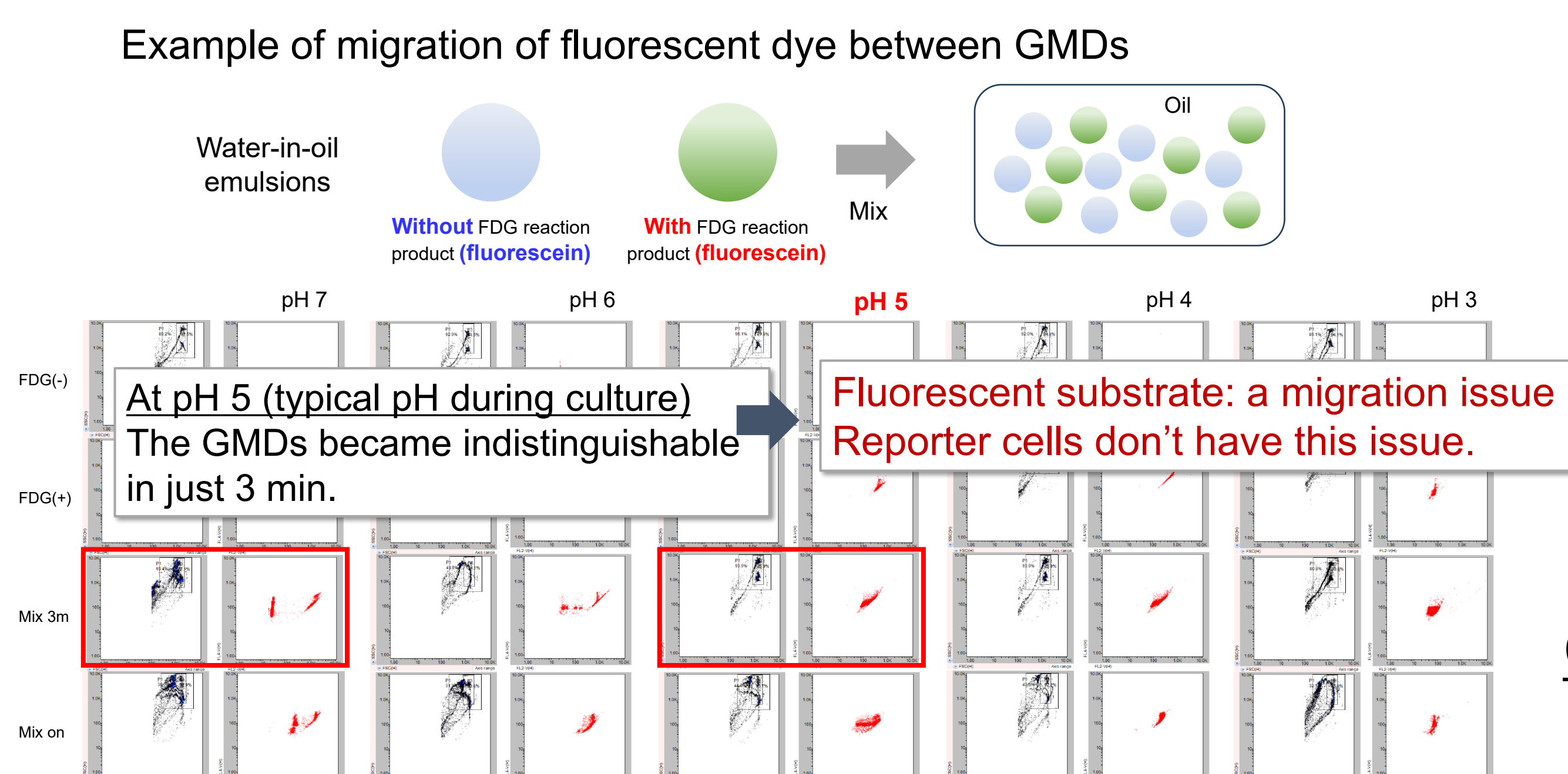
3. Screening procedure using reporter cells



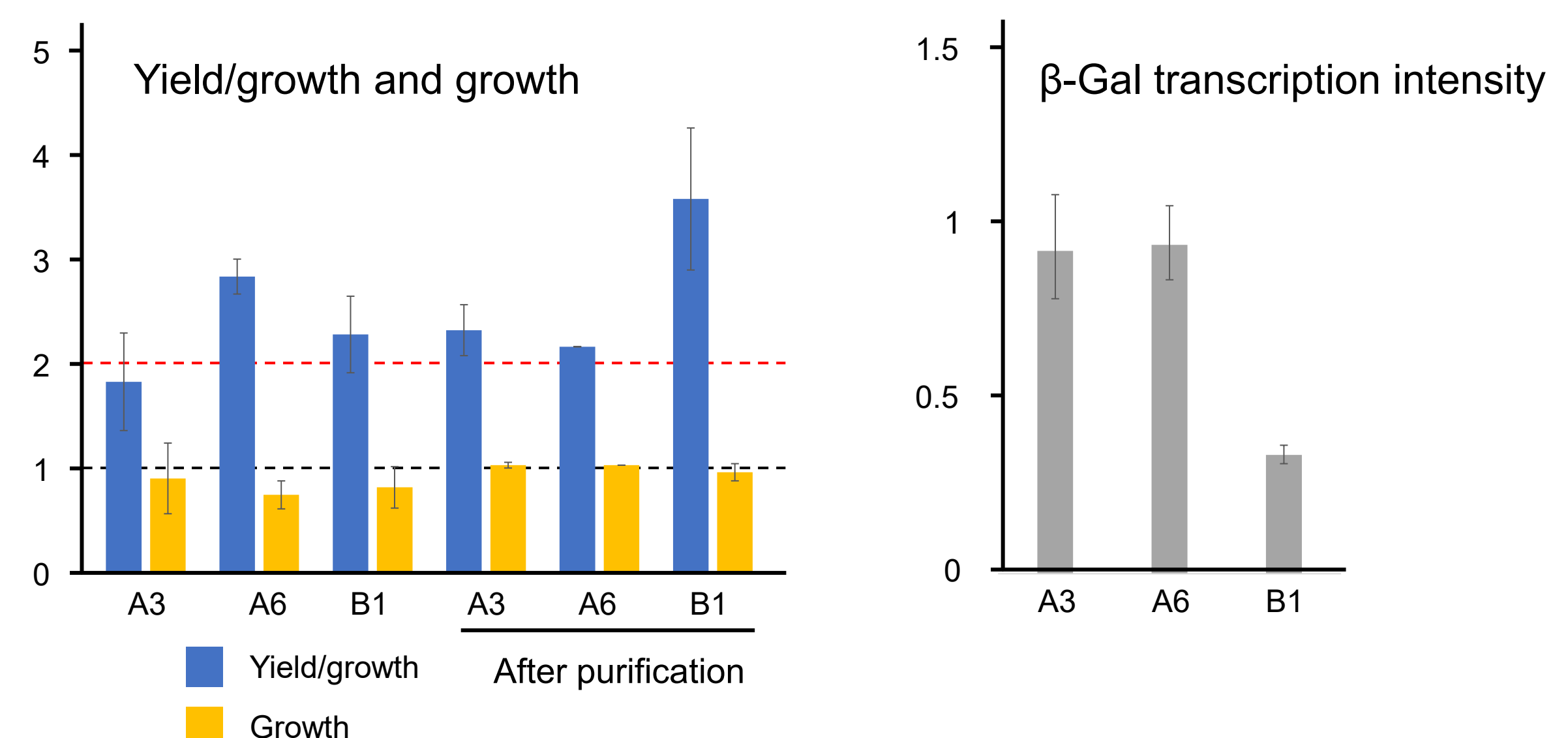
4. Analysis of individual high-yield strains of β -Gal



5. Example of migration of fluorescent dye between GMDs



6. Purification of high-yield cells and transcription analysis



Conclusions

- We obtained mutant strains exhibiting several times higher secretory production within a week.
- Suitable mutant strains can be selected from numerous strains with improved productivity.
- High-performance reporter cells for compounds such as GABA, Trp, and Glc have been constructed (new construction and screening condition evaluation take approximately two months).