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From rational to random approaches, towards the expression of a cryptic gene cluster in *Enterococcus faecalis*

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Context:

- Human microbiota are a promising source of novel antimicrobials, including **Ribosomally synthesized and post-translationally modified peptide** such as lasso peptides
- A putative transcriptionally silent lasso peptide biosynthetic gene cluster (*sat*) was identified in *Enterococcus faecalis*
- The peptide, named **satanycin**, is extremely difficult to produce, preventing its characterization

Objective: activate and enhance *sat* cluster expression in *E. faecalis* to increase satanycin production

Methods:

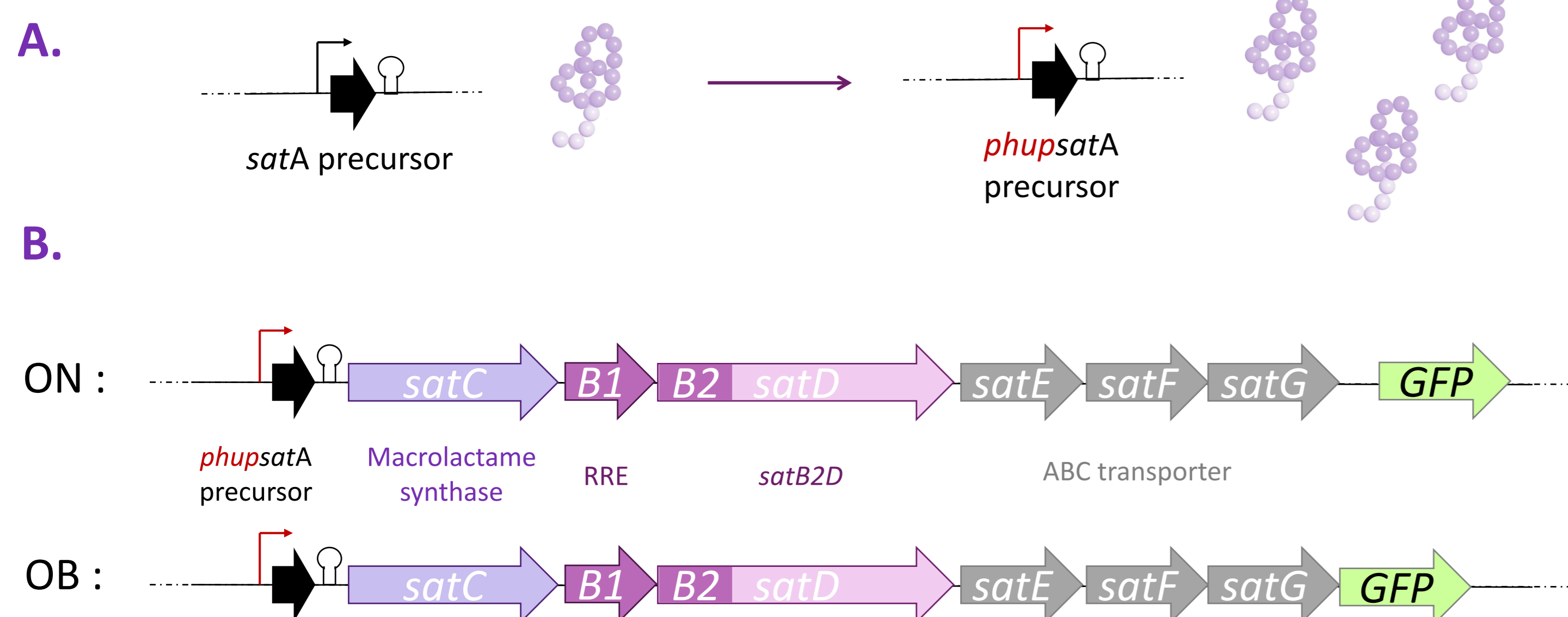


Figure 2. Targeted genetic approach by substituting the native *sat* promoter with the constitutive *hup* promoter (A), transcriptional and translational fusions using GFP (B)

- Green fluorescent protein (GFP) inserted either in operon with *sat* genes (transcriptional fusion, ON strain) or fused to *satG* (translational fusion, OB strain) to reflect *sat* cluster
- Peptide successfully detected, but production levels remained too low to allow for purification

Results:

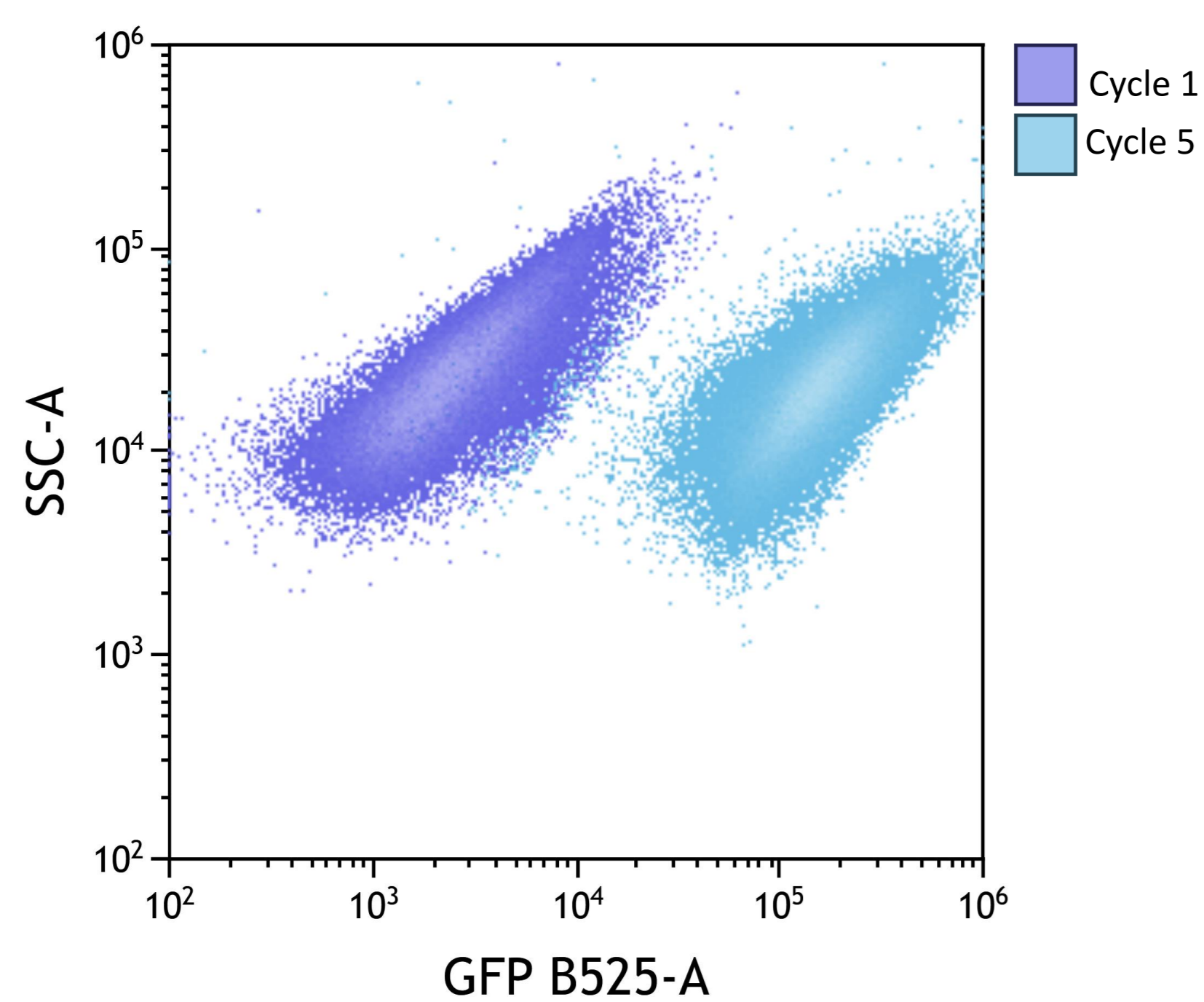


Figure 4. Fluorescence emission scatter plot for the OB2 strain (CytoFLEX SRT, B525-A Channel)

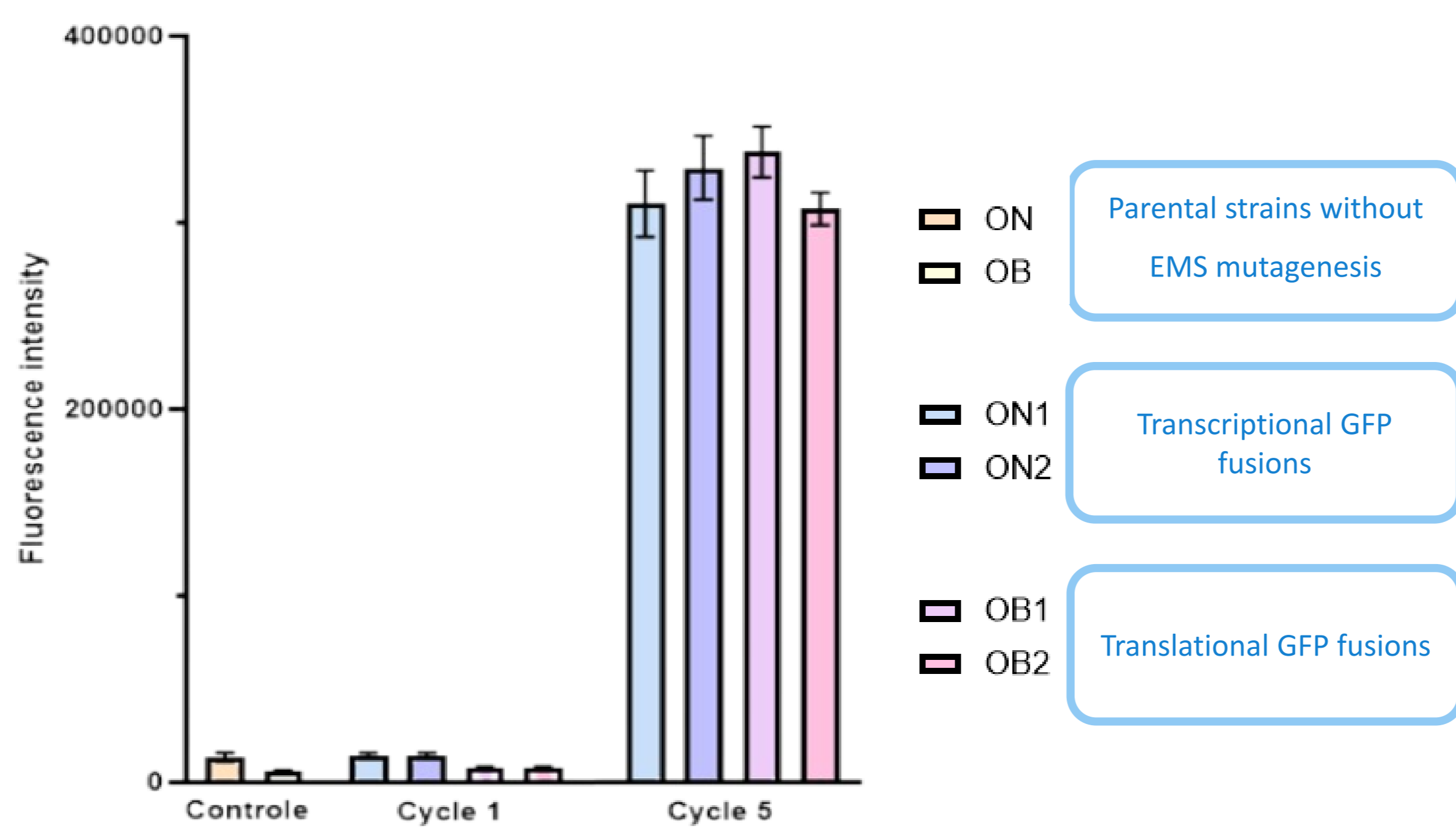


Figure 5. Measurement of GFP fluorescence emission at 515 nm before and after five cycles of mutagenesis and cytometry sorting

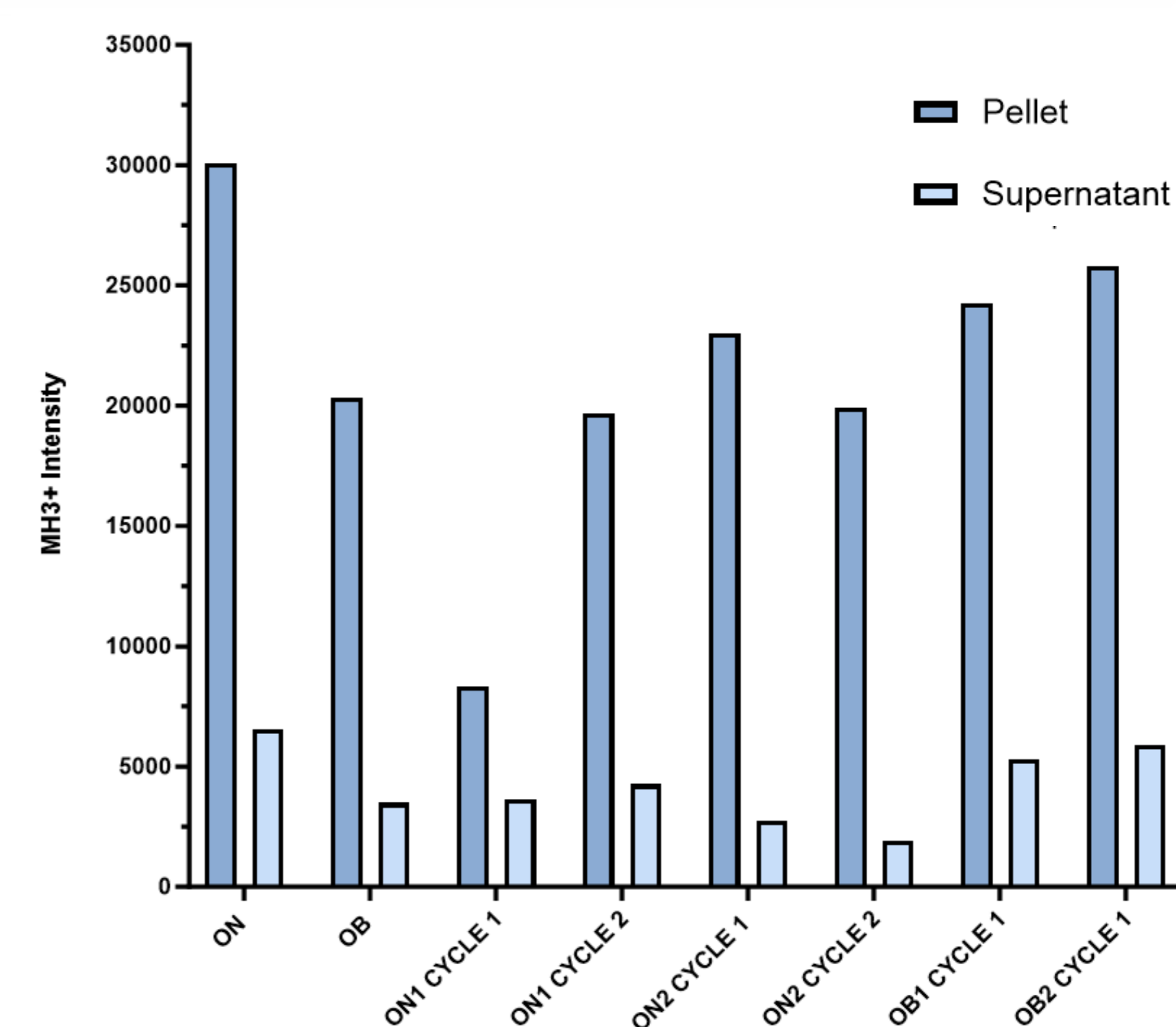


Figure 6. Peptide production levels measured by mass spectrometry

- Significant shift demonstrates increased GFP fluorescence per cell, reflecting the effectiveness of our screening method

- Successive cycles of mutagenesis and fluorescence-based sorting enabled the selection of mutants with enhanced GFP expression and potentially increased satanycin production

- Strains were tested but only a few mutants produced the peptide and production levels are still not sufficient enough to allow for purification

Conclusion:

- Promoter engineering combined with iterative random mutagenesis and fluorescence screening successfully activated and enhanced expression of the cryptic *sat* cluster in *E. faecalis*
- Potential overproducing mutants have been identified, illustrating the value of combining rational and stochastic approaches to activate silent pathways
- Complementary approaches must be considered in order to finally succeed in purifying satanycin and enable its functional characterisation

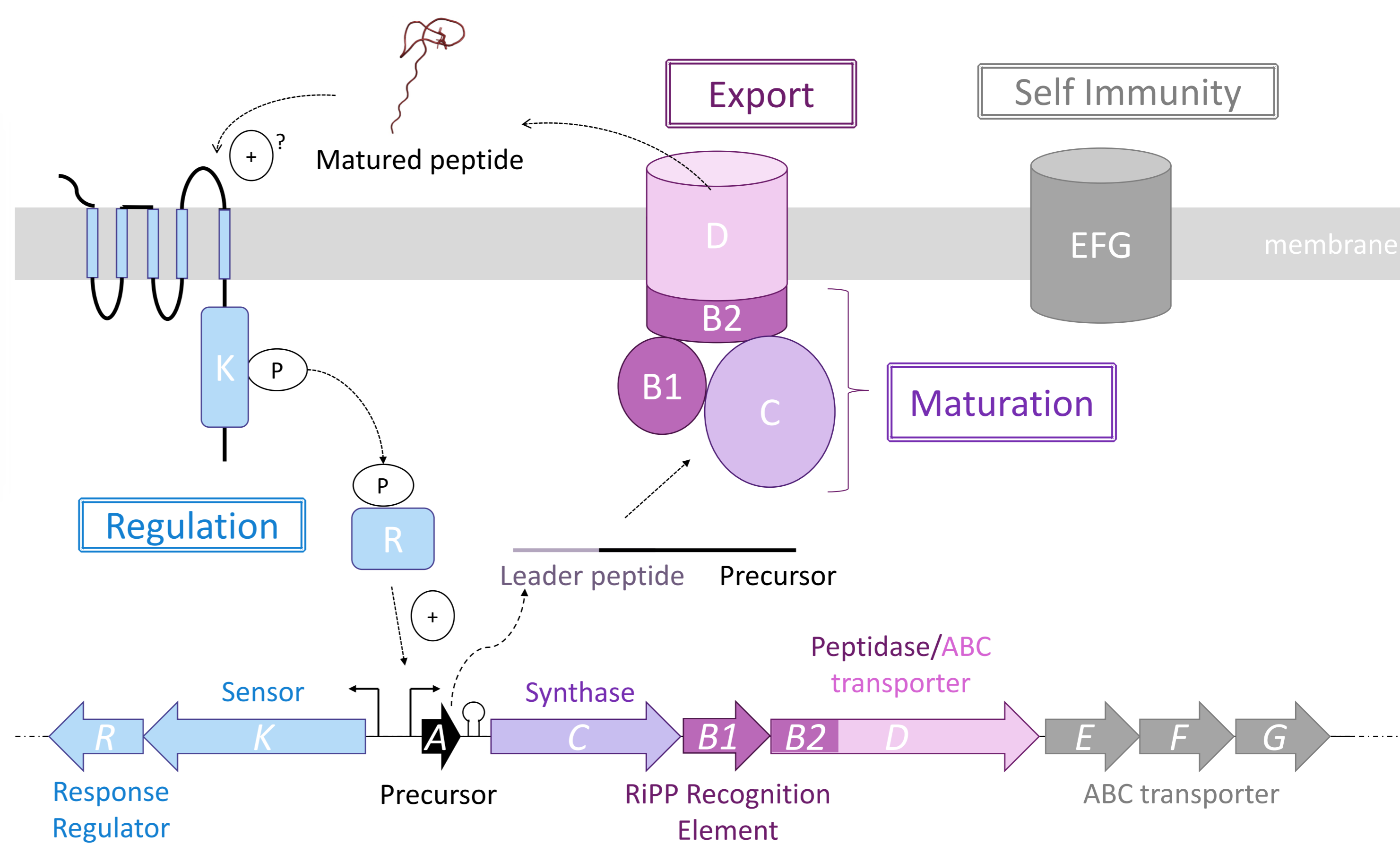


Figure 1. Organization of the satanycin biosynthetic gene cluster and its regulatory system

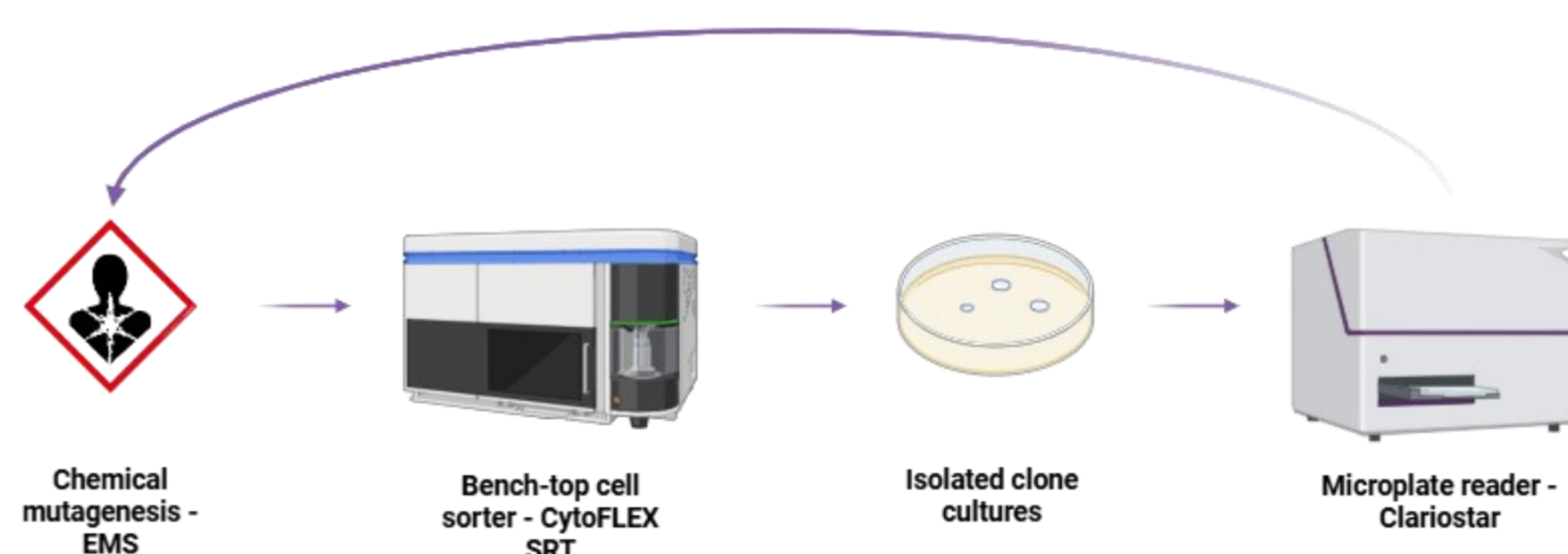


Figure 3. Schematic representation of a mutagenesis cycle coupled with flow cytometry screening based on *sat*-GFP fusion