

- **Title and abstract**
 - Does the title clearly reflect the content of the article? [] Yes, [] No (please explain), [] I don't know
 - Does the abstract present the main findings of the study? [] Yes, [] No (please explain), [] I don't know
- **Introduction**
 - Are the research questions/hypotheses/predictions clearly presented? [] Yes, [] No (please explain), [] I don't know
 - Does the introduction build on relevant research in the field? [] Yes, [] No (please explain), [] I don't know
- **Materials and methods**
 - Are the methods and analyses sufficiently detailed to allow replication by other researchers? [] Yes, [] No (please explain), [] I don't know
 - Are the methods and statistical analyses appropriate and well described? [] Yes, [] No (please explain), [] I don't know
- **Results**
 - In the case of negative results, is there a statistical power analysis (or an adequate Bayesian analysis or equivalence testing)? [] Yes, [] No (please explain), [] I don't know
 - Are the results described and interpreted correctly? [] Yes, [] No (please explain), [] I don't know
- **Discussion**
 - Have the authors appropriately emphasized the strengths and limitations of their study/theory/methods/argument? [] Yes, [] No (please explain), [] I don't know
 - Are the conclusions adequately supported by the results (without overstating the implications of the findings)? [] Yes, [] No (please explain), [] I don't know

The article by Pourcelot E. et al. aims to design yeast microbial communities that represent the diversity of wine fermentation environments. Along with this, the aim is to develop a high-accuracy method to monitor the population dynamics of microbial consortia during the fermentation process. The work fluorescently labels *S. cerevisiae* and 6 non-*Saccharomyces* species. The work is well written and of high quality and shows great rigour in each of the methodologies used. It should be noted that the authors provide the scientific community with five new strains of fluorescently labelled yeast, a valuable material that can be used for future research. In addition, the clarity and quality of each of the scripts provided in the supplementary material is appreciated.

In the abstract it is mentioned that mixed fermentations were performed with two sugar concentrations (200 and 280 g/L). What is the result of this experiment. Is it relevant? If so, mention the result; if not, eliminate it from the abstract.

Eliminate sentence (L33-35) and change it to one that starts from a positive perspective. This helps and motivates the reader to continue reading.

The introduction makes clear the need to explore population dynamics and the role of microbial diversity in the wine fermentation process. The use of differential cultures, qPCR and metabarcoding are mentioned as examples. However, no clear examples are given as to

why the use of flow cytometry is superior to the techniques mentioned above. Clear arguments or examples should be added as to why this experimental strategy was selected.

This will give value and weight to the article, motivating researchers to use this experimental design over others. Again, I consider this to be possible only due to the clarity of the methodology presented in the paper.

Across the text, some words present typing errors (e.g. L89, L190, this could be due to a format issue in the platform).

Include at the bottom of Table 1 the meaning of the acronym CIRM and SPO.

L113- Add the full name of the TDH3 gene.

In supplementary figure 1, given the low variability of the measurements of auc, k, and r, it is not possible to differentiate the colors of the boxplots. Add in the figure caption meaning of D6, B8, A1, BA1 and C3.

Figure 1 shows how to separate each of the strains according to the different markers incorporated. What was the inoculation ratio in the example set up?

In the fermentations, 6 ml (scaled) is collected per time point and 1,5 ml in the 250, does this volume affect the fermentation itself?