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# Application of flow cytometry based cell sorting for the analysis of *Lactococcus lactis*

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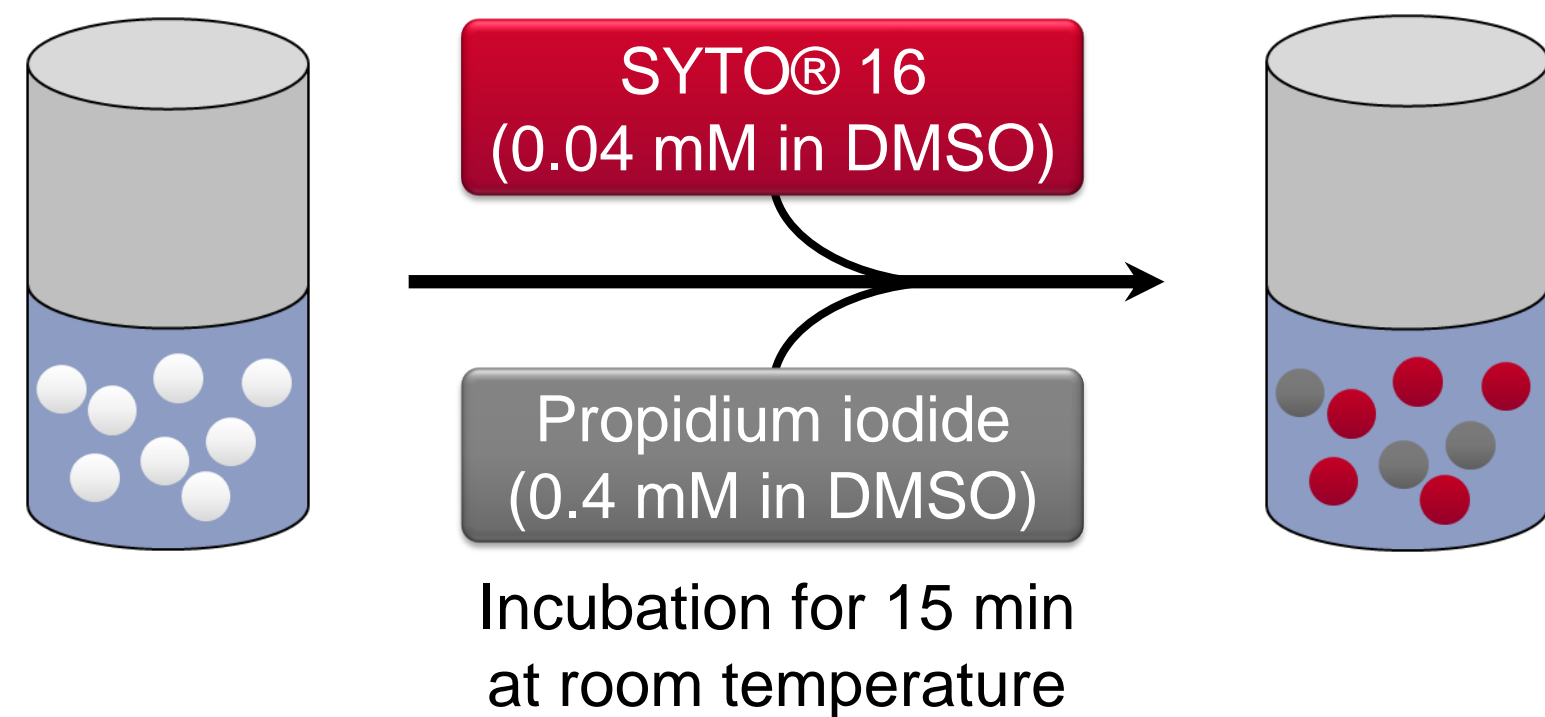
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## Flow cytometry based cell sorting

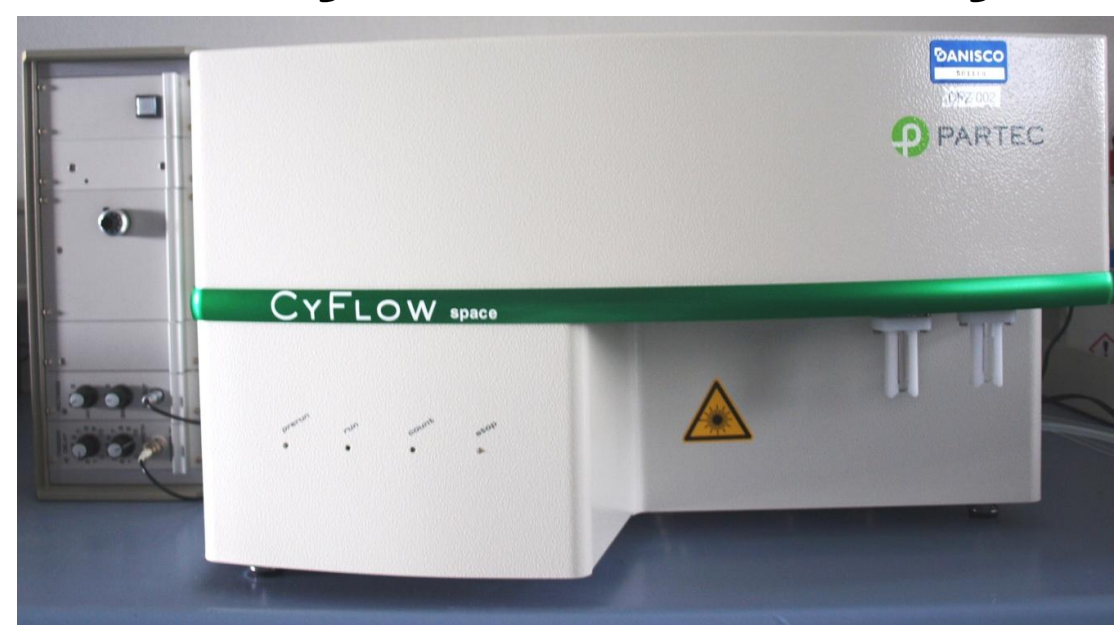
### Staining of cells

Prediluted sample in sodium chloride solution

*Lactococcus lactis* subsp. *cremoris*

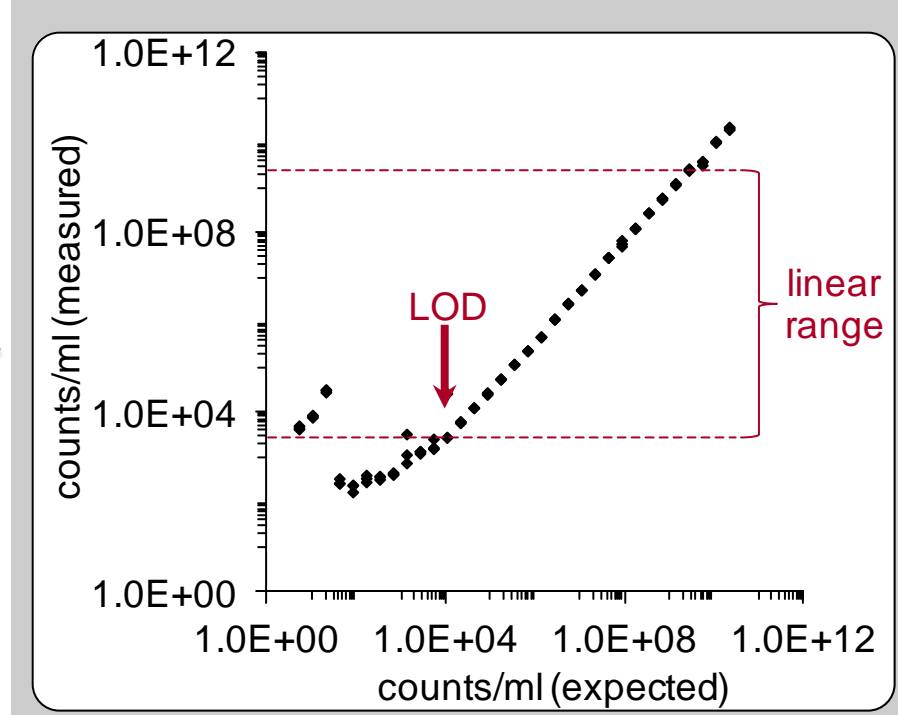


### Flow cytometric analysis

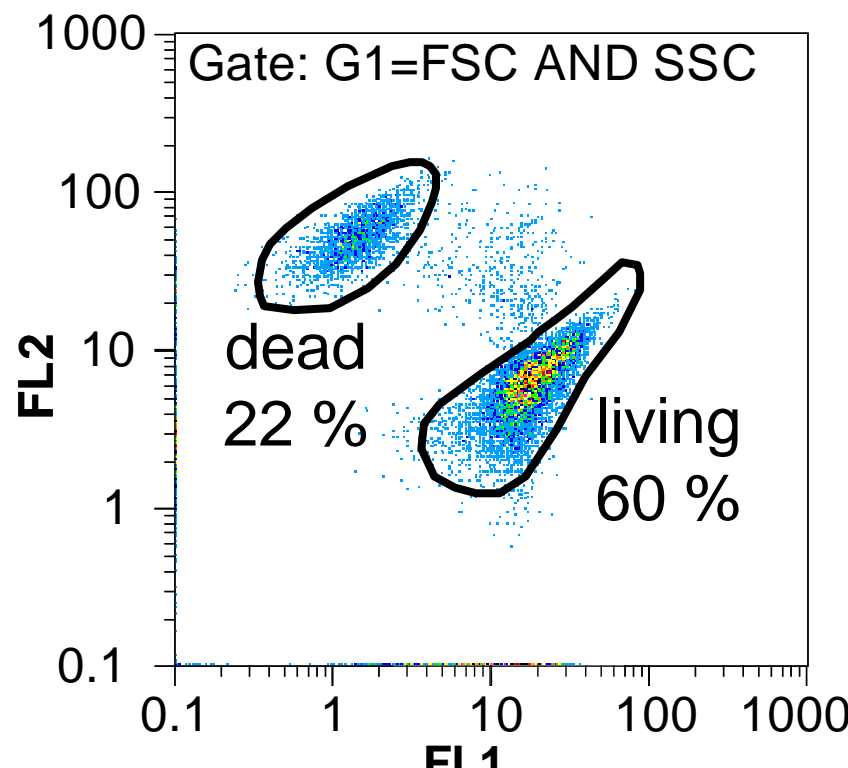


CyFlow® Space + Particle and Cell Sorter

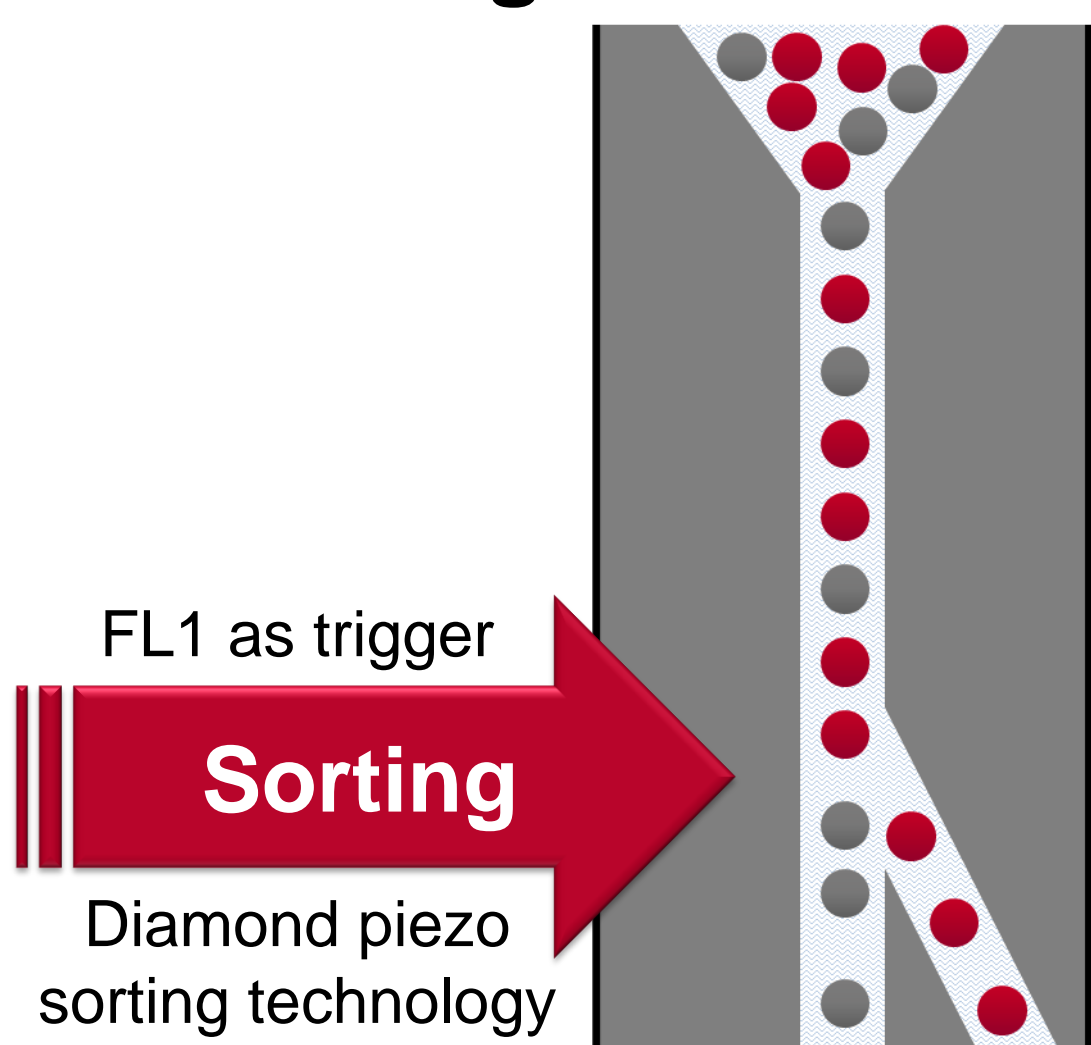
Limit of detection for cells



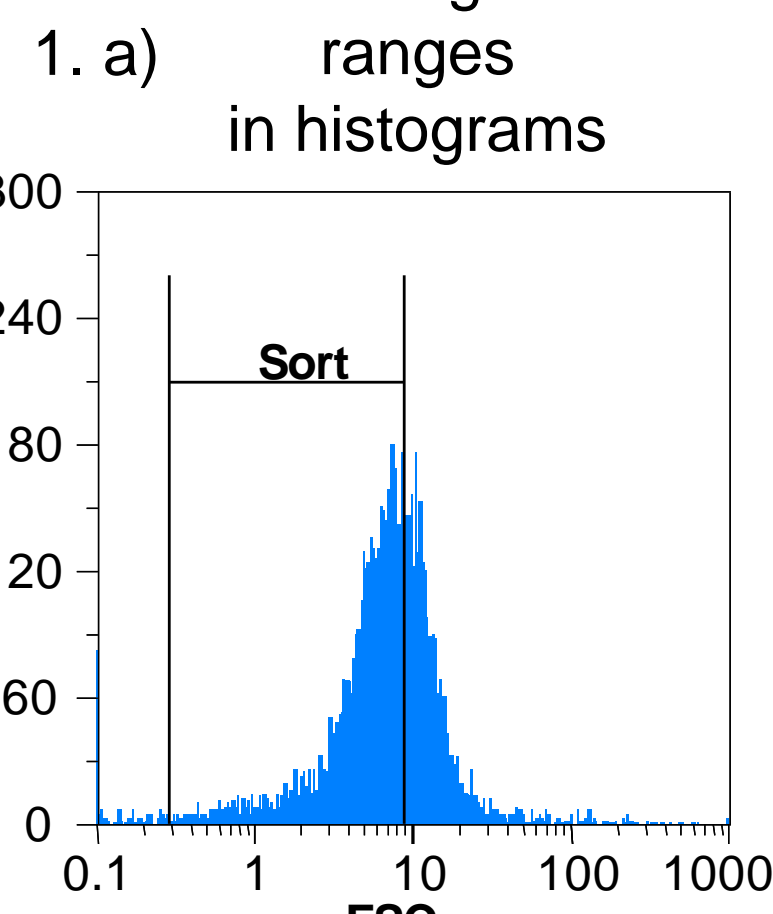
Staining with SYTO® 16, 4 µl/s



### Cell sorting

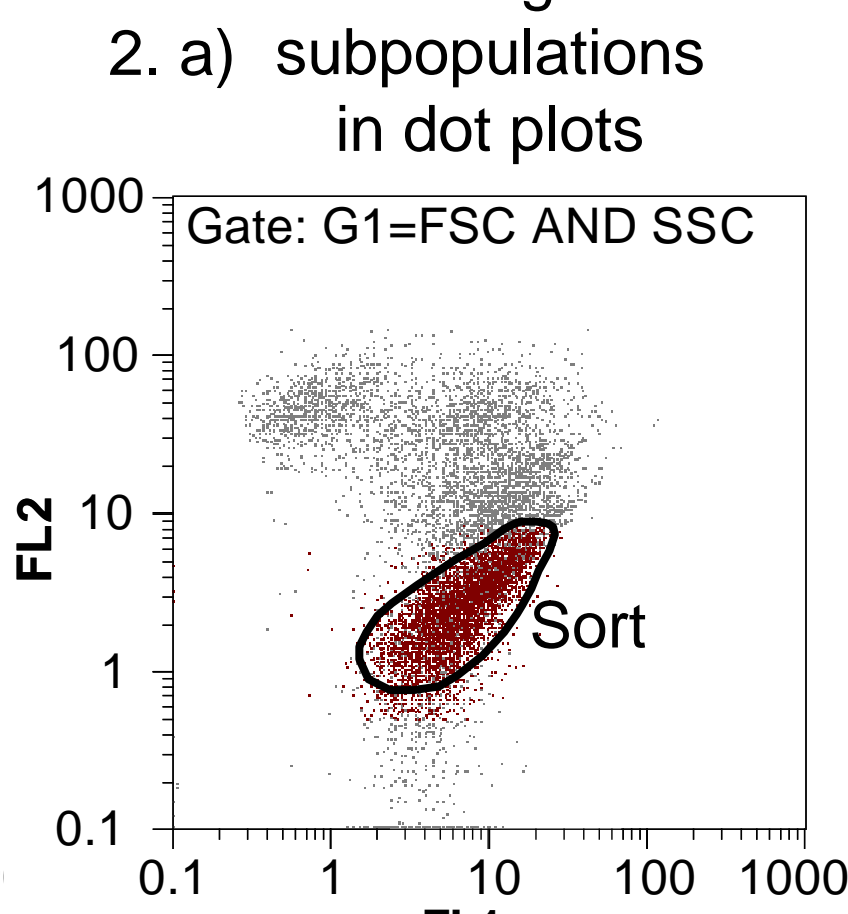


Sorting of ranges in histograms



Sorting rate 130 to 150 cells/s

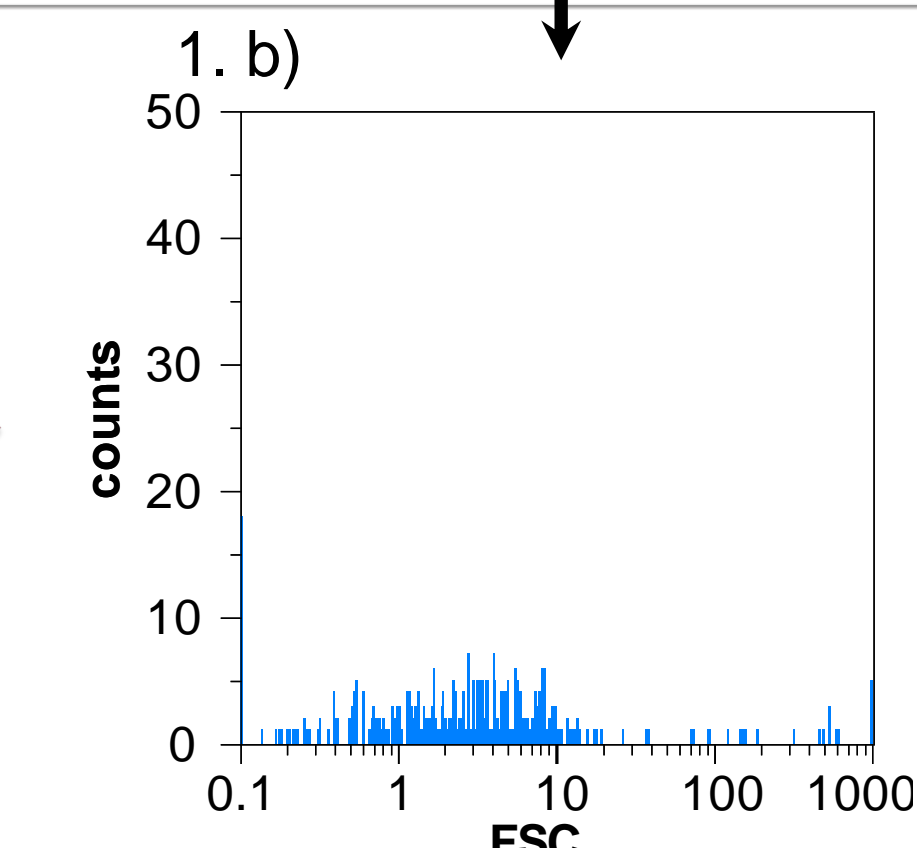
Sorting of subpopulations in dot plots



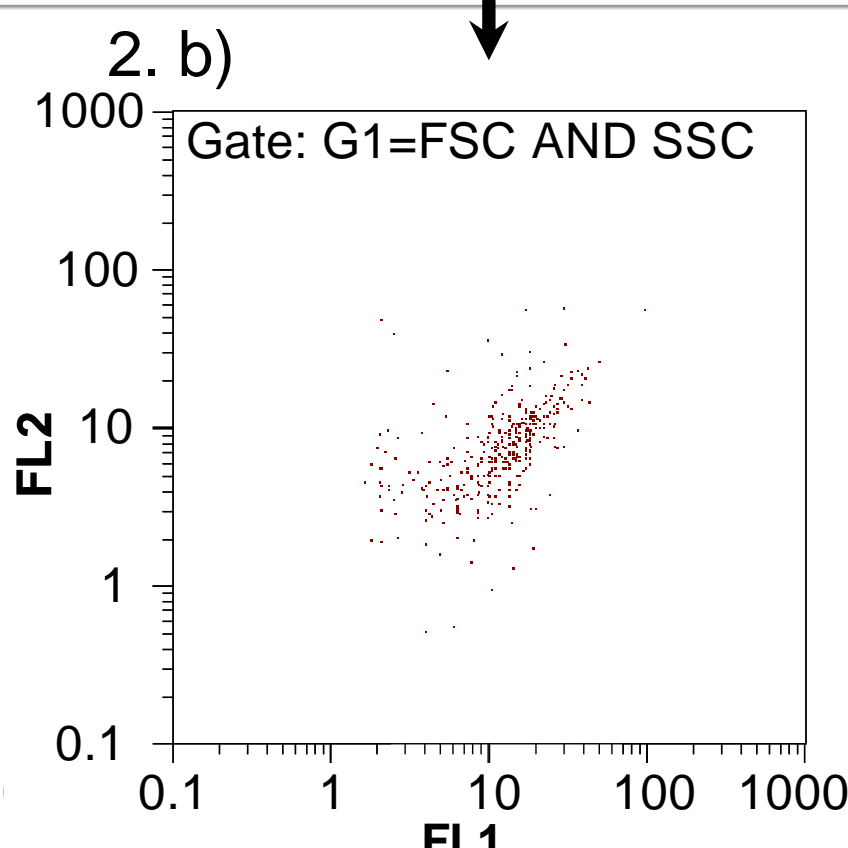
Sorting rate 140 to 160 cells/s

### Analysis of sorted cells

Flow cytometric analysis after re-staining



Cell counts 1.1 · 10<sup>3</sup> counts/mL  
Recovery rate 58 %  
Purity 81 %

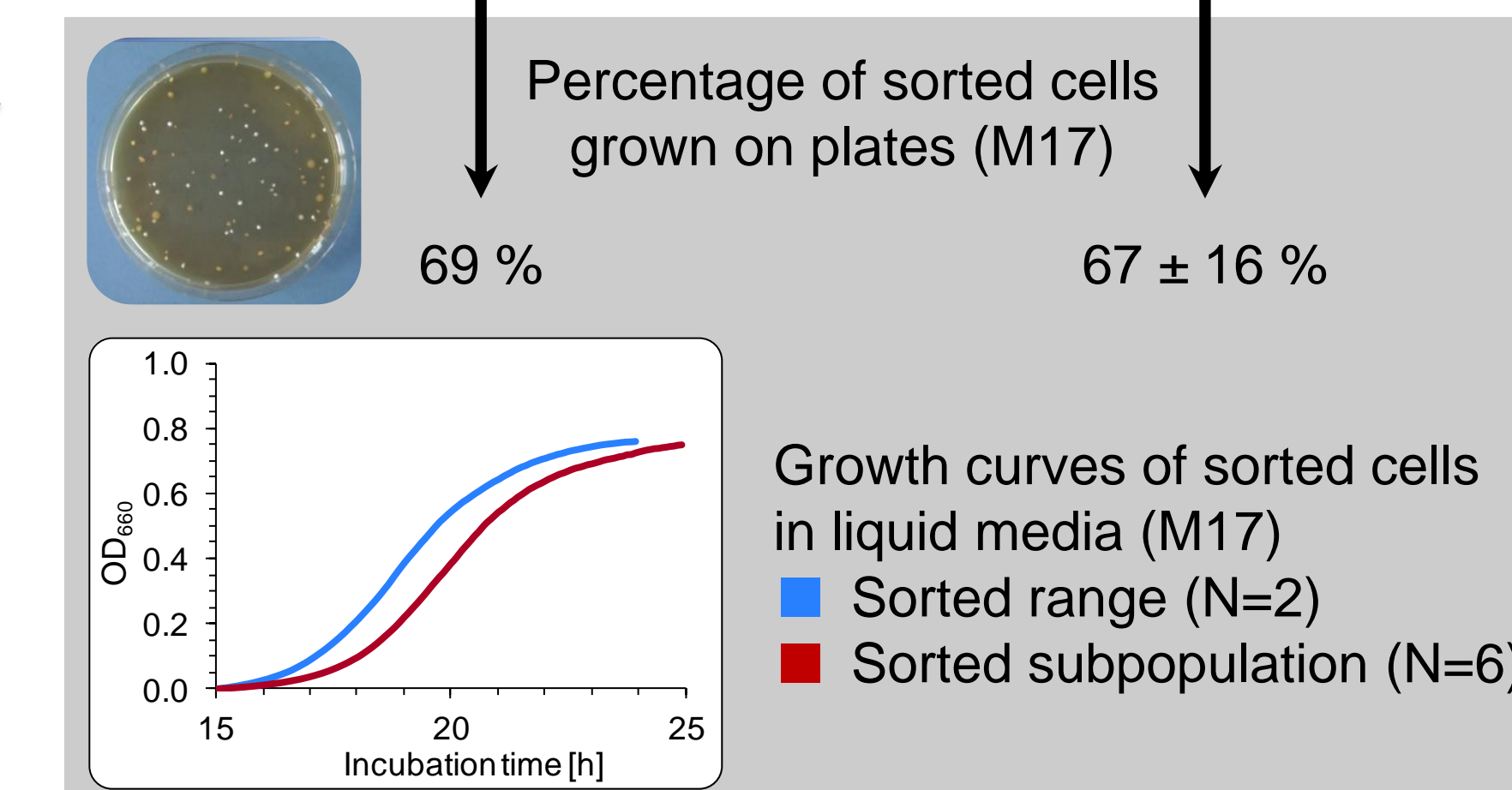


Cell counts 1.8 · 10<sup>3</sup> counts/mL  
Recovery rate 45 ± 9 %  
Purity 69 ± 12 %

Growth on plates and in liquid media

Metabolic and physiological activity

Link to function of initial population



## Introduction

After overcoming sensitivity limitations and through the development of fluorescent dye technology, even bacterial cells can now be differentiated from background noise during flow cytometric analysis. Moreover, multiparameter flow cytometry in combination with cell sorting offers the possibility not only to recognize but also to physically separate individual cells on the basis of user-defined characteristics.

In this study, the impact of staining followed by flow cytometry based cell sorting on the physiological state of *Lactococcus lactis* subsp. *cremoris* was investigated. In particular, the influence of flow diversion sorting systems on these cells has not yet been evaluated.

## Conclusions

Suitable combinations of staining and sorting conditions with minimal impact on the physiological state of *Lactococcus lactis* subsp. *cremoris* were identified.

- Various fluorescent dyes and staining protocols were tested to characterize the physiological state.
- Instrument and sorting settings were optimized for cell sorting in terms of recovery rate and purity.
- No influence of piezo based sorting on cell growth of this strain was found; survival was verified by agar plating and cultivations (small-scale).

The best results regarding cell sorting performance and viability were obtained for SYTO® 16 and propidium iodide. Selective sorting of ranges and subpopulations was successful. Growth of cells after staining and sorting was demonstrated for 'living' and 'damaged' subpopulations.

## Outlook

Sorting and analysis of subpopulations will be used to understand how stress conditions during production, processing, and storage affect the physiological state of *Lactococcus lactis*. Using this knowledge, process conditions will be optimized to improve the survival and stability in both process and product. The robustness of *Lactococcus lactis* strains plays a significant role for their application as starter cultures in manufacturing of high-quality dairy products.