

An in-droplet assay for the functional selection of antibodies from yeast libraries

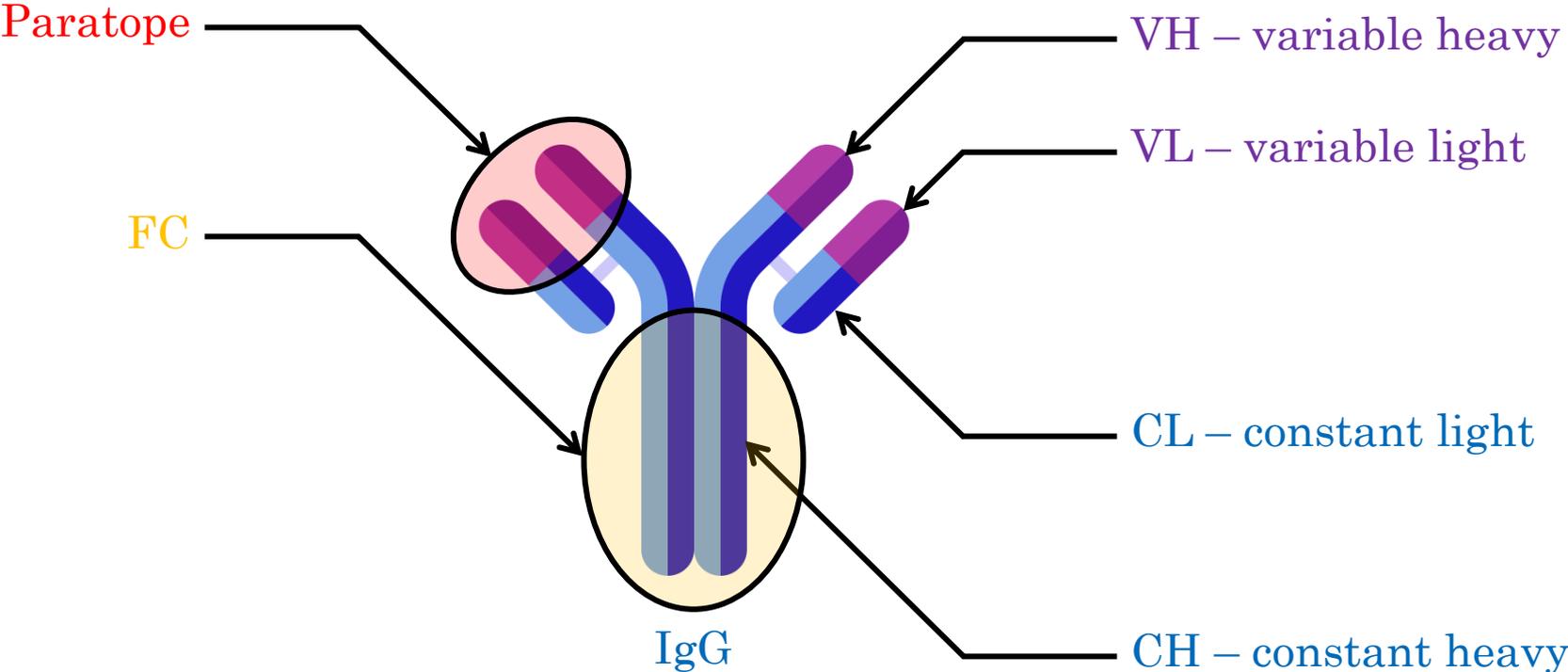
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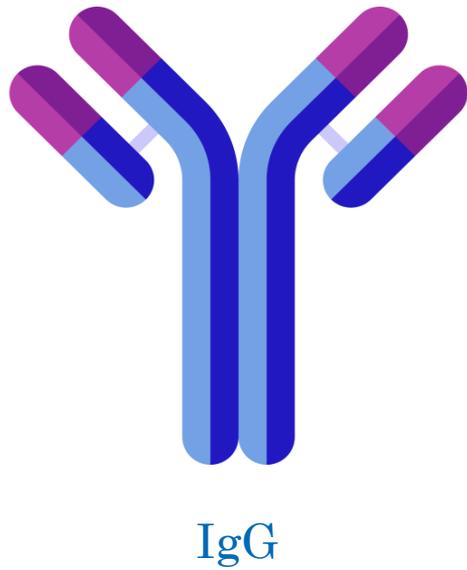
¹ Sanofi, Vitry-sur-Seine, LMR France, Microfluidic Team

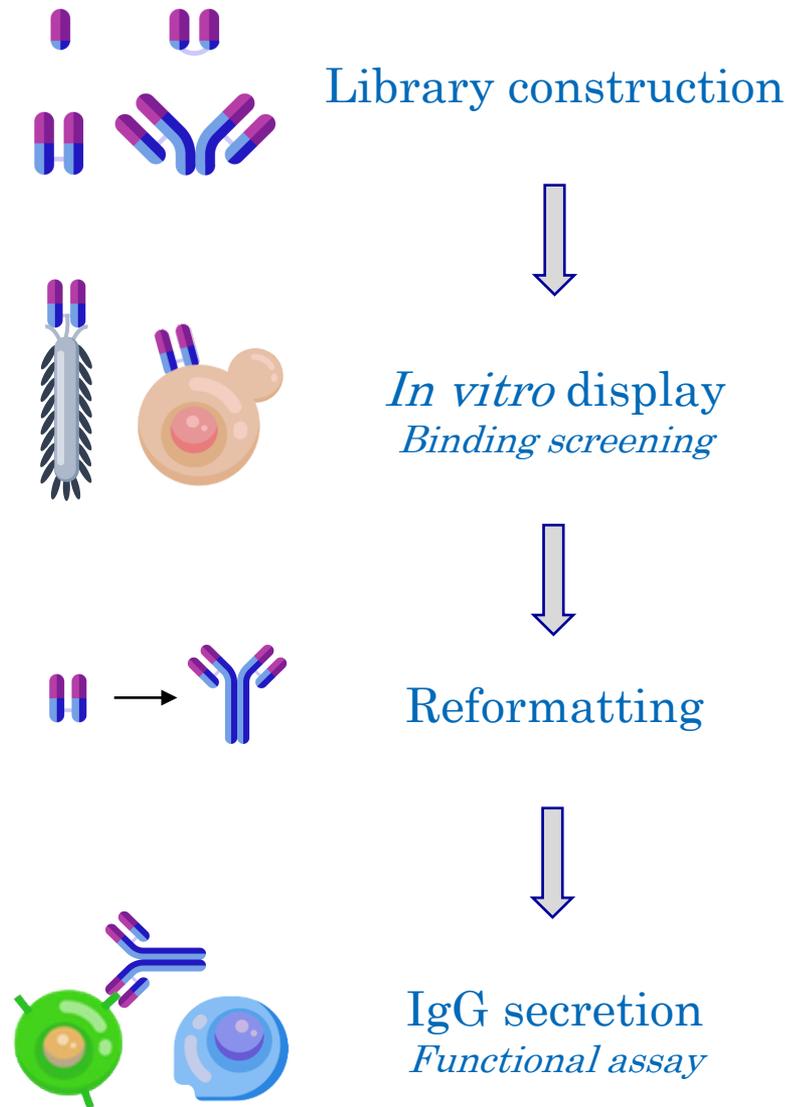
² INRAE, Jouy-en-Josas, Micalis Institute, BIMLip Team

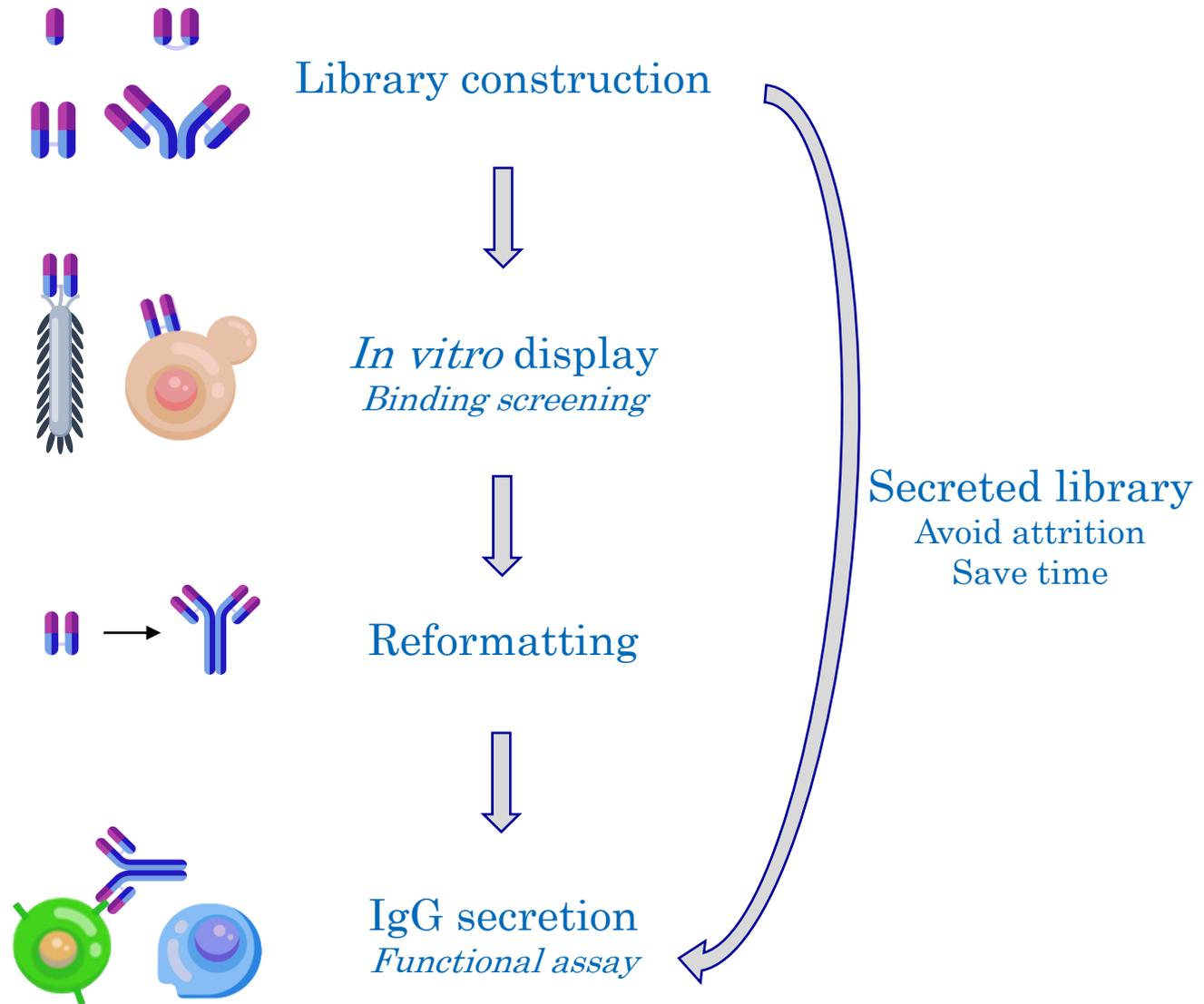
The structure of antibodies



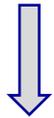
The structure of antibodies



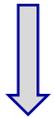




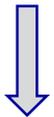
Library construction



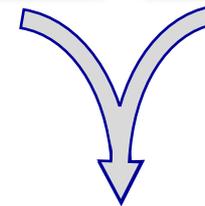
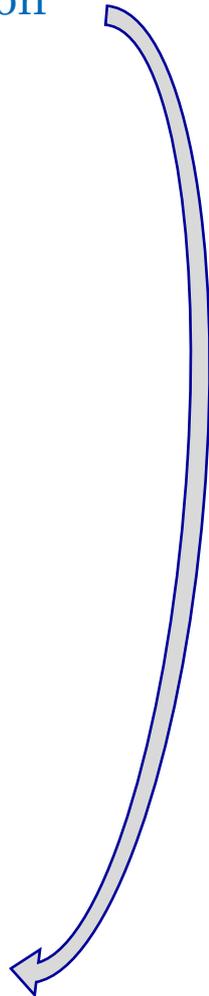
In vitro display
Binding screening



Reformatting



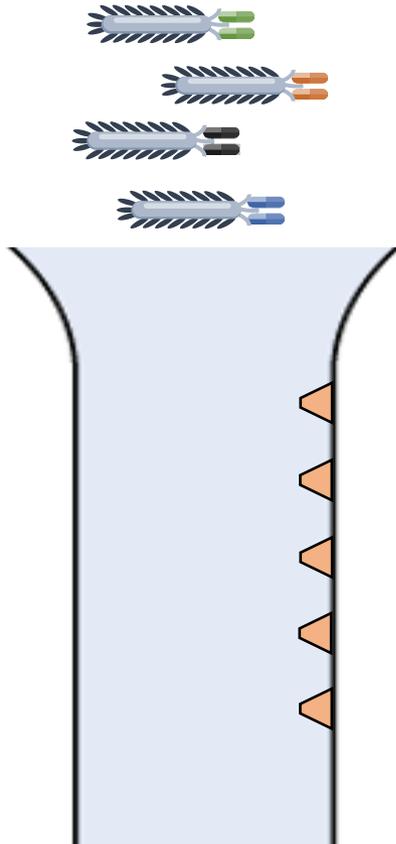
IgG secretion
Functional assay



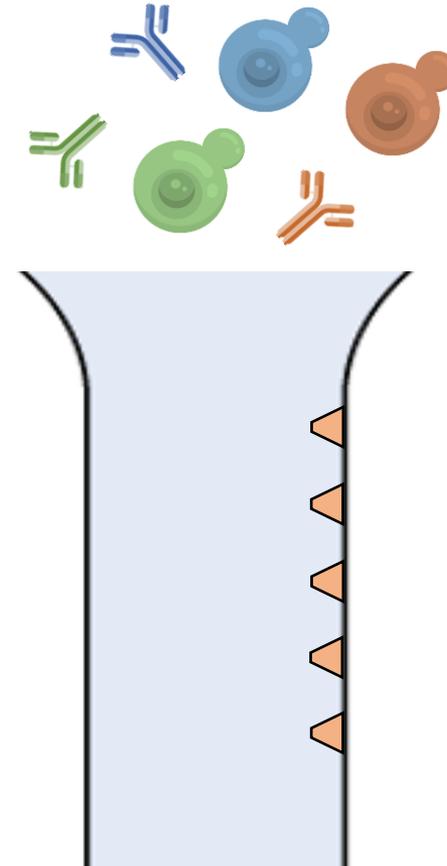
Secreted library
Avoid attrition
Save time



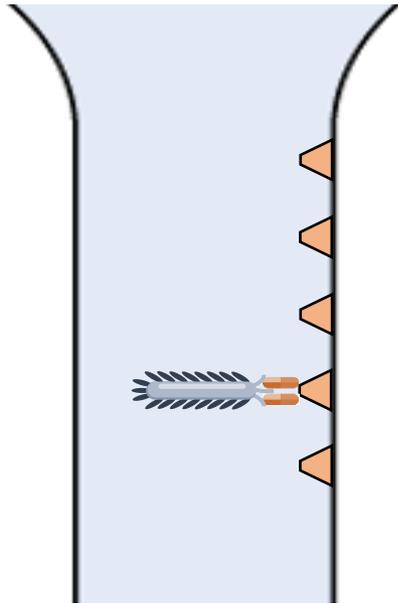
Display Technologies



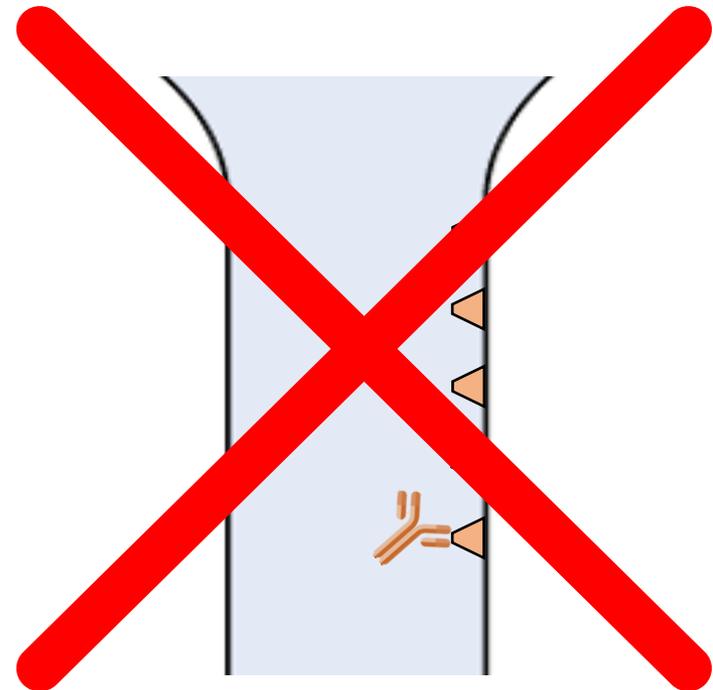
Secretion Technologies

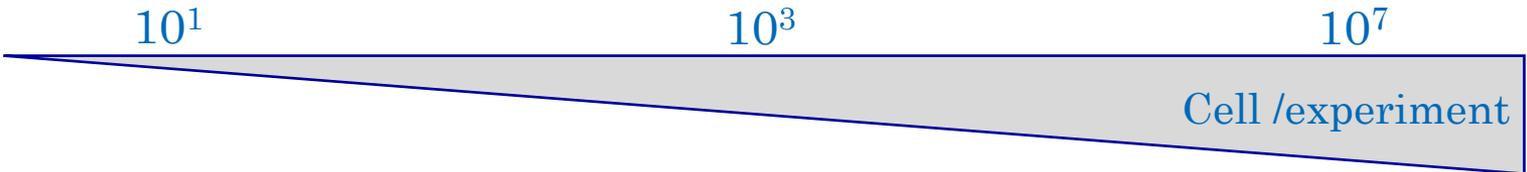


Display Technologies

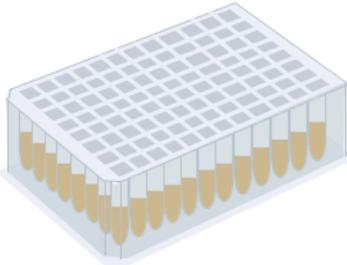


Secretion Technologies

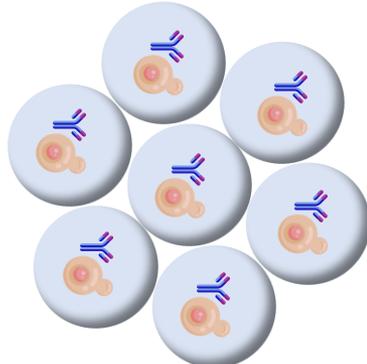




Tubes

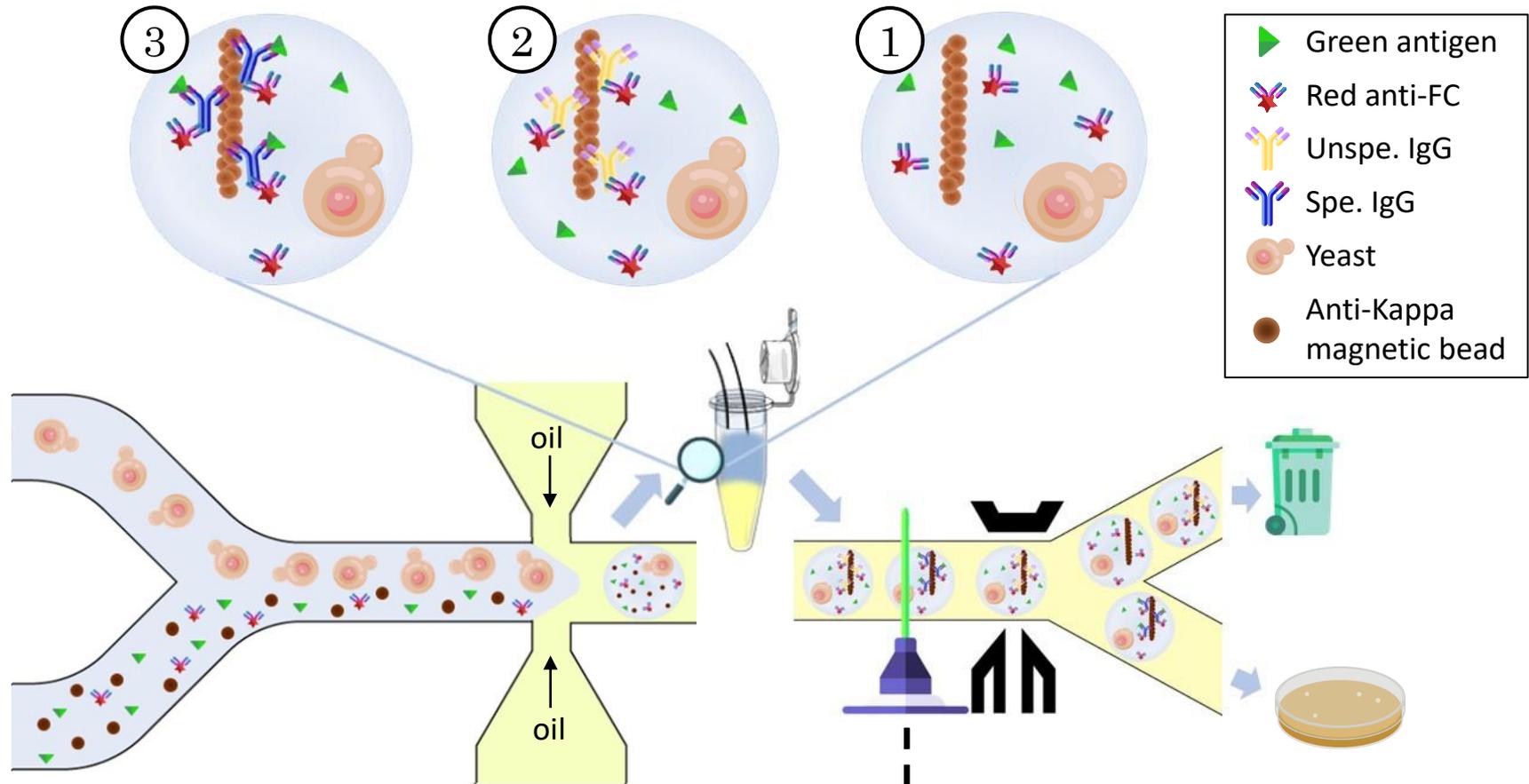


Well Plates

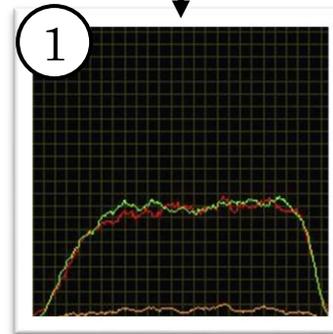
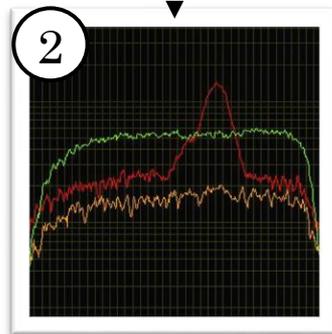
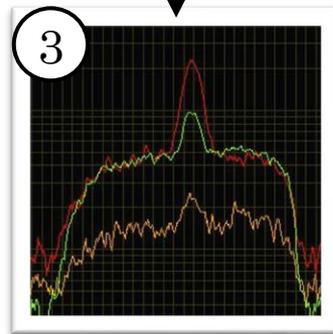


Droplet Microfluidics

Bioassay in droplet



- Survival?
- Production?
- Discrimination?
- Recovery?

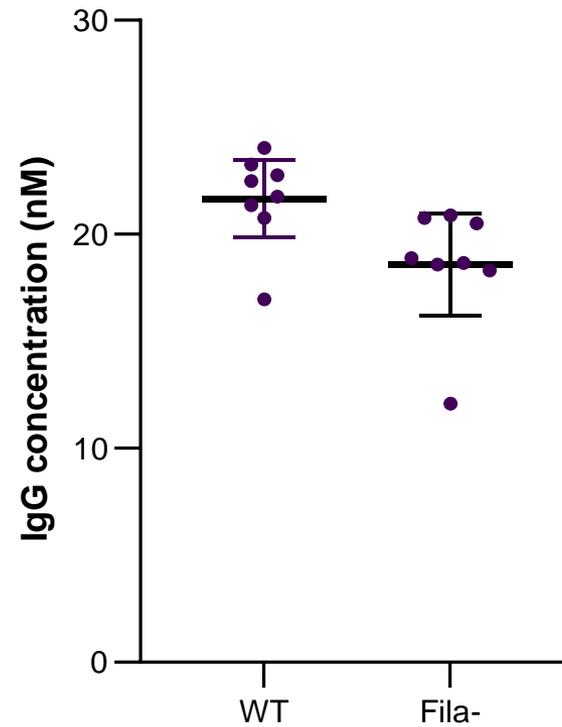
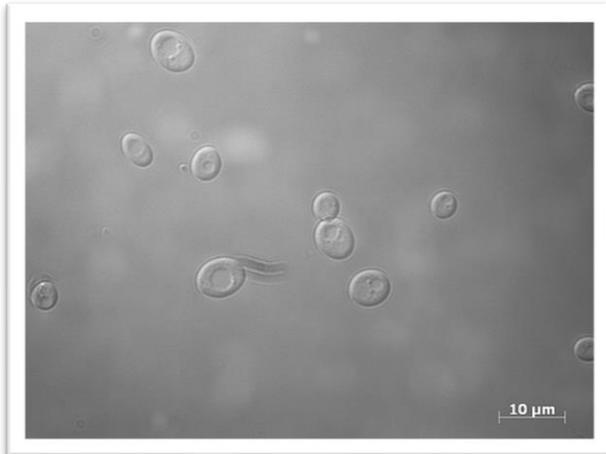


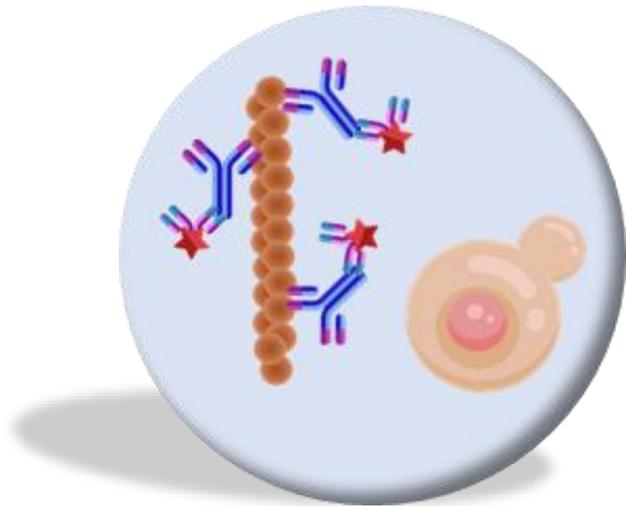
IgG secreting yeast

Yarrowia lipolytica WT

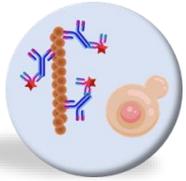


Yarrowia lipolytica Fila-





- Survival?
- Production?



- Survival?
- Production?

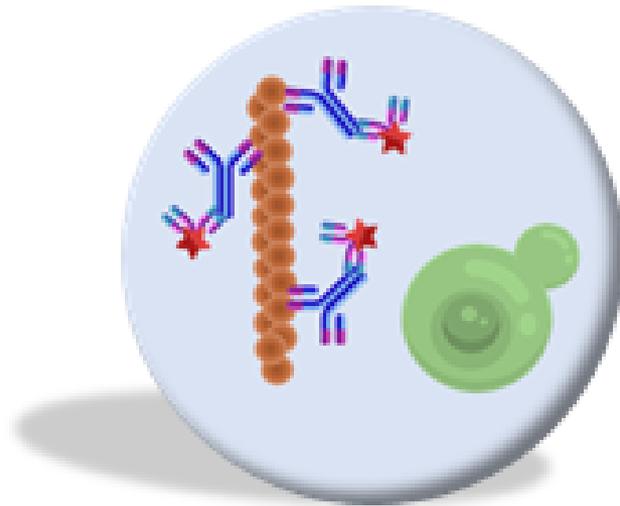
After 24 hours



6 hours recording

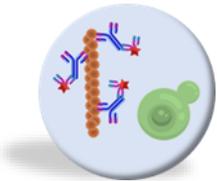


➤ Yeasts grow and secrete IgG!

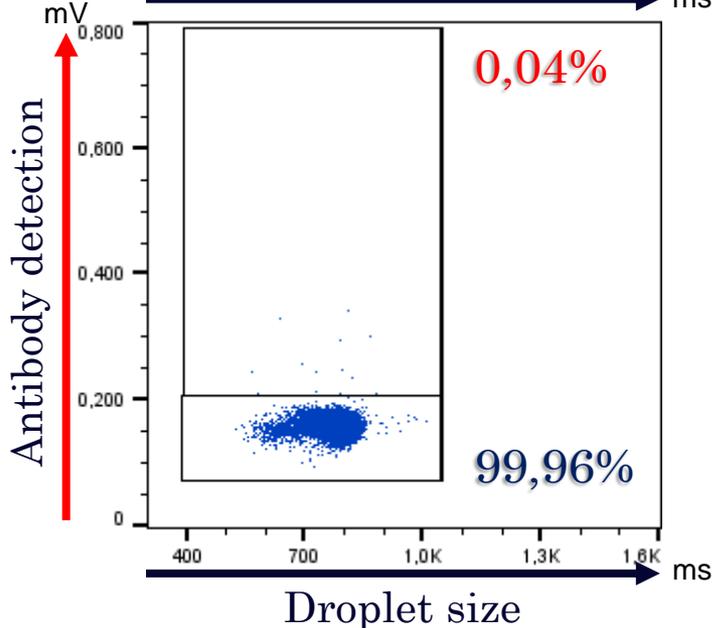
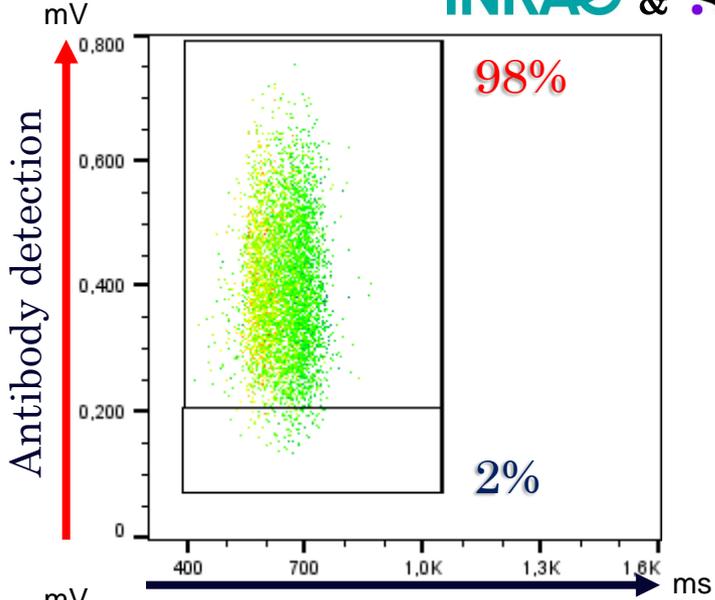
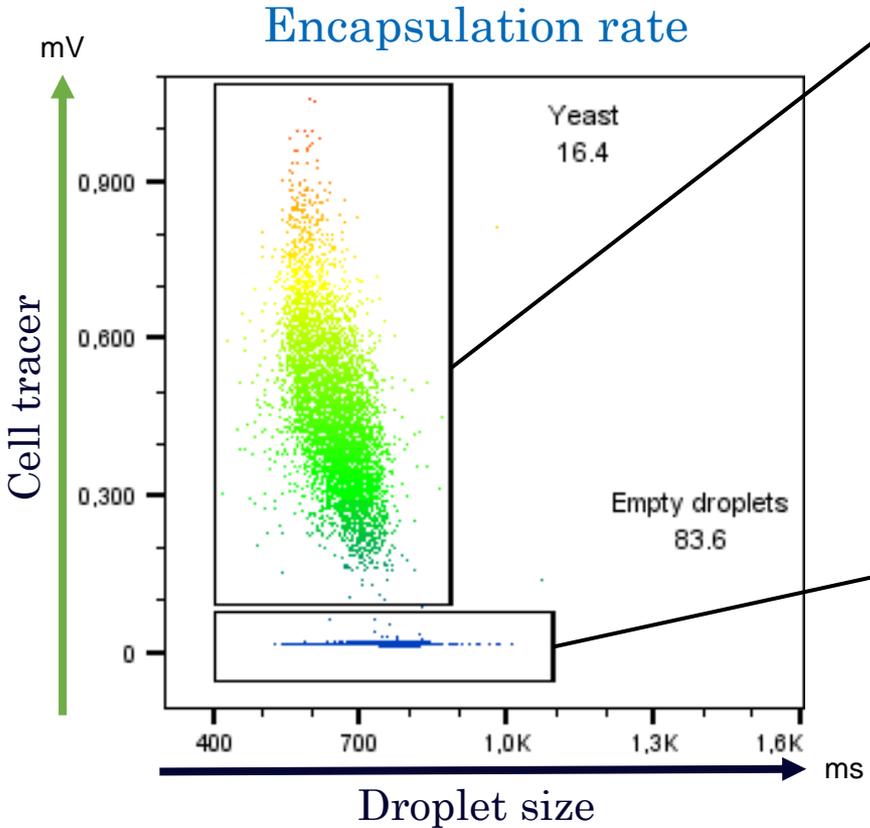


➤ Production yield ?

Secretion efficiency

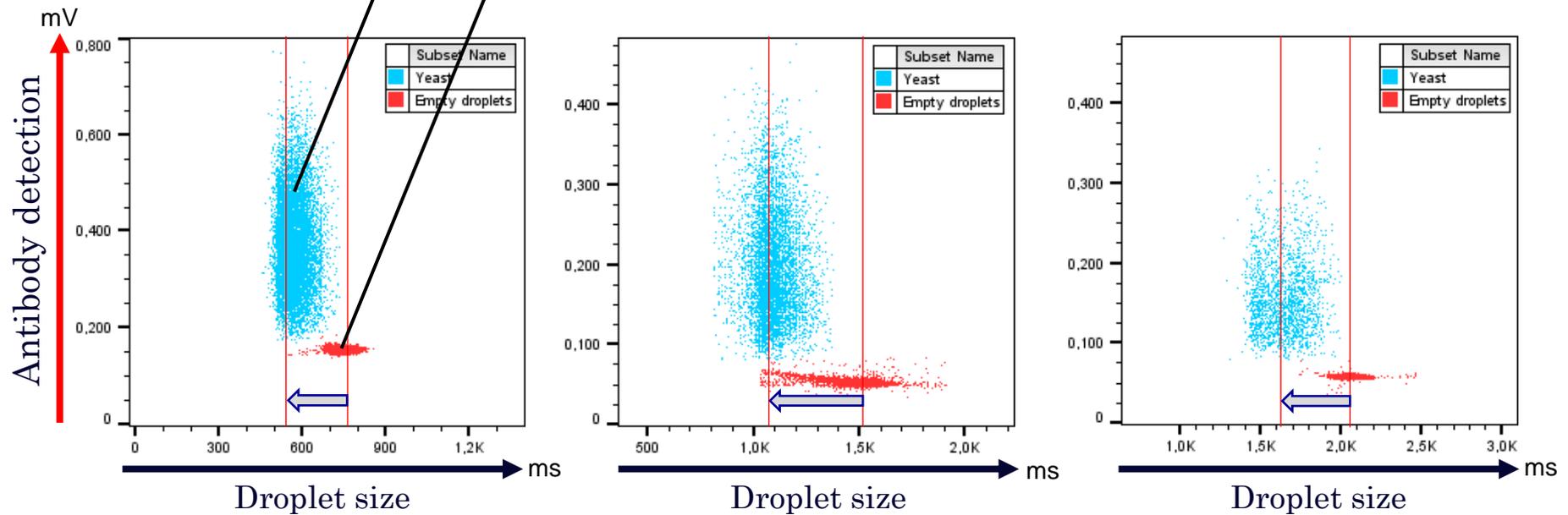
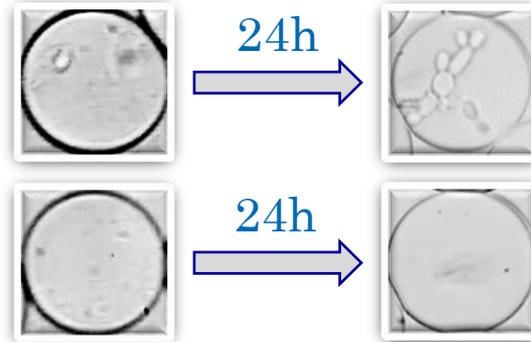
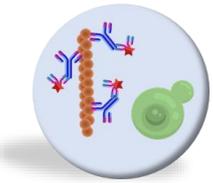


➤ Production yield ?

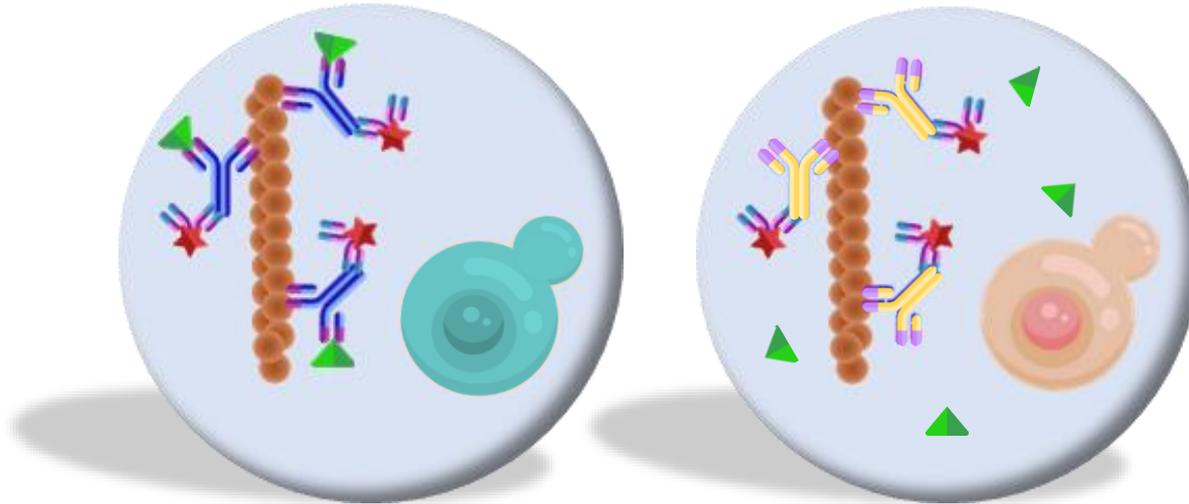


➤ 98% of the yeasts secrete IgG!

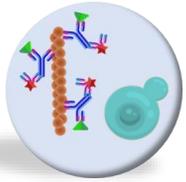
Droplet size decrease



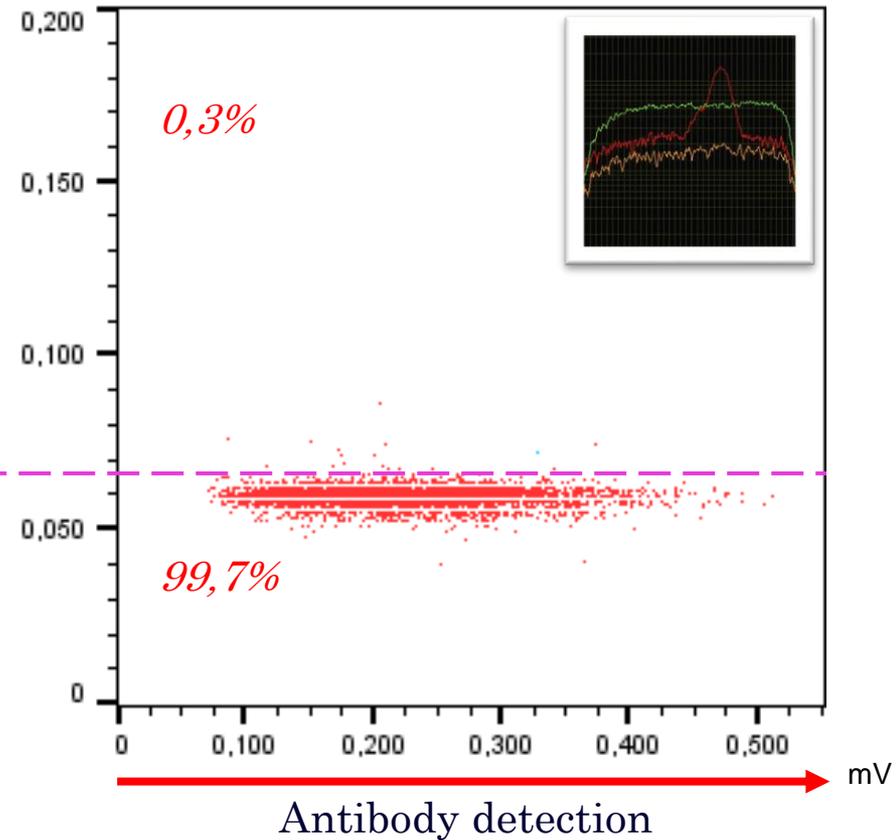
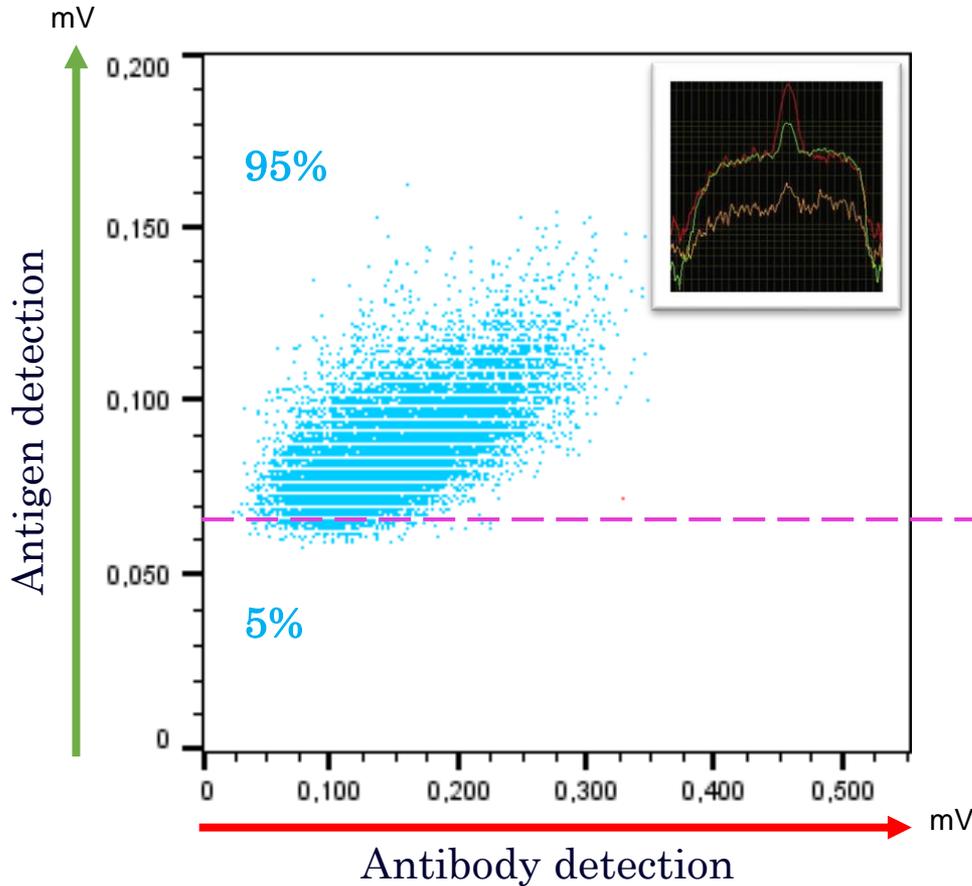
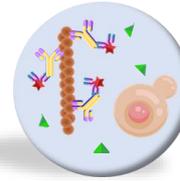
➤ Free cell tracer!



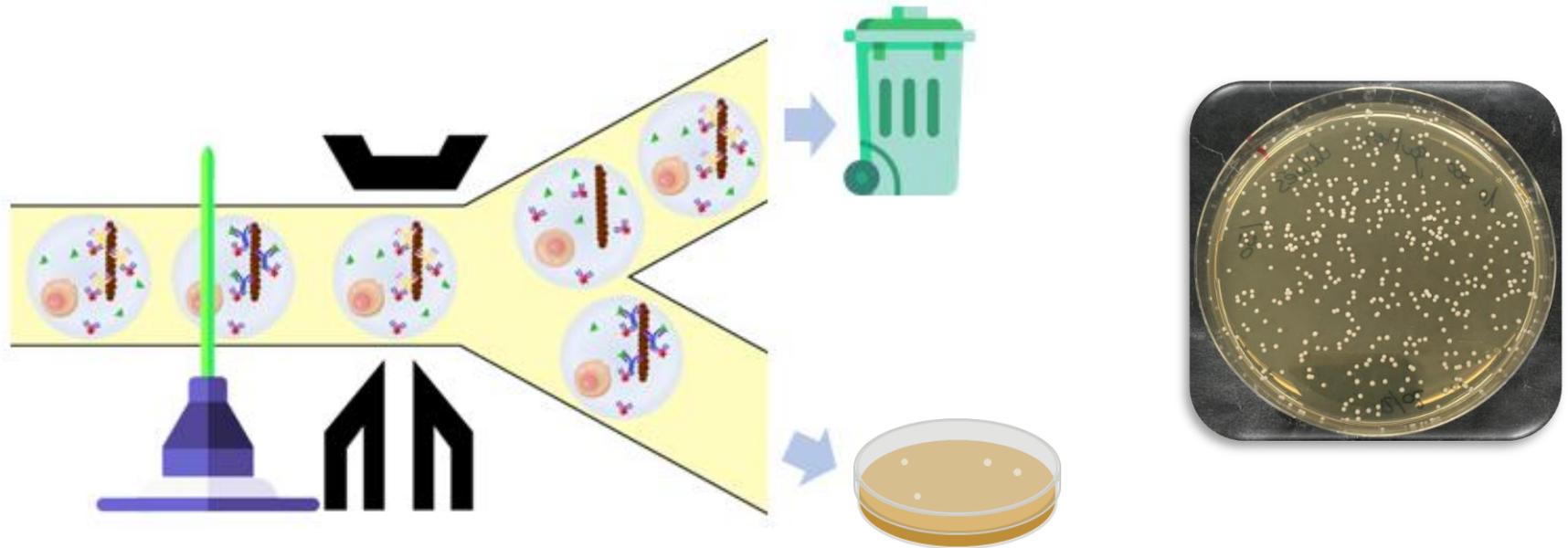
➤ Discrimination?



➤ Discrimination?



➤ IgG are functional!

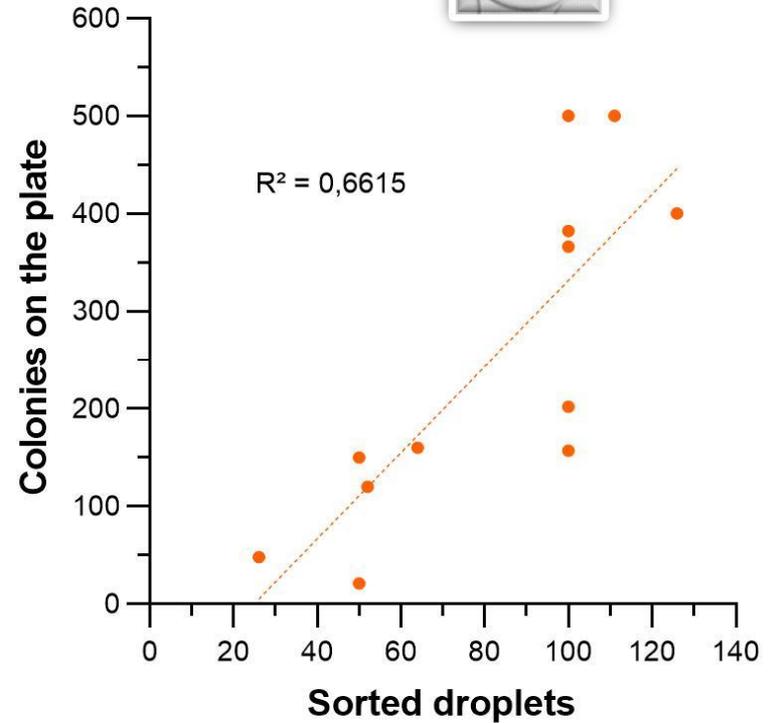
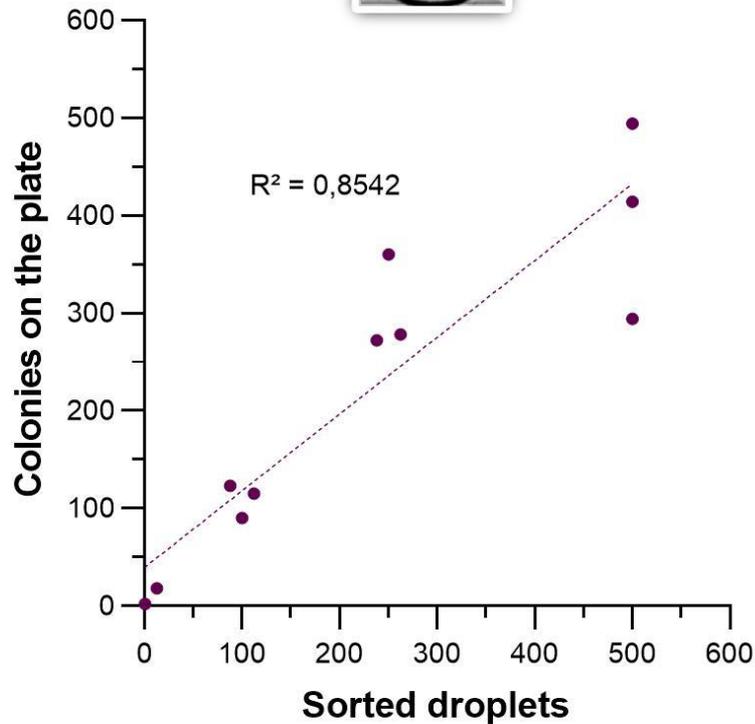
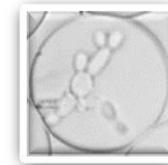


➤ How many droplets can we sort?

➤ Recovery?

Droplet incubation

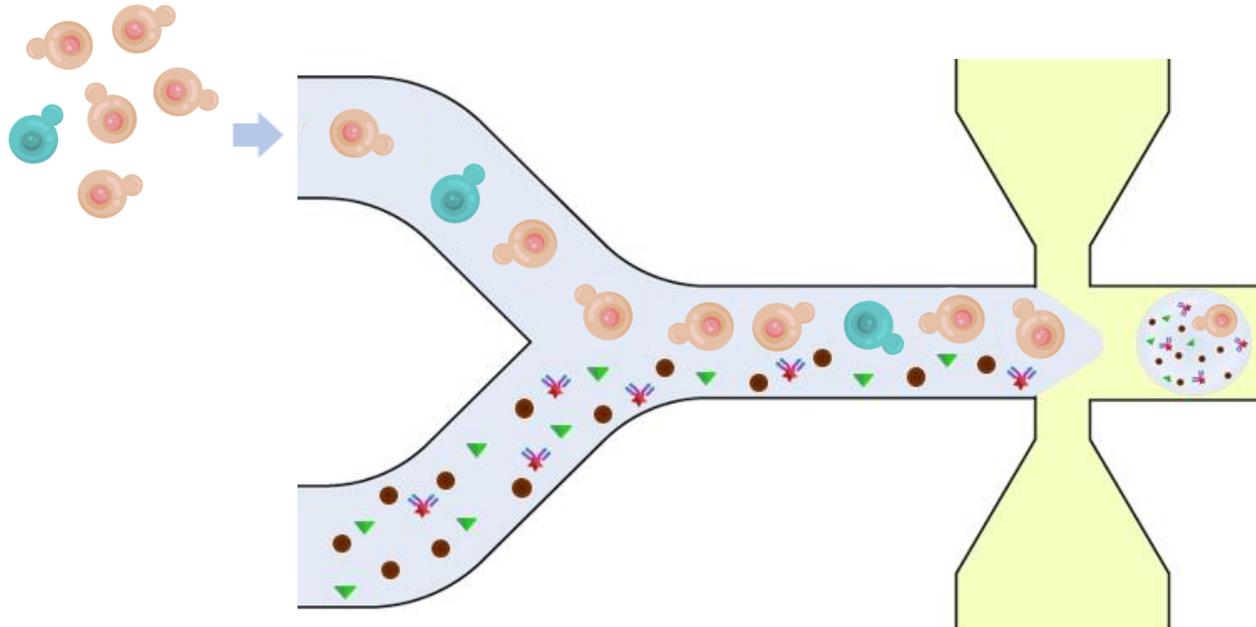
- 1 hour
- 24 hours



➤ Rare events can be recovered!

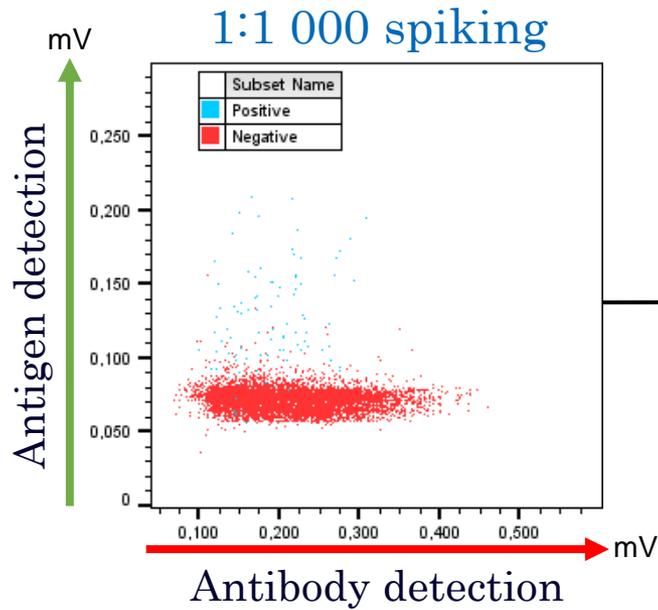
Positive yeast spiking

Dilution
1:1 000 / 1:10 000

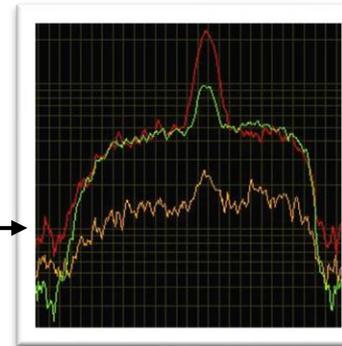


➤ Objective: find rare events

Positive yeast spiking

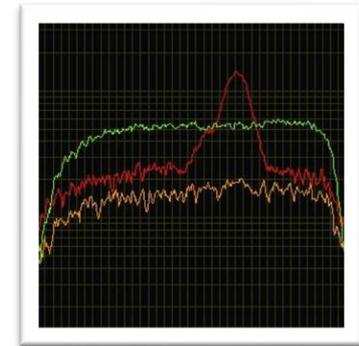


True positive

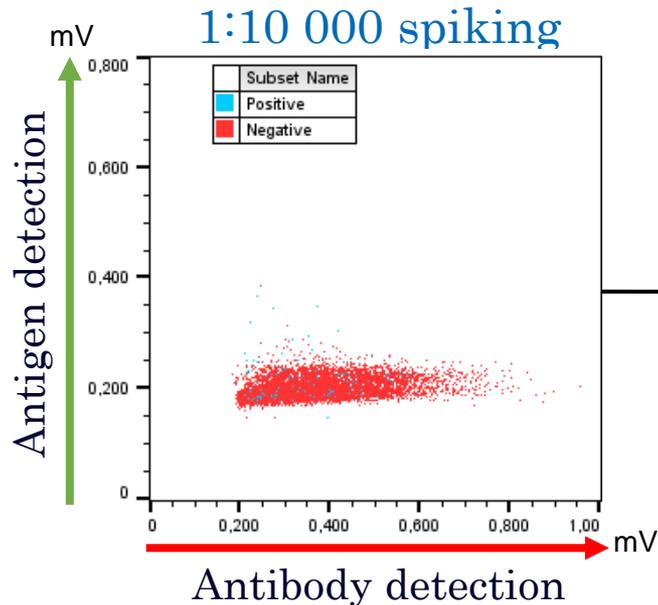


96%

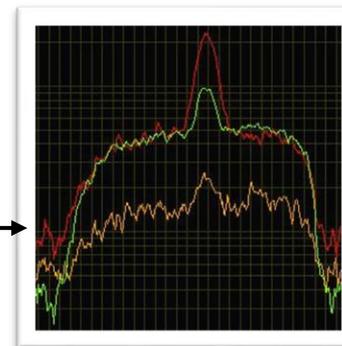
False positive



4%

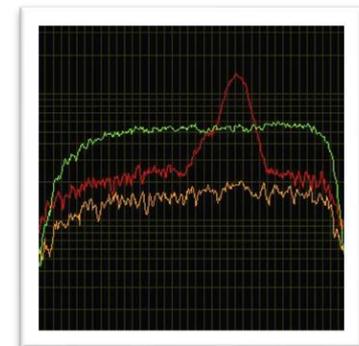


True positive



30%

False positive

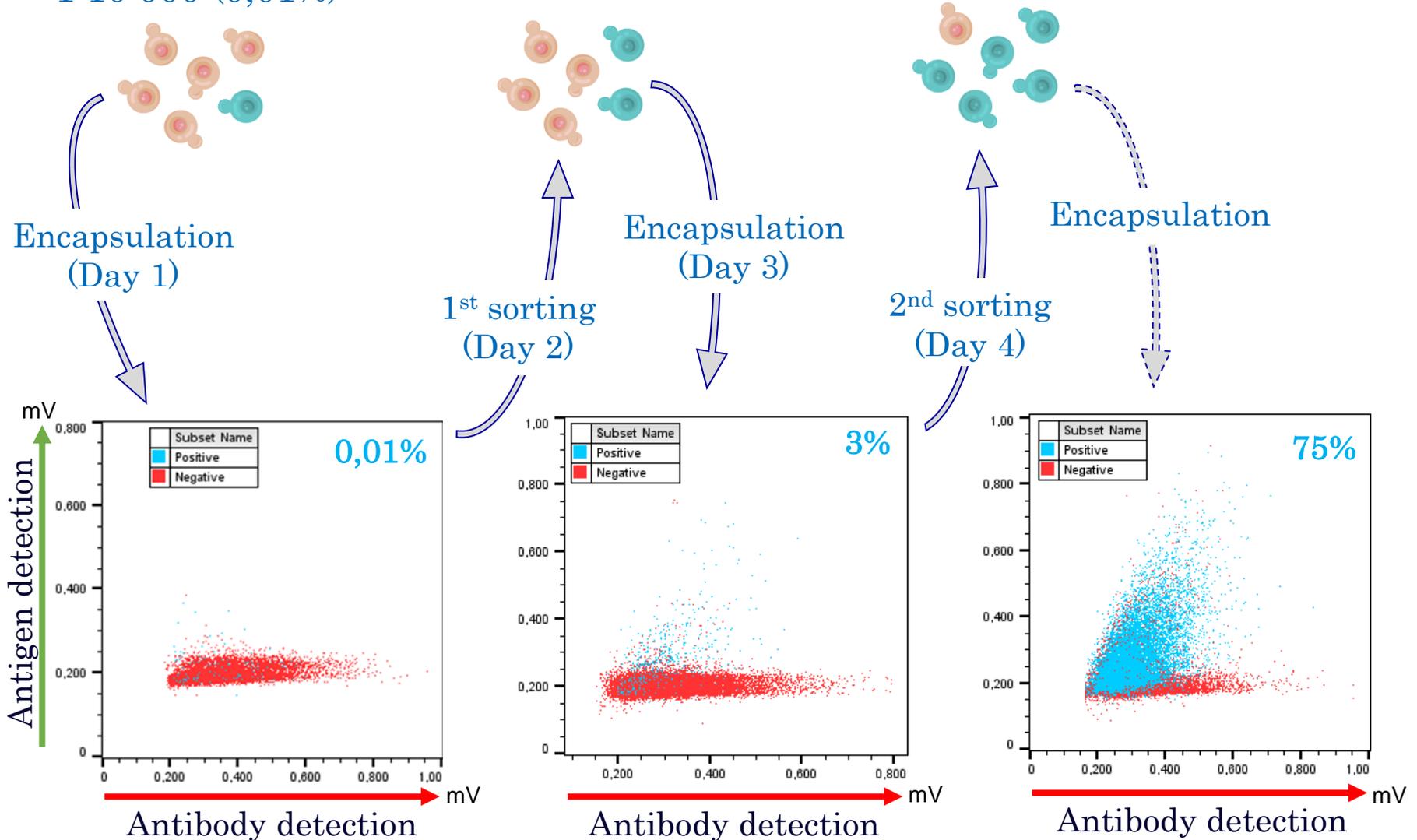


70%

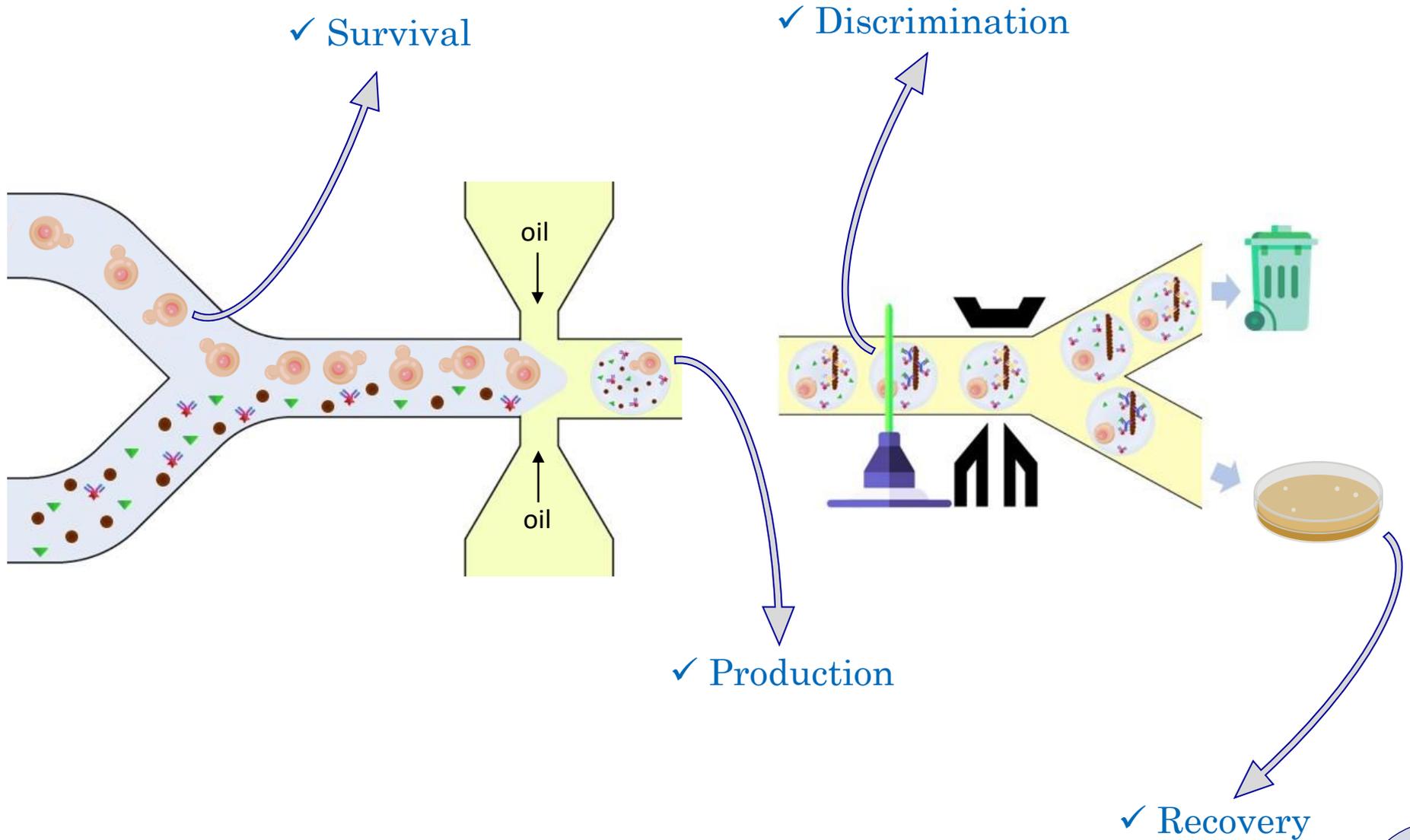


Two-round spiking

1:10 000 (0,01%)



➤ 7500-fold enrichment (in progress)



Thank you

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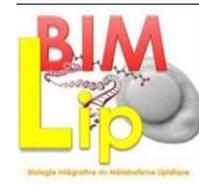
Microfluidic team

- Guillaume Mottet
- Micaela Vitor
- Helene Erasmus
- Cécile Plaire
- Viacheslav Rusanov



BIMLip team

- Jean-Marc Nicaud
- Léa Vidal
- Tristan Rossignol
- Djamila Onesime



Sanofi

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- Bruno Dumas
- Isabelle Maury
- Anne Brie
- Eric Senechal
- Steven Blanchard

- Nadine Michot
- Evelyne Deschamps
- Theo Pizette
- Nathalie Couteault
- Laurent Maton
- Cendrine Lemoine

Thesis committee

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- Samy Gobaa
- Eric Dugat-Bony
- Pierre Larraufie