

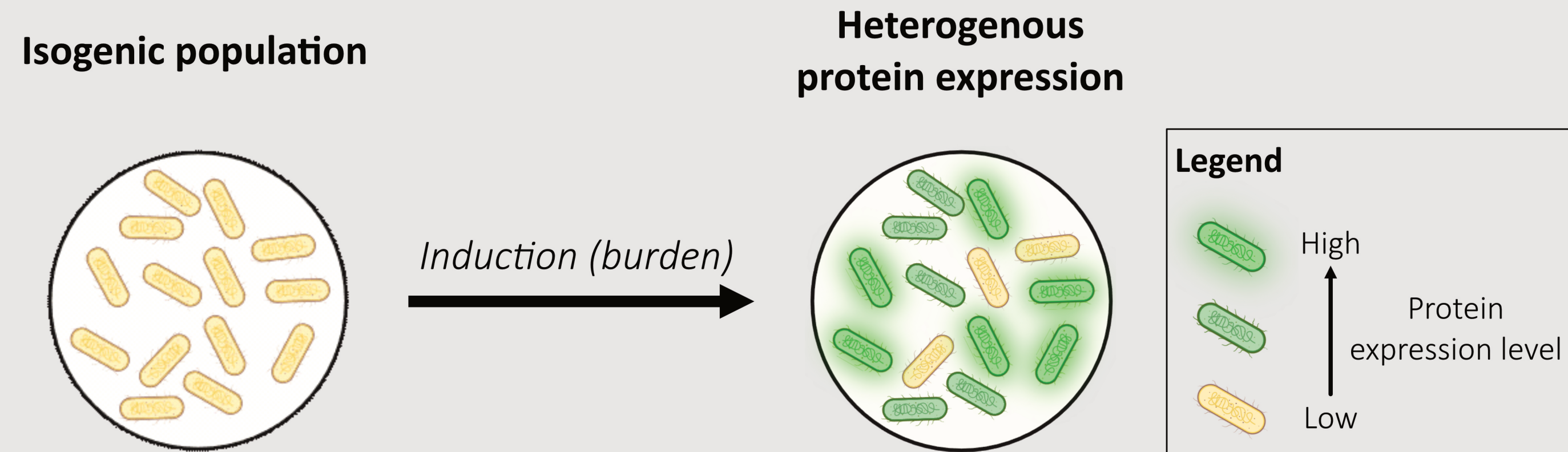
INVESTIGATING PHENOTYPIC ESCAPE TO ENHANCE ROBUSTNESS IN CONTINUOUS MICROBIAL BIOPROCESSES

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1 CONTEXT

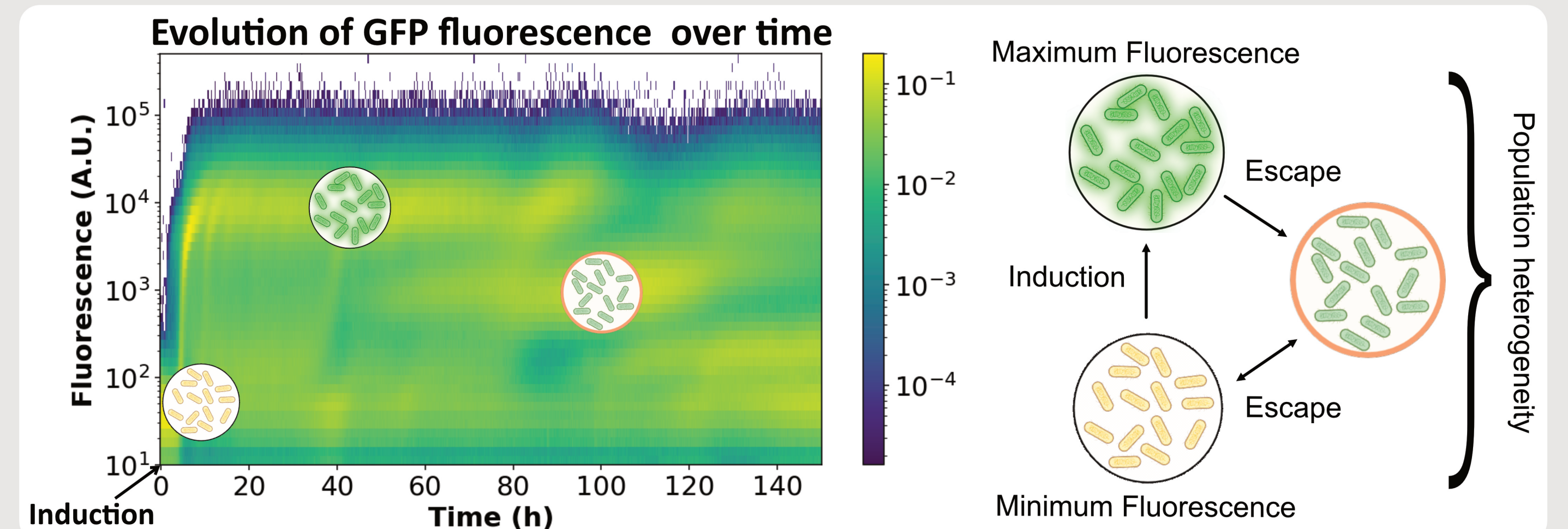
Recombinant protein production imposes a significant **metabolic burden** (= reallocation of cellular resources) on bacterial cells. This burden leads to gene expression **instability** within isogenic populations.



As a result, individual cells produce **different** amounts of recombinant protein, which can be tracked using **GFP** as a reporter. This **heterogeneity** arises from multiple mechanisms, including **phenotypic escape**, which is the focus of this study.

2 PRELIMINARY OBSERVATIONS

Using **flow cytometry**, we observe **population instability** during continuous cultivation [1]. Upon induction, a subpopulation strongly expresses GFP, while others partially or fully **escape induction** over time.



The escape may be mutational or phenotypic. This project focuses on **phenotypic escape**, defined as a **reversible** and **non-genetic** change in gene expression.

3 OBJECTIVES

CONTROL POPULATION DYNAMICS OF *ESCHERICHIA COLI* BL21 (DE3) IN CONTINUOUS CULTIVATION THROUGH PHENOTYPIC ESCAPE.

- A** Characterize the **phenotypic escape** mechanism.
 - ↳ Identify cultivation conditions promoting and preventing phenotypic escape.
 - ↳ Disentangle phenotypic and mutational escape.
- B** Exploit the **phenotypic escape** to control and stabilize population dynamics in continuous cultures.

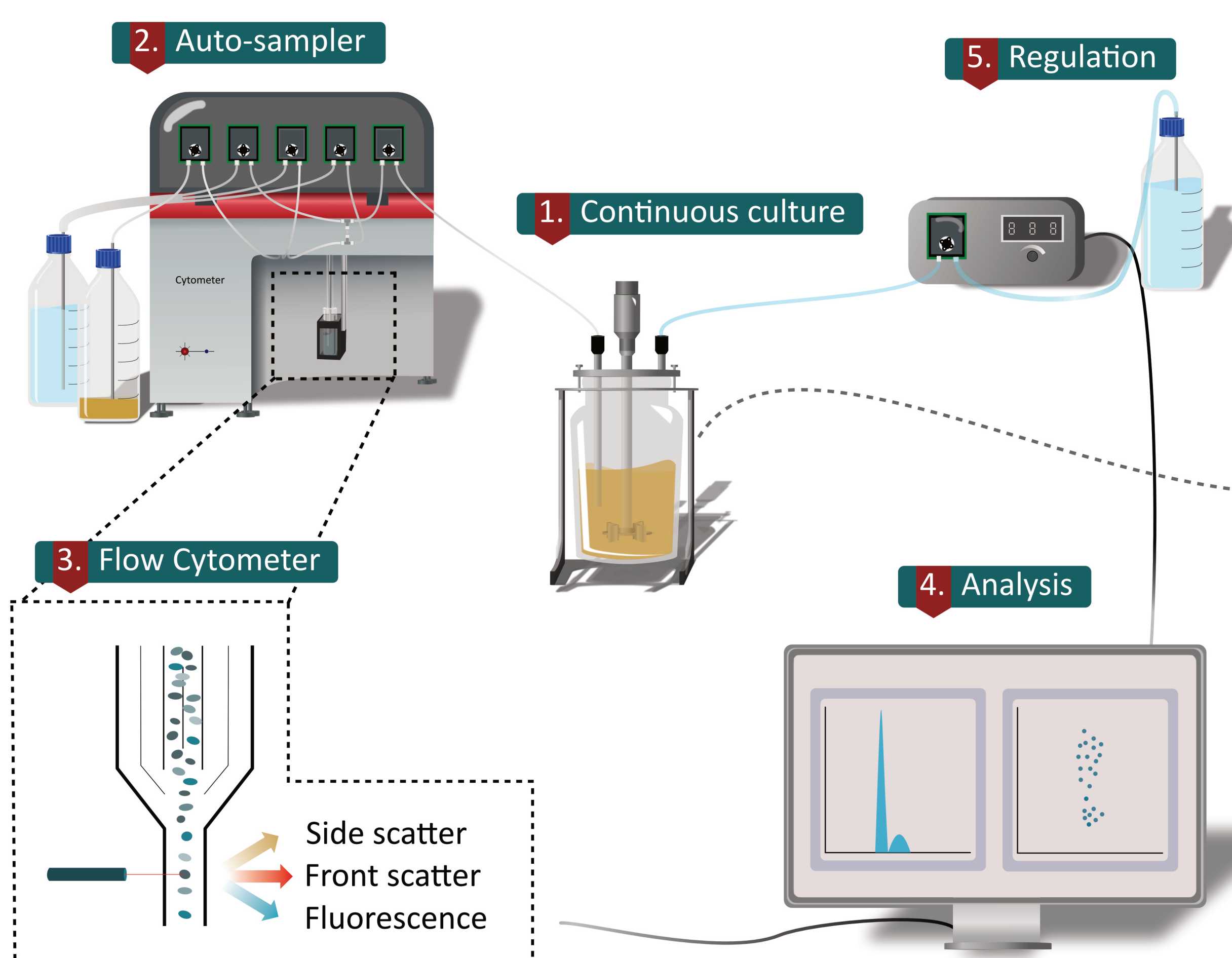
4 EXPERIMENTAL STRATEGY

E. coli BL21 (DE3) pET28::GFP is used as a **burdensome** recombinant model.

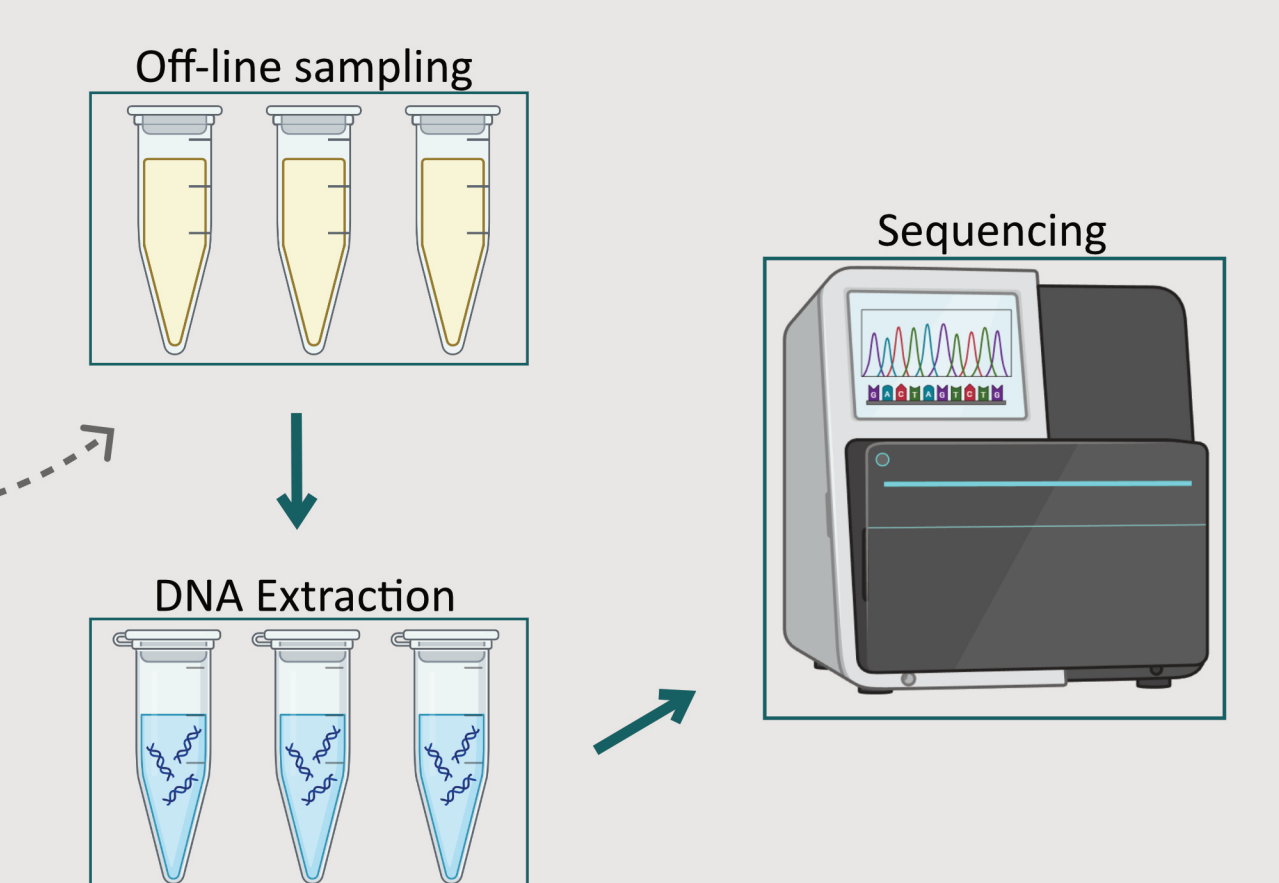
GFP enables easy monitoring of gene expression at the **single-cell level** using flow cytometry.

High-throughput Biolector pre-screening to assess growth rate and GFP dynamics under multiple cultivation conditions.

The **Segregostat** enables **online**, single-cell monitoring using **flow cytometry** and allows a direct **regulation** of culture conditions in continuous **bioreactors**.

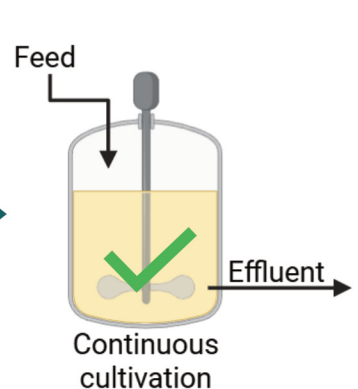


To distinguish **phenotypic escape** from **mutational escape**, genomic **sequencing** is performed on **off-line** culture samples.

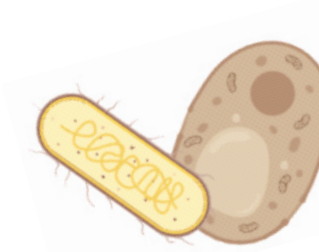


Combined with **flow cytometry** data, **mutation** analysis helps elucidate the **origin** of the observed expression **heterogeneity**.

5 PERSPECTIVES



Improve the **stability** and **productivity** of continuous bioprocesses.



Enable **robust** microbial **co-culture** for the production of more **complex molecules** [2].

