APPLICATION NOTE

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# Accelerating bacteria transformation and cloning workflows using microbial single-cell isolation with the B.SIGHT

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## Abstract

The isolation of single strains is an important step for establishing an expression system from transformed bacteria or yeast. The isolation can be achieved using manual or automated colony picking, however, this approach is time consuming. Here we present a workflow that uses a two step approach that cut down on cost and duration relative to colony picking. The workflow uses the B.SIGHT to isolate single strains after transformation. The system is able to dispense single bacteria onto SBS plate format, with image confirmation, both into liquid culture and onto solid media. The transformation and cloning workflow presented here can be easily adapted to any workflow and any strains with varying traits.

## Introduction

Microbial cell factories have been identified as key tools for establishing a sustainable bioeconomy. In industrial biotechnology, genetically modified pathways in yeast or bacteria help researchers search for highly valuable proteins. The isolation of single strains is an important step for protein variant screens in biocatalysis or the recombinatorial creation of new pathways. The variants within these libraries are transformed into the expression hosts, the resultant transformed cells are then isolated and screened. Isolation is performed using colony picking which is a very labor-intensive

task. These libraries contain $10^3$ to $10^{12}$ variant strains and therefore can only be handled in an automated way, which is not feasible by manual colony picking. In recent years, picking robots have addressed this challenge. Despite automation, colony picking still contain a lot of time consuming steps. Cells must first be streaked onto a selective agar medium, and after waiting for growth, they can then be picked and transferred into liquid culture.

The B.SIGHT, developed by CYTENA, overcame the drawbacks of this two-step approach and isolated individual cells directly from the liquid medium after transformation. This enabled us to circumvent the problem of fast-growing non-producers outgrowing metabolically-strained high producers. It also enabled us to accelerate the entire workflow. In this study, we demonstrated a workflow and protocol for bacteria transformation, automated isolation and sorting that accelerated the process compared to existing colony picking robots.

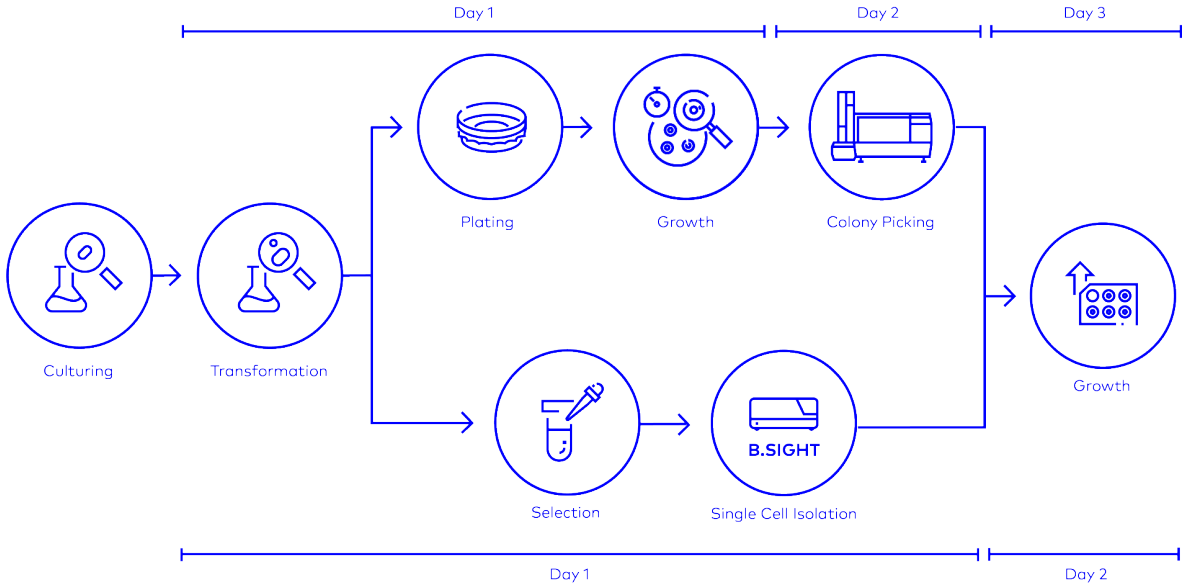


**Figure 1.** *The transformation and colony isolation workflow with the B.SIGHT. Traditional workflows using colony picking robots take up to 3 days (top). The B.SIGHT workflow (bottom) takes 2 days with significantly fewer plates, thereby accelerating the workflow, decreasing costs and reducing the impact on the environment.*

# Materials and Methods

## Sample preparation

NEB E.coli Turbo Competent cells were transformed according to protocol with 100 ng pET16b plasmid containing a codon-optimized coding sequence for mScarlett under control of the late stationary-induced promotor for glpT. The cells were subsequently preselected in SOC medium containing 100 µg/mL Ampicillin to select successfully transformed cells. Samples were dispensed using the B.SIGHT every two hours. The samples were each diluted to an OD600 of 0.005 and dispensed onto either LB agar plates or directly into LB medium in 384-well plates. Cells were then incubated overnight at 37°C.

## Single-cell isolation principle

The B.SIGHT is an automated laboratory instrument that enables microbial cell isolation using high-resolution imaging [1]. The sample is loaded into a cartridge where it pools into a 20 µm nozzle. A dispenser chip rapidly generates small droplets on demand when the silicon membrane is deflected by a piezo actuated piston. The dispenser chip's nozzle region is imaged continuously during

operation. A cell detection algorithm analyzes each passing particle until it confirms that the next droplet is a single cell with correct parameters and allows the cell to dispense into one well of the 96- or 384-well plate below. Unwanted droplets (ie, void droplets or droplets with multiple cells) are removed by vacuum prior to dispensing (Figure 2A). A series of images of the nozzle region is automatically stored for each deposited droplet so single-cell ejection can be verified (Figure 2B). Single microorganism isolation with the B.SIGHT is routinely used for cultured yeast, bacteria, microbiome and environmental samples in institutes around the world [2,3].

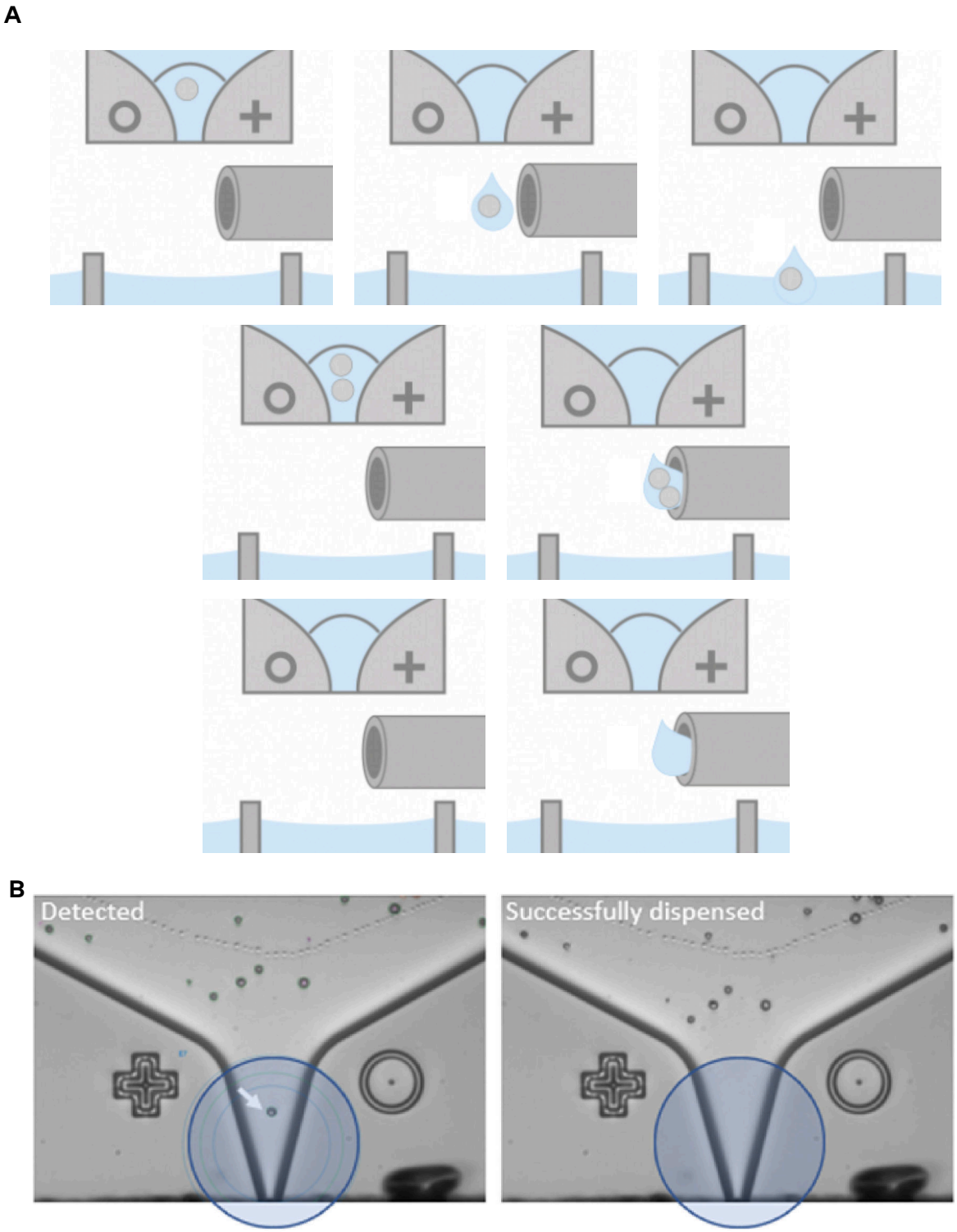


**Figure 2.** *Single microorganism isolation principle with the B.SIGHT. A) Schematic showing how the B.SIGHT allows droplets to be dispensed if the high resolution nozzle image revealed a single cell. Droplets with potentially more than one cell or no cells are ejected and immediately deflected using a pneumatic shutter. B) Nozzle images showing a single yeast cell detected by the device and successfully dispensed in a subsequent frame.*

# Results and Discussion

## Workflow for transforming and cloning bacteria using microbial single-cell isolation

The B.SIGHT was used to improve and accelerate the transformation of the E.coli isolation workflow. We used off-the-shelf NEB Turbo Competent E.coli cells and transformed them with a pBR322-based ampicillin-resistant plasmid containing the cds for a red fluorescent protein under control of a late stationary phase activated promoter of the gene glpT. The transformation mixture was recovered in ampicillin containing SOC medium to allow outgrowth of successfully transformed cells. During this outgrowth, the number of non-transformed cells stayed the same while transformed cells were able to propagate and become enriched in the sample over time. After this recovery, the cells were dispensed onto a non-selective LB agar plate.

The optimal duration of the outgrowth phase was determined 4 to 6 hours into the recovery time, which was sufficient for this application (Figure 3A). The transformation rate was high after 2 hours of recovery, showing that the non-performant cells were efficiently killed (Figure 3B). After only 2 hours of recovery in selective SOC medium, sorted cells consisted of more than 80% successfully transformed cells. After 6 hours, more than 95% of the dispensed cells were successfully transformed (Figure 1B).

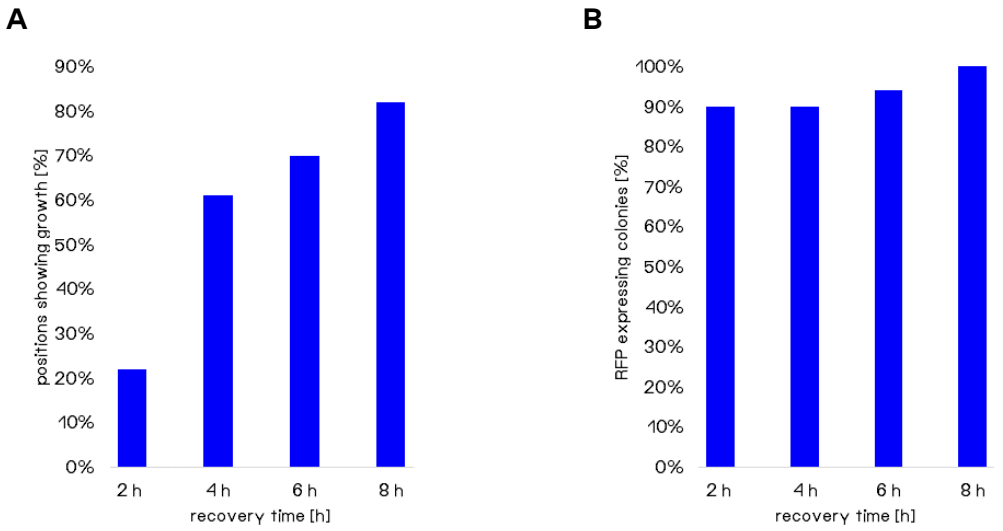


**Figure 3.** *B.SIGHT integration in automated transformation and separation of cells. A) Successful growth of colonies from individual dispensed droplets on LB-agar. B) Successful transformation of colonies that have grown from single bacteria in individual dispensed droplets.*

The B.SIGHT processes 96-well plates in approximately 5 minutes and 384-well plates in approximately 15 minutes using its automated dispensing mode. Speed varies with the cell concentration in the loaded sample. Under optimized conditions, cell growth can be observed in 75% of the cases on LB agar and is higher in liquid medium.

A single-cell isolation platform should be able to guarantee clonality by avoiding dispensing two cells in the same dispensing event. The B.SIGHT high resolution optics allow the detection and enables the dispensing of single bacteria. In addition, the system can be set up to dispense empty droplets that contain no cells which can be used to check the dispensing quality. The system's high-resolution optics ensure that clumped cells can be distinguished and removed from the dispensed library in order to avoid having mixed strains in the wells. Additionally, the B.SIGHT's gating parameters enable sorting based on morphology such as shape, size and fluorescence (Figure 4).

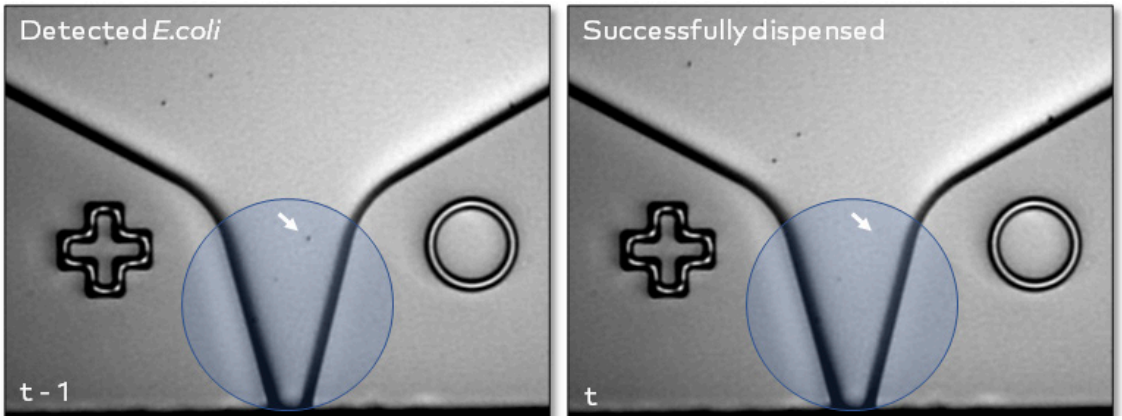


**Figure 4.** *Identified single E. coli cell at the nozzle of the B.SIGHT cartridge. The inner circle represents the detection volume that will be dispensed into the well as a single droplet. The selection criteria according to size and shape were recognized and as evident by the empty exclusion zone the cell can safely be dispensed with any cross contamination by other cells.*

# Conclusion

The B.SIGHT is used to isolate single strains for use in transformation and microbial screening methods. The transformation and cloning workflow presented in this study can be easily adapted to any workflow that uses strains with varying traits. The B.SIGHT is flexible in its ability to deposit single cells with image confirmation, both into liquid culture and onto solid media. Additionally, its small footprint is compatible with laminar flow hoods.

# In summary

1. The B.SIGHT workflow provides fast and reliable single-cell isolation of bacteria after transformation.

2. The workflow can be adapted to a wide range of applications such as creating mutant libraries or developing cell factories with new pathways.

3. The workflow presented here can be adapted for any bacterial strains with varying traits.

4. The workflow provides high efficiency and a high degree of automation that accelerates transformation and cloning workflows.

5. Fully automated and high-throughput cloning of bacteria enhances bacterial cultivation and saves time and costs, while minimizing manual labor. The workflow takes less time and uses significantly fewer plates for the same number of clones compared to colony picking robots.

# References

1. Riba J, Gleichmann T, Zimmermann S, et al. Label-free isolation and deposition of single bacterial cells from heterogeneous samples for clonal culturing. Scientific Reports. 2016; 6: 32837. DOI: 10.1038/srep32837

2. Wiegand S, Dam HT, Riba J, et al. Printing microbial dark matter: using single cell dispensing and genomics to investigate the patescibacteria/candidate phyla radiation. Frontiers in Microbiology. 2021; 12. DOI: 10.3389/fmicb.2021.635506

3. Afrizal A, Hitch TCA, Viehof A, et al. Anaerobic single-cell dispensing facilitates the cultivation of human gut bacteria. Environmental Microbiology. 2022. DOI: 10.1111/1462-2920.15935

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