

CHALLENGES IN AUTOMATION OF MICROFLUIDIC ULTRA HIGH THROUGHPUT SCREENING FOR CELL LINE AND MICROBIAL STRAIN DEVELOPMENT

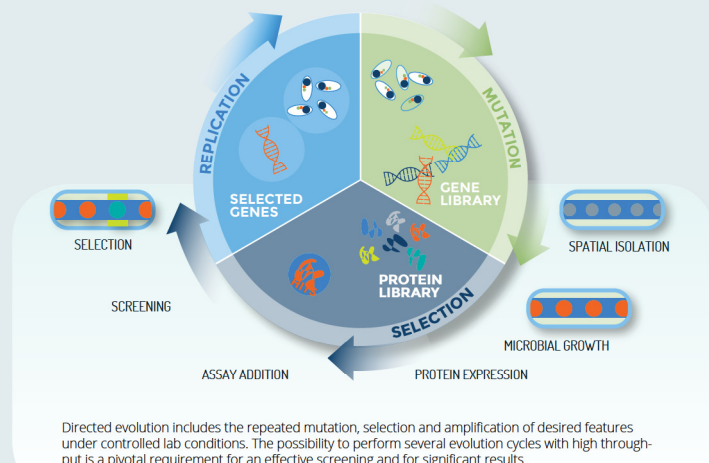
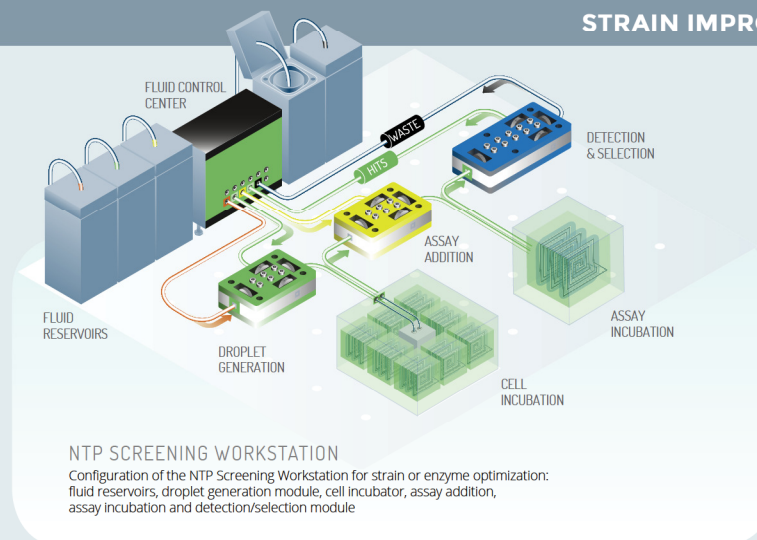
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Directed Evolution is a powerful tool for improving and optimizing microbial strains or cell lines for pharmaceutical and industrial applications. We present an innovative microfluidic approach for screening cellular and gene libraries with the entire screening workflow fully au-

tomated. The microfluidic setup offers a number of advantages over workflows based on microtiter plates, in particular low consumption of samples and reagents, a better comparability of flow and growth parameters for scale-up processes and an ultrahigh-throughput regime.

To demonstrate that the NTP (Nano Titer Pipe) workstation is suitable for optimization approaches such as screening in directed evolution, handling, flow characteristics, growth and detection limits of a number of different microorganisms were tested.

STRAIN IMPROVEMENT AND OPTIMIZATION VIA DIRECTED EVOLUTION



VERSATILE APPLICATIONS IN SCREENING SETUPS

BROAD RANGE OF MICROORGANISMS



Lactococcus lactis



Bacillus subtilis



Saccharomyces cerevisiae



Xanthophyllomyces dendrorhous

Droplet Generation

Droplets are generated with an active dosing system with valves and pressure control, resulting in reproducible and identical conditions for each droplet. The droplet size can be controlled by adjusting the frequency of the droplet generation.

Droplet Size

Droplet size is adjustable and can be between 20 nl and 100 nl. This volume range has been proven to be advantageous regarding growth conditions of microorganisms.

Applied Growth Media, e.g.:

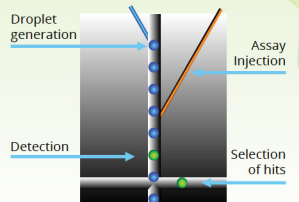
Complex growth media (LB-Medium, YM-Broth)

Synthetic media

Natural growth media (milk)

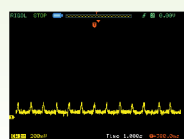
3 Assay Addition and Assay Incubation

As basis of many biological and chemical functionalities, assay reagents can be added individually to each or a predefined sequence of droplets. The volume of the assay addition is variable and can be adjusted between 50 and 100% of the sample volume.

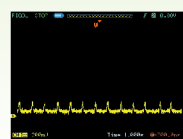


Detection

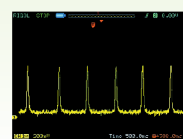
To detect cells or enzymes featuring the desired characteristics, several detection methods have been applied: highly sensitive fluorescence intensity with a picomolar detection limit, fluorescence anisotropy, bioluminescence or light scattering.



Bioluminescence



Fluorescein 10 pM

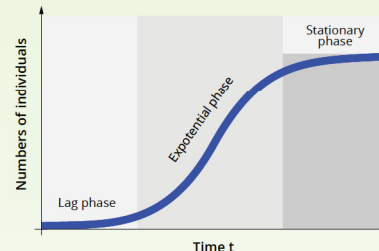


Fluorescein 100 pM

Cell Incubation

The workstation's incubation system enables the incubation of cells with or without assay addition. Incubation time is flexible, incubation conditions (time, temperature, gas supply) are controllable and identical for each sample. Because the system is closed, evaporation and contamination are avoided. As each droplet is incubated under controlled conditions, detection and selection is possible at any time in the growth cycle.

TIME-RESOLVED DETECTION IN LOG-PHASE:



CULTIVATION IN NANOTITERPIPES

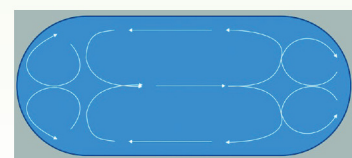


Cell viability

Cell viability in the pipe system is high, with growth rates comparable to fermenter incubation. Temperature, O₂ and CO₂ transfer are controlled and mixing during transport through the system is optimal.

Simulation of flow conditions inside a droplet

The results of simulating exact flow conditions within a droplet while it is in motion confirm real picture analysis. They show continuous fluid mixing within moving droplets.



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Selection

Selection and separation of the detected hits is based on a user defined hit threshold.

Based on statistical methods, the workstation then sets the detection level automatically. Alternatively, the user can manually set and adjust the detection level in real time.

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Summary and Outlook

Experiments performed with the microfluidic NTP screening workstation showed very promising results. Several different strains of microorganisms in a variety of media were tested for stability of generated droplets and for flow and growth conditions in the system. Detection limits of fluorescence dyes in the detection module

of the workstation have been shown to be in the picomolar range. Flexibility and adjustability of the system combined with technical stability and a fully automated workflow allow for exploiting the complete potential of directed evolution with an extremely high throughput of up to 10 mio. samples per day (data shown elsewhere).

A wide variety of applications is possible. In particular screening of filamentous fungi, which is of high industrial relevance and has been shown by other authors to be possible in nl-sized droplets, is a highly promising application and will be evaluated by the authors in near future.