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The application of microfluidic-based technologies in the cycle of metabolic engineering

Xiaoyan Ma, Yi-Xin Huo*

College of Life Science, Beijing Institute of Technology, 5 South Zhongguancun Street, Haidian District, Beijing, 100081, China

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ABSTRACT

The process of metabolic engineering consists of multiple cycles of design, build, test and learn, which is typically laborious and time-consuming. To increase the efficiency and the rate of success of strain engineering, novel instrumentation must be applied. Microfluidics, the control of liquid flow in microstructures, has enabled flexible, accurate, automatic, and high-throughput manipulation of cells in liquid at picoliter to nanoliter scale. These attributes hold great promise in advancing metabolic engineering in terms of the phases of design, build, test and learn. To promote the application of microfluidic-based technologies in strain improvement, this review addressed the potentials of microfluidics and the related approaches in DNA assembly, transformation, strain screening, genotyping and phenotyping, and highlighted their adaptations for single-cell analysis. As a result, this facilitates in-depth understanding of the metabolic network, which in turn promote efficient optimization in the following cycles of strain engineering. Taken together, microfluidic-based technologies enable on-chip workflow, and could greatly accelerate the turnaround of metabolic engineering.

Contents

1. Introduction

The development of cell factories undergoes multiple cycles of design, build, test and learn. Each cycle is likely to improve the yield, efficiency and the stability of the strain, and ultimately, the desired strain capable of industrial production is acquired. However, the robust and complex metabolic network is usually insusceptible to manipulations, thus a single cycle of strain development can take months and typically nearly a decade with millions of dollars spent to meet with industrial requirements [1]. This greatly

* Corresponding author.
E-mail address: huoyixin@bit.edu.cn (Y.-X. Huo).

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hinders the advancement of sustainable and green industry.

To speed up the process of strain construction and exploit the potential of cell metabolism, a series of techniques have been developed and applied to different levels of metabolic engineering, such as the tuning of the promoter strength at the gene level [2], the elimination of the competing pathways [3], and the identification of additional beneficial enzymes by omics analysis of the whole cell system [4]. However, conventional methods are often limited by the throughput, precision, cost, convenience and the compatibility with other assays. These defects were overcame by microfluidics, which is the technology of manipulating and controlling the flow of liquids inside micrometer-sized channels, reagent wells and reaction chambers. It usually appears in two forms: stream of fluids and droplet. The latter is comprised of two immiscible fluids such as buffer and oil, droplets are formed via shearing one into the other [5]. The microfluidics are characterized by laminar flow, surface effects, short diffusion length and small volume [6,7]. Laminar flow induces a predictable stream behavior and minimal mixing, and surface effects facilitate capillary liquid drawing, interfacial transport and reactions. Meanwhile, the minute length and volume scale enable rapid changes in temperature and chemical composition. These attributes ensure accurate control over the flow at picoliter to nanoliter scale under automated mode, hence higher throughput and precision (Table 1). Along with reduced cost and labor, as well as flexible integration with sensors, actuators, and controllers, the microfluidic methods exhibit promising applications in the systems level of strain optimization, offering unprecedented opportunities for improving yield and strain stability [8].

To encourage the implement of microfluidics in metabolic engineering, we reviewed the revolutions brought by this approaches in the key processes of strain development including design, build, test and learn. Meanwhile, we emphasized the realization of single-cell analysis for metabolic engineering thanks to the combined utilization of microfluidics and the downstream approaches. We believe that the microfluidic technologies could accelerate the cycle of metabolic engineering toward a rapid turnaround by following its own “Moore's law”, just like the electronic industry.

2. Microfluidics in the realization and decision-making of metabolic design

The process of metabolic engineering begins with the step of design. It includes the selection of host and the creation of the synthetic pathways capable of overproducing the chemicals of interest. The blueprint of metabolic pathways is usually drafted *in silico*, with the aid of computer algorithms to design the most feasible and efficient routes using the data from a set of databases.

Although the design phase does not involve with *in vivo* or *in vitro* operations, it is linked with microfluidic-based technologies (Fig. 1). Their application in metabolic design was attributed to the high-throughput and high-resolution of microfluidics. Due to their higher throughput than conventional approaches, combining microfluidics with supporting algorithms will reduce labor in the process of metabolic design [9]. For instance, microfluidic trapping devices have recently been used to characterize the biophysical properties of transcription factor binding sites at a throughput up to 4000 [10]. With the aid of computational tools capable of detecting motifs in large dataset [11], the proper binding strength could be easily selected and incorporated into the genetic design. The advantage of high-resolution enables precise design of the metabolic pathway. For instance, metabolic information collected at the scale of single cells by using droplet microfluidics considers the variation of individuals rather than the metabolic state of the whole cell population. This increases the accuracy of the estimated coefficients in the flux balance analysis and may also uncover new pathways, enabling more reliable calculation of the theoretical yield and the allocation of metabolites [12], thus guiding the design of the optimal metabolic pathway that achieves the highest yield. Moreover, the resources, rules and tools for metabolic designs are increasing rapidly, which broadened the diversity of chemicals that could be produced by metabolic engineering [13]. This also calls for coupled improvement of the analytical technologies to fulfill the design space in order to exploit the potentials of metabolic engineering in modern industry. Compared with conventional approaches, microfluidics is one of the most cutting edge technologies featuring high sensitivity, accuracy, high-throughput and other advantages, which can expedite the realization of metabolic design. At last, the initial design is mostly always flawed. The errors or bottlenecks must be identified and revised in the subsequent redesigning. Microfluidics enables the integration of the omics platform [14]. Data acquired from the systematic studies of metabolomics, proteomics, genomes and transcriptomes provide quantitative information of the interactions within the complex cell network, offering new strategies for strain improvement. For example, the integration of transcriptomic and proteomic analysis has been used to uncover the competing pathway of phosphoenol pyruvate carboxykinase in the production of threonine, deletion of the related targets increased the productivity of threonine by more than 40% [15].

3. Microfluidics facilitates strain building

The build step introduces exogenous pathways into the host and directs the metabolic fluxes towards the production of desired chemicals in accordance to the prior design. This phase involves the synthesis, assembly and transformation of DNA into the chassis host, for which microfluidic platforms have recently been introduced to expedite this process.

3.1. DNA assembly

To obtain novel pathways, fragments of DNA encoding different functions must be assembled into the vector. The traditional manual procedures were laborious and time-consuming. Although the automatic robotic techniques have reached the level of high-throughput, their application was limited by the high cost in equipments and consumable assays [16]. Fortunately, microfluidics overcome these defects by reducing processing time, reagents consumption (100-fold), DNA loss, and by offering facile control

Table 1
The characteristics of microfluidics compared with conventional approaches.

Approaches	Sample volume	Throughput	Integration	Manipulation	Cost	Labor	Solution mixing	Molecule adsorption[a]
Conventional	Microliter	Low (usually $<10^7$)	Complex	Difficult	High	High	Fast	Difficult
Microfluidics	Pico to nanoliter	High (can reach 10^9)	Flexible	Easy	Low	Low	Slow	Easy
Pros or cons	✓	✓	✓	✓	✓	✓	✗	✗

[a] Molecule adsorption is the adsorption of hydrophobic molecules to the surface due to the large surface-to-volume ratios of microfluidic devices, which may affect the concentrations of reagents.

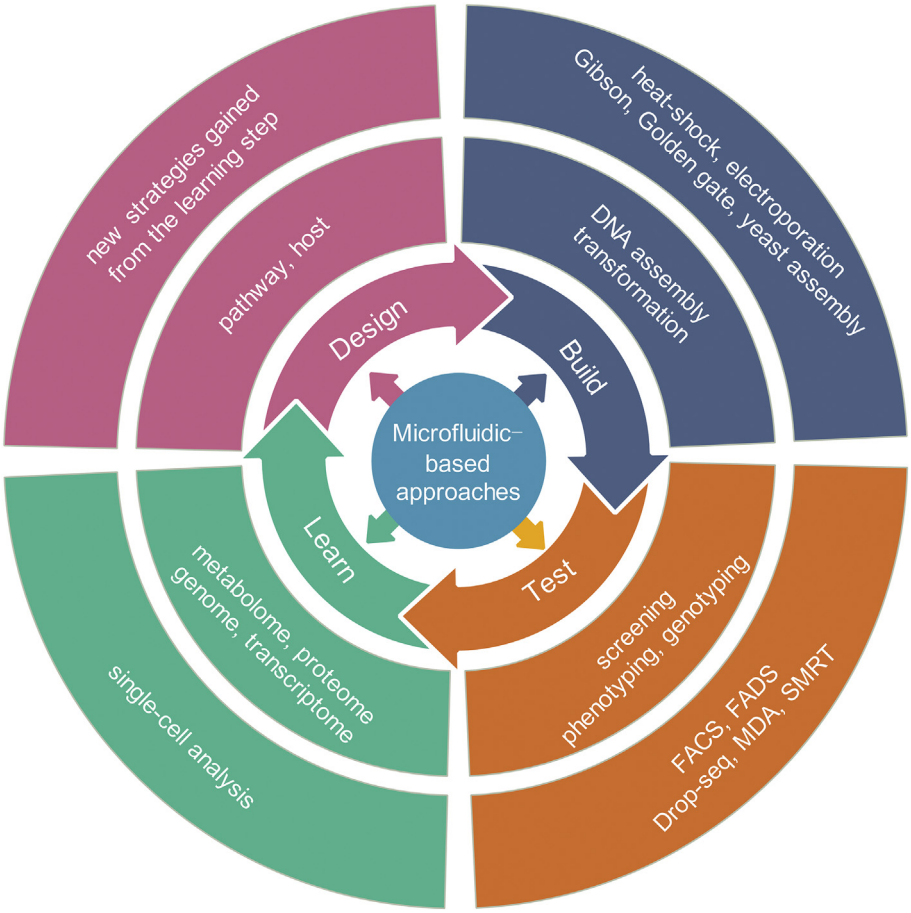

Fig. 1. The application of microfluidic-based approaches in the cycle of metabolic engineering. The inner circle indicates the steps where microfluidic approaches could be applied; the middle circle shows the specific contents; the outer circle lists the combined strategies or methods. FACS: fluoresence-activated cell sorting, FADS: fluoresence-activated droplet sorting, MS: mass spectrometry, MDA: multiple displacement amplification, SMRT: single molecule, real-time sequencing.

over multiple flows simultaneously. For example, microfluidic chips with different functional components have been designed to integrate DNA digestion and ligation into a single run [17]. Digital microfluidic devices imbedded with electrodes have achieved even higher precision and reproducibility [18]. These platforms have been successfully adopted to generate large libraries of plasmids following protocols such as Gibson assembly, Golden gate assembly, and yeast assembly [19].

3.2. Transformation

After construction, the plasmids must be transferred into the cell to perform functions. The efficiency of transformation ensures the size of the genetic libraries, which correlates positively with the probability of the occurence of the desired mutant. By incorporating either heat-shock or electroporation procedures, transformation using microfluidic platforms have been proved to achieve at least the comparable efficiencies for *E. coli* and *S. cerevisiae* as the tube-based approaches [19,20]. Moreover, the microfluidic-based approaches are much cheaper and convenient. Further optimization such as the utilization of magnetic beads can improve the transformation efficiency up to 2-fold [21]. These advantages allowed routine construction of large genetic libraries.

4. Increased efficiency and accuracy in the test step

The test step validates the efficacy of the design and build phases in achieving the desired properties. It includes the characterization, screening and analysis of the engineered strain in terms of cell phenotype and genotype.

4.1. High-throughput screening

The build process generates large genetic libraries with high diversity, which have to be screened based on the phenotypic or genotypic characteristics of the cells. Since the occurence of the target properties can be rather infrequent, usually 10^{-4}, the size of the mutant libraries is typically 10^5, with maxima up to 10^{12} [22]. Accordingly, high-throughput analysis is essential for detecting the desired mutant from such a large pool. Traditional screening was performed using microtiter well plates, and the contents of the metabolites of interest are measured as indicators for screening. This method is low-throughput, time consuming, laborious and costly, thus is less likely to acquire the optimal strains.

In contrast, microfluidic platforms can achieve high efficiency by using the fluorescent properties of the cells as reporters. For small molecules that emit no spectral signature, fluoresence could be realized by linking the fluorescent gene to the gene of interest. Another strategy is to use biosensors. These are substrates capable of entering cells or attached to the cell membranes, and can be converted to fluorescent reporters by enzymatic reactions to reflect the contents of the desired metabolites [5]. These enabled the application of fluoresence-activated cell sorting (FACS) for the screening of genetic libraries [22]. The FACS is suitable for stream-

based microfluidics and works as microscaled flow cytometry, which increases the throughput to 10^8 variants per day [23]. However, this method only allows the detection of intracellular metabolites, or metabolites attached to the cell membrane without diffusing into the fluid. For secretory metabolites, the latest fluoresence-activated droplet sorting (FADS) serves the purpose [24,25]. The droplet microfluidics isolates single cell into individual compartment and preserves the unique phenotypic characteristics of the cell. By employing electrical, acoustic, magnetic, optical, hydrodynamic and mechanical approaches [26], the FADS droplet microfluidics has enabled a high screening rate of 10^6 cells per hour. Further modification such as device parallel can increase the throughput by another three orders of magnitude [27]. Due to this merit, microfluidic platforms have been successfully applied to strain screening. For instance, yeast strains capable of over-consuming xylose were acquired using microfluidics from a library of 10^5 clones [27], and at a rate of 300 droplets per second, strains with high α-amylase yield were isolated in a total of 10^6 cells within 3 h [25].

Another major advantage of microfluidics is the facilitation of fine-scale spatial and temporal control of the cells. By using microwells, microchambers, droplets, hydrogels and other contact or contactless trappings, cells could be easily manipulated in zero to three dimensional spaces [5,26]. This enables batch cultivation of single or multiple cells in the upstream or downstream of the microfluidics workflow. For instance, the droplet trapping has been used for short-term culturing of the single cells, allowing secretory metabolites to accumulate and ready for FADS screening [27,28]. Moreover, the high controllability of microfluidics enables facile changes in the microenvironment around the cell. This could be adapted to optimize strain stability, such as the ability to withstand concentrated end product, as well as the ability to sustain high productivity over the course of cell growing phases.

4.2. Single cell analysis

Strain screening is often coupled with phenotype and genotype of the cells to validate the manipulations of the pathway, which enable global analysis of the cellular metabolites. Conventionally, the measurement is based on the average information of a whole cell population. However, cells are highly heterogeneous systems. Due to stochastic gene expression, cell cycle, aging, epigenetic regulation and microenvironments, they can present unique properties that differ significantly from the mean levels in terms of gene translation and expression, even for isogenic populations in identical environments [26,29]. Thus, single cell analysis at the systems level can provide new possibilities to uncover rare mechanisms of the metabolic regulations, which may lead to great improvement in strain performances.

The feasibility of single cell analysis in metabolic engineering depends on two factors: cell sorting and downstream assays. The throughput issue concerning cell sorting is better fulfilled by automated droplet microfluidics using FADS method, in comparison to the conventional approaches such as optical tweezers, serial and microwell dilutions [30]. On the other hand, the sensitivity, fidelity and throughput become the major challenges for downstream measurements. Fortunately, these issues have been addressed by the recent development in analytical technologies.

The analysis of proteomics and metabolomics for single cells has been realized thanks to the increased sensitivity and resolution of the latest mass spectrometry (MS). The MS has been the ideal device to identify and quantify the complex mixtures containing thousands of analytes in biological samples [31]. The detection limit of the latest MS devices has reached attomole level [32], which is suitable for the analysis of proteins and metabolites from single cells. However, the low throughput of MS-based approaches still confines their utilization in large-scale analysis. Recent solutions such as mass spectrometry imaging has increased the number of measured samples per run [33]. Nevertheless, future development of MS-based approaches should incorporate both the sensitivity and throughput. One promising solution is the integration of microfluidics, by reducing sample volume, it improves MS sensitivity and resolution, and facilitate parallel detection for large-scale analysis. This will allow unrestricted access to diverse types of trace molecules, which could be more relevant to the desired metabolic characteristics. The protein expression, enzymatic activity and metabolite levels can also be extracted from the identical cells, enabling the establishment of direct metabolite-protein interactions, and subsequent evaluation of their feedbacks on gene expression and signaling [34].

For the measurements of cell genome and transcriptome, one strategy to increase throughput is double barcoding, which tags both the cell and the genetic molecules. The method is based on droplet microfluidics and is hence referred to drop sequencing (drop-seq) [35]. Take transcriptome for instance, the cell barcode usually consists of 12 bases that has the potential to distinguish 4^{12} (16,777,216) individual cells, for each cell barcode, a unique molecular identifier of 8 bases is then attached, enabling it to mark 4^8 (65,536) different mRNAs in a single cell. The barcode and cell are co-encapsulated in a droplet, after the binding of mRNAs and reverse transcription. The transcriptome from a single cell is tagged uniquely, allowing parallel sequencing in a single run [35,36]. The current throughput for drop-seq is 10^4 cells per day, although still an order of magnitude lower than the typical size of a mutant libraries, it already shortened the processing time by > 100-fold comparing with the existing methods [35], and has realized routine genotyping of single cells. This can rapidly reveal significant differences in the gene transcription level among individual cells, aiding parallel validation of the efforts in modifying targeted genes. The comparative analysis of genome and transcriptome among different cells helps to identify the key genetic factors that endow the desired phenotypes, facilitating directed manipulation of other strains in the process of inverse metabolic engineering [37]. Moreover, the single-cell omic analysis captures a “snap-shot” of the cell status, a series of “snap-shots” will create a dynamic record of the cellular metabolism, offering timing and causal analysis of the metabolic regulation mechanisms.

The fidelity of the amplified genome is crucial for single-cell analysis since the initial copy number is only one. The traditional PCR-based method is highly biased due to its low coverage and high rate of amplification error [30], thus is not suitable for single-cell analysis. A better strategy is to use isothermal approaches such as multiple displacement amplification (MDA) [38]. This method generates greater genome coverage with lower error rate compared to PCR-based methods. However, the lack of uniformity for MDA method may introduce bias in the final data. To compensate these defects, hybrid methods such as multiple annealing and looping-based amplification cycles (MALBAC) has been proposed to strike a balance between the PCR-based and the MDA methods. The MALBAC achieves medium coverage and error rate with high uniformity, and is considered an alternative for MDA method [30]. In terms of single cells, the amplification bias for both MDA and MALBAC methods are rather similar, but with different preferences. The former one has much lower error rate whereas the latter is better at detecting variations in copy numbers. In all, the amplicon-based methods are intrinsically flawed for single cell genotypic analysis.

The third-generation sequencing technologies realized an amplicon-free, single molecule-based direct DNA or RNA sequencing [39], which eliminates the bias resulted from the

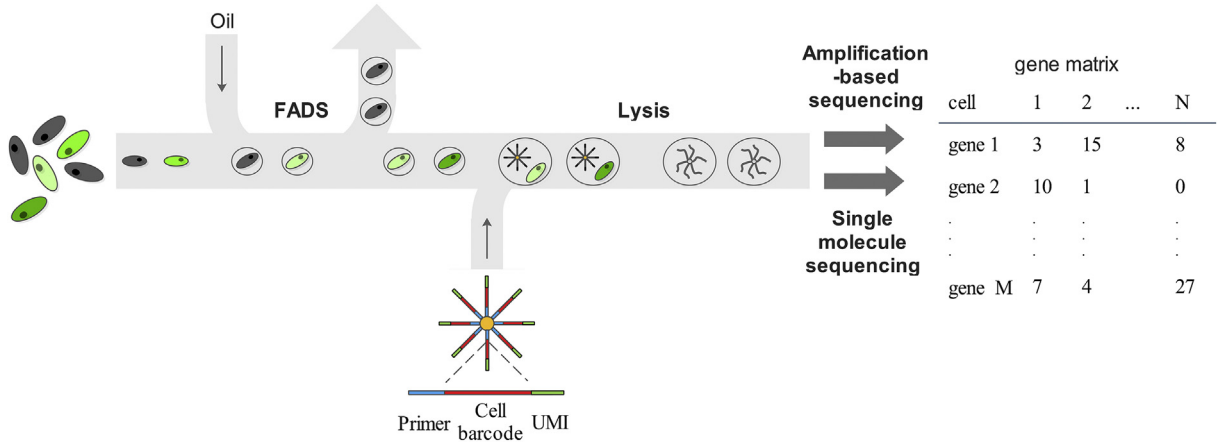

Fig. 2. The schematic of the integration of droplet microfluidics, cell barcoding and sequencing techniques for single cell genetic analysis. FADS: fluoresence-activated droplet sorting; UMI: unique molecular identifier.

amplification step. One of the most well-known methods is the single molecule, real-time (SMRT) sequencing. It adopts a sequencing-by-synthesis approach in which a circular template, usually a DNA molecule, is targeted by a single DNA polymerase. The type of nucleotide is identified when it is incorporated by the DNA polymerase, according to changes in the fluorescence of the nucleotide. Another advantage of SMRT is the long reads length up to 20 kb [40], which facilitates accurate genome assembly. The error in SMRT sequencing derives primarily from insertions, followed by deletions, and a small chance of mismatch, which are all random and will not increase with the increase of read length [41]. It has been claimed that the SMRT was of low accuracy [42], in fact, this only applies to a single read (~90% accuracy) [43]. By increasing sequencing depth and implementing a circular consensus sequence, the SMRT can achieve a final accuracy up to 99.999% [44,45].

An alternative single molecule sequencing method is the nanopore sequencing, in which the nucleotide is identified by detecting the changes in current when strands of DNA pass through the nanopore [46]. Besides advantages of amplification-free and long read length, this method also offered a high portability and easy preparation of the templates [47]. However, compared with SMRT, the accuracy of nanopore sequencing requires further improvement [48]. Nevertheless, supported by microfluidic-based cell sorting and efficient barcoding, the single molecule sequencing techniques hold great promise for single cell genotypic analysis (Fig. 2).

5. Microfluidics enables deep learning

Learn is usually neglected in current metabolic engineering practice, yet perhaps the most crucial step to ensure successful engineering. The learn phase investigates the test results and guides subsequent strain design and build. This step typically lacks robust, targeted and systematic data to generate global profiles of the metabolic networks [1,14]. Fortunately, the development in microfluidic-based and related approaches encourages reexamination of this process.

The microfluidics revolutionized the learning of metabolic engineering by providing big data, increasing specificity, and deepening the systematic analysis to the level of single cells. The high-throughput construction of genetic libraries increases the probability of positive mutations, and the subsequent screening techniques facilitate the refinement of targeted information. The single cell analysis may be the most significant advancement for metabolic engineering. It enables a closer look at the diverse regulatory mechanism of the cell, and reveals the rare but key pathways which are easily masked by the mean data. Together with the facile manipulation of cells in microfluidic platforms, it is able to study the genetic and phenotypic responses of cells to different micro-environments and cell cycles. For instance, microfluidic coupled with optical tweezers was able to manipulate the microenvironment around single cells in less than 2 s, and has been used to study the intracellular responses of single yeast cells to changes in glucose availability [49]. Using microfluidic bioreactor, single cells such as yeast can also be cultivated for generations in constant environment, allowing unbiased investigation of the effects of cell cycle on cell biochemical heterogeneity [50,51]. Findings from these studies can offer new insights for pathway modeling, design and strain construction, and ultimately increase the rate of success.

6. Outlook and conclusions

The microfluidic-based technologies are characterized by high-throughput, small volume, easy control, low cost, and flexible integration with other assays. These qualify microfluidics as the ideal choice for metabolic engineering, which covers the steps of DNA assembly, transformation, strain screening, genotyping, phenotyping, and in-depth systems analysis. Moreover, the combined application of fluorescent-based cell sorting, barcoding and single molecule sequencing opens the gate of single cell analysis for metabolic engineering, which hold great promise in the near future. The era of microfluidic-based metabolic engineering has just begun, and the microfluidic platforms should move toward modularity to speed up its application. This will allow standard components for various steps such as strain build, screening and sequencing to be assembled into an engineering line for different purposes. The seamless and flexible integration of functional units can avoid extra procedures for sample handling and transferring, and enable automation. Taken together, the microfluidic-based technologies hold the potential to miniaturize the process of metabolic engineering to on-chip scale, and greatly reduce the turnaround of the engineering cycle.

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References

[1] Nielsen J, Keasling JD. Engineering cellular metabolism. Cell 2016;6:1185–97.
[2] Alper H, Fischer C, Nevoigt E, Stephanopoulos G. Tuning genetic control through promoter engineering. P Natl Acad Sci USA 2005;36:12678–83.
[3] Kind S, Jeong WK, Schröder H, Zelder O, Wittmann C. Identification and elimination of the competing N-acetyldiaminopentane pathway for improved production of diaminopentane by *Corynebacterium glutamicum*. Appl Environ Microbiol 2010;15:5175–80.
[4] Zhang W, Li F, Nie L. Integrating multiple 'omics' analysis for microbial biology: application and methodologies. Microbiology 2010;2:287–301.
[5] Vasdekis AE, Stephanopoulos G. Review of methods to probe single cell metabolism and bioenergetics. Metab Eng 2015:115–35.
[6] Squires TM, Quake SR. Microfluidics: fluid physics at the nanoliter scale. Rev Mod Phys 2005;3:977–1026.
[7] Kou S, Cheng D, Sun F, Hsing IM. Microfluidics and microbial engineering. Lab Chip 2016;3:432–46.
[8] Lan EI, Liao JC. Microbial synthesis of n-butanol, isobutanol, and other higher alcohols from diverse resources. Bioresour Technol 2013:339–49.
[9] Medema MH, van Raaphorst R, Takano E, Breitling R. Computational tools for the synthetic design of biochemical pathways. Nat Rev Micro 2012;3:191–202.
[10] Fordyce PM, Gerber D, Tran D, Zheng J, Li H, DeRisi JL, et al. De novo identification and biophysical characterization of transcription-factor binding sites with microfluidic affinity analysis. Nat Biotech 2010;9:970–5.
[11] van Hijum SAFT, Medema MH, Kuipers OP. Mechanisms and evolution of control logic in prokaryotic transcriptional regulation. Microbiol Mol Biol Rev 2009;3:481–509.
[12] Orth JD, Thiele I, Palsson BO. What is flux balance analysis? Nat Biotech 2010;3:245–8.
[13] Lee JW, Na D, Park JM, Lee J, Choi S, Lee SY. Systems metabolic engineering of microorganisms for natural and non-natural chemicals. Nat Chem Biol 2012;6:536–46.
[14] Petzold CJ, Chan LJG, Nhan M, Adams PD. Analytics for metabolic engineering. Front Bioeng Biotechnol 2015:135.
[15] Hermann T. Using functional genomics to improve productivity in the manufacture of industrial biochemicals. Curr Opin Biotechnol 2004;5:444–8.
[16] Hillson NJ, Rosengarten RD, Keasling JD. j5 DNA assembly design automation software. ACS Synth Biol 2012;1:14–21.
[17] Wang A-B, Cheng C-W, Lin IC, Lu F-Y, Tsai H-J, Lin C-C, et al. A novel DNA selection and direct extraction process and its application in DNA recombination. Electrophoresis 2011;3–4:423–30.
[18] Choi K, Ng AHC, Fobel R, Chang-Yen DA, Yarnell LE, Pearson EL, et al. Automated digital microfluidic platform for magnetic-particle-based immunoassays with optimization by design of experiments. Anal Chem 2013;20:9638–46.
[19] Shih SCC, Goyal G, Kim PW, Koutsoubelis N, Keasling JD, Adams PD, et al. A versatile microfluidic device for automating synthetic biology. ACS Synth Biol 2015;10:1151–64.
[20] Gach PC, Shih SCC, Sustarich J, Keasling JD, Hillson NJ, Adams PD, et al. A droplet microfluidic platform for automating genetic engineering. ACS Synth Biol 2016;5:426–33.
[21] Chung Y-C, Chen Y-S, Lin S-H. Enhancement for gene transfection of low-descent-velocity bacteria using magnetic attraction in electroporation chip. Sens Actuators B 2015:261–7.
[22] Dietrich JA, McKee AE, Keasling JD. High-throughput metabolic engineering: advances in small-molecule screening and selection. Ann Rev Biochem 2010;1:563–90.
[23] Yang G, Withers SG. Ultrahigh-throughput FACS-based screening for directed enzyme evolution. ChemBioChem 2009;17:2704–15.
[24] Kim HS, Guzman AR, Thapa HR, Devarenne TP, Han A. A droplet microfluidics platform for rapid microalgal growth and oil production analysis. Biotechnol Bioeng 2016;8:1691–701.
[25] Sjostrom SL, Bai Y, Huang M, Liu Z, Nielsen J, Joensson HN, et al. High-throughput screening for industrial enzyme production hosts by droplet microfluidics. Lab Chip 2014;4:806–13.
[26] Fritzsch FSO, Dusny C, Frick O, Schmid A. Single-cell analysis in biotechnology, systems biology, and biocatalysis. Annu Rev Chem Biomol Eng 2012;1:129–55.
[27] Wang BL, Ghaderi A, Zhou H, Agresti J, Weitz DA, Fink GR, et al. Microfluidic high-throughput culturing of single cells for selection based on extracellular metabolite production or consumption. Nat Biotech 2014;5:473–8.
[28] Debs BE, Utharala R, Balyasnikova IV, Griffiths AD, Merten CA. Functional single-cell hybridoma screening using droplet-based microfluidics. P Natl Acad Sci USA 2012;29:11570–5.
[29] Vasdekis AE, Stephanopoulos G. Single-cell phenotypic screening in inverse metabolic engineering. In: Lu C, Verbridge SS, editors. Microfluidic methods for molecular biology. Switzerland: Springer; 2016. p. 189–204.
[30] Gawad C, Koh W, Quake SR. Single-cell genome sequencing: current state of the science. Nat Rev Genet 2016;3:175–88.
[31] Nilsson T, Mann M, Aebersold R, Yates JR, Bairoch A, Bergeron JJM. Mass spectrometry in high-throughput proteomics: ready for the big time. Nat Methods 2010;9:681–5.
[32] Kandiah M, Urban PL. Advances in ultrasensitive mass spectrometry of organic molecules. Chem Soc Rev 2013;12:5299–322.
[33] de Rond T, Danielewicz M, Northen T. High throughput screening of enzyme activity with mass spectrometry imaging. Curr Opin Biotechnol 2015:1–9.
[34] Wurm M, Schöpke B, Lutz D, Müller J, Zeng AP. Microtechnology meets systems biology: the small molecules of metabolome as next big targets. J Biotechnol 2010;1–2:33–51.
[35] Macosko Evan Z, Basu A, Satija R, Nemesh J, Shekhar K, Goldman M, et al. Highly parallel genome-wide expression profiling of individual cells using nanoliter droplets. Cell 2015;5:1202–14.
[36] Klein Allon M, Mazutis L, Akartuna I, Tallapragada N, Veres A, Li V, et al. Droplet barcoding for single-cell transcriptomics applied to embryonic stem cells. Cell; 5: 1187–1201.
[37] Çakar ZP. Metabolic and evolutionary engineering research in Turkey and beyond. Biotechnol J 2009;7:992–1002.
[38] Walker A, Parkhill J. Single-cell genomics. Nat Rev Micro 2008;3:176–7.
[39] Thompson JF, Milos PM. The properties and applications of single-molecule DNA sequencing. Genome Biol 2011;2:1–10.
[40] Eid J, Fehr A, Gray J, Luong K, Lyle J, Otto G, et al. Real-time DNA sequencing from single polymerase molecules. Science 2009;5910:133–8.
[41] Carneiro MO, Russ C, Ross MG, Gabriel SB, Nusbaum C, DePristo MA. Pacific biosciences sequencing technology for genotyping and variation discovery in human data. BMC Genomics 2012;1:1–7.
[42] Shapiro E, Biezuner T, Linnarsson S. Single-cell sequencing-based technologies will revolutionize whole-organism science. Nat Rev Genet 2013;9:618–30.
[43] Xu M, Fujita D, Hanagata N. Perspectives and challenges of emerging single-molecule DNA sequencing technologies. Small 2009;23:2638–49.
[44] Chin C-S, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, et al. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat Methods 2013;6:563–9.
[45] Guo X, Lehner K, O'Connell K, Zhang J, Dave SS, Jinks-Robertson S. SMRT sequencing for parallel analysis of multiple targets and accurate SNP phasing. G3-Genes Genomes Genet 2015;12:2801–8.
[46] Eisenstein M. Oxford Nanopore announcement sets sequencing sector abuzz. Nat Biotech 2012;4:295–6.
[47] Hyun B-R, McElwee JL, Soloway PD. Single molecule and single cell epigenomics. Methods 2015:41–50.
[48] Loman NJ, Watson M. Successful test launch for nanopore sequencing. Nat Methods 2015;4:303–4.
[49] Eriksson E, Sott K, Lundqvist F, Sveningsson M, Scrimgeour J, Hanstorp D, et al. A microfluidic device for reversible environmental changes around single cells using optical tweezers for cell selection and positioning. Lab Chip 2010;5:617–25.
[50] Kortmann H, Chasanis P, Blank LM, Franzke J, Kenig EY, Schmid A. The Envirostat - a new bioreactor concept. Lab Chip 2009;4:576–85.
[51] Roman GT, Chen Y, Viberg P, Culbertson AH, Culbertson CT. Single-cell manipulation and analysis using microfluidic devices. Anal Bioanal Chem 2007;1:9–12.