Trends in Biotechnology

CellPress REVIEWS

Review

High-Throughput Screening Technology in Industrial Biotechnology

Weizhu Zeng,[1,2,4] Likun Guo,[1,2,3] Sha Xu,[1,2,3] Jian Chen,[1,2,3,4] and Jingwen Zhou[1,2,3,4,*]

Based on the development of automatic devices and rapid assay methods, various high-throughput screening (HTS) strategies have been established for improving the performance of industrial microorganisms. We discuss the most significant factors that can improve HTS efficiency, including the construction of screening libraries with high diversity and the use of new detection methods to expand the search range and highlight target compounds. We also summarize applications of HTS for enhancing the performance of industrial microorganisms. Current challenges and potential improvements to HTS in industrial biotechnology are discussed in the context of rapid developments in synthetic biology, nanotechnology, and artificial intelligence. Rational integration will be an important driving force for constructing more efficient industrial microorganisms with wider applications in biotechnology.

Challenges in Breeding Industrial Microorganisms

Industrial biotechnology uses microorganisms and enzymes to produce numerous important products such as chemicals, fuels, enzymes, antibiotics, materials, and healthcare products. The microorganisms employed in industrial biotechnology are commonly called **cell factories** (see Glossary) [1,2]. Naturally isolated microorganisms can rarely be directly used for industrial-scale production because of low yields and weak tolerance to harsh industrial conditions. Various strategies have been developed to overcome these problems, including reconstructing cell factories through modifying the physiological functions of the microorganism at the gene level, and by providing optimum environments for strain growth and product accumulation [3–5].

To increase the accumulation of target products, several random mutagenesis and rational engineering approaches, including physical and chemical mutagenesis [6], **adaptive laboratory evolution (ALE)** [7], and **genetic and metabolic engineering** [8,9], have been developed. It is crucial to develop methods for rapidly screening microbial strains in a large library of mutants because the probability of a beneficial mutation can be very low ($<1/10^5$). The efficiency of conventional screening is significantly limited by low throughput and slow detection methods, leading to high costs for screening large numbers of mutants [10,11]. Therefore, HTS procedures have been widely used to isolate high-producer microbial cell factories (Figure 1, Key Figure). Two aspects need to be carefully considered in the selection of industrial microorganisms: how to expand the scope of the screening (generation of a diverse screening library and enabling technologies for expanding the search range), and how to enhance the recognition of target products (analytical methods to detect specific target products biosynthesized from mutated or engineered microorganisms). Recent reviews have summarized commonly used strategies for mutant library construction [12] and analysis [13]. This review mainly focuses on current strategies and future perspectives of HTS procedures ranging from mutant library generation, enabling technologies for expanding the search range, to the analytical methods used for HTS. Some of the most recent advances and benefits of HTS technology are summarized in Box 1.

Highlights

High-throughput screening (HTS) provides more possibilities for both rational and semirational engineering of microorganisms to obtain better industrial strains.

Various mutagenesis and directed evolution approaches have been developed and applied to constructing more diverse mutant libraries and screening these libraries for more powerful industrial microorganisms.

In addition to detection using UV/visible and fluorescence spectroscopy, screening techniques based on electrochemical sensors and biosensors, as well as on Raman, IR, and near-IR spectroscopy, are being developed to fulfill the diverse requirements of HTS.

Advances in automated systems make HTS less labor-intensive and smarter. Accomplishments in microfluidics and cell sorting have significantly accelerated screening speed and have decreased the cost of screening.

[1]National Engineering Laboratory for Cereal Fermentation Technology, Jiangnan University, 1800 Lihu Road, Wuxi, Jiangsu 214122, China
[2]Key Laboratory of Industrial Biotechnology, Ministry of Education, School of Biotechnology, Jiangnan University, 1800 Lihu Road, Wuxi, Jiangsu 214122, China
[3]Jiangsu Provisional Research Center for Bioactive Product Processing Technology, Jiangnan University, 1800 Lihu Road, Wuxi, Jiangsu 214122, China
[4]Website: http://lbbe.jiangnan.edu.cn/

*Correspondence: zhoujw1982@jiangnan.edu.cn (J. Zhou).

Diverse Screening Library: Mutagenesis and Evolution

Enrichment and Acclimation

A variety of microorganisms exist in nature, including archaea, bacteria, yeasts, actinomycetes, and fungi. These microorganisms often accumulate useful products. This can be enhanced by long-term adaptation to environmental factors including pH, temperature, osmotic pressure, radiation, and mutagenic substances. Therefore, microbial strains accumulating specific fermentation products are first enriched and acclimated from the natural library [14]. The general procedure for screening for a specific strain in a natural library contains the following steps: (i) collecting samples rich in the target strain, (ii) enriching the target strain with a specific medium, (iii) separating pure species, (iv) determining the performance of the separate strains, and (v) identifying the most promising strain through sequencing analysis [15,16].

Mutagenesis Technologies

Mutagenesis has been extensively applied to the refinement of industrial microorganisms and has significantly enhanced many industrial biotechnology processes (Figure 2A). Some commonly used physical mutagenesis approaches include UV light, α-rays, β-rays, γ-rays, and X-rays, as well as chemical mutagenesis approaches using alkylating agents, base analogs, and antibiotics [12]. In recent decades some novel mutation methods such as **atmospheric and room temperature plasma (ARTP)** [17–19] and **heavy ion irradiation** [20,21] have been extensively used for generating industrial microorganisms. Compared with traditional methods, these two methods present several advantages such as higher mutation rates, more random mutation sites, and they do not require toxic and harmful chemicals [21,22]. Currently, mutagenesis is still considered to be a valid route for generating industrial microorganisms, particularly secondary metabolite producers such as actinomycetes and fungi.

Adaptive Laboratory Evolution (ALE)

ALE is based on an important feature of microorganisms: the ability to adapt to different environmental conditions through beneficial mutations in different genes and regulatory regions [23,24]. Typical applications of this method include (i) adapting microorganisms to tolerate harsh environmental stresses such as high osmotic pressure, low/high pH, or high/low temperature [25,26]; (ii) improving the capacity of strains with respect to growth rate, substrate consumption, or product accumulation [27,28]; and (iii) activating potential pathways to synthesize nonnative products or consume nonnative substrates [29,30]. eVOLVER [31] and the microdroplet microbial culture (MMC) system [32] have been designed for microbial high-throughput cultivation and adaptive evolution. eVOLVER is an in-vial continuous cultivation system that allows characterization of adaptive niches for yeast and bacteria. It can efficiently balance throughput, reproducibility, and quality. A minichemostat system equipped with multiple sensors was constructed to characterize yeast physiology under different conditions [33]. MMC is based on combining microdroplet-generating chips with a detection module [34,35]. These systems can achieve large-scale HTS at reduced cost. In addition, based on ALE, genome replication engineering-assisted continuous evolution (GREACE) has been employed to improve L-lysine production in *Escherichia coli* [36] (Figure 2B). ALE could be integrated with strategies of metabolic engineering and **synthetic biology** to construct more efficient microbial cell factories.

Directed Evolution

The biosynthesis of a target product involves a series of enzymatic reactions. A practical route to improve the accumulation of a target product is by enhancing enzyme activities. **Directed evolution** is a powerful method for engineering enzymes and has been widely employed in industrial biosynthesis processes. It can be divided into nonrational design, rational design, and semirational design according to the methods used for building mutant libraries [37,38].

Glossary

Adaptive laboratory evolution (ALE): an approach to change the characteristics of a microorganism through adaptation to a particular environment under selective pressure for a prolonged period.

Atmospheric and room temperature plasma (ARTP): a novel random mutagenesis method based on radio-frequency atmospheric-pressure glow discharge plasma jets which can easily break down C–N bonds, amino groups, and P–O bonds.

CasEMBLR: a marker-free method of multigene assembly based on CRISPR/Cas9 that is mainly used *in vivo* in *S. cerevisiae*.

Cell factories: artificially designed systems of microbial metabolism for converting renewable substances to target products including chemicals, fuels, enzymes, antibiotics, materials, and healthcare products.

COMPASS: combinatorial pathway assembly is a high-throughput cloning method for the balanced expression of multiple genes in *S. cerevisiae*.

CRISPR/Cas: clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas), a genetic editing system developed from the adaptive immune system in archaea/bacteria.

Directed evolution: an approach that mimics the evolutionary process in nature to quickly establish a mutant library from which mutants with desired characteristics can be obtained by high-throughput screening (HTS).

ePathBrick: a synthetic biology platform in *E. coli* for optimizing pathway configuration and for efficiently generating pathway diversity based on a series of engineered ePathBrick vectors.

Flow cytometry (FC): a biological technique applied to achieve high-speed counting and sorting of single cells or other biological particles in suspension based on labeled fluorescence signals.

Genetic and metabolic engineering: a discipline of engineering that genetically modulates living cells or organisms to increase the production of particular desired substances.

Heavy ion irradiation: a random mutagenesis method based on charged particles with an atomic number >2, such as ^{12}C, ^{40}Ar, and ^{58}Ni, which can easily damage single- and double-stranded DNA.

Nonrational design is a random evolution strategy that does not require in-depth understanding of enzyme sequence and structure, and the main models include error-prone PCR, DNA shuffling, and saturation mutagenesis (Figure 2C). Rational design is based on smart computation strategies to simulate the kinetics and evolution of natural enzymes, through which target mutants can be screened in a more efficient way [39,40]. Semirational design is established by combining nonrational and rational designs. A series of semirational strategies have been devised, and semirational design is currently the most widely applied route because it takes advantage of sequence space and screening scale [41].

Random Assembly-Based Strategies

To construct strain libraries in a more rational manner, many random assembly-based strategies have been established, such as the random assembly of promoter–5′-UTR (5′-untranslated region) complexes with genes [42], multiplex automated genome engineering (MAGE) [43], **ePathBrick** [44,45], **CasEMBLR** [46], and **COMPASS** [47] (Figure 2D). Random assembly of genes with promoter–5′-UTR complexes is the most straightforward strategy to optimize the biosynthesis pathway of a target product [48]. MAGE, which was initially developed in *E. coli*, can achieve large-scale programming and evolution through rapid and continuous modification of multiple sites on the chromosome. Its upgraded version CRMAGE has been developed based on **CRISPR/Cas9** and λ Red-mediated recombineering [49]. In addition, EMAGE has been established for eukaryotes such as *Saccharomyces cerevisiae* [50]. The ePathBrick method provides a versatile platform for the rapid design and optimization of metabolic pathways, based on a vector featured in four isocaudamer pairs. CasEMBLR, which can integrate DNA fragments with homologous sequences into specific sites of the genome, is a marker-free method for multigene assembly *in vivo* for *S. cerevisiae*. COMPASS equipped with multilocus CRISPR/Cas9-mediated modification has been employed for the production of β-carotene and coproduction of β-ionone and biosensor-responsive naringenin. These random assembly strategies can be useful for constructing large-scale libraries with numerous different functions and regulatory units which could potentially interact with each other in a flexible manner.

Expanding the Search Range: Enabling Technologies

Spectroscopy Equipment

Implementation of HTS is usually based on accurate high-throughput detection equipment. Microplate readers are the most commonly used equipment in HTS. Upgraded versions include a multilabel plate reader – a single desktop device with multiple detection modes [51]. A detection wavelength in the range 200–1000 nm can be used on a multilabel plate reader to detect absorbance, fluorescence intensity, time-resolved fluorescence, fluorescence polarization, and chemiluminescence [52,53]. This approach been widely applied for detecting cell apoptosis or growth, metabolite content, enzyme activity, etc. [38,54,55]. In addition, researchers have also developed devices that can detect an extended range of chemicals based on Raman [56], Fourier transform IR [57], and near-IR [58] spectra. Some of these devices have been further developed for high-throughput single-cell detection, analysis, and sorting by exploiting fluorescence-activated cell sorting (FACS), **microfluidics**, and optical tweezers [59,60].

Automated Systems

Automation is a significant feature of HTS and is the basis of micro-quantitative experiments and large-scale analyses. HTS integrates a series of continuous automated experimental operations. The equipment involved in classic HTS mainly includes a colony picker, a liquid-handling system, and a multifunctional microplate reader which can be connected and operated under the control of a software system [61,62]. In addition, a series of accessory devices have been modified or redesigned for integration into a HTS system, including PCR machines, centrifuges, refrigerators,

Microbioreactor: a fermentation platform for rescreening following HTS and for the development of initial processes for optimization.
Microfluidics: actuation of small volumes of fluid or droplets, typically at the picoliter to microliter scale, that are manipulated in confined microchannels.
Sandwich lid: a lid designed for microtiter plates (MTPs) which consists of a fixation layer and membrane layer to ensure sufficient oxygen supply and decrease cross-contamination.
Synthetic biology: an interdisciplinary branch of biology and engineering that attempts to develop artificial biological systems by analyzing, understanding, modeling, and simulating with the help of mathematical and computational methods.

Key Figure

Cell Factories for Product Synthesis Facilitated by High-Throughput Screening (HTS)

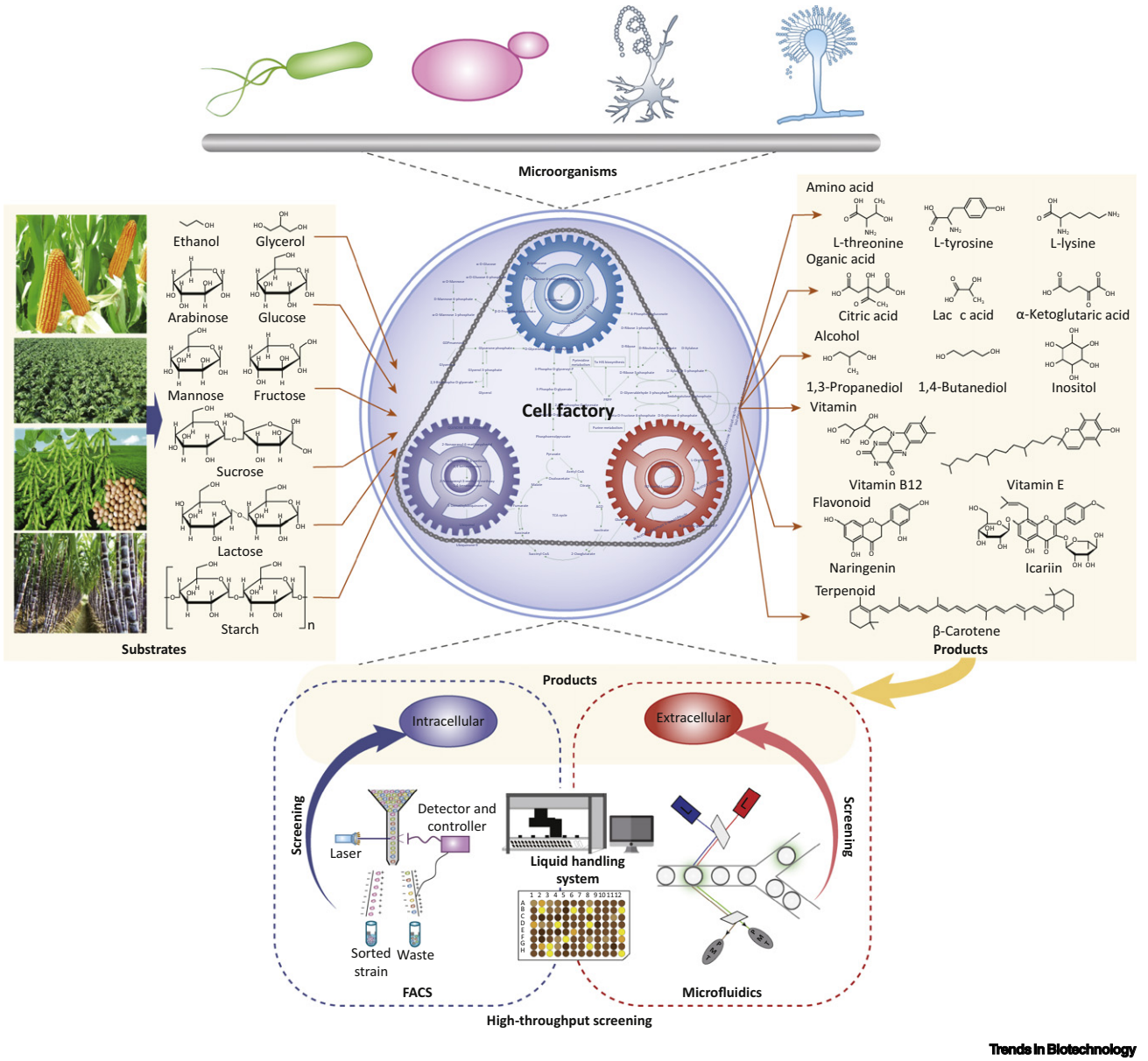


Figure 1. Based on cell factories, substrates (ethanol, glucose, starch, etc.) are converted into a series of products (amino acids, organic acids, alcohols, vitamins, flavonoids, terpenoids, etc.). Common microbial cell factories include bacteria, yeasts, actinomycetes, and fungi. The performance of these microorganisms in accumulating target products can be improved by HTS for intracellular products based on fluorescence-activated cell sorting (FACS) and screening of extracellular products based on microfluidics.

and sterilization and packaging systems. In the past several years, different automated screening pipelines have been established based on FACS and microfluidics for selecting high-performance industrial producers [11,63,64] (Figure 3).

Box 1. Advantages of HTS Technology

HTS is well-established at the molecular and cellular levels. It combines automated and micro-quantitative experiments with analysis of large-scale data [106]. Automated steps include sampling, dilution of samples to a suitable range for detection, transfer of samples and chromogenic agents, mixing samples, washing cells, development of chromogenic or enzyme-linked reactions, color or fluorescence detection, data analysis, and collection of improved strains [61,62]. HTS was firstly developed in the early 1990s, and the subsequent increase in throughput further stimulated the development of technologies in assay miniaturization, automation, and robotics. HTS has now evolved into ultra-HTS screening (uHTS) [107,108]. HTS has become a routine laboratory technique and is widely applied in basic and applied studies in industrial biotechnology [109,110]. The scope of HTS has been considerably expanded with the development of new equipment such as colony pickers [63], liquid handling systems [111], fluorescence-activated cell sorting (FACS) [10], and droplet microfluidics [112].

Compared with traditional screening methods, HTS has significant advantages [11,113], including: (i) more efficient automated operation – advanced equipment has improved automation of HTS, which prevents potential contamination and human error; (ii) fewer human resources are required – the establishment of automated operation systems and cultivation and analysis modes using microplates and FACS has significantly reduced labor costs; (iii) more sensitive and accurate – new assay methods have resulted in fast and accurate screening by detecting changes related to target metabolite content; and (iv) lower sample volumes are required – reliable quantification requires only several microliters (in microplates) or even nanoliters (in droplets), leading to significant decreases in the costs of culture media and reagents.

FACS

Flow cytometry (FC) can quickly analyze multiple parameters of a single cell and rapidly classify target groups in multiple ways. Information from cells can be accurately detected using FC, including cell density and size, internal complexity, and other fluorescence signals. FC is a promising technique for HTS owing to its ability to perform simultaneous quantitative and qualitative analyses [65]. FACS equipped with FC was developed to screen target cells at the single-cell level with high purity and throughput. A series of commercial FACS instruments have emerged and have become an indispensable tool in fundamental biological research and industrial applications. FACS is well known for its ultra-high sorting speed, reaching up to 10^6 cells/s [66]. However, the main disadvantage of FACS is its high nonspecific background, and FACS-screened strains need to be further rescreened by a microplate-based HTS process.

Most target products in industrial biotechnology are extracellular products that avoid intracellular feedback inhibition and can also withstand downstream processing. FACS can only be used for the analysis of intracellular or membrane-bound products that generate fluorescence signals that correlate with the target compounds [67], and the use of FACS for analyzing extracellular products is challenging. Microfluidic FACS, which combines microfluidics and cell sorting, can overcome the limitations of traditional FACS, and has attracted considerable interest owing to its cost-effective identification and separation methods [64,68].

Microfluidics Technology

Microfluidics is a technique for manipulating fluids at the microliter to picoliter scale. It is an emerging interdisciplinary technology involving chemistry, fluid physics, microelectronics, materials, and biology. Microfluidics technology generally employs a microfluidic chip, which is known as a 'lab on a chip' owing to its ability to cover the most basic functions of biological and chemical laboratories. Based on microfluidic chip technology, a droplet-based microfluidic system has been developed for independent single-cell analysis. This has been used in several applications, such as screening for overconsuming/secretory phenotypes and directed enzyme evolution, given its advantages that include high sensitivity, quantitative readout, and accuracy [67,69,70].

Microfluidic chips can be designed that achieve packaging of single cells in a single droplet. Provision of substrates that generate fluorescence signals proportional to product concentration can facilitate FACS analysis of the packaged droplets. The screening efficiency of droplet-based microfluidic systems can be significantly enhanced by combining them with advanced methods

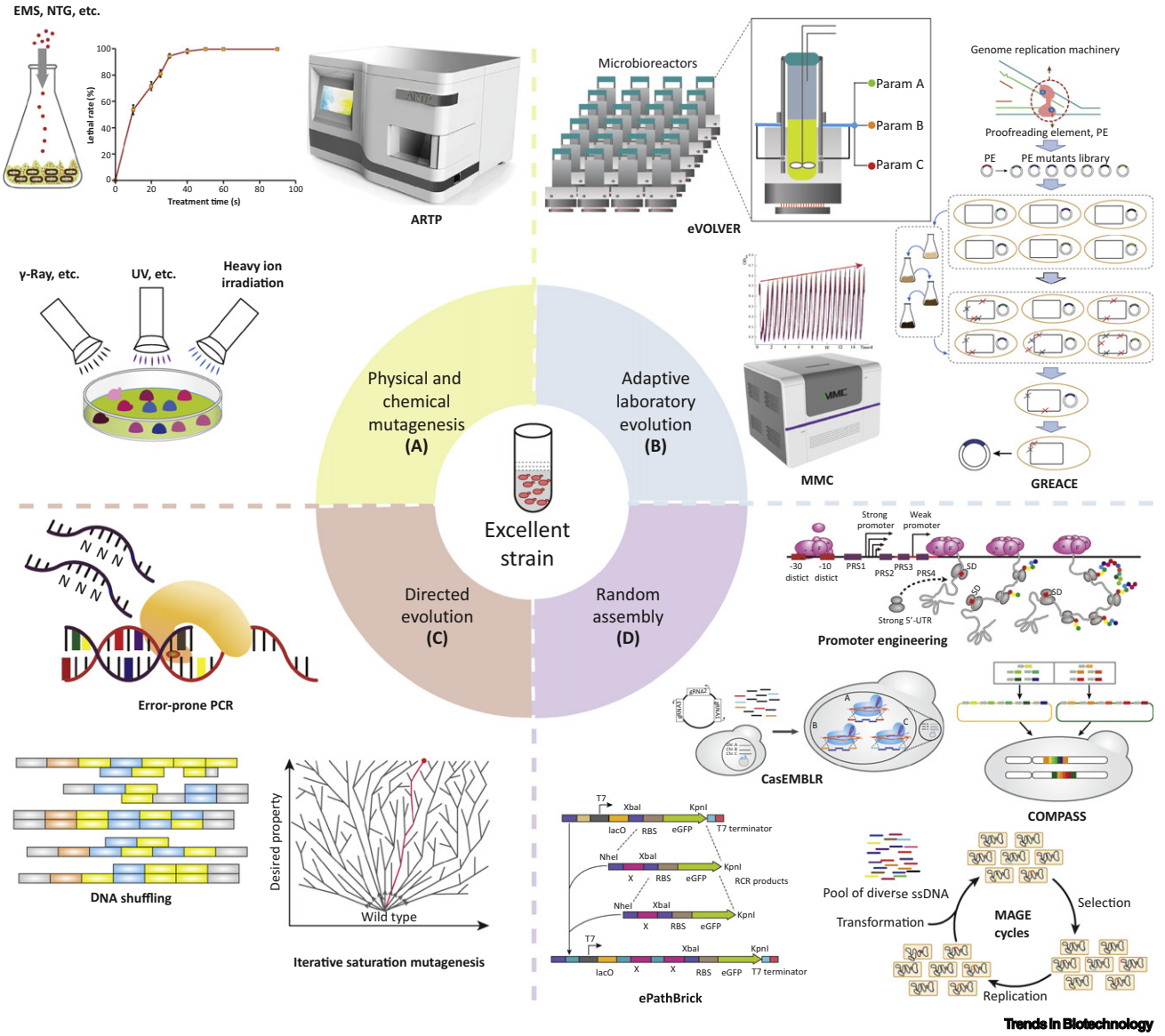


Figure 2. Strategies for the Construction of Diverse Strain Libraries. A variety of methods have been used for constructing diverse strain libraries for high-throughput screening (HTS) to select high-performance strains. (A) Physical and chemical mutagenesis, including ARTP, ultraviolet, γ-rays, EMS, NTG, etc. (B) Adaptive laboratory evolution, involving the improved method GREACE. (C) Directed evolution, such as error-prone PCR, DNA shuffling, and iterative saturation mutagenesis. (D) Random assembly strategies such as ePathBrick, MAGE, promoter engineering, and CasEMBLR. Abbreviations: ATRP, atmospheric and room temperature plasma; EMS, ethyl methane sulfonate; GREACE, genome replication engineering assisted continuous evolution; MAGE, multiplex automated genome engineering; MMC, microbial microdroplet culture; NTG, nitrosoguanidine; Param, parameter; RBS, ribosome binding site; ssDNA, single stranded DNA.

of fluorescent labeling and sorting [11]. In addition, some detection techniques, such as mass spectrometry, Raman spectroscopy, and capillary approaches, can be integrated into a droplet-based microfluidic system for further expansion of the universality of the screening procedure [60,71,72]. Until recently the most obvious disadvantage of the microfluidic droplet method is that the encapsulating speed for obtaining uniform droplets is around 10^3–10^4/s, which is far below that of FACS. Tailor-made microfluidic chips and high-performance droplet-stabilizing surfactants can alleviate this problem [11,69].

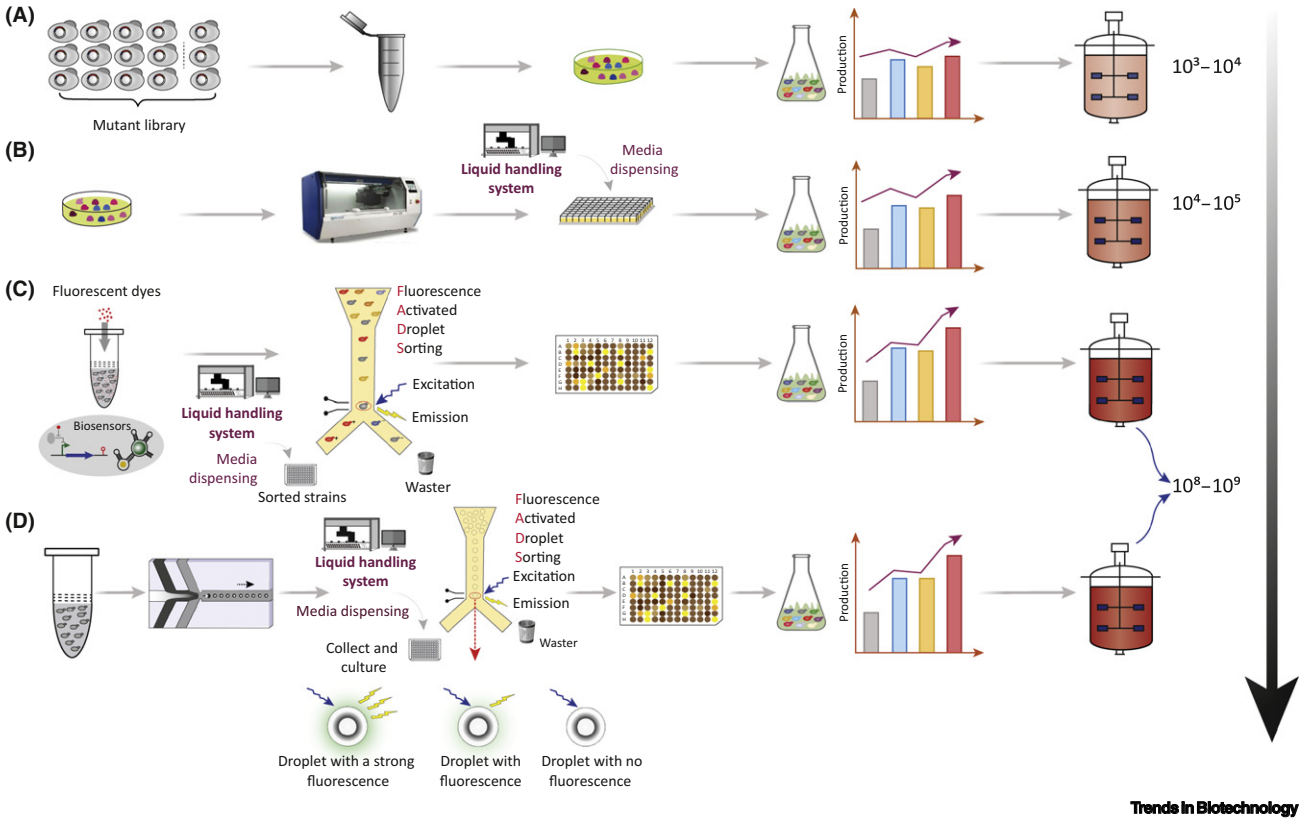
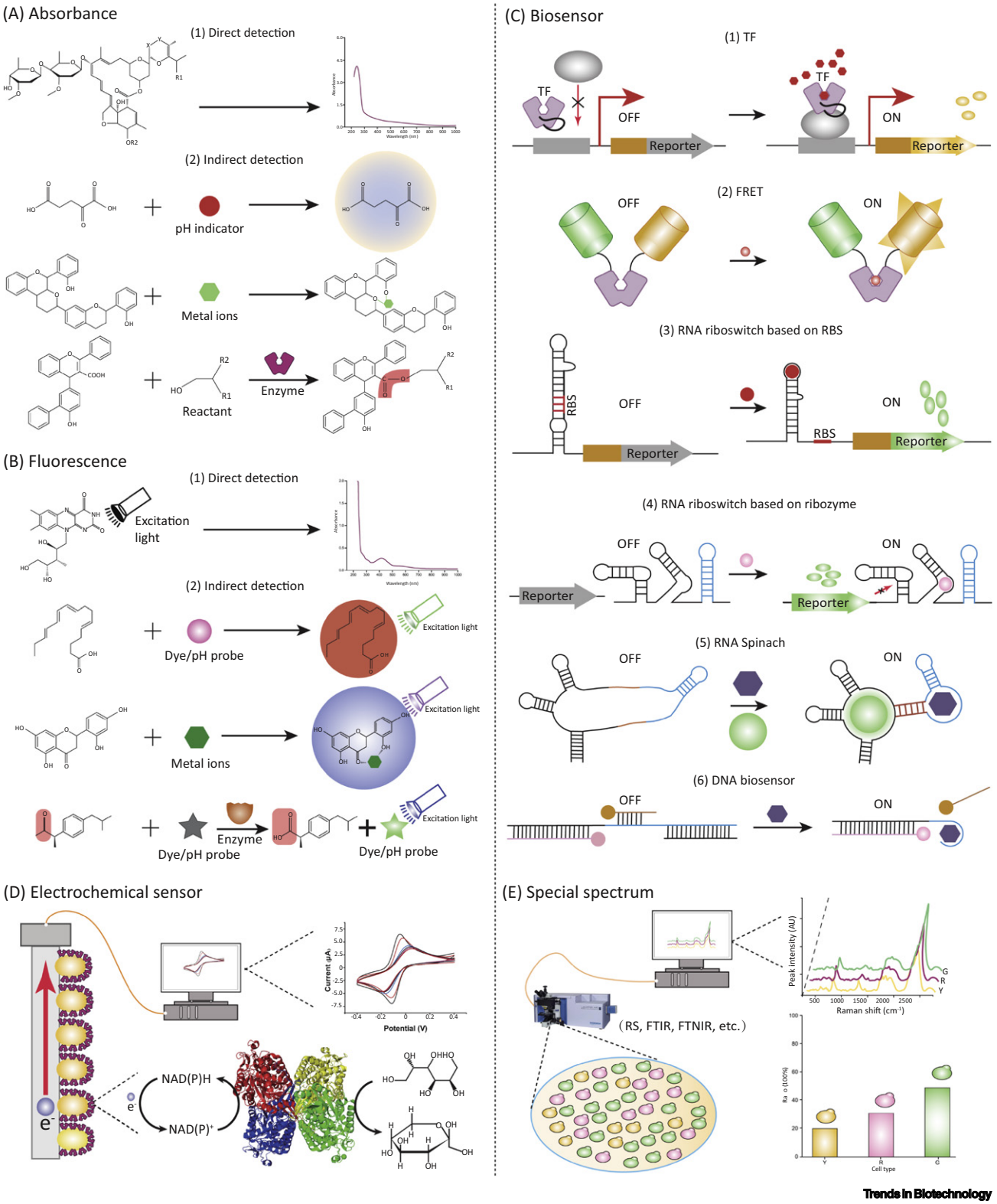


Figure 3. Pipelines with Different Screening Throughputs. Compared with traditional screening procedures, the screening scale and efficiency of high-throughput screening (HTS) has gradually been improved based on the use of colony pickers, fluorescence-activated cell sorting (FACS), and droplet microfluidics. (A) Pipeline of traditional screening based on the spread plate method, with a screening scale of ~10^3–10^4. (B) Pipeline of HTS based on a colony picker, with a screening scale of ~10^4–10^5. (C) Pipeline of ultra-HTS (uHTS) based on FACS, with a screening scale of ~10^8–10^9. (D) Pipeline of uHTS based on microfluidics and FACS, with a screening scale of ~10^8–10^9 and improved efficiency.

High-Throughput Culture Systems

Miniaturization is an important characteristic of HTS because it can reduce the amounts of biological and chemical samples that are required for analysis [63,73]. In general, high-throughput analysis is conducted in 96- or 384-well microtiter plates (MTPs), whereas high-throughput culture is mainly performed in deep 24-, 48-, and 96-well MTPs because of the need for culture medium and oxygen. High-throughput culture in MTPs would obviously increase the scope for screening target strains. To achieve a mixing effect in MTPs comparable with that of shake flasks, shakers for MTP cultures should reach 800–1000 rpm, whereas a typical flask shaker speed is ~100–300 rpm. In addition, **sandwich lids** are also useful for long-term MTP culture because both the rate of evaporation of fermentation broth and cross-contamination between wells can be significantly reduced [48,74].

An ideal microculture device should generally be able to accurately reproduce large-scale cultivation conditions and reflect the physiological metabolism and production capacity of the microorganism [75,76]. Traditional rescreening is usually performed in shake flasks and bioreactors in sequence. However, the process parameters in shake flasks are difficult to measure and regulate in real time, thus seriously limiting the amplification of an improved strain in a bioreactor [77]. **Microbioreactors** have been designed for high-throughput optimization to replace traditional two-step fermentation in shake flasks and bioreactors by a one-step integrated process. The



(See figure legend at the bottom of the next page.)

evolved design is further equipped with a waterless temperature-control system, microvalve-controlled feeding, and noninvasive optical pH and dissolved oxygen (DO) biosensors. However, these techniques need further improvement, especially regarding the detection range and sensitivity of pH and DO electrodes in the very limited culture volume [78,79].

Highlighting Target Compounds: Detection Methods

UV/Visible Spectroscopy-Based Screening

For practical applications of HTS, numerous screening methods based on UV/visible spectroscopy have been established. These can be divided into direct and indirect detection methods (Figure 4A). Products with a relatively complex molecular structure or innate color properties, such as avermectin [10], cephalosporin C [74], lycopene [80], and *p*-coumaric acid [81], can be screened through direct measurement of absorbance. Some products that have no obvious absorbance characteristics for direct detection can be detected by inclusion of pH indicators, chelation with metal ions, or coupling with enzymatic or chemical reactions. For example, if a strain accumulates a single type of organic acid as the target product, a screening procedure can be established by adding suitable pH indicators [82]. A low pH not only indicates that the strain accumulates more organic acid, but also shows that the strain has the capacity to tolerate a lower pH. For more specific detection of a target organic acid, such as pyruvic acid, a screening process can be established by adding a chelation ion that generates a detectable yellow complex [63]. In addition, screening methods have been developed based on chemical or coupled enzyme reactions which establish quantitative relationships between target metabolite concentration and chromogenic substances such as ATP/ADP [83] and $NAD(P)^+$ [84]. Some typical HTS methods and their applications in screening industrial microorganisms are listed in Table 1. A large number of selected strains with improved performance have been successfully applied in industrial-scale production.

Fluorescence Spectroscopy-Based Screening

Similarly to UV/visible spectroscopy-based HTS, fluorescence spectroscopy-based HTS can be divided into direct and indirect detection methods according to the characteristics of the target product (Figure 4B). For instance, riboflavin has fluorescence excitation/emission wavelengths at 450/518 nm, and *Yarrowia lipolytica* mutants with increased riboflavin production could be screened based on fluorescence intensity at 518 nm [54]. The use of fluorescent dyes/probes for screening is a common approach, such as labeling dead or live cells with dyes [10], analyzing lipids and single-cell oils with Nile red [85,86], and quantifying menaquinone and polymalic acid with rhodamine [87,88]. Interestingly, before screening based on Nile red, the density of *Y. lipolytica* was used for prescreening of more efficient lipogenic strains through the identification of floating cells [89]. Methods based on chemical or enzyme-coupled reactions that generate fluorescent signals have also been widely used for the efficient selection of improved industrial microorganisms [38,48]. HTS based on fluorescence can achieve ultra-HTS (uHTS) because it permits an increase in speed and a reduction in scale comparable with FACS. Fluorescence spectroscopy may be further expanded to biosensor-based HTS.

Figure 4. General Strategies for Establishing High-Throughput Screening (HTS). Different strategies have been employed to highlight the target products for establishing HTS for screening high-performance microorganisms. (A) Absorbance-based strategies include (1) direct detection based on the molecular structure or an innate color property of the target product; (2) indirect detection by, for example, the addition of a pH indicator, metal ion chelation, and coupling with enzymatic or chemical reactions. (B) Fluorescence-based strategies include (1) direct detection based on innate fluorescence of the target product; (2) indirect detection using a dye/pH probe, metal ion, and coupling with enzymatic or chemical reaction. (C) Electrochemical Sensor (ES)-based strategies. (D) Biosensor-based strategies including (1) transcription factors (TFs); (2) fluorescent proteins (FPs) and detection by Förster resonance energy transfer (FRET); (3) RNA riboswitch based on ribosome binding sites (RBS); (4) RNA riboswitch based on ribozymes; (5) RNA Spinach; (6) DNA biosensors. (E) Special spectrum-based strategies such as Raman (RS), Fourier transform IR (FTIR), and Fourier transform near-IR (FTNIR) spectroscopy.

Table 1. Applications of HTS for Selecting High-Performance Industrial Microorganisms

Strain	Product	HTS analysis method	Outcome	Refs
Bacteria				
Escherichia coli				
E. coli BL21 (DE3) treated with site-directed mutagenesis	*p*-Coumaric acid	Direct detection of the visible light spectrum of the product	Improved the titer by 65.9%	[81]
E. coli BL21 9G3-CHST7 modified by promoter engineering	(2*S*)-Naringenin	UV fluorescence spectrum based on interactions with metal ions	Improved the titer to 191.9 mg/l	[48]
E. coli BL21(DE3) 3-2F9 modified by directed evolution	L-DOPA	Absorbance caused by a colorimetric reaction	Improved the titer by 36.5%	[114]
E. coli BL21 (DE3)	Succinic acid	Absorbance and fluorescence produced by a coupled enzyme reaction	Development of a method for screening high-producer strains	[115]
E. coli BL21 (DE3) modified by directed evolution	Cyclohexylamine oxidase	Fluorescence produced by a coupled enzyme reaction	Improved the catalytic efficiency by 960 fold	[38]
E. coli 10G modified by directed evolution	Esterase	Fluorescence following staining with different dyes	Improved enzyme enantioselectivity by >700-fold	[11]
E. coli JCL260 Δ*lysA* pSA65/9 modified by genetic engineering and random mutagenesis	Isobutanol	Biosensor with a partner strain based on absorbance detection	Improved the titer by fivefold	[116]
E. coli pPCC1251 modified by metabolic engineering	Salicylate	Biosensor-based fluorescence (TF sensor/reporter: AraC/GFP)	Improved the titer by 123%	[117]
E. coli HDH4/*E. coli* HDH6-D12 modified by RBS construction/ random mutagenesis	L-phenylalanine	Biosensor-based fluorescence (TF sensor/reporter: TyrR/YFP)	Improved the titer by 180%/160.2%	[118]
E. coli ThrH-27(pTZL2) subjected to ARTP mutagenesis	L-threonine	Biosensor-based fluorescence for primary screening (TF sensor/reporter: cysJp and cysHp/GFP) and a chromogenic reaction for secondary screening	Improved the titer by 17.95%	[119]
E. coli DL2-LYS2(pAG)-MU1 subjected to ARTP mutagenesis	L-lysine	Biosensor-based fluorescence for primary screening (TF sensor/reporter: pA/GFP) and a chromogenic reaction for secondary screening	Improved the titer and yield by 21.0% and 9.0%, respectively	[120]
E. coli S3 modified by genetic engineering	Triacetic acid lactone	Biosensor-based fluorescence (TF sensor/reporter: AraC/GFP)	Improved the titer by ~20-fold	[121]
E. coli M3-4AT modified by eqPCR treatment	Resveratrol/(2*S*)-naringenin	Biosensor-based fluorescence (TF sensor/reporter: TtgR/RFP)	Improved the titer by 48.0%/49.1%	[122]
E. coli BL21/MG1655(DE3)	(2*S*)-naringenin	Biosensor-based fluorescence (riboswitch sensor/reporter: RNA aptamer/GFP)	Development of a HTS method for metabolic engineering applications	[123]
E. coli BL21 modified by feedback-regulated evolution of phenotype	Lycopene	Biosensor-based fluorescence (TF sensor/reporter: AraC DNA-binding domain fused to IPP enzyme/MutD5)	Improved the titer by 3–17-fold	[124]
E. coli BL21 modified by feedback-regulated evolution of phenotype	L-tyrosine	Biosensor-based fluorescence (TF sensor/reporter: AraC DNA-binding domain fused to IPP enzyme/MutD5)	Improved the titer by up to fivefold	[124]
E. coli BL21(DE3) pLysS	*p*-Coumaric acid	Surface-enhanced Raman scattering	Establishment of a high-throughput platform for screening of high-producer strains	[125]
Bacillus subtilis				
B. subtilis F126 subjected to ARTP mutagenesis	Uridine	Direct detection of absorbance	Improved the titer by 8.7-fold	[126]

Table 1. (continued)

Strain	Product	HTS analysis method	Outcome	Refs
B. subtilis THY-15 isolated from a natural library	Surfactin	Chromatic visualization based on a color indicator and mediator	Improved the titer to 1240 mg/l	[16]
B. subtilis 168/pMA5-S17G/A90S/R156S/K272A modified by genetic engineering and eqPCR	L-asparaginases	Chromogenic reaction measured at 450 nm	Improved the activity by 2.1-fold	[110]
B. subtilis WB600-mut12 subjected to ARTP mutagenesis	Alkaline α-amylase	Transparent rings in prescreening on plates and a chromogenic reaction in 96-well microplates	Improved the extracellular titer by 37.9%	[127]
B. subtilis 4511 modified by genetic engineering	Riboflavin (vitamin B_2)	Biosensor-based fluorescence [riboswitch (ribozyme) sensor reporter: RNA aptamer/GFP]	Obtained a high riboflavin producer	[128]
Corynebacterium glutamicum				
C. glutamicum ATCC13032 treated with chemical mutagenesis	L-lysine	Biosensor-based fluorescence (TF sensor/reporter: LysG/YFP)	Improved the titer by up to 185-fold	[129]
C. glutamicum A36-pDser subjected to ARTP mutagenesis	L-serine	Biosensor-based fluorescence (TF sensor/reporter: NCgl0581/YFP)	Improved the titer by 35.9%	[6]
C. glutamicum Δ*ace*E modified by ALE	L-valine	Biosensor-based fluorescence (TF sensor/reporter: Lrp/YFP)	Improved the titer by up to 100%	[130]
C. glutamicum ATCC 13032 modified by directed evolution	*N*-acetyl-L-glutamate kinase	Biosensor-based fluorescence (TF sensor/reporter: LysG/YFP)	Decreased sensitivity to L-arginine inhibition by 20-fold	[131]
Others				
Bacillus sp. N16-5-EA modified by directed evolution	Thermostable alkaline pectate lyases	High-temperature treatment	Improved the t_{50} and activity by 24-fold and 23.3%, respectively	[132]
Bacillus coagulans IIIB5 subjected to ARTP mutagenesis	L-lactic acid	pH indicator for primary and secondary screening, and absorbance for tertiary screening	Improved the titer by 46.10%	[133]
B. coagulans E11 subjected to ARTP mutagenesis	L-lactic acid	pH probe for high-throughput microdroplet sorting and absorbance in rescreening	Improved the titer by 52.0%	[134]
Lactobacillus paracasei S4 subjected to ARTP mutagenesis	L-lactic acid	pH indicator	Improved the titer by 18.9%	[78]
Lactococcus lactis JC017 treated with mutagenesis	Riboflavin (vitamin B_2)	Innate fluorescence of product	Improved the titer by 86.1%	[135]
Flavobacterium sp. treated with ion beam mutagenesis	Menaquinone	Fluorescence caused by reaction between menaquinone and the dye rhodamine 123	Positive mutants identified	[88]
Salmonella typhimurium MC5–2 subjected to ARTP mutagenesis	Vitamin B_{12}	Biosensor-based fluorescence (riboswitch sensor/reporter: RNA aptamer *btuB*/GFP)	Improved the titer by 21.9%	[136]
Yeasts				
Saccharomyces cerevisiae				
S. cerevisiae CYIB-DID2 modified by genetic engineering	β-Carotene	Direct detection of absorbance	Improved the titer by 2.1-fold	[137]
S. cerevisiae YastD-03 modified by directed evolution and metabolic engineering	Astaxanthin	Direct detection of absorbance	Improved the activity of β-carotene ketolase by 2.4-fold and the titer of astaxanthin to 47.18 mg/l	[138]

(continued on next page)

Table 1. (continued)

Strain	Product	HTS analysis method	Outcome	Refs
S. cerevisiae XZ-11 modified by ALE	Urea	Detection of absorbance caused by a chemical reaction	Decreased urea accumulation by 47.9%	[24]
S. cerevisiae YXM29- ISPSM4 modified by directed evolution	Isoprene	Detection of cell concentrations indirectly inhibited by precursor	Improved the titer by 1.55-fold, reaching 3.7 g/l in a fermenter	[139]
S. cerevisiae YPH500 modified by genetic engineering	Cellulase	Fluorescence produced by a coupled enzyme reaction	Enrichment of positive cells by 12-fold	[140]
S. cerevisiae EBY100 modified by directed evolution	Glucose oxidase	Fluorescence produced by a GFP fusion protein	Improved the mean enzymatic activity by 44%	[141]
S. cerevisiae modified by directed evolution	Muconic acid	Biosensor-based fluorescence (TF sensor/reporter: ARO9/YFP)	Improved the titer by threefold	[142]
S. cerevisiae modified by genetic engineering	3-Hydroxypropionic acid	Biosensor-based fluorescence (TF sensor/reporter: FapR/RFP)	Improved the titer by 120%	[143]
S. cerevisiae	Medium-chain fatty acids	Biosensor-based fluorescence (GFP coupled to a G protein)	Establishment of a HTS method for screening fatty acid producer	[144]
S. cerevisiae BY4742 modified by directed evolution	*N*-acetylglucosamine	Biosensor-based fluorescence [RNA riboswitch (*glmS* ribozyme) sensor/reporter: RNA aptamer/cell growth]	Improved the titer by fivefold	[145]
S. cerevisiae modified by directed evolution	L-tyrosine	Biosensor-based RNA Spinach (sensor/reporter: RNA aptamer/fluorescence)	Improved the titer by 28-fold	[70]
Yarrowia lipolytica				
Y. lipolytica WSH-Z06-C6 subjected to ARTP mutagenesis	α-Ketoglutaric acid	pH indicator	Improved the flask titer by 51.8% and by 45.4% in a fermenter	[82]
Y. lipolytica PO1f subjected to EMS mutagenesis and ALE	Riboflavin (vitamin B_2)	Innate fluorescence of product	Improved the titer by 32/54-fold	[54]
Y. lipolytica E26E1 modified by iterative evolutionary engineering	Lipogenesis	Identification of high-producer strains by detection of floating cells, and plate screening using fluorescence following Nile red staining	Improved the titer by 55%	[89]
Y. lipolytica NCIM 3589-YlE1 subjected to chemical mutagenesis	Lipid	Fluorescence following Nile red staining	Improved the titer by 1.49-fold	[85]
Y. lipolytica Y203A modified by metabolic evolution	Single-cell oil	Fluorescence following Nile red staining	Improved the titer by 1.5-fold	[86]
Y. lipolytica JMY4510 expressing gene *xlnc* generated by eqPCR	Thermostable xylanase	Fluorescence produced by a coupled enzyme reaction	Improved the activity by 4.7-fold	[146]
Pichia pastoris				
P. pastoris GS115/XynA-4 subjected to ARTP mutagenesis	Xylanase	Fluorescence produced by a fluorogenic substrate	Improved the activity by 1.3-fold	[147]
P. anomala GS2-3 modified by genome shuffling	Sugar alcohols	Absorbance produced following reaction with a chemical reagent	Improved the titer by 32.3%	[148]
Others				
Candida glabrata H6 subjected to ARTP mutagenesis	Pyruvic acid	Absorbance produced by a chemical reaction	Improved the titer by 32.2% in shake flasks and by 35.4% in a 3 l fermenter	[63]
Actinomycetes				
Actinomyces JN537 subjected to ARTP mutagenesis	Acarbose	Antibiotic susceptibility	Improved the titer by 62.5%	[149]

Table 1. (continued)

Strain	Product	HTS analysis method	Outcome	Refs
Streptomyces avermitilis S-233 subjected to mutagenesis	Avermectin B1a	Direct detection of absorbance	Improved the titer by 23.8% in a 500 l bioreactor	[150]
Acremonium chrysogenum W-6 subjected to UV and LiCl treatment	Cephalosporin C	Direct detection of absorbance	Improved the titer by 50% in MTPs and by 200% in a 50 l bioreactor	[74]
Saccharopolyspora erythraea T-13	Erythromycin	Detection of absorbance based on a bacterial indicator	Establishment of a HTS method for screening high-producer mutant strains	[151]
S. avermitilis ATCC 31267-G8 subjected to ARTP mutagenesis	Avermectin	Fluorescence detection in prescreening followed by absorbance detection	Improved the flask titer by 18.9% and by 20.6% in a fermenter	[10]
Streptomyces albus S12 subjected to ARTP mutagenesis and ribosome engineering	Salinomycin	Agar block diffusion method in plate screening, and a bacterial indicator in a HTS procedure	Improved the titer by twofold	[152]
Fungi				
Aspergillus niger GS3-3 modified by genome shuffling	Transglycosylation activity	Detection of nonfermentable reducing sugar	Improved the activity by 194.1%	[153]
Aspergillus niger H11201 subjected to $^{12}C^{6+}$ ion treatment	Cellulase	Direct detection of absorbance	Improved the β-glucosidase activity by 63.23%	[154]
Aspergillus niger DS03043 subjected to ARTP mutagenesis	Glucoamylase	pH indicator	Improved enzyme activity by 70%	[155]
Aspergillus niger IV-7-C6 subjected to ARTP mutagenesis	Gluconate	pH indicator for rapid qualitative assay and absorbance for quantitative assay	Improved the titer by 32.8%	[156]
Mucor plumbeus VI02019/*Mucor hiemalis* VI01993	Fatty acid	FTIR	FTIR results showed strong correlation with gas chromatography analysis	[157]
Rhizopus oryzae ZJU11 subjected to UV treatment	Fumaric acid	Colonies based on size and color on plates	Improved flask titer by 205% and by 160% in a fermenter	[158]
Trichoderma reesei modified by metabolic engineering and ARTP mutagenesis	Cellulase	Fluorescence produced by recombinant red fluorescent protein (DsRed)	Improved the activity of endoglucanase by 1.6–2.3-fold	[159]
Blakeslea trispora NRRL 2895 subjected to ARTP mutagenesis	Lycopene	Color intensity	Improved the titer by 55%	[80]
Mortierella alpina D20 subjected to ARTP and diethyl sulfate mutagenesis	Arachidonic acid	Colonies based on size and color on plates	Improved the titer by 40.61%	[160]
Auerobasidium pullulans AH-21 subjected to ARTP mutagenesis	Polymalic acid	Fluorescence of the pH probe rhodamine	Improved the titer by 13.8%	[87]
Aurantiochytrium sp. collected from a natural library	Lopd/docosahexaenoic acid/carbohydrate	FTIR	Obtained two hyperproducers from 109 strains	[57]

Biosensor-Based Fluorescence Spectroscopy Screening

HTS of microorganisms is often limited because target products or key intermediates cannot be detected by direct or indirect color or fluorescence reactions, and biosensor-based screening

strategies have been proposed as an alternative [90,91]. A biosensor consists of a sensor and a reporter. The sensor identifies specific intracellular metabolites, while the reporter generates quantitative signals through a series of programmed genetic circuits linked to the sensor signal. A series of biosensors have been developed for uHTS based on this principle. Biosensors are generally of two types – protein-based biosensors and nucleic acid-based biosensors. Most protein-based biosensors are based on transcription factors (TFs) and engineered fluorescent proteins (FPs), whereas nucleic acid-based biosensors mainly include RNA riboswitches, RNA Spinach, and structure switching-based DNA biosensors [92,93] (Figure 4C).

TFs are natural sensory proteins that bind to the enhancer or promoter regions of target genes and regulate their expression in response to environmental changes or key intracellular signals. TF-based biosensors have become one of the most common and valuable types of biosensors, and have been widely designed for HTS of metabolites including amino acids, organic acid, flavonoids, sugars, and lipids [94]. The scope of TF-based biosensors has further broadened by the construction of hybrid or fusion TF biosensors and *in vitro* TF biosensors [95]. FPs can also be engineered to detect specific metabolites by introducing a ligand-binding domain. Although FP biosensors have been used in several different modes, including Förster resonance energy transfer (FRET), circularly permuted, and destabilizing domain-coupled fluorescence, the application of FP-based biosensors for HTS of industrial microorganisms has been rarely reported [96,97]. RNA riboswitches targeting the regulatory domain of an mRNA can detect various metabolites via RNA aptamer domains, thereby regulating the transcription and translation of the encoded protein. Bacterial and eukaryotic ribosome binding site (RBS)- and ribozyme-based biosensors are widely used for metabolite screening and strain engineering, particularly in *E. coli* and *S. cerevisiae*. RNA Spinach-based biosensors have been developed as RNA 'aptamers-in-droplets' for enhancing the tyrosine production and recombinant protein secretion in *S. cerevisiae* [70]. In addition, DNA biosensors have been designed for sensing metabolites; however, they have rarely been applied for HTS in industrial biotechnology [92,96]. Nevertheless, HTS based on biosensors is a growing trend in the generation of industrial microorganisms.

Electrochemical Sensor (ES)-Based Screening

ES devices are based on a change in electric current generated by biochemical reactions occurring on the surface of an electrode. ES electrodes have significant advantages because they are extremely sensitive and selective for their targets, display rapid responses, and have potential for miniaturization [98]. They incorporate a biological recognition element (sensor) and a transducer that transforms the biological reaction into a measurable electrochemical signal (reporter). ES devices are generally of four types depending on the electrode technique employed: amperometric, potentiometric, conductometric, and impedimetric. The electrode material is the most important factor affecting ES performance, and ES design has incorporated advanced nanomaterials such as carbon nanotubes, metal and metal oxide nanoparticles, silica nanoparticles, and semiconductor material-based nanoparticles [99]. ES devices are currently widely applied in clinical diagnosis, the analysis of harmful components in food, and environmental monitoring [100]. In HTS, ES techniques have been employed to enhance the activity of enzymes associated with NAD(P)H or H_2O_2, mainly oxidoreductases and oxidases [101,102] (Figure 4D); however, few studies have addressed ES applications for the detection of particular fermented products in industrial biotechnology.

Screening Based on Advanced Spectroscopic Techniques

To enhance the sorting efficiency and obtain industrial producers with specific target phenotypes, spectroscopic techniques based on advanced instrument platforms such as Raman, Fourier

transform IR (FTIR), and Fourier transform near-IR (FTNIR) spectroscopy are being applied in industrial biotechnology (Figure 4E). Raman spectroscopy is based on the Raman effect, and has advantages of rapid, sensitive, nondestructive, and real-time detection. A HTS Raman spectroscopy platform was recently established for label-free screening of single cells [103] and biocatalysts [104]. Similarly to Raman spectroscopy, FTIR and FTNIR spectroscopy are also nondestructive analytical methods with the advantages of high throughput and rapid and automated detection. These two analytical methods have been employed for screening *Aurantiochytrium* sp., *Mucor* sp., and *S. cerevisiae* [57,58,105]. These spectroscopic techniques and their advanced imaging technologies integrated with confocal laser scanning microscopy have great potential in industrial HTS.

Concluding Remarks and Future Perspectives

MTP-based HTS techniques have improved both automation and throughput for obtaining industrial microorganisms with better phenotypes, resulting in less labor-intensive and more efficient workflows. FACS- and microfluidics-based HTS techniques, in combination with different fluorescent probes and biosensors, have significantly improved screening efficiency from 10^5/day to 10^{11}/day. These advanced techniques expand the scope of screening and enhance the recognition of target products. However, an enzyme harboring 1000 amino acid residues could theoretically generate up to 19^{1000} variants, and current screening throughput is far below this magnitude. Further expansion of the screening range of microorganisms faces numerous challenges (see Outstanding Questions), and the development of screening techniques that are more efficient than FACS-based methods, using nanotechnology and electronic techniques, is an important goal. Nanotechnology is an emerging technology in which the nanopores and nanoprobes have been used to analyze tumor cells and pathogenic bacteria. Combined with the microfluidics and other measurement techniques, these technologies have great potential for constructing more sensitive biosensor systems to screen a greater diversity metabolites or enzymes, and further enhance screening efficiency.

Many efficient analytical methods, especially those based on biosensors, have been successfully used for HTS to isolate microbial cell factories with improved performance. However, these strategies are only suitable for specific target products. Developments in synthetic biology and genome-editing tools are likely to expand the scope of biosensors to specifically respond to a wider range of small molecules. Furthermore, because the performance of industrial microorganisms in droplets and MTPs may differ from that in bioreactors, a reliable scale-down process that can simulate industrial-scale conditions will be vital to ensure more accurate final screening results. Furthermore, advances in artificial intelligence and the advent of big bio-data are set to revolutionize current screening pipelines and further reduce the costs of HTS.

Outstanding Questions

How can we obtain more rationally designed HTS biosensors based on synthetic biology and diverse editing tools for the construction of more efficient cell factories with specific phenotypes?

Can we incorporate nanotechnology into HTS to improve the efficiency of high-throughput detection and high-throughput culture?

How can we integrate microbioreactors into the HTS pipeline, not only for secondary screening but also to implement scale-down optimization simulating industrial-scale fermentation?

How can we incorporate recent advances in artificial intelligence and big bio-data to upgrade current HTS strategies? How can we make HTS more reliable and universally applicable?

In addition to advanced spectroscopy-based screening methods, can we establish more efficient screening models based on electric, magnetic, quantum, or other processes?

Microfluidics and cell-sorting strategies have been developed for several decades, and their screening efficiency has been limited to the range of $\leq 10^{10}$/h. However, a single enzyme harboring 1000 amino acid residues could have 19^{1000} potential variants, and screening mutants across the whole proteome would involve an exponentially larger number of variants. How can we further expand the screening range in a shorter time to deal with genome-scale mutagenesis screening?

Acknowledgments

This work was supported by the National Key Research and Development Program of China (2017YFC1600403, 2017YFC1600405), the National Outstanding Youth Foundation (21822806), the National Natural Science Foundation of China (31670095, 31770097), the Fundamental Research Funds for the Central Universities (JUSRP51701A), the Distinguished Professor Project of Jiangsu Province, the National First-Class Discipline Program of Light Industry Technology and Engineering (LITE2018-08), and the 111 Project (111-2-06).

References

1. Lee, S.Y. *et al.* (2019) A comprehensive metabolic map for production of bio-based chemicals. *Nat. Catal.* 2, 18–33
2. Chen, X. *et al.* (2018) DCEO biotechnology: tools to design, construct, evaluate, and optimize the metabolic pathway for biosynthesis of chemicals. *Chem. Rev.* 118, 4–72
3. Rugbjerg, P. and Sommer, M.O.A. (2019) Overcoming genetic heterogeneity in industrial fermentations. *Nat. Biotechnol.* 37, 869–876
4. Wehrs, M. *et al.* (2019) Engineering robust production microbes for large-scale cultivation. *Trends Microbiol.* 27, 524–537

5. Lee, S.Y. and Kim, H.U. (2015) Systems strategies for developing industrial microbial strains. *Nat. Biotechnol.* 33, 1061–1072
6. Zhang, X. *et al.* (2018) Integration of ARTP mutagenesis with biosensor-mediated high-throughput screening to improve L-serine yield in *Corynebacterium glutamicum*. *Appl. Microbiol. Biotechnol.* 102, 5939–5951
7. Choe, D. *et al.* (2019) Adaptive laboratory evolution of a genome-reduced *Escherichia coli*. *Nat. Commun.* 10, 935
8. Meyer, F. *et al.* (2018) Methanol-essential growth of *Escherichia coli*. *Nat. Commun.* 9, 1508
9. Cao, M. *et al.* (2018) Genetic and metabolic engineering for microbial production of poly-gamma-glutamic acid. *Biotechnol. Adv.* 36, 1424–1433
10. Cao, X. *et al.* (2018) Enhanced avermectin production by *Streptomyces avermitilis* ATCC 31267 using high-throughput screening aided by fluorescence-activated cell sorting. *Appl. Microbiol. Biotechnol.* 102, 703–712
11. Ma, F. *et al.* (2018) Efficient molecular evolution to generate enantioselective enzymes using a dual-channel microfluidic droplet screening platform. *Nat. Commun.* 9, 1030
12. Zhou, S. and Alper, H.S. (2019) Strategies for directed and adapted evolution as part of microbial strain engineering. *J. Chem. Technol. Biotechnol.* 94, 366–376
13. Leavell, M.D. *et al.* (2020) High-throughput screening for improved microbial cell factories, perspective and promise. *Curr. Opin. Biotechnol.* 62, 22–28
14. Held, T. *et al.* (2019) Survival of the simplest in microbial evolution. *Nat. Commun.* 10, 2472
15. Pandey, B.R. *et al.* (2019) Isolation, growth, enzyme assay and identification via 16S rRNA full sequencing of cellulolytic microbes from Nepal for biofuel production. *Renew. Energy* 132, 515–526
16. Yang, H. *et al.* (2015) A novel high-throughput and quantitative method based on visible color shifts for screening *Bacillus subtilis* THY-15 for surfactin production. *J. Ind. Microbiol. Biotechnol.* 42, 1139–1147
17. Wang, L.Y. *et al.* (2010) Novel mutation breeding method for *Streptomyces avermitilis* using an atmospheric pressure glow discharge plasma. *J. Appl. Microbiol.* 108, 851–858
18. Zhang, X. *et al.* (2014) Atmospheric and room temperature plasma (ARTP) as a new powerful mutagenesis tool. *Appl. Microbiol. Biotechnol.* 98, 5387–5396
19. Zhang, X. *et al.* (2015) Quantitative evaluation of DNA damage and mutation rate by atmospheric and room-temperature plasma (ARTP) and conventional mutagenesis. *Appl. Microbiol. Biotechnol.* 99, 5639–5646
20. Hu, W. *et al.* (2017) Recent advances of microbial breeding via heavy-ion mutagenesis at IMP. *Lett. Appl. Microbiol.* 65, 274–280
21. Jiang, A-L. *et al.* (2018) Enhanced production of L-lactic acid by *Lactobacillus thermophilus* SRZ50 mutant generated by high-linear energy transfer heavy ion mutagenesis. *Eng. Life Sci.* 18, 626–634
22. Ban, S. *et al.* (2019) Improving hydrogen production of *Chlamydomonas reinhardtii* by reducing chlorophyll content via atmospheric and room temperature plasma. *Bioresour. Technol.* 275, 425–429
23. LaCroix, R.A. *et al.* (2017) A model for designing adaptive laboratory evolution experiments. *Appl. Environ. Microbiol.* 83, e03115-16
24. Zhang, W. *et al.* (2018) Adaptive evolution relieves nitrogen catabolite repression and decreases urea accumulation in cultures of the Chinese rice wine yeast strain *Saccharomyces cerevisiae* XZ-11. *J. Agric. Food Chem.* 66, 9061–9069
25. Song, P. *et al.* (2018) Enhancement of pneumocandin B-0 production in *Glarea lozoyensis* by low-temperature adaptive laboratory evolution. *Front. Microbiol.* 9, 2788
26. Pereira, R. *et al.* (2019) Adaptive laboratory evolution of tolerance to dicarboxylic acids in *Saccharomyces cerevisiae*. *Metab. Eng.* 56, 130–141
27. Mundhada, H. *et al.* (2017) Increased production of L-serine in *Escherichia coil* through adaptive laboratory evolution. *Metab. Eng.* 39, 141–150
28. Pfeifer, E. *et al.* (2017) Adaptive laboratory evolution of *Corynebacterium glutamicum* towards higher growth rates on glucose minimal medium. *Sci. Rep. UK* 7, 16780
29. McCloskey, D. *et al.* (2018) Adaptive laboratory evolution resolves energy depletion to maintain high aromatic metabolite phenotypes in *Escherichia coli* strains lacking the phosphotransferase system. *Metab. Eng.* 48, 233–242
30. Graf, M. *et al.* (2019) Continuous adaptive evolution of a fast-growing *Corynebacterium glutamicum* strain independent of protocatechuate. *Front. Microbiol.* 10, 1648
31. Wong, B.G. *et al.* (2018) Precise, automated control of conditions for high-throughput growth of yeast and bacteria with eVOLVER. *Nat. Biotechnol.* 36, 614–623
32. Tan, Z.L. *et al.* (2019) In vivo continuous evolution of metabolic pathways for chemical production. *Microb. Cell Factories* 18, 82
33. Bergenholm, D. *et al.* (2019) Construction of mini-chemostats for high-throughput strain characterization. *Biotechnol. Bioeng.* 116, 1029–1038
34. Jakiela, S. *et al.* (2013) Bacterial growth and adaptation in microdroplet chemostats. *Angew. Chem. Int. Ed.* 52, 8908–8911
35. Jian, X. *et al.* (2019) *Microbial microdroplet culture system (MMC): an integrated platform for automated, high–throughput microbial cultivation and adaptive evolution*. Published online December 20, 2019. http://doi.org/10.1101/2019.12.19.883561
36. Wang, X. *et al.* (2019) GREACE-assisted adaptive laboratory evolution in endpoint fermentation broth enhances lysine production by *Escherichia coli*. *Microb. Cell Factories* 18, 106
37. Packer, M.S. and Liu, D.R. (2015) Methods for the directed evolution of proteins. *Nat. Rev. Genet.* 16, 379–394
38. Debon, A. *et al.* (2019) Ultrahigh-throughput screening enables efficient single-round oxidase remodelling. *Nat. Catal.* 2, 740–747
39. Huang, P-S. *et al.* (2016) The coming of age of de novo protein design. *Nature* 537, 320–327
40. Ebert, M.C.C.J.C. and Pelletier, J.N. (2017) Computational tools for enzyme improvement: why everyone can – and should – use them. *Curr. Opin. Chem. Biol.* 37, 89–96
41. Cheng, F. *et al.* (2015) Directed evolution 2.0: improving and deciphering enzyme properties. *Chem. Commun.* 51, 9760–9772
42. Zhou, S. *et al.* (2017) Obtaining a panel of cascade promoter–5'-UTR complexes in *Escherichia coli*. *ACS Synth. Biol.* 6, 1065–1075
43. Si, T. *et al.* (2017) Automated multiplex genome-scale engineering in yeast. *Nat. Commun.* 8, 15187
44. Lv, Y. *et al.* (2017) Engineering of an H_2O_2 auto-scavenging in vivo cascade for pinoresinol production. *Biotechnol. Bioeng.* 114, 2066–2074
45. Zha, J. *et al.* (2018) Metabolic engineering of *Corynebacterium glutamicum* for anthocyanin production. *Microb. Cell Factories* 17, 143
46. Jakociunas, T. *et al.* (2015) CasEMBLR: Cas9-facilitated multiloci genomic integration of *in vivo* assembled DNA parts in *Saccharomyces cerevisiae*. *ACS Synth. Biol.* 4, 1226–1234
47. Naseri, G. *et al.* (2019) COMPASS for rapid combinatorial optimization of biochemical pathways based on artificial transcription factors. *Nat. Commun.* 10, 2615
48. Zhou, S. *et al.* (2019) Fine-tuning the (2S)-naringenin synthetic pathway using an iterative high-throughput balancing strategy. *Biotechnol. Bioeng.* 116, 1392–1404
49. Ronda, C. *et al.* (2016) CRMAGE: CRISPR optimized MAGE recombineering. *Sci. Rep. UK* 6, 19452
50. Barbieri, E.M. *et al.* (2017) Precise editing at DNA replication forks enables multiplex genome engineering in eukaryotes. *Cell* 171, 1453–1467
51. May, M. (2018) Microplate reader update speed, sensitivity, accuracy, and optional modes lead the market. *Am. Lab.* 50, 28–29
52. Swasey, S.M. *et al.* (2018) Adaptation of a visible wavelength fluorescence microplate reader for discovery of near-infrared fluorescent probes. *Rev. Sci. Instrum.* 89, 095111

53. Mauri, M. *et al.* (2019) Deconvolution of luminescence cross-talk in high-throughput gene expression profiling. *ACS Synth. Biol.* 8, 1361–1370
54. Wagner, J.M. *et al.* (2018) A comparative analysis of single cell and droplet-based FACS for improving production phenotypes: riboflavin overproduction in *Yarrowia lipolytica*. *Metab. Eng.* 47, 346–356
55. Hanson, K.M. and Finkelstein, J.N. (2019) An accessible and high-throughput strategy of continuously monitoring apoptosis by fluorescent detection of caspase activation. *Anal. Biochem.* 564, 96–101
56. Schie, I.W. *et al.* (2018) High-throughput screening Raman spectroscopy platform for label-free cellomics. *Anal. Chem.* 90, 2023–2030
57. Yu, X-J. *et al.* (2019) High-throughput biochemical fingerprinting of oleaginous *Aurantiochytrium sp*. strains by Fourier transform infrared spectroscopy (FT-IR) for lipid and carbohydrate productions. *Molecules* 24, 1593
58. Morita, H. *et al.* (2014) A new screening method for recombinant *Saccharomyces cerevisiae* strains based on their xylose fermentation ability measured by near infrared spectroscopy. *Anal. Methods UK* 6, 6628–6634
59. Pilat, Z. *et al.* (2018) Microfluidic cultivation and laser tweezers Raman spectroscopy of *E-coli* under antibiotic stress. *Sensors-Basel* 18, 1623
60. He, Y. *et al.* (2019) Ramanome technology platform for label-free screening and sorting of microbial cell factories at single-cell resolution. *Biotechnol. Adv.* 37, 107388
61. Doerr, M. *et al.* (2016) Fully automatized high-throughput enzyme library screening using a robotic platform. *Biotechnol. Bioeng.* 113, 1421–1432
62. Du, G. *et al.* (2016) Microfluidics for cell-based high throughput screening platforms – a review. *Anal. Chim. Acta* 903, 36–50
63. Luo, Z. *et al.* (2017) A high-throughput screening procedure for enhancing pyruvate production in *Candida glabrata* by random mutagenesis. *Bioprocess Biosyst. Eng.* 40, 693–701
64. Qin, Y. *et al.* (2019) A fluorescence-activated single-droplet dispenser for high accuracy single-droplet and single-cell sorting and dispensing. *Anal. Chem.* 91, 6815–6819
65. Stavrakis, S. *et al.* (2019) High-throughput microfluidic imaging flow cytometry. *Curr. Opin. Biotechnol.* 55, 36–43
66. Tan, Y. *et al.* (2019) Directed evolution of an alpha 1,3-fucosyltransferase using a single-cell ultrahigh-throughput screening method. *Sci. Adv.* 5, eaaw8451
67. Wang, B.L. *et al.* (2014) Microfluidic high-throughput culturing of single cells for selection based on extracellular metabolite production or consumption. *Nat. Biotechnol.* 32, 473–478
68. Ren, L. *et al.* (2018) Standing surface acoustic wave (SSAW)-based fluorescence-activated cell sorter. *Small* 14, 1801996
69. Sesen, M. *et al.* (2017) Droplet control technologies for microfluidic high throughput screening (mu HTS). *Lab Chip* 17, 2372–2394
70. Abatemarco, J. *et al.* (2017) RNA-aptamers-in-droplets (RAPID) high-throughput screening for secretory phenotypes. *Nat. Commun.* 8, 332
71. Chen, P. *et al.* (2019) Microfluidics towards single cell resolution protein analysis. *TrAC Trends Anal. Chem.* 117, 2–12
72. Steyer, D.J. and Kennedy, R.T. (2019) High-throughput nanoelectrospray ionization-mass spectrometry analysis of microfluidic droplet samples. *Anal. Chem.* 91, 6645–6651
73. Lin, Y. *et al.* (2019) Rational high-throughput screening system for high sophorolipids production in *Candida bombicola* by co-utilizing glycerol and glucose capacity. *Bioresour. Bioprocess.* 6, 17
74. Tan, J. *et al.* (2013) High-throughput system for screening of cephalosporin C high-yield strain by 48-deep-well microtiter plates. *Appl. Biochem. Biotechnol.* 169, 1683–1695
75. Janakiraman, V. *et al.* (2015) Application of high-throughput mini-bioreactor system for systematic scale-down modeling, process characterization, and control strategy development. *Biotechnol. Prog.* 31, 1623–1632
76. Tajsoleiman, T. *et al.* (2019) An industrial perspective on scale-down challenges using miniaturized bioreactors. *Trends Biotechnol.* 37, 697–706
77. Kreye, S. *et al.* (2019) A novel scale-down mimic of perfusion cell culture using sedimentation in an automated microbioreactor (SAM). *Biotechnol. Prog.* 35, e2832
78. Tian, X. *et al.* (2018) Application of 8-parallel micro-bioreactor system with non-invasive optical pH and DO biosensor in high-throughput screening of L-lactic acid producing strain. *Bioresour. Bioprocess.* 5 UNSP 20
79. Sandner, V. *et al.* (2019) Scale-down model development in ambr systems: an industrial perspective. *Biotechnol. J.* 14, 1700766
80. Wang, Q. *et al.* (2014) Mutation breeding of lycopene-producing strain *Blakeslea trispora* by a novel atmospheric and room temperature plasma (ARTP). *Appl. Biochem. Biotechnol.* 174, 452–460
81. Zhou, S. *et al.* (2016) Characterization of mutants of a tyrosine ammonia-lyase from *Rhodotorula glutinis*. *Appl. Microbiol. Biotechnol.* 100, 10443–10452
82. Zeng, W. *et al.* (2015) A high-throughput screening procedure for enhancing alpha-ketoglutaric acid production in *Yarrowia lipolytica* by random mutagenesis. *Process Biochem.* 50, 1516–1522
83. Guo, C. *et al.* (2018) Developing a colorimetric assay for Fe(II)/2-oxoglutarate-dependent dioxygenase. *Anal. Biochem.* 548, 109–114
84. Agu, C.V. *et al.* (2018) Development of a high-throughput assay for rapid screening of butanologenic strains. *Sci. Rep. UK* 8, 3379
85. Katre, G. *et al.* (2017) Mutants of *Yarrowia lipolytica* NCIM 3589 grown on waste cooking oil as a biofactory for biodiesel production. *Microb. Cell Factories* 16, 176
86. Unrean, P. and Champreda, V. (2017) High-throughput screening and dual feeding fed-batch strategy for enhanced single-cell oil accumulation in *Yarrowia lipolytica*. *Bioenergy Res.* 10, 1057–1065
87. Li, H. *et al.* (2016) A novel rhodamine-based fluorescent pH probe for high-throughput screening of high-yield polymalic acid strains from random mutant libraries. *RSC Adv.* 6, 94756–94762
88. Liu, Y. *et al.* (2016) A high-throughput screening strategy for accurate quantification of menaquinone based on fluorescence-activated cell sorting. *J. Ind. Microbiol. Biotechnol.* 43, 751–760
89. Liu, L. *et al.* (2015) An evolutionary metabolic engineering approach for enhancing lipogenesis in *Yarrowia lipolytica*. *Metab. Eng.* 29, 36–45
90. Eggeling, L. *et al.* (2015) Novel screening methods – biosensors. *Curr. Opin. Biotechnol.* 35, 30–36
91. Dekker, L. and Polizzi, K.M. (2017) Sense and sensitivity in bioprocessing – detecting cellular metabolites with biosensors. *Curr. Opin. Chem. Biol.* 40, 31–36
92. Lim, H.G. *et al.* (2018) Design and optimization of genetically encoded biosensors for high-throughput screening of chemicals. *Curr. Opin. Biotechnol.* 54, 18–25
93. Chen, X. *et al.* (2019) Visualizing RNA dynamics in live cells with bright and stable fluorescent RNAs. *Nat. Biotechnol.* 37, 1287–1293
94. Cheng, F. *et al.* (2018) Transcription factor-based biosensors in high-throughput screening: advances and applications. *Biotechnol. J.* 13, 1700648
95. Silverman, A.D. *et al.* (2019) Deconstructing cell-free extract preparation for *in vitro* activation of transcriptional genetic circuitry. *ACS Synth. Biol.* 8, 403–414
96. Lin, J-L. *et al.* (2017) Enabling tools for high-throughput detection of metabolites: metabolic engineering and directed evolution applications. *Biotechnol. Adv.* 35, 950–970
97. Nadler, D.C. *et al.* (2016) Rapid construction of metabolite biosensors using domain-insertion profiling. *Nat. Commun.* 7, 12266
98. Yousefi, M. *et al.* (2019) Aptasensors as a new sensing technology developed for the detection of MUC1 mucin: a review. *Biosens. Bioelectron.* 130, 1–19
99. Majdinasab, M. *et al.* (2019) Optical and electrochemical sensors and biosensors for the detection of quinolones. *Trends Biotechnol.* 37, 898–915

100. Silva Lima, H.R. *et al.* (2018) Electrochemical sensors and biosensors for the analysis of antineoplastic drugs. *Biosens. Bioelectron.* 108, 27–37
101. Oyobiki, R. *et al.* (2014) Toward high-throughput screening of NAD(P)-dependent oxidoreductases using boron-doped diamond microelectrodes and microfluidic Devices. *Anal. Chem.* 86, 9570–9575
102. Aymard, C. *et al.* (2017) High-throughput electrochemical screening assay for free and immobilized oxidases: electrochemiluminescence and intermittent pulse amperometry. *ChemElectroChem* 4, 957–966
103. Wang, X. *et al.* (2017) Raman-activated droplet sorting (RADS) for label-free high-throughput screening of microalgal single-cells. *Anal. Chem.* 89, 12569–12577
104. Westley, C. *et al.* (2016) Label-free surface enhanced Raman scattering approach for high-throughput screening of biocatalysts. *Anal. Chem.* 88, 5898–5903
105. Kosa, G. *et al.* (2017) FTIR spectroscopy as a unified method for simultaneous analysis of intra- and extracellular metabolites in high-throughput screening of microbial bioprocesses. *Microb. Cell Factories* 16, 195
106. Nishioka, K. *et al.* (2018) Unbiased shRNA screening, using a combination of FACS and high-throughput sequencing, enables identification of novel modifiers of Polycomb silencing. *Sci. Rep.* 8, 12128
107. Harris, S.R. *et al.* (1991) Complement C5a receptor assay for high throughput screening. *J. Recept. Res.* 11, 115–128
108. Mayr, L.M. and Bojanic, D. (2009) Novel trends in high-throughput screening. *Curr. Opin. Pharmacol.* 9, 580–588
109. Kang, A. *et al.* (2017) High-throughput enzyme screening platform for the IPP-bypass mevalonate pathway for isopentenol production. *Metab. Eng.* 41, 125–134
110. Li, X. *et al.* (2018) Simultaneous cell disruption and semi-quantitative activity assays for high-throughput screening of thermostable L-asparaginases. *Sci. Rep.* 8, 7915
111. Machillot, P. *et al.* (2018) Automated buildup of biomimetic films in cell culture microplates for high-throughput screening of cellular behaviors. *Adv. Mater.* 30, 1801097
112. Feng, W. *et al.* (2018) Droplet microarrays: from surface patterning to high-throughput applications. *Adv. Mater.* 30, 1706111
113. Longwell, C.K. *et al.* (2017) High-throughput screening technologies for enzyme engineering. *Curr. Opin. Biotechnol.* 48, 196–202
114. Zeng, W. *et al.* (2019) Integrating enzyme evolution and high-throughput screening for efficient biosynthesis of L-DOPA. *J. Ind. Microbiol. Biotechnol.* 46, 1631–1641
115. Sun, L. *et al.* (2013) A double-enzyme-coupled assay for high-throughput screening of succinic acid-producing strains. *J. Appl. Microbiol.* 114, 1696–1701
116. Saleski, T.E. *et al.* (2019) Syntrophic co-culture amplification of production phenotype for high-throughput screening of microbial strain libraries. *Metab. Eng.* 54, 232–243
117. Qian, S. *et al.* (2019) Biosensor-guided improvements in salicylate production by recombinant *Escherichia coli*. *Microb. Cell Factories* 18, 18
118. Liu, Y. *et al.* (2017) Biosensor-based evolution and elucidation of a biosynthetic pathway in *Escherichia coli*. *ACS Synth. Biol.* 6, 837–848
119. Liu, Y. *et al.* (2015) Developing a high-throughput screening method for threonine overproduction based on an artificial promoter. *Microb. Cell Factories* 14, 121
120. Wang, Y. *et al.* (2016) Evolving the L-lysine high-producing strain of *Escherichia coli* using a newly developed high-throughput screening method. *J. Ind. Microbiol. Biotechnol.* 43, 1227–1235
121. Tang, S-Y. *et al.* (2013) Screening for enhanced triacetic acid lactone production by recombinant *Escherichia coli* expressing a designed triacetic acid lactone reporter. *J. Am. Chem. Soc.* 135, 10099–10103
122. Xiong, D. *et al.* (2017) Improving key enzyme activity in phenylpropanoid pathway with a designed biosensor. *Metab. Eng.* 40, 115–123
123. Xiu, Y. *et al.* (2017) Naringenin-responsive riboswitch-based fluorescent biosensor module for *Escherichia coli* co-cultures. *Biotechnol. Bioeng.* 114, 2235–2244
124. Chou, H.H. and Keasling, J.D. (2013) Programming adaptive control to evolve increased metabolite production. *Nat. Commun.* 4, 2595
125. Morelli, L. *et al.* (2017) Surface enhanced raman scattering for quantification of p-coumaric acid produced by *Escherichia coli*. *Anal. Chem.* 89, 3981–3987
126. Fan, X. *et al.* (2017) Improvement of uridine production of *Bacillus subtilis* by atmospheric and room temperature plasma mutagenesis and high-throughput screening. *PLoS One* 12, e0176545
127. Ma, Y. *et al.* (2015) Significantly improving the yield of recombinant proteins in *Bacillus subtilis* by a novel powerful mutagenesis tool (ARTP): alkaline alpha-amylase as a case study. *Protein Expr. Purif.* 114, 82–88
128. Meyer, A. *et al.* (2015) Optimization of a whole-cell biocatalyst by employing genetically encoded product sensors inside nanolitre reactors. *Nat. Chem.* 7, 673–678
129. Binder, S. *et al.* (2012) A high-throughput approach to identify genomic variants of bacterial metabolite producers at the single-cell level. *Genome Biol.* 13, R40
130. Mahr, R. *et al.* (2015) Biosensor-driven adaptive laboratory evolution of L-valine production in *Corynebacterium glutamicum*. *Metab. Eng.* 32, 184–194
131. Schendzielorz, G. *et al.* (2014) Taking control over control: use of product sensing in single cells to remove flux control at key enzymes in biosynthesis pathways. *ACS Synth. Biol.* 3, 21–29
132. Zhou, C. *et al.* (2015) Directed evolution and structural analysis of alkaline pectate lyase from the alkaliphilic bacterium *Bacillus* sp strain N16-5 to improve its thermostability for efficient ramie degumming. *Appl. Environ. Microbiol.* 81, 5714–5723
133. Lv, X. *et al.* (2016) High-throughput system for screening of high L-lactic acid-productivity strains in deep-well microtiter plates. *Bioprocess Biosyst. Eng.* 39, 1737–1747
134. Zhu, X-D. *et al.* (2019) High-throughput screening of high lactic acid-producing *Bacillus coagulans* by droplet microfluidic based flow cytometry with fluorescence activated cell sorting. *RSC Adv.* 9, 4507–4513
135. Chen, J. *et al.* (2017) Finding the needle in the haystack-the use of microfluidic droplet technology to identify vitamin-secreting lactic acid bacteria. *Mbio* 8, e00526-17
136. Cai, Y. *et al.* (2018) Engineering a vitamin B-12 high-throughput screening system by riboswitch sensor in *Sinorhizobium meliloti*. *BMC Biotechnol.* 18, 27
137. Li, J. *et al.* (2017) Discovery of several novel targets that enhance beta-carotene production in *Saccharomyces cerevisiae*. *Front. Microbiol.* 8, 1116
138. Zhou, P. *et al.* (2017) Alleviation of metabolic bottleneck by combinatorial engineering enhanced astaxanthin synthesis in *Saccharomyces cerevisiae*. *Enzym. Microb. Technol.* 100, 28–36
139. Wang, F. *et al.* (2017) Combining Gal4p-mediated expression enhancement and directed evolution of isoprene synthase to improve isoprene production in *Saccharomyces cerevisiae*. *Metab. Eng.* 39, 257–266
140. Ostafe, R. *et al.* (2013) Flow cytometry-based ultra-high-throughput screening assay for cellulase activity. *Anal. Biochem.* 435, 93–98
141. Kovacevic, G. *et al.* (2019) Development of GFP-based high-throughput screening system for directed evolution of glucose oxidase. *J. Biosci. Bioeng.* 127, 30–37
142. Leavitt, J.M. *et al.* (2017) Biosensor-enabled directed evolution to improve muconic acid production in *Saccharomyces cerevisiae*. *Biotechnol. J.* 12, 1600687
143. Li, S. *et al.* (2015) Development of a synthetic malonyl-CoA sensor in *Saccharomyces cerevisiae* for intracellular metabolite monitoring and genetic screening. *ACS Synth. Biol.* 4, 1308–1315
144. Mukherjee, K. *et al.* (2015) GPCR-based chemical biosensors for medium-chain fatty acids. *ACS Synth. Biol.* 4, 1261–1269
145. Lee, S-W. and Oh, M-K. (2015) A synthetic suicide riboswitch for the high-throughput screening of metabolite production in *Saccharomyces cerevisiae*. *Metab. Eng.* 28, 143–150
146. Beneyton, T. *et al.* (2017) Droplet-based microfluidic high-throughput screening of heterologous enzymes secreted by the yeast *Yarrowia lipolytica*. *Microb. Cell Factories* 16, 18

147. Ma, C. *et al.* (2019) Gel microdroplet-based high-throughput screening for directed evolution of xylanase-producing *Pichia pastoris*. *J. Biosci. Bioeng.* 128, 662–668
148. Zhang, G. *et al.* (2015) Genome shuffling of the nonconventional yeast *Pichia anomala* for improved sugar alcohol production. *Microb. Cell Factories* 14, 112
149. Ren, F. *et al.* (2017) Highly improved acarbose production of *Actinomyces* through the combination of ARTP and penicillin susceptible mutant screening. *World J. Microbiol. Biotechnol.* 33, 16
150. Song, X. *et al.* (2017) Mutation breeding of high avermectin B-1a-producing strain by the combination of high energy carbon heavy ion irradiation and sodium nitrite mutagenesis based on high throughput screening. *Biotechnol. Bioprocess Eng.* 22, 539–548
151. Tan, J. *et al.* (2013) A high-throughput screening strategy for accurate quantification of erythromycin. *J. Taiwan Inst. Chem. Eng.* 44, 538–544
152. Zhang, K. *et al.* (2019) Combinatorial effect of ARTP mutagenesis and ribosome engineering on an industrial strain of *Streptomyces albus* S12 for enhanced biosynthesis of salinomycin. *Front. Bioeng. Biotechnol.* 7, 212
153. Li, W. *et al.* (2014) Genome shuffling of *Aspergillus niger* for improving transglycosylation activity. *Appl. Biochem. Biotechnol.* 172, 50–61
154. Jiang, B-L. *et al.* (2017) A high-throughput screening method for breeding *Aspergillus niger* with $^{12}C^{6+}$ ion beam-improved cellulase. *Nucl. Sci. Tech.* 28, 1
155. Zhu, X. *et al.* (2017) Development of a method for efficient cost-effective screening of *Aspergillus niger* mutants having increased production of glucoamylase. *Biotechnol. Lett.* 39, 739–744
156. Shi, F. *et al.* (2015) A qualitative and quantitative high-throughput assay for screening of gluconate high-yield strains by *Aspergillus niger*. *J. Microbiol. Methods* 109, 134–139
157. Shapaval, V. *et al.* (2014) Fourier transform infrared spectroscopy for the prediction of fatty acid profiles in *Mucor* fungi grown in media with different carbon sources. *Microb. Cell Factories* 13, 86
158. Huang, L. *et al.* (2010) High-throughput screening of high-yield colonies of *Rhizopus oryzae* for enhanced production of fumaric acid. *Ann. Microbiol.* 60, 287–292
159. Gao, F. *et al.* (2018) A versatile system for fast screening and isolation of *Trichoderma reesei* cellulase hyperproducers based on DsRed and fluorescence-assisted cell sorting. *Biotechnol. Biofuels* 11, 261
160. Li, X. *et al.* (2015) Enhanced arachidonic acid production from *Mortierella alpina* combining atmospheric and room temperature plasma (ARTP) and diethyl sulfate treatments. *Bioresour. Technol.* 177, 134–140