Chapter

Design and Implementation of High Throughput Screening Assays for Drug Discoveries

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Abstract

The process of drug discovery is challenging and a costly affair. It takes about 12 to 15 years and costs over $1 billion dollars to develop a new drug and introduce the finished product in the market. With the increase in diseases, virus spread, and patients, it has become essential to invent new medicines. Consequently, today researchers are becoming interested in inventing new medicines faster by adopting higher throughput screening methods. One avenue of approach to discovering drugs faster is the High-Throughput Screening (HTS) method, which has gained a lot of attention in the previous few years. Today, High-Throughput Screening (HTS) has become a standard method for discovering drugs in various pharmaceutical industries. This review focuses on the advancement of technologies in High-Throughput Screening (HTS) methods, namely fluorescence resonance energy transfer (FRET), biochemical assay, fluorescence polarization (FP), homogeneous time resolved fluorescence (HTRF), Fluorescence correlation spectroscopy (FCS), Fluorescence intensity distribution analysis (FIDA), Nuclear magnetic resonance (NMR), and research advances in three major technology areas including miniaturization, automation and robotics, and artificial intelligence, which promises to help speed up the discovery of medicines and its development process.

Keywords: Drug discovery and development, High-Throughput Screening, fluorescence resonance energy transfer, biochemical assay, fluorescence polarization (FP), homogeneous time resolved fluorescence (HTRF), Fluorescence correlation spectroscopy (FCS), Fluorescence intensity distribution analysis (FIDA), Nuclear magnetic resonance (NMR), miniaturization, automation and robotics, and artificial intelligence

1. Introduction

It takes about 12 to 15 years and costs over $1 billion dollars to develop a new drug and introduce the finished product in the market. Moreover, the process is highly complex and costly as huge investments are made into technology [1, 2]. There is a need to reduce costs, increase efficiency and introduce drugs conveniently and faster to the market by higher throughput methods. The High-throughput screening method screens millions of chemical and biological compounds in a short interval of time. It is an automated process and screens many biological or chemical compounds for their therapeutic potential. The

High-throughput screening method efficiently accelerates the discovery of drugs, which are of potentially great therapeutic promise compared with other screening methods [3–5].

This review highlights the types of High-throughput screening assays and different detection techniques such as fluorescence resonance energy transfer (FRET), biochemical assay, fluorescence polarization (FP), homogeneous time resolved fluorescence (HTRF), Fluorescence correlation spectroscopy (FCS), Fluorescence intensity distribution analysis (FIDA) [6], Nuclear magnetic resonance (NMR), and research advances in three major technology areas including miniaturization, automation and robotics, and artificial intelligence, which has shown great promise to speed the discovery of medicines and its development process [7, 8].

1.1 Understanding high-throughput screening process for the discovery of drugs

In the high-throughput screening process, many compounds are screened to find potential candidate compounds that efficiently affect a biological target. These so-called candidate compounds are referred to as 'hits.' As an example, the high-throughput screening process successfully identified a potent pan-SRC kinase inhibitor now known as 'Dasatinib, BMS-354825' for the biological target of diabetes. The high-throughput screening processes involve various detection methods such as robotics or plate readers and corresponding software to process and analyze the data obtained. Once the 'hits' are discovered, further analysis in the high-throughput screening process can enable one to understand the potential optimization of the 'hits' achieved during the first screening round [9–11].

It is essential to understand that the high-throughput screening method alone cannot wholly evaluate a potential drug as toxicity studies are further needed for this evaluation. Instead, high-throughput screening is essential to eliminate the time that would have been wasted on investigating compounds that had little or no desired effect on the biological target. Through the utilization of this automated screening process, millions of compounds can together be screened and compounds with no or poor effects eliminated [10].

Basically, in the process of high-throughput screening HTS high number of effectors and biological modulators are screened and assayed against specific and selected targets. The high-throughput screening HTS process ensures that the time taken to screen large compound libraries is reduced and the whole drug discovery process is speeded up. In this manner, the high-throughput screening HTS method can be capable of screening more than thousand compounds per day [12]. It is ideally a mechanism-based approach where compounds are screened to provide improved drugs. High-throughput screening process precisely focuses on single mechanism contributing to identification of target specific compounds. The high-throughput screening HTS assays helps to screen various types of libraries such as genomics, protein, combinatorial chemistry, and peptide libraries [10]. The high-throughput screening HTS and assay method includes various steps such as preparation of reagents, target identification, compound management, assay development, and high throughput library screening, which are performed with extreme care and precision. The detail steps are as follows [13].

Firstly, targets are selected. There are presently around 500 targets that are being utilized by various companies. Among these targets, cell membranes receptors, mostly G-protein coupled receptors are commonly used and comprise the largest group with 45% of the total, followed by Enzymes (28%), hormones (11%), unknowns (7%), ion-channels (5%), nuclear receptors (2%), and DNA (2%). Off late pharmaceutical companies are interested to analyze compounds that interfere or modulate the function of GPCRs [13].

Undoubtedly, the integration of different compound libraries with wide chemical diversity in the high throughput screening method is a potential solution for massive drug discovery. The identification of good hits via the high throughput screening method can effectively reduce the time frame of discovering drugs in the process. However, for the screening technique to be successful various factors are dependent on it. Various factors that are essential to be considered are quality and number of validated targets, diversity and number of compounds, and the capability to screen compounds in a cost effective and timely manner using robust informative assays. Not just that, there are certain limitations of the high throughput screening method that defines the pharmacological properties of active compounds such as, synthetic chemistry for lead optimization and the low throughput of secondary assays. This major drawback causes a major hindrance to the overall identification rate of potential candidates for clinical evaluation. Due to this reason, researchers and scientists are trying to develop better technological solutions to overcome these challenges.

1.2 Application of high throughput screening (HTS) in drug discoveries

Applications of High-throughput screening (HTS) method in drug discoveries are detailed below

1.3 In screening

- For screening of novel biological active compounds
- Various natural products
- Combinatorial libraries such as peptides, chemicals etc.
- Biological libraries
- RNA chips
- DNA chips
- Protein chips

Generally, High-throughput screening process is carried pout via a microliter plate. Today, modern micro plates for HTS assays are performed in automation-friendly microliter plates with a 96, 384, 1536 or 3456 well format. The so called wells successfully comprise of experimentally useful matter, often an aqueous solution of dimethyl sulfoxide (DMSO) and some other chemical compound, the latter of which is different in each well across the plate [14].

Today, in most of the drug discovery labs, the collection of libraries has increased from 400,000 to more than 1 million compounds. In order to screen these high numbers of compound libraries automated 384 wells or higher density single compound test formats are used. Ideally, the primary screen is responsible and designed for rapid identification of hits from this library of compounds. The aim is to achieve a minimum number of false positives and maximum number of confirmed hits. Not to mention, the hit rates generally range between 0.1 – 5%, depending on the assay. The hit range or number also depends on the cutoff parameters that are set by the researchers, or the dynamic range of a given assay. These Primary screens run in multiples of single compound concentrations. The results of

the primary screening method are expressed in terms of percent activity as a negative (0 percent) and a positive (100 percent) control. The achieved Hits are further retested, generally independently from the first assay. After retesting, if a compound displays the same activities, it is accepted as a confirmed hit, and the process go through secondary screening or lead optimization. The results obtained from the secondary screening method are used to decide and further filter the substances that will make it on to clinical trials [13].

The combination of screening methods with bioinformatics, allows potential drugs to be efficiently and quickly screened, and hence discovering drugs at a faster speed and, which can be explored in more detail. The Initial screening of these compounds for their binding ability is the main role of high-throughput screening method. The high-throughput screening process generally involves developing tests, or assays, where in the potential compounds are made to bind with proteins, causing visible change that can be automatically read by a sensor. Generally, this change is achieved by light emissions by a fluorophore in the reaction mixture. The way the process works out is that, fluorophores are attached to target proteins in such a way that its ability to fluoresce is diminished (quenched) when the protein binds to another molecule. Then a different system measures the difference in polarization, which is a property of light, emitted by unbound versus bound fluorophores. Usually, bound fluorophores are highly polarized and hence can be easily detected by sensors. Various detection technologies for high throughput s screening are available today these includes time-resolved fluorescence (TR-FRET), fluorescence resonance energy transfer (FRET), fluorescence polarization, luminescence and absorbance. Not to mention these methods required efficient, highly sensitive, and versatile multi-mode micro plate readers [13].

Generally, libraries are referred as sets of compounds produced by combinatorial chemistry. Depending on how the solid-phase are handled, these compounds may be either mixtures or individual compounds. In biological assays range of compounds present in the libraries are tested as follows.

- Test of mixture in solutions
- Test of individual compounds in solutions
- Test of compounds on the beads

1.4 Test of mixture in solutions

In this test method the compounds are cleaved from the beads and tested in solution. Sometimes, it is a tedious task to find the compounds that are active by observing the pharmacological screen. In order to carry out successful identification of the most active compounds, it is essential to resynthesize the componenets individually. In this manner the process of screening and resynthesizing in an iterative manner is one of the most successful and simple methods for the identification of most potent components from libraries.

1.5 Test of individual compounds in solutions

A second method of testing compounds is the separation of the beads manually into individual wells and cleaving the compounds from the solid-phase. These compounds are then tested as individual entities.

Method	Traditional Screening	High – Throughput Screening
Assay Format	Single Tube	Array Format 96-Well
Assay Volume	~1 ml	50-100 µl
Compound Used	~5-10 mg	~1 µg
Assay Compounds	Added Singly	Added Simultaneously
Mechanical Action	1:1	1:96
Compound/Solution	Dry/Custom Solution	In Solution/DMSO
Assay Speed	Slow & Laborious	Fast and Efficient (~1 min/step/96-well plate)
Screening Ability	20-50 Compounds/Week/Lab	1000-10,000 Week/Lab
Diversity Screening	Limited Number	Unlimited Number

Table 1.
Based on 96 well format.

1.6 Test compounds on the beads

Another method for screening is testing on the beads. This is carried out by the application of fluorescent assay or colorimetric technique. In this process appropriate beads can be chosen by fluorescence or color, and picked out by micromanipulation. Further the the product structure of the active compounds, if a peptide, can be determined by sequencing on the bead. Whereas, non-peptide structures can be identified by one of the tagging methods.

1.7 Applications

High-throughput technology finds wide applications in areas other than drug development. These include the following

- Genomics
- Protein Analysis
- DNA Sequencing

1.8 Traditional screening vs. high-throughput

Traditional methods were time consuming as compared to high-throughput discovery. The table below demonstrates the differences between traditional methods and high-throughput methods. As shown in **Table 1**, screening ability of high-throughput screening ability increased 50 times on the low end, and 200 times on the high end as compared to traditional screening [3]. Leading to an increase of efficacy and accuracy, in High-throughput screening method. Moreover in high-throughput screening method minimal amounts of test compounds are used [12].

2. Types of high throughput assays

Assays are segregated into cell-based assays and biochemical assays. Biochemical assays are further segregated into homogeneous and heterogeneous assays [15].

2.1 Homogeneous assay

Homogeneous assay measurement is a single step process, where reagents are added in a single stage or multiple steps. Steps involved are fluid addition, incubation and readings. The homogeneous assay measurement is characterized by the interaction between the surrounding environment and the analyte. The homogeneous assay measurement method can be coupled with different detection techniques such as fluorescence, radiometric etc. for HTS. The advantageous feature of the homogeneous assay measurement method is that it is simple and involves minimum steps thereby contributing to reduce cost and minimum robotic complexity required in automation. One of the drawback of the homogeneous assay measurement method is that there are interference in measurements as it is carried out in the presence of other assay components and having a signal to background ratio of less than 10 [15].

2.2 Heterogeneous assays

Heterogeneous assays measurement method is a bit more complicated than the homogeneous assay method as it involves a few additional steps such as filtration, centrifugation etc. These additional steps ensures that the component(s) to be measured are separated from rest of the components, which may cause interference in assays measurement method and hence contributing to high signal to background ratio. A thumb rule always followed is that when homogeneous assay fails or high signal to background ratio is required, heterogeneous assay measurement method is generally carried out [15].

2.3 Biochemical assays

Biochemical assays are protein, enzyme-based, or receptor assays that utilizes a designated target in a more purified form. Generally speaking, biochemical assays are frequently carried out using scintillation proximity assay (SPA), radiometric, colorimetric fluorescence detection techniques. In the Scintillation Proximity Assay technology binding reactions are assayed without carrying out the filtration or washing process step. In this technique radioactive labels emitting electrons at about 10 μm in water are used to carry out the assay. Generally, SPA technique is a preferred method for all surface cell receptors when high binding and low receptor density is required [15].

One of the biochemical assays technique is Fluorescence resonance energy transfer. This technique is further summarized in the next sections [15].

2.4 Fluorescence resonance energy transfer (FRET)

FRET technique is a non-radiative quantum mechanical process, where energy is transferred from an excited donor fluorophore to a suitable acceptor fluorophore. Here, energy from incident light is absorbed onto the donor fluorophore and it is transferred to nearby acceptor molecule [15].

There are certain conditions that must be fulfilled for an effective FRET assay:

i. Ensuring effective overlap between the donor molecules fluorescence emission spectrum and the acceptor chromophore's excitation or absorption spectrum. The degree of overlap is termed as spectral overlap integral (J).

ii. The acceptor and donor fluorophore must be as close to each other (typically 1-10 nanometer).

iii. Ensuring significant difference in the extent of quenching of the starting material and product.

iv. Ensuring that the transition dipole orientations of the acceptor and donor are almost parallel. (SANGEETA SAINI, Syed Arshad Hussain)

v. Limitation of Fluorescence resonance energy transfer technique FRET:

vi. It needs an external illumination for the initiation of fluorescence transfer to occur.

vii. Possibility of direct excitation of the acceptor or to photo-bleaching. To avoid this, Bioluminescence Resonance Energy Transfer technique (or **BRET**) is used, which uses a bioluminescent luciferase from sea pansy *Renillaren iformis*to produce an intialpoton [15].

2.5 Fluorescence polarization (FP)

The Fluorescence polarization technique is widely used in high throughput screening method. In this technique when light is irradiated to the fluorophore, it gets excited and emits light in same polarized plane. The fluorophore remains steady throughout the excitation state. However, if the fluorophore changes its position i.e., it rotates during excitation state, it emits light in different plane (depolarized). Larger molecule tends to show little movement while smaller molecule rotates quickly and giving high and low polarization value [15, 16].

2.6 Applications

i. The Fluorescence polarization technique has been used in ligand/receptor studies, Tyrosine Kinase Assays etc.

ii. The Fluorescence polarization technique has also successfully found application in quantifying biochemical properties such as attachment of proteins to nucleic acid, protein denaturation etc. [15].

2.7 Homogeneous time resolved fluorescence (HTRF)

The HTRF method is a combination of time resolved measurement (TR) of fluorescence and standard FRET technology, thereby allowing the elimination of short-lived background Fluorescence, which occurs due to interfering materials in the sample and allowing a delay of approximately 50 to 150μseconds between the initial excitation and fluorescence measurement. Homogeneous time resolved fluorescence method utilizes europium cry ptate (Eu3 + cryptate) as fluorescent energy donor, which are rare earth complexes consisting of a macrocycle within which a Eu3+ ion is tightly embedded. This cage behaves as an antenna, collecting and transferring energies to the Eu3+ ion that eventually releases this energy with a specific long lived fluorescent pattern. Cryptate comprise of cross-linked allophycocyanin or XL665, a phycobilli protein pigment purified from red algae as acceptor [15].

In a recent research study HTRF was used as a screening application for the assay of tyrosine kinase and screening against tumor necrosis factor receptor in a 384-well microplate.. It was observed that (HTRF) was similar and related to fluorescence intensity techniques. The detector was gated for a short time period of

(10 ns) - > and the initial burst of fluorescence was not measured. Post this gating period the longer lasting fluorescence of the sample was measured. HTRF is effectively used to enhance sensitivity levels [15].

Applications of HTRF and FRET methods:

- In studies of molecular interaction and immunoassays.
- There exist many reagents pre-labeled with HTRF donors, which can be adapted to many assays.

2.8 Fluorescence correlation spectroscopy (FCS)

FCS is a confocal fluorescence where parameters of prime importance are the fluctuation of the fluorescence intensity, occurring from noise as well as biological, chemical, and physical effects on the fluorophore. Changes in chemical and physical properties, like equilibria, reactions, complexation, quenching, and like molecular motion, photophysical interactions, changes in conformation affects the overall emission [15].

2.9 Applications

1. Enabling the determination of various molecular interactions.
2. Enabling the study of conformation changes.
3. Enabling efficient diffusion analysis by concentration and aggregation measurements.
4. Enabling applications in binding assays and enzymatic assays.
5. Enabling structural and molecular dynamics of fluorescent proteins in vivo and in vitro.

2.10 Fluorescence intensity distribution analysis (FIDA)

The FIDA technique involves the monitoring of the sample's fluorescence intensity with a heterogeneous brightness profile. Hence, it help to determine the concentrations and specific brightness values of a number of individual fluorescent species in a solution [15].

2.11 Nuclear magnetic resonance (NMR)

Nuclear magnetic resonance based screening is an effective method to help identify leads of low molecular weight organic compound which bind to the protein targets. The screening method provides information on the binding location and affinity of potential lead compounds. This method is especially useful in the designing of highly selective lead structures or when hallow binding pockets need to be targeted. However, the technique is extremely sensitive toward loose fragment binders offering comparatively high throughput.

This Nuclear magnetic resonance NMR based screening method not only provides information of the binding site or the conformation of the bound ligand but it also reveals information related with docking of the ligand to the protein's binding pocket [15].

3. Cell based assays

Cell-based assays in HTS method are classified under following classes: [6].

3.1 Second messenger assays

The Second messenger assays are used to efficiently measure fast, transient fluorescent signals occurring in matter of seconds or milliseconds. It monitors signal transduction from activated cell-surface receptors. Many fluorescent molecules are known to respond to changes in intracellular.

Calcium ion concentration, membrane potential and various other parameters, hence they are The second messenger assays are made of fluorescent molecules as these molecules are known to respond to changes in intracellular Calcium ion concentration, membrane potential, and various other parameters, So, the fluorescent molecules are used in second messenger assays for receptor stimulation and ion-channel activation. The development of hydrophobic voltage-sensitive probes and FRET-compatible microplate instrumentation has helped the advancement of the screening technique for ionchannel drug discovery [15].

3.2 Reporter gene assays

The reporter gene assays monitors cellular responses at translation levels. It is responsible for indicating the absence or presence of gene products and in turn reflecting changes in a signal transduction pathway. The quantifications of reporters are generally performed by various bio-chemical methods i.e., by measuring enzymatic activities. Typically, plasmids are used as reporter genes [15].

3.3 Cell proliferation assays

Cell proliferation assays are responsible for monitoring the overall growth or no growth responses of the cell to external stimuli. Cell proliferation assays can be quickly and easily employed for automation [15].

3.4 Statistics

It is essential to achieve quality hits with high degree of confidence in drug discovery process. Errors occur or issues arise when analytical method for hit selection is repeated under similar conditions. In real case scenarios obtained results differ from each other and there occurs variability in the system under studies. Using statistical tools in analyzing screening experiments is the correct approach for the interpretation of screening data, and hence supporting making right decisions [15].

4. Breakthrough technologies

4.1 Automation and robotics

In the high-throughput screening process, large numbers of samples are screened, microplates having up to 3456 wells are generally used to hold the samples. Primarily, automation plays an essential role in the high-throughput screening process enabling millions of compounds to be rapidly screened in shorter

time periods, as opposed to laboratory benchtop investigation of compounds by researchers in the same period [2, 17].

However, there are various challenges faced in automation. For instance, the reagents quantity added to each well of a microplate has to be minimized in order for the potential compounds screening experiment to be designed within the constraints presented by automation. Other challenges include limiting the adjustments that can be made to compounds through the screening process. The means that only one single injection of compound is applicable to target samples. Hence, further adjustments cannot be made to how compounds are added to samples as the experimental design is would no longer be suitable for automation process [2].

Automation is generally categorized into three common modes namely, batch, semi-automated, and integrated. These three modes differ in functions such as walk away capabilities, flexibility, complexity and numbers of tasks. For example, in the batch mode, scientist still need to load stacks of plates, which are further limited to fewer steps in the process. On the other hand, Integrated automation, is a more sophisticated process, which is capable out of performing multiple scheduled steps facilitated by a robotic mover, further allowing non-manual operation for long periods [2, 18].

Often the automated solution requires the operator to be well skilled with the automation process. If not so, specialized training are provided from the equipment. Batch automation are often performed with little specialized training of the operators. Today automation has evolved and become more democratized as compared to the scenario ten years ago. This trend reduces the need for specialized training of the operators in the future and further making such solutions commercially available [2].

A further process involved in high-throughput screening is a robotic configuration. Often a robotic system is incorporated into high-throughput screening platforms to accelerate the time by which data is acquired [2].

The system essentially would be able to perform multiple functions such as adding reagents, transferring microplates, mixing samples, and incubating samples at specific temperatures. This enables both experimental times to be reduced and the elimination of any error that could potentially be brought about if the process was carried out manually [2].

4.2 Miniaturization

Today researchers aim to reduce the cost the process even more than current high-throughput screening technology. Miniaturization is a technology where smaller or lesser sample quantities are utilized to provide results, in the aim to reduce cost of using more samples. However, smaller quantities must provide reliable results. Development in miniaturization introduces higher sensitivity microplates for high-throughput screening, which can reliably measure signals from small sample sizes and overcoming the challenges of the initial miniaturization technology [2].

However, it is predicted that in the further miniaturization of the screening process can be achieved in future, thus reducing costs of the process even more than current high-throughput screening technology [2].

Most of the steps in HTS lead discovery are influenced by miniaturization. First step in miniaturization is increasing the density of plate well to more than a 96-well standard. Target densities of about 384, 1536, and 3854 wells per plate are available. Hence, higher throughput screening (HTS) achieved through this method of

miniaturization majorly reduces the reagent costs as reaction volumes decreases from 10 to 20 mL in the well of a 384-well plate down to <2 mL in the well of a 1536-well plate [2, 19].

It is quite challenging to handle fluids in miniaturized assays, nevertheless it is a crucial parameter for performance. It is difficult to dispense compounds that are stored or solubilized in organic solvents, in a fast, controllable, and accurate manner. Additional issues include effective mixing, clogging, and evaporation that need to be resolved. Ebner states that "One of the most common problems that high-throughput labs have to address is spatial or edge effects." When poor cellular growth occurs at the perimeter of the wells as compared to growth of the cells in the rest of the plate, the phenomena is called as edge effect. As a consequence, these challenges tend to restrict the plate density to about 384-wells. Microfluidic technology, is a more extreme form of miniaturization, helps to addresses some of these known fluid handling challenges. Microfluidic chips replaces the liquid handling mechanics with channels connected to liquid reservoirs while providing the benefits of reduced volumes. In most cases, the devices comprise of integrated tools including electrodes built-in and combines multiple operational steps [7, 20].

Microfluidic devices are also capable of isolating single cellsthat can be further cultured on the chips. This ability of microfluidic devices eliminates cellular heterogeneity on cancer cell populations as an example. Traditional drug screening methods see response information from an average of all cells. The microfluidic solution allows analysis of a single cell's antidrug response. In addition to this cell-on-chip model, recent advances have led to tissue-on-chip and organ-on-chip models which are still early in development [21]. Someday, these chip models may provide an alternative to animal models [22]. Because they are early in development, they are not high- throughput solutions today. But they show great promise to speed determination of drug activity, optimal combinatorial drug screening and toxicity testing in the future [2].

4.3 Artificial intelligence

Artificial intelligence (AI) has found great applications in medicinal chemistry for designing compounds and the discovery of drugs since the 1960s. A well-known Machine-learning tool like quantitative structure–activity relationship modeling has played a very important role to help in the identification of various useful target molecules from millions of compounds. Today, the application of Artificial intelligence has expanded onto drug discovery and tasks including image analysis, robotics control, and logistics. Artificial intelligence has also expanded its application in the process of drug discoveries namely hit identification, target selection, lead optimization, efficiently helping in preclinical and clinical trial studies [2, 23–25].

New applications of Artificial intelligence in drug discovery process now lets the researchers and scientists supervise the system as opposed to driving the system manually. Moreover, Artificial intelligence combined with robotic systems provides automation of the design, build, test, and learn (DBTL) cycle, resulting in a system for designing experiments, executing it, data analysis, hence, the optimization and execution of experiments iteratively. Consequently the application of artificial intelligence decreases the number of experiments to be performed and helps to generate the best possible optimization. In practice such systems have been developed and demonstrated at the University of Illinois. The new fully-automated system outperformed traditional screening methods by 77% and evaluated less than 1% of possible variants [2, 26].

Following are the advantageous features of Artificial intelligence in drug discovery applications [2, 4].

i. Helps in cutting down of the time by more than half to develop a potential drug candidate from lead molecule.

ii. Predicted molecules by Artificial intelligence are Helpprecise.

iii. No time is wasted on testing irrelevant molecules, as compared to traditional methods that test 90% of the irrelevant molecules [27].

iv. Currently, Artificial intelligence is capable of discovering novel compounds that are more selective and potent. This is achieved by quicker speed of high quality screening data sets at an affordable cost as compared to the expensive and slower screening method alone.

An area where artificial intelligence plays an essential role is the field of personalized or "precision" medicine [28]. Precision medicines are basically growing drugs in the industry. In the development process of personalized medicine collections of healthy and diseased human samples are needed [4]. Usually, the samples are sequenced using next-generation sequencing technique, resulting in the generation of massive data. The application of Artificial intelligence and methods of deep learning helps in the efficient analysis of big data sets [25].

5. Conclusions

The primary goal of high-throughput screening processes is to screen through a library of compounds, and help in the identification of candidates that affect the target in a desired way. This phenomena is referred as "hits" or "leads". Generally, hits are achieved by using various technologies including liquid handling devices, plate readers, robotics, and software for data processing. Today automation and robotics has been widely accepted in the drug discovery process and great progress continues to be made in this area. Automated process provides better process consistency and hence, better data quality. Alternatively, automation not only allows scientists to walk away freely and pursue other tasks, but it also allows trail of traceability if any questions arise. The process of automation minimizes human errors [29].

To sum it up, HTS processes does not particularly helps in the identification of drugs, because HTS cannot assess several properties that are critical for developing new drug. For example, HTS method cannot evaluate properties like bioavailability and toxicity. Instead, the primary role of HTS assays is to help in the identification of "leads" and provide suggestions for their optimization. Hence, the results from HTS assays helps to reveal the initial point for further steps in the drug discovery process, including drug design. HTS assays also helps to understand the interaction or role of a particular biochemical process.

Hence, the HTS method should be accepted as a technology that scans biological library quickly and efficiently excluding compounds showing no effect in the analysis. Various academic institutions and mainly industries use high-throughput screening method to screen large number of compounds on a daily basis. Various detection techniques FCS, NMR, HRTF etc., contribute to the screening of compounds in large number.

According to market analysis, the global (HTS) market size was at 15.3 billion USD in 2020 and is projected to reach 26.4 billion USD by 2025, growing at a CAGR of 11.5% in the forecast period. Market growth is driven by factors including improving research and development spent by biotechnological and pharmaceutical companies, advancements in high throughput screening technologies, availability of funding from government, and capital investments from various bodies. As we all are aware of the outbreak of the corona virus, in response to this, various biopharmaceutical, pharmaceutical companies, and small startups have stepped forward to develop solution to this issue. Scientists and researchers were able to find list of molecules that could target COVID-19. As per the latest reports, there are 79 available vaccine candidates, out of which 20 vaccine candidates are in the third stage of clinical trials. Out of the 20 vaccine candidates, eleven of them have been authorized in various countries. Researchers and scientists have taken the initiative to speed drug discovery process by using high throughput screening method and found few promising drugs that can be used against COVID-19 namely Remdesivir, Chloroquine & Hydroxychloroquine, Lopinavir & Ritonavir, and Lopinavir with Ritonavir plus Interferon beta-1a. There has been an increase in drug discovery projects in efforts to treat COVID-19, which is the driving force for the growth of the high-throughput screening products market [30].

Off late, pharmaceutical & biotechnology industries are collaborating with various academic and research institutions to implement drug discovery more efficiently. The industries and the institution works hand in hand as institutions perform target identification and validation of research, while industries carry out high throughput screening assay development and screening campaigns. In this manner the industries and research institutes, benefits from this collaboration [30].

However, there are certain hindrances to the growth of the high throughput screening market. The commercially available assay platforms are applicable for the already established target classes namely G-protein coupled receptors, ion-channels, nucleic acids, and enzymes. However, today there exists addition of target classes such as transmembrane receptors, transporters, signaling pathways, protein–protein interactions, protein-RNA interactions, and protein-DNA interactions leading to numerous complexities (such as protein instability and reagent variability) in the field of assay development & target identification and being a barrier to the growth of the high throughput screening market [30].

Owing to this expansion of new target classes, researchers and scientist must be encouraged to invest their efforts in developing new essay platforms [30].

6. Executive summary

- The chapter highlights the different phenomenal concepts of HTS including fluorescence resonance energy transfer (FRET), biochemical assay, fluorescence polarization (FP), homogeneous time resolved fluorescence (HTRF), Fluorescence correlation spectroscopy (FCS), Fluorescence intensity distribution analysis (FIDA), Nuclear magnetic resonance (NMR).

- The chapter also illustrates the various types of HTS applications.

- A key factor for successful HTS protocols, optimization of precise conditions and environments for the production of optimal “hits”.

- HTS is the selected experimental method for drug sensitization by screening thousands of drug compounds offered as libraries in a single day.
- Applications of HTS may include but not limited to, novel drug discoveries, natural products, biological libraries, DNA & RNA chips, and protein chips.
- Screening ability of high-throughput screening ability increased 50 times on the low end, and 200 times on the high end as compared to traditional screening.
- Different assays can be used for end-results such as cell-based assays, second messenger assays, reporter gene assays, and cell proliferation assays.
- Artificial intelligence (AI) has been widely used in HTS for selection of hits and potential targets.

7. Future directions

- HTS for bioactive small molecules can be identified through marine natural products to address ectopic issues.
- Artificial intelligence is coming to be more precise in the identification and selection of hits and potential targets.
- Recent research papers have pave the way for using HTS to identify novel potential small molecule inhibitors that can be used to inhibit bacterial, viral or parasitic replication.
- Recent HTS protocols have highlighted the importance of using HTS in the identification of modulators and activators of important pathways.
- Recent reports of HTS using spheroid 3D gastric carcinoma cells unleash new trends of using 3D cells in high-content imaging.

DOI: http://dx.doi.org/10.5772/intechopen.98733

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