TRACKING HTS ASSAY DEVELOPMENT TIME

opportunity for improving drug discovery

High throughput screening assays are developed more quickly now due to advances in technology, improved liquid handling and sensitive detection, as well as increased communication between scientists in high throughput labs and therapeutic areas. Increased availability of commercial reagents, target proteins and engineered cell lines will relieve current bottlenecks for further improvement.

By Dr Jennifer Hartigan, Cindy Liu and William Downey

If time is money, then real progress has been made in high throughput screening (HTS) technology in the past two years – HTS assays are being developed more quickly. According to the recent report *High Throughput Screening 2010: Effective Strategies, Innovative Technologies, and Use of Better Assays* based on interviews with 52 HTS directors at pharmaceutical companies and government-sponsored institutes and published by HighTech Business Decisions, the time it takes to develop HTS assays is now less than six months on average. Several factors contribute to this improvement: experience on the part of the scientists with technology and targets, new technology such as two-antibody assays, sensitive detection and accurate liquid handling, and commercially available reagents including antibodies, assay kits and a broader selection of fluorophores.

Faster assay development

The directors of high throughput screening labs report that assay development time has improved; HTS assays now take an average of 5.1 months to develop. The range of times is large, varying between one and 18 months; however, most of the directors (84%) interviewed for the study report assay development time of six months or less. The distribution of assay development time is shown in **Figure 1**.

Assay development time has generally decreased in the past two years. Only 10% of the directors say their assay development time has increased. The remaining directors say assay development time has decreased (52%) or stayed the same (38%) in the past two years. This change in assay development time is shown in **Figure 2**.

HTS directors mention several reasons that have contributed to this decrease, including:

Figure 1: Distribution of assay development time in months

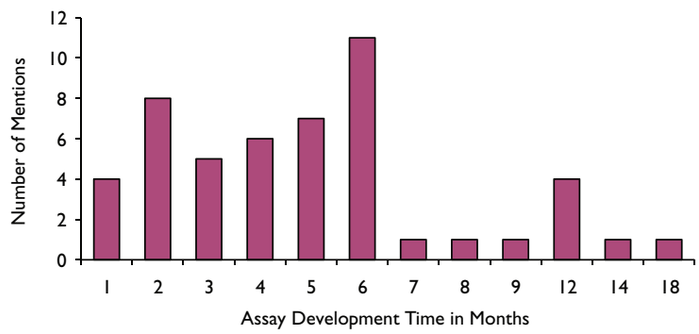
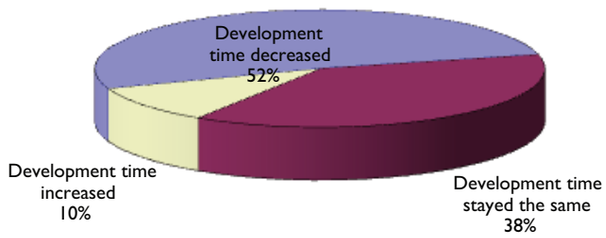


Figure 2: Change in assay development time over the past two years



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- Experience and knowledge of the target.
- Implementing high throughput automation earlier.
- New technologies and platform approaches.
- Availability of custom or commercial reagents and assays.

Experience with HTS and the target

High throughput screening has become a well-established technology in the past decade, and directors and their staff have become experienced meeting the unique miniaturised and automated demands of HTS. Familiarity with a target and knowledge of its active site dynamics and other physical properties of the proteins facilitates assay development. The following comments from the HTS lab directors describe the effect of experience on assay development.

"Assay development time has decreased a little bit because people working with the assays are more knowledgeable."

HTS laboratory

"When I worked at a small biotech company, assay development time took two to three weeks because we were working on similar target classes."

Non-commercial laboratory

Implementing high throughput automation earlier

HTS directors have seen that it is important to incorporate automation and robust screening assay formats from the beginning of assay development. Early communication with the therapeutic or target identification groups diminishes delays caused by the need to generate reagents. The comments below describe how early implementation of HTS speeds assay development.

"We start off with a robot-friendly assay so assay development is very fast, and there's no adapting the assays to the high throughput instrumentation."

HTS laboratory

"It used to be that targets were proposed, approved, and then we would have to wait for reagents and assays to be generated before we could start screening. Now when the target ID group finds a target, they let us know about three months ahead, and we start generating reagents and developing assays ourselves. This has completely eliminated reagents and assays that are inappropriate in a high throughput format, and it has decreased our assay development time by 2.5 months."

HTS Laboratory

New technologies and platform approaches

New technology has enhanced screening making it faster with better quality. Liquid handling is more accurate and automated scheduling has improved. One HTS vendor noted: "Most of the mechanical and software problems have been solved, so the challenge isn't a matter of moving the wells around." As a consequence of experience and familiarity with the targets, lab directors are able to develop some standardised approaches to assay development. The following remarks expand on some of the standardisation directors are implementing.

"Assay development time has decreased because we have set up a process in which we do a full high throughput characterisation of the proteins we use."

HTS laboratory

"Certain parts of our assay development are repeated and we've developed sort of a template. We have also limited our target choices to fit into this assay development template."

HTS laboratory

Availability of custom and commercial reagents and assays

The availability of commercial reagents and assay kits is another time-saving tool that has influenced assay development, as one director commented, the time "depends on the availability of tools. If we can purchase an enzyme commercially, then it may take three to four months". Custom or commercial assays are enabling labs to save time on internal development thus speeding targets into screening. One director noted below that previously established assays are helpful.

"There's a big variation, which depends on the complexity of the assay, biochemical or cell-based, whether there is already an assay in the literature or commercially available. Assay development time has decreased over the past two years due to the availability of commercial assays."

HTS laboratory

Roadblocks preventing further improvement

Despite the improvements made in assay development, some roadblocks continue to present challenges as summarised in **Figure 3**. Generating reagents, both proteins and cell lines, continues to be the most significant roadblock to further improvements in assay development times (44%). Outsourcing reagent production has had a beneficial impact on development time. Several directors mention they prefer to purchase these reagents and find assay development is inhibited if they are unable to find commercially available products.

The assays themselves sometimes cause trouble, with 21% of directors reporting that novel or complex targets take more time, and 17% reporting that developing assays robust enough for an automated system is problematic. The challenge is to fulfill all criteria: low signal-to-noise ratio, uniform signal across the entire plate, and reproducibility.

Figure 3: Roadblocks preventing faster assay development

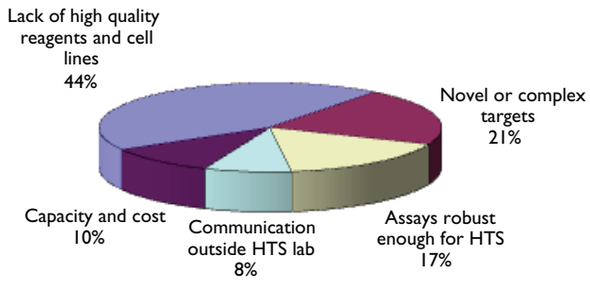

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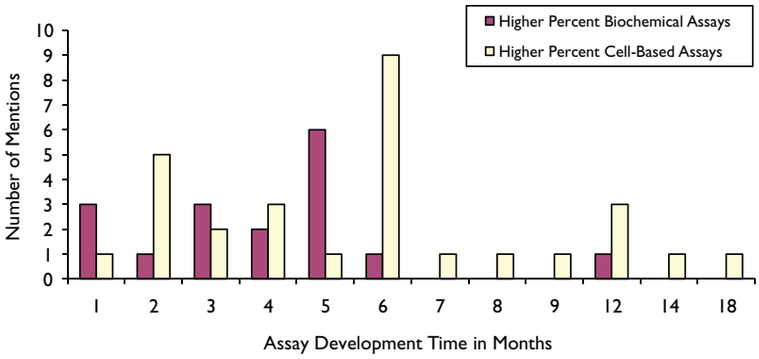
Figure 4: Distribution of cell-based and biochemical assays with respect to development time



Several directors commented below that the biology in the assays can be surprisingly complex.

"We think of single molecular targets, but often that is not really how things work, so you don't get what you expect."

HTS laboratory

"Physiological relevance is our greatest assay development roadblock. We spend a lot of time convincing teams to do a screen in the most physiological relevant system. We have the most difficulty with the translation of our *in vitro* results to *in vivo* systems."

HTS laboratory

Interestingly, several directors said communication with the scientists bringing the assay into HTS is a challenge. These directors, who are mostly at core academic screening centres, are providing service to their university communities, and as such, do not participate as early in assay development as directors at more integrated companies. One of these academic centre directors describes the benefits of early collaboration.

"For us, one of the things is communicating to the investigator the ways in which HTS is different from a bench top experiment. Controlling for temperature is more challenging and so is the time needed to initiate and read the assay. With our automation, we do things in batches and we can't just read the results whenever we want to."

Non-commercial laboratory

More time needed for cell-based assays

Assay complexity contributes to development time and cell-based assays are notoriously more complex than biochemical assays. The HTS 2010 study respondents can be divided into two groups: labs that perform a higher percentage of biochemical assays (17 labs) and labs that perform a higher percentage of cell-based assays (29 labs). The distribution of each of the two groups, with respect to their assay development time, is illustrated in **Figure 4**, and reveals that labs using predominantly biochemical assays have shorter development times, an average of 4.1 months, whereas labs using more cell-based assays have longer development times, an average of 6.2 months. Further, the 10% of directors who reported increasing assay development times in the past few years also report increasing their percentage use of cell-based assays.

Solutions for improvement

Innovative tools and enhancements to current technology will positively impact assay development. Already, HCS and patch clamp instruments are more robust with faster throughput, and label-free technology has opened up opportunities for intractable targets. One HTS director described the contribution of innovations in technology.

"More complex assays are needed now to find hits. HCS is probably the most important. Now we can do phenotype assays in high throughput. We are no longer limited to doing follow-up assays on hits from biochemical assays. We can screen targets directly in a smart, multiplexed way. Population patch clamp technology is also important and mass spectrometry (MS) is coming to HTS. With our UPLC device, we can screen 10,000 data points in a week. With two instruments in tandem, we can screen our complete library of 250,000 compounds in six weeks, which makes it competitive."

HTS laboratory

Increased availability of commercial reagents and further outsourcing of reagent production will also benefit assay development. Currently, 35% of HTS labs outsource cell line development or protein production according to the HTS 2010 study. In addition to outsourcing reagent production, a few of the HTS directors currently outsource some custom assay development. The comments below reveal some of the motivation for outsourcing.

"We prefer to outsource protein production, cell line development and scale up. We outsource items that we don't have enough infrastructure for and requires no innovation on our part."

HTS laboratory

"We outsource cell production because it is cheaper. We outsource compound profiling to another company because of their expertise. We outsource assay development to gain access to certain technology."

HTS laboratory

Several directors commented they do not currently outsource assay development, but would be interested if something unique became available.

"We would be interested in outsourcing custom assay development if a unique solution for us came along, but no one in the biotech industry supplies our specialised needs."

HTS laboratory

"There was one company that approached us with an assay and it was really intriguing, and it wasn't something we could do, so we contracted them to do it."

HTS laboratory

Custom assay development

Faced with an increased need for assays that probe targets in a more natural but complex environment, screening directors are asking HTS vendors for help in developing customised assays. The vendors often have specialised expertise in assay development through validating their own commercial reagents and assay kits. In the comments below, several vendors describe how they provide customised solutions for their clients.

Thermo Scientific HCSOnDemand™ offers managed services for cellular imaging to accelerate research and drug discovery. Mike Anhalt, Product Manager at Thermo Scientific, says, "At the core of HCSOnDemand™ lays our expertise in developing multiplexed imaging assays used to quantitatively monitor the cellular changes in cells after drug treatments. Cell physiology parameters such as morphology, viability, migration, nuclear fragmentation and response to stress can be monitored and then measured in parallel and correlated with protein localisation, post-translational modifications and abundance. To run a timely and efficient project, multiple review milestones are created within the schedule and a lead scientist is assigned to each project. This allows for direct and efficient communication to the client. In addition to assay development, screening services are also available, which is ideal for expanding capacity for time/resource critical projects" (**Figure 5**).

According to Melissa Stolow, Market Development Leader, Cell Systems Division, Discovery Assays and Services at Life Technologies, drug discovery scientists are faced with a variety of challenges to overcome in assay development. These include the reduction of internal resources and increased pressure for biologically relevant assays. "Project goals are critical," said Dr Stolow, "and we have developed a dedicated infrastructure to design and manage these to a successful outcome. We offer effortless project initiation and execution, managed by a dedicated project manager committed to proactive communication. With our suite of enabling technologies such as Jump-In™ and BacMam we can significantly reduce assay development time and

Figure 5
Array scan

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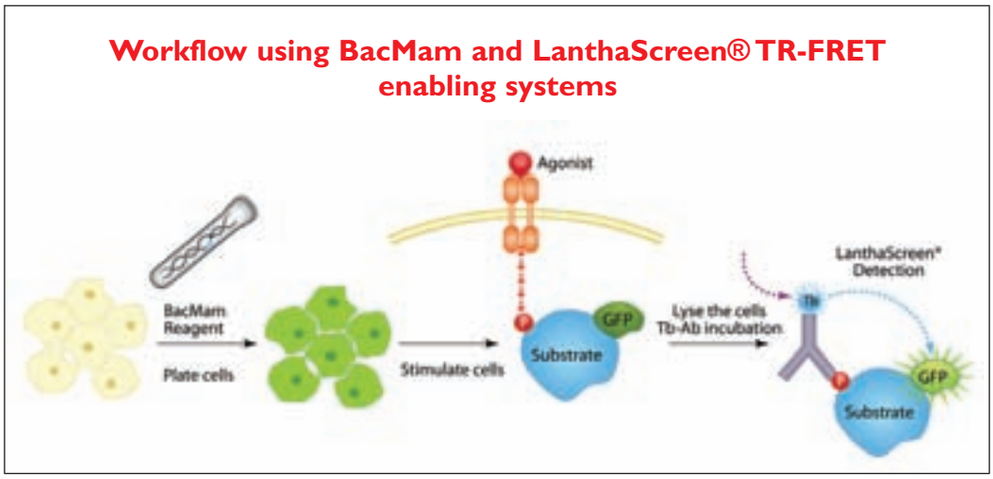

Figure 6
Example workflow for custom assay development using BacMam and LanthaScreen® TR-FRET enabling systems from Life Technologies

create assays in more relevant cell systems, such as human primary cells" (**Figure 6**).

"Cisbio offers customised assay solutions based on its proprietary HTRF® technology (www.htrf.com) to enhance and accelerate the HTS drug discovery process," said François Degorce of Cisbio Bioassays. The Tag-lite platform is a combination of HTRF® detection with SNAP-tag self-labelling technology (New England Biolabs). "Tag-lite enables the investigation of biological events at the cell membrane by offering plasmid generation encoding for tagged receptors, custom design and synthesis of fluorescent ligands for binding assays, generation of multimode cell lines, delivery of pre-labelled ready-to-use cells for receptor ligand binding and internalisation assays. This unique solution enables researchers to address the whole biological complexity of membrane receptors, from structure to function, through a customised approach" (**Figure 7**).

"At BioFocus, we have developed more than 350 assays for drug and target discovery," said William Spearing, Marketing Communications Manager. "As technologies advance, more approaches for difficult targets become available for screening. Every BioFocus assay is optimised for the specific application required, ranging from fragment screening using biophysical methods to identify low molecular weight hits, to phenotypic screening in disease relevant assays. For a disease such as Amyotrophic Lateral Sclerosis, which affects motor neurons specifically, BioFocus is screening in human stem cell-derived motor neurons, which has only become a possibility with the recent advances in stem cell biology" (**Figure 8**).

Conclusion

Assay development time has improved over the past several years due to the skill of screening scientists, robust and precise automated instruments, and high quality compounds and reagents. HTS directors use several strategies to speed development time including more communication with other departments, developing platform approaches where possible, and outsourcing production of protein and stable cell lines. Unfortunately, roadblocks to improved assay development persist. Producing proteins and cell lines internally is slow

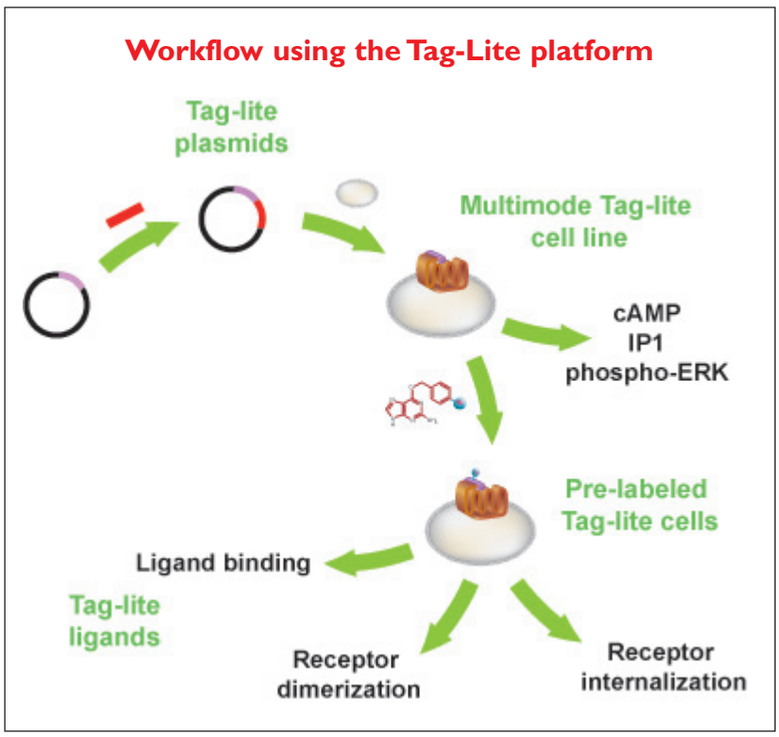

Figure 7: Cisbio provides Tag-lite plasmids, multimode Tag-lite cell line, pre-labelled Tag-lite cells and Tag-lite ligands customised on request

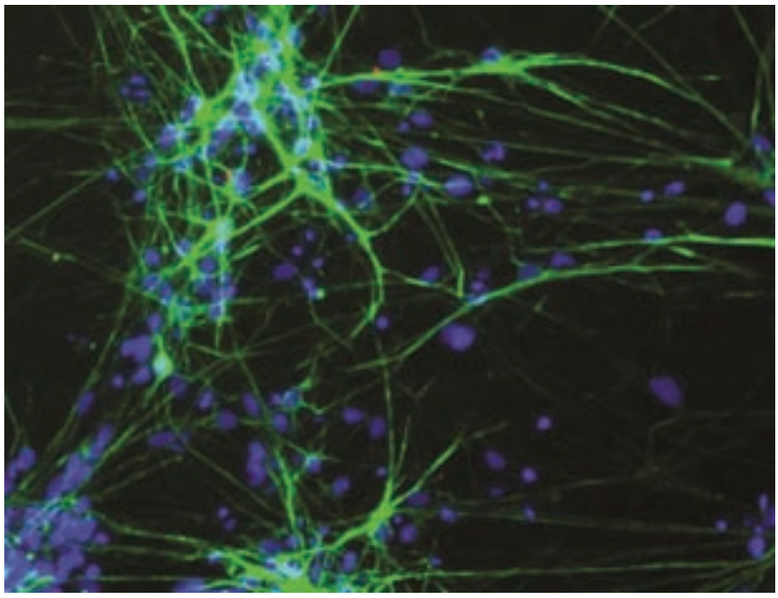

Figure 8: Validation of the human motor neuron platform. Motor neurons differentiated from human ES cells in 96-well format stained with neurofilament marker SMI-32 (green) and nuclear stain Hoechst (blue)

and commercial reagents are not available for every target. In addition, developing cell-based assays continues to be challenging, yet these more complex assays are considered critical for generating physiologically relevant hits. Solutions are coming as vendors provide a wider variety of validated reagents and assays and develop software and instruments that are easier to programme for high throughput screening assays. DDW

William Downey *is President,* ***Cindy Liu*** *is Managing Director and* ***Dr Jennifer Hartigan*** *is an analyst at HighTech Business Decisions, a consulting firm specialising in customised market analysis, industry reports and customer loyalty studies for suppliers serving the pharmaceutical and biotechnology industries. The company recently published the report* High Throughput Screening 2010: Effective Strategies, Innovative Technologies and Use of Better Assays. *www.hightechdecisions.com*