

Kinase assays for drug discovery and development

Reaction Biology Corporation has developed an array of high-quality assays including a panel of 336 kinases for drug discovery and development. RBC's services are particularly suited for research in the oncology field.

Reaction Biology Corporation was founded in 2001 to license technology out of the University of Pennsylvania that facilitated the miniaturisation of HTS assays. In the process of developing that technology, RBC's scientists created new innovations in the field of radiolabeled kinase assays. The new methods allowed for miniaturisation of this platform to the nanolitre scale. In 2007, RBC began providing services on this new assay platform, called Kinase HotSpotSM.

In creating its original technology, RBC developed much expertise at nanolitre-scale liquid handling. In the process of evaluating the latest techniques in this area, the company was able to create a combination of nanolitre techniques that allowed a dramatic scale-down of the conventional radiolabeled kinase assay. Once the platform was validated, RBC embarked on an intensive programme of buying in kinases from various suppliers and picking the best ones to create assays. In most cases the Kinase HotSpot platform uses standard kinases and substrates, but in other cases RBC has worked with vendors to express special enzymes or has customised the assays for clients that have proprietary enzymes. Currently, RBC has 336 validated kinases for the platform, the largest number available for the activity-based kinase assay, and is increasing the number constantly.

Upgrading kinase screening

"Kinases are among the most intensely studied drug targets, so pharmas are continuously looking to upgrade their kinase screening technologies," says Haiching Ma, RBC's chief technology officer. "There is an array of kinase assay methods, but only three major types of kinase assays –

fluorescent-based, binding, and radioisotope-based – are commonly used for profiling services. Fluorescent-based assays are typically constructed with a wide range of labeling or tagging strategies that can cause interference in detecting activity signals, in addition to difficulties in assaying compounds that are themselves fluorescent. The specifically designed substrate may also interfere with the actual enzyme activity or the mode of inhibitor binding. Binding assays do not measure actual enzyme activity, but only competitive binding with a known probe. Protein folding, probe selection and labeling or tagging can all affect the new inhibitor's binding mode. Most researchers prefer to look for enzyme inhibition using the activity-based assay and, in the case of kinases, the activity-based assay using a radioisotope for detection is the 'gold standard'. This assay has been in use for a long time, but has been expensive, and companies generally do not want to deal with radioactive material. Therefore the assay has usually been relegated to use only late in the preclinical discovery process for only a few compounds.

"RBC's kinase screening forms the majority of our business. Its enhancements to the traditional ³³P assay have reduced costs by a factor of five or more, and have reduced radioactive waste considerations by an order of magnitude. Accordingly, RBC can offer its proprietary HotSpot radiolabeled assay at a fraction of the cost previously available. This means that researchers can get more and better kinase assay data earlier in the discovery process, and not only avoid wasting time and money on compounds that ultimately don't work, but also may rescue compounds that could be lost in the early

discovery process.

"We have developed a 'Wide Field' Discovery platform, which uses early profiling to accelerate discovery. For example, a client with a small compound library wanted to find an efficient way to discover kinase inhibitors, without multiple HTS runs. RBC used its HotSpot profiling service to quickly focus and profile the library," says Ma.

In addition to miniaturisation, the efficiencies in the RBC HotSpot platform allow the company to complete 100,000 profiling assays per week, and to offer seven-day turnaround for contract orders. The company has more than 150 clients ranging from Big Pharma to start-ups.

"Through applying our technology, we have expanded our services into many enzyme target areas, including the epigenetic factors such as histone and DNA methyltransferases. A few special targets currently without reliable assays are also under development through collaborating with clients," Ma adds.

Conventional vs parallel profiling

The conventional preclinical drug discovery process is shown in Fig 1. A single target is selected for an HTS run. If a hit is found, it is then confirmed with IC₅₀. Medchem and SAR are used to derive analogues, and the process from IC₅₀ is repeated. Eventually, cell testing and a wider profiling is done to determine if the compound is a 'Lead' or a 'Loser'. The issue with this method is that it is linear and non-parallel. Ma says that by looking for one target at a time and then profiling only at the end, much time is wasted.

The 'Wide Field' discovery method using

Meet Haiching Ma of RBC

Dr Haiching Ma is Chief Technology Officer of Reaction Biology Corporation. He has more than 20 years' biomedical research experience with publications in assay development, drug screening, chemical microarray, enzymology, protein engineering, gene delivery, and stem cell biology. Dr Ma received his PhD in Pharmacology from the Medical School of

the University of Pennsylvania and continued his postdoctoral training there in the Institute for Medicine and Engineering. He also holds an MS in Chemistry and a BS in Entomology. He is the Principal Investigator for multiple SBIR awards and an RO1 award from NIH to RBC. He acts regularly as a reviewer for many NIH study sections and a variety of scientific journals.



Biotech Drug Development

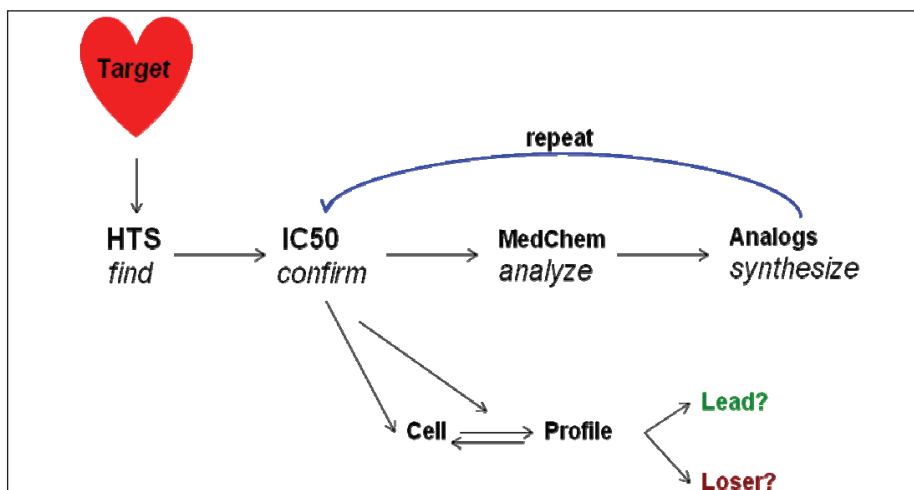


Fig 1. The conventional preclinical drug discovery process. "By looking for one target at a time and then profiling at the end, much time is wasted," says RBC's CTO, Dr Haiching Ma

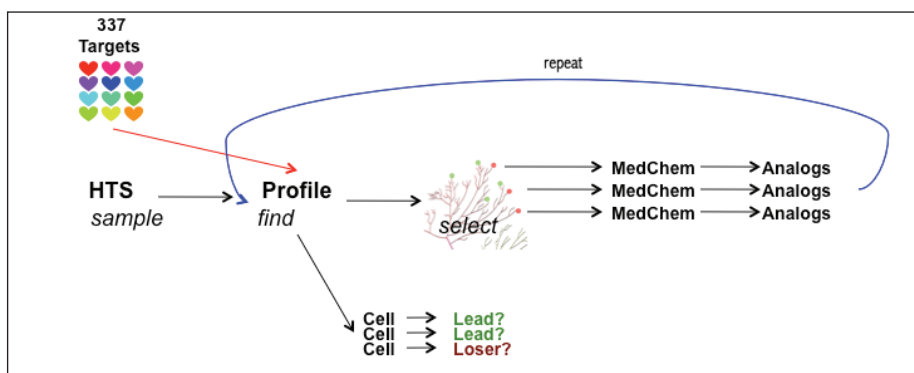


Fig 2. The 'Wide Field' discovery method using RBC's HotSpot profiling. This method uses parallel discovery enabled by inexpensive profiling to find inhibitors that would otherwise be missed

RBC's HotSpot profiling is shown in Fig 2. The library goes through one HTS run for an interesting kinase target, which may not be the actual final target. The inhibitors from this HTS run are reselected and grouped based on the structure and potency information. In this example, 11 inhibitors were selected and profiled against 260 kinases in parallel. Thus, instead of looking at only one kinase against the library, the researcher was looking at a focused subset of the library against hundreds of kinases.

"From this simple run, RBC discovered that few compounds were weak inhibitors for the initial target, but very potent and selective to certain new targets which may never have been examined in the conventional discovery process," explains Ma. "One compound that became immediately visible was a highly potent JAK3 inhibitor, which has proven to be the start of an exciting new scaffold of lead candidates. This 'Wide Field' method uses parallel discovery enabled by inexpensive HotSpot profiling to find inhibitors that would otherwise be missed.

"RBC has seen researchers using this technology to pull profiling earlier and earlier into the discovery process. Finding that a

lead compound lacks specificity at late stage can delay an entire programme. If candidates are profiled earlier, time will not be wasted on non-specific compounds, and dead-end leads can be avoided. Compounds for new targets can also be discovered, which may start a fresh discovery programme that may not be in the manager's original blueprint."

RBC has offered customised assay development to further harness HotSpot's capabilities for clients with proprietary or custom-expressed enzymes. In addition, combining HotSpot profiling with an accelerated medchem/SAR/synthesis cycle can dramatically shorten the time for preclinical discovery, and enable parallel paths with multiple scaffolds. RBC also performs substrate competition, cofactor competition, and binding reversibility testing to enhance this process.

Chief technology officer Ma has presided over the successful development of the HotSpot profiling platform. In addition, he has started to establish research partnerships, and the company has at least one discovery partnership with a European partner. Each partner brings to the mix a different skill-set, in an effort to create a

'virtual pharma' for preclinical discovery. RBC has partnered with other companies to create new assay classes and target categories for screening. Its development of methyltransferase targets includes one such partnership. Others are pending for new target classes.

Low-cost/high-quality benefits

"The only constant in the drug discovery sector is change," observes Ma. "Recent economic upheavals have only increased the pace at which Big Pharma companies are reconsidering their fundamental business structure and strategies. RBC has seen the benefit of being a low-cost/high-quality outsourced data provider in this environment. In some cases, while corporate-wide procurement decisions have ground to a halt, RBC has been able to work within individual lab budgets to provide more profiling without increasing costs. Globally we continue to see borders being of less and less significance, as RBC services more than 150 clients from four continents. As a consequence of increased demand for our services, we are moving to a new facility in Malvern, which will double the size of our labs.

"We expect the pharmaceutical business to continue to have cyclical waves of consolidation and spin-offs, both intentionally and unintentionally created by downsizing. The trend towards buying in innovation will only continue, since small companies are more cost-efficient and nimble at creating new discoveries. In addition, we see an increasing trend towards 'virtual pharmas', with cross-functional teams of companies and individuals using Internet-based communication and extremely discrete small expenditures to create discovery programmes at a cost at least an order of magnitude lower than large pharmaceutical companies can attain," he says. ^{sp2}

Further information

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