Technology Briefing

Kinase hurry up

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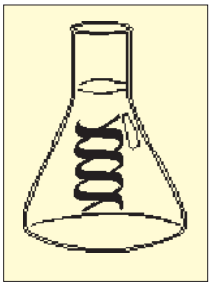
Ambit Biosciences Corp. has figured out how to modify phage expressed in E. coli to create a rapid and reproducible quantitative kinase binding assay. KinomeScan screens 170 kinases at a time against 10 compounds per day with typical turnaround to customers of two to five days.

This week, the company was to announce service deals with Pfizer Inc. (PFE, New York, N.Y.), GlaxoSmithKline plc (LSE:GSK; GSK, London, U.K.) and Bristol-Myers Squibb Co. (BMY, New York, N.Y.), building on top of its existing deal with Roche.

"We expect to increase throughput by at least a factor of 10 and add more kinases this year," President and CSO David Lockhart told BioCentury. Ambit (San Diego, Calif.) can move quickly because its binding assay is more scalable than traditional enzyme activity assays, he said.



Services provided by companies such as Invitrogen Corp. and Serologicals Corp. also can test hundreds of kinases, but typically are lower throughput and take a couple weeks to get the data to customers. The competitors argue, however, that their methods provide biological data that Ambit's approach would miss.

KinomeScan measures the binding of compounds at or near the functionally important ATP site of kinases. The assay is based on competition with known ATP site binders.

To express the kinases used in KinomeScan, Ambit uses a heavily modified T7 phage particle expressed in E. coli.

Lockhart noted that it's unnatural for a bacteriophage to have a human protein attached to its surface, and also that viruses mutate under selective pressure. "We did a lot of work to find out why that happened and fix it for our purposes. The proteins that we intend to make are made consistently and with no mutations, or specifically chosen mutations."

Ambit has a license to phage display technology from Dyax Corp. (DYAX, Cambridge, Mass.).

The technology is scalable because binding assays for all of the company's 170 kinases are performed under similar conditions. By contrast, traditional assays use radioactivity or fluorescence to show when the enzymes are modulated through binding.

"You have to make and purify the proteins in significant amounts and then activate them in order to perform activity assays – they are very difficult to make, purify and re-fold. We don't have to do any of that," Lockhart said. "We let the E. coli and the virus make the protein for us. And since it's a binding assay, we don't need a substrate. And because of the way the proteins are tagged we don't need radioactivity or fluorescence."

Other companies offering kinase screening services include Upstate Group (Charlottesville, Va.), which was acquired last year by Serologicals (SERO, Atlanta, Ga.).

"The main difference is that Ambit is doing competition binding assays, and ours are based on kinase activity," said Greg Moore, business development director for drug discovery at Upstate. "A compound binding to kinases can't be directly correlated to inhibition or activation of the kinases. For example, it could just be binding to a non-active site or an allosteric site. Upstate's assays are based on activity — we use the gold standard radiometric and fluorescence based assays."

"Our technology is more traditional and, I think, more enzymatically rigorous," said Brian Pollock, CSO for the drug discovery solutions group at Invitrogen (IVGN, Carlsbad, Calif.). "We can compare compounds with non-ATP competitive mechanisms of action. A lot of kinase inhibitors don't compete for the ATP binding site and can still block kinase activity." Ambit's technology may miss such compounds, Pollock said.

Pollock also noted IVGN's kinases are expressed "in a more normal cellular environment, since we use a eukaryotic expression system." The company uses a commonly used insect cell line called SF9 that is derived from butterflies.

"If you're expressing kinases in phage-infected bacteria, then the conditions are not the same," added Chris Armstrong, business area manager for screening services at IVGN. "For example, you don't get the same post-translational modifications, like phosphorylations, that we get using SF9."

Lockhart defended the utility of Ambit's approach. "We've shown that measuring binding at the ATP site is highly correlated with function, and that's not surprising because kinases need to bind ATP to function," he said.

Activity assays also have limitations that can affect their accuracy, Lockhart said. "Activity assays have to have the kinase substrate, and people almost never use a natural substrate. Also, the ATP concentrations used in activity assays are almost never the natural ATP concentrations, and the concentrations are usually different from assay to assay. They also only measure the active form of the kinase, and not other biologically relevant forms. Gleevec, which is the best known kinase inhibitor, works by binding the inactive form of its target, Abl."

Nevertheless, Lockhart, Moore and Pollock all agreed that activity and binding methods can be complementary. In addition, each company has some unique kinases that the others do not have. There are expected to be about 500 kinases encoded in the human genome, Lockhart said.

Upstate has a portfolio of 165 kinases and generally returns data from screens to customers in one to three weeks, Moore said. IVGN has 70 human kinases, which will increase to more than 100 by February, and also has typical turnaround times of two weeks. Upstate and IVGN haven't disclosed their kinase screening deals.

In addition to service deals, Ambit is using KinomeScan for internal discovery and expects to have a small molecule inhibitor of Flt-3 receptor tyrosine kinase in the clinic by mid-2006. "You can think of it as a Gleevec-like molecule for chronic myelogenous leukemia," Lockhart said.

Ambit's first compound — not a kinase inhibitor — is a molecule for stroke and neuroprotection that the company plans to put into the clinic this year.