c&en | WHITE PAPERS

A FRESH START: HOW DNA-ENCODED LIBRARIES ARE REINVENTING HIT IDENTIFICATION

DNA-encoded library (DEL) screening technology is changing the game of hit identification during early-stage drug discovery by enabling the testing of billions of molecules—at the same time and in a single Eppendorf tube—against a biological target.[1]

A 100,000-FOLD INCREASE

Almost every small-molecule drug discovery program kicks off with the identification of a starting compound—one that exhibits activity against the intended biological target. As the program progresses toward preclinical testing, this structure is optimized to fine-tune its biological performance. Starting molecules can be rationally designed from the bottom up, informed by previous drug discovery efforts, but many are found by screening large libraries of diverse molecules against the target of interest.

High-throughput screening (HTS) is the most widely used screening technology today. These automated systems can typically screen up to 100,000 molecules

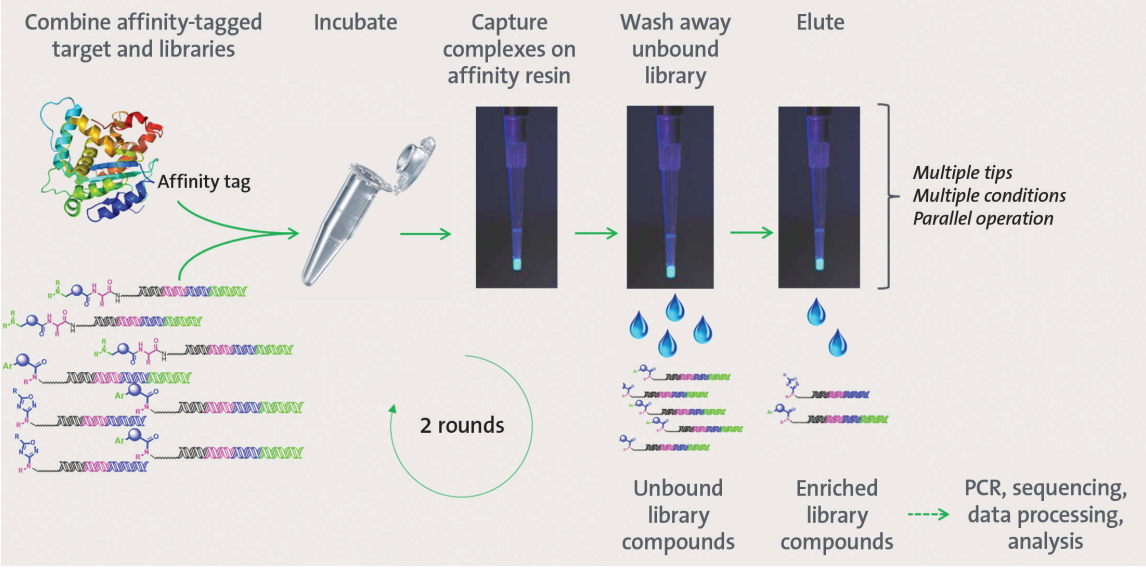
Credit: Shutterstock

(individually, in multiwell arrays) against a biological target in a single experiment. One to two million compounds are typically tested this way before a starting molecule—referred to as a hit—is selected.

In contrast, typical library collection used for a DEL screen can contain as many as a 100 billion pooled molecules. "All of these can be screened simultaneously in a single compartment," says Anthony Keefe, the senior vice president of innovation at X-Chem, a Waltham, Massachusetts–based provider of DEL technology and services. "With a vastly increased number of compounds tested, the likelihood of finding a hit goes up," he adds. But this isn't just a numbers game: the chemistry matters, particularly molecular weight (see box "Getting the most out of a DEL screen," page 6). Hits from an encoded library that is populated with lead-like compounds are more likely to progress, according to Keefe, who adds that such libraries are a focus at X-Chem.

DEL technology is also significantly cheaper per molecule screened than traditional compound libraries. Because it does not require a major investment in automation, DEL provides "a cost-effective way to screen very large compound sets in pools rather than arrays," says Ian Storer, the head of hit discovery at AstraZeneca.

Another advantage of DEL is that by its very nature, it "can identify hit compounds to targets without a known functional activity," Storer says. This gives it an edge, as HTS usually relies on detecting changes in the activity of a target. Since DEL screens are instead based on target affinity, they can be applied to targets without known or traditional activity.



The application of DELs to hit identification is based on affinity for the target. This figure shows how entire library collections are incubated with a protein target in free solution. The target is subsequently captured on a matrix, and nonbinding library members are washed away; this is followed by amplification and sequencing of bound library members to permit their identification.

Source: X-Chem.

DELVING INTO DEL

The concept behind DEL screening is simple. Combinatorial chemistry is used to rapidly build huge libraries of diverse compounds from a set of chemical building blocks. Each of the individual building blocks is encoded by a unique short DNA tag, which means every molecule in the final library has a unique combination of tags—a DNA barcode.

To carry out the screen, the entire DEL is placed in a single Eppendorf tube and incubated with the biological target, typically a protein. Compounds with an affinity to the target will bind to it. After the mixture has come to equilibrium, the protein and bound library members are captured on a matrix. Unbound library molecules in the tube are washed away, and the bound molecules are identified by amplifying and then sequencing their DNA barcodes. Next, the hit molecule is resynthesized without its DNA tags and its biological activity confirmed.

The idea of a DEL screen was first floated in 1992 by Richard Lerner and Sydney Brenner, who were both at the Scripps Research Institute in California.[2] They drew their inspiration from conceptually similar work with proteins and RNA—the latter enabled by polymerase chain reaction (PCR)—all reported in the previous decade. DEL screening wasn't fully enabled until the mid-2000s, when the advent of next-generation gene sequencing methods slashed the price of reading the DNA barcodes by a factor of 1 million. "It was the advent of new gene sequencing techniques that permitted this platform to truly work," Keefe says.

In 2017, when reflecting on the progress the field has made since they presented their original concept, Lerner and Brenner wrote, "By linking the power of genetics and the power of chemistry a new field has emerged. … Scientists can now do selections on the benchtop that previously required access to large and complex high-throughput screening centers."[3]

Today, many pharmaceutical companies use DEL screens to generate hits in early-stage drug discovery. Some outsource this work to DEL technology experts like X-Chem; others have brought DEL screening capabilities in-house, although many with their own screening facilities still source DELs from external suppliers.

The expense of HTS is considerable, and most large pharmaceutical companies run only a few such projects a year. To avoid wasting this valuable resource, it is important to be confident of the tractability of a target before undertaking an HTS. Because a DEL screen's cost and reagent consumption needs are relatively low, some organizations are beginning to use a DEL screen as a tractability assessment metric. The output of such an assessment then informs the screening strategy, which may include HTS for sufficiently tractable hit molecules. Genentech recently published a paper describing how it adopted this strategy.[4]

THE COVALENT COMEBACK

The discovery of covalent drugs—those that form covalent bonds with their target protein—is one area where DEL is proving invaluable. Many of our oldest and most used drugs—including aspirin, penicillin, and omeprazole—are covalent. But screening for new covalent drugs fell out of fashion, and, as a result, the vast majority of compounds in traditional HTS libraries do not bind to targets covalently. By the early 2000s, covalent drugs were back in vogue because the covalent approach offered advantages such as enhanced potency, selectivity, and prolonged action. Fourteen covalent drugs gained approval from the US Food and Drug Administration between 2011 and 2019,[5,6] which represents >5% of the drugs approved in that period.

"We're pioneers of extending the DEL approach to the discovery of covalent irreversible molecules," Keefe says. X-Chem has a dedicated electrophilic DEL collection comprising over 100 billion compounds with the same broad chemical diversity as its noncovalent DEL collection. Each library molecule in this library deck contains an encoded covalent warhead capable of forming covalent bonds with nucleophilic residues within protein targets. "Covalent DEL is well suited for hit generation of targeted covalent inhibitors owing to the observable affinity-based binding and irreversible nature of the selection methodology," says Ying Zhang, the vice president of discovery at X-Chem.

This library collection's utility has already proved its worth against a handful of anticancer drug targets, including Bruton's tyrosine kinase.[7]

DRUGGING THE UNDRUGGABLE

For biological targets that evade hit identification using traditional screening approaches, DEL screens are finding success. The protease-activated receptor 2 (PAR2) is one such example. This G-protein-coupled receptor has long been known to play an important role in inflammatory responses, pain, and some cancers.

PAR2 activation occurs when its N-terminus is cleaved by an extracellular protease. The released peptide can then bind to and activate the receptor. No drugs have been approved that target PAR2.

In 2017, a collaboration of scientists from AstraZeneca, Heptares Therapeutics, and X-Chem published in *Nature* the details of two different antagonists bound to PAR2.[8] One of the compounds, AZ3451, was identified by X-Chem scientists using a DEL screen, and they managed to solve the crystal structure of it binding to a hitherto unknown remote allosteric site on PAR2.

"The unbiased nature of a DEL screen allowed the discovery of a number of ligands that were shown to comprise both agonists and antagonists and led to the discovery of a novel allosteric binding site," AstraZeneca's Storer says. "The ability of a single screening technology to deliver such a rich output is a great example that showcases the power of DEL."

Allosteric binding is believed to prevent the structural rearrangement needed for PAR2's activation. A follow-up study demonstrated in vivo anti-inflammatory activity for both molecules and concluded that their mechanisms have high potential for the design of future PAR2-targeted therapeutics.[9]

REMOTE OPPORTUNITIES

As described above, DEL screening can be used to identify molecules that interact with sites remote from the active site—including exosites. Blocking an exosite may modulate the protein's biological function. "The protein active site still remains accessible and fully functional, unlike an allosteric modulation," says Ghotas Evindar, vice president and head of hit discovery and lead generationat Exo Therapeutics. "Exosite modulation allows you to block the desired single [mechanistic] pathway, but not any other affiliated pathways that could lead to potential side effects."

Exo was founded in 2020 based on the DEL technology developed to find exosite inhibitors.[10,11] Much of this foundational work focused on efforts to target an exosite in insulin degrading enzyme. This enzyme breaks down both insulin and glucagon, two molecules with opposing effects on the body. Targeting the exosite rather than the active site enables insulin binding to be inhibited while preserving glucagon activity.

Exo has since moved beyond diabetes treatments and is using DEL screening to discover exosite modulators for enzymes that are relevant to oncology, inflammation, and immunology therapeutics. "Our first pass [screening technology] is always DNA-encoded libraries," Evindar says. The firm has four drug leads currently advancing toward clinical trials.

PUTTING IT TO THE TEST

No drugs that started with a DEL screen have so far reached the pharmacy shelf. This isn't surprising, as the use of DEL screening didn't take off until the mid-2000s, and on average it takes around 12 years for a drug to travel from bench to bedside. In addition, less than 14% of all drug candidates that enter clinical trials are eventually approved.[12]

There are, however, multiple drug candidates in Phase 1 and Phase 2 trials that were found with the help of DEL technology. Examples include X-165, an autotaxin inhibitor that was cleared by the FDA for Phase 1 trials against idiopathic pulmonary fibrosis, a progressive lung-scarring disease. This drug discovery program started with a DEL screen that identified a series of potent autotaxin inhibitors. "Optimization of the hits proceeded rapidly and efficiently given the rich SAR [structure activity relationship] information from the DEL screen," Zhang says. "This further illustrates the potential of DEL technology to identify hits and also to accelerate hit-to-lead optimization by virtue of the richness of data within the selection output." Successful development of hit series from this screen by X-Chem scientists led to the clinical candidate X-165.[13]

X-Chem's Keefe predicts that the use of DEL technology will continue to accelerate. He also expects more researchers to start out with a DEL screen rather than turning to it when more traditional approaches have failed to identify suitable compounds. "It becomes the first thing they do, rather than the last thing they do," Keefe says. The increased use of this technology will inevitably result in an increasing number of molecules on our pharmacy shelves whose roots originate from a DEL screen.

GETTING THE MOST OUT OF A DEL SCREEN

1) Secure an adequate amount of active target

A DEL screen requires around 1 mg of the target enzyme or other type of protein. "You need to know how to express and purify the enzyme in a form that is highly active and properly folded," says Anthony Keefe, the senior vice president of innovation at X-Chem, a Waltham, Massachusetts–based provider of DEL technology and services. "You want the vast majority of what's in the [Eppendorf] tube to be an active protein."

2) Choose your library carefully

"It is extremely important to have access to a library collection that delivers molecules suitable for lead identification and subsequent development," says Ying Zhang, vice president of discovery at X-Chem. Library compounds that cover a highly diverse chemical space and possess desirable physicochemical properties found in lead- and druglike compounds provide the best chance for screening success.[14] Such libraries embrace a philosophical approach that prioritizes access to large numbers of building blocks in combination with proprietary schemes to deliver desirable lead-like compounds across a broad and diverse chemical space, Zhang says. X-Chem has pioneered this philosophy and has a large number of libraries that conform to it. These libraries are now accessible to all through X-Chem's ReadiDEL program.

3) Consider your need for exclusivity

X-Chem offers DEL screening on both an exclusive and nonexclusive basis. With the exclusive service, DELcore, clients receive proprietary licenses for confirmed hit compounds. This means that no hit molecule would ever be included in compounds provided to a different client, Keefe says.

The nonexclusive option provides clients with a significant cost reduction, which makes it a favorite of start-ups and other small companies, Keefe says. It was established at the suggestion of Ghotas Evindar, the executive director of drug discovery at Exo Therapeutics in Watertown, Massachusetts. Exclusivity is unnecessary, Evindar says. "Even if you give the same molecule from a DEL screen to two different medicinal chemists, the output of their medicinal chemistry will be two drug candidates that are quite different from one another."

REFERENCES

1. Robert A. Goodnow Jr., Christoph E. Dumelin, and Anthony D. Keefe, "DNA-Encoded Chemistry: Enabling the Deeper Sampling of Chemical Space," *Nat. Rev. Drug Discov.* 16 (Feb. 2017): 131–47, https://doi.org/10.1038/nrd.2016.213.
2. Sydney Brenner and Richard A. Lerner, "Encoded Combinatorial Chemistry," *Proc. Natl. Acad. Sci. U.S.A.* 89, no. 12 (June 15, 1992): 5381–3, https://doi.org/10.1073/pnas.89.12.5381.
3. Richard A. Lerner and Sydney Brenner, "DNA-Encoded Compound Libraries as

Open Source: A Powerful Pathway to New Drugs," *Angew. Chem. Int. Ed.* 56, no. 5 (Jan. 24, 2017): 1164–5, https://doi.org/10.1002/anie.201612143.
4. Peter S. Dragovich et al., "Small-Molecule Lead-Finding Trends across the Roche and Genentech Research Organizations," *J. Med. Chem.* 65, no. 4, (Feb. 24, 2022): 3606 –15, https://doi.org/10.1021/acs.jmedchem.1c02106.
5. Bethany Halford, "Covalent Drugs Go from Fringe Field to Fashionable Endeavor," *Chem. Eng. News* 98, no. 43 (Nov. 9, 2020): 28 https://cen.acs.org/pharmaceuticals/drug-discovery/Covalent-drugs-fringe-field-fashionable/98/i43.
6. Fandi Sutanto, Markello Konstantinidou, and Alexander Dömling, "Covalent Inhibitors: A Rational Approach to Drug Discovery," *RSC Med. Chem.* 11 (July 2020): 876–84, https://doi.org/10.1039/D0MD00154F.
7. J. P. Guilinger et al., "Novel Irreversible Covalent BTK Inhibitors Discovered Using DNA-Encoded Chemistry," *Bioorg. Med. Chem.* 42 (May 2021): 116223, https://doi.org/10.1016/j.bmc.2021.116223.
8. Robert K. Y. Cheng et al., "Structural Insight into Allosteric Modulation of Protease-Activated Receptor 2," *Nature* 545 (May 2017): 112–5, https://doi.org/10.1038/nature22309.
9. Amanda J. Kennedy et al., "Protease-Activated Receptor-2 Ligands Reveal Orthosteric and Allosteric Mechanisms of Receptor Inhibition," *Commun. Biol.* 3 (Dec. 2020): 782, https://doi.org/10.1038/s42003-020-01504-0.
10. Juan Pablo Maianti et al., "Anti-diabetic Activity of Insulin-Degrading Enzyme Inhibitors Mediated by Multiple Hormones," *Nature* 511 (July 2014): 94–8, https://doi.org/10.1038/nature13297.
11. Juan Pablo Maianti et al., "Substrate-Selective Inhibitors that Reprogram the Activity of Insulin-Degrading Enzyme," *Nat. Chem. Biol.* 15 (2019): 565–74, https://doi.org/10.1038/s41589-019-0271-0.
12. Chi Heem Wong, Kien Wei Siah, and Andrew W. Lo, "Estimation of Clinical Trial Success Rates and Related Parameters," *Biostatistics* 20, no. 2 (April 2019): 273–86, https://doi.org/10.1093/biostatistics/kxx069.
13. John W. Cuozzo et al., "Novel Autotaxin Inhibitor for the Treatment of Idiopathic Pulmonary Fibrosis: A Clinical Candidate Discovered Using DNA-Encoded Chemistry," *J. Med. Chem.* 63, no. 14 (July 23, 2020): 7840–56, https://doi.org/10.1021/acs.jmedchem.0c00688.
14. Ying Zhang and M. A. Clark, "Design Concepts for DNA-Encoded Library Synthesis," *Bioorg. Med. Chem.* 41, no. 1 (July 2021): 116189, https://doi.org/10.1016/j.bmc.2021.116189.

AMERICAN CHEMICAL SOCIETY
1155 Sixteenth Street, NW,
Washington, DC 20036