

Freedom to evolve

Researchers at the Complutense University of Madrid are using directed evolution techniques to develop new enzymes for a variety of bioprocessing applications. Taking advantage of the walkaway liquid handling capabilities of a Freedom EVO® 75 workstation, the university's Enzyme Biotechnology Group – headed by Dr Isabel de la Mata – has been able to investigate ~4,000 *Rhodococcus* mutants for penicillin acylase and N-acyl homoserine lactone acylase activity, conducting over 190,000 assays in the primary screen alone.



The Enzyme Biotechnology Group – part of the Biochemistry and Molecular Biology Department I – at the Complutense University of Madrid, Spain, has been investigating industrial applications of enzymes for over 30 years. One of the group's current research areas is the investigation of enzymes with potential applications in the synthesis of therapeutics, as Dr de la Mata explained: "We are interested in enzymes with novel activities for use in the production of semi-synthetic antibacterial agents, such as β -lactam antibiotics, to overcome the resistance mechanisms which are becoming

increasingly prevalent in nosocomial pathogens. We are also using this approach to develop novel antifungal agents, such as echinocandins, as there are currently very few effective therapeutic antifungals."

"We use directed evolution techniques to look for mutations which modify the substrate specificity or catalytic activity of the target enzyme, as well as its stability in differing environmental conditions, such as variations in pH or temperature or presence of organic solvents. The success of these molecular evolution methods depends on the ability to investigate large numbers of clones, and so we needed a stable micro-organism expression system suitable for high throughput screening. Unfortunately, many of the enzymes we study cannot be expressed in the commonly used *E. coli* models, and so we have had to develop an alternative system

using *Rhodococcus* sp. strain T104. *Rhodococcus* is far more difficult to work with than *E. coli* but, using our Freedom EVO 75 platform, we have been able to develop a robust high throughput assay for screening the mutants in a 96-well microplate format."

The group's Freedom EVO 75 platform is equipped with an 8 Plus 1 Access™ arm, a MIO™ incubator and temperature-controlled carriers for assay plates and reagents, allowing walkaway processing and reproducible results. Rodrigo Velasco, a PhD student in the group, commented: "Thanks to the throughput capabilities of the Freedom EVO platform, each sample could be simultaneously assayed under several operational conditions, which would be very complex and difficult to achieve manually. This provides invaluable information to help us design the downstream stages of the research."



Left to right: Rodrigo Velasco, Miguel Arroyo and Isabel de la Mata with the group's Freedom EVO 75



Dr de la Mata continued: “Using the Tecan platform we have been able to develop a sensitive, high throughput colorimetric screening method to identify, for example, new acylase specificities. In the first round of screening we investigated over 4,000 individual clones, each tested in duplicate, looking at a variety of conditions: two pH values, two temperatures and seven different substrates. This experimental design resulted in over 190,000 individual assays, which would have been impossible to perform manually, but using the Freedom EVO we were able to process the samples faster than we could analyze the results! The primary screen gave us a number of promising candidates with characteristics of interest for technological applications, which were then taken forward to create a second generation of mutants for screening. This second round of testing consisted of around 27,000 assays, and yielded several highly efficient biocatalysts for further investigation, all within two years of beginning the project.”

“We have been very happy with the performance of the Freedom EVO workstation and the assistance we have received from Tecan. This type of project simply wouldn't have been possible to perform manually, and the technical support has been excellent,” Dr de la Mata concluded.

To find out more about Tecan's Freedom EVO 75, visit www.tecan.com/freedomevo75

To learn more about the Complutense University of Madrid, go to www.ucm.es

“We were able to process the samples faster than we could analyze the results!”



Werner Hälgl, Corporate IP Coordinator

Leading the debate

When people hear the term ‘intellectual property’ (IP), they usually only think of patents. However, IP also includes trademarks, design rights, internet domain names, copyrights and trade secrets, all of which can play an important role in a company's ability to meet the needs of customers. As a leading manufacturer of laboratory automation technologies, Tecan constantly strives to offer faster, more robust and more efficient solutions to address the specific needs of the life sciences market. Innovation is a crucial aspect of what we do as a company, and supporting this with a strong IP portfolio is an important part of ensuring we offer our customers the very best technical solutions and support.

Although the end user may not be aware of it, every Tecan system is packed with unique, proprietary technologies which help to ensure more efficient and reliable performance, from the Spark™ 10M's Humidity Cassette – which minimizes evaporation during assays – to Adaptive Signal Technology™, a new generation of liquid level sensing introduced with the Fluent™ workstation. The software and its features are also protected by copyright law. Trademarks and registered designs are another important aspect of IP. These don't just stop another supplier from using a product's name or ‘look and feel’, they are an important way of offering the customer a ‘guarantee’ that they are buying a genuine Tecan product, backed by our liquid handling expertise and application knowledge.

This innovation, quality and integrity is especially important for our Partnering Business. Our OEM customers rely on Tecan to develop and build high quality, effective automation solutions that offer trouble-free operation and high quality results. This helps to ensure a positive experience for end users and to protect our partners' reputations and market positions.

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