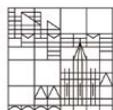


Nanoliter liquid transfers aid search for new antibiotics

Researchers at Germany's University of Konstanz have established a fluorescence-based method for screening small molecules to identify potential antimicrobial agents. Using the Freedom EVO® workstation's MultiChannel Arm™ 384, the University's Screening Center has developed an innovative technique allowing reliable liquid transfers down to 80 nanoliters.

Universität
Konstanz



Most antimicrobial agents that target the ribosome as their mode of action act on the fully assembled ribosome, preventing translation at different points of the cycle; some are bacteriostatic, others bacteriolytic. However, a team at the University of Konstanz, Germany, is taking a new approach, looking earlier in the process and trying to identify drug candidates that might inhibit assembly of the ribosome in the first place. Dr Rainer Nikolay, a post-doctoral researcher in the Department of Molecular Microbiology, explained: "I wanted a research project that had clear medical or pharmaceutical relevance, and decided to develop a fluorescence-based screening method for drug candidates that block ribosome formation, preventing protein translation and ultimately stopping bacterial growth. With the rise of antimicrobial resistance, this is a hot topic, and can also give us a better insight into how ribosomes form at the basic molecular level."

The project makes full use of the University's Screening Center, a service facility within the Faculty of Biology that is equipped with a Freedom EVO workstation and has access to a library of over 38,000 compounds. Silke Müller, a biologist in the screening facility, has extensive experience of performing large-scale library screens using the system, and worked closely with Rainer to establish the automated screening method. Silke

added: "Our Freedom EVO is equipped with a MultiChannel Arm 384 (MCA 384), two Monitored Incubator Options (MIO™) and an Infinite® F500 microplate reader, which allows us to be very flexible in the types of assays we can perform. We work with a wide range of samples – enzymes, mammalian cells, bacteria – using various assay formats including serial dilutions, time-course assays, and virtually any kind of high throughput screening assay that the groups here want to do. The results we get are very reproducible and the system itself runs very reliably."

To analyze the ribosome assembly *in vivo*, the team used chromosomal gene knock-in techniques to create an *E. coli* strain harboring large and small ribosomal subunits labeled with the fluorescent proteins EGFP

and mCherry respectively. This reporter strain showed growth properties and translation apparatus that were similar to the wild type, allowing alterations in ribosome assembly to be detected using the ratio of EGFP to mCherry fluorescence*. Rainer described the principle of the assay: "Only intact ribosomal subunits exhibit fluorescence activity so, by measuring the fluorescence intensity of both proteins, we could identify potential subunit-specific assembly defects. Based on this principle, we developed a screening assay in 384-well microplates, using 80 µl of cell suspension per well. This small reaction volume is ideal for screening applications – allowing large-scale studies to be performed cost effectively – but can require wasteful predilution of the 10 mM stock solutions from the compound library."



Dr Rainer Nikolay and Silke Müller



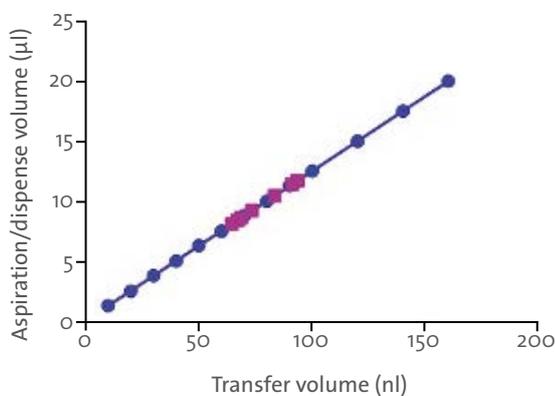
To achieve a final compound concentration of 10 μM without the need for dilution of the stock solution, the team developed a novel liquid transfer technique based on the MCA 384. Silke explained: “The minimum transfer volume specified for the MCA 384 is 500 nl, but we needed to achieve reliable transfer of just 80 nl of liquid. Using fixed steel tips, I developed a technique similar in principle to a pin tool device, relying on the residual liquid volume on the inside and outside of the tips following several aspiration and dispense cycles to provide very low volume transfers. Although the exact aspiration/dispense volume and number of cycles needs to be optimized for each assay buffer, this method has proved highly reliable, with CVs of $\sim 10\%$ for 80 nl transfers.”

“With Silke’s help, we figured out all the different parameters,” Rainer continued. “Everything now works smoothly and the system is very reliable. The two MIOs make it possible to run 12 plates daily, taking several fluorescence measurements at reasonable time intervals. We believe this is the first method to directly detect ribosome assembly defects *in vivo* in a high throughput compatible format and, as a result, we discovered some promising compounds that we would like to characterize further.”

To find out more about Tecan’s drug discovery solutions, visit www.tecan.com/drugdiscovery

To find out more about the University of Konstanz Screening Center, visit cms.uni-konstanz.de/screening-center/screenings

*Rainer Nikolay, Renate Schloemer, Sabine Schmidt, Silke Mueller, Anja Heubach and Elke Deuerling. Validation of a fluorescence-based screening concept to identify ribosome assembly defects in *Escherichia coli*. *Nucl Acids Res* (2014) **42** (12): e100.



A calibration curve is performed for each assay buffer, demonstrating the excellent reproducibility of the liquid transfer technique

“This method has proved highly reliable, with CVs of $\sim 10\%$ for 80 nl transfers.”