# Investigating protein targets and cellular pathways in yeasts

Scientists at the Donnelly Centre for Cellular and Biomolecular Research have chosen generations of Tecan microplate readers to monitor the effects of environmental or drug perturbation on molecularly barcoded yeasts.

The Donnelly Centre for Cellular and Biomolecular Research, based at the University of Toronto, Canada, is an interdisciplinary research institute that houses scientists from a wide variety of backgrounds, integrating the fields of biology, computer science, engineering and chemistry, as well as leading areas of biomedical research. Corey Nislow, Associate Professor at the Donnelly Centre's Banting and Best Department of Medical Research (BBDMR) faculty, explained: "The Donnelly Centre houses faculty from many different departments, adopting a multidisciplinary approach to experimental studies with the intention of cross-pollinating between bioinformaticians, genomic and proteomic scientists. My own laboratory, and the laboratory of my collaborator Dr Guri Giaever, is responsible for running a next generation chemogenomics facility, and our work involves monitoring the growth of large pools of molecularly barcoded yeast, simultaneously screening 6,000 different mutants. The presence of a unique mutant barcode identifier enables us to distinguish each one of the mutants and deconvolute a complex sample pool at the end of an experiment. This simplifies the procedure, allowing the pool to be treated as a simple culture and challenged with different environmental or drug perturbations."

Corey continued: "Guri, Michael Proctor (a research scientist working at Stanford University at the time) and myself first developed a workstation for this in 2000, consisting of four Tecan GENios™ microplate readers to monitor the growth of the culture, integrated with a PerkinElmer multiprobe. The basic principle is the same for this and the second generation system we later created using Tecan's Safire™ reader and Freedom EVO® 200 liquid handling workstation. Based on a predetermined parameter, the liquid handler samples and reinoculates the culture, keeping it in logarithmic phase for up to 100 generations and allowing subtle effects on different mutants to be investigated. Initially, the liquid handler moves some of the culture to fresh media and, at the time of transfer, saves a sample so that the abundance of each strain can be decoded at the end of the experiment by microarray hybridization or next generation sequencing. The

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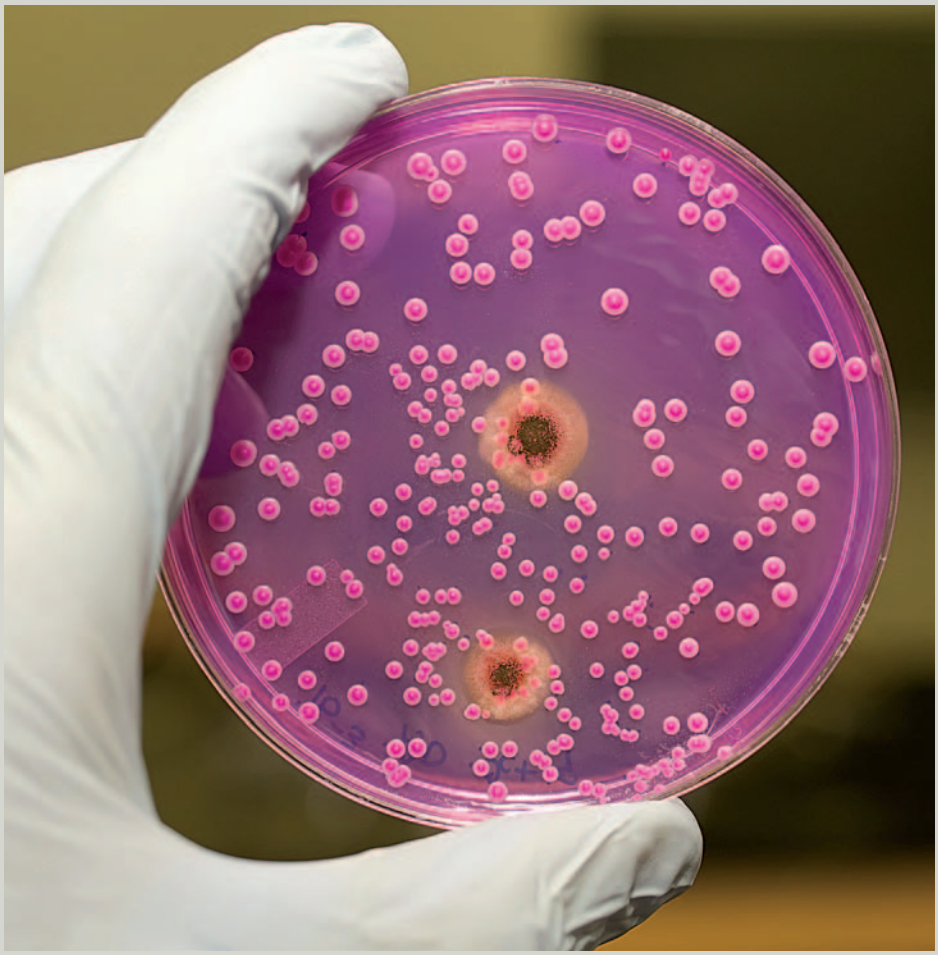
Corey Nislow and Guri Giaever with their Freedom EVO liquid handling workstation

readers monitor both optical density and fluorescence – 95 % of the time we monitor optical density, but occasionally we study the readout from a fluorescent reporter – and each well of each plate is independently monitored. Essentially, we chart the abundance of every strain under a particular condition and, based on the abundance of the different mutants, infer the particular protein targets or cellular pathways that are important for culture survival under those circumstances. In the absence of these protein targets or cellular pathways, the culture is sensitive to that particular condition."

"For the first system we chose the GENios reader because it was the only instrument at the time that had a sufficient orbit to keep yeast cells well suspended and that could maintain temperature without condensation. However, although this system is still in use, the workflow means that the readers are effectively serving as shaking incubators, operating 24 hours a day, and shaking and reading at 15 minute intervals. The process of ejecting each plate to allow the liquid handler to sample the culture and return the plate takes a minute each time and is very much a rate-limiting step. In contrast, the second generation screening system is equipped with six shaking incubators, and the Safire microplate reader is just used to read! The speed of the Safire reader allows six to eight plates to be accommodated without any additional waiting time, and we can now interrogate model organisms that require light, such as the model algae *Chlamydomonas*; this would previously have been impossible in the dark of the reader. In addition, using dedicated shaking incubators has enabled us to increase capacity."

"We didn't just 'settle on' Tecan, we purposely selected the Company, and so did all of our collaborators, who loved the growth curves they saw from our work. Every time we ran large screens we needed to confirm individual strains, and that required a server rack full of readers. We have remained with Tecan and, between our Stanford site and Toronto, have 24 GENios systems, which are all still running! The flexibility of the Tecan systems is a big advantage, particularly the smooth information transfer."



"As well as increasing throughput, our Tecan instruments have given us new avenues of exploration. We are now focusing on data collection and the introduction of new organisms, developing simple barcoding strategies for other organisms and investigating *E. coli* strains associated with Crohn's Disease. We also plan to study more model organism genomes, *de novo* genomes, and will be doing a lot of next generation sequencing library preparation. In the future, we plan a third generation system, with two new generation Tecan readers and twelve shaking incubators, which will further increase our capabilities," concluded Corey.

To find out more on Tecan's detection solutions, visit **www.tecan.com/detection**

To find out more about the Donnelly Centre, visit **tdccbr.med.utoronto.ca**

The GENios and Safire systems have been superceded by the Infinite® range of microplate readers, which features even more enhanced capabilities and is ideal for the assays and methods described above.