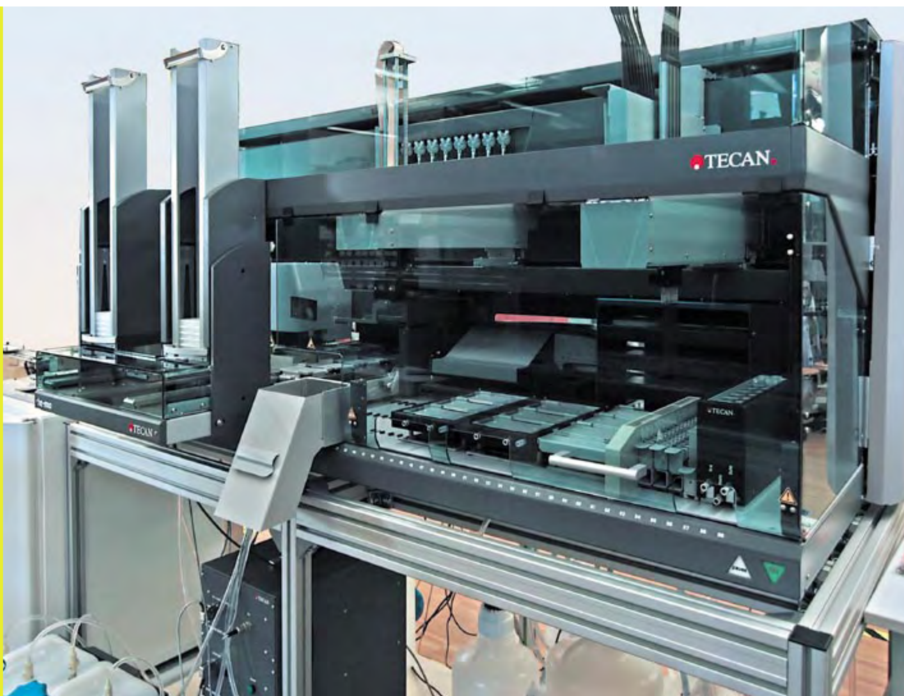
High throughput screening of infectious virus entry in mammalian cells

Researchers at the Institute of Molecular Systems Biology (IMSB)*, ETH Zurich, have two Freedom EVO® 200 liquid handling workstations that have been custom developed for high throughput screening of infectious virus entry in mammalian cells. The large-scale study aims to create a profile of all the mammalian genes involved in infection by 11 major viruses, and is estimated to be complete within a year.



Custom developed Freedom EVO 200 liquid handling workstation

Can you imagine not having to worry about viral infections – no more flu, no risk of chicken pox, maybe even the end of HIV? This could only be possible in a world where viruses are less able to develop resistance to anti-viral therapies. It might sound too good to be true – but it's a very real dream for Dr Lucas Pelkmans, assistant professor at the IMSB, and his colleagues. Their theory is simple: rather than try to attack the virus, you change the entry mechanisms that viruses use to infect mammalian cells – almost like changing the door locks. Since the entry mechanism proteins are expressed by the host cells and not by viruses, it would be difficult for a virus to adapt to the change.

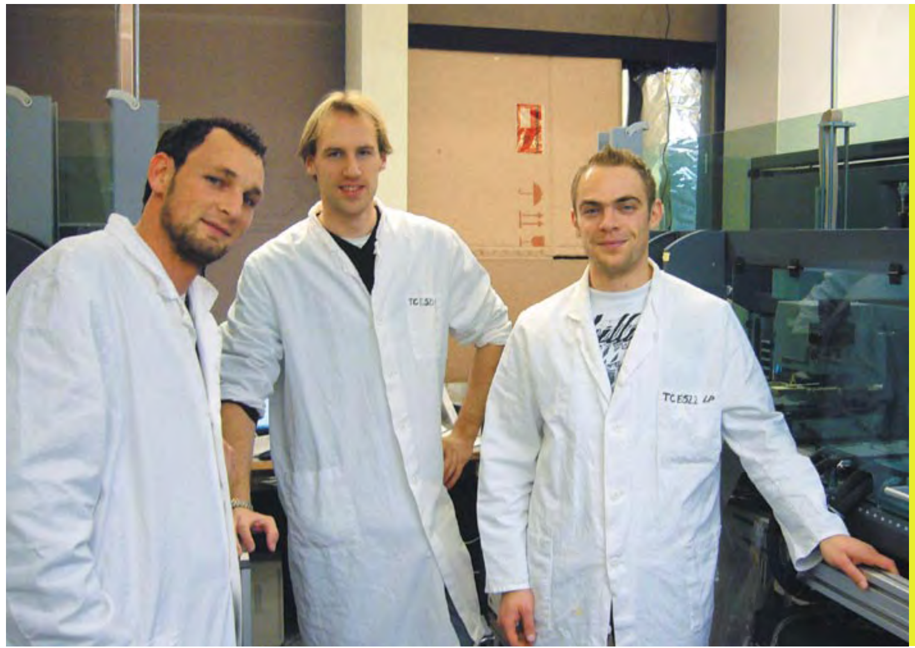
Such anti-viral therapies are a long way off, but the team at the Swiss Federal Institute of Technology (ETH), Zurich, is already identifying the specific sets of host genes that different viruses depend on to infect cells. Endocytosis – the process by which cells absorb material – can occur in a variety of ways, and it is well established that viruses make use of many endocytic pathways for host entry. Major mechanisms include the clathrin-mediated uptake route, caveloae-mediated endocytosis, lipid-raft mediated endocytic pathways and macropinocytosis.

Dr Pelkmans is looking at how viruses hijack these pathways and has recently embarked on a genome-wide study to individually silence 7,000 mammalian genes using RNA interference (RNAi), and measure how each silencing affects the abilities of 11 different viruses, including influenza, herpes and SV40, to infect mammalian hosts. The 7,000 genes have been chosen for their potential involvement in the process, based on protein structure and DNA sequence. By systematically silencing each gene, a corresponding decrease or increase in viral transfection of cells can be measured, giving a functional host genetic profile of virus entry for each virus type studied, which directly reflects how the virus enters and infects the cell.

This huge project depends on a fully automated system that transfects thousands of cells with short interfering RNA (siRNA) molecules. Genetically silenced and control cells are then infected with the viruses and the corresponding viral transfection rates are analyzed. The laboratory has two Tecan Freedom EVO 200 workstations that have been custom developed to fully automate the procedure.

The first workstation carries out all the transfection and infection assays in 384-well plates using a Te-MO™ 384-channel pipetting head. A reverse transfection procedure is used, where the siRNAs and reagents are plated out in advance; these plates can be stored at −80 °C for several months. The workstation then plates out cells (from HeLa cell lines) into the wells and transfects them with siRNAs (Qiagen). The cells are then automatically infected with viruses, and fixed and stained for analysis.

The second Freedom EVO platform has two fully integrated Cell Works microscopes and a Te-Stack™ stacker module. The entirely automated workstation loads the 384-well plates onto the microscopes to image the cells and, when the imaging is complete, each plate is removed and replaced with a new one. The captured images are then analyzed to provide a quantitative measurement of the proportion of cells that are infected with the virus. The total number of cells is automatically counted, based on nuclear staining (DAPI), and the total number of infected cells is also determined, based on expression of a reporter gene carried by the virus, such as green fluorescent protein, or antibody detection of a viral-specific protein. Dividing the total number of infected cells by the total number of cells provides an infection index; the control indices are compared with those obtained for cells that have been subjected to RNAi for a single gene, to indicate that gene's importance in viral infection. The viral infection rates of the laboratory's control cells are normally around 10%, allowing an infection increase or decrease of up to one order of magnitude to be measured as a result of the RNAi.



From left to right: Berend Snijder, Manuel Bauer and Raphael Sacher, with their Freedom EVO 200

All measurements are performed in triplicate, 10,000 cells are measured per well, and at least three different siRNAs are used per gene, to exclude false negatives or false positives. Analyzing the effects of silencing 7,000 genes on one virus, in triplicate and with three independent siRNAs, requires 207 384-well plates; it takes about two weeks using the automated set-up and including all data analysis. A reliable barcoding system and sophisticated LIMS are essential for managing the process!

For Dr Pelkmans and his colleagues, the fully automated and integrated set-up is critical to the project. In particular, the fact that even the imaging and analysis are fully automated saves a vast amount of time and labor, as well as maintaining consistency.
"When the system is fully operational, we hope to be able to just press 'start' and the system will screen 207 plates, requiring only little manual intervention. Most of the time, we really don't have to worry about whether it's working or not, because it is," said Dr Pelkmans. "We have already seen some very exciting results; the approach clearly allows us to group viruses together in a way that you would not be able to predict from classical virus groupings."

It seems that the real complexity of a viral infection does not come from the virus, but from the host. A typical entry pathway involves at least 300–500 human genes, with complicated mechanisms that include membrane trafficking, microtubule-mediated transport, cytoskeletal and cell signaling – all of which are important for successful viral infection. New ways of grouping viruses together will help us to better understand the real complexities of viral entry and, in the future, it may be possible to develop anti-viral approaches that target host cellular genes instead of viral components.

> "We have already seen some very exciting results"

*The IMSB is part of a Swiss-wide initiative, Systems X, which aims to develop and enhance systems biology research in Switzerland.