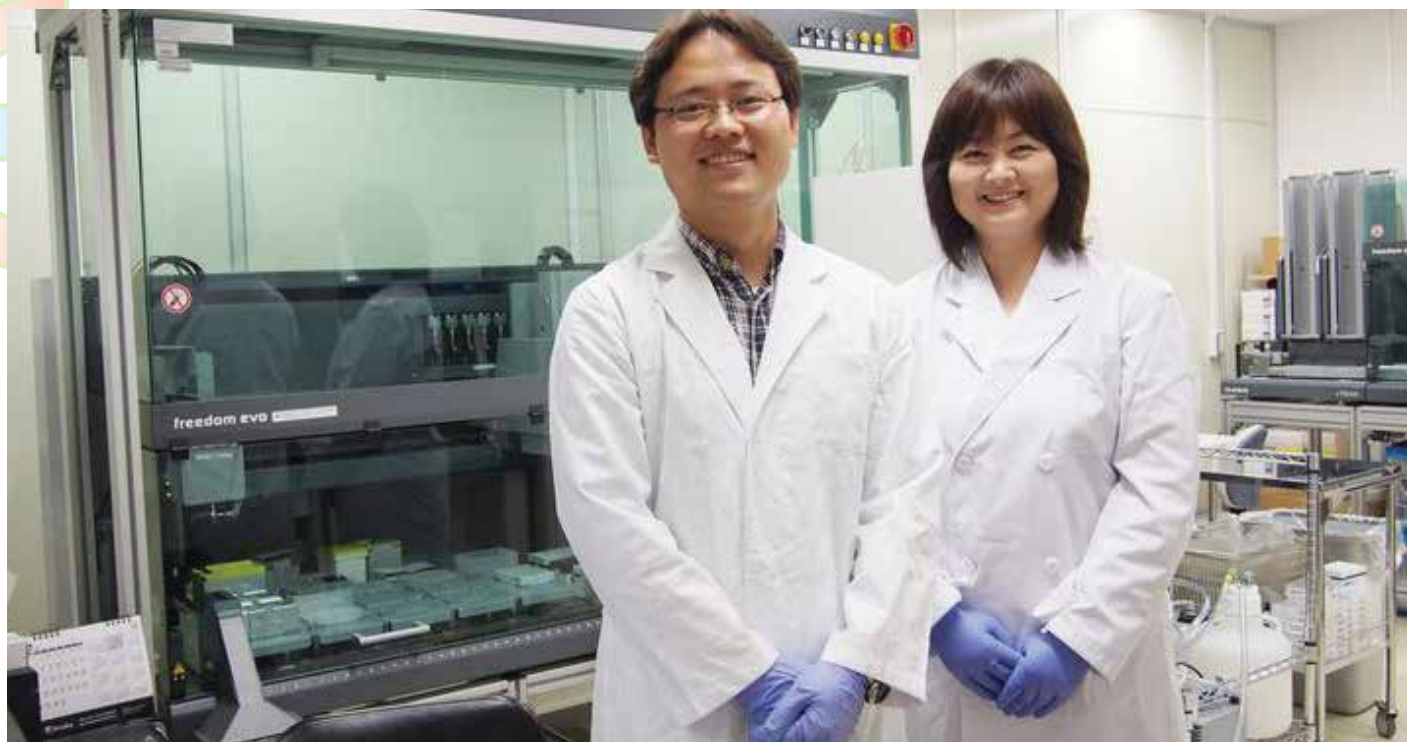


Boosting drug discovery with efficient transfection technology

The Life Sciences Laboratory of the Central Research Institute at Ishihara Sangyo Kaisha Ltd has used Tecan's Freedom EVO® 200 workstation to develop a successful automated protocol for efficient siRNA transfection, paving the way for efficient high throughput screening of small RNA libraries.



Dr Lin Bangzhong and Ms Tomona Yamaguchi with the Freedom EVO 200

“Automation has allowed us to significantly widen our products' applications, and we have now started larger scale investigations.”

Under the theme 'science to protect life', Ishihara Sangyo Kaisha Ltd in Osaka, Japan, conducts research and development of bio-reagents that contribute to progress in medical and life sciences. Working jointly with Professor Yasufumi Kaneda of Osaka University and the university's venture enterprise, the Company has developed GenomONE™, a series of unique kits for transfection, cell fusion and IgG antibody

introduction. The core technology of this product range is a new type of non-viral vector that uses the cell membrane fusion activity of envelope membrane components from the hemagglutinating virus of Japan (HVJ). The genome of the virus has been completely inactivated while still maintaining the viral envelope's ability to introduce its contents directly into target cells. Various physiologically active substances can be put into this envelope for transfection into cells.

“My work involves optimizing a transfection system for siRNA using the HVJ Envelope (HVJ-E) vector,” explained Ms Tomona Yamaguchi, research scientist at the Central Research Institute. “We have developed reagents and protocols specialized for siRNA, and have had considerable success with various cells that have, until now, been difficult to transfect; non-adherent

immune cell strains, particularly primary culture T-cells and B-cells, can now be transfected, and many GenomONE users have reported success with cell types that they could not transfect using other methods, such as lipofection and electroporation. By successfully transfecting the desired substances in appropriate amounts and still keeping the cells in good condition, the HVJ-E method gives investigators a smooth progression towards *in vivo* experimental stages.”

The team, again in association with Osaka University, has now successfully developed an automated HVJ-E transfection and functional assessment system using a Freedom EVO 200 liquid handling platform and Infinite® M1000 microplate reader, as Ms Yamaguchi described: “We started in January 2013, performing a series of experiments to confirm that we could

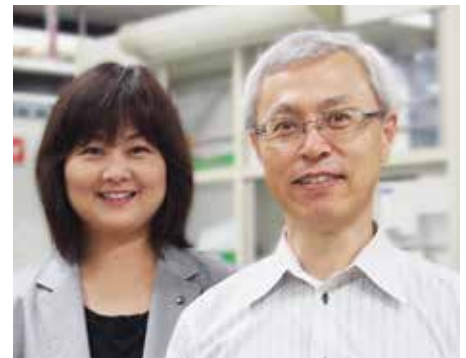
reproduce the results from manual processing and, by March, we had established an optimized automated test system.”

Dr Bangzhong Lin of Osaka University Office for University-Industry Collaboration programmed and operated the Freedom EVO 200. He commented: “For a high throughput screening (HTS) protocol such as this, it was essential to perform automated dispensing operations under sterile conditions, and this was made possible with the installation of a HEPA filter with an integrated incubator and Carousel HS™. It was also critical that minute volumes of two types of solutions were mixed within each well of a 96-well plate. This was achieved by a combination of the precise positioning of the pipetting tips during dispensing using both a Liquid Handling Arm and a MultiChannel Arm™ 96, and by expelling the droplets at high speed. Following incubation, the Infinite M1000 was used for quantitative colorimetric assessment of cell growth.”

Ms Yamaguchi highlighted the benefits of switching from manual operation: “Automation has eliminated both the variability in the results caused by differences

in the skill of the experimenter, and the potential for human error. I was particularly impressed by the way the Freedom EVO transfers plates; the movement is really smooth and there is very little vibration. Using the Freedom EVO has halved the processing time, plus we have increased the number of assay plates we are able to process, which was limited by manual handling. The quantity of consumables we use has also been reduced by our automated protocol. Automation has allowed us to significantly widen our products’ applications, and we have now started larger scale investigations, including screening large siRNA libraries for candidate molecules that may become drug discovery targets. I certainly aim to use the Freedom EVO again in future projects, whenever automation could potentially play a part.”

Dr Fuminori Kato, general manager at the Central Research Institute, added: “With the advent of techniques like next generation sequencing, it has become possible to detect and identify small RNAs that were not previously known, suggesting that many more original small RNAs are still to be discovered in the near future. By



Ms Tomona Yamaguchi and Dr Fuminori Kato

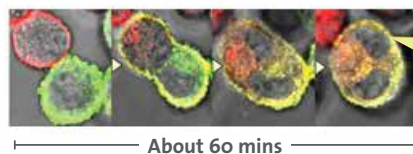
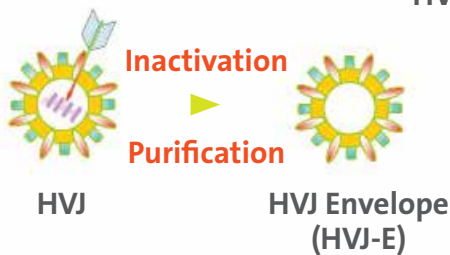
combining GenomONE with the automation of the Freedom EVO, we have been able to demonstrate HTS of immune cells for the first time. We anticipate that the GenomONE series and Freedom EVO 200 will make a great contribution towards the research of this quickly expanding group of biological molecules, as well as propelling applied research in the field of medical diagnosis.”

To find out more on Tecan’s genomic solutions, visit www.tecan.com/genomics

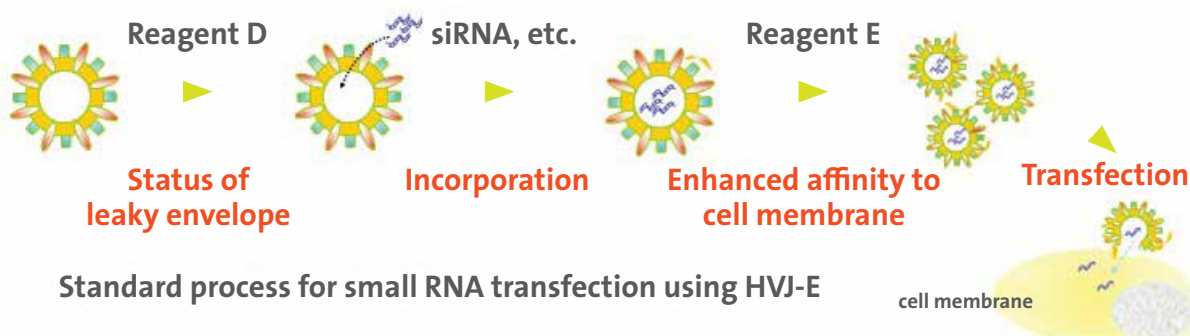
To find out more about Ishihara Sangyo Kaisha Ltd, visit www.iskweb.co.jp/eng/index.html

What is HVJ-E?

*HVJ; Hemagglutinating Virus of Japan (Sendai virus)



Cell fusion using HVJ-E!



Standard process for small RNA transfection using HVJ-E

The Freedom EVO has enabled the Company to optimize a transfection system for siRNA using the HVJ Envelope vector (schematic provided by Ishihara Sangyo Kaisha Ltd)