

Automation of time-consuming washing and detection steps accelerates screening of protein-protein interactions

The Protein Interaction Screening Unit at the German Cancer Research Center, Heidelberg, has automated the co-immune precipitation method for investigating protein-protein interactions (PPIs) using Tecan's HydroFlex™ washer and Infinite® F200 microplate reader, increasing throughput up to 100-fold compared to manual methods, and greatly accelerating PPI screening.



Tecan's HydroFlex washer for processing of magnetic beads

The German Cancer Research Center (DKFZ) in Heidelberg carries out basic scientific research into mechanisms of cancer development and identification of cancer risk factors, feeding these results into new approaches in the prevention, diagnosis and treatment of cancer. DKFZ has several core facilities offering specialized services internally and externally, including the Protein

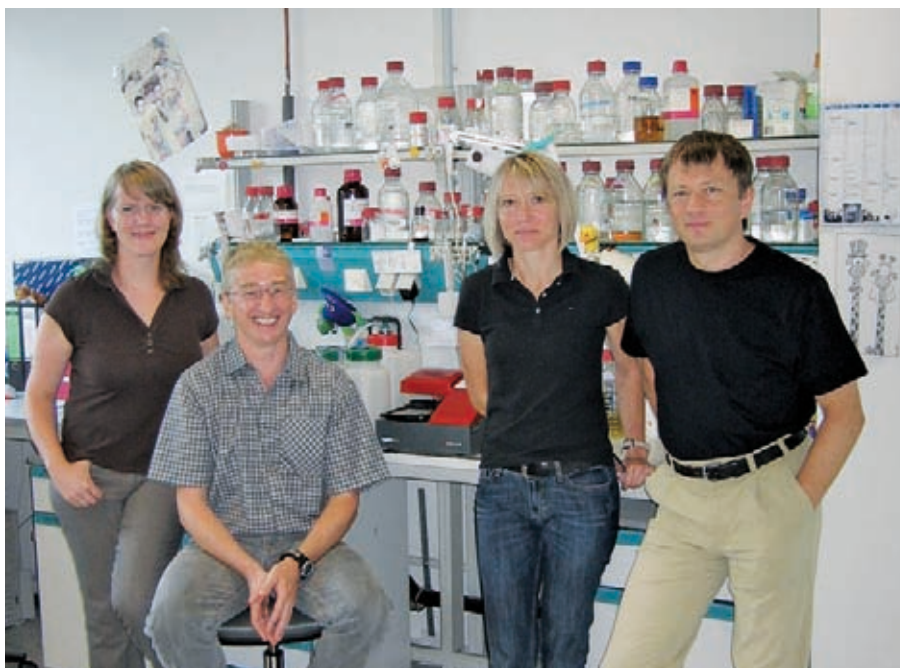
Interaction Screening unit, led by Dr Manfred Koegl. Automation is playing an important role in improving the services these facilities offer, as Manfred explained: "Researchers for many decades have increased their understanding of proteins by looking for PPIs, largely relying on the successful and cost-effective Yeast Two-Hybrid (Y2H) method. However, the Y2H method has several disadvantages

for investigating mammalian systems so we have concentrated on developing more physiological methods that are equally fast and cheap. I believe that the most promising of these methods that could take over from the Y2H method within five years is co-immune precipitation of two proteins from transiently transfected cells (Barrios-Rodiles *et al.*, 2005). A particularly important advantage of this method is that it can be easily automated."

The team at DKFZ has dedicated two Tecan instruments to automating its co-precipitation assay: a HydroFlex washer with smart-MBS option for processing magnetic Dynal® beads; and an Infinite 200 microplate reader with Connect™ stacker, allowing processing of batches of up to 50 microplates of any appropriate format without any manual intervention.

Dr Koegl described the basis of the method developed by his team: "The 96-well microplate containing a suspension of magnetic beads and proteins of interest in each well is washed in the HydroFlex and transferred, using the Connect stacker, to the Infinite 200 reader for the luminescence measurements."

Optimizing the washing stage was critical but made straightforward by the flexibility of the HydroFlex. "We discovered that the protein complexes could fall apart if washing goes on for too long,



(L to R) Kerstin Hettler, Frank Schwarz, Christiane Rutenberg and Manfred Koegl

resulting in the loss of some PPIs, but the HydroFlex washes the magnetic bead suspension very quickly and thoroughly. Two wash cycles effectively reduce the background from the initial one million counts to that of the instrument, but we found that routinely washing four times eliminates false positives due to spurious PPIs and still doesn't result in any loss of beads. It is easy to test these wash variables on the HydroFlex so we can be sure that we are working under optimal conditions, and this ease-of-handling and speed of wash are, for us, the most important features of the washer."

A major advantage of automation is the throughput his team can now achieve, as Dr Koegl explained: "Washing one 96-well microplate takes less than five minutes so we can easily wash 10 microplates in less than an hour. We are currently testing only 200-400 PPIs in one experiment, but I expect that further automation will increase the throughput to 1,000 PPIs per day. Manually, using the standard co-immune precipitation protocol, 12 PPIs are typically done per day, followed by gel electrophoresis and Western blotting."

"A further advantage of the precipitation method over the Y2H method is that it uses mammalian cells, HEK293 (Human Embryonic Kidney cells) that can be stimulated with certain triggers so that PPIs form under physiological conditions. PPIs are regulated in time and space, for

example, proteins that interact in signal transduction only do so when that signal transduction pathway is stimulated."

"The precipitation method uses two tagged proteins: one is affinity-tagged with Protein A which binds to an invariant part (FC) of any immunoglobulin (IgG) molecule for fast and efficient purification; and the second protein is tagged with a luciferase for detection of the protein-protein complex. HEK293 cells are transfected to express the protein of interest and proteins being investigated for binding to the protein of interest, so protein-protein complexes form inside the cell. Cells are lysed by a mild detergent, and magnetic beads coated with IgG are added. The Protein A-tagged protein binds to the magnetic beads and if it forms a complex with the luciferase-tagged protein, the latter will be indirectly bound to the beads. Before washing, the raw extract contains over one million counts per well. The washing steps follow incubation, removing all proteins not bound to the protein of interest, reducing the luciferase count down to below 50 per well if no proteins bind to the proteins of interest, and representing a 20,000-fold purification in the shortest possible time. Those proteins still bound to the protein of interest after washing are detected by above-background levels of bioluminescence measured by the Infinite reader. Wells

with above-background luciferase activity, detected as bioluminescence, point to an interaction between the two tagged proteins present."

"The effectiveness of this method is due to luciferase being so exquisitely sensitive in its detection. Each well of a 96-well microplate contains a different potential binding partner for the affinity-tagged protein we are analyzing, so we can test for 96 different PPIs in one microplate and we usually test one protein against many. For example, if I have a protein that I suspect has a role in transcriptional regulation, then I screen it against many different known transcriptional co-regulators."

Dr Koegl concluded: "Before we release our new automated co-immune precipitation method, we are aiming to assemble large arrays of around 1,000 proteins that we can offer to customers for testing in their PPI screening assays. Our objective is to form arrays from public clone collections arranged in groups according to cellular pathways and processes, such as proteins involved in certain signal transduction pathways, or in DNA repair. This information, together with the increased throughput the co-precipitation assay offers, will give our fellow scientists an alternative to methods like Y2H screening."

Dynal is a registered trademark of Dynal Biotech ASA, Oslo, Norway

References:

Barrios-Rodiles M, Brown KR, Ozdamar B, Bose R, Liu Z, Donovan RS, Shinjo F, Liu Y, Dembowy J, Taylor IW, Luga V, Przulj N, Robinson M, Suzuki H, Hayashizaki Y, Jurisica I, Wrana JL. High-throughput mapping of a dynamic signaling network in mammalian cells. Science. 2005 307:1621-5

For more information on Tecan's HydroFlex as well as the corresponding application note, visit www.tecan.com/hydroflex

For more information about Tecan's Infinite 200 multi-mode reader, visit www.tecan.com/Infinite200