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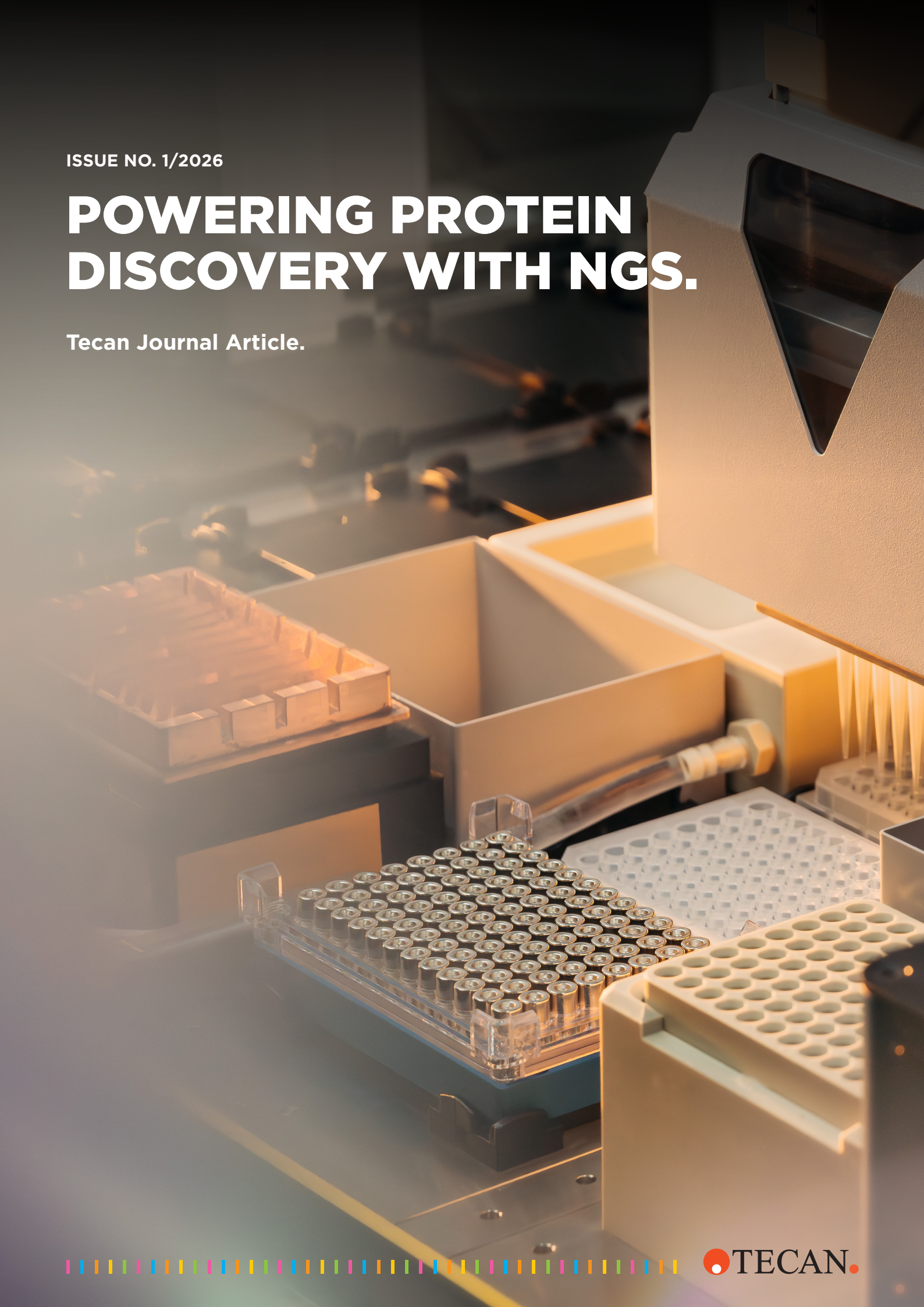
POWERING PROTEIN DISCOVERY WITH NGS.

Tecan Journal Article.



TECAN.

Proteomics workflows have long been plagued by inefficiencies, low coverage and the inability to detect low abundance targets in biological samples, lagging behind equivalent genomics workflows. SomaLogic, now part of Standard BioTools Inc., changed the paradigm for protein measurement by using modified DNA aptamers – called SOMAmer®

Reagents – to allow NGS-based measurement of proteins. Illumina® has used it sequencing expertise to develop an automated proteomics solution based on this revolutionary approach, allowing the quantification

Proteomics technologies have advanced significantly in recent years, expanding capabilities across numerous applications, including the discovery and validation of disease-specific biomarkers. Illumina's Protein Prep solution is one such technology, which enables large-scale, sensitive and highly multiplexed quantification of proteins, offering an alternative to mass spectrometry or antibody- based approaches. This pioneering solution was the result of a remarkable collaboration, combining SomaLogic's SOMAmer Reagents with Illumina sequencing and Tecan automation to deliver a high throughput platform with the potential to accelerate proteomics research worldwide.

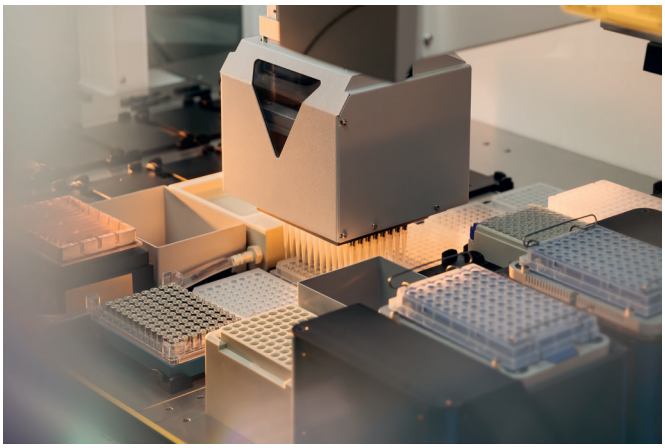
Michael Dorwart, Director of Research at Illumina, was involved in the development of this solution from the very first discussions to its recent launch. Michael explained the project's aim: "Our goal was to develop a single workflow that seamlessly integrated SomaLogic's technology with our NGS capabilities. We partnered with Tecan to help us to automate the first stage – the Illumina Protein Prep assay – which uses SOMAmer reagents with human serum or plasma samples."

"One of the biggest challenges in the project was adapting to the changes we encountered along the way; proteomics is an incredibly fast-paced field, so we must remain agile to ensure we keep addressing the needs of our customers. This proved easy to do with the team at Tecan, who allowed us to modify the features on the instrument right through its development. We ultimately chose a Fluent ® Automation Workstation equipped with a Multiple Channel Arm™ 96, a Robotic Gripper Arm™ and a Fluent ID™ barcode scanner, as well as several custom modules that Tecan developed for us. Normally, this type of assay uses an enzymatic cleavage step, but our version harnesses a unique wavelength of light, which Tecan helped us design an automated workflow for. Total hands-on time is only around four hours across the entire workflow, which takes approximately 2.5 days from running the assay through to NGS library preparation, sequencing and analysis."

"Thanks to Tecan's OEM capabilities, we were able to package the entire workflow – including Illumina library preparation and sequencing – into a streamlined solution for our customers," Michael continued. "Working with Tecan was an extremely positive experience; the internal teams are clearly very good at what they do, both on the business and technical sides. Overall, I don't know how it could have gone better.

"The Translational Genomics Research Institute (TGen) – a large, non-profit medical research institute in Phoenix, Arizona – was one of the first labs outside of the UK to implement the platform, and was part of Illumina's early access program for the system, actively participating in the development of the assay. Patrick Pirrotte, Associate Professor and Director of the Mass Spectrometry and Proteomics Facility at TGen, explained the workflow and it's benefits in more detail: "After we transfer the sample from the vials onto the assay plate, the workstation takes over. Proteins bind to the SOMAmer Reagents to form a complex, which then binds to streptavidin beads. UV light is used to cleave these complexes from the beads, and polyanionic competitors are introduced, which prevent rebinding of non-specific complexes, helping the assay to achieve better specificity. A second bead binding step is then carried out, and the beads are eluted. Following this assay, the samples are converted to sequencing-ready libraries."

The difficulty of detecting low abundance proteins in blood samples has been a major hurdle in the development of this type of assay, but this has largely been overcome. Patrick said: "The high dynamic range of proteins in plasma and serum samples is usually a real issue in proteomics studies. For example, albumin is normally by far the most abundant protein, which is of no interest to our research. In contrast, tumor-secreted proteins – including interleukins and cytokines – are usually present at concentrations 10 to 12 orders of magnitude lower. It's like searching for a needle in a haystack. Mass spectrometry has traditionally been used for this purpose, and we still use it regularly when we only have limited samples to run, but the problem is depth of coverage and, because data analysis takes a lot longer, the workflow often extends over four or five days. The Illumina solution allows us to quantify thousands of proteins in parallel, and reproducibility is brilliant. Just to give you an idea, from plate to plate – even spaced out by months – we are achieving CVs for most analytes of three to four percent or lower. This is why I am advocating people towards this solution; this is simply not something we can achieve with mass spectrometry. A large reason for this is the consistency of the automated platform, while the assay itself is also incredibly well refined."



"We've run thousands of plasma samples so far across multiple different studies. One study focuses on the research and development of an early detection pan-cancer assay designed to rule out the presence of a malignancy, which could then guide further investigations into specific diagnoses and care pathways at an earlier stage. The first part of the assay identifies a signature to rule in/out cancer, followed by malignancy staging if it was indeed positive. We've been quite successful in the discovery stage, and we're now expanding into validation, increasing the number of patients and samples that we'll run on the Tecan platform," Patrick concluded.

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