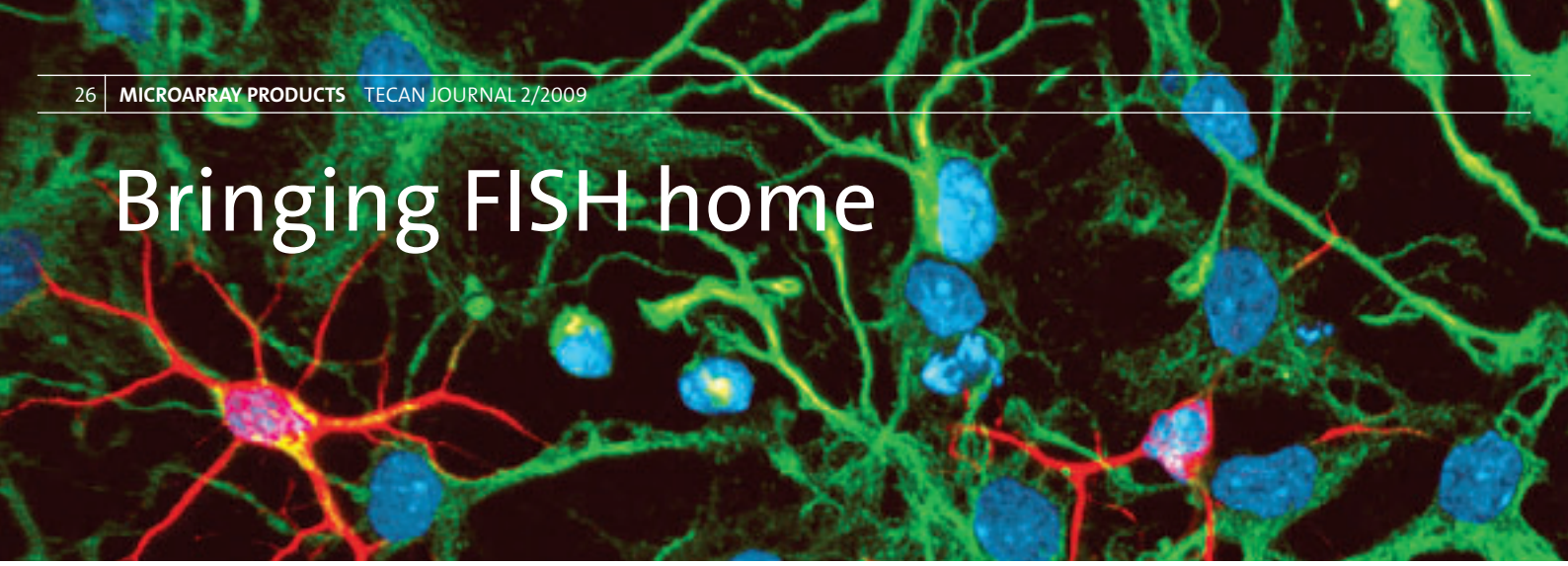


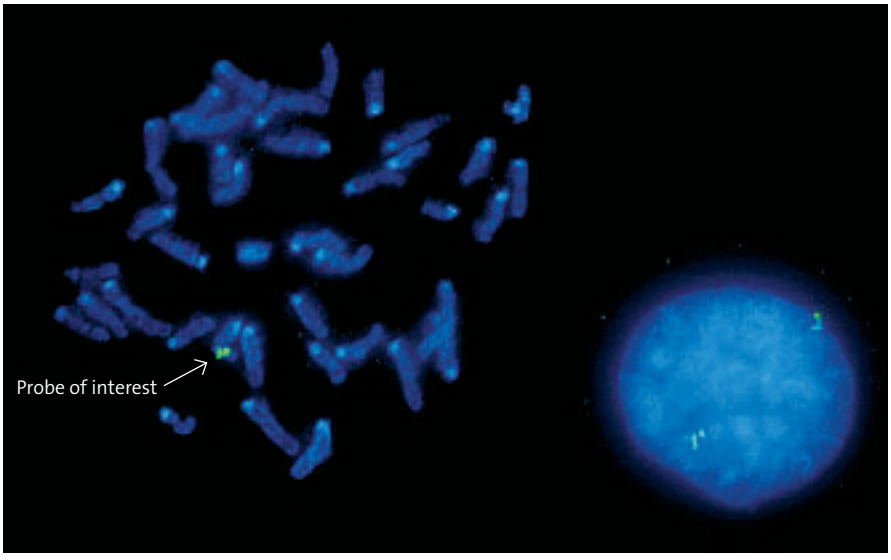
Bringing FISH home

Researchers at Medarex in California, USA, have semi-automated their hybridization process for FISH analyses with the HS 400™ Pro Hybridization Station from Tecan, allowing in-house screening of all their transgenic mouse strains.

(from l to r): Maria Galou-Lameyer and Poonam Sharma with the HS 400 Pro Hybridization Station.

Medarex, a biopharmaceutical company in California and New Jersey, USA, specializes in the discovery, development and potential commercialization of human antibody-based therapeutics for the treatment of life-threatening or debilitating diseases such as cancer, inflammatory, autoimmune and infectious diseases. The challenge faced by researchers is to convert the many thousands of potential disease-causing proteins – revealed by mapping of the human genome – into potential targets for new treatments. For many targets monoclonal antibodies may be the answer, and central to the research at Medarex is the production of high-affinity human monoclonal antibodies derived from transgenic mice. These strains of mice, distinguished by the particular antibodies they generate, need to be characterized. Immunogeneticists Maria Galou-Lameyer and Poonam Sharma, members of Medarex's animal biology laboratory, explained the role of fluorescence *in situ* hybridization (FISH) in initial strain characterization.

"An important step in the characterization of our transgenic mice is using FISH, a cytogenetic technique, to visualize the location of specific nucleic acid sequences on metaphase chromosomes prepared from individual cultured cells. A fluorescently-labeled probe, complementary to the target sequence, hybridizes to its counterpart within the chromosome, and the resulting fluorescence is detected using a microscope.



Metaphase chromosome spread of a transgenic mouse. FISH with the probe of interest reveals the transgene insertion in green, while genomic DNA fluoresces blue with DAPI. The insertions are also visible in interphase nuclei.

From FISH analysis of metaphase cells, we can establish the location and approximate copy number of the transgene in the mouse's genome. The location of transgene insertion in the mouse genome can be critical to the level of expression, ultimately affecting both the quality and quantity of human antibodies produced in the engineered mice. The ability to raise high affinity antibodies to a wide range of therapeutic targets can be challenging, as not all targets have the same degree of immunogenicity. It is hoped that FISH techniques can help us to understand the relationship between transgene integration patterns in our mice, and their ability to mount a robust antibody response. FISH analysis also helps us to track the integration status of mice harboring multiple but unique transgenes. All of this work is ultimately about making better mice, and hence better therapeutic antibodies."

Dr Sharma continued: "Our FISH analysis begins with fibroblast cultures prepared from tail tips of very young mice. Metaphase preparations of these cultured cells are spread onto slides, then processed in our HS 400 Pro Hybridization Station. We chose this system because we have had plenty of good experience with systems from Tecan, so were very confident that this hybridization station would be reliable and deliver excellent consistency from experiment to experiment. The HS 400 Pro Hybridization Station automates several steps including pre-hybridization, addition of biotin-labeled probe, hybridization, post-hybridization washing and the addition of NeutrAvidin conjugate for visualization of the biotin-labeled probe. Four slides are processed per run, with a total run time of 21 hours, and we currently use the hybridization station three or four times a week. We need to process between 40 and 50 slides per mouse strain, giving a high workload during the characterization stage, however, no further screening is required once we have obtained good metaphase spreads to establish the integration pattern of our transgenes."

"The HS 400 Pro handles our current throughput needs very efficiently. It is an excellent workflow-based system, and has enabled us to start implementing in-house FISH analyses. This set-up will allow us to look back at specific transgenic strains, some of which were created several years ago, as well as newer uncharacterized strains. FISH analysis adds valuable information to the overall picture of the mouse genotype, which ultimately informs us about its phenotype. Having the HS 400 Pro has allowed us to work to our own schedule instead of being forced to rely on external contractors, where we only have limited control over throughput and experimental variables. Overall, it's a very useful tool."

Leading the debate

Leading the debate in this issue of the Tecan Journal is Ralph Beneke, marketing product manager at Tecan for five years.

In this auspicious year we celebrate the bicentenary of Charles Darwin's birth and the 150th anniversary of the publication of his seminal work 'On the Origin of Species'. What better a time to reflect on how the study of heredity and genetics has itself evolved, and what an impact this is having on society.

Research depends on outstandingly innovative technologies making new discoveries everyday and, with applications encompassing clinical diagnostic and therapeutics, food testing, environmental monitoring and forensics, society benefits from genomics in so many diverse ways and its influence is closer to home than many realize. Genomics is moving forward so quickly that, for example, the latest generation sequencing technology is measuring at the level of single molecules – a truly amazing breakthrough. Technologies like high density microarrays are adding value to high throughput sequencing by their capability for fast front-end sequence capturing. At the same time, all genomic microarrays are capitalizing on new insights gained from ultra-high throughput sequencing providing better sequence and assay quality.

The technology may change but it brings with it new challenges. How do we analyze the vast amounts of data this latest technology creates? How do we separate the noise from what is truly meaningful? And how can we rely on what we're looking at? New bioinformatics tools and hardware are constantly being developed to deal with these new issues, but they are still part of the bigger picture that cannot be ignored. One remaining constant is that of good sample quality – however good the technology is, if you put garbage in, you get garbage out.

Following along this theme, this year's Tecan Symposium will focus on applied genomics and will present an ideal opportunity for researchers in these fields to discuss the future of this discipline.

Email talk@tecan.com to tell us what you think about this or another life science topic of your choice.