Getting under the skin of stem cells

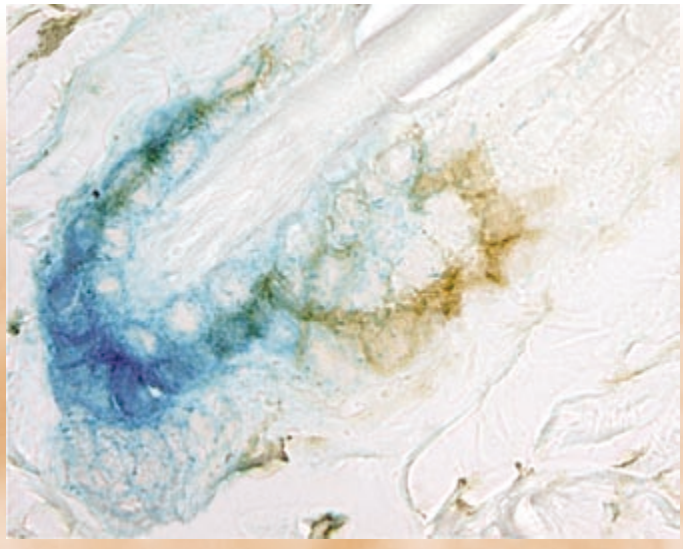
Researchers in the Center for Biosciences at the Karolinska Institute in Stockholm, Sweden, have been using an Infinite® 200 microplate reader and NanoQuant Plate™ for the characterization of a newly isolated population of skin stem cells in mice.

Researchers in the Center for Biosciences at the Karolinska Institute, Sweden, have recently isolated skin stem cells displaying several exciting properties which challenge previous assumptions. The study, published in Nature Genetics*, demonstrated that these stem cells (Lgr5+ cells) are the most primitive skin stem cells yet described, able to divide actively and migrate through the skin tissue during follicle growth and regression. These cells are partly regulated by the well characterized 'Hedgehog' signaling pathway, and are of particular interest to the wound-healing process and the development of basal cell carcinomas (BCC), the most prevalent form of skin cancer.

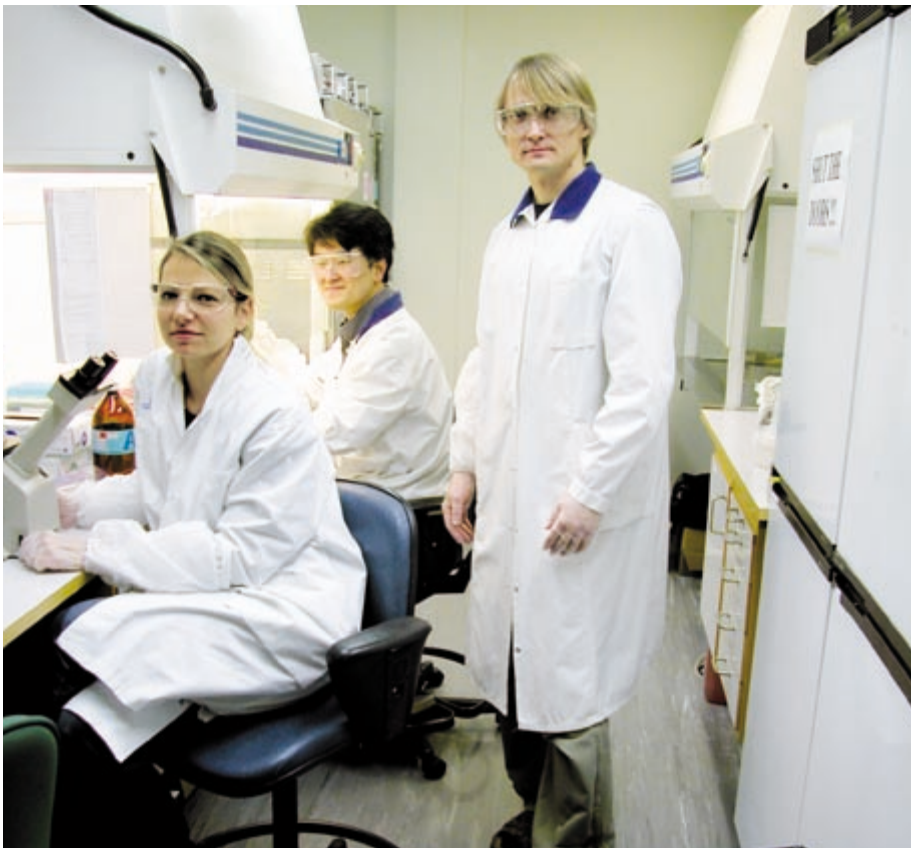
Characterization of gene expression in these cells has been particularly difficult due to their scarcity. Dr Maria Kasper, from the Karolinska Institute's environmental toxicology group, explained: "These cells are extremely rare, and so the experimental material we obtain is especially valuable; 100,000 sorted cells only renders 150 ng of RNA. Once this material has been isolated, it is used to identify signaling pathways involved in stem cell regulation by qPCR.

The significance and reliability of the data obtained is dependent on both the quantity and integrity of the extracted RNA, as qPCR using very low starting concentrations of RNA is particularly sensitive to these variables. Therefore, it is very important for us to achieve exact quantification of extracted samples with minimal loss of material."

Traditional photometry-based systems for evaluating RNA samples are dependent on comparing absorbance measurements at multiple wavelengths, but most readers cannot produce reliable absorbance readings from small volumes of RNA sample. However, using Tecan's NanoQuant Plate in conjunction with a multi-functional Infinite 200 microplate reader offers the necessary sensitivity for reliable low volume RNA purity analysis. Maria commented: "Using this system ensures that only minimal material is required for reliable evaluation of our valuable RNA samples and allows us to investigate more than 30 selected genes by qPCR from the initial 150 ng of isolated RNA."



Lgr5 expression in the mouse hair follicle. Immunohistochemical staining showing localization of Lgr5 (blue) and CD34 (brown) expressing cells.



Maria Kasper, Takashi Shimokawa and Viljar Jaks

The NanoQuant Plate is a 16-channel quartz optic for measurement of low concentrations and low sample volumes, with quick and simple washing procedures to eliminate cross-contamination. Specifically designed to give outstanding performance and a very high rate of reproducibility, the NanoQuant Plate is intended for a broad range of applications requiring sample volumes as low as 2 µl. Combined with the Infinite 200 microplate reader, this system allows accurate absorbance measurement of DNA/RNA concentrations as low as 1ng/µl, helping to conserve valuable samples.

Maria's colleague, Dr Viljar Jaks, continued: "Having the NanoQuant Plate in the laboratory has been very useful for our investigations. Because it is integrated into the Infinite microplate reader, it is very easy to use and the i-control™ software is intuitive. Although our studies are not high throughput by nature, the 16-channel plate format allows us to analyze entire experimental sets together."

"Accurate quantitation allows us to normalize RNA concentrations very precisely for further analysis of gene expression using a variety of methods, helping to identify the pathways contributing to the behavior of these stem cells. Elucidation of these regulatory mechanisms could help us to understand the role of keratinocyte stem cells in various forms of tissue growth. One example of this is the wound healing process. Because hair follicles are laid down during embryonic development, they are not formed in human scar tissue when new skin is grown. Therefore, the next important step is to identify a similar stem cell population in human hair follicles. If we can understand how these stem cell populations are regulated, then we may be able to induce proliferation to produce new hair follicles." Maria added: "Another application of great interest is the development of basal cell carcinoma – the most common form of skin cancer – the origins of which have not yet been determined. It is still not clear whether BCC originates in the interfolicular epidermis or the hair follicles, but using Lgr5 as a marker of hair follicle stem cells offers a potentially powerful tool to investigate the genesis of this cancer. Deregulation of the pathways that control these cells can cause sustained activation of their proliferation and disturb their normal differentiation process, which increases the risk of cancer formation."

(* Nature Genetics 40, 1291 - 1299 (2008))

The Infinite 200 and NanoQuant Plate for analizing small volumes of RNA sample