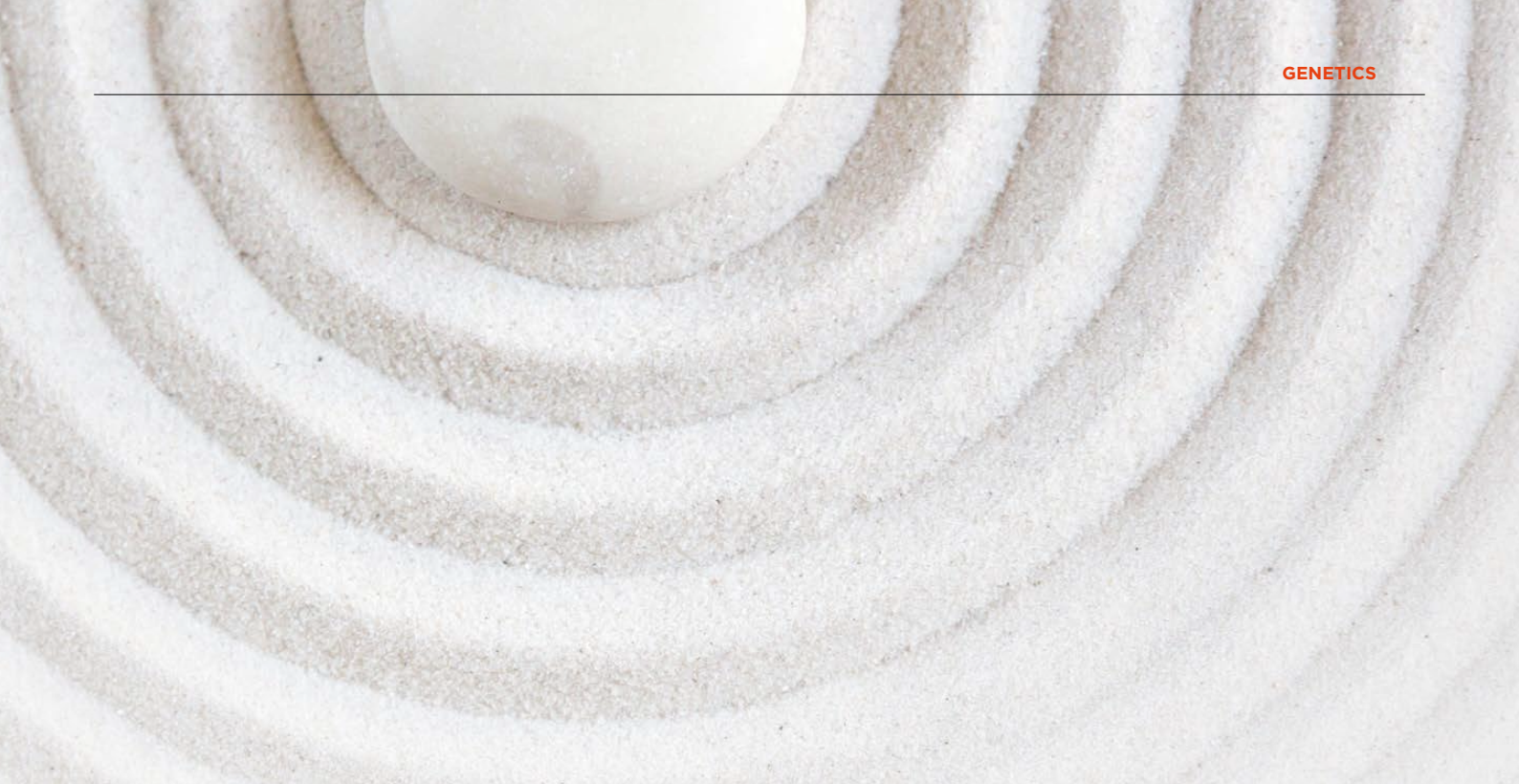
Analyzing poor quality RNA: how low can you go?

NGS is a vital tool used for studying the structure and function of DNA for multiple applications. However, there are several challenges commonly encountered when using this technique, particularly when working with degraded or trace levels of RNA. These issues motivated research staff at Kazusa DNA Research Institute in Japan to search for library preparation kits that would enable high quality sequencing for its customers when working with low quality samples.

Kazusa DNA Research Institute (KDRI) in Kisarazu, Chiba Prefecture, was established in 1994, making it the world's first organization specializing in DNA-related research. Since then, the institute has been involved in many human and plant genome projects, and is recognized as a global leader in genetic studies. It performs NGS on human, plant and bacterial samples provided by clients from diverse sectors. This includes the agriculture industry – where genetic analysis is crucial for breeding commercially important plant species with desired traits, and for standardizing the quality of plant seeds – and medicine, where KDRI runs NGS to support the early detection of rare diseases and the identification of novel cancer markers and precursors.

KDRI assessed the capability of the Revelo™ RNA-Seq High Sensitivity library preparation kit to process low-input and degraded or trace level RNA samples prior to NGS. This flexible, end-to-end library preparation solution produces rRNA libraries from 10 250 pg to 10 ng of total RNA using Tecan's proprietary SPIABoost™ technology. Dr Kazuto Kugou, a researcher in the Laboratory of Gene

Dr Kazuto Kugou is part of the KDRI team using the Revelo kit for genetic research



Sequencing Analysis at KDRI, explained why the center made this decision: "We chose to trial the Revelo kit because it was specifically formulated for the preparation of highly degraded samples – for instance, samples with an RNA Integrity Number of just 1.8 – and those containing only trace amounts of RNA. We frequently encounter these types of specimens, and typically handle low throughput applications, so the Revelo system seemed like the perfect solution for us and we were keen to try it out."

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Dr Kugou described some of the advantages of the Revelo kit over the alternative options they had previously tested in the lab: "The library preparation tools offered by other companies all require an additional DNA purification step in order to remove adapter dimers. In contrast, the Revelo solution includes DNase treatment, and this one simple step is enough to remove all the adapter dimers completely. The kit therefore enabled us to prepare a large number of clean libraries within a short timeframe using only a few PCR cycles, reducing PCR bias and providing unbiased 5' to 3' transcript coverage."

Part of the lab's role is to sequence RNA-Seq libraries and map the sequencing data onto a human reference sequence. This had proven difficult in the past when using poor quality specimens, but the team noticed that sufficient quality data for analysis was obtained during the trial of the Revelo kit. "We are always concerned about the mapping rates we will achieve from samples with highly degraded or trace level RNA, but when we used the Revelo system to prepare the libraries, we observed unique mapping rates of over 80 percent," said Dr Kugou.

"Our institute aims to be a world leader in all aspects of DNA research, and we strive to contribute to society through medicine, agriculture, industry and education. We are excited to see what new breakthrough solutions the company develops over the coming years to further advance the field of genetic analysis," Dr Kugou concluded.

For research use only. Not for use in clinical diagnostics.

To find out more about the Revelo RNA-Seq High Sensitivity library preparation kit, go to lifesciences.tecan.com/revelo-rna-seq-high-sensitivity

For more information about the Kazusa DNA Research Institute, visit www.kazusa.or.jp/en