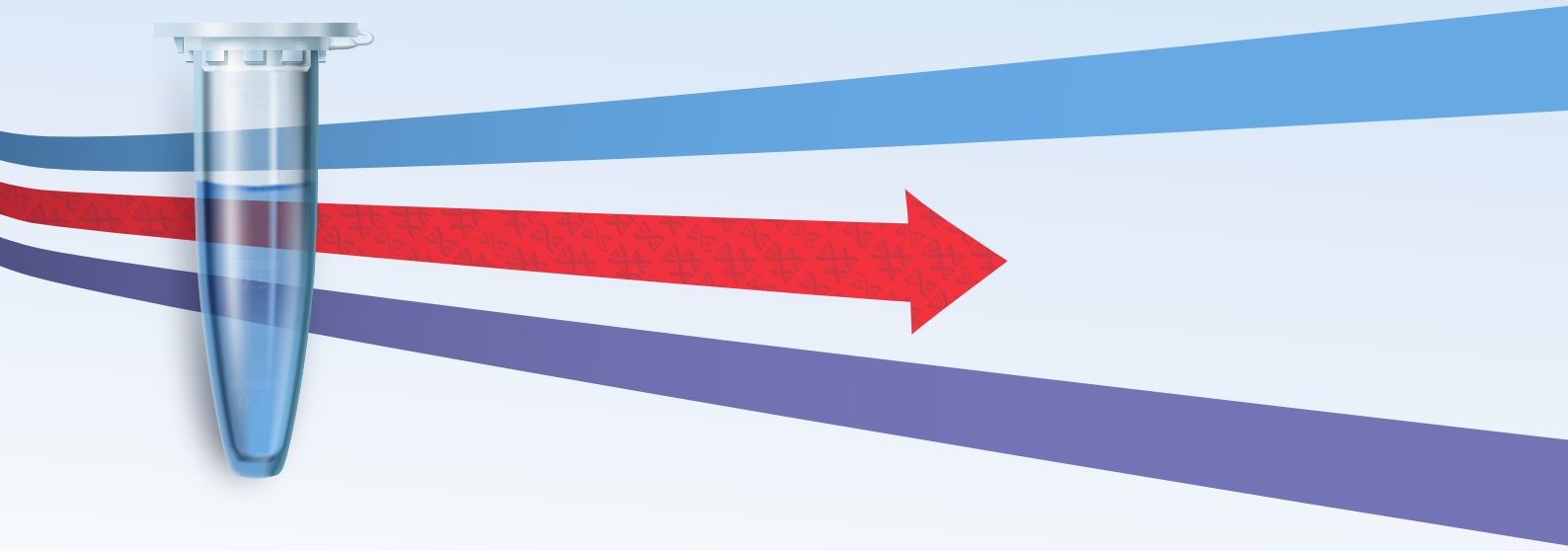


Next-generation sequencing

Spotlight showcase: Ion AmpliSeq transcriptome assays for translational research

Kazuko Sakai, PhD, Senior Lecturer, Kindai University

Transcriptome analysis has evolved significantly from early techniques like northern blotting to more advanced methods such as next-generation sequencing (NGS) and single-cell RNA sequencing (RNA-Seq). The comprehensive insights provided by transcriptome analysis are pivotal for translational and clinical research implementation, accelerating our understanding of relevant biomarkers and their impact on disease progression.

In recent years, basic science, translational, and clinical researchers have utilized transcriptome NGS assays for various research applications, including cancer research, immunology, neurological research, and more (Figure 1).

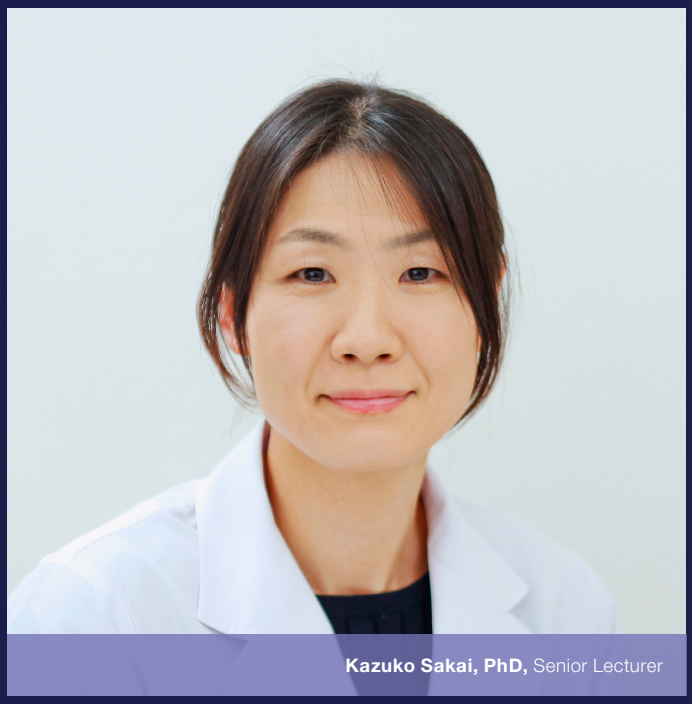
The growing use of transcriptome analysis on NGS platforms is driven by its ability to provide detailed and comprehensive insights into gene expression. Continual advancements in NGS technology further enhance the capabilities and applications of transcriptome analysis, solidifying its role as a critical tool in modern biology and medicine. Let's hear from an expert in the field about why and how she has been integrating transcriptome NGS into her lab's workflows, and the significant value it brings to her research.

Cancer research	Immunology	Neurological disorders	Rare diseases	Developmental biology
Identifying gene expression patterns in tumors	Understanding the biology of immune responses and researching new vaccines	Studying gene expression changes in neurological diseases	Discovering genetic causes to drive research on targeted therapies	Investigating gene expression during development and differentiation

Figure 1. Some of the clinical research applications that have utilized NGS-based transcriptome assays.

The Faculty of Medicine at Kindai University excels in medical research and education, particularly in genome biology. Renowned for pioneering genetic research, the Department of Genome Biology extensively uses NGS for transcriptome research to accelerate cancer biomarker discovery, advance research in genetic disorders, and develop personalized medicine. Their expertise in transcriptome analysis via NGS drives significant contributions to clinical research and healthcare innovations.

In this interview, Kazuko Sakai, senior lecturer in the Department of Genome Biology, Kindai University Faculty of Medicine, shares insights into the motivations, key findings, and future goals of transcriptome research.



Kazuko Sakai, PhD, Senior Lecturer

Thermo Fisher Scientific: What are the goals of your research lab?

Kazuko Sakai: Our translational research focuses on biomarker analysis. We use genomic analysis of human specimens to identify predictive markers of drug efficacy and clinical outcomes, with the ultimate goal of advancing discovery research for cancer therapies.

Thermo Fisher: How has NGS influenced your research work?

Sakai: Our experience with NGS has been transformative since we started using the Ion PGM™ System in 2013 and the Ion GeneStudio™ S5 System subsequently. We were impressed by the vast amounts of genetic data these NGS platforms could generate and analyze quickly, though data processing was initially challenging. The comprehensiveness of NGS is invaluable for exploratory research and discovering new biomarkers.

Thermo Fisher: There are several transcript profiling methods available (Figure 2); what motivated you to add whole-transcriptome analysis to your lab?

Sakai: We chose the Ion AmpliSeq™ Transcriptome Human Gene Expression Kit over microarrays for gene expression analysis because of its lower cost and straightforward workflow. While microarrays required complex, time-consuming workflows, the ease of NGS sample preparation reduced handling time significantly, with results consistent with those of microarrays or TaqMan™ Assays.

We primarily use FFPE samples, and the Ion AmpliSeq workflow is ideal for its ability to handle fragmented samples and its low sample input requirement. For Ion AmpliSeq transcriptome analysis, 10 ng of RNA is needed for FFPE samples, while 1 ng suffices for cell lines and frozen samples. This low RNA requirement is particularly beneficial for small biopsy samples for lung cancer research. In contrast, other NGS assays typically require 50–250 ng or more RNA.

Kazuko Sakai received financial compensation from Thermo Fisher Scientific for her participation in this interview.

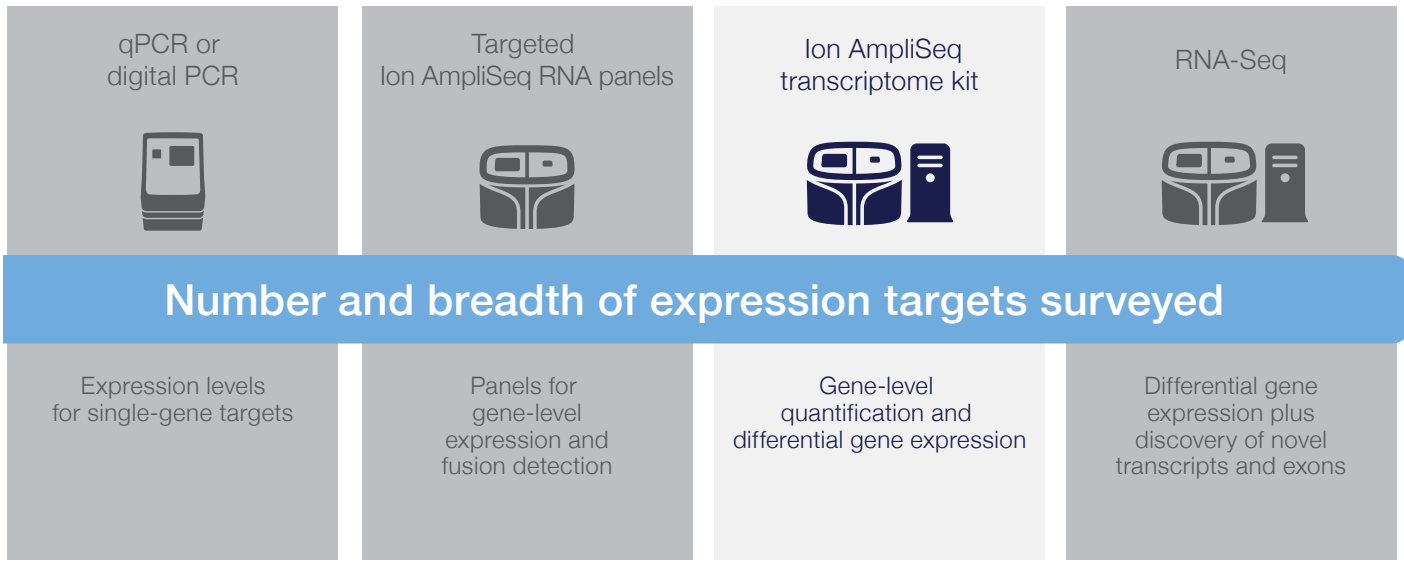


Figure 2. Transcript profiling options. RNA transcript analysis solutions range from multiplex PCR and RNA sequencing of individual targets to unbiased, global transcript profiling by whole-transcriptome analysis.

Thermo Fisher: What do you do to follow up after running the transcriptome NGS kit?

Sakai: With this panel, we have successfully confirmed the relative gene expression levels for multiple genes [1,2]. Sometimes we use reverse transcription polymerase chain reaction (RT-PCR) or immunohistochemistry (IHC) for confirmatory testing. In other cases, the NGS results could also be used as a predictive model for FFPE samples [3].

We could set up custom panels to improve throughput and reduce costs for further clinical research. When target genes and their pathways were clear, the small panel had significant advantages in cost and measurement time.

Thermo Fisher: How has your experience been with custom Ion AmpliSeq solutions in general?

Sakai: The advantage of the Ion AmpliSeq workflow is that there are many predesigned panels and community panels for gene mutation analysis. I typically receive my custom panel designs within 24 hours, and the panel is delivered to my lab 1–2 weeks after I place the order. If there are any technical difficulties, we receive prompt technical support, and difficulties are almost always resolved within a day.

> "The handling time is excellent with the Ion AmpliSeq transcriptome kits, due to the ease of sample preparation."

Looking to customize a targeted NGS panel to meet your unique research needs?

Visit Ion AmpliSeq™ Designer at **ampliseq.com** or talk to a representative to help you select the design strategy that best fits your needs.

Thermo Fisher: What do you like most about the transcriptome sequencing workflow on the Ion GeneStudio systems?

Sakai: We like the following points:

- Library preparation, sequencing, and data analysis can be completed as fast as in 3 days.
- It is easy to evaluate the quality of analysis, in our experience.
- The data analysis is simple and straightforward using Ion Reporter™ Software or Applied Biosystems™ Transcriptome Analysis Console (TAC) Software, so it is easy to make decisions regarding quality assurance.

The Ion Chef™ and Ion GeneStudio S5 systems with the Ion 550™ Chips have simplified the NGS process, enabling us to consistently obtain high-quality data, regardless of user experience. These instruments are crucial for our NGS analysis, allowing the processing of up to 8–10 samples at a time.

We have two Ion Chef and two Ion GeneStudio S5 instruments, allowing for parallel operation that significantly reduces analysis time, especially for large panels. While automated library construction using the Ion AmpliSeq™ Kit for Chef DL8 is easy and effective for quality assurance in tests and for smaller numbers of samples, we currently perform library preparation manually due to the volume of specimens (10–100 samples per preparation batch) and cost considerations.

> "With the Ion Chef and Ion GeneStudio S5 systems, we can now obtain a certain amount of high-quality data, regardless of the level of experience with NGS instruments."

Thermo Fisher: Are there other assays you plan to implement in your lab?

Sakai: We continue using the transcriptome kit for its simplicity, low sample requirement, and cost-effectiveness, though it would be more comprehensive if it could detect mitochondrial and miRNA gene expression like RNA-Seq. The Ion AmpliSeq™ Microbiome Health Research Assay is also intriguing for studying human tissue interactions and developing new biomarkers. We're interested in using it for analyzing host–microbiota interactions, relevant in aging and cancer research.

1. Hayashi H et al., *J Clin Invest.* 2024 Apr 1;134(7):e168318. doi: 10.1172/JCI168318
2. Aoki T et al., *Liver Cancer.* 2023 Oct 9;13(3):285-305. doi: 10.1159/000533818
3. Hayashi H et al., *JAMA Oncol.* 2020 Dec 1;6(12):1931-1938. doi: 10.1001/jamaoncol.2020.4643

Ordering information

Description	Cat. No.
Ion AmpliSeq Transcriptome Human Gene Expression Panel, Chef-Ready Kit	A31446
Ion AmpliSeq Transcriptome Human Gene Expression Kit	A26325
Ion Chef System	4484177
Ion GeneStudio S5 System	A38194

Learn more about Ion AmpliSeq transcriptome solutions at **thermofisher.com/ampliseq-transcriptome**