Gaining Insight into Genetic Disease

Dr. Kenjiro Kosaki furthers his clinical research and expands the genetic analysis services he provides with the TruSight™ One Sequencing Panel.

Introduction

A pediatrician by training, Kenjiro Kosaki, M.D. is also a clinical geneticist. His research focuses on congenital malformation syndrome, a combination of physical anomalies affecting more than one body part. What started as the focus of a fellowship at the University of California, San Diego, has turned into a 20-year exploration of the genetic underpinnings of this syndrome. Currently the Director of the Center for Medical Genetics at the Keio University School of Medicine in Tokyo, Dr. Kosaki is conducting clinical research using the latest next-generation sequencing (NGS) tools, including the MiSeq® System and the TruSight One Sequencing Panel.

iCommunity spoke with Dr. Kosaki about how Illumina NGS systems and products are transforming his research and enabling him to meet the medical genetics needs of the Keio University School of Medicine.

Q: What genomics tools did you first use for your research?

Kenjiro Kosaki (KK): Before the NGS era, I characterized patients with congenital malformation syndrome based on clinical presentation and genetic data. For molecular characterization, I used denaturing high-pressure liquid chromatography (DHPLC) to screen for exons associated with congenital malformation syndrome and then sequenced those exons using Sanger sequencing. The whole process took a long time.

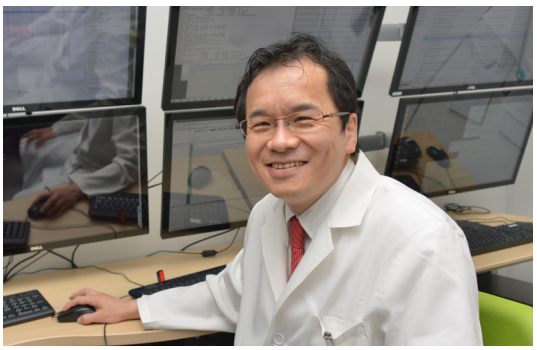
Q: When did you start using Illumina sequencing systems?

KK: I began using the Genome Analyzer® System about four years ago to perform exome analysis. I also used it to develop a custom panel of genes linked to congenital malformation syndrome. About three years ago, I became involved in the Japanese government's NGS program and obtained one of the first MiSeq Systems imported to Japan.

Q: Why were you interested in using the TruSight One Sequencing Panel for your research?

KK: I originally developed my own custom panel that covers 500 genes identified in the classic textbook, *Smith's Recognizable Patterns of Human Malformations*[1], or by the International Classification of Skeletal Dysplasia Society[2]. I used the Agilent SureSelect kit to create the panel, which required an initial design fee to create a panel for about 96 samples.

> "The TruSight One Sequencing Panel simplifies quality control issues by enabling us to use one rather than multiple panels to conduct our research."



Kenjiro Kosaki, M.D. is the Director of the Center for Medical Genetics at the Keio University School of Medicine in Tokyo.

The TruSight One Sequencing Panel covers all 500 of these genes. It's an off-the-shelf rather than a custom product, which means that there are no design fees to add to the cost.

Q: What are the advantages of the TruSight One Sequencing Panel?

KK: Coverage of the TruSight One Sequencing Panel appears uniform throughout all 500 target genes (Figures 1 and 2). It simplifies quality control issues by enabling us to use one rather than multiple panels to conduct our research. The researchers and technicians in my lab are very confident using it and like the ease of sample preparation. They also like the simplicity and speed of the MiSeq System.

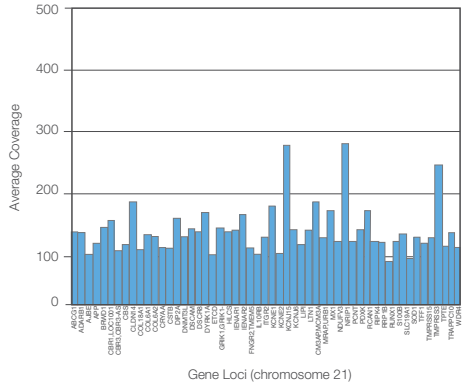
Q: What level of performance are you seeing for the TruSight One Sequencing Panel?

KK: I use NGSrich open source software[3] to evaluate Illumina sequencer target enrichment performance. It runs on UNIX and is written in Java. It allows me to see genome-wide performance through all the targets, rather than exome by exome. Using the software, the performance of my custom panel and the TruSight One Sequencing Panel are comparable.

Q: Do you use VariantStudio software to analyze the results?

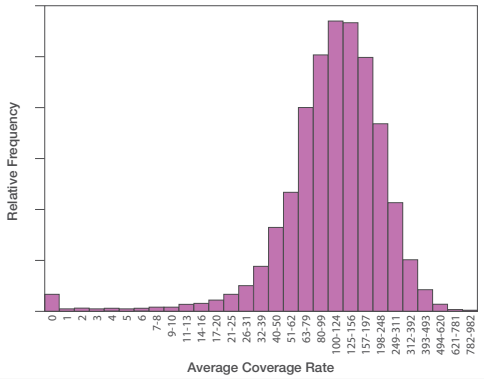
KK: With the introduction of VariantStudio, I can let go of the data analysis and enable my team to deal with the data. There is no need to write Linux scripts for variant analysis. VariantStudio provides a final list of potential pathogenic variants and that's very helpful for clinicians like myself. I think it is excellent software.

Figure 1: Average Coverage of Gene Loci within Chromosome 21



TruSight One Sequencing Panel performance across Chromosome 21 genes showing even coverage across the targets with few outliers.

Figure 2: Coverage Across All TruSight Sequencing Panel Genomic Regions



Frequency of coverage in the TruSight One Sequencing Panel across all regions, showing the vast majority of targets at greater than 20× coverage and a tight read distribution around mean coverage.

Q: What makes VariantStudio so special in your eyes?

KK: While I enjoy working with command-line languages, the process is not appealing to my laboratory staff. Command-line programming and understanding the details about the generation of good quality BAM and VCF files is a black box to most researchers and clinicians. VariantStudio eliminates the need for command-line scripts to further process the data.

VariantStudio also enables us to build a local database of neutral and pathogenic variants and the software will allow us to extract and report on only those variants. That keeps the data focused on what is relevant for our clinical research.

> "VariantStudio provides a final list of potential pathogenic variants and that's very helpful. I think it is excellent software."

Q: Is the low sample requirement (50 ng) of the TruSight One Sequencing Panel an advantage for your research?

KK: For my congenital malformation syndrome research, we're extracting genomic DNA from the umbilicus and can easily obtain the sample amounts we need. We've started working on tumor samples, where it's difficult to get large amounts of genomic DNA. The fact that the TruSight One Sequencing Panel requires low DNA sample input is helpful in these situations.

Q: What do you think the key benefit of running a TruSight One Sequencing Panel is over performing whole-exome sequencing?

KK: Exome sequencing is effective in identifying potential variants. However, to establish the pathogenicity of a variant we have to perform trio analysis—sequencing parental and the affected child's DNA. Exome sequencing three samples is more expensive and isn't cost-effective for routine daily use.

The TruSight One Sequencing Panel consists of more than 4,800 genes associated with known clinical phenotypes. It allows us to sequence a single sample instead of three, and establish the pathogenicity of a variant within one of those known regions. We're importing the data from HGMD Professional[4] to VariantStudio. If we have a hit from the HGMD Professional database, we feel confident that we've identified a potential pathogenic variant.

When I began using VariantStudio in October 2013, I incorporated most of the variant data that I'd accumulated over the past four years. This included data from HGMD Professional, normal variants observed in Japanese people, and in-house neutral variant data. That was an easy process and showed the great degree of flexibility that's embedded in the VariantStudio software. We can use the software in a sophisticated manner without having to bother with the complexity of many of the variant analysis programs on the market, such as SnpEff.

Q: How do you see the TruSight One Sequencing Panel impacting your future research?

KK: Up until three years ago, I was in the pediatrics department of the hospital studying malformation syndromes. As the Director of the Center for Medical Genetics of the medical school, I now serve a larger community of clinicians.

While I have Sanger sequence primers for my congenital malformation syndrome research, I don't have primers for other fields of genetics study. With the help of the TruSight One Sequencing Panel, I can easily perform analyses on other genes that are relevant or important in the field of medical genetics, without having to develop a new analytic system of primers, sequencing tools, and workflows. It allows me to serve the needs of other experts in the medical school more effectively.

Thanks to the nature of NGS, I don't have to worry about quality control issues, keeping the primers refrigerated, and the other pitfalls of customized processes. I highly recommend the TruSight One Sequencing Panel for people conducting medical genetics research.

> "The idea of having a weapon against all the medically relevant genes is mindboggling, and I think it will drastically change how we assess patients suffering from undiagnosed disorders."

Characterizing syndromes is easy when the phenotypic presentation is clear and reflects the classic description of the congenital disease. We don't need exome analysis to confirm what we already know.

Reaching an accurate characterization is exceptionally difficult when the clinical presentation is atypical. I have published several articles about diagnostic odysseys that finally ended with the help of NGS panels[5–7]. The TruSight One Sequencing Panel now provides us with a tool to detect and identify variants in patients with an atypical presentation, alerting us that there is more there than meets the eye.

Q: When you first got into medicine, could you have imagined a day when you'd be performing these types of genetic analyses?

KK: Never, in my wildest dreams would I have believed that such tools would be available. The concept that there would be tools to assess the presence of all the known causative genes of human genetic disorders was inconceivable when I first became a physician. The idea of having a weapon against all the medically relevant genes is mindboggling, and I think it will drastically change how we assess patients suffering from undiagnosed disorders.

I find the identification of causative variants in patient samples an enjoyable challenge. Most physicians find it overwhelming, especially those who are not clinical geneticists. Those physicians will appreciate the simplicity of the TruSight One Sequencing Panel. We all have the goal to combat genetic disease and we finally have molecular tools to help us do that.

I hope your company continues to excel in this field and develop more innovative products like the TruSight One Sequencing Panel and VariantStudio software.

References

1. Jones K, Jones M, and del Campo M (2013) Smith's Recognizable Patterns of Human Malformation. Elsevier: 1016 pages.
2. Warman ML, Cormier-Daire V, Hall C, Krakow D, Lachman R, et al. (2011) Nosology and classification of genetic skeletal disorders: 2010 revision. Am J Med Genet 155: 943–968.
3. Frommolt P, Abdallah AT, Altmüller J, Motameny S, Thiele H, et al. (2012) Assessing the enrichment performance in targeted resequencing experiments. Human Mutat 33: 635–641.
4. Biobase Biological Databases, www.biobase-international.com/product/hgmd, April 16, 2014.
5. Takenouchi T, Hida M, Sakamoto Y, Torii C, Kosaki R, et al. (2013) Severe congenital lipodystrophy and progeroid appearance: Mutation in the penultimate exon of FBN1 causing a recognizable phenotype. Am J Med Genet 161: 3057–3062.
6. Takenouchi T, Shimizu A, Torii C, Kosaki R, Takahashi T, et al. (2014) Multiple café au lait spots in familial patients with MAP2K2 mutation. Am J Med Genet 164: 392–396.
7. Takenouchi T, Matusuzaki Y, Yamamoto K, Kosaki K, Torii C, et al. (2014) SOX9 dimerization domain mutation mimicking type 2 collagen disorder phenotype. Eur J Med Genet (in press).

Illumina, Inc. • 1.800.809.4566 toll-free (U.S.) • +1.858.202.4566 tel • techsupport@illumina.com • www.illumina.com

FOR RESEARCH USE ONLY

Pub. No. 1070-2014-001 Current as of 07 July 2014

illumina®