Customer case study
Field of study: Oncology

With a Focus on Patient Care, UC Davis Scientists Track Cancer Mutations in NGS Data

At an NCI-designated comprehensive cancer center, Cliff Tepper uses Ingenuity® Variant Analysis™ to analyze tumor samples and circulating DNA for clinically relevant mutations. Whether the data is from gene panels or whole exomes, the QIAGEN application delivers useful results that are easy to share with oncologists.

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As technical director of the Genomics Shared Resource at the University of California, Davis, Comprehensive Cancer Center, Cliff Tepper routinely faces a series of challenges: closely monitoring new technologies; adapting platforms for translational use; interrogating a broad array of cancers; and tailoring results reports for investigators with diverse expertise ranging from molecular biology to clinical oncology.

To meet these challenges, Tepper works closely with scientists and physicians — both at the cancer center and at other institutions — and keeps an eye on peer-reviewed research for the latest information about technologies and cancer studies. He also invests in leading informatics solutions to get the best results for his clients. Recently, he began using Ingenuity Variant Analysis from QIAGEN, an application that he calls “phenomenal.”

Two recent projects highlight the utility of Ingenuity Variant Analysis. In one, Tepper was able to home in on important variants detected in circulating tumor DNA — variants seen at such low levels they were missed by other analytical solutions. In the other, the application helped Tepper quickly analyze mutations from exome data derived from bladder cancer samples.

Translating Results

The UC Davis Comprehensive Cancer Center got its prestigious designation from the National Cancer Institute in 2001, and Tepper’s core facility was one of the first shared resources for investigators there. Run through the Department of Pathology and Laboratory Medicine, the core lab has a real need to churn out high-quality results that can be understood by scientists and physicians alike.

“We try to provide a comprehensive genomic solution to all of the investigators that want to use our core, whether it’s a physician who doesn’t have a lab or a highly skilled molecular biologist,” Tepper says. His team can help clients with everything from sample acquisition to data analysis, relying on a host of cutting-edge genomic technologies in between. “We do a lot of bioinformatics analysis, and provide support for results interpretation as well as manuscript and grant preparations,” he adds.

As an NCI-designated cancer center, there’s a big emphasis on demonstrating that results of these studies could make a difference in medicine down the road. “The NCI wants to see that what we’re doing can be translated into the clinic and have an impact on some aspect of patient care,” Tepper says.

One approach that has proven very promising is using targeted sequencing panels that cover a host of genes implicated in different types of cancer. Tepper uses QIAGEN’s GeneRead DNAseq Targeted Panel for human cancer, which looks across 160 commonly mutated cancer-relevant genes in more than 740 kb of sequence. This helps put the focus on key mutations in each tumor, rather than the site of origin or genes associated with just one form of cancer. “Many aberrations apply to all cancers,” he says, “so we’re going to a more pan-cancer approach for our studies.” That

universal view of cancer is one way he and his team are ensuring that the work they do will ultimately make a difference for patients.

Once the targeted panel is used to enrich for relevant cancer genes, Tepper and his colleagues sequence the samples and submit the results to the GeneRead DNAseq Variant Calling Pipeline for sequence alignment, annotation, and variant classification. "We just upload raw data, and in the morning it's completely analyzed for variants," Tepper says. "It's a very nice analysis solution."

Diving into Variants

Once Tepper gets initial results from the portal, he turns them over to Ingenuity Variant Analysis, a tool he recently brought into the core facility. "That's been working very well," he says. "It's actually phenomenal — it takes the data we have analyzed and helps us prioritize the variants based on their functional impact and whether they've been found to be associated with cancers." The application also gives the team critical information about how commonly those variants are seen in the general population to allow for quick exclusion based on normal human genetic diversity.

In a recent study that Tepper presented in a poster at this year's annual meeting of the American Association for Cancer Research, his team used Variant Analysis to filter variants found in circulating tumor DNA from patients with pancreatic cancer. The project aimed to generate proof-of-principle data to understand the utility of circulating DNA for this type of cancer in samples from a cohort of nearly 30 patients. To figure out whether KRAS mutations found in circulating DNA — associated with a poor patient prognosis —were truly representative of the tumor, they also sequenced tumor samples from three of the patients.

"In general what we found was that if we manually looked through the data, the assay does pick up the KRAS mutations in all of the samples," Tepper says. The challenge is that with some analysis tools, standard threshold settings are too high to detect variants at the low levels seen in the data. "Sometimes these mutants weren't being called even though the reads were there," he adds. "Much of the sequencing is expended on the background normal DNA, and this decreases the sensitivity for detecting the tumor-derived mutations."

But loading the data into Variant Analysis revealed the whole picture. "We can see the mutations there," Tepper says. "We got a good idea of what somatic mutations were present in the tumor just based on the circulating DNA." The ability to easily adjust thresholds and change filters in the application is a big advantage for the genomics core team. They can set up a filter cascade for various traits they're interested in, such as depth of coverage, sequencing quality, common variants, as well as more specialized filters for variants implicated in cancer, mutations associated with cancer therapeutics, and expression levels. Essential information, such as gene location and base change, is shown clearly. And the tool's inclusion of the COSMIC database makes it a cinch to pick

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out known somatic mutations. In a separate study, Tepper and his team generated whole exome data from patients with bladder cancer. "We used Variant Analysis to help us determine the somatic mutations in the tumor and filter away potential germline variants," he says. "It was helpful for quickly consolidating all that information and applying it to our sample set." In that study, he imported RNA-seq data from the same samples into Variant Analysis to determine whether mutations were likely to be expressed.

Tepper also finds that Variant Analysis saves time by letting him see numerous samples at once, organizing and summarizing data for all of them. Variants can easily be compared across multiple samples, with functional impact information readily accessible as well. "It has it all right there for you," he says. "The interface is much easier to go through than the standard Excel file."

With his translational focus, though, the real payoff for Tepper is generating results that can be easily understood by all of his clients. A clinical pathologist who looked over the Variant Analysis results "liked the presentation a lot because it is easy to examine the data, and it's very clear what it means as well," Tepper says. "The interface is very user-friendly and you can organize the columns based on the things you're most interested in seeing. That helps us share this data with oncologists."

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