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Prepping for Targeted Sequencing Cancer Trial, UCSD Publishes First Human Data on MiSeq

by Monica Heger

IN ANTICIPATION of a clinical trial involving targeting sequencing of patients with metastatic breast cancer, researchers at the University of California, San Diego, have published some of the first human data using the Illumina MiSeq instrument.

The team has developed a targeted sequencing assay, called ultra-deep targeted sequencing, or UDT-Seq, focusing on 42 cancer genes. It first tested the performance of the assay on the Illumina Genome Analyzer using four calibration samples obtained from the Coriell Institute. Those samples had previously had their exomes sequenced, so the positions of coding polymorphisms were known.

The team then tested UDT-Seq on six tumor samples and also assessed its performance on the MiSeq using the four Coriell calibration samples. The results were published in *Genome Biology* this week.

Olivier Harismendy, an assistant adjunct professor in the division of genome information sciences at UCSD, told *Clinical Sequencing News* that the team is collaborating with researchers at the University of California, Irvine, to use the assay in a pilot study of 40 patients with breast cancer.

For the pilot study, which will kick off in January, the team will use a very similar assay to the one published in *Genome Biology*, which targets 71 kilobase pairs of mutational hotspots in 42 cancer genes, sequencing to several thousand-fold coverage.

The team has not yet decided on a platform that it will use for the study, and is weighing both the MiSeq and Life Technologies' Ion Torrent PGM. They are also considering several

different enrichment techniques, including RainDance, Illumina's TruSeq kit, and Life Tech's AmpliSeq Cancer Panel that was designed for the PGM.

Harismendy said the goal of the clinical trial is to guide the next course of treatment for patients, whether it is identifying a drug candidate that has been approved for breast cancer, an off-label drug, pharmacogenomic markers for toxicity, markers predictive of response or resistance, or a clinical trial that the patient is eligible for.

Institutional review board approval is still pending and has so far collected about 20 samples for research purposes.

The sequencing will not be done in a CLIA-certified lab, nor does the UCSD Cancer Center have plans in the immediate future to establish a CLIA-certified lab, said Harismendy. Instead, he said, they have identified a number of different reference laboratories around the country that can use approved tests to validate actionable mutations identified via sequencing.

"We're still in the translational research aspect of this," said Harismendy. Obtaining CLIA certification is "something that a diagnostics company would do, or a genetics lab within the pathology department."

He said that he is aiming for a three- to four-week turnaround time, including the validation of the results. Additionally, there are two levels of consent built into the process.

Patients originally consent to the sequencing and return of results, but before the results will be returned to the physician, Harismendy's team will prepare a preliminary report for the physician saying that it found something of significance. The patient would then have the option to continue receiving the full results or to opt out.

"We leave the option to the patients to continue and receive results or decline because maybe in the meantime they have changed their mind or they don't want to receive some sort of mutation, for example, a germline mutation," Harismendy said.

In the case that the patient opts to receive results, the CLIA-validated list of mutations will then be transferred to the treating physician, who will share them with the patient. The list will also be stored in the patient's electronic medical record.

Harismendy said that the team is also working to implement an automated alert system, so that as new clinical trials open up for a patient's mutational profile, the physician will be notified.

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A Targeted Approach

For cancer sequencing, there are divided opinions as to whether a targeted approach or a more comprehensive approach that includes some mix of exome, whole-genome, and transcriptome sequencing is the most effective.

Harismendy said that the UCSD team decided to go for a targeted approach because it “wanted to be able to analyze heterogeneous samples.” Heterogeneity in cancer samples arises from many different causes. For instance, samples often contain normal cells, microphages, or even immune cells, so only a subset will actually be tumor cells.

In research projects like the Cancer Genome Atlas, this heterogeneity is not as problematic, Harismendy said, because samples can be selected for their high tumor content. However, “in the clinic, very few samples are that clean.”

Additionally, cancers differ between tumor types. For some cancers, “just because of the way they grow, you don’t get a homogenous tumor mass,” he said. Tumor cells often grow in a very dispersed fashion, mixing in with stroma cells, so preparing those samples to get high tumor content is time-consuming and expensive.

“We wanted to compensate for that by going very deep in our sequencing, so that we can take the sample as it is ... and still detect important mutations in the cancer cells,” Harismendy said.

In order to do that, the team is sequencing to several thousand-fold coverage and will report mutations down to a 5 percent frequency. The assay itself can detect variants down to 1 percent, said Harismendy, but not as confidently.

The other reason for choosing a targeted approach is that there are “still very few actionable mutations that are known,” Harismendy said. Most mutations identified by whole-exome or whole-genome sequencing still have unknown function.

And even all the actionable mutations are not yet routinely screened in cancer patients. For instance, PIK3CA mutations can be targeted by PI3 kinase inhibitors. But “our cancer center doesn’t by default screen for that mutation,” said Harismendy. It will however, be included in the targeted assay, and the “fact that we’re going to run this assay in a very standard way will increase the chances of [identifying] these patients, who may be eligible for a clinical trial,” he said.

Other genes in the panel will include key genes that are mutated across a broad range of cancers, such as TP53, as well as pharmacogenomic markers and germline mutations that are predictive of inherited forms of breast cancer such as the BRCA 1/2 mutations. Additionally, as a proof of principle, the team is including the gene for cystic fibrosis.

Harismendy said that following the initial pilot of 100 breast cancer patients, he would like to expand the study to include additional patients from a wider range of cancers, such as gastrointestinal and lung, but that will depend on funding.

While the entire gene panel has not yet been chosen, it will

be very similar to the 42-gene panel the team discussed in the *Genome Biology* paper, but with some modifications to focus more on breast cancer-specific genes and also pharmacogenomic markers, Harismendy said.

In the paper, the team’s gene list included hotspots that were selected from the COSMIC database, which covered 53 percent of all mutations, for a total of 71.1 kilobase pairs.

The team originally validated the panel on the Illumina Genome Analyzer, using RainDance for target enrichment. They tested the analysis first on four Coriell DNA samples with known coding polymorphisms. The samples were pooled four times to create four different samples with a range of relative concentrations.

Sequencing of the samples resulted in 24,000-fold coverage depth per sample and demonstrated an average sensitivity of 89.1 percent for variants down to 1 percent prevalence. Sensitivity was increased to greater than 94 percent, when variants at 5 percent prevalence or above were considered.

The team next decided to test whether multiplexing would be more cost effective and found that pooling 2, 4, 8, 16, and 32 samples per lane would lead to coverage depths of 12,000, 6,000, 3,000, 1,500, and 750, respectively. Sensitivity is above 92 percent at 3,000-fold coverage or higher.

Next the assay was tested on primary and xenograft samples, including from a primary colon adenocarcinoma and its matched xenograft; a primary breast carcinoma and its xenograft; an ovarian carcinoma xenograft; a sarcoma xenograft; and matching germline DNA from all four patients’ blood.

Analysis was restricted to mutations at 5 percent prevalence or higher, and a subset of the mutations was then validated using Sanger sequencing or Snapshot, a single-base extension assay designed by Applied Biosystems.

Mean coverage for each of the cancer samples was around 24,000-fold. Of the mutations that were followed up with Sanger sequencing or single-base extension, all were validated except for three that were detected at a prevalence of below 5 percent.

Finally, the team tested the assay on the MiSeq. It ran the same calibration samples, pooling all four samples in one run, achieving an average depth of 1,571-fold per amplicon.

Not only was the sequencing much quicker on the MiSeq, but compared to the GA data, there was a “significant reduction of the substitution rate, especially at the end of the reads for A and T reference bases,” the authors wrote.

They attributed the improvements to better chemistry and faster cycling time. The improvements resulted in a six-fold reduction in the number of positions deemed noisy, and the assay’s sensitivity improved from 85 percent to 90 percent when comparing the data at equivalent coverage depth.

“With an improved performance when run on the Illumina MiSeq, the UDT-Seq assay is well suited for clinical applications to guide therapy and study clonal selection in heterogeneous samples,” the authors concluded.