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Personalized Cancer Therapies through Precision Diagnostics

Dr. Robert Daber uses TruSeq® Custom Amplicon to conduct genomic testing that can inform prognosis and therapeutic choices.

Dr. Robert Daber is Technical Director of Clinical Genomics at Penn Medicine's Center for Personalized Diagnostics (CPD). He is a board-certified clinical cytogeneticist with expertise in bioinformatics, SNP arrays, and next-generation sequencing (NGS). He oversees the clinical diagnostics laboratory, using NGS to identify distinct molecular changes and genomic signatures in the DNA of tumor specimens to provide treating oncologists with the knowledge they need to best treat their patient's disease.

Q: What are the goals of the Center for Personalized Diagnostics?

Robert Daber (RD): Our goal at the CPD is to identify as many clinically actionable mutations that are present in a tumor specimen as possible so that clinicians can better treat their patient's disease, either by understanding the prognosis, or by identifying appropriate clinical trials or approved targeted therapies.

Q: What applications are you using TruSeq Custom Amplicon for?

RD: We used TruSeq Custom Amplicon to develop a liquid tumor panel, our hematologic malignancy panel. It was designed primarily to identify important molecular changes across genes previously studied in acute myeloid leukemia (AML). A seminal paper was published in the New England Journal of Medicine in early 2012 that demonstrated the importance of 16 genes in this disease. As a result, we designed our custom panel and offered it as both a clinical test and research tool to clinicians at our institution. Since February, we've analyzed over 150 AML patients clinically and have found the assay to be very robust. We have also had a few research projects use this panel. The capture reproducibility is so robust that we have been able to design copy number calling algorithms to identify chromosomal imbalance in the regions interrogated by the panel, including being able to call gender on each sample. We have amplicons on the sex chromosomes and we're able to quantitate dosage and determine if it's male or female. This provides us with a nice QC metric after performing a run, to ensure that there were no sample or plate mix-ups during processing. That's really nice in a clinical setting. We have also had incredible sensitivity with detecting both point mutations and insertions/deletions with this assay. In addition to this panel, we have a second version with expanded content under validation, as well as a genome-wide copy number panel we've been calling SNPseq.

> "The lowest [input amount] we've used is 8 ng total DNA and the results were beautiful."

Robert Daber, Ph.D., is Technical Director of Clinical Genomics at Penn Medicine's Center for Personalized Diagnostics.

Q: What other amplicon panels are you using?

RD: Aside from our Hematologic malignancy panel, which includes 33 genes, we have also validated the TruSeq Amplicon - Cancer Panel for use on all of our solid tumors. We're validating our second custom panel, which is an expanded heme malignancy panel with 68 genes that addresses acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), myeloproliferative neoplasms (MPN), and chronic lymphocytic leukemia (CLL). A single-tube, ~3,700 amplicon assay* is also under development. This assay interrogates the genotype of evenly spaced single nucleotide polymorphisms (SNPs) across the genome to provide us with genome-wide copy number coverage.

Q: Why did you choose TruSeq Custom Amplicon?

RD: TruSeq Custom Amplicon allowed us to build our own hematologic malignancy assay, and the off-the-shelf TruSeq Amplicon - Cancer Panel addressed our solid tumor needs.

Q: How has TruSeq Custom Amplicon changed what you are able to do in your laboratory?

RD: The biggest impact is that it's a one-day protocol. From a workflow perspective, in one working day, a technician can analyze anywhere from 1 to 96 samples. We try to keep batches under 48, but we've successfully generated 48 libraries in a single day without

* This amplicon panel is part of an ongoing research collaboration. TSCA supports custom designs up to 1,536-plex.

any problems. This has allowed us to tackle larger projects with a few hundred samples, and turnaround results fairly quickly. On the clinical side, we've been able to validate these assays and offer them as clinical tests, running nearly 600 clinical samples since February. As the demand continues to grow, we have plenty of margin for growth because the assay is so scalable. There's really no extra steps when you add more samples to a run plate until you pass the 96 sample mark in a day. At that point, the bottleneck becomes reviewing the data and generating reports. With the genes interrogated across our two panels, we are finding disease associated mutations in about 75% of the samples we test. That's a fairly high yield.

Q: What do you look for in a new assay and how did TruSeq Custom Amplicon measure up?

RD: First, we look for coverage of desired regions. If we can't capture what we want to sequence, then the assay isn't going to work for us. Using TruSeq Custom Amplicon, we captured over 95% of our desired target region. This was achieved by sequencing to a minimum mean depth of coverage of 1,000×, but on average we've been doing somewhere between 2,000× and 4,000×.

Second, the assay needs to be performed in one day in our clinical setting, and ideally scalable to a large number of samples in that one-day period. With TruSeq Custom Amplicon, we can scale to 96 samples in a day.

Next, we look for the percent of usable data after running the assay, sequencing, and filtering the off-target reads and low-quality data. With our optimized protocol, we're hitting over 90% useable data on a regular basis, with over 95% of reads on target.

Lastly, it comes down to reproducibility of mutation capture. We use a customized informatics solution that incorporates both DNA and library QC with our informatics. In terms of mutation detection, it is incredibly reproducible. We've performed over 40 clinical runs with the same positive control and no template control and we've had 100% mutation concordance for that positive control in every repeat analysis. This is with calling variants down to a 5% allele frequency burden.

Q: What have you been able to accomplish with TruSeq Custom Amplicon?

RD: The nature of the assays, having the same starting and ending sequence for every amplicon, allows us to write some robust custom programs and identify large (10–90 base pair) insertions and deletions that have a hard time mapping with routine alignment software. We sequence 186 base pairs in both directions in our assay and struggle with finding larger indels when processing that data with standard analysis pipelines. Due to the nature of the TruSeq Custom Amplicon assay, and having the primer sequence at the beginning of every read, we can go through and find those mutations using software we have developed called Garbage Picker. This program effectively searches through the trash, or the data that cannot be mapped properly to the human genome due to the large alterations in the sequence reads, and flags those amplicons with enrichment over normal background noise.

Q: What's the smallest amount of DNA that you've tried with good results?

RD: The lowest we've used is 8 ng total DNA and the results were beautiful. We've analyzed 23 samples where we used less than 25 ng of total DNA and another 15 where we used less than 100 ng of input DNA and have had beautiful results. The assay can perform very well on low input amounts, but it's critical to understand the quality of the DNA. Good quality DNA as determined by our QC assay will have success even at low input amounts. Poor quality DNA will fail even if over 250 ng is used.

Q: Did TruSeq Custom Amplicon meet your expectations?

RD: Yes. As a clinical lab, we have high standards for performance. If we don't have high accuracy, reproducibility, sensitivity, and specificity, we can't offer it as a test. We really need something that is reproducible and robust if we are going to offer it with the potential of helping to inform patient care.

Q: What are your future plans for the TruSeq Custom Amplicon?

RD: We have a few larger panels with expanded content either in validation or under active development. We want to push this assay to its limits.

Q: What advice do you have for other researchers considering TruSeq Custom Amplicon?

RD: QC, QC, QC. This is a robust assay, but you need to understand the quality of the DNA you are putting into the assay. Understanding how well the DNA will amplify, specifically for samples that were fixed in formalin, is critical to achieving robust performance. We perform two QC assays on every sample before starting an assay, and this has really empowered us to push the technology beyond its stated limitations. You can have very reproducible results if you're not analyzing poor quality samples.

> "As a clinical lab, we have high standards for performance. If we don't have high accuracy, reproducibility, sensitivity, and specificity, we can't offer it as a test."

Learn more about TruSeq Custom Amplicon at www.illumina.com/TSCA.

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