

Interview

Alternative Splicing Brings Variety to Life

How can a complex organism be made from a very limited number of genes? Alternative splicing. But how is alternative splicing directed? And what do changes in splicing patterns mean? These are the questions foremost in Dr. Brenton Graveley's mind.

i: What fascinates you about alternative splicing?

BG: Alternative splicing is a way of generating multiple proteins from a single gene. Very little attention has been paid to alternative splicing compared to transcription. Most techniques used to study transcription tell you whether the gene is on or off, but they don't tell you what flavor of the gene is actually expressed, which is very important to the biology of the organism.

It is known that at least 15% of mutations that cause any type of disease are due to mutations that disrupt the normal splicing process. If you can correct the splicing error, you can potentially provide therapeutic intervention. Splicing plays an important role in protein diversity as well as different biological processes. For instance, in fruit flies, it is the key step in sex determination. If you switch the splicing of just two genes, it switches their sexes and sexual orientation.

i: What about the DASL® Assay appealed to you for studying splicing events?

BG: I started using the DASL Assay during a collaboration with Joanne Yeakley (Illumina). I had seen Jo use it working with rat splicing. I was very impressed with the consistency of the results that you get with the BeadArray™ BeadChips and the ease of use compared to other platforms.

The DASL Assay offered advantages in many ways. By designing the probes in the assay correctly, I could analyze the alterna-

tive splicing of a large number of genes. Of even greater interest was that you could do it in many, many samples at once in a 96-well format. For me, that was the key. I wanted to analyze splicing changes in a large number of conditions. We bought the BeadStation and have been doing lots of DASL Assays. We got into the VeraCode® platform because it was a way to do the same thing, but on more focused gene sets.

i: Can you tell us about the primary experiment you performed on the BeadStation?

BG: Our original goal was to identify splicing changes resulting from knocking out different RNA binding proteins in the genome. Instead of knocking out one protein and doing a genome-wide array to find everything that changes, we took a different approach. We looked at a representative set of genes, knocked everything out, and looked across the regulatory space as opposed to the regulated space. It's allowed us to look at a different dimension than other people have. Instead of just comparing wild-type and knockout, we are looking at all knockouts.

i: Is this approach giving you a different type of insight?

BG: Yes. For instance, there are about 75,000 alternative splicing events in the human genome. Before we started this study, we knew of about 20 proteins that could regulate splicing. And that just



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doesn't make any sense. Something else must be going on. So we looked through the *Drosophila* genome for any genes encoding proteins that could potentially bind RNA. We knocked all of these genes out to see what proteins were involved in splicing. We went from knowing about 20 splicing regulatory proteins to knowing about 100 or more proteins that can control splicing. At the same time, we figured out what targets they regulate. It's definitely increased our understanding of what can control splicing. I think that the data set we have is really going to be paradigm shifting as far as how people think about splicing regulation.

i: Why did you choose VeraCode technology for your lower plexity studies?

BG: Our initial project used the DASL Assay on the BeadStation at 1,536-plexity. That let us look at 1,500 exons in the fly genome. It was great for that. Then we wanted to focus on a single gene, *Dscam*, which can generate 38,000 alternative, differently spliced variants. Fortuitously, *Dscam* happens to have 95 alternatively spliced exons, which fit perfectly into the 96-plex of the VeraCode platform. So we could generate an oligo pool specific to that one gene.

It was also an experimental advantage to stay with Illumina because I didn't have to invent anything new. I simply moved the same assay over to the VeraCode platform. Convenient. I didn't even have to design new oligos. So that's very easy. In addition, with other platforms, you get your RNA and they say, "here's a protocol and you have to buy ten different items from ten different vendors and hope it might work." With the VeraCode technology, you just get your RNA, buy the Illumina kit, label it, and it works. It's very user-friendly.

i: How do data produced with BeadArray and VeraCode technologies compare?

BG: They are completely different platforms in many ways. Overall, we got the same qualitative results using both the BeadStation and the VeraCode technology. I feel the reproducibility of the data is very high between the two platforms.

i: You've recently decided to pursue sequencing studies. Why did you choose Illumina's Genome Analyzer to perform these experiments?

BG: The main reason for choosing the Genome Analyzer is the existing user base. It's out there. It's established. Everybody's using it. And the main bottleneck, once you get your data, is analyzing it. So, having a large number of users who are developing data analysis solutions for Genome Analyzer data is a huge advantage. In addition, it's easy to use—just plug everything in and push go.

i: What are your plans with the Genome Analyzer?

BG: We've been working with a lab at the Salk Institute to study small RNAs involved in planarian regeneration. We previously found about 60 miRNAs using traditional methods and have increased this number to over 100 with the Genome Analyzer. Moreover, we have used deep-sequencing to identify piRNAs in planarians and, even though we've gotten hundreds of thousands of reads, basically none of them were duplicates. The diversity of these RNAs is simply amazing and, even better, they are expressed in stem cells. So we're going to be looking in greater depth at the small RNAs and microRNAs in these fascinating organisms.

I really want to use it to look at splicing. For instance, we're going to be doing a lot of paired-end cDNA sequencing. But there are so many things you can do with this platform. I wake up in the night thinking, "Hey! We have to do that!" Actually, one of the cool things we are doing involves *Dscam*. Although *Dscam* can theoretically make 38,000 different isoforms, only slightly over 200 have been shown to be expressed. We've come up with a protocol using a combination of exon-specific PCR, barcoded primers, and paired-end reads that allows us to count the real number of isoforms that exist. Very cool.

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i: How do you see the three different platforms working together?

BG: Each platform offers different opportunities. From the VeraCode, you can have very low plexity with very high throughput. On the BeadStation you can have higher plexity, still high throughput. And with the Genome Analyzer, you get lower throughput, but the data you get out of it are phenomenal and inexpensive.

ADDITIONAL INFORMATION

Please visit www.illumina.com to learn more about Illumina's gene expression analysis solutions, including VeraCode technology and the Genome Analyzer.

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