UK Laboratory Uses SNP Array to Expedite and Enhance Cytogenetics Analysis

Ease of use and high-quality data support a smooth transition from oligo microarrays to the Infinium® CytoSNP-850K BeadChip.

Introduction

With a career spanning almost three decades at the Northern Genetics Service of the Newcastle upon Tyne Hospitals, United Kingdom,[1] clinical cytogeneticist Simon Andrew Zwoliński, PhD, has used a wide range of cytogenetics technologies. Working mostly with postnatal samples, he is sensitive to the need for timeliness and accuracy in his methodology.*

Dr. Zwoliński has always been open-minded about evolving his approach and the technologies that he uses in his work. He began his career using G-banding and fluorescence *in situ* hybridization (FISH), moved to bacterial artificial chromosome (BAC) comparative genome hybridization (CGH) arrays, and then oligo arrays. In 2015, he became interested in SNP-based arrays. After comparing the Illumina CytoSNP-850K BeadChip with the Agilent CGH + SNP and Affymetrix CytoScan SNP arrays, he chose the Infinium CytoSNP-850K BeadChip. His team validated the array on the NextSeq® 550 System, and made a smooth transition to using the CytoSNP-850K BeadChip in a month.

iCommunity spoke with Dr. Zwoliński about the beneficial qualities of the CytoSNP-850K BeadChip and how it has increased his team's efficiency.

Q: What sparked your interest in genetics?
Simon Andrew Zwoliński (SAZ): I was originally trained as a botanist, specializing in plant breeding and the evolution of crops. I then went on to do a Masters in Science in applied genetics at Birmingham and earned a PhD in pure genetics from Imperial College in London. I was fascinated with the ability to predict meiotic segregation patterns in *Ascobolus immerses*.

Q: What has it been like to experience all the changes in technology throughout your career?
SAZ: When I started my career, it was all Mendelian genetics. Then people started identifying sequences of DNA, and soon after the whole human genome was sequenced. This had a profound influence on diagnostic genetics. Performing cytogenetics was like "spot the difference," looking for small changes under a light microscope. Nowadays, cytogenetics is computer-based and we're interpreting actual gene content of copy number changes. Most clinical scientists don't even think about Mendelian genetics today and I'm a little sad about that.

Q: How have new technologies like next-generation sequencing (NGS) helped identify the variants related to previously unknown genetic syndromes?
SAZ: NGS has identified pathogenic genes and associated phenotypes, so now we are able to use that information in our array interpretation.

Q: Has the speed of these discoveries increased over the past few years?
SAZ: The speed of discovery has increased exponentially. There were about 20 syndromes that we could identify when we first started performing arrays in 2006. We're now heading towards 500 syndromes that we can identify with arrays, and some of those are still unnamed.

Q: What types of cytogenetic testing do you perform in your lab today?
SAZ: At the moment, we still carry out G-banding to look for balanced rearrangements and use FISH and a few types of staining techniques for cytogenetic testing. However, most of our work now is with SNP arrays.

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*The Northern Genetics Service is a National Health Service laboratory that performs its own validation on research use only (RUO) products for diagnostic purposes according to the International Standards Organization (ISO)

Q: What have been some of the advantages and disadvantages of each of the different cytogenetic testing technologies that you've used?
SAZ: When I started in 1988, all that we had available was G-banding and a method called C-banding. It was a whole-genome screen, but it was gross in the information it captured. We were looking at a resolution of about five megabases. Our abnormality identification rate was lower than 5%. As we progressed to FISH, we were limited to knowing if the sample represented a specific syndrome. FISH would give no more information than a "yes" or a "no."

Then we worked with many other technologies that came and went quickly. One was called M-FISH, which assigned different colors to each chromosome so we could see translocations invisible by G-banding. We also tried multiplex ligation-dependent probe amplification (MLPA) for telomeric rearrangements.

We started using BAC arrays in 2006 and moved to oligo arrays in 2010. In 2016, we transitioned to SNP arrays, and these are by far the most powerful and accurate arrays for determining abnormalities. In our experience, we found that we had a 1 in 20 chance of finding an abnormality using G-banding. As new technologies were introduced, our chances of detecting abnormalities increased. Wiith the CytoSNP-850K array, we now find an answer for 1 in 4 samples. That's a significant difference.

Q: What do SNP arrays offer that oligo CGH arrays cannot?
SAZ: Oligo arrays and BAC arrays can only identify copy number changes, whereas SNP arrays can identify copy neutral changes such as uniparental disomy (UPD), loss of heterozygosity, and lack of heterozygosity. SNP arrays are powerful tools and don't require extra work to gain the additional information.

> "The CytoSNP-850K arrays were so good, and appeared so reliable and consistent, that we completed the transition in just four weeks."

Q: What SNP arrays did you consider?
SAZ: We looked at all the commercial SNP array suppliers including Agilent, Affymetrix, and Illumina. We went with the CytoSNP-850K BeadChip because we thought it was the best platform in terms of resolution, design, and cost. We also knew that Illumina was reliable from our past experiences and we enjoyed a good relationship with the company.

Another factor in our decision was that the BlueFuse® Multi Software that we use to analyze SNP arrays was already familiar to us. This was a significant selling point, as the software is of high quality and very user-friendly.

Q: How did you make the transition to the CytoSNP-850K BeadChip?
SAZ: When we decided to switch to the CytoSNP-850K BeadChip, we had meetings for about three months to persuade the managers at our institute that it was a good decision to transition from an oligo to a SNP array.

I estimated that it would take about eight weeks to validate the array platform and another four weeks to run the oligo and SNP arrays simultaneously. I thought this would be the length of time required to make the transition and to train our team. However, the CytoSNP-850K arrays performed so reliably and consistently that we completed the transition in just four weeks. In February 2016, we were using oligo arrays, and by the beginning of March 2016 we were only using SNP arrays.

> "We are now picking up SNP abnormalities that we were unable to identify with oligo arrays."

Q: How did you validate the CytoSNP-850K BeadChip?
SAZ: To validate the CytoSNP-850K array, we ran 32 historical abnormal samples. In every case, the SNP array not only picked up the abnormality, but was far more precise in determining the breakpoint than the oligo array. The sizes of the abnormalities all fell between the minimum and the maximum that we'd estimated from the oligo arrays. The data validated the SNP arrays and showed us how good the oligo arrays had been originally in terms of giving a range of sizes.

When we chose those original 32 samples we made sure that they represented a range of genetic abnormalities, including deletions, duplications, and triplications. We also chose samples with nullisomy, trisomies, very small abnormalities, and samples where there were known sex chromosome abnormalities in both male and female subjects. Occasionally, even in the 32 samples, we found more information than we had obtained from the oligo arrays. Some copy number changes that looked simple on an oligo array were found to be more complex on the CytoSNP-850K array.

Q: What tissue types did you use for your CytoSNP-850K BeadChip validation studies?
SAZ: We mostly used blood and also performed validation on malignancy samples. In particular, we looked at what an amplification of an oncogene looked like using the two technologies. In addition, we analyzed some placental samples, where we knew that there was maternal cell contamination, and that was easily picked up by the CytoSNP-850K BeadChip. We also analyzed some prenatal samples to make sure that this technique worked with all the tissue types we use routinely.

Q: How long did the validation studies take?
SAZ: Because the CytoSNP-850K BeadChip was so good and identified genetic abnormalities with precision and accuracy, we only spent three weeks validating the array. The oligo and SNP arrays were run in parallel for a couple of weeks, however we hardly looked at the oligos because we were so confident in the CytoSNP-850K BeadChip data.

We saved some oligo slides and some of the reagents in case we ever needed them. Finally, we realized we weren't going to use them anymore, so we offered them to another lab that was still performing oligo arrays. Presently, we have no intention of going back to using oligo arrays.

Q: How long did training take to familiarize your team with running the NextSeq 550 System and CytoSNP-850K BeadChip?
SAZ: The Illumina staff was fantastic and trained us over a two-week period to perform the practical setup and analysis. It was remarkable how quickly our team caught on to the workflow. We've not encountered any problems, and have trained the whole team in the last few months.

A significant benefit of the successful transition to the CytoSNP-850K BeadChip is the reduced amount of hands-on time required. We're analyzing 64–128 samples a week with the CytoSNP-850K BeadChip. If we were trying to perform that many analyses with oligo arrays, we simply wouldn't have the manpower. Even for only 64 samples a week, it would have taken at least three people to perform the analyses with oligo arrays.

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Q: How do the CytoSNP-850K BeadChip results compare with the aCGH results in terms of resolution?
SAZ: The difference between the two technologies is significant. Because of the International Standard of Cytogenomics (ISCA) v2 oligo array design, many syndromes were not covered well. One of the positive things about SNP arrays is that they are designed to include all the known syndromes and every targeted gene is covered. We are now picking up SNP abnormalities that we were unable to identify with oligo arrays.

Q: Was there a difference in DNA concentration requirements between oligo and SNP arrays?
SAZ: There is a difference, but it hasn't had an impact on us. We used to spend a large amount of time cleaning up the DNA for oligo arrays and we don't have to do that anymore. We're processing the samples with the CytoSNP-850K BeadChip exactly as they come off the robot, and that's saving a significant amount of work and time. As a result, our turnaround time has improved.

Q: Can you detect loss of heterozygosity with the CytoSNP-850K BeadChip?
SAZ: The CytoSNP-850K BeadChip provides us with a more accurate determination of copy number changes and copy neutral changes. It is useful to have the allele frequencies and in-depth information about heterozygosity. SNP arrays provide built-in confirmation when we have a deletion or a duplication that we can see by the log R ratio. When we were analyzing samples with oligo arrays, we realized that most of the calls made by the software were not real. We had to guess that they were not real based on how many times we had seen the call in our cohort of subjects. When we perform a SNP array today, we can see whether it's real or not by the B allele frequency, and that gives us confidence in the result. In addition, there are over 100 array features on the CytoSNP-850K BeadChip for some syndrome regions, compared with just three or four features on an oligo array.

Q: Is there a difference in detecting amplifications or mosaicism with the CytoSNP-850K BeadChip versus oligo arrays?
SAZ: When we were using oligo arrays, we expected to see a theoretical log 2 ratio of +0.6 for a duplication and -1.0 for a deletion. However, it was only a theoretical value and there was always a degree of variation. Sometimes we would get a log 2 ratio that wasn't +0.6 or -1.0 and that would give us a clue about mosaicism, but we could only confirm that by FISH. Now we can recognize mosaicism and multiple copies clearly by deviations in the B allele frequency.

It is difficult to know what degree of mosaicism can be detected accurately, but I estimate we have identified levels for deletions below 10%. With the duplications, the detection of mosaicism is much more difficult, and my guess is we will be able to see a level of about 20%. For multiple copies, we can see how many there are by looking at the distinct pattern within the B allele frequency chart.

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Q: Did you have any concerns about transitioning to the CytoSNP-850K BeadChip?
SAZ: One of the things that we were worried about when we moved to the CytoSNP-850K BeadChip was the increased number of calls we might obtain. In reality, the number of calls is no different than what we obtained with oligo arrays. The difference is that with the CytoSNP-850K BeadChip, nearly all the calls are real. It is easy to decide whether they are benign or pathogenic because the benign ones are usually located where there are no significant genes or exons.

While the SNP arrays can identify UPD or lack of heterozygosity for some syndromes such as in Prader-Willi, Russell Silver, or Beckwith-Wiedermann, we are also picking up nonsignificant

whole-chromosome UPD. This requires sensitive reporting and we are still on a learning curve. We are also concerned about identifying consanguinity, which might be known only to one of the parents.

Q: What are the next steps in your lab?
SAZ: We will focus on consolidating our work with the CytoSNP-850K arrays and are beginning to use the NextSeq 550 System for sequencing gene panels for malignancy samples. In the future, we hope that NGS will help us detect genetic variants associated with autism and epilepsy.

When we moved to SNP arrays, various research groups within the university requested our SNP services to analyze cell lines. In the past, they either didn't perform array analyses for their cell lines or they were sending them out to expensive private service companies.

We were expecting to perform 2000 cytogenetic analysis cases per year, and we've already carried out more than 2000 cases in the eight months since we started using SNP arrays. We have increased our throughput and efficiency, and we're happy about that!

Learn more about the Illumina products mentioned in this article:

Infinium CytoSNP-850K v1.1 BeadChip, www.illumina.com/products/by-type/clinical-research-products/infinium-cytosnp-850k.html

NextSeq 550 System, www.illumina.com/systems/nextseq-sequencer.html

BlueFuse Multi Software, www.illumina.com/clinical/clinical_informatics/bluefuse.html

References

1. The Newcastle upon Tyne Hospitals, Northern Genetics Service. www.newcastle-hospitals.org.uk/directorates/northern-genetics-service. Accessed on December 21, 2016.

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