Scientist spotlight

All chromosomal microarrays are not created equal

GeneDx is a world leader in genomics with expertise in rare and ultra-rare genetic disorders research and an extensive genetic analysis menu. As part of their comprehensive molecular cytogenetics analysis, they recently switched to the Applied Biosystems™ CytoScan™ HD Array Kit, a high-resolution whole-genome chromosomal microarray (CMA) for prenatal and postnatal analysis.

Thermo Fisher Scientific spoke with Dr. Jeanne Meck and Stephanie Warren about their journey to implement the CMA platform utilizing a hybrid microarray consisting of both copy number and single-nucleotide polymorphism (SNP) probes. During this process, GeneDx clearly identified that not all arrays are the same. Dr. Meck and Ms. Warren also touch on the importance and impact that different array types or designs can have.

Thermo Fisher Scientific: What attributes of a hybrid SNP CMA platform made it the best choice for GeneDx?

Dr. Meck: Many of the reasons come back to the DNA. The hybrid SNP array that we implemented tolerates lower-quality DNA, allowing us to analyze buccal (70% of our postnatal cases) and uncultured prenatal samples. It has been particularly useful during the SARS-CoV-2 crisis, where we have increased our use of at-home collection of buccal samples for our studies.

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Also, the new hybrid SNP microarray requires less DNA. This is especially good for buccal and uncultured prenatal samples. Our clients often order multiple research tests, so efficient use of DNA is important. We have been able to reduce the amount of sample necessary by 40%, going from 100 nanograms to a current 60 nanograms.

Jeanne Meck, PhD, FACMG, director, prenatal diagnosis and cytogenomics, GeneDx

Stephanie Warren, assistant director, microarray operations, GeneDx

This also results in a reduced need for culturing. Recent GeneDx statistics show that we culture fewer than 10% of our samples derived from amniotic fluid and chorionic villus sampling (CVS), which reduces turnaround time (TAT) by nine days.

With the new hybrid SNP array, our quality control metrics are more clearly defined. With the previous system, we had more gray areas, which resulted in additional time being spent communicating about what was or wasn't considered "passing." Now we save this time.

The new system provides fewer inconclusive results and fewer repeats compared to our prior platform. This saves GeneDx time, money, and resources. Ultimately, our lab is more efficient, providing results in a shorter TAT.

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Thermo Fisher Scientific: What impact do microarrays have in your lab for prenatal and postnatal analysis?

Dr. Meck: Microarrays play a very large role at GeneDx for both prenatal and postnatal analysis. CMA has been recommended by the American College of Medical Genetics and Genomics since 2010 for postnatal analysis to support studies in congenital anomalies, developmental delay, or intellectual disability, while in 2016 the American College of Obstetrics and Gynecology and the Society for Maternal-Fetal Medicine recommended CMA samples for studies in pregnancies with a fetus having abnormalities detected by ultrasound.

The relatively short TAT for CMA could be well suited to aid research studies in the prenatal setting where time is of the essence. Furthermore, CMA could be an excellent research tool since: (1) results can usually be obtained rapidly, and (2) it directly addresses the increased risk for aneuploidy as a follow-up to analysis showing an elevated risk for Down syndrome, trisomy of chromosomes 13 or 18, and sex chromosome aneuploidies, as well as microdeletion syndromes and other copy number variation (CNV) studies.

CMA offers answers rapidly and with high sensitivity. With a high-density hybrid array like we are using, we can identify very small deletions and duplications down to the level of single exons for some genes. Furthermore, the breakpoints for CNVs are generally quite accurate since there is a good density of probes within introns and intergenic regions, unlike other technologies. Having the most accurate breakpoints possible is important, even if it is just for future interpretation, since we may learn of enhancers in the region or relevance of a gene not currently known to be important.

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CMA is a whole-genome test analysis and, although phenotype is taken into account when reporting CNVs, we still report all potentially relevant CNVs regardless of the reason since there may be some phenotypic features not observed at the time of analysis but may become evident later in life.

Running parental samples along with proband is generally not important for CMA, although it can be useful for classification and interpretation of variants of uncertain significance. After proband analysis and in the context of a positive finding, parental analysis may be important to provide scientific insight regarding the recurrence risk in families.

Thermo Fisher Scientific: Based on your experience, with quite a few microarray options available, do you think all microarrays are equivalent?

Dr. Meck: All microarrays are not equivalent. One should take the time to determine which type of array is being used, the level of coverage, the reporting thresholds, and their performance with certain specimen types (difficult specimen types include buccal and uncultured prenatal specimens).

There are two arrays that we have lots of familiarity with at GeneDx, and they represent the basic types of arrays available: (1) a custom-designed aCGH with or without SNPs and (2) a hybrid SNP array.

These two types of arrays are very different with respect to

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probe density, the number of probes used to make a deletion or duplication call, and the method of identifying regions of homozygosity, uniparental disomy, and the information obtained from genotyping, when needed. Our strong preference for CMA is the hybrid SNP array since it provides detailed, accurate information about both CNVs and runs of homozygosity (ROH).

Furthermore, allelic (SNP) tracks support the copy number calls and have reduced the number of confirmations in our studies; if there are sufficient SNP probes in the CNV region, it can also show the deletion or duplication. The allele difference and B allele frequency tracks are also very important for seeing mosaicism. They make it much easier to identify and estimate the level of mosaicism, and visualization of results on these tracks often eliminates the need for confirmation. In summary, we have increased confidence about the calls due to the large number of probes with our hybrid SNP platform.

Thermo Fisher Scientific: What factors drove your decision to change from your previous platform to the one you have now?

Dr. Meck: When the proportion of specimen types other than blood (buccal, uncultured amniotic fluid, and CVS) started increasing in our studies, we realized we needed an array platform that could give us accurate and reliable results on suboptimal specimen types, i.e., with degraded DNA. For prenatal research samples, we wanted to get results more reliably from uncultured amniotic fluid, CVS, and products of conception (POC) to decrease our TAT.

Also, many of our research clients request that we find multiple test analyses for them, so the amount of DNA required is of utmost importance and the hybrid SNP array uses less DNA than some other array platforms.

On the new system, we love the analysis tools for the region of homozygosity calling because both the probes and the algorithm are more reliable. We can identify triploidy with much more confidence. We also see fewer inconclusive analysis results on buccal swab specimens compared to our previous platform.

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For all specimen types, we wanted more sensitive and reliable information to aid the studies in regions of homozygosity and uniparental disomy (UPD) via genotyping.

Thermo Fisher Scientific: What are the main benefits that you see with your current hybrid SNP CMA platform?

Dr. Meck: The new hybrid SNP system is faster, has improved calling, is more efficient, and has lower costs. The increased speed and the decreased costs were not readily apparent at first but have turned out to be a significant reason we are so happy with this platform.

You need to look at the total cost for using it in day-to-day scenarios. This means that if we need less repeat analysis, have reduced culture needs, and need less time assessing the quality of data or analyzing calls, then the overall cost of analysis is lower. Our confidence factor is higher when we do not have to repeat analyses for calls that may or may not be real.

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Our previous platform was difficult to troubleshoot and sometimes required repeating the array two or three times. Reduced repeats also increases the efficiency of our research lab personnel. So, even though the hybrid SNP protocol takes an extra day, the high percentage of time that we get a high-quality result the first time more than makes up for it.

Another point in support of the increased research study efficiency is the platform's tolerance for lower-quality DNA and the reduced need for confirmation of CNVs based on additional info from allele difference and B-allele frequency (BAF) tracks.

The array is off-the-shelf, so when our stock is low, we do not need to wait for a custom design to be produced. Additionally, the manufacturer provided a track of polymorphic regions that allowed us to filter out these common CNVs right from the beginning without having to compile the research analysis data from our own experience.

Thermo Fisher Scientific: Do you have any advice for other labs that are contemplating changing their current setup or platform or looking to bring CMA in-house?

Ms. Warren: Whether bringing CMA in-house or changing platforms, the biggest advice we can offer is to consider the whole picture and not just any perceived disadvantage, such as protocol timing or cost—these two points were our biggest concerns.

The benefits that we have talked about, such as DNA tolerance, decreased repeat rate, reduced need for alternative confirmation methods, and the ease and intuitiveness of the analysis software, outweigh the disadvantages.

The system we chose is very widely used in the industry, making protocols and polymorphic regions well-defined and readily shared. This allowed the technical and analytical validation and transition to be relatively painless.

Thermo Fisher Scientific: Can you summarize the key points you learned and provide words of wisdom from your journey converting from one platform to another?

Ms. Warren: It can be time-consuming at first, but well worth it. Utilize the vendor partner's help when starting the assay and with training. Don't think you have to go it alone—work with the vendor specialist when challenges arise.

To request a consultation by a technical expert, **contact us**

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