TruSeq® Targeted RNA Expression on the MiSeq® System Uncovers Effect of CNV Linked to Schizophrenia

Frank Middleton, Ph.D., at SUNY-UMU developed a focused gene panel to screen for expression alterations caused by a newly discovered CNV in schizophrenia using the MiSeq System.

Frank Middleton, Ph.D., is an Associate Professor at SUNY Upstate Medical University (UMU) with a primary appointment in the Department of Neuroscience and Physiology, and appointments in the Psychiatry and Biochemistry and Molecular Biology departments. Dr. Middleton teaches courses on biostatistics, experimental design, and systems biology, as well as a neuroscience course for the medical school. He established, and now manages the university's high-throughput genomics core facility. The facility provides internal and external users access to microarrays, real-time PCR, and next-generation sequencing (NGS) systems, such as the MiSeq System. Recently, Dr. Middleton used the core facility's MiSeq System and a TruSeq Targeted RNA Expression (TREx) panel to perform targeted RNA sequencing of a focused set of genes, screening for schizophrenia-related expression alterations.

Q. What is the focus of your research?

Frank Middleton (FM): I'm most interested in diseases that affect how you think and how you move. I study how schizophrenia, Parkinson's disease, attention deficit hyperactivity disorder, and drugs of abuse affect the brain's dopamine circuitry, which regulates thinking and moving. I have about four major projects going on in my lab related to these topics at any one time.

Q: What is your approach in studying neurological disorders?

FM: In the discovery phase, we try to uncover potential causes or contributors to disease processes. Sometimes we use human genetic assays, and human or rodent genetic data. We'll use biomarker, post-mortem brain, and epigenetic data in the discovery phase. Once we find something interesting, we'll test potential mechanisms using cell culture or rodent models. We employ a combination of molecular, neuroanatomical, and often behavioral measures in our outcomes.

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Frank Middleton, Ph.D., a neuroscientist by training, teaches, runs the core facility, and has his own research laboratory at SUNY-UMU.

Q: How has the MiSeq System contributed to these studies?

FM: NGS with the MiSeq System has contributed a great deal, enabling novel findings in schizophrenia. We studied a large, five-generation pedigree that had many affected subjects. Using SNP genotyping arrays we found what looked to be a copy number variant (CNV), but we couldn't narrow down the region closer than a few thousand base pairs where it began and ended. We simply didn't have complete confidence in the findings until we performed whole-genome sequencing (WGS) using the Illumina HiSeq® System. WGS narrowed down the CNV to single-base resolution and pinpointed exactly where the CNV started and ended. That initiated a whole new line of research studies using targeted RNA expression sequencing. We characterized changes in expression in schizophrenia subjects who have this CNV, and compared that data with subjects who don't have the CNV from the same family and outside of the family.

Q: How did you use targeted RNA expression sequencing in the study?

FM: In our follow-up studies on the CNV, my Ph.D. student Parisa Afshari and I created a custom panel using DesignStudio® and the TREx assay. It contained about 370 of the best candidate genes that have been identified for schizophrenia, bipolar disorder, autism, and other neuropsychiatric disorders. That candidate gene list was informed by looking at the results of large genome-wide association studies (GWAS), large meta-analyses of candidate genes, and large-scale expression studies. It would be cost-prohibitive to do this by custom PCR or PCR arrays. With TREx on the MiSeq System, we

could put all the genes of interest into one assay. It's ideal for profiling a moderate number of samples at a time, with several hundred gene targets. In just a few days, we received data on dozens of subjects generated from only a few MiSeq runs. The MiSeq System truly maximizes the amount of data you get for the amount that you invest, both in terms of time and money.

Q: Did you uncover anything interesting?

FM: In the TREx follow-up profiling, we looked at all the potential responses in candidate genes related to schizophrenia, genes related to important neurotransmitter pathways in the brain, and in glutamate-related genes. All these neurotransmission-related genes revealed that the cells that have this CNV appear to be changing in a very consistent way. There's an entire glutamate hypothesis of schizophrenia that's well established in the field, and the CNV we identified, a large hemi-deletion in a glutamate transporter gene, fit within the biological framework.

Just looking at the TREx data and the most significant follow-up findings, you can see that specific glutamate neurotransmitter receptor types are involved in the cellular response to this mutation. It turns out that those exact same neurotransmitter receptor subtypes have been targeted by the latest-generation neuropsychiatric medications that have been developed for schizophrenia. This is an incredible convergence of our discovery and follow-up work with a completely independent line of work pinpointing the most important neurotransmitters to manipulate with schizophrenia medications.

Q: What does this finding mean for the neuroscience field?

FM: It underscores that clinical researchers who are targeting these neurotransmitter receptors are definitely on the right path. The early results from the clinic also support that the disease can be manipulated at this level. For our study to hit exactly the same potential mechanism as a therapeutic target is a nice fit.

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Q: How else can targeted RNA expression sequencing be applied?

FM: Targeted RNA expression sequencing could be used as a high-throughput validation method for a broader screening technology. In whole transcriptome profiling, either by microarray or by RNA-Seq, you spend a lot of time and money probing the entire genome for all your samples. After statistical analyses, you hope to identify a small set of genes that are meaningful and important. That subset becomes your focus and you can use targeted RNA expression sequencing to validate changes observed using another orthogonal technique.

TREx Study Parameters

- **Read length of sequencing run: 50 bp single reads**
- **Analysis method: MiSeq Reporter quantified reads and FASTQ alignment in third-party software (Strand-NGS, Partek Flow)**

The most powerful contribution, however, is using targeted RNA expression sequencing as another screening tool. It gives you a focused set of genes to generate data in different contexts in single MiSeq runs. If you performed your screening study correctly, then you can take those several hundred genes and profile them in different contexts. These could represent other experimental manipulations that focus on just what you consider to be the relevant genes from your broader screening. I consider TREx on the MiSeq System to be essential for the type of broad, functional gene network profiling that current systems biology-based research has moved toward.

Q: Are you ever challenged with low starting material?

FM: Sometimes we start with samples of cells that are isolated using laser capture micro-dissection (LCM) of specific brain regions or blood samples where the cells are low in number. The TREx assay enabled us to perform targeted RNA expression sequencing with as little as 25 ng or 30 ng of total RNA, and we've been very pleased with the data quality. We are satisfied with what we've been able to do, whether it's DNA, RNA, or small RNA, in terms of low starting material.

Q: What do you like best about the MiSeq System?

FM: What we like the most about the MiSeq is that we don't need a separate cluster generation instrument. We like having the ability to put the cartridge in and the MiSeq generates the clusters and performs sequencing all in one box. With fewer instruments to maintain, we have more system up-time, which translates into lower costs, faster assays, and higher-quality data. We also like the ability to upload MiSeq data into BaseSpace® and make it readily accessible for downstream analysis tools. It's a seamless process and everyone on my team has been very happy with it. The MiSeq System has been a great resource.

Q: How is the MiSeq System reliability?

FM: It certainly helps having a reliable instrument in place that's so easy to use. The MiSeq System has been very easy for us to maintain. We have confidence that it's not going to require user intervention in the middle of an overnight sequencing run and that it's going to generate high-quality data. We've been so impressed with the MiSeq; it's been a very positive experience. We'll definitely keep the MiSeq System going full speed in our core facility.

Learn more about the MiSeq System at www.illumina.com/miseq

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