

# Spotlight on Science



Mining the post-mortem human brain for neurodegenerative markers: high-throughput deep sequencing of cell type specific transcriptomes

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## What is the main focus of your research?

Cerevance is focused on answering fundamental questions about the complexity of the central nervous system and its diseases. We are especially interested in identifying changes in gene expression associated with neurodegenerative disorders where effective disease management or therapeutics are not available.

## What are the advantages of the Nuclear Enriched Transcript Sort Sequencing (NETSseq) technique?

NETSseq is a technique that enables us to isolate nuclei from specific cell populations derived from post mortem human brain. This allows us to isolate enough RNA to generate high quality and deep

whole transcriptomic data for each of these populations. This enables us to compare statistically meaningful numbers of patients and controls within a short time-frame, even for relatively rare cell populations.

## Why is the whole transcriptome approach important for your studies?

CNS diseases have largely defied elucidation via animal model-based efforts and shallower (or heterogeneous tissue)-based transcriptomic methods. We are looking for new information here, something significant that has gone unnoticed against background noise in the past. To achieve this, we feel it is essential to develop a deep and accurate gene expression profile for a wide range of target cell types. From there we can extend to our bioinformatic analysis to elucidate networks of interacting genes. Deep sequencing from pooled cell populations enables us to identify subtle changes that would be missed by shallower approaches.

## What challenges did you face while optimizing this technique?

To establish NETSseq as a high throughput platform we reviewed every aspect of the basic protocol from the perspective of the constraints imposed by automated liquid handling systems. This involved rigorous evaluation of automation friendly RNA extraction and amplification systems from a range of suppliers. Our samples are available in limited quantities and of variable quality, so it was crucial to identify products that maximise yield and reproducibility at every step. In some cases, this has even necessitated customising commercial products to meet our specific needs.

## How has Trio RNA-Seq enabled this research?

Initially we trialled the NuGEN Ovation RNA-Seq V2 kit, with its Single Primer Isothermal Amplification (SPIA) technology, alongside a rival product and found that it returned a better performance when challenged

with very low concentration and degraded input RNA. The Trio RNA-Seq kit, which is a complete workflow including SPIA amplification and post-library transcript depletion, became available during this evaluation period and it was immediately apparent that the product was designed with automated liquid handling in mind. This was exactly what we needed, and NuGEN have been very helpful in supplying custom scripts for our Agilent Bravo systems. This kit has allowed us to attain our goal of high throughput while maintaining the inherent data integrity that plate based platforms offer. Being configured as an integrated solution simplifies our workflows and minimises variability between runs and between samples.

### **What are the implications and ultimate goal of your work?**

By taking a unique approach to studying the brain transcriptome and using human, not animal, samples, we are discovering a great deal about brain substructures

and the impact of disease states on individual cell types. Insights into the processes underlying neurodegenerative disorders are leading us to new candidates for disease intervention. Ultimately the dream is to identify therapeutic agents that can modify causative factors and halt progression of some of these devastating neurological conditions.

**To learn more about the research at Cerevance, please visit:**

<https://www.cerevance.com/>