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

SPOTLIGHT ON SCIENCE

Steven Sheardown, PhD
Head of Molecular Biology and Cell Engineering, Cerevance
Cambridge, United Kingdom

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 brains. It 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 according to 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 customizing commercial products to meet our specific needs.

How has Trio RNA-Seq™ enabled this research?

We initially trialed the Tecan Ovation® RNA-Seq V2 kit, with its Single Primer Isothermal Amplification (SPIA®) technology, alongside a rival product, and found that it returned better results when challenged with very low concentration, 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 this product was designed with automated liquid handling in mind. This was exactly what we needed, and Tecan has 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 minimizes 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/

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Australia +61 3 9647 4100 **Austria** +43 62 46 89 330 **Belgium** +32 15 42 13 19 **China** +86 21 220 63 206 **France** +33 4 72 76 04 80 **Germany** +49 79 51 94 170
Italy +39 02 92 44 790 **Japan** +81 44 556 73 11 **Netherlands** +31 18 34 48 17 4 **Nordic** +46 8 750 39 40 **Singapore** +65 644 41 886 **Spain** +34 93 595 25 31
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