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Application Note

Dynamic Neuronal Exosomal Biomarkers in Cell Culture Medium

Multiple partnerships with Exosome Diagnostics, a Bio-Techne brand, highlights capabilities for exosome approaches to support characterization through cell culture of progenitor cells and differentiated cells. Studies are broadly applicable across research areas but specifically showcase capabilities of exosome RNA as dynamic biomarkers in neuronal progenitors and differentiated cells.

Exosome Diagnostics can:

1. Characterize gene expression changes in progenitor and differentiated cells through ex vivo cell culture samples
2. Dynamically characterize distinct cell populations through exosomes
3. Ensure reproducibility and quality control for cell culture analysis

Contents

Overview 1

Neuronal Progenitors & Differentiated Cells 2

Introduction 3

Background and Results 3

Conclusions..7

References 7

Overview

Capabilities:
Drug target engagement, treatment response stratification, trial monitoring, companion diagnostics development, etc.

Sample:
Data demonstrates feasibility in serum/plasma, CSF, urine and other biofluids.

Analyte:
Exosomes contain Protein, RNA and DNA with information related to metabolomics, lipidomics and glycomics.

Quality:
Bio-Techne is able to provide clinical workflow solutions with CGMP and GCLP capabilities.

Expertise:
Exosome Diagnostics has extensive intellectual property related to exosome-based diagnostics, and the longest running company providing solutions around exosome diagnostics.

Dynamic Exosomal Biomarkers from Neuronal Progenitors and Differentiated Cells

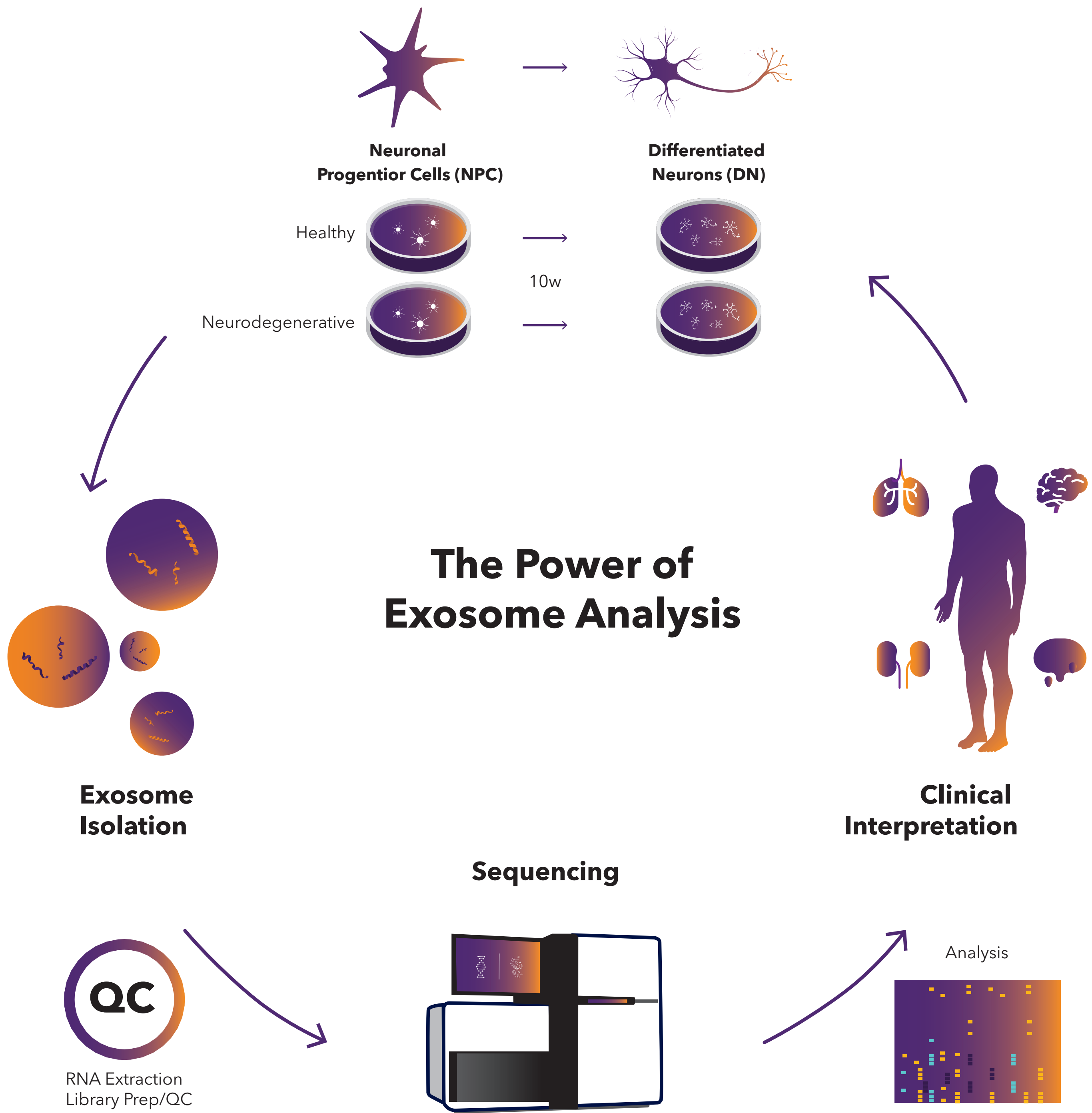


FIGURE 1 Neuronal progenitor cells (NPCs) from Healthy and Neurodegenerative cells were cultured for 10 weeks with distinctive media conditions to induce differentiated neurons (DN). Exosomes were isolated from NPCs and DN cells, RNA extracted, library prepped, quality control (QC) performed, sequenced, and analyzed. Analyzed cells are used for clinical interpretation of the progenitor and differentiated cells to inform culture decisions.

Introduction

Tremendous diagnostic power resides in exosomes as demonstrated by numerous peer-reviewed studies[1-5] and existing commercial products, most notably the ExoDx Prostate test.[6-7] Exosomes are powerful diagnostic tools because they:

- Are numbered in the billions per milliliter of plasma, urine and other biofluids.[1]
- Are released from all cells reflective of the content from the cell of origin.
- Pass through the blood-brain barrier.[8]
- Are rich and stable sources of RNA, DNA and proteins.
- Allow characterization with multi-analyte analyses in native and modified state (e.g.methylation).
- Can be separated by tissue-specific markers to analyze an enriched signature.
- Are highly stable packages of RNA, allowing samples to be stored up to 25 years.

Background and Results

Exosomes are data rich, containing information on various types of RNA, protein and DNA. Numerous studies have indicated that exosomes are critical for communication between different neural cell types.[8] Crosstalk between neuron, glial and other cells occur through exosomes, and specifically microglia have been observed to internalize oligodendrocyte-derived exosomes by macropinocytosis, and most of these microglia were MHC class II negative and did not activate immunological responses.[8-11] Positive and negative feedback loops of exosome communications are being studied for both autocrine and paracrine pathways. Exosome communications both influences and can be a method of characterization for the differentiation of neuronal progenitors to neurons. Similar signaling events can be found for glial progenitors to astrocytes and oligodendrocytes. Cell culture media and other biofluids can be utilized for characterization of the host cells they initiate from and the dynamic state of progenitor or differentiation profiles that they possess.

1. **Characterize Gene Expression Changes in Progenitor and Differentiated Cells Through Non-Invasive Cell Culture Samples**

Neuronal progenitors cultured with differentiation medium for 10 weeks gives rise to an enriched neuronal profile as seen by both the gene expression data (FIGURE 2A) and violin plots (FIGURE 2B). Expression profiles of neuron specific genes are observed distinctly for both healthy and neurodegenerative cells. Furthermore, the genes most distinctly changed in samples is observed in neuronal development and established profiles (FIGURE 2C). Data indicates the power of monitoring dynamic changes in cellular development and differentiation through non-invasive cell culture medium using isolated exosomes.

A deeper look at the pathways demonstrated in FIGURE 2C to be upregulated indicates that differentiated cells (DN) over progenitor cells (NPCs) have particular emphasis on pathways associated with neuronal function, development and differentiation.

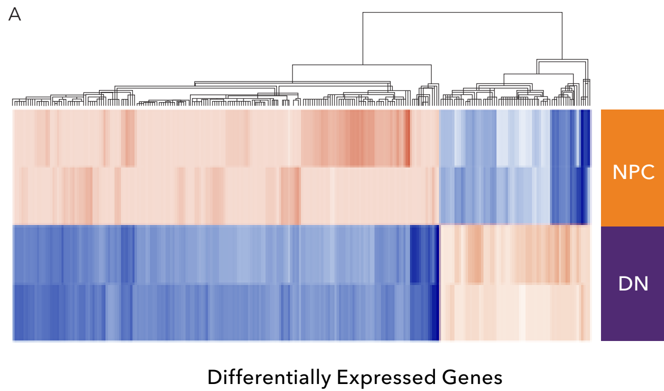
2. **Dynamic Characterization of Distinct Cell Populations Through Exosomes**

Exosomes isolated from progenitor and differentiated cells need to be proven distinct at individual points throughout their differentiation. There are distinct populations at the very least for the NPC and the fully differentiated DN based on gene expression analysis of exoRNA (FIGURE 1). Dynamic gene expression profiles can be seen shifting over the 10 weeks of differentiation creating distinct populations at the initial stage of progenitor cells and those differentiated after culture. FIGURE 3A demonstrates a tissue-of-origin deconvolution analysis of exoRNA isolated gene profiles indicating that differentiated (DN) neuronal cells are most closely aligned with brain tissues compared to all other types of tissues. Furthermore, FIGURE 3B indicates that differentiated (DN) cells are most closely associated with brain sub-compartments including thalamus, cerebral cortex and basal ganglia; while NPCs display a gene expression profile more similar to broader subsets of cells.

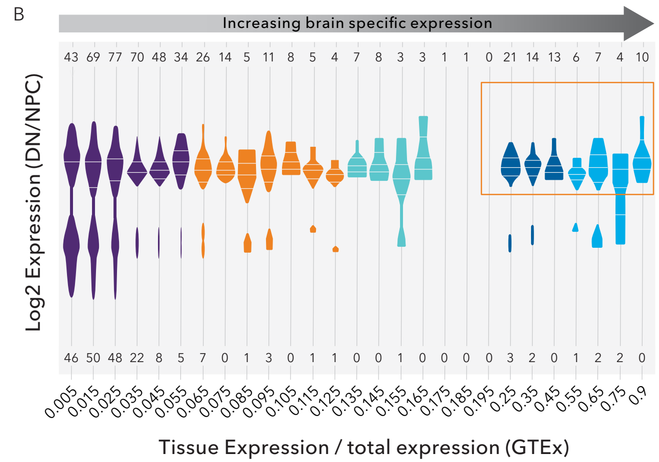
3. **Ensure Reproducibility and Quality Control for Cell Culture Analysis**

Experimental reproducibility and quality control is critical when monitoring and characterizing cellular differentiation through exosomes from the cell culture medium. Replicates of various cell culture samples indicate little variability from sample to sample while as expected there are some observed changes from sample type to sample type for genomic mapping or gene detection (FIGURE 4A/B). Further evidence of the distinct populations and experimental reproducibility is shown in FIGURE 4C where transcriptome-wide analysis finds biological replicates to have a better correlation than comparisons between differentiation states (DN vs. NPCs). Lastly, the linear correlation for spike-in sample controls demonstrate workflow reproducibility FIGURE 4D.

Comparing Gene Expression Profiles between NPCs (Neuronal Progenitor Cells) and DNs (differentiated neurons)



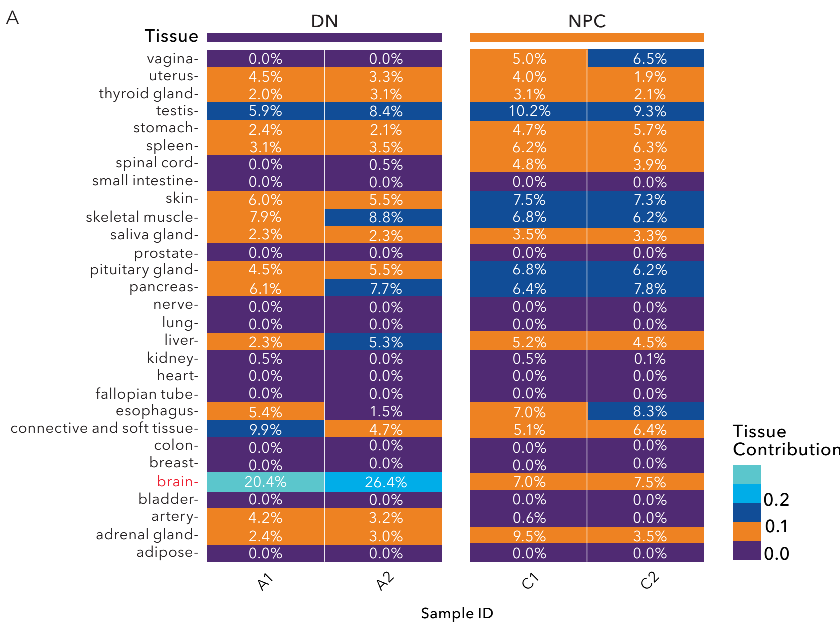
Comparing the Proportion of Brain-Enriched RNA between NPCs (Neuronal Progenitor Cells) and DNs (differentiated neurons)



Pathway Analysis - Upregulation of Neuronal Development and Differentiation Related Pathways in DNs (differentiated neurons) over NPCs (Neuronal Progenitor Cells)

FIGURE 2 **(A)** Differential gene expression was matched to major pathways through GO analysis based on exoRNA. **(B)** The violin plot displays gene expression patterns in fully differentiated neurons (DN) vs. neuronal progenitor cells (NPCs). **(C)** GO analysis captures the change in differentiation status of the source cells in both control and neurodegenerative samples albeit to different content.

Deconvolution analysis: Human Tissues



Deconvolution Analysis: Brain Sub-compartments

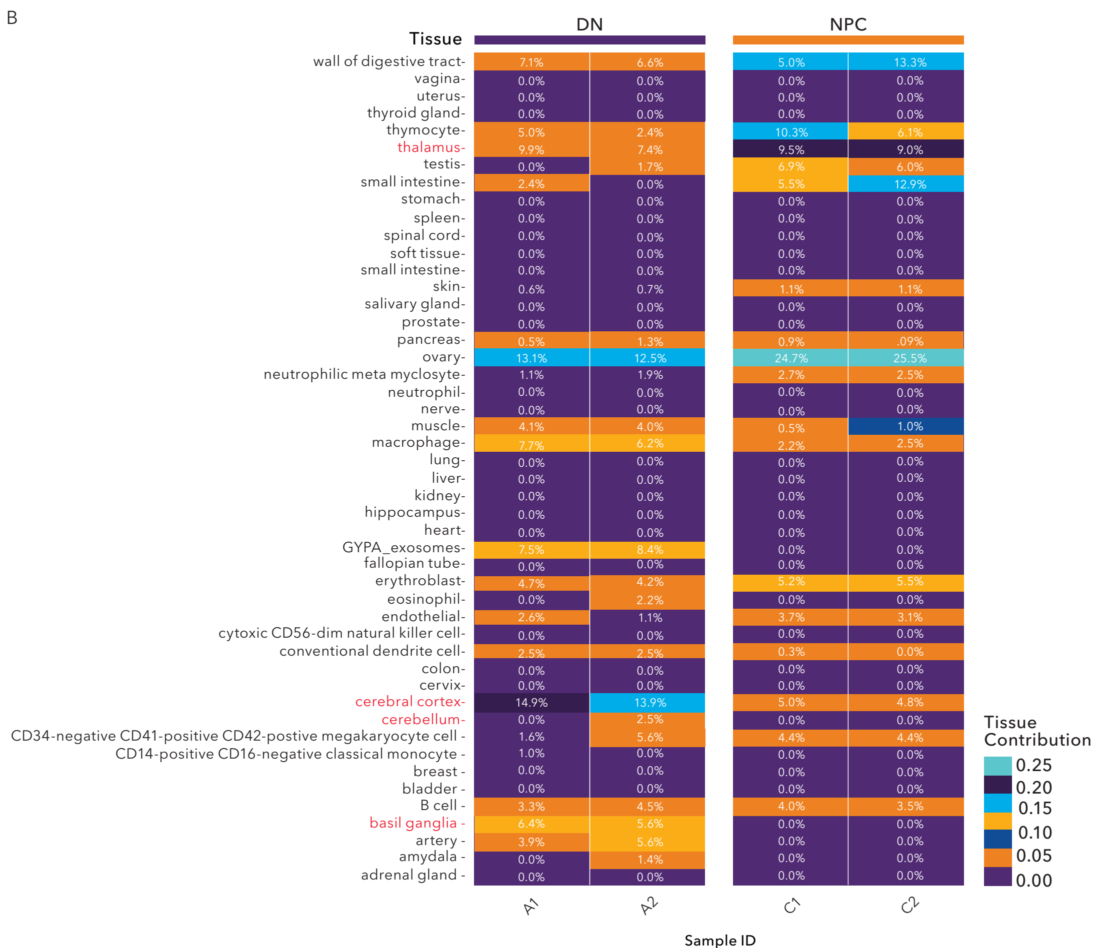
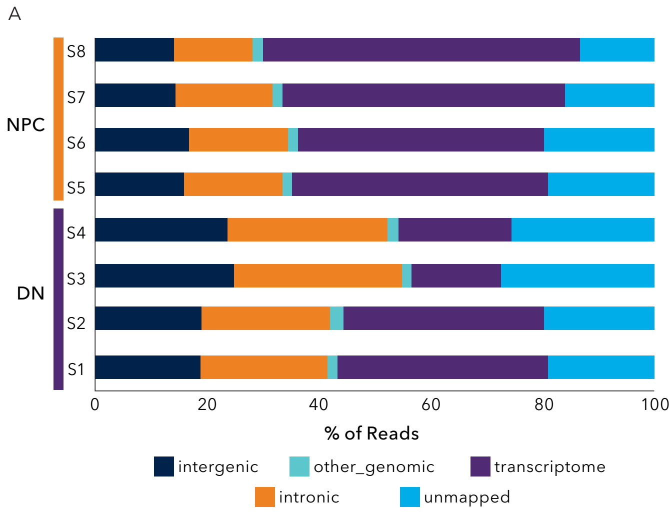
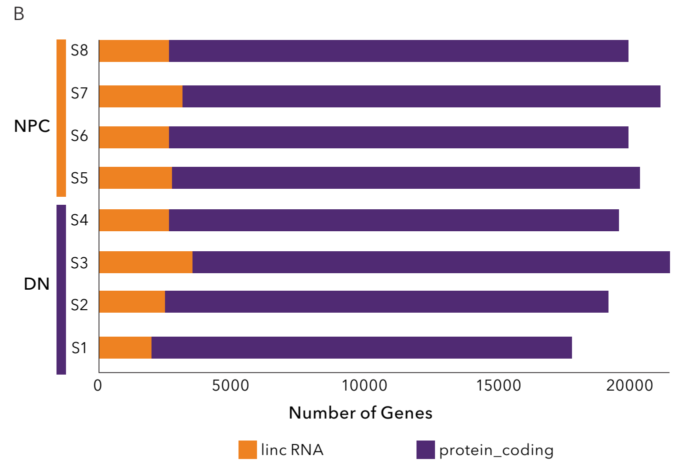


FIGURE 3 **(A)** Enrichment of brain related genes in differentiated neurons when compared to NPCs (Neuronal Progenitor Cells). **(B)** More in-depth analysis revealed enrichment of brain sub-compartments specific genes up in differentiated neurons when compared with NPCs.

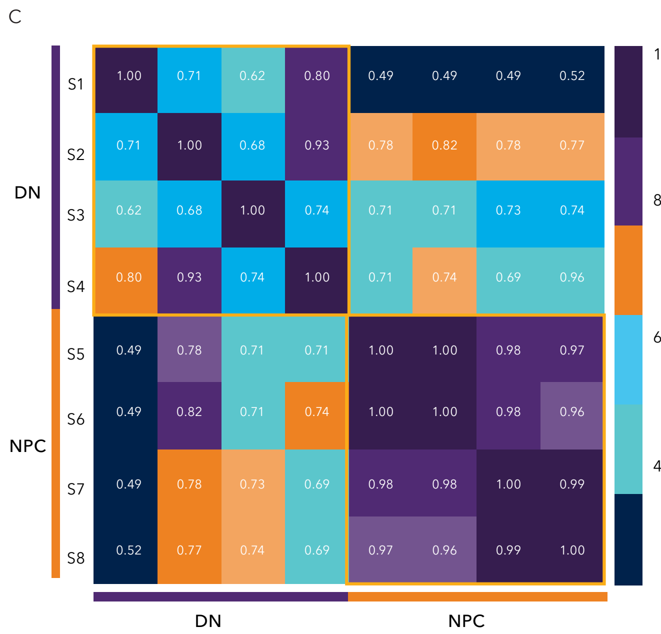
Mapping Statistics



Gene Detection



Pearson's Correlation of Gene Expression (Whole Transcriptome)



Pearson's Correlation of ERCC Spike-ins

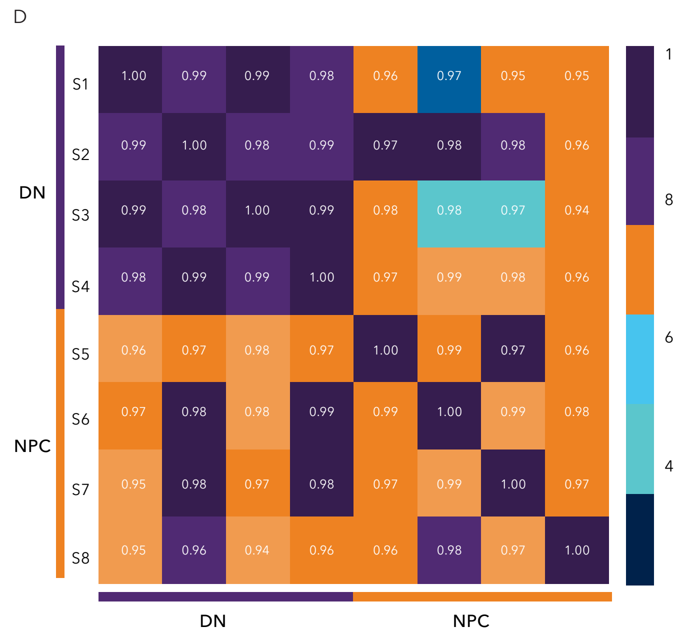


FIGURE 4 Reproducibility is indicated in exosomes isolated from neuronal progenitor (NPCs) and fully differentiated cells (DN). exoRNA isolated from the cells indicated were analyzed for **(A)** mapping statistics within the genome, **(B)** gene detection of protein-coding genes and long non-coding RNA. **(C)** Transcriptome-wide correlation of gene expression (Pearson's) is higher between biological replicates within each differentiation stage than between differential states of the same sample. **(D)** Pearson's correlation of ERCC spike-ins demonstrating reproducibility of the workflow.

Conclusions

Plasma, CSF, urine, and potentially other biofluids are all sample options that can be used to characterize multiple disease indications in more detail. FIGURE 5 indicates the power of a longitudinal sampling of cell culture media to determine the dynamic state of cell differentiation. The power of non-invasive or *ex vivo* monitoring technique as demonstrated in the data helps to develop biomarkers and companion diagnostics. Whether you are looking to stratify patient responses to a therapy, understand resistance to a therapy or understand the potential for segmentations/ stratifications of a patient population previously thought to be homogenous, then leveraging our exosome-based platform may be the perfect solution. The implications for exosomes in biomarker research are limitless. Exosome Diagnostics is uniquely suited with the patented technology, experience, and a pipeline already established to delivering custom projects in a timely manner.

Comparing: Neurodegenerative vs. Healthy Cells

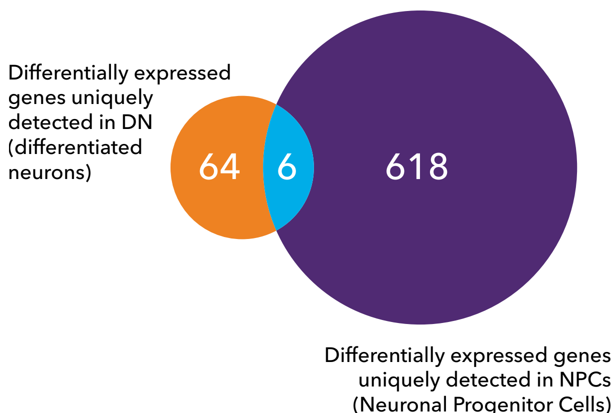


FIGURE 5 The gene expression profiles of exRNA isolated from differentiated neurons and progenitor cells (NPCs) indicate that when diseased vs healthy samples are compared, there is a total of 688 differentially expressed genes, 618 only seen in NPCs while 64 seen only differentially expressed in differentiated neurons while 6 are shared between NPCs and differentiated neurons.

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