Q&A ATCC® *Excellence in Research* Webinar "Neural Progenitor Cells – Potent Models of Normal and Disease Neurobiology"

General Questions

1. Will we be able to download the presentation?
   This presentation will be available to watch on demand on the ATCC website, or click here.

2. Does ATCC offer dopaminergic neuron, astrocyte, and oligodendrocyte differentiation media and protocols?
   ATCC is currently offering a dopaminergic differentiation media (ATCC® ACS-3004) with associated protocols for differentiating NPCs down dopaminergic neuron, astrocyte, and oligodendrocyte lineages. Optimized media for astrocyte and oligodendrocyte lineage differentiation may be available in the future.

3. Often neuronal cultures require the use of plates coated with a substrate such as poly-d-ornithine or poly-l-lysine. Does NPC culture require a gel coating or substrate?
   Yes. ATCC NPC protocols require that the culture dishes be coated with CellMatrix Basement Membrane Gel (ATCC® ACS-3035).

4. In the paper that you presented showing the tau mutant disrupts vesicle trafficking, did the authors show that the effect of the N279K mutant was neuronal rather than global (Wren MC, *et al.* Frontotemporal dementia-associated N279K tau mutant disrupts subcellular vesicle trafficking and induces cellular stress in iPSC-derived neural stem cells. *Mol Neurodegen* 10:46, DOI 10.1186/s13024-015-0042-7. PMID: 26373282)?
   Findings from this paper show accumulation of flotillin-1 and EEA1, and reduction of Lamp1, in the cytoplasm of differentiated neural cells with the N279K mutation compared to the wild type neural cells. The evidence that this phenotype was restricted to neurons came when Wren and colleagues monitored flotillin-1, EEA1, and Lamp1 expression in normal fibroblasts compared to fibroblasts from the N279K patients. They observed no difference in the expression of those proteins in the normal and mutant fibroblasts, which enabled them to conclude that the effects were isolated to a neuronal phenotype. Similarly, if a researcher using ATCC NPCs wanted to test whether or not a phenotype was neuronal or global, they could run the same experiment in comparison to the ATCC control iPSC lines BYS0112 (ATCC® ACS-1026™) or BXS0117 (ATCC® ACS-1031™).

5. What is the recommended seeding density for NPCs?
   For optimal cell recovery, it is critical to seed NPCs at high seeding densities for post-thaw and for subculturing. We recommend that you seed NPCs at a density of 80,000 cells/cm^2 post-thaw and 40,000 cells/cm^2 for subculture. In addition, optimal seeding densities are also critical for NPC differentiation. Please follow the suggested seeding densities in the ATCC protocols for tri-lineage differentiation.

6. Is there an effect of passage number on neural differentiation?
   We have not seen any differences in passage number on neural differentiation. Early and late passage NPC cells have similar differentiation potential.

7. Is there a difference between neural stem cells (NSCs) and NPCs?
   Both NSCs and NPCs are multipotent cells that can be differentiated into neurons, astrocytes, and oligodendrocytes. However, there is a difference in proliferative capacity of NSCs and NPCs. NSCs have the ability to self-renew and proliferate without limit, while NPCs exhibit limited proliferative capacity. The non-stem cell progeny of NSCs are referred to as NPCs.

8. Have you differentiated NPCs to GABAergic or glutamatergic neurons?
   We have not attempted differentiating NPCs into either GABAergic or glutamatergic lineages yet. It is highly recommended to search for optimal and reliable protocols in the literature for differentiation of NPCs into GABAergic or glutamatergic neurons.

9. What is ATCC's source of antibodies against nestin, Pax-6, and tyrosine hydroxylase (TH)?
   ATCC uses EMD-Millipore antibodies against nestin, Pax-6, and TH. Detailed protocols for NPC expansion and dopaminergic neuron differentiation, as well as the sources of these antibodies, will be available on the ATCC NPC product webpages.