

life lab

PRODUCTS, INFORMATION, AND SCIENTAINMENT
ISSUE 20 | SPRING 2017

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AN INTERVIEW WITH DR. DAVID KUNINGER

Senior Manager, R&D (Cell Biology)
Thermo Fisher Scientific

Neurobiology research is benefiting from the enormous advances in stem cell biology

The advent of induced pluripotent stem cell (iPSC) technology has enabled investigators to develop and explore areas of human biology previously limited by a number of reasons, including lack of available tissue or appropriate cell lines. iPSCs can now be routinely derived from donor tissue (typically a skin biopsy or small amount of blood), expanded to very large numbers, and then used to create discrete cell types through specific differentiation protocols. Because so much of the value of pluripotent stem cells is derived from their ability to produce valuable downstream cell types, much effort has been applied to improving the efficiency and specificity of these differentiation protocols. The ability to create functional human neurons (and associated neural cell types) from iPSCs has fundamentally expanded how scientists approach questions in neurobiology and opens the way for therapeutic applications of stem cell-derived neurons.

ARE THERE ANY PARTICULAR AREAS OF NEUROSCIENCE YOU ARE EXCITED ABOUT?

“There is so much interesting and important work going on these days. One aspect that stands out is the ability to use stem cell-derived neurons for disease modeling, which typically starts with iPSCs containing a specific disease-associated mutation, and differentiating these cells to specific types of neurons. Companion, normal, or healthy iPSCs are included as controls and together this can create powerful models for understanding the mechanisms of disease and for use in drug discovery and screening. Neurological diseases with a clear genetic component, such as ALS (amyotrophic lateral sclerosis) and Parkinson’s disease, are particularly well suited for these types of studies.”

TO THAT END, WE RECENTLY RELEASED A NEW STEM CELL DIFFERENTIATION KIT, WHICH ENABLES THE CREATION OF DOPAMINERGIC NEURONS FROM HUMAN iPSCS. WHAT’S SPECIAL ABOUT THIS?

“The product we developed is designed to recreate the very specific population of dopamine-producing neurons that degenerate in individuals with Parkinson’s disease. These neurons are derived from unique set of progenitor cells found in the midbrain, floor plate region of the human brain. Our kit faithfully reconstitutes both the formation of these progenitors and their maturation to dopamine-producing neurons. One significant benefit we identified during development was conditions that enable the expansion and cryopreservation of these midbrain, floor plate progenitors,

thus enabling researchers to readily scale up experiments and pause a lengthy differentiation workflow (through cryopreservation) if desired.”

WHAT ARE LIMITATIONS TO USING STEM CELL-DERIVED NEURONS?

“One trait that’s common with stem cell-derived cell types, neurons included, is that they are much more similar to the equivalent fetal human cells. Or put another way, they aren’t representative of cells in adult humans and as such, they don’t always represent ideal models for studying human physiology and pathophysiology. This is a clear gap.

“Another practical limitation is the intrinsic variability in the efficiency of differentiation when pushing an iPSC to become a cell type of interest. For example, protocols to create iPSC-derived neurons often lead to mixed populations of cells; this can include proliferating progenitors and postmitotic neurons, and over time the cultures on neurons can be overgrown by progenitors.

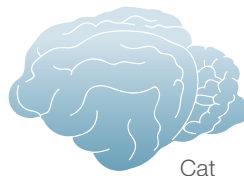
“We often encountered this in our internal work and are excited to have just launched a new product, Gibco™ CultureOne™ Supplement, designed to specifically suppress the outgrowth of neural stem cells, a common multipotent cell type that is an intermediate in many iPSC neural differentiation protocols. An added benefit, at least with the stem cell-derived neurons we’ve looked at so far, is that treatment with the CultureOne reagent also improves electrophysiological responses or activity of stem cell-derived neurons.”

thermofisher.com/stemcells

JUST FOR



Sheep



Cat



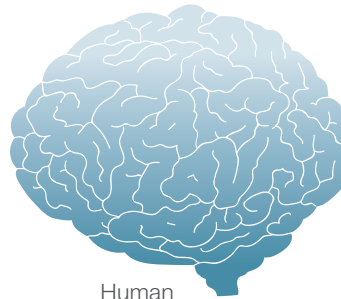
Rabbit



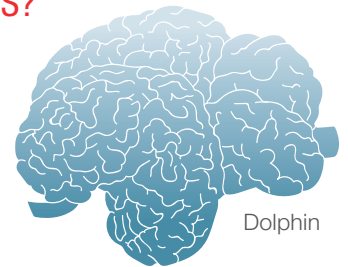
Rat



Chimpanzee



Human



Dolphin

—
1 cm

Rat

Rabbit

Cat

Sheep

Chimpanzee

Human

Dolphin

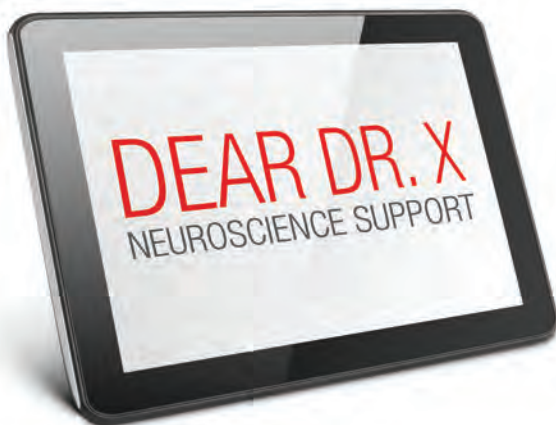
EVER WONDERED HOW OUR BRAIN COMPARES TO OUR OTHER MAMMALIAN FRIENDS?

HOW'S THAT CUPPA JOE WORKING FOR YOU?

We all need something to get us through the workday. Deadlines are looming, and the most popular boost to help us along is caffeine, with 85% of the US population consuming it every day in the form of coffee or other beverages. Coffee's effectiveness as high-performance brain fuel makes it our work best friend. What most people don't know is that coffee's primary active ingredient, caffeine, is the most commonly used psychoactive drug. We all love it too, so don't feel bad.

The caffeine in coffee acts as a mild stimulant to the central nervous system. Studies have shown that, depending on level of intake, caffeine can help to improve mental performance, alertness, attention, and concentration. When you have a paper due or a grant proposal awaiting, it helps get you to the finish line.

There is also currently research into the effectiveness of caffeine in reducing neurodegenerative disorders such as Alzheimer's disease and Parkinson's disease. There are multiple studies available on coffee and neurodegenerative disorders, so next time you have a minute, brew your favorite blend and take a look. It's fascinating to think our favorite warm, comforting drink has medicinal qualities as well as being delicious.



Having difficulties with your experiment? We are dedicated to your success. Get back on track with our expert recommendations for commonly encountered problem scenarios.



After I labeled neurons with Invitrogen™ MitoTracker™ Red CMXRos dye, they were dead the next day. Is this expected?

The MitoTracker dyes should be imaged soon after staining because over time those dyes can be toxic.



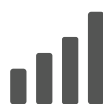
I keep getting low transduction efficiency when using Invitrogen™ CellLight™ labeling reagents on my neurons. What can I do to improve the efficiency?

Neurons are more difficult to transduce than many other cells. The main way to improve transduction is to label with a higher number of particles per cell. For primary neurons, it can also help to transduce them at the time of plating rather than on established cultures. There can also be a slower onset of expression in neurons and peak expression often occurs on day 2 or 3 rather than 16 hours after transduction.



I have labeled my neurons with an Invitrogen™ Alexa Fluor™ conjugated biocytin to look at transport, but I wanted to examine only retrograde transport and biocytin appears to be moving retrograde and anterograde. What should I do?

Observing both types of transport is typical for biocytin. The conjugated cholera toxin subunit B products have been observed to travel only retrogradely.



I labeled my neurons with Invitrogen™ Dil stain and then fixed and permeabilized, and now I have no signal. What did I do wrong?

Dil is a lipophilic dye that resides mostly in lipids in the cell; when cells are permeabilized with detergent or fixed using alcohol, this strips away the lipid and the dye. If permeabilization is required, Invitrogen™ Vybrant™ CM-Dil cell labeling solution can be used because this binds covalently to proteins in the membrane. Some signal is lost upon fixation/permeabilization, but enough signal should be retained to make detection possible.



I stained my cells with a lipophilic cyanine dye, like Dil, but the signal was lost when I tried to follow up with antibody labeling. Why?

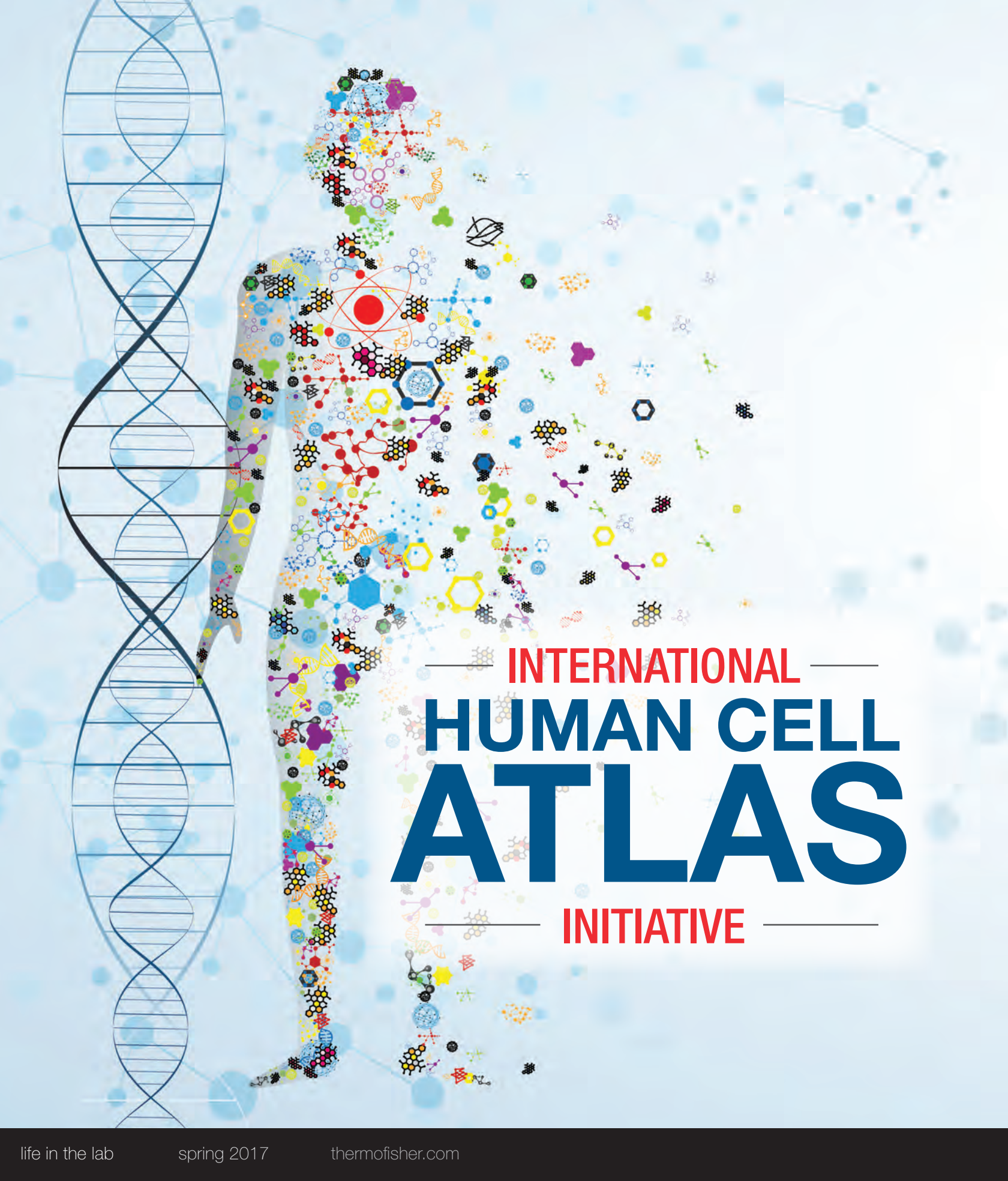
Since these dyes insert into lipid membranes, any disruption of the membranes leads to loss of the dye. This includes permeabilization with detergents like Triton™ X-100 detergent solution or organic solvents like methanol. Permeabilization is necessary for intracellular antibody labeling, leading to loss of the dye. Instead, a reactive dye such as CFDA SE should be used to allow for covalent attachment to cellular components, thus providing better retention upon fixation and permeabilization.



I used one of your Invitrogen™ FluoroMyelin™ stains and noticed it stains cells other than glial cells. Is there something wrong with the product?

FluoroMyelin stain is a lipid stain. Therefore, any lipid can be stained by it, but there is a higher lipid content in myelin so it will stain much more intensely than other membranes.

To find out more, go to thermofisher.com/neurosupport



INTERNATIONAL
**HUMAN CELL
ATLAS**
INITIATIVE

An ambitious global initiative to create a Human Cell Atlas—a description of every cell in the human body as a reference map to accelerate progress in biomedical science—was discussed at an international meeting in London on October 13–14, 2016. Ultimately, the Human Cell Atlas would revolutionize how doctors and researchers understand, diagnose, and treat disease.

The first project of its kind, and as ambitious in scope as the Human Genome Project—which cataloged the first full human DNA sequence—the Human Cell Atlas aims to chart the types and properties of all human cells, across all tissues and organs, to build a reference map of the healthy human body.

The meeting, convened by the Broad Institute of MIT and Harvard, Wellcome Trust Sanger Institute, and Wellcome Trust, brings international experts together to decide on the elements of the first phase of the Human Cell Atlas initiative.

By making the Atlas freely available to scientists all over the world, scientists hope to transform research into our understanding of human development and the progression of diseases such as asthma, Alzheimer’s disease, and cancer. In the future, the reference map could also point the way to new diagnostic tools and treatments.

The human body is made of trillions of cells—the fundamental units of life—which divide, grow, and acquire distinct functions in the embryo, eventually leading to different cell types (such as skin cells, neurons, or fat cells) that form the various tissues of the body. These tissues come together to form organs such as the lungs and the brain.

Previous knowledge of cells has come from looking at them under a microscope, or more recently by analyzing clumps of hundreds or thousands of cells and finding the average properties. However, to see the true picture for every cell type, it is necessary to first separate the cells and then find out what molecules are produced in each. These molecules include sets of RNA messages, called the transcriptome, which help give each cell its own identity and distinguish it from the many other cell types found in the body.

A few years ago, measuring this complex and extensive information would have been impossible, but recent technological advances in the field of single-cell genomics can separate individual cells from different tissues and organs, and measure the transcriptome or other important molecules from each of them.

“The cell is the key to understanding the biology of health and disease, but we are currently limited in our understanding of how cells differ across each organ, or even how many cell types there are in the body. The Human Cell Atlas initiative is the beginning of a new era of cellular understanding as we will discover new cell types, find how cells change across time during development and disease, and gain a better understanding of biology.”

Dr. Sarah Teichmann, FMedSci, Head of Cellular Genetics at the Wellcome Trust Sanger Institute

“We believe that a successful description of all the cells in the healthy human body will impact almost every aspect of biology and medicine in the decades to come. We now have the tools to understand what we are composed of, which allows us to learn how our bodies work and to uncover how all these elements malfunction in disease. By creating this atlas through an open, international effort, we are building a new research tool for the whole community.”

Dr. Aviv Regev, Chair of the Faculty at the Broad Institute

“Since sequencing the human genome, there have been some incredible advances in the field of genetics, but we still know surprisingly little about our individual cells and how they can vary across organs and body systems. We now have the technological capabilities, and the ability to carry out science at an unprecedented global scale, to bring an atlas of every human cell type within our reach. The knowledge we gain has the potential to transform our understanding of the human body and some of the most serious diseases of our time.”

Dr. Michael Dunn, Head of Genetics and Molecular Sciences at Wellcome

Key supporters of this project include:

Dr. Cori Bargmann from the Chan Zuckerberg Initiative; Prof. Sten Linnarsson from the Karolinska Institute, Sweden; Dr. Piero Carninci and Dr. Jay W. Shin from the RIKEN Center for Life Science Technologies, Japan; Prof. Michael Stratton and Dr. Peter Campbell, Wellcome Trust Sanger Institute; Prof. Alexander van Oudenaarden and Prof. Hans Clevers, Hubrecht Institute, Netherlands; Prof. Eric Lander, Broad Institute of MIT and Harvard; Prof. Jonathan Weissman, University of California-San Francisco (UCSF) and Howard Hughes Medical Institute; Prof. Arnold Kriegstein, UCSF; Dr. Nir Hacohen, Massachusetts General Hospital; Prof. Gary Nolan, Stanford School of Medicine; Prof. Euhd Shapiro and Dr. Ido Amit, Weizmann Institute of Science, Israel; Prof. Dana Pe’er, Sloan Kettering Institute; Prof. Chris Ponting, University of Edinburgh and the Sanger Institute; and Prof. Steve Quake, Stanford University and the Chan Zuckerberg biohub.

News article by The Sanger Institute, October 14, 2016



NEUROSCIENCE AND NONCODING RNA ANALYSIS

MagMAX *mirVana* Total RNA Isolation Kit

About 80% of the mammalian genome gives rise to noncoding RNA (ncRNA) (Kellis et al., 2013). MicroRNA (miRNA) are small noncoding RNA about 21–25 nucleotides long. Long ncRNA (lncRNA) up to 100,000 nucleotides long have been observed. What role do these RNA play in biology/disease?

Despite the complexity, early research has revealed that ncRNA may function as regulators of protein coding genes, other RNA, and key mammalian developmental pathways. Of interest to neuroscientists, dynamic spatial and temporal expression patterns of ncRNA (Moreau, et al. 2013, Dong et al., 2015) during brain development suggest ncRNA play vital roles in the early nervous system development. Multiple lines of research suggest that neurodevelopmental disorders, such as Prader-Willi syndrome, Angleman syndrome, and Fragile X syndrome, may arise from dysregulation of mechanisms under the control of ncRNA (Esteller 2011).

How will you start your ncRNA studies? Not all sample preparation kits can recover small and large ncRNA, so specialized technologies to purify ncRNA across the size range is critical to success. The Applied Biosystems™ MagMAX™ *mirVana*™ Total RNA Isolation Kit is phenol-free and automation-ready, allowing neuroscientists to effortlessly capture all the RNA from a variety of sample types. The kit uses magnetic bead technology to recover high-quality RNA and can be performed manually or using a magnetic particle handler to automate sample preparation.

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NANODROP ONE INSTRUMENT ENABLES ALS RESEARCH

Hear from Alyssa Grantham on how the Thermo Scientific™ NanoDrop™ One instrument supports research on amyotrophic lateral sclerosis (ALS) and nociception.

nanodrop.com/news.aspx



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EVERYDAY HERO Quyên Hoang, PhD

Associate Professor,
Indiana University School of Medicine and
Stark Neurosciences Research Institute,
Indianapolis, IN, USA

Research area: Structural biology of neurodegenerative disease and structure-based drug design, with a focus on understanding the mechanism of Parkinson's disease by studying the structure and function of disease-associated proteins.

The challenge: LRRK2, a high molecular weight protein consisting of several domains, is the central focus of Professor Hoang's research. A significant proportion of patients afflicted with Parkinson's disease have mutations within LRRK2, which explains why many pharmaceutical companies are interested in identifying compounds that have the ability to alter its activity. Professor Hoang took a novel approach: rather than identify compounds that bind and inhibit the kinase domain of LRRK2—like many pharmaceutical companies have been attempting to do—his method of inhibiting kinase activity relies on identifying compounds that bind the neighboring GTPase domain,

which regulates the activity of the protein's kinase domain. To accomplish his research objectives, his laboratory employs a variety of structural biology techniques (e.g., X-ray crystallography, CD spectroscopy), which require ample amounts of protein. Prior to the availability of the Gibco™ Expi293™ Expression System, the best option for expressing LRRK2 was by using HEK293T cells. Unfortunately, the HEK293T system exhibits significantly lower relative performance, which makes it difficult for researchers like Professor Hoang to perform all of their structural studies.

How he tackled it: The Expi293 Expression System enabled Professor Hoang to generate 10x more protein than his laboratory is accustomed to getting with HEK293T. "As an X-ray crystallographer, we need a lot of protein; so we look for other systems that can express a higher level of protein so we can measure activity as well as determine the structure."

The happy result: "We get significantly more protein and it's equally pure," says Professor Hoang. The advantage of using the Expi293 Expression System is clear: 10x more protein translates to significant cost and time savings. For example, using HEK293T cells, he obtained 12.5 μ g of LRRK2 at a cost of \$154 USD. However, the Expi293 Expression System enabled him to obtain 135 μ g at a cost of \$217 USD, saving his lab \$10.71 USD per μ g.



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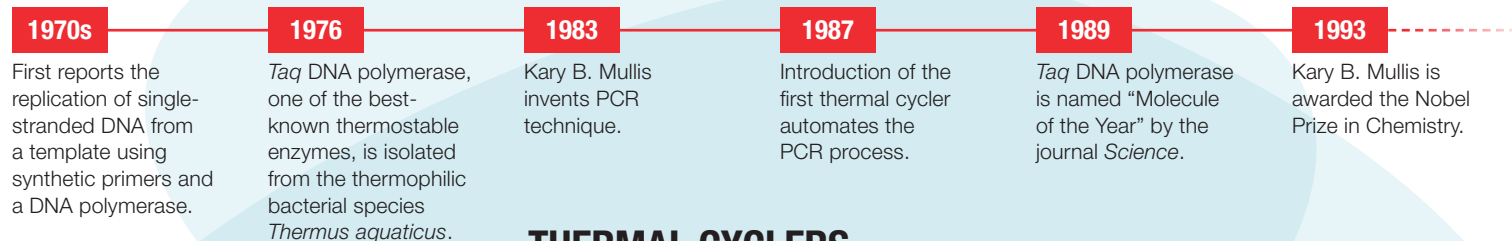


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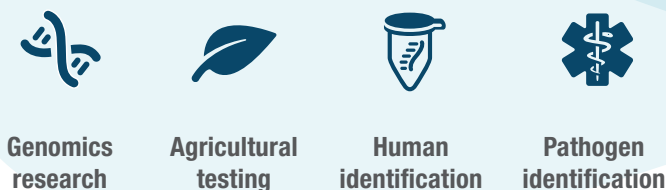
THE HISTORY OF PCR (polymerase chain reaction)



THERMAL CYCLERS (current Applied Biosystems™ technology)



APPLICATIONS (research-based)



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3 Vandenberg, et al., used the Veriti 96-well Fast Thermal Cycler for their research, "Novel Insights into the Multiple Sclerosis Risk Gene *ANKRD55*."

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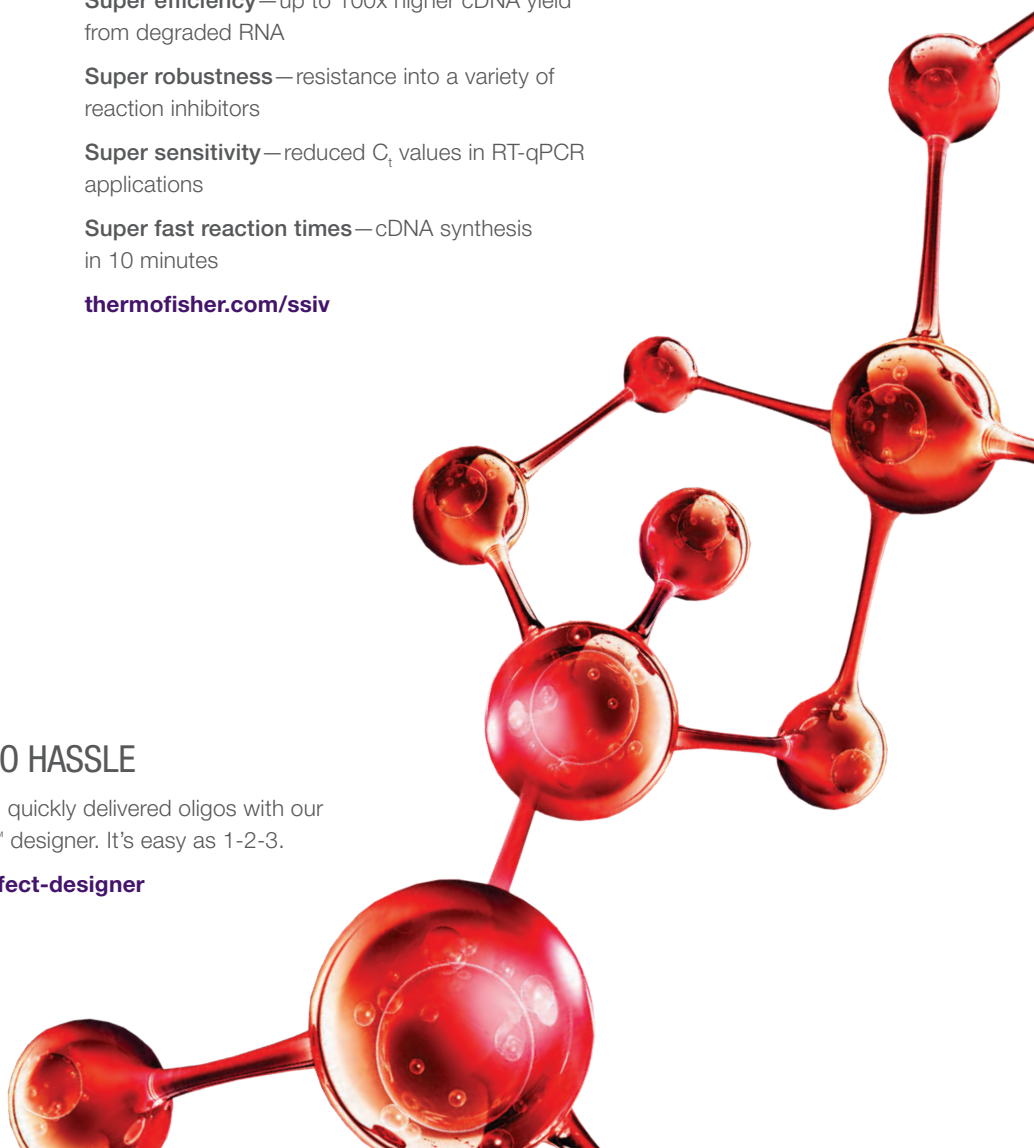
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IT'S IN THE BLOOD

A test for Alzheimer's disease

Dr. Lesley Cheng

Lesley's groundbreaking research is a milestone for not only degenerative diseases but health conditions of all types. It's a step towards personalized medicine. Get a glimpse of the future.

DETECTING ALZHEIMER'S DISEASE BIOMARKERS WITH NEXT-GENERATION SEQUENCING

A hallmark sign of Alzheimer's disease is the presence of amyloid plaques, which collect in the brain and destroy neurons. Even after this neurodegeneration sets in, however, Alzheimer's disease-induced dementia does not manifest for another decade. By the time cognitive deficits are first detected, it is often too late to treat the disease with any significant impact.

Alzheimer's disease is the most common cause of dementia, and as the world's population ages, the disease will only become more prevalent. Despite its pervasiveness, there are currently no objective diagnostic tests for Alzheimer's disease; rather, doctors primarily rely on cognitive testing and family history to diagnose it. But, to prevent Alzheimer's disease from overburdening patients and families in the future, scientists must develop objective methods to detect and treat Alzheimer's disease before the cognitive deficits set in.

Dr. Lesley Cheng, a postdoctoral scholar in Prof. Andrew Hill's laboratory at La Trobe University in Melbourne, Australia, is developing a novel blood test for detecting Alzheimer's disease as soon as amyloid plaques first appear in the brain. Her work began with discovering 16 biomarkers that are strongly associated with Alzheimer's disease and can be differentiated from healthy genetic variations. Then, with the help of next-generation sequencing (NGS) technology from Thermo Fisher Scientific, Dr. Cheng developed

a blood test that collects circulating exosomes, so she could sequence the microRNAs (miRNAs) contained within and detect those that express Alzheimer's disease markers.

Dr. Cheng gave an inspirational talk at TEDx Melbourne about her breakthrough work, where she described how this NGS technology is enabling her lab to develop an early detection test.

As pioneers and world leaders in biomarker detection via exosome analysis, the Hill Lab quickly realized the utility of the Ion Personal Genome Machine™ (PGM™) System for NGS to advance their research. As such, in mid-2011, they were one of the first labs in Australia to purchase the Ion PGM System, and since then, Dr. Cheng has been using this system to refine her early detection method for Alzheimer's disease. She uses the PGM system for deep sequencing, and she has devised methods to sequence nucleic acids from small-volume samples.

Using the PGM System, Dr. Cheng and her lab have become leaders in using exosomes to detect Alzheimer's disease, as well as other neurodegenerative disorders such as Parkinson's disease. A year ago, the lab expanded their throughput capabilities by purchasing the Ion Chef™ and Ion S5™ Systems. "Having our own Ion Chef and Ion S5 instruments in the lab means we can sequence when we want, no different than with any other essential instrument of the lab," says Dr. Cheng.

In future studies, Dr. Cheng will use Applied Biosystems™ TaqMan® miRNA Assays, Invitrogen™ *mirVana*™ miRNA Mimics, and CRISPR-Cas9 technology to validate her miRNA candidates in cell cultural models. The Ion S5 System will continue to play a critical role in those studies, enabling Dr. Cheng to examine the consequences of disease-associated miRNA knockdowns and identify potential therapeutic targets.

Today, Dr. Hill's group continues to push the boundaries of discovery and implementation using these sequencing systems. With NGS technology, Dr. Cheng has been able to produce libraries from small miRNA yields extracted from exosomes or small serum samples, and this capability, she says, has attracted collaborators from all over Australia.

As she has collated more miRNA panels for specific diseases, she has discovered discrete biomarkers for each one. Not only does this provide novel routes to diagnosis, but provides her lab with the ability to dissect specific pathological pathways for each disease. In the future, systems from Thermo Fisher Scientific can be used to quickly sequence exosomal miRNA sequences to diagnose not only neurodegenerative disease, but also any other disorder with defined miRNA biomarkers.

TEDxMelbourne
TEDx Talks
 Published on August 30, 2016

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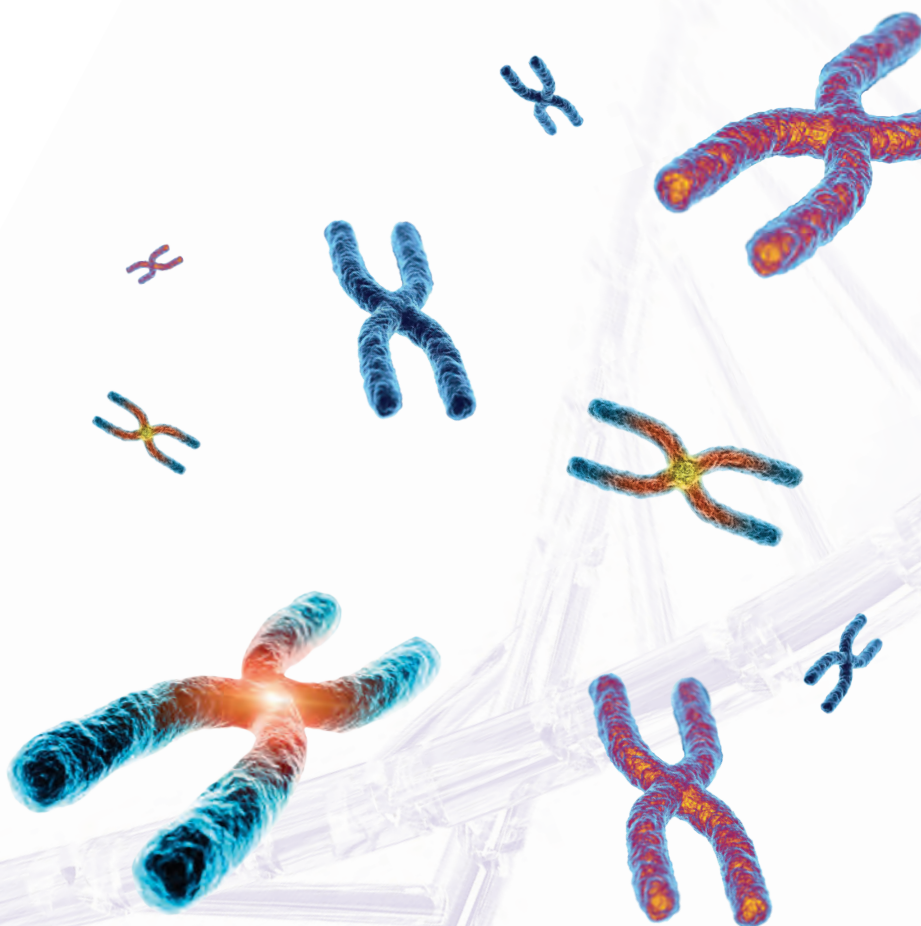
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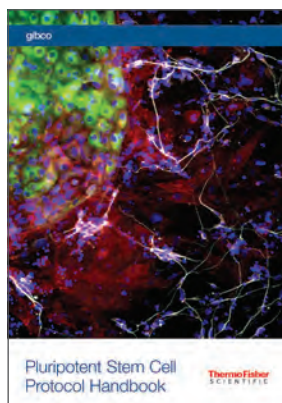
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SUCCESSFUL NEUROBIOLOGY EXPERIMENTS START HERE

Over 20 protocols from our experienced scientists are available in our updated Gibco™ Neurobiology Protocol Handbook.

thermofisher.com/neurohandbook



NEUROBIOLOGY RESEARCH MADE SIMPLE

STEP 2

Modify

- Transfection
- Reprogramming

Transfecting primary neurons or reprogramming stem cells can be challenging due to low efficiencies. Low efficiencies result in additional work downstream. Improve your outcomes with the right reagents and protocols for your cells.

thermofisher.com/transfection

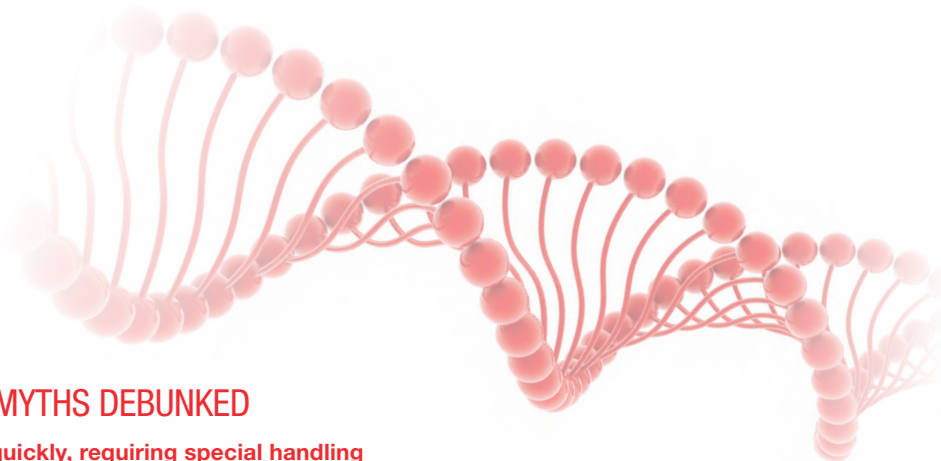
TRANSFECTION

Invitrogen™ Lipofectamine™ MessengerMAX™ Transfection Reagent

- Fast protein expression without the risk of genomic integration
- Optimized for transfection of neurons and primary cells
- Impressive performance: 60%+ transfection efficiency in neurons and primary cells
- Up to 10x higher cleavage efficiency using mRNA CRISPRs

thermofisher.com/transfection





COMMON mRNA TRANSFECTION MYTHS DEBUNKED

Myth 1: mRNA is unstable and degrades quickly, requiring special handling

- A few simple precautions can help ensure mRNA stability: use RNase-free reagents and tips, aliquot and store mRNA at -80°C , and keep mRNA on ice when in use

Myth 2: mRNA is difficult to prepare

- The Invitrogen™ mMESSAGING mMACHINE™ T7 Ultra Transcription Kit provides 20–40 μg of ready-to-use mRNA
- Once prepared, the mRNA can be safely stored for later use in multiple transfection experiments

Myth 3: Transfection is much easier to perform with DNA than mRNA

- The protocol for Lipofectamine MessengerMAX reagent is straightforward, requiring the same basic steps as protocols for DNA transfection reagents, and we offer a positive control GFP mRNA that can be used to evaluate the system
- For difficult-to-transfect nondividing or primary cells, mRNA transfection obviates the need for nuclear entry and improves protein expression and homogeneity

Myth 4: Exogenous mRNA elicits an immune response

- Chemically modified mRNAs, with 5-methylcytidine and pseudouridine modifications, dramatically reduce innate immune response and improve mRNA translation



REPROGRAMMING AND DIFFERENTIATION

Human neural cells can be accessed through the reprogramming of easily obtained fibroblasts into stem cells. These can then be differentiated into neural cells for further analysis. Increase your cell yield by at least 2-fold by combining high-efficiency reprogramming kits with optimized differentiation reagents.

- Invitrogen™ CytoTune™-iPS 2.0 Sendai Reprogramming Kit (Cat. No. A16518)
- Gibco™ PSC Neural Induction Medium (Cat. No. A1647801)
- Gibco™ PSC Dopaminergic Neuron Differentiation Kit (Cat. No. A3147701)

thermofisher.com/reprogramming

NEUROBIOLOGY TRANSFECTION GUIDE

The field of neurobiology is rapidly growing, with researchers providing new insights into a variety of areas essential for human health, including memory, mood disorders, aging, and disease. As the field moves toward more physiologically relevant models such as primary neurons and small animal models, the need for new nucleic acid delivery tools is rapidly increasing. Use our helpful Neurobiology Transfection Guide to assist you in this effort.

thermofisher.com/transfection

NEUROBIOLOGY RESEARCH MADE SIMPLE

STEP 3

Analyze

- Imaging
- Western blotting
- ELISA

We offer a diverse collection of reagents and kits for neuroscience research, including fluorescent probes for dissecting neural anatomy and function, neuronal tracers, and a vast selection of neurobiology-related primary antibodies and kits.

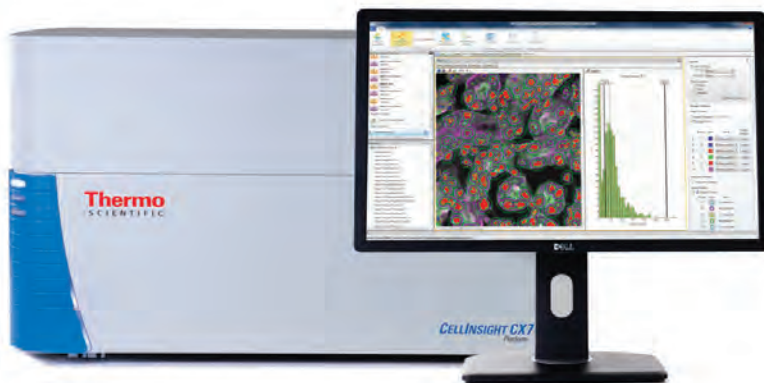
thermofisher.com/neuro

GET THE MOST FROM YOUR PRECIOUS SAMPLES

Thermo Scientific™ CellInsight™ CX7
High-Content Analysis (HCA) Platform

Acquire and analyze images simultaneously with this instrument's widefield, brightfield or confocal capability.

thermofisher.com/CX7



DITCH THE CLICKER

Invitrogen™ Countess™ II FL Automated Cell Counter

Count cells, monitor fluorescent protein expression, and measure cell viability.

thermofisher.com/countess



Antibody-based tools for neurodegenerative disease research handbook

Get access to detailed protocols for a wide range of experimental techniques, including western blotting, immunofluorescence, flow cytometry, ELISA, and more.

thermofisher.com/neurodegenerativehandbook

Antibodies for all applications

Over 74,000 primary and secondary antibodies ready to pair up.

thermofisher.com/antibodies

Popular ELISA kit products

- Invitrogen™ Amyloid beta 40 ELISA Kit, Human (Cat. No. KHB3482)
- Invitrogen™ Amyloid beta 42 ELISA Kit, Human (Cat. No. KHB3442)
- Invitrogen™ Tau ELISA Kit, Human (Cat. No. KHB0041)

thermofisher.com/elisa

Invitrogen™ fluorescent reagents

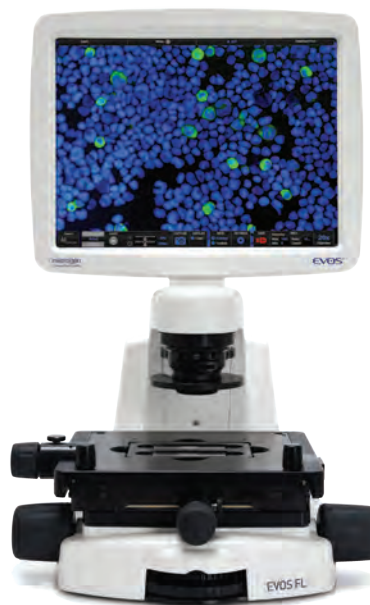
Trusted products with a legacy bibliography.

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Western blotting systems

Complete solutions for the western blotting workflow.

thermofisher.com/western





REVEALING SYNAPTOGENESIS WITH HIGH-CONTENT ANALYSIS

Synapse studies are key in many neuroscience applications. Using high-content analysis (HCA) methods, you can automatically determine the presence of synapses by measuring the colocalization of a presynaptic marker with a postsynaptic marker, and correlating with neuronal and neurite morphology.

THERMO SCIENTIFIC™ CELLINSIGHT™ CX7 NEURO APPLICATION

Synapses allow neurons to communicate with each other via the release of neurotransmitters that open ion channels or activate second messenger systems. The molecular network between synapses controls signal transmission and plasticity to regulate neuronal growth, differentiation, and death. The modulation of neurite and synaptic structures are closely studied as they relate to the pathological process of neurological diseases or neurotoxicity, or in neurodevelopment.

thermofisher.com/hcs-apps

KEY REAGENTS FOR NEURONAL CELL IMAGING

Cell imaging reagents for neuroscience target key neuronal cell processes. Products include a Neurite Outgrowth Assay, Synaptogenesis Assay CellRox Assay for reactive oxygen species studies, Live/Dead Viability Assay, and more.

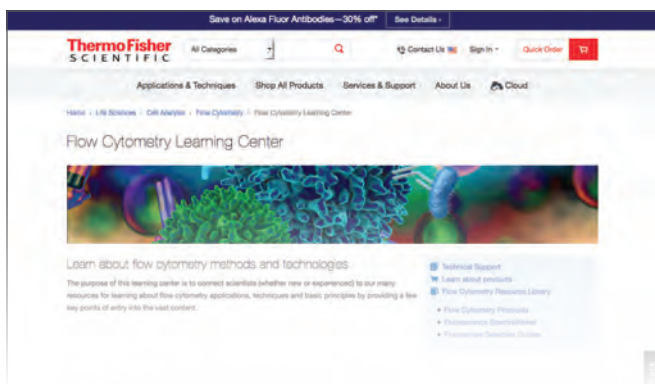
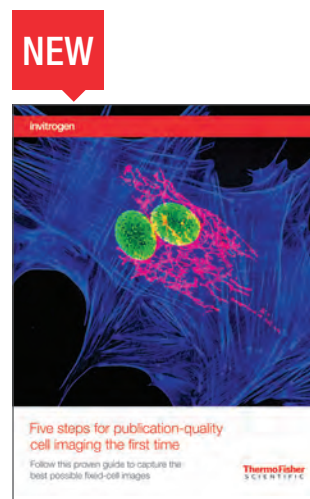
See the complete list in our tools for neuroscience research brochure at thermofisher.com/neuro



5 STEPS FOR PUBLICATION-QUALITY IMAGES

Our “Five Steps for Publication-Quality Cell Imaging” website and guide detail the steps, reagents, and imaging platforms necessary to capture the best-quality images and results. The easy-to-follow guide also offers best practices and solutions for fixed cell imaging.

thermofisher.com/5-steps



LEARNING CENTERS OFFER RESOURCES FOR BOTH NEW AND EXPERIENCED SCIENTISTS

Scientists of all experience levels will gain valuable insights and in-depth information by accessing our many resources for learning about cell analysis and flow cytometry applications, techniques, and basic principles. These portals provide key entry points into the vast content.

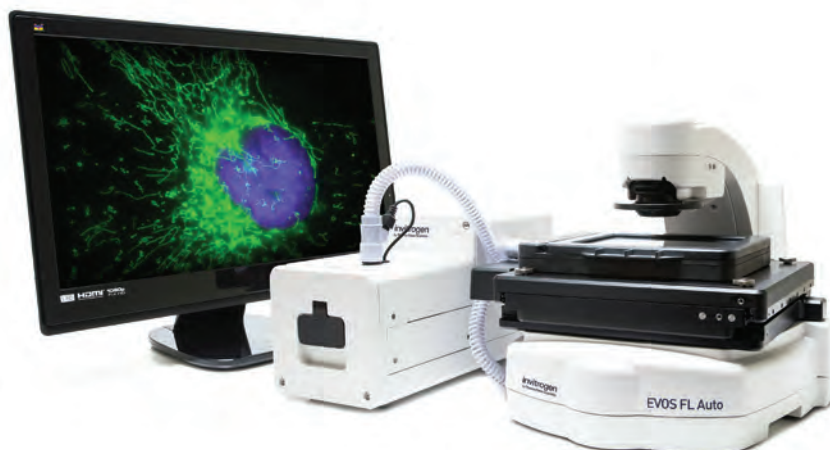
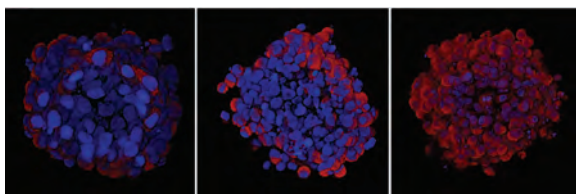
thermofisher.com/flowlearning

HOW TO AVOID HYPOXIC CONDITIONS

Autophoxia application

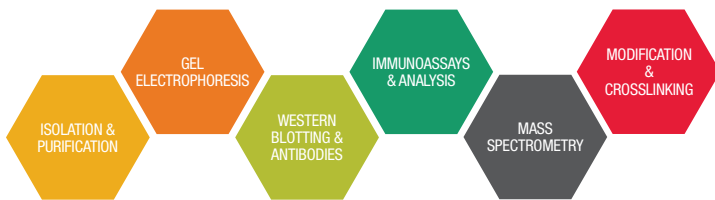
Reduced oxygen—hypoxic conditions—can have a significant impact on neuronal cell growth and function. The Invitrogen™ EVOS™ FL Auto Cell Imaging System with Onstage Incubator provides the controlled environmental conditions and high-quality imaging that is needed to study the effects on neuronal, tumor, and many other cell types under adverse conditions.

thermofisher.com/evos



NEURO RESEARCHERS: **QUANTIFY** AND DETECT PROTEINS WITH CONFIDENCE

Wherever you are in your protein biology workflow, we've got the right products and educational resources to help you on your way.



Characterize your neuronal cell population by accurately quantifying or imaging intracellular proteins using protein analysis tools designed for single or multiplex protein biomarker analysis.

thermofisher.com/proteinbiology

NEUROBIOLOGY IMMUNOASSAYS

For Alzheimer's disease and Parkinson's disease research

Invitrogen™ neuro ELISA and neuro 3-plex Luminex® kits are designed to deliver accurate, sensitive, and fast quantitative determination in sample types such as cerebrospinal fluid (CSF), cell culture supernatants, primary neurons, and brain tissue samples.

Targets include:

- Aβ 40/42 (amyloid beta)
- Tau (total and phospho-specific)
- α-synuclein

thermofisher.com/elisa

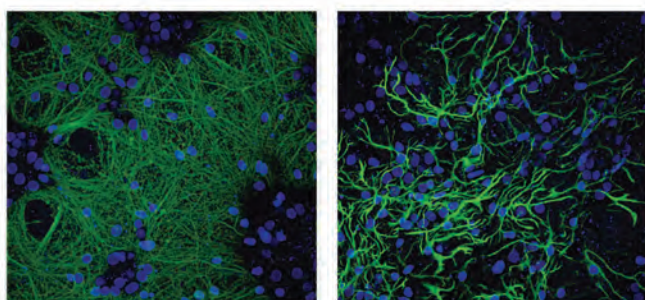


GET HIGHER SIGNAL-TO-NOISE RATIO**NEW****Alexa Fluor Plus secondary antibodies**

Proven to provide up to 4x greater sensitivity, the unique, proprietary chemistry of new Invitrogen™ Alexa Fluor™ Plus secondary antibodies is designed to help you detect and visualize low-abundance targets.

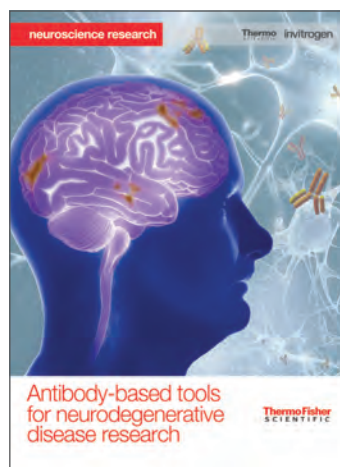
Alexa Fluor Plus secondary antibody enables brighter images with a higher level of detail.

To see the visible difference, go to thermofisher.com/alexafluorplus



**Alexa Fluor 488
secondary antibody**

**Alexa Fluor Plus 488
secondary antibody**



ANTIBODY-BASED TOOLS FOR NEURODEGENERATIVE DISEASE RESEARCH

This 68-page handbook includes a brief overview of the current state of neurodegenerative disease research, with an emphasis on evaluating protein biomarkers. Download this free handbook for access to protocols covering a wide range of techniques, including western blotting, immunofluorescence analysis, flow cytometry, ELISA, and more.

thermofisher.com/neuroantibody-hb

EVERYDAY HERO Dr. Gerold Schmitt-Ulms, PhD

**Associate Professor,
Tanz Centre for Research in Neurodegenerative Diseases,
University of Toronto, Ontario, Canada**

Area of research: Our work contributes to two strands of investigations at the interface of neurodegenerative disease research and cutting-edge protein science: the development of methods for the study of neurodegenerative disease proteins, and their application to generate insights from which novel angles for disease diagnosis or intervention can be derived.

His challenge: More than 30 years ago prion proteins (PrP) were discovered as the causative agents of a subset of devastating neurodegenerative diseases that include Creutzfeldt-Jakob disease (CJD) in humans and bovine spongiform encephalopathy (BSE) in cattle. Although much has been learned about these diseases, the normal role of the cellular prion protein (PrPC) has remained elusive.

How he tackled it: We applied CRISPR-Cas9 genome editing technology to generate mammalian cells, which can no longer express PrPC. We then compared by global proteome analyses wild-type and PrPC-deficient cells. To this end, we made use of a workflow that relied on isobaric labeling of peptides with Thermo Scientific™ TMT™ Technology reagents for relative quantitation and capitalized on the outstanding speed, resolution, and sensitivity of a Thermo Scientific™ Orbitrap Fusion™ Tribrid™ Mass Spectrometer for protein identification.

The happy result: We observed that levels of the neural cell adhesion molecule 1 (NCAM1) were reduced more than three-fold and its modification with polysialic acids was profoundly impaired in PrPC-deficient cells. NCAM1 polysialylation is known to contribute to a wide range of biological phenomena, including cell migration, circadian rhythm, neurogenesis, and the myelin ensheathment of peripheral nerves. These activities were independently reported to be perturbed in PrPC-deficient mice, consistent with the interpretation that the control of NCAM1 polysialylation is a key role of PrPC.

invitrogen



DISCOVER THE ATTUNE NxT FLOW CYTOMETER

The Invitrogen™ Attune™ NxT™ Flow Cytometer combines precision with performance in a benchtop flow cytometer that's configurable with up to 4 lasers and 16 parameters of detection.

Do more. Find more. Explore more.

- **Empower your research**—superior level of data fidelity at speeds up to 10x faster
- **Enable new applications and sample types**—investigate difficult sample types like tumors with clog-resistant fluidics
- **Enjoy walk-away automation**—convert between tubes and plates in seconds

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CHALLENGE

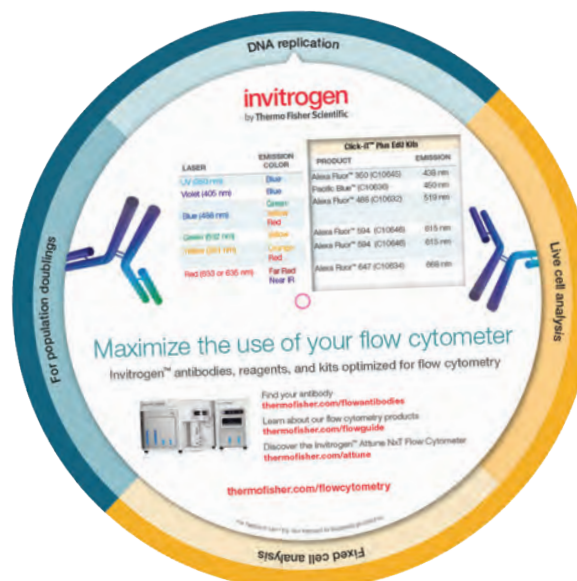
THE STATUS FLOW

“THE RESULTS ARE STRIKING”

Dr. James W. Jacobberger of Case Western Reserve University, a leader in multiparametric cell cycle studies, participated in a demo of the Attune NxT Flow Cytometer and walked away stunned.

Learn how the Attune NxT Flow Cytometer is revamping the flow workflow.

thermofisher.com/attune



MORE THAN JUST DOTS

Flow cytometry contributing to many types of research:

- **Cancer stem cells**—investigation by Dr. Jordi Petriz
- **Immuno-oncology**—immunotherapy: checkpoint inhibitors, therapeutic antibodies, and chimeric antigen receptor T cells (CAR T)
- **CRISPR**—analyzing editing efficiencies using flow cytometry

Watch the webinars at thermofisher.com/flowcytometry-webinars

DESIGNING A FLOW EXPERIMENT?

Don't reinvent the wheel, spin this one. Use this reagent selection wheel to identify which products match the laser excitation and emission channel of your panel.

Request your wheel at thermofisher.com/flowtoolkit

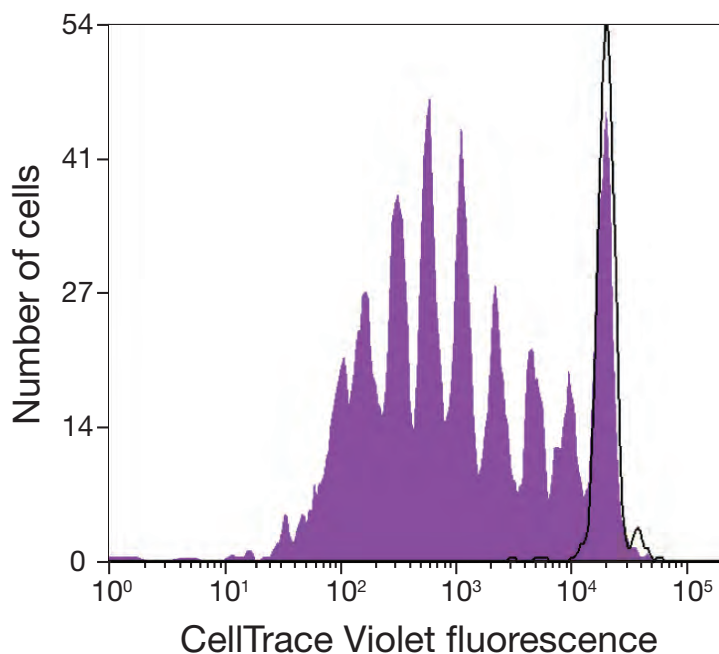
SIMPLE, ROBUST GENERATION STAINING

Dr. Andrew Filby assesses peak resolution of Invitrogen™ CellTrace™ Violet dye

“CellTrace Violet dye is the best reagent for tracking proliferation in any amenable cell type by fluorescent dye dilution and flow cytometry. Compared to CFSE, which is cytotoxic to cells when used at higher concentrations, CellTrace Violet dye labels cells brightly with low toxicity and is faithfully distributed to daughter cells ensuring the best possible peak resolution.”

~ Andrew Filby, Flow Cytometry Core Facility Manager and ISAC SRL Emerging Leader, Newcastle University

Read the paper at thermofisher.com/celltrace



JUST THIS MOMENT

MINDFULNESS AND MEDITATION

How often have you looked at slides through the microscope and your thoughts have been miles away? Have you ever been sitting at the bench pipetting and preparing a PCR and wondered if you had really added your forward primer to all your samples (I'll put my hand up to this one)? How much time have you spent needlessly worrying about a presentation?



Work can be stressful, regardless of your position. We all have deadlines, goals to reach, all while trying to maintain the work-life balance.

So what are some of the tried and true relaxation tools you can add into the mix to give your brain the rest it deserves? Some people find taking a walk or hitting the gym a great way to de-stress, but more people are adopting the practice of mindful meditation.

MINDFUL MEDITATION

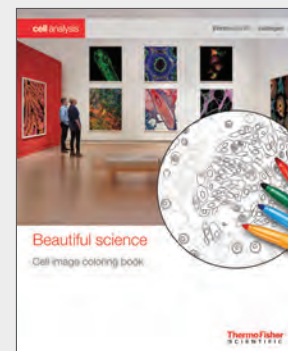
So what is mindful meditation? Isn't that an oxymoron, are you not supposed to switch off rather than be "mindful"?

It refers to the practice of focusing your awareness on the present moment to bring about a state of calm and serenity.

HERE ARE THE TOP TIPS TO GET STARTED:

1. Set aside some time regularly where you can practice.
2. Find a comfortable and peaceful place to give yourself space.
3. Relax into your breath, soften your gaze, or close your eyes.
4. Find peace in the present moment. This is what the practice is all about. Enjoy the moment and limit distractions.
5. When distractions do arise, acknowledge them, release them, and focus on the moment once again.
6. When you are ready to end your practice, slowly become aware of your surroundings and bring yourself back into a state of motion.

There are many resources for guided meditation, which can help get you started on the path to enlightenment. Check out your app store for tools that can help you on your journey to a less stressful life.



BEAUTIFUL SCIENCE CELL IMAGING

Coloring Book and Marker Set

For hours of fun, creative expression, join millions of adults all around the world who are rediscovering the simple bliss of coloring.

This free book's 30 unique and incredible coloring pages are inspired from actual cell images submitted by customers around the world. True to the broad spectrum of cells and their complex structures, pages include fantastic designs of tissue, neurons, villi, nerves, osteosarcoma, hepatocytes, astrocytes, endothelial cells, mesenchymal stem cells, dendritic cells, and more, sure to captivate colorists of all ages.

thermofisher.com/color



CARTOON CAPTION CONTEST

Check out the Invitrogen™ Facebook page, where we host a cartoon caption contest every few weeks. Keep an eye out for upcoming cartoons at:

[facebook.com/invitrogen](https://www.facebook.com/invitrogen)

Thanks to all those who have participated, and congratulations to our most recent winner!



WE'RE HERE TO HELP YOU SAVE

How far can you stretch your research budget?

Below are some current promotions on some of our most loved products.

There are even more promotions for the products found in this issue.

See them at: thermofisher.com/lifeinthelabpromos

Cell culture

Free hoodie

Spend \$2,500 and get a Gibco™ Life of Cells hoodie, or get up to 35% off your purchase—more than 1,000 products qualify for this offer.



To see details, go to thermofisher.com/gibcopromo

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Save up to 50% on RT-qPCR products

Achieve success in your gene analysis research and save up to 50% on the tools you need for your RT-qPCR workflow.

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Buy Invitrogen™ protein gels and add on protein clean-up, purification, and isolation reagents. Use promotion code **RPPR01** at checkout for instant savings.

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Use promotion code **P1311746** (US) or **P1311747** (Canada) at checkout. For details and to see a complete list of qualifying products, go to thermofisher.com/antibodypromo

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With the addition of eBioscience products to our Invitrogen™ portfolio of immunoassay products, you can choose from a large, unique menu of highly published ELISA and Luminex® assays.

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