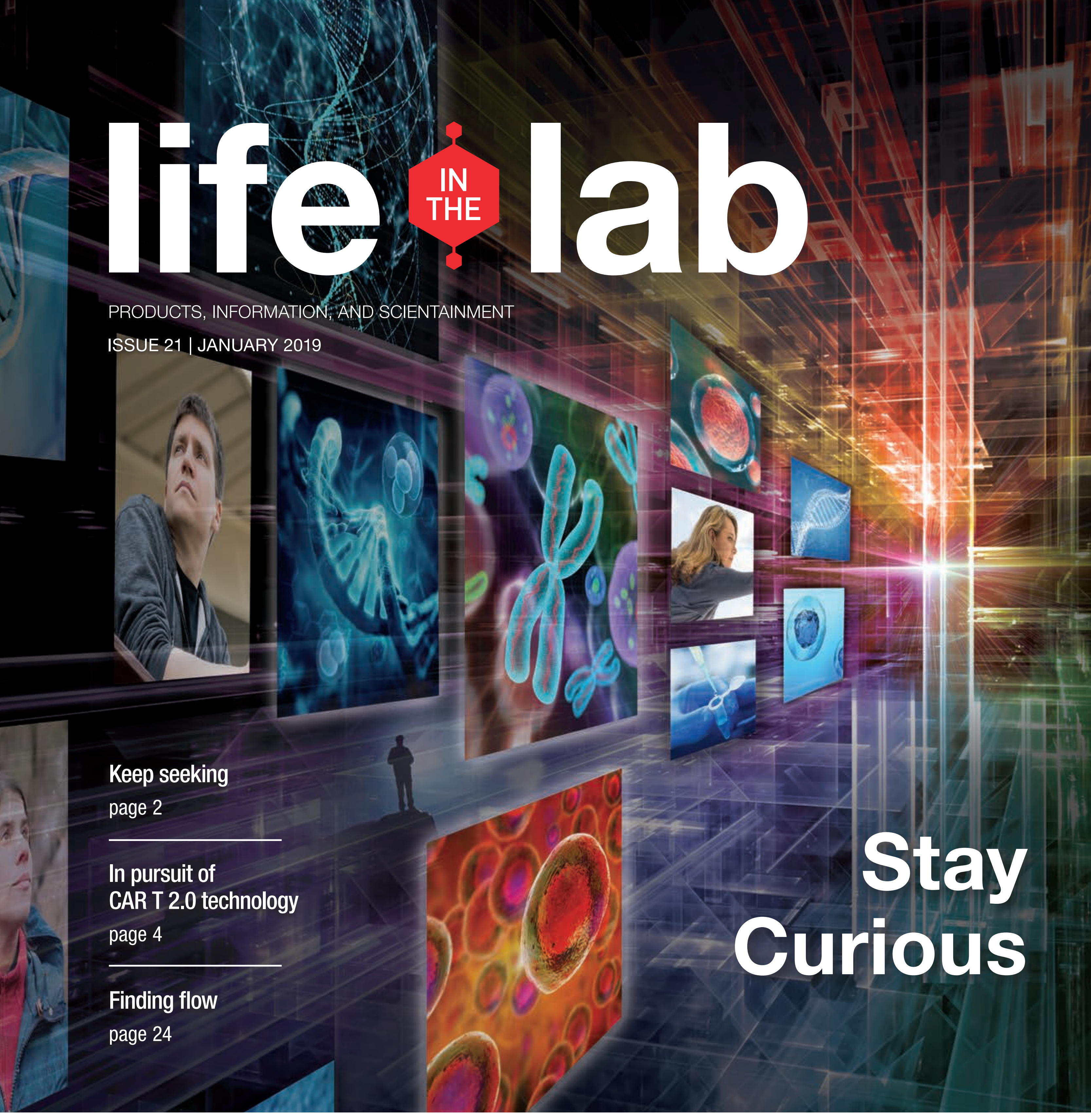

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ThermoFisher SCIENTIFIC

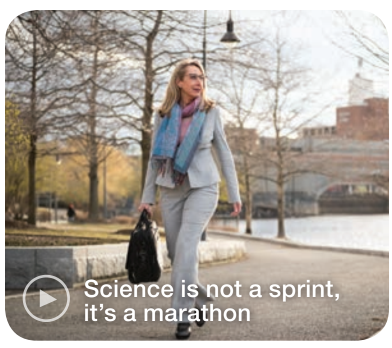
KEEP SEEKING

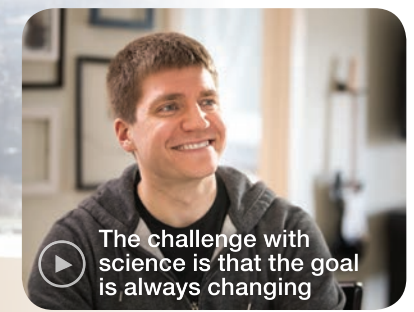
FACING THE CHALLENGES OF SCIENCE

Scientific progress is marked by both breakthroughs and failures. We asked fellow scientists how they learn from their setbacks, connect abstract ideas, and persevere through this long, noble, and very human journey. What we heard was amazing.

“Science is not a sprint, it’s a marathon.”

“I really put a lot of effort every single day in making sure that I am filling myself up; because if I don’t, I can’t be creative in the lab, I can’t perform. When you want to be innovative, you have to fail; you have to know where that edge is. When you are hitting a wall, just step back, take a walk, get air: it opens up a creative space for other things to happen. What keeps me going day in and day out is hope.” –Christina Waters



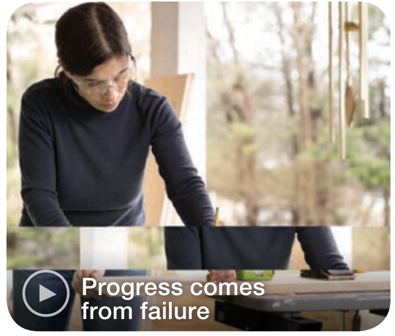


“The challenge with science is that the goal is always changing.”

“A bad day is: nothing works. A good day is: you finally got it to work. So whenever I hit a wall, the first thing I always go to is music. I fell asleep with a big old smile on my face and just went in the lab the next day just feeling like, ready to roll, recharged, ready to go. Maybe I’ll find some great cure, or maybe I‘ll move the science just a little bit forward. That really gets me out of bed every day, knowing that I am part of that fight.” –Justin Slawson

“Progress comes from failure by pushing through.”

“I was strictly analytical and scientific; and as I got more sophisticated in doing research, I wanted to be more creative. When I get stuck in my lab I kind of go to these places that draw me to a creative space. Progress comes from failure by pushing through. You need to overcome the fear of failure because you fail all the time when you are learning. The best scientists I know, the ones that make those incredible breakthroughs, are incredibly creative.” –Sarah Dykstra



Watch the series at
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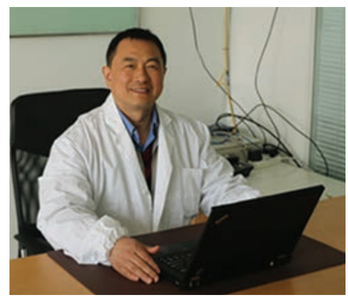
IN PURSUIT OF CAR T 2.0 TECHNOLOGY

The story of one physician-turned-researcher's journey down the CAR T innovation continuum



Dr. Lei Yu, MD, PhD,
Director of the Institute of the Biomedical Engineering and Technology (iBET), Shanghai Engineering Research Center of Molecular Therapeutics and New Drug Development, School of Chemistry and Molecular Engineering, East China Normal University, China; Shanghai Unicar-Therapy Bio-Medicine Technology Co., Ltd.

Since last year's personalized immunotherapy success story surrounding **Emily Whitehead**, CAR T cell therapy has embarked on a new era. There's an exciting surge of scientific effort toward understanding chimeric antigen receptor (CAR) technology and its potential to advance cures. Enter Dr. Yu, equal parts medically trained surgeon and astute researcher. His cross-cultural efforts between China, the US, and Canada are driven by a passion for translational medicine rooted in gene therapy research. While recounting his endeavor to bridge the gap between the bench and the clinic, Dr. Yu reflects on the promise of an innovative CAR T cell therapy 2.0 and why he continues to pursue a more precise immunotherapy.

You have an impressive résumé—it starts in medicine and branches out into basic research. What inspired you?

After conducting cardiac surgery on many patients, I noticed that almost every patient has to go back to the ICU. Infection and sepsis are hard to control within patients undergoing this type of surgery. It led me to the study of how to control and prevent these infections in the hospital.

Can you take us through your scientific journey?

Helping patients was my motivation so I applied for a PhD in medical microbiology and immunology at the University of Alberta [Canada]. My focus was vaccine research. After graduation, I thought, what next? At that time, gene therapy was quite hot. I applied to UCSD, Theodore Friedmann's lab. Professor Friedmann is a pioneer of gene therapy and he coined the "gene therapy" term in 1972. We used genetic engineering to increase safety in the design of viral vectors. Afterward, I studied gene delivery with Professor Sam Wang Kim. As I continued to develop gene therapy technology, I partnered with a Japanese company to set up a location

in Oceanside, California. We started with five employees and have grown to 115.

It sounds like you are really driven to help patients. Also, your work carries across multiple countries; can you please tell us how your work in China and the US impacts translational research?

In China, they have many patients and also they have a faster clinical trial process. In the US, sometimes we're waiting for patients and the process can be long and slow. [So] I'm back in China to set up training for more of the scientists within translational medicine. I try to make the technologies get to clinic faster. This is the reason why I'm in basic research and translational medicine and pharmaceutical development.

Your approach is from a number of perspectives. Why is that?

I feel this is helping me to be multidisciplinary. Currently, many of the life science technologies need more and varied scientific knowledge to design and to reduce the risk of failure. Also, the industry as a whole challenges me to think more systematically, like a systems engineer. You cannot just take care of one part and forget another. You need to consider the big picture to move ahead. I feel this gives me a greater chance of success for helping people.

What's your proudest moment so far?

Working at a hospital, you see very sad scenes. That motivates me to research gene therapy technology—gene therapy from the vector part, the gene part, the genetics part, and also the clinical part. Gene therapy has been around for 30 years. CAR T cell therapy is a form of gene therapy. This is why I say it's the right timing for us. I've worked with a patient who was close to death; but after the infusion of the CAR T cells, the patient was very much alive and was even able to stand. This is why I try to do my best, and have branched out from lymphoma and myeloma to leukemia and solid tumors. So many patients are waiting, and there are many lives to save. I'm so happy with what I do because almost 87% of my patients have been saved.

You've mentioned CAR T cell therapy. What's CAR T 2.0?

I think of the first CAR T cell therapy as version 1.0 because usually they just modify CAR T molecules. This has already proven to be successful. However, even though we can continue to improve their therapeutic effect, we cannot prove their safety. How do you balance it? This is why we think about how to go about making the T cell more fit for reduced risk by increasing safety. You cannot just modify the CAR T molecules because if you modified the CAR T molecules you may reduce risk but also you'll reduce efficacy.

For solid tumors, they have a microenvironment that is not good for the activity of CAR T cells. We consider how to make CAR T cells that can ignore inhibitory effects present in the tumor microenvironment (TME). For example, PD-1 is mainly expressed by the T cell. PD-L1 is mainly expressed by the cancer cell and also the TME. Even though you have a lot of the PD-L1 in the tumor tissues, if we can reduce the PD-1 expressed on T cells, then they can go into the tumor uninhibited and keep functioning. This is why we are also using silencer RNA to study this as we continue to genetically engineer T cells that are better fit for their environment. This is what we call 2.0.

You need to consider the big picture to move ahead. I feel this gives me a greater chance of success for helping people.

What are the biggest challenges you face as you work toward CAR T 2.0?

Lots of issues here. Many of the current manufacturers producing modified CAR T cells are simply from basic research backgrounds. For industry, I think we require more of a single-use technology so we can avoid contamination. Second, simplification of the process is important. Third, cells from each patient are different so it's challenging to make controls. In this case, you have to have a very experienced scientist observe cells to make a judgment call. Or, using AI is promising because that sort of system will remove variability. In Chinese medicine, for example, an experienced doctor who is very old and with lots of experience would like to pass down knowledge to their student. However, it's difficult to pass down personal intuition and feeling. But if you can tell a machine to "understand" by accumulating data, you can design this machine and make the passing on of knowledge easier by modern methods.

What is your perspective on the future of CAR T and its impact?

I think safety is still a problem. It's hard to reduce the risk and make CAR T therapy more benign so you can treat the patients, but also not harm them. This is still a clinical bottleneck or pain point. Another challenge is finding the right targeting molecule. It's important to distinguish exactly what the CAR T is fighting. Biomarker discovery is challenging because you have to look at the specific cancer, numerous biomarkers, and the TME. For example, you have to look at overexpression. It means a cancer cell can make 100 times more biomarkers than a normal cell because the normal cell is still healthy. Also, neoantigens: these antigens change so frequently. Once you hit the right target, you may hit 100 cancer cells but another 100 will change. This is why it's quite difficult. I really think this is where T cell receptor (TCR) therapy in combination with CAR T therapy could help. This is maybe where the future lies.

Is there anything outside of the science that really drives you and motivates you?

I like reading, swimming, and walking. This is also my time to think. I've read and written lots of kung fu stories about teaching someone how to fight a bad guy and helping weaker people. This is the same as a doctor or scientist: the goal is to treat the patients, to save the patients. This can be summed up in two purposes: one purpose is to try to make the people live longer; the second is to try to make their quality of life better. In other words, to make patients happy. Healthy, happy. This is why I use my knowledge and greatest effort to reach this goal one step at a time.

thermofisher.com/cartcelltherapy

SYNTHETIC BIOLOGY, GAMING, AND FOOD SAFETY

Seeking answers through unconventional approaches

According to the World Health Organization (WHO), **one in 10 people** worldwide are affected by a foodborne illness every year. Aflatoxin, a poisonous substance released by mold that grows on grain, is a major contributor to the global foodborne health crisis. **Four and a half billion people** are chronically exposed to this naturally occurring poison, which can lead to stunted growth among children (low physical growth and intellectual development), liver cancer, and death. Several approaches for managing and degrading aflatoxins are currently in practice, but none are widely considered to be effective. Scientists think an enzyme can be created to attack and degrade aflatoxin, which would decrease its toxicity by several orders of magnitude. As a result, a group of uncommon collaborators including Mars Incorporated, the University of California at Davis, Thermo Fisher Scientific, the University of Washington, Northeastern University, the Partnership for Aflatoxin Control in Africa (PACA), and the Food and Agriculture Organization of the United Nations (FAO) have come together to tap into the power of online, gamified protein folding with hopes that it can expedite progress toward a solution.

Unique solution: To address the dangers of aflatoxin, Mars asked scientists at Thermo Fisher and UC Davis to help develop a way to destroy or mitigate the poison. The aflatoxin initiative launched on World Food Day (October 16, 2017) with a game-a-thon demo where players used a crowdsourcing computer game called Foldit to attempt to restructure a protein enzyme that can be used to destroy aflatoxin.

In less than one year, players have designed more than 1.6 million models to potentially degrade aflatoxin. The time these players have taken to morph the 3D molecules equates to approximately 80,000 player hours—that's equivalent to a labor force of around 100 full-time employees working on the problem for one year.

SYNTHETIC BIOLOGY AS A FORCE FOR FOOD SAFETY

Over the past year, there have been 12 gaming rounds of the aflatoxin puzzle released on the Foldit platform. After each game round, the best-scoring models are picked for the next round of the process—analysis. Scientists at the Siegel Lab at UC Davis analyze the protein structures for their amino acid sequence and send the information to Thermo Fisher. Thanks to advances in synthetic biology technologies, the amino acid information is translated and optimized into biology's digital code: DNA. This DNA is physically produced by the synthetic biology team here at Thermo Fisher, leveraging our proprietary oligo capabilities and Invitrogen™ GeneArt™ Gene Synthesis to encode for the newly designed proteins. Future DNA synthesis runs will use our miniaturized semiconductor-based nucleic acid synthesis platform. It can generate 35,000 individually selectable oligos manufactured at the same time, which are then stitched together to make up the code for the enzyme. Once created, the synthesized DNA molecules are sent back to the Siegel Lab to see if—when expressed and folded into real proteins—they have the ability to detoxify aflatoxin. The scientists are particularly interested in targeting aflatoxin's susceptible lactone ring, because this allows the synthesized DNA to form an enzyme capable of performing lactone hydrolysis on aflatoxin B1 under industrial conditions where only water needs to be present to perform the desired chemical reaction. Chemical degradation of this lactone ring through enzymatic hydrolysis has the potential to decrease aflatoxin mutagenicity by more than 400-fold.

> Synthetic biology is a key discipline for a sustainable future.

"It is our hope that we can mitigate this serious health concern in the developing world by joining forces and leveraging the capabilities and know-how of each member in this unorthodox partnership," says Helge Bastian, vice president and general manager of synthetic biology at Thermo Fisher Scientific. "By utilizing Thermo Fisher's proprietary and industry-leading gene synthesis technologies and platforms, we also want to demonstrate that synthetic biology is a key discipline for a sustainable future. Synthetic biology is bringing life science know-how closer to the consumer and has the potential to alleviate societal issues and to make our world a healthier, cleaner, and safer planet."

Adapted from: Sponsor Feature in Nature 562, 7727 (18 October 2018)

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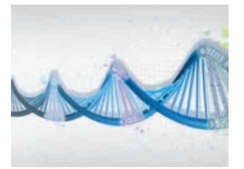

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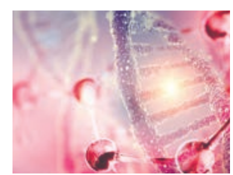

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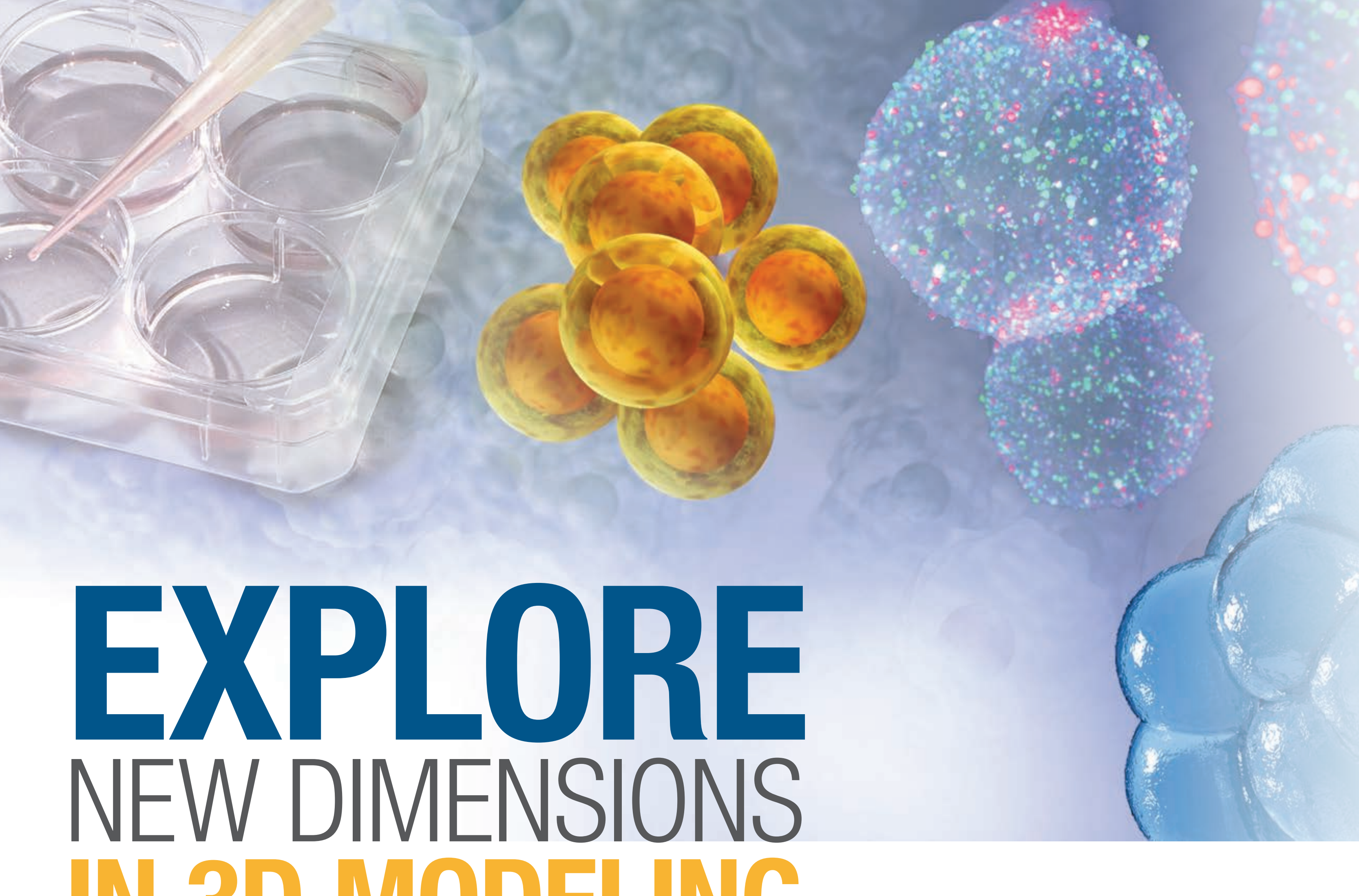

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These three-dimensional (3D) models not only mimic cell functions, but allow for more robust testing of compounds and produce more representative analysis.

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HISTORICAL HIGHLIGHTS OF RESEARCH ON 3D MODELS

1957

At the Rockefeller Institute for Medical Research, American developmental biologist Aron Moscona showed that dissociated cells cultured together created aggregates—3D structures.

1970s

Robert Sutherland and his colleagues at the University of Western Ontario in Canada coined the term "spheroids" for the structures that Moscona described.

IN-LAB OBSERVATIONS FROM LAURA BROUTIER, THE GURDON INSTITUTE, UNIVERSITY OF CAMBRIDGE

"I am really interested in how we can use organoids to model tumor pathology. So far, we are only using 2D cell lines and animal models; but now, thanks to the organoid technology published (notably in prostate cancer, colon cancer, and pancreatic cancer research areas), we have a better input in this pathology. Now we can maintain the cell-to-cell and cell-to-matrix interactions and improve our work on the response of drugs."

See the rest of her story and others below.

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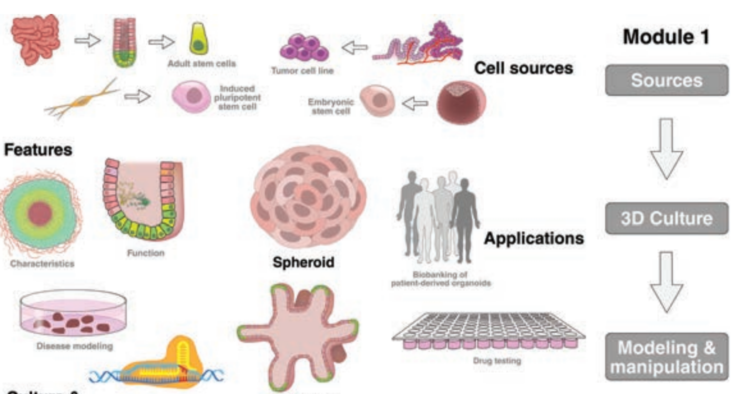

WHAT PRODUCTS SHOULD I USE TO CULTURE 3D MODELS?

As the most cited products in peer-reviewed papers about organoids and spheroids, Gibco™ media, supplements, and matrices are widely used in the growth, differentiation, and maturation of cellular 3D models. This is where a culture system can be developed to suit specialized cell types like stem cells or cancer cell lines.

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1980

A team led by Mina Bissell at Lawrence Berkeley National Laboratory published a paper highlighting the importance of the extracellular matrix.

2008

Yoshiki Sasai and his team at RIKEN (research institute) demonstrated that stem cells can be coaxed into balls of neural cells that self-organize into distinctive layers.

TODAY

Nature Methods names organoids as the 2017 Method of the Year, marking a significant increase in research publications over the last decade.

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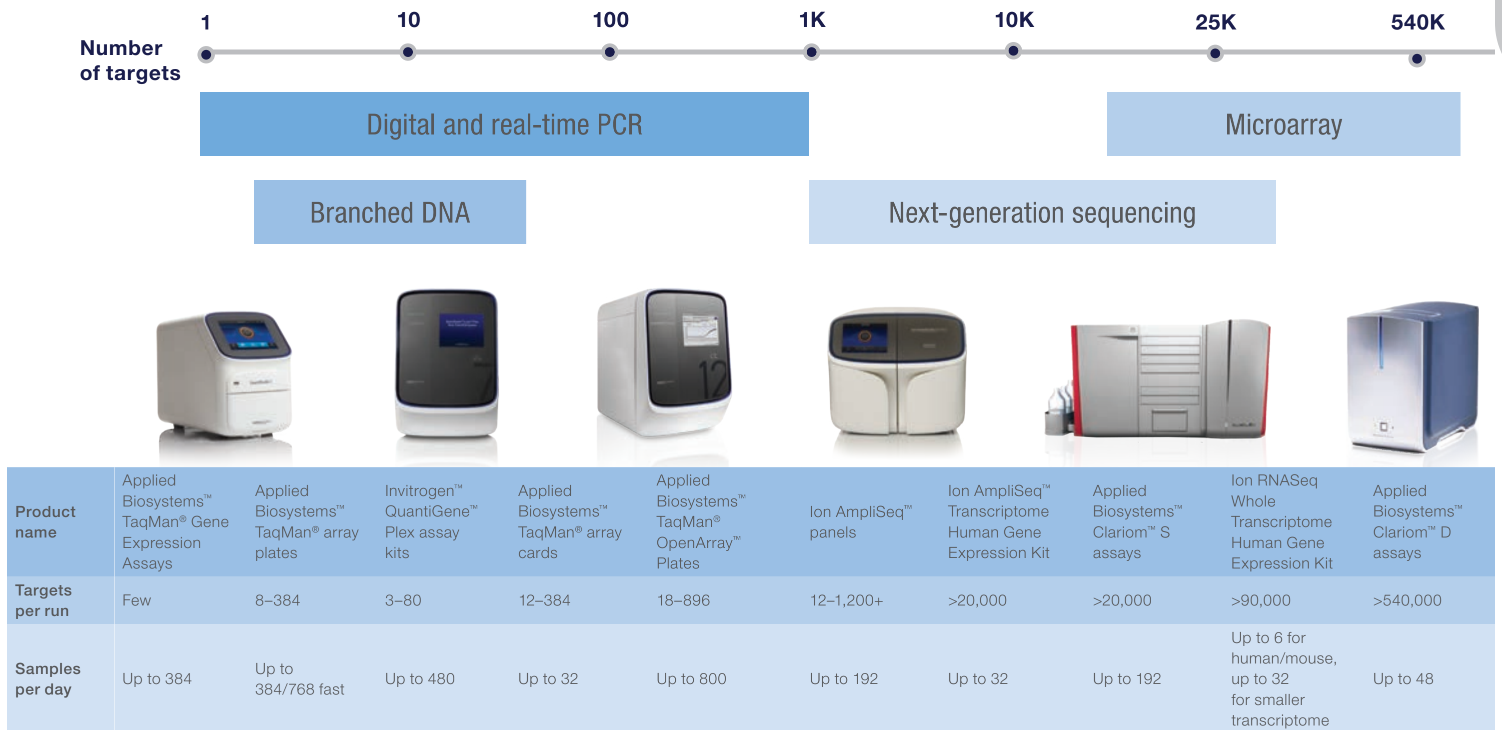
OVERVIEW OF GENE EXPRESSION SOLUTIONS



Product name	Applied Biosystems™ TaqMan® Gene Expression Assays	Applied Biosystems™ TaqMan® array plates	Invitrogen™ QuantiGene™ Plex assay kits	Applied Biosystems™ TaqMan® array cards	Applied Biosystems™ TaqMan® OpenArray™ Plates	Ion AmpliSeq™ panels	Ion AmpliSeq™ Transcriptome Human Gene Expression Kit	Applied Biosystems™ Clariom™ S assays	Ion RNASeq Whole Transcriptome Human Gene Expression Kit	Applied Biosystems™ Clariom™ D assays
Targets per run	Few	8–384	3–80	12–384	18–896	12–1,200+	>20,000	>20,000	>90,000	>540,000
Samples per day	Up to 384	Up to 384/768 fast	Up to 480	Up to 32	Up to 800	Up to 192	Up to 32	Up to 192	Up to 6 for human/mouse, up to 32 for smaller transcriptome	Up to 48

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In the webinar "Investigating the Genetics of Human Macrophage Response and Resistance to *Mycobacterium tuberculosis*," Audrey Papp, the technical director at the Ohio State University, discusses research to identify genetic variants that influence cellular responses and patterns of M.tb infection and growth in human alveolar macrophages.

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"There are those who seek knowledge for the sake of knowledge; that is Curiosity. There are those who seek knowledge to be known by others; that is Vanity. There are those who seek knowledge to serve; that is Love." –Bernard of Clairvaux

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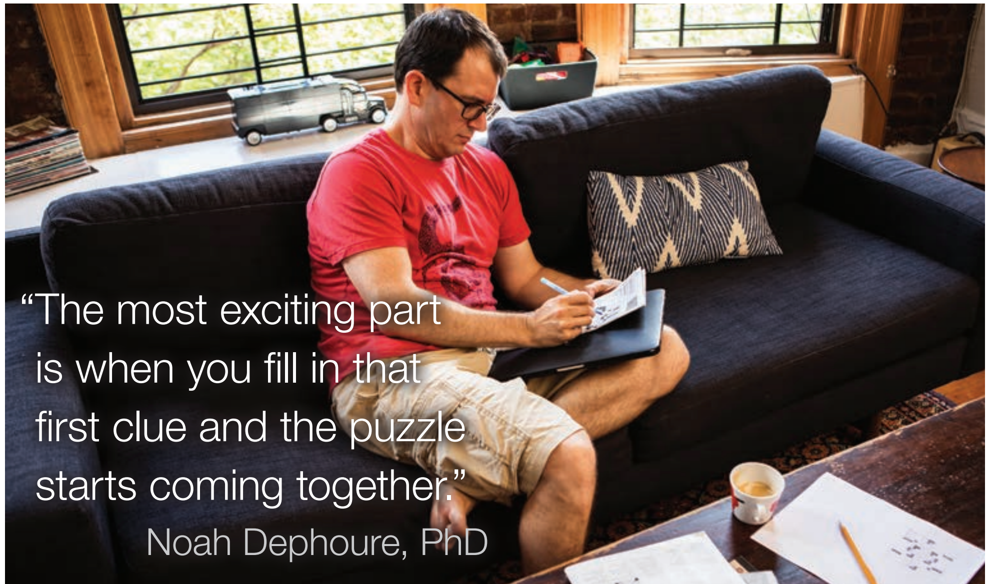
FROM LAB MANAGERS

Lab managers share their wisdom on how to be more productive and efficient in the lab, while making the most out of your lab environment

1. Play it safe—honor health and safety concerns in your lab
2. Know something—even just a little bit—about what everyone does in the lab
3. Stock up on nonperishable items when your lab is in good financial standing
4. Make friends with local sales representatives to score the latest offers, product samples, etc.
5. Get along with the people in the lab and respect one another—great labmates make for good laughs and solid company during late nights
6. Include all people working in the lab on duty rotations—from undergrads to senior postdocs
7. Befriend on-site building and supply center staff
8. Cultivate relationships with neighboring labs—you never know when you may need a favor
9. Understand your PIs and help them with grant applications
10. Enjoy yourself—discovery is a journey

SOLVING THE PROTEIN PUZZLE



"The most exciting part is when you fill in that first clue and the puzzle starts coming together."
Noah Dephoure, PhD

Human cells are a chatty bunch. Most of the time that's a good thing, as messages can be relayed, for example, to start an immune response to unwelcome bacteria. Other times, however, inter-cell communications generate the wrong messages, creating pathological changes that can result in cancer and other diseases. Researchers understand many aspects of this communication process, but a complete grasp of such signaling remains an unfinished puzzle.

Fortunately, Noah Elias Dephoure, a biochemist who has dedicated his life to solving puzzles both in the lab and in his free time, has his sights set on unraveling the mystery of how cell signaling can be harnessed to fight cancer. At his laboratory at Weill Cornell Medical College in New York City, he specializes in the study of protein kinases—enzymes that modify proteins to change their function or location.

Delving into the possibilities of protein kinases and working through the New York Times crossword puzzle are both key parts of what continues to drive Dephoure. Fortunately, over the years the tools that he uses in the lab have made that research a bit less complicated.

"In the past, if you wanted to figure out what proteins were present in your sample, you had to work really hard to make special tools just to identify one of them," Dephoure says. "Now, you can break them all up, throw them in the mass spectrometer, and identify and quantify thousands of proteins at a time."

Dephoure uses Thermo Scientific™ Tandem Mass Tag (TMT™) reagents to identify and quantify proteins by mass spectrometry.

"Tools from Thermo Fisher Scientific continue to enable improvements that have a serious impact on the quality and precision of the data we require. Where we once could examine 500 peptides at a time, now we can study tens of thousands," he says.

His goal is ultimately to understand signaling events that underlie basic cellular biology and human disease research. "We are still only scratching the surface of biological understanding. There are over 500 protein kinases encoded in the human genome, and we probably only know what about 100 of them do," the biochemist muses. "Solving that puzzle could lead to breakthroughs in cancer therapies that attack the disease by stimulating the immune system."

More peer inspiration

Meet the flow guy
Steve McClellan

Connecting rare kids with rare bears
Christina Waters

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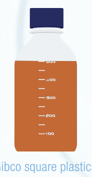

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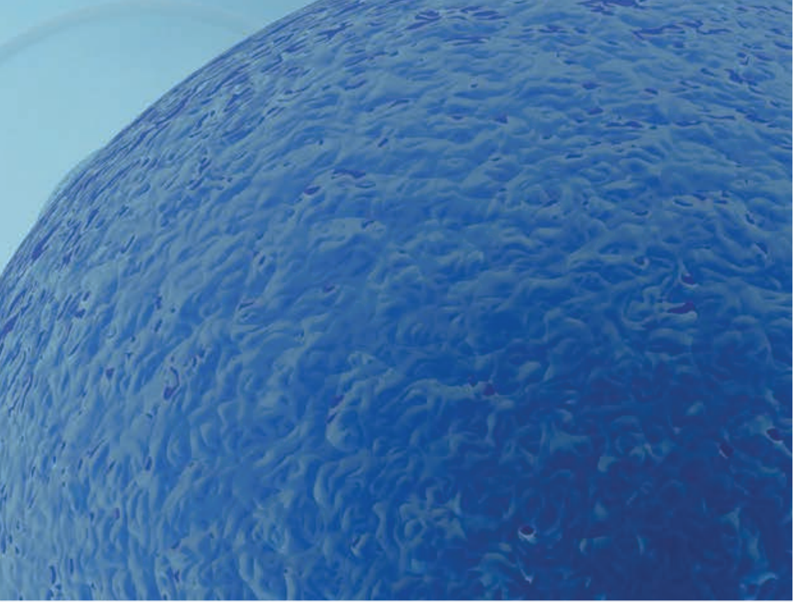
thermofisher.com/fbs

FBS category	Description
Value FBS	Sera for standard research applications • Up to 50 qualified tests including 9 CRF virus testing, endotoxin, and performance • Triple 0.1 micron filtration
Premium FBS	Sera with the least risk of BSE and low viral load • Meets USP/EP guidelines • Up to 90 qualified tests including EMA virus testing; USP/EP mycoplasma, endotoxin, and performance; biochemical/hormonal profiling; Oritain™ fingerprinting • Triple 0.1 micron filtration
Specialty FBS	Sera qualified for specialty research • Specific assays, including stem cell research, immunoassays, antibodies, and others



7 REASONS TO BUY GIBCO FBS RIGHT NOW

Global, vertically integrated supply chain for continuity of supply and risk mitigation

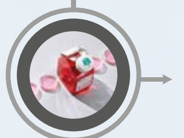
International Serum Industry Association (ISIA)–certified for traceability since 2014

cGMP–ISO 13485 and/or ISO 9001 facilities

Differentiated workflow solutions, from specialty serum to innovative packaging like the aliquot-free Gibco™ One Shot™ FBS 50 mL bottle

FBS "fingerprinting" technology—first FBS supplier to develop origin reassurance like no other

Gibco™ iMATCH™ technology—multi-parametric matching tool minimizes lot variation and eliminates need for testing



Better together—maximize reproducibility by pairing our FBS with other Gibco media and our Thermo Scientific™ Nunc™ plastics

NEW GIBCO™ HEAT STABLE RECOMBINANT HUMAN bFGF PROTEIN

Engineered for greater stability in cell culture conditions, sustaining >80% activity for at least 72 hours, whereas native bFGF is highly unstable with a half-life of <8 hours.

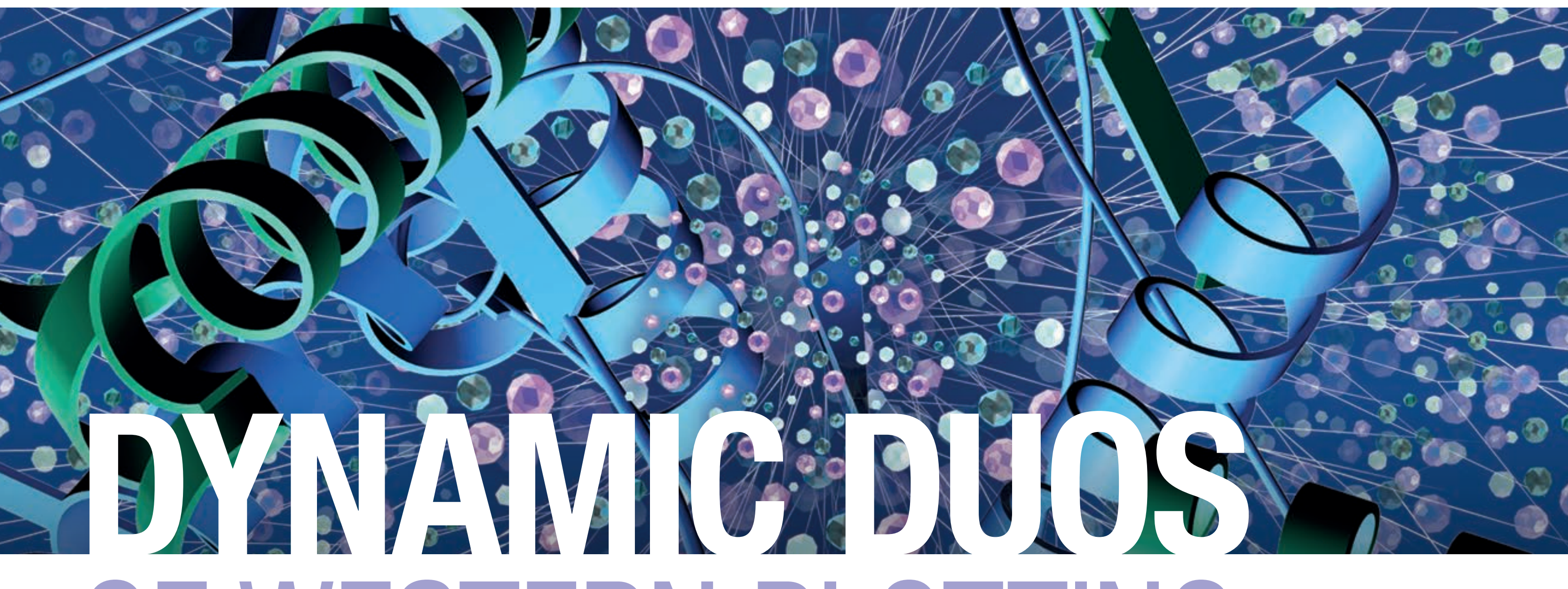
Learn more at
thermofisher.com/heatstablebFGF

FLEXIBLE TRANSFECTION OPTIONS FOR GENOME EDITING

Consider transfecting your difficult-to-transfect cells, like primary or stem cells, via electroporation with the Invitrogen™ Neon™ Transfection System. When traditional lipid-based reagent delivery of your CRISPR-Cas9 system is giving you problems, the Neon Transfection System is up for the challenge.

When you need a lipid-based reagent or if you need a high-throughput transfection solution, try the Invitrogen™ Lipofectamine™ CRISPRMAX™ Cas9 Transfection Reagent.

thermofisher.com/crisprtransfection



DYNAMIC DUOS OF WESTERN BLOTTING

Is your western workflow in need of a hero? Enlist these power pairs to come to the rescue.

INVITROGEN™ BOLT™ BIS-TRIS PLUS GELS AND THE INVITROGEN™ iBLOT™ 2 DRY BLOTTING SYSTEM

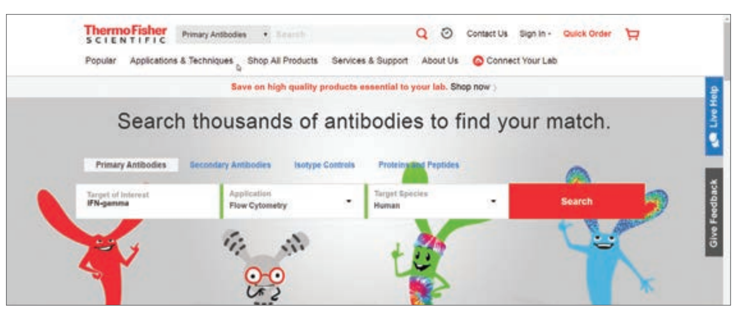
Have the need for speed? Bolt Bis-Tris Plus gels offer the benefits of Bis-Tris chemistry, allowing you to load up to 60 uL of sample with run times of just 20 minutes. Tag-team with the iBlot 2 Dry Blotting System, which utilizes unique, ready-to-use dry transfer stacks for transfer times of only 7 minutes. Separate and transfer your proteins in a flash!

thermofisher.com/bolt
thermofisher.com/iblot2

THERMO SCIENTIFIC™ SUPERSIGNAL™ WEST PICO PLUS SUBSTRATE AND THE INVITROGEN™ iBIND™ WESTERN DEVICE

With our innovative automated western blot processing system—the iBind Western Device—you can simply load primary antibody, secondary antibody, and wash solutions, then walk away. In less than 3 hours, your blot is ready for final detection. Solutions are processed using sequential lateral flow technology, with no batteries, shakers, trays, or timers required. Team up with our most versatile chemiluminescent HRP substrate with a great balance of sensitivity, signal duration, and signal intensity for a smooth and efficient immunodetection workflow.

thermofisher.com/chemisubstrates
thermofisher.com/ibind



PRIMARY AND SECONDARY ANTIBODIES

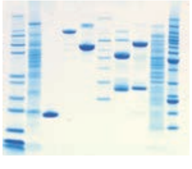
Find the perfect pair of primary and secondary antibodies for your western blot detection. We offer thousands of options in over 50 research areas such as cancer, epigenetics, immunology, and neuroscience. Find your match today.

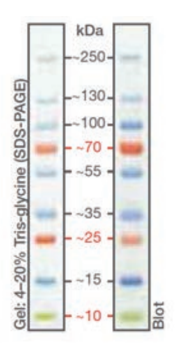
thermofisher.com/antibodies

THERMO SCIENTIFIC™ PAGERULER™ PLUS PRESTAINED PROTEIN LADDERS AND INVITROGEN™ NUPAGE™ BIS-TRIS GELS

You can trust the neutral pH of the NuPAGE Bis-Tris gels to resolve and maintain the integrity of your protein and provide you with straight lanes and publication-quality data. Combine them with the popular PageRuler Plus Prestained Protein Ladder (molecular weight range of 10–260 kDa) to monitor your gel run and transfer.

thermofisher.com/nupage
thermofisher.com/proteinladders





THE INVITROGEN™ iBRIGHT™ FL1000 IMAGING SYSTEM AND INVITROGEN™ ALEXA FLUOR™ PLUS SECONDARY ANTIBODIES

We've saved the best for last…are you ready to take your western blots to the next level with fluorescent multiplexing? Recruit our easy-to-use iBright FL1000 Imaging System—designed to capture high-resolution, high-sensitivity fluorescent western blots—and our Alexa Fluor Plus conjugated secondary antibodies with enhanced sensitivity and low background. With this power combo, you can detect multiple proteins in the same blot.

thermofisher.com/chemisubstrates
thermofisher.com/ibright

Alexa Fluor Secondary

TO OPTIMIZE UV-VIS DNA/RNA QUANTIFICATION

Speed up genomic workflows with these microplate-based methods for quantifying nucleic acids

Quantifying nucleic acids by direct photometric quantification at 260 nm is widely popular, as it doesn't require reagents, incubation steps, or standards. Samples can also be recovered if needed. However, the photometry method has limitations: the detection limit isn't as low as fluorometric methods, and it may not be as sensitive as desired for certain applications. Here are some tips for better exploitation of photometric UV-Vis quantification of nucleic acids.

Format comparisons			
Sample format (measurement device)	**Cuvettes**	**Microplates**	**µDrop Plate**
Required volumes	50 µL to 1–2 mL	15–100 µL (for 384-well plates) 30–150 µL (for 96-well plates)*	2–10 µL
Theoretical LOD for dsDNA (using Multiskan Sky instrument)	0.45 µg/mL (1.0 cm pathlength)	0.9 µg/mL (0.5 cm pathlength)**	9 µg/mL (0.05 cm pathlength)
Theoretical maximum measurable concentration of dsDNA (using Multiskan Sky instrument)	125 µg/mL (1 cm pathlength)	250 µg/mL (0.5 cm pathlength)**	2,500 µg/mL (0.05 cm pathlength)
Throughput	+	+++	++

* Applicable to small-area UV microplate.
** Pathlength when pipetting 50 µL of sample in 384-well UV plate.

1 SAMPLE FORMAT CAN IMPROVE DETECTION LIMIT

The limit of detection (LOD) is the lowest amount of nucleic acid that can be separated from the background. The LOD is dependent on whether the measurement is done using a microplate, a cuvette, or the proprietary Thermo Scientific™ µDrop™ Plate. Cuvettes provide the best sensitivity (lowest LOD), but require the largest sample volume with the lowest throughput. For the highest throughput, microplates are the best choice and they provide an intermediate sensitivity (see table above).

2 BLANKING IS ESSENTIAL

Photometric measurement devices always have some background absorption, driving the need to perform blank subtraction. For instance, when using the µDrop Plate, blanks can be included at the same time as the samples or they can be measured before the samples in exactly the same sample positions. Blank subtraction can be automatically performed with Thermo Scientific™ SkanIt™ Software, which controls Thermo Scientific™ microplate readers.

3 WAVELENGTH "BLANKING" IS ALSO IMPORTANT

Wavelength blanking is actually a background correction, and not a blank (although it is often referred to as "blanking") but it is important to perform. Wavelength blanking is recommended at 320 nm. This is a wavelength at which the absorbance of nucleic acids is minimal, and thus high 320 nm values would indicate unwanted contamination in the measured sample.

4 MINIMIZE THE IMPACT OF EVAPORATION

With small volumes, evaporation will result in higher concentrations and misleading estimations of the nucleic acid concentrations, especially when using low volumes in the µDrop Plate or the Thermo Scientific™ NanoDrop™ spectrophotometer. Effects of evaporation can be minimized by pipetting as rapidly as possible and measuring the samples immediately after pipetting.

5 ELIMINATE EXTRA WORK BY DILUTING SAMPLES

Know your detection range—undiluted samples can only be quantified within the so-called detection range. The lower limit in this range is given by the LOD (discussed in Tip #1), while the upper end is determined by the linear range of the instrument used. This value is also dependent on the sample format; thus, increasing the maximum concentrations can also be done by changing the sample format (see table above).

Note: the µDrop Plate allows for both cuvette and microvolume samples to be read using Thermo Scientific™ Varioskan™ LUX and Multiskan™ Sky microplate readers.

Learn more at **thermofisher.com/platereaders**

invitrogen

MATCHMAKING MUSTS IN MULTIPLEXING

Webinar: elevate your flow cytometry panel design to get more complete data and increase your productivity

The correct control can make or break your flow multicolor panel. One of the biggest challenges in multiparameter flow cytometry is selecting the right combination of fluorophores and antibody conjugates so that the need for compensation and spillover adjustments is kept to a minimum while the quality and accuracy of the data are not compromised.

In this webinar, you'll learn the value of including the correct controls and reagent titration and better understand the importance of fluorescence spillover and finding your fluorescence spectral balance.

Join Carol Oxford, field applications scientist at Thermo Fisher Scientific, to learn:

- Why it's important to titrate each reagent; building the right panel is an iterative process
- Important concepts like spillover and spreading to aid in fluorochrome choice for multicolor design
- Tips for making sure your experiment is successful

Watch the webinar today at **thermofisher.com/flowdesignwebinar**

SEEK PRODUCTIVITY INSPIRATION

P	E	R	S	I	S	T	E	N	C	E	Y	B	K	B	P	L
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A	Y	V	D	C	D	I	T	N	I	Y	R	O	C	H	Q	H

Find the 15 hidden words to inspire more productivity

exploration	ingenious
breakthrough	insight
inspiration	curiosity
persistence	triumph
productivity	discovery
innovation	impactful
motivation	pioneer
creativity	

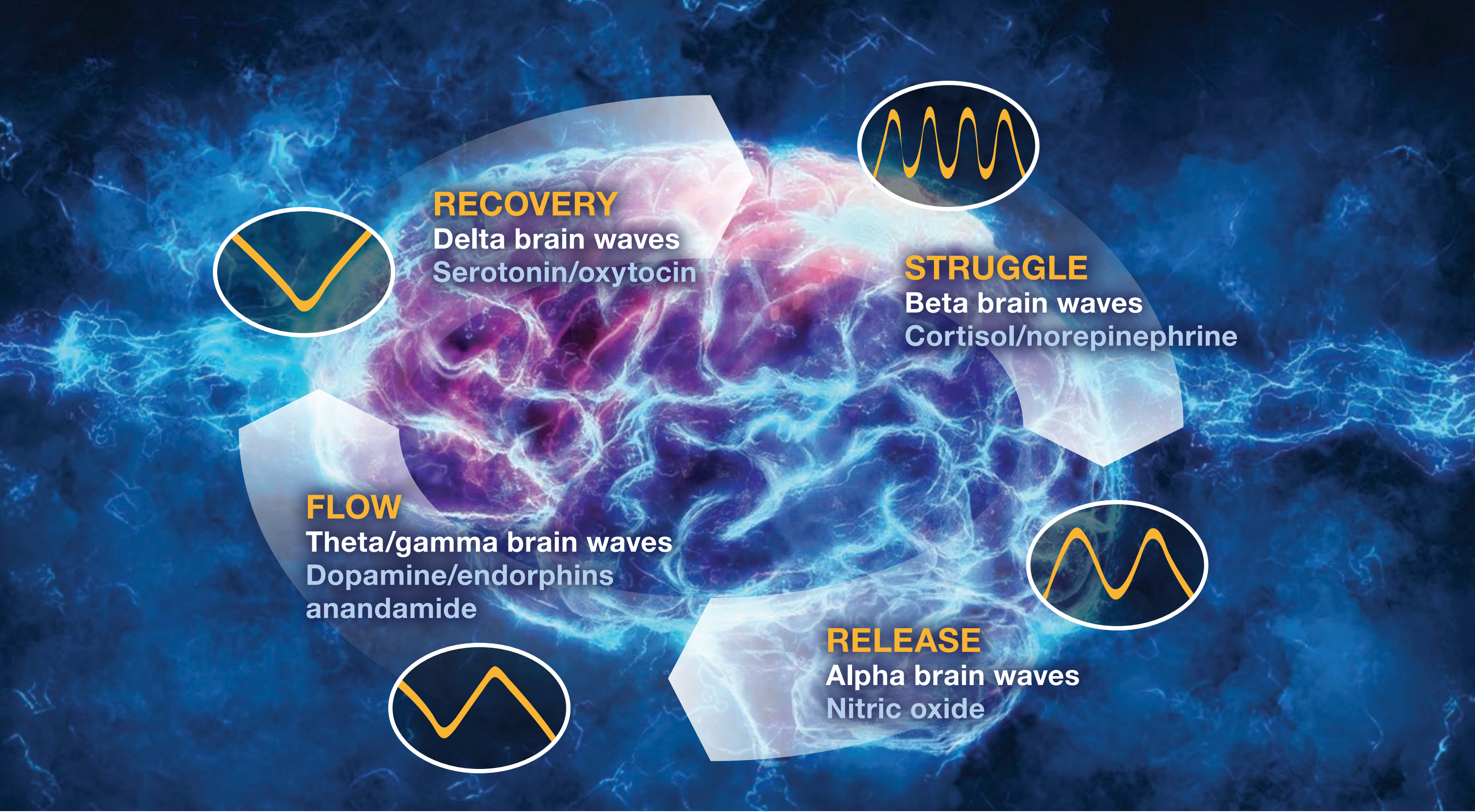
FOUR MOBILE LAB APPS TO **FUEL YOUR RESEARCH**

DailyCalcs—science calculator
Want to turn your smartphone into a science calculator? The DailyCalcs app can calculate molarity, dilution, formula weight, transfection, unit conversions, culture vessel data, media conversions, and specific productivity.

Cell Culture—learn, experiment, calculate, and streamline your bench time
The Cell Culture app will streamline your bench time by recording cell culture data in real time and exporting it to your computer.

CloningBench—lab experiment tools
The CloningBench app is an essential tool to help you perform your cloning experiments. With valuable selection guides, calculators, and timers, this app will simplify your experiment design.

Sanger Sequencing
Check out the Sanger Sequencing app to learn more about the basic principles of Sanger sequencing, as well information on workflows and applications.



FINDING FLOW

What does your scientific research have in common with adventure sports? They both have triggers that get you into a focused, zen-like state called flow. This article discusses these triggers in the context of researchers like you.

We spoke with Steven Kotler, science writer, director of the Flow Genome Project, and author of "The Rise of Superman: Decoding the Science of Ultimate Human Performance." Kotler's research and writing on the flow state are based on decades of work done by Mihaly Csikszentmihalyi (pronounced "Me-high Chick-sent-me-high"), and he views the state of flow through different lenses, including the insane, accelerated achievements in adventure sports.

Kotler describes flow as an optimal state of consciousness where we feel and perform our best. He writes, "Action and awareness merge. Our sense of self vanishes. Our sense of time distorts, and performance goes through the roof." Sound familiar to you?

As a scientist, you may already know about transient hypofrontality—that as focus tightens, the brain stops multitasking and the effect of the efficiency exchange going on deactivates swaths of the neocortex, distorting our ability to compute time. Ever find yourself in the lab at a late hour and wonder where the time went?

While flow may be incredibly desirable, it is also elusive. Over twenty triggers to flow have been identified; but risk is one of the fastest paths to get there, inducing a flood of endogenous neurochemicals that make exogenous opiates look like watered-down fakes. Kotler says adventure sport athletes are better at hacking flow because the stakes are unequivocally higher and it can be a situation of "flow or die." For you as a scientist, this is the risk equivalent of "publish or perish."

THE SCIENCE OF FLOW

While athletes rely mostly on environmental triggers, as a scientist you can also access the flow state to up your game. There are environmental, internal, creative, and social triggers to flow; to initiate it, you must first go through four biologically regulating phases:

1. **Struggle:** where you amp up focus, so stress hormones like cortisol and norepinephrine are pumped into your system, increasing focus and helping remove unwanted background. Struggle is probably very familiar to you as a scientist from trying to problem-solve, address setbacks, etc.
2. **Release**: where nitric oxide floods your system, signaling stress hormones to decline and making way for the final ingredients of this neurochemical cocktail achieved in the flow state.
3. **Flow:** dopamine, endorphins (blocking the pain of long lab hours and that darn stool) and anandamide are released, which helps elicit the lateral thinking to connect the dots that's so vital to scientific insight.
4. **Recovery:** at last, once flow is finally reached you settle into recovery. Serotonin, the "feel good" neurochemical, bonds you with a love for your work and (in group flow) for your team.

To see an illustration of this, check out how some scientists engage the "Release" phase, and how they learn from their observations, connect abstract ideas, and persevere through the cycles of breakthroughs and failures: **thermofisher.com/keepseeking.**

Why should you want more flow for you and your labmates?

As a scientist, you probably don't ignore things that can give you an edge to accelerate your research, so why ignore the triggers that quantitatively impact your creativity, motivation, and learning?

Creativity soars in flow, and there can be significant amplification of performance and productivity; this is why the best labs accelerate to become even better. Their ability to utilize individual and group flow and see creative solutions faster, gives them the edge.

Facebook, Google, and other companies leverage flow because it impacts creativity and innovation, so of course scientists should access the science of the brain in the quest to accelerate research.

So, what are these triggers and how do you hack flow?

Risk is one, but don't worry—you don't need to throw yourself into a 50-foot wave to leverage this trigger. The brain reacts similarly to both physical and emotional risk, so even speaking up in a big meeting or giving a speech at a friend's party can be enough to induce flow. As a scientist, some risk is already present in the form of the potential loss or gain of peer respect, of resources, of betting on an idea. Also, ask yourself: "Is my lab leveraging the advantage of the 'fail forward' culture of Silicon Valley?" If your team doesn't support the ability to take risks, and you're not actively incentivizing risk, you may be limiting the ability to get flow going in the lab. As a lab, you share goals and there is already some risk; but are you tapping in to the advantage of group flow, like a jamming jazz band?

Increase the novelty, unpredictability, and complexity in your environment, and as a result flow, innovation, and creativity will increase as well.

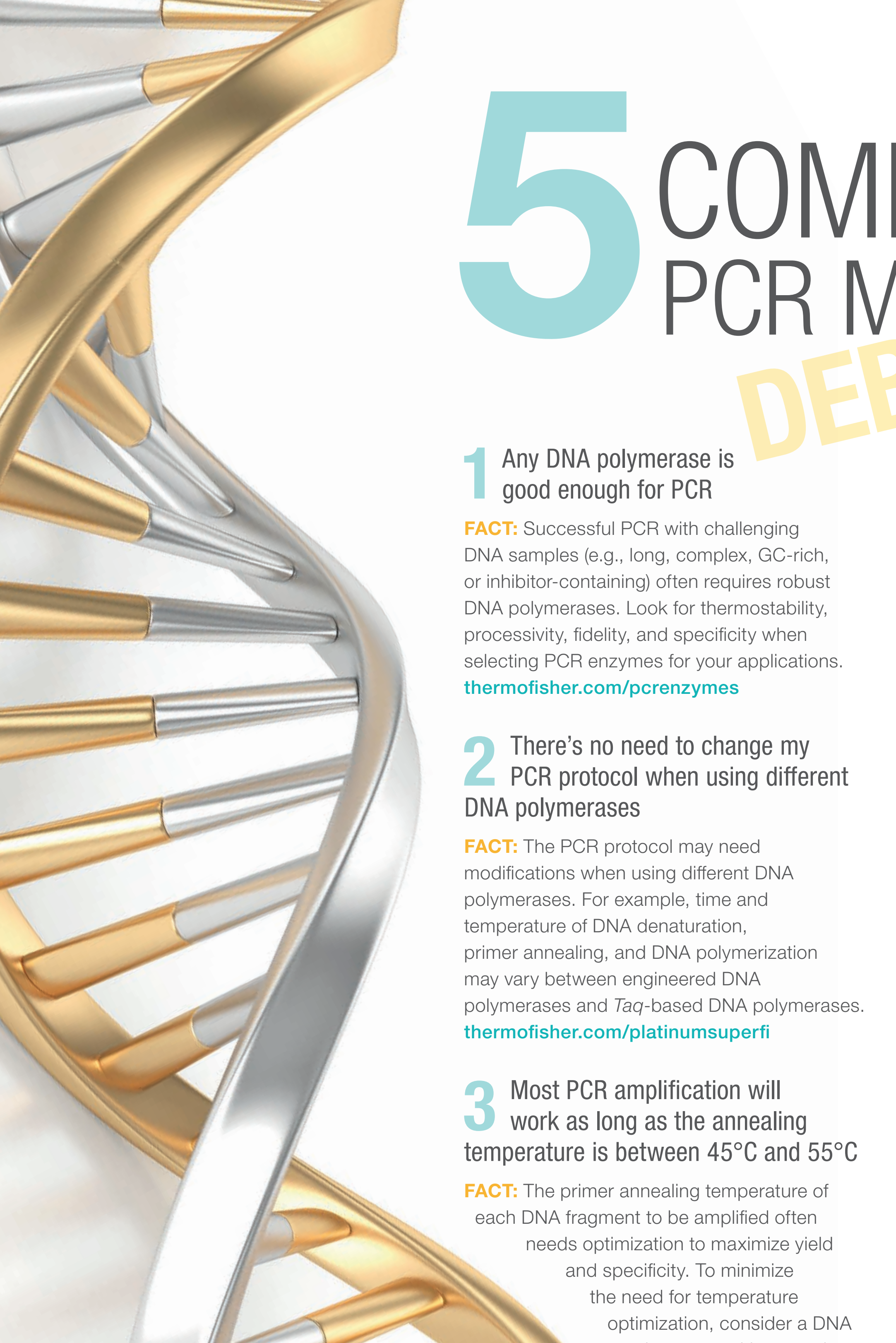
Kotler says, "Increase the novelty, unpredictability, and complexity in your environment, and as a result flow, innovation, and creativity will increase as well." As a scientist you may be thinking: Are you crazy? I need less, not more, unpredictability and complexity. But consider this. As a supplier of tools to you, we at Thermo Fisher Scientific are trying to reduce unpredictability and complexity. We hope this opens the opportunity for you to find novelty—for example, by stepping out of habits and routines once in a while. Autopilot and routines have their place (for example, when you're performing PCR for the 1,000th time), but they won't get you to the flow state. Look for opportunities to try something new (like trying magnetic beads instead of slurry for your immunoprecipitation), which forces you to think about getting to the result in a different way. Kotler says that even brushing your teeth with your nondominant hand will help get you going in the right direction, so try pipetting with your opposite hand. Essentially, going against the grain demands new focus and ups the degree of novelty.

One of our favorite flow hacks is leveraging free-writing. Write out a challenge or question you have with actual pen and paper. Then go do something completely different and relaxing that requires focus away from the question—slackline, yoga, or guitar. Then come back to your question and write, without stopping, everything that comes to mind no matter how it sounds in your head. Release is all about taking your mind off the problem. Michael Jordan practiced like crazy but often played golf before a big game.

Altruism, also known as "helper's high," is another trigger. Simply helping out a labmate can move you toward flow.

Remember, there are over twenty triggers of flow, and many are a great fit for use in the lab. If you want to know more, pick up the book or check out **flowgenomeproject.co/about**. Knowledge is power; and if flow strengthens optimal performance, then understanding the triggers of flow—both where it comes from and why it comes—can help you achieve optimal performance more often.

Reference: The Rise of Superman: Decoding the Science of Ultimate Human Performance. Steven Kotler, 2014



5 COMMON PCR MYTHS DEBUNKED

1 Any DNA polymerase is good enough for PCR

FACT: Successful PCR with challenging DNA samples (e.g., long, complex, GC-rich, or inhibitor-containing) often requires robust DNA polymerases. Look for thermostability, processivity, fidelity, and specificity when selecting PCR enzymes for your applications.
thermofisher.com/pcrenzymes

2 There's no need to change my PCR protocol when using different DNA polymerases

FACT: The PCR protocol may need modifications when using different DNA polymerases. For example, time and temperature of DNA denaturation, primer annealing, and DNA polymerization may vary between engineered DNA polymerases and *Taq*-based DNA polymerases.
thermofisher.com/platinumsuperfi

3 Most PCR amplification will work as long as the annealing temperature is between 45°C and 55°C

FACT: The primer annealing temperature of each DNA fragment to be amplified often needs optimization to maximize yield and specificity. To minimize the need for temperature optimization, consider a DNA polymerase with a reaction buffer that allows universal primer annealing at 60°C.
thermofisher.com/platinumiitaq

4 Gradient thermal blocks are the best technology available for optimization of primer annealing

FACT: Due to heat interaction between lanes, temperatures across a gradient thermal block follow more of a sigmoidal curve instead of a true linear gradient when setting up optimization of primer annealing in PCR. For more precise control of temperatures, consider thermal cyclers with three or more segmented metal blocks, each with a separate heating and cooling element.
thermofisher.com/veriflextechnology

5 Any PCR plastics are good to use in fast PCR protocols

FACT: PCR plastics with low profiles (reduced heights) and ultrathin walls (50% thinner than the standard) are specifically designed for running fast PCR. The shorter design minimizes the air space above the reaction, reducing the effects of evaporation and enhancing thermal conductivity. Thinner walls further reduce the thermal barrier, leading to faster and more robust reactions.
thermofisher.com/pcrplasticsselection

Find more technical resources on PCR enzymes, PCR plastics, and thermal cyclers at **thermofisher.com/pcreducation**

PRESENTATION TIPS

How to speak about your successes

Your personal brand is developed by the way you craft and convey your scientific journey.

PITCH

We mean the "elevator pitch." If you had 30–60 seconds to relay your story and scientific success, what would you say?

1. Ask yourself what are the three things you'd want a stranger to understand about you and your work.
2. When you start your pitch, start with the punchline. The sooner you can get to the most important things you want to get across, the better.
3. Start strong and finish strong. First impressions have power. Decide ahead of time how you would like to tidily end the conversation. Examples: extend an invitation to connect on the LinkedIn™ platform or ask for a business card.

POSTER

A scientific poster can be a researcher's point of pride. It's snapshot of your most recent success and a convenient visual aid to guide spectators through your scientific journey.

1. Capture attention by using your poster title as an advertisement for your poster. Details can be included in your conclusion.
2. Speaking of the conclusion, consider elevating it—visually. Conclusions often get buried, but they can be the very element that drives the point home for spectators.
3. Images and data speak volumes. Hone in on this by using figure legends and data titles to quickly provide your spectators with a "takeaway" message.

POWERPOINT

Comprehensive and flexible, the PowerPoint™ presentation format allows you to tailor your story to the audience. Plan ahead using these best practices:

1. Have empathy for your audience. Do you know who you will be presenting to? Design your content around the following questions: How much background will they need? How much context? What will be most interesting for them? What do you need them to know?
2. Use one idea per slide. It's said that if it takes more than 10 seconds for the audience to comprehend your slide, you've lost effectiveness in your presentation.
3. Keep it simple. It's tempting to use word art and special effects, but these can often be distracting. Choose a consistent color palette and add images if they add value. Leverage the power of white space.

LEARNING

Taking research to the next level through the power of education

Looking to try a different molecular biology technique? Need to brush up on your expertise? What about a review of molecular biology basics? The Invitrogen™ School of Molecular Biology is your one-stop resource hub for the latest techniques in PCR, reverse transcription, cloning and competent cell applications, and electrophoresis. Providing rich and reliable technical content, this learning resource is designed for both new and experienced molecular biologists.

Discover what you can learn at **thermofisher.com/molbioschool**

IMPACTING CUSTOMERS

The Invitrogen School of Molecular Biology is an excellent source of educational content that is helping the world understand the fundamentals of molecular biology. In the last two years:

- Over 1 million scientists around the world have accessed our content >2 million times
- The Molecular Biology Handbook has been downloaded >23,000 times

The content within the school is designed to be informative and educational, and the response from researchers has been phenomenal:

“Keep up the exceptional work! The PCR courses are remarkable!”

“Very interesting article”

“Wow thanks”

“Marvelous”

“Very educative”

Researchers use the content in a variety of ways, and on a global scale. Scientists in Japan, for example, have used the school’s educational modules in various ways:

“We would like to broaden the scope of our research”

“We are launching a new system and used this to review understanding and confirm operational procedures”

“Education of newcomers in the laboratory”

“Check the basics and see what you don’t know”

“To help you troubleshoot your current experiment”

Regardless of the need, the Invitrogen School of Molecular Biology is an excellent tool to help you and your lab expand your current skills within molecular biology. Be sure to explore the capabilities.

thermofisher.com/molbioschool

EDUCATION IN ACTION

Sometimes nothing explains a scientific principle better than a visual presentation. In the last two years, almost 3,000 researchers have been helped by our educational webinars; and on the YouTube™ video community, the PCR basic videos alone have been viewed >300,000 times. Here’s what actual users have said:

“I wish bio textbooks explained as well as these videos do”

“Wow this video was very well done. Thanks for educating me on what a PCR is in a quick and easy-to-understand way”

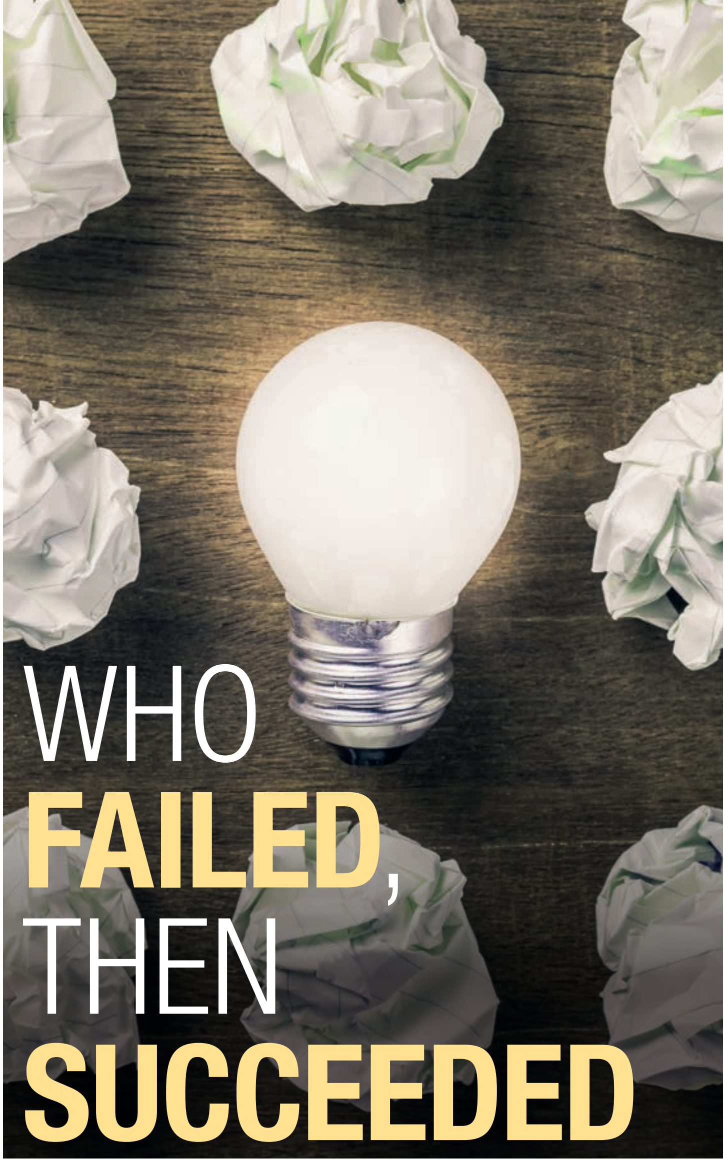
“Very very helpful”

“Simple and awesome”

Ready to learn more? Explore our offering of over 65 molecular biology videos and webinars, with topics including all the basics, protocols, troubleshooting, and useful tips and tricks to help you get answers faster.

thermofisher.com/molbiowebinars
thermofisher.com/molbiovideos

FIVE SCIENTISTS WHO FAILED, THEN SUCCEEDED



Scientific progress is driven by both breakthroughs and failures. It is said that failing fast and often will get you closer to achieving your goal.

Basic research can be littered with experimental blunders, human error, and the fizzle of theories. However, the idea of failure can be used as a motivational tool. Harnessed correctly, you can pave your way to success. *Scientific American*™ magazine reports on keeping a log of unsuccessful publishing applications to—as Melanie Stefan of Edinburgh Medical School puts it—"remind you of some of the essential parts of what it means to be a scientist—and it might inspire a colleague to shake off a rejection and start again." What defines a great scientist is how they learn from their observations, connect abstract ideas, and persevere through this long, noble, and very human journey. The whole of the scientific community is in this together. Read on to see how five famous scientists failed and then, ultimately, succeeded.

Rosalind Franklin

While deep in her X-ray diffraction work on DNA, she discovered there were two forms, wet and dry, that produced very different photos. Though a helical structure was observed, her mathematical analyses indicated otherwise. It was not until over a year later, through tenacious diligence, that she was able to resolve the two and conclude that there was a double helix structure in DNA.

Charles Darwin

He was harangued by his father for giving up on medical school. However, island-hopping on the Galápagos turned out to be a perfectly productive use of time, as made evident by what is now considered a core tenet of biology: the theory of evolution and natural selection.

Marie Curie

In her pursuit of science, she failed to realize just how damaging radioactivity is; but the credit for its discovery in 1898 goes to her. Furthermore, she developed the portable X-ray machine and used radium to cure disease. Perhaps most notably, in 1903 she became the first woman to win a Nobel Prize for her discovery.

Vera Rubin

She was disheartened, and her passion for astronomy rattled, when she was turned down by Princeton. Nevertheless, she continued on to Georgetown and earned a PhD. She was the first to make the observation that the speed of orbit of outlying stars matched the speed of stars in the center of the galaxy. It debunked the gravitational dogma of her time.

Thomas Edison

A teacher labeled him "stupid" early on; and although some of his over 1,000 patents were of questionable utility, many are now commodities we use daily, like the light bulb. He is quoted as saying, "I didn't fail 1,000 times. The light bulb was an invention with 1,000 steps."

RADIATING FROM THE HEART

Renu Chandra Segaran,
BS in Medical Studies from
the University of South Wales,
Research Assistant at Singapore
Nuclear Research and
Safety Initiative

If you could change anything about society's perception of science and scientists, what would it be?
That scientists aren't boring people that just sit in a lab and repeat experiments. We have our fun side and other interests too.

How would you explain your current scientific work to a nonscientist?
My current research involves studying the effects of radiation on the brain. Many people have heard about the negative effects of high-dose radiation and there has been a lot of fear surrounding incidents like the Fukushima nuclear power plant incident. My work is about trying to understand radiation better because at certain controlled doses, radiation does have therapeutic effects. It is also about obtaining a better understanding of radiation and our natural environment, so that we can minimize the risks to humans.

What's your favorite memory you have of your work?
Just spending time with my lab buddies, through the successful and unsuccessful experiments. We learn to laugh and cheer each other on. We also get to know each other as individuals, and learn about other things outside the lab through mixing with lab buddies of different cultures and nationalities.

Complete this sentence: If I wasn't a scientist, I would be a...
Photographer or an artist, capturing the beauty of nature around us.

Which science icon inspires you?
Albert Einstein. He once said that sometimes you're a golfer but sometimes you need to play tennis—you need a fellow scientist to challenge your ideas so that you can improve.

It's about serving humanity, not just now, but in the future.

Why did you choose to pursue science?
I have a strong interest in research and believe that it helps us find answers to many things we don't understand about life. While we may not always get answers immediately or the journey may be challenging, I feel that it's one that requires a lot of determination, and something that can pave the way for future generations. It's about serving humanity, not just now, but in the future.

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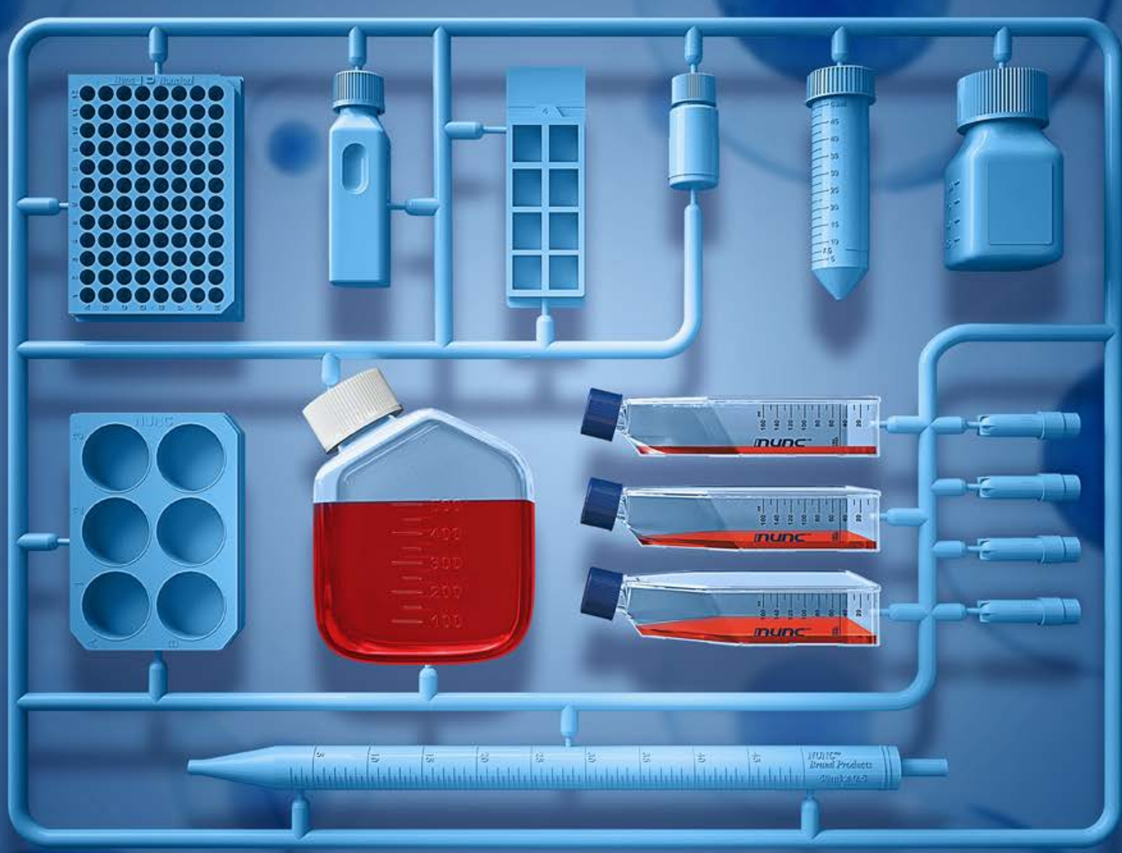

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