Text: Louise Hagbard, Ph.D. **Photo:** James Mayfield

STEM CELL INNOVATORS

TENNEILLE LUDWIG

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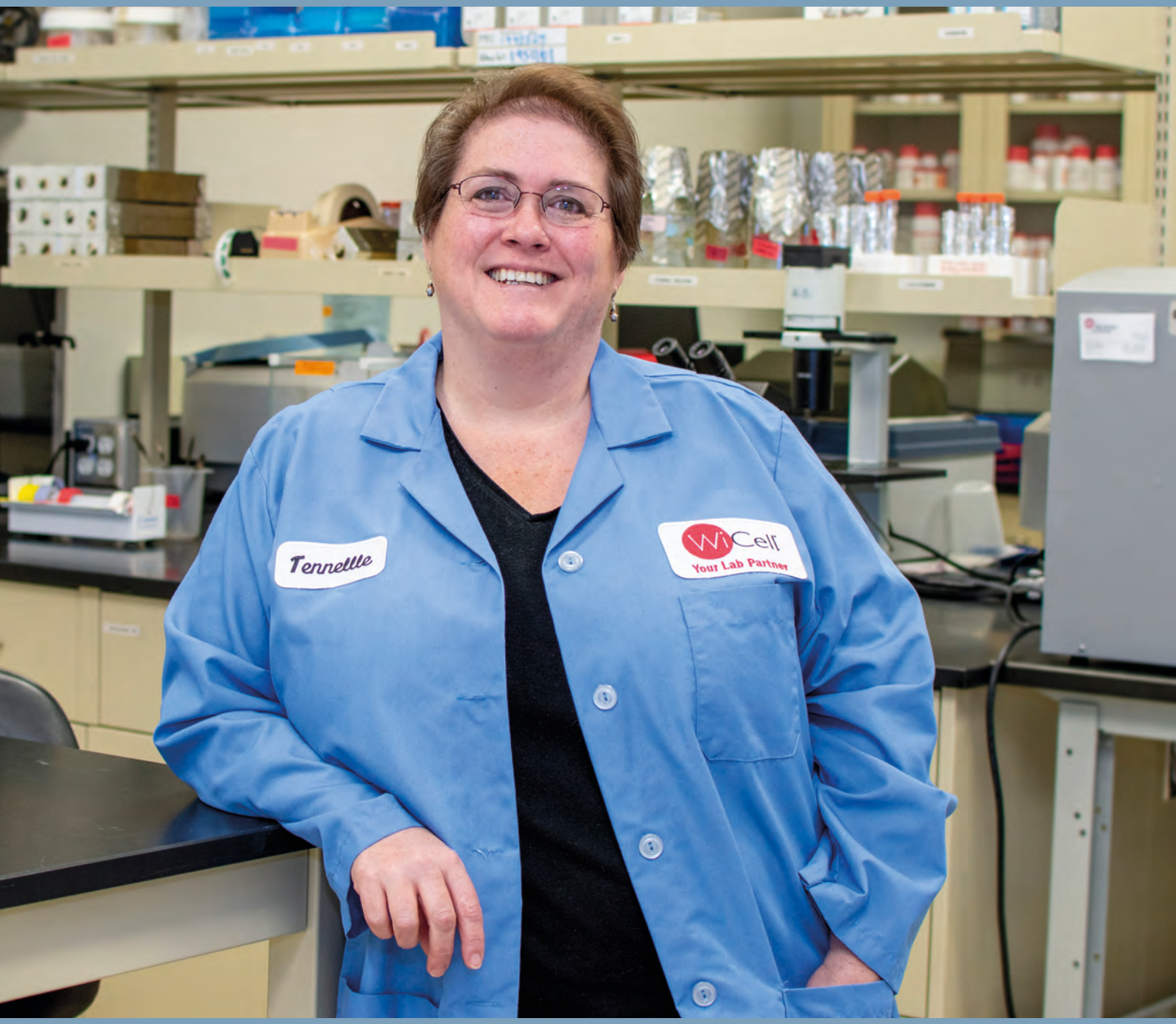
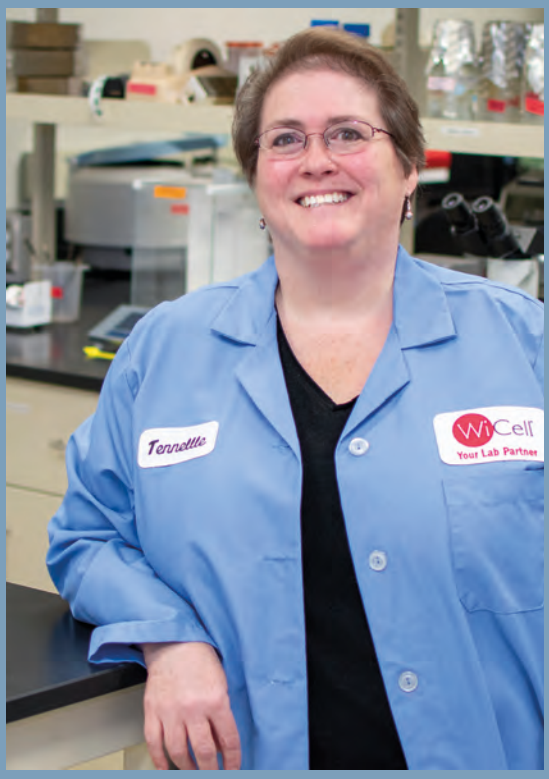


TENNEILLE LUDWIG, Ph.D.

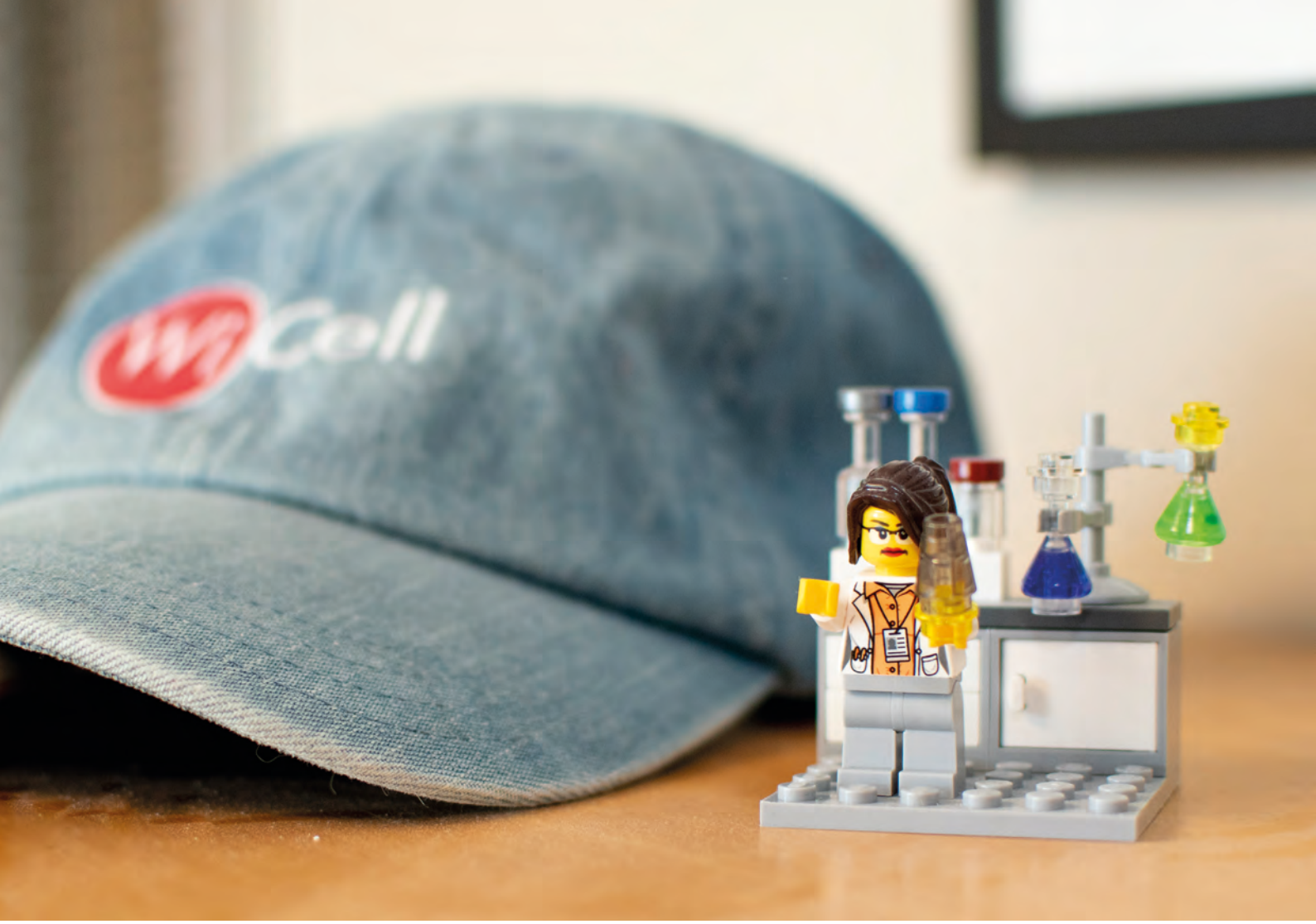
DIRECTOR AT WICELL STEM CELL BANK
MADISON, WISCONSIN, USA



ABOUT: Dr. Tenneille Ludwig is the Director of the WiCell Stem Cell Bank overseeing the banking, distribution, and core services operations at WiCell. Dr. Ludwig obtained a Ph.D. in embryology and developmental biology from UW–Madison in 2001. Between 2001-2007 she worked in Dr. James Thomson's laboratory where her work on the optimization of cell culture conditions resulted in the development of the first defined, feeder-independent culture system for human embryonic stem cells (TeSR/mTeSR). From 2005 to 2010 Dr. Ludwig served as the director of distribution for the U.S. National Stem Cell Bank operated at WiCell. Dr. Ludwig is a member of the Stem Cell and Regenerative Medicine Center at the University of Wisconsin and has served as a Scientific Advisor to multiple boards. She serves on the steering committee for the International Stem Cell Banking Forum (ISCBF) and operates one of the Core Laboratories for the International Stem Cell Initiative (ISCI).

WiCell

Recognizing the potential of Dr. James Thomson's human embryonic stem cells, and aware that regulations surrounding their use in a university setting were unclear, the Wisconsin Alumni Research Foundation established WiCell in 1999 as a safe haven for the advancement of stem cell research in the politically charged environment of the time. WiCell is a nonprofit, supporting organization of the University of Wisconsin–Madison and is a global leader in cell banking, characterization, testing, and distribution of stem cell lines. The organization also provides clinical grade pluripotent stem cell lines, quality control testing, and cell banking services. From 2005 through 2010, WiCell hosted the National Stem Cell Bank, which collected, banked, characterized and distributed the 21 hES cell lines approved for federal funding in the U.S. by then President George W. Bush. With the invention of iPS cells, WiCell expanded its cell line offerings to include both iPS and modified cell lines, forming the WiCell Stem Cell Bank. Today, WiCell's Stem Cell Bank is home to a growing number of cell lines offered across a variety of culture platforms. The collection contains cell lines submitted from researchers around the globe and includes the original National Stem Cell Bank lines as well as many modified cell lines useful as research tools.



How did you get into stem cell research?

"I was working on the University of Wisconsin campus finishing up my Ph.D. when Jamie (James Thomson) derived his original hES cells. My work at the time was in embryo development, specifically media optimiza-tion for embryo culture. When I completed my Ph.D. I started looking for postdocs, and they said, 'What do you want to do?' I'm like, 'Well, obviously I'd like to work with Jamie,' but one does not just call Jamie Thomson up. Clive Svenson, now at Cedars-Sinai, was working in Wisconsin at the time and looking for post-docs so I interviewed. We had a good talk, but he wouldn't offer me the position. He said, 'You're good, you're bright, I like you, but you don't know a thing about the brain. You'd be a benefit for Jamie. You should call him.' Then I spoke to Jeff Jones who was working in an IVF clinic and who's the first author on Jamie's paper and asked if he knew anybody who was looking in the stem cell field, and he said, 'You know, you should really call Jamie.' So, I sat down that night at 8:00 and I wrote an email to Jamie with my CV attached and essentially said, 'Hi, you don't know me. This is who I am. I'm really interested. I know you don't have anything in your lab, but if you know anybody in the field who's looking for a postdoc, please pass my resume along.' At 8:00 in the morning, I had an email back from

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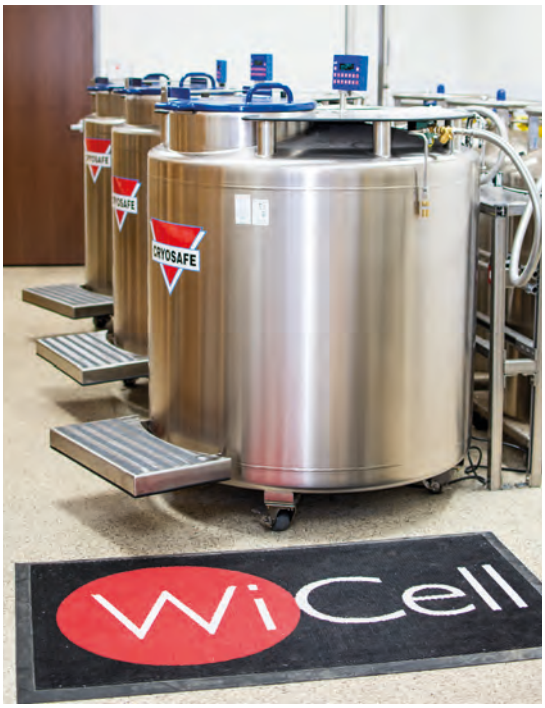
him that said, 'Come see me in my office at 10:00.' I went to talk with him and made an idiot of myself because I didn't know nearly enough about stem cells as I should have. The field was really young, and he asked, 'Why do you want to be in this?' I just started rambling, 'I want to save the world and cure diseases. It's just fascinating and the potential and ...' He stopped me, 'What I really need is someone to develop media.' ... 'I can do that.' This was around late September, and he said, 'Well, I would really like to bring you on, but I can't because I don't have grant funding for you until November 1st.' It was six weeks away – just 6 weeks. 'You know, I can wait. I'll just wait. I'll be here. I'll start November 1.' He said, 'Okay, we'll see you then' and that was that. At that point I had been looking for a post doc for 3.5 months, and I had just turned down an offer to go work for Harlan, the mouse company. And it wasn't the first position I had passed on. It was one of the moments where you turn down a job and think, 'Oh my God, I'm going to starve. That was a terrible decision.' I was living in a friend's house, sleeping in their spare room because I didn't have a place to live and didn't have a job, and then Jamie picked me up. He said, 'We need a serum-free, feeder-independent media within the next 5 years. That's your job.' We had it in 4. He carved out a portion of the lab for my exclusive use so we could keep things particularly clean, gave me great techs to help with the heavy lifting, a talented team to review data and bounce things off, and the freedom to do my thing – essentially all the resources needed to be successful. Roots and wings, right? I used to joke that since we finished a whole year early, I should have gotten that time off as a bonus! I don't think he was as amused as I was with the idea."

How did you come to end up where you are today?
"People ask me 'When did you know you wanted to be a scientist?' I have no idea. I started out wanting to be a veterinarian. At each turning point, each disappointment, each triumph, I just made the best decision I could about the next step based on what I knew, and what was in front of me at the time. For me it wasn't a single big decision. It was a series of defined small decisions that ultimately led to where I am now. You know, in most of my career, a lot of it has been serendipity. It's been about being in the right place at the right time or talking to the right person and getting just the right piece of information. A lot of it's very, very hard work, but a lot of it's being present and having a bit of



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HIGHLIGHTED PUBLICATION

Derivation of human embryonic stem cells in defined conditions.
Ludwig T.E et al.
Nat Biotechnol., 2006, doi: 10.1038/nbt1177

This article reports the development of TeSR1, a serum-free medium that supports the derivation and long-term feeder-independent culture of human embryonic stem cells. The authors describe the derivation of new human ES cell lines under defined condition that includes protein components solely derived from recombinant sources or purified from human material.

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luck. There are a lot of people out there who are working hard, and sometimes you get what you get because you stumbled your way into it, you know?"

Don't be so modest, you also had the personality that gets you along the way, right? You made it happen.

"That's very kind. Yes, to a point we made it happen... and I had some luck along the way. I don't want for one moment to suggest that other groups weren't working as hard, weren't as determined, and weren't doing excellent quality research. The reality of our field is that sometimes you work your butt off, and someone else gets there first. We worked very hard, we did good science, and we had a bit of luck in being first to publish. And then it was licensed and ended up being first to market – and first to market makes a huge difference in adaptation and adoption of the media."

"The other part of it is being nice to people – building bridges and relationships. You be nice to the administrative assistants who are helping with the grants, and the lab managers, and the contracts managers, and the guy who empties the trash can and the people who sweep the floors because generally they have been around forever, and they know how it all really works. They hear all the things and have all the information. And they can make things easier for you, or harder for you. And many times they have the ear of the people that you may need help from, you know? In this competitive field, it never hurts to be kind - to have people rooting for you."

When did you start working at WiCell?

"When I started, unlike now, Jamie's lab and WiCell were very interconnected. WiCell was initially founded as sort of a safe haven to do the stem cell research off campus. In the US at the time, there wasn't a clear understanding of whether or not stem cell research was going to be considered embryo research, and embryo research can't be funded by any federal money. The universities are pretty highly federally funded, so to do any sort of stem cell research on campus would have jeopardized all of the government grants at the university, not just stem cell stuff, but everything. The university could not have any stem cell research on campus until there was clarity on the position of the federal government. WARF put $1,000,000 into establishing WiCell off campus, and that was where the stem cell research happened."

"It was practically invisible. You would never know it was there. You could walk by it all day and never know it. It had no signs. It was just off campus in a little residential area. It was actually on top of the telephone substation, and you walk in and it's like this little clandestine hallway, and you go up the stairs and bam, there's the lab. The room itself was terrible - like a bunker. Only small windows, way up high so practically no natural light and no way to see what's going on outside. You could tell if it was overcast, and that was it. It was hot in the summer and cold in the winter. But it was close to campus, easily accessible to the research community, and fully outfitted with all the equipment you needed, with plenty of space for all the researchers that wanted to try this new technology. So, as rough as it was, it was a gift, and it was the place to be. It made for some really interesting collaborative relationships, because you had bioengineers and microbiologists and medical researchers from different areas of campus that normally would not have interacted together all in one place. It was pretty vibrant, even though it wasn't that glamorous. WiCell existed in that space for a very long time."

"WiCell opened another lab at University Research Park off campus to teach the education course, and bank and distribute cell lines. Jamie's lab at the time was still at the Primate Center on campus, in limited space. As my work was different enough from what was going on in the bulk of the lab, it made sense for me to be the one to move as space got more limited, so my group migrated out to the WiCell Research Park facility. I had a lab space and technicians physically separate from Jamie's lab, but I was still very much part of Jamie's lab. At one point, after 6+ years, with grant funding for the media project ending, Jamie and I talked about the focus of his lab moving away from media development. He asked if I would consider working

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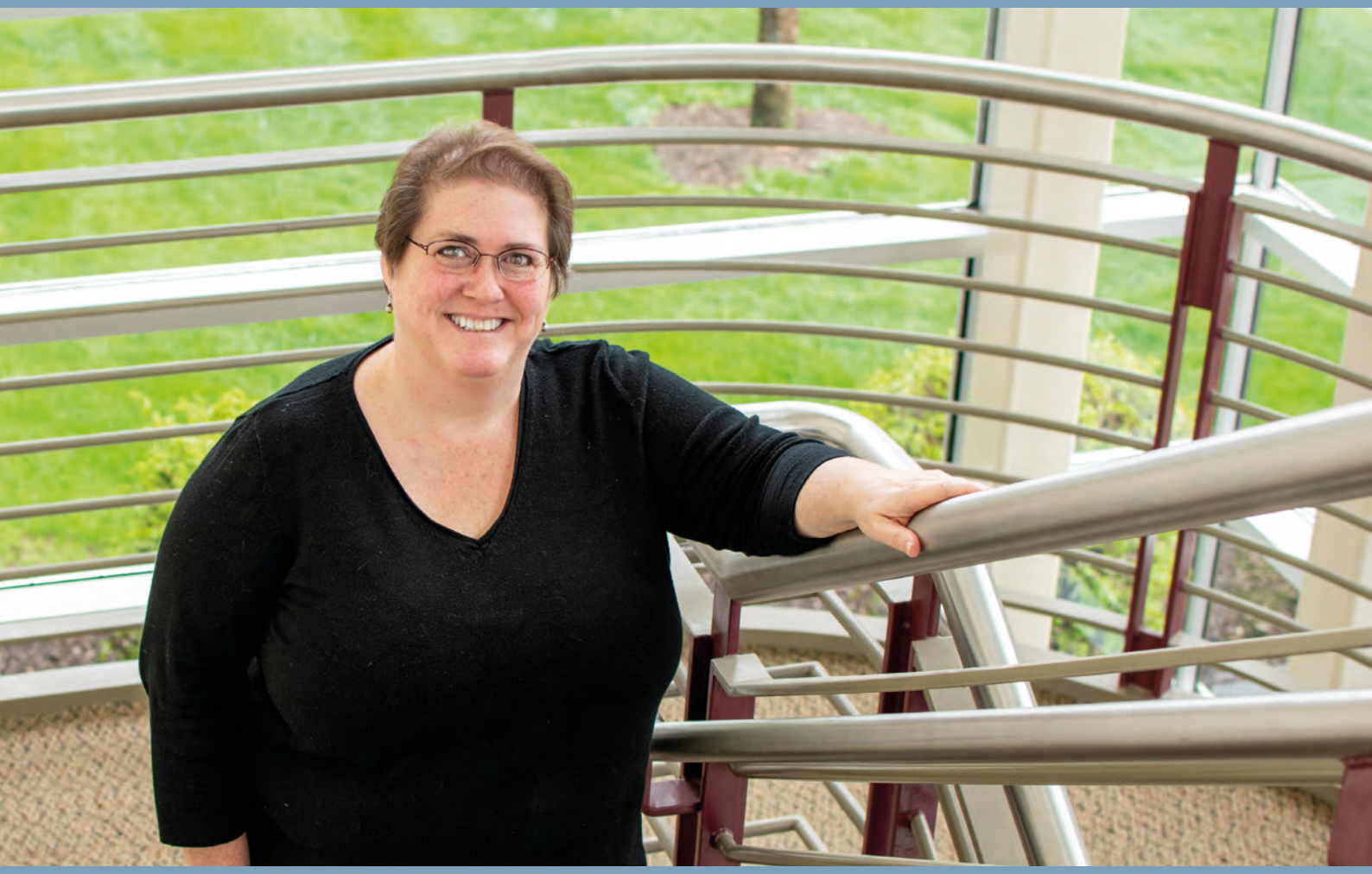

directly for WiCell. It made a lot of sense at the time. WiCell and UW won the National Stem Cell Bank contract, so there was funding there to continue some of the work, and be more involved in the quality control, characterization, and customer service associated with the stem cell bank. My office didn't change, my staff didn't change, my lab didn't change, just the name on my paycheck. It was sort of seamless to slide into WiCell at that point. The job has changed over the years, from more of a research position to more of a service position. As much as I loved the active research, I love what I'm doing now. This is the job I hope to retire from. I don't want to go anywhere else. I tell my boss they'll have to drag me out by my cold, dead feet."

Tell me a bit about your job!

"It's a really nice environment to work in. It's a flexible, collaborative work environment. You really feel that we are a team pulling together to help bring the field to its full potential."

"I lead the WiCell Stem Cell Bank portion of WiCell. I see it as more of a service position, which I really like.

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I've never been the person who had to have my name first on the paper. Professionally sometimes it's necessary, but personally I don't really care. I don't want to say I'm not highly competitive, but I can be thrilled with other people's success knowing we had a little piece in it, even if other people don't know we had a little piece in it. Personal satisfaction has always been more important to me than public praise. I like the idea of getting other people what they need to do the work that they do. It's a good fit for me, makes me

“Any time you’re using feeders or serum, you’ve got a biological product that’s going to be highly variable lot to lot, batch to batch, and that’s going to affect research. With a more defined system, you have more confidence that the differences that you’re seeing are due to your treatment and not a random effect of a variability in a biological product.”

happy. I spend a lot of time working with people with the university and other people in the general scientific community finding out, ‘What do you need? What cell lines do you need? What characterization do you need? How can we help you get where you’re going? What can I do to make your job easier?’”

“I travel quite a bit and represent the organization at national and international meetings. While the actual ‘getting there’ isn’t always great, I do get the opportunity to visit with colleagues all over the world, and hear first hand about new and innovative research taking place. I also get to dedicate some of my time to working with larger international consortia like the International Stem Cell Initiative (ISCI) and the International Stem Cell Banking Forum (ISCBF).”

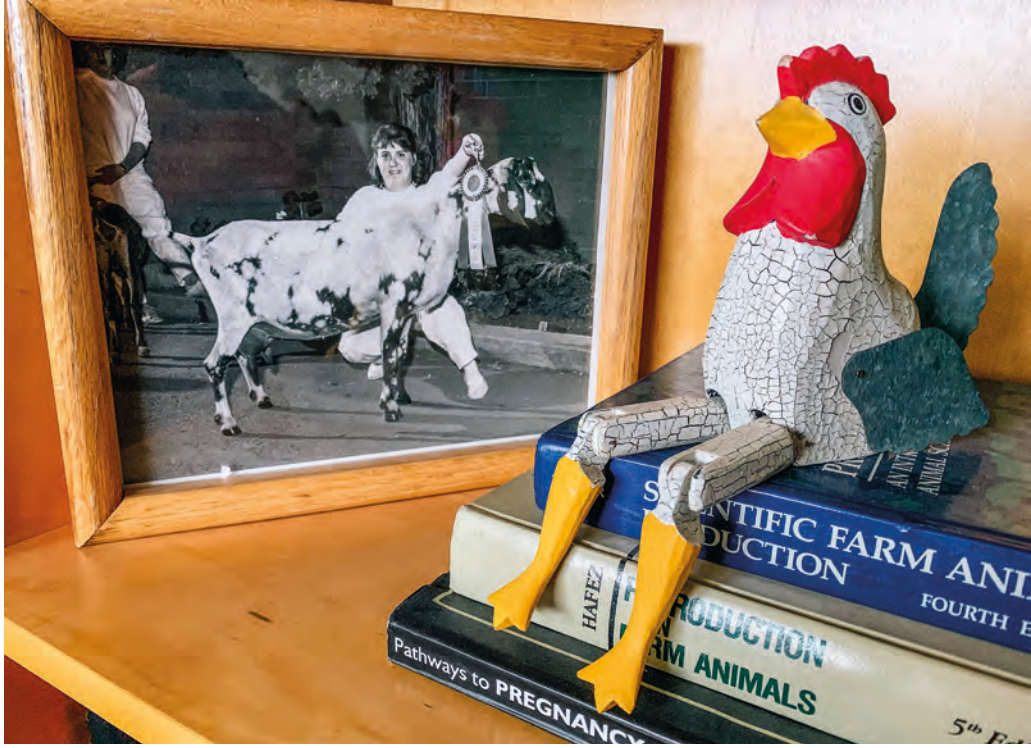
If you look at your career, what are you most proud of? Is it to have developed serum-free media?

“That’s certainly right up there. Clearly, I am biased, but I like TeSR and it makes me happy every time I see someone give a talk and mention that they used it in their research, and I know it’s working well for them. Yeah. It’s my baby. It’s still my baby. But I think I’m equally proud of the work we’ve done here at the Bank. We’ve moved form 21 lines available to more than 1,400 at the moment with more arriving all the time. To have generated and curated a collection this vast with the high level of quality we have is something I’m very proud of. And with the additional services we’ve grown to offer – contract banking, quality control testing – we’ve come a long way in the last 20 years. We have a great team of passionate people focused around a singular goal: to make a positive difference in research that will ultimately improve the human condition. So the Bank is also my baby. My second baby. And I would add to that the work with the ISCBI and ISCBF. I enjoy the time spent working with other scientists from around the globe discussing issues that we all face, and how to investigate and overcome them. I’m quite proud of the work that we’ve done as a part of those groups, and I’m both proud and honored to serve on the steering committees for both organizations. Actively engaging as a scientific community and working towards consensus standards is critical to the continued development of this relatively new field, and I'm pleased to be involved in that process.”

What was the biggest reason for Thomson and you, why did you want to go feeder-free?

“It creates a cleaner platform for research and ultimately clinical translation. If you’re doing a lot of this research, you don’t know what the feeders are contributing to the culture system, and so it’s difficult to sort out. The biological variability is huge, right? Any time you’re using feeders or serum, you’ve got a biological product that’s going to be highly variable lot to lot, batch to batch that’s going to affect research. You have more confidence that the differences that you’re seeing are due to your treatment and not a random effect of a variability in a biological product.”

“Jamie was thinking in terms of more defined systems early on. In our grants, which the US government had offered for the development of the original ES cell lines approved by then President Bush, he had included media optimization and moving to more defined media formulations as he considered these essential to the progression toward clinical application. Jamie recognized that instantly. The thing that he did that surprised folks was insist that the formulation for TeSR be publicly available as opposed to proprietary. We published the whole formulation, and step-by-step directions for how to make it. A lot of people were surprised by that at the time, but the reason was very research-focused. If people were going to use it, they needed to know what was in it because they needed to be able to evaluate whether or not what was in it was going to affect their research results. So, the entire formulation for TeSR was published. Likewise with Jamie



→ "The picture in the frame is in the bookshelf in my office. Reminders of whence I came. The picture was taken in the summer of 1985 at the Washington State Fair in Puyallup. That's a 17 year old me with my grand old Nubian doe Shalimar after winning a Championship ribbon for Dairy Goat Fitting and Showing.

"The biggest hurdle that everybody's going to face is financial."

and Kai's later work, the entire formulation for E8 was published. So, Jamie's real focus with the media work was to enable research, to give the researchers the resources and the information to be able to advance the field."

Regarding regenerative medicine and all the different protocols in trials right now, do you think regenerative medicine will be as big as we hope? Will it be an off-the-shelf treatment in the end?

"It seems pretty clear to me that it will at some point. I don't know how far out that is. I think it still may be further than the public will be happy with, but I think it's obvious based on the results already seen in current trials, particularly some of the ones with macular degeneration and spinal injury already. Even looking forward to those on the horizon like Lorenz Studer's work on Parkinson's. We haven't seen that trial yet, but it appears to be very promising. Personally, I think that the trials that are currently out there, many of them will be tremendous successes, but even if they weren't, they're such fabulous steps forward that it seems pretty clear that there will be off-the-shelf pluripotent stem cell-based treatments in the future. However, I think the bigger impact is going to come from what we learn about basic developmental biology."

In your opinion, what will be the biggest hurdle to overcome for the development of a successful cell-based treatment?

"Depending on the treatment, the hurdles are going to be different, biologically or scientifically. The biggest hurdle that everybody's going to face is financial. The amount of money that it takes to go through the clinical trial process and the need for venture capitalist investors to see a short-term return on dollars makes the funding harder and harder to come by even when it's very promising. Tumorigenicity testing, we've got to come up with a better way to do that. Right now, having to do it in small and large animal models takes years and hundreds of thousands of dollars. So, if we could develop a predictive test that didn't involve large animal models, that would be a major breakthrough."

Do you have any personal view on the regulatory system around pluripotent stem cells research?

"I don't have an issue with regulators or compliance. I think it's important and necessary, and it's designed to protect the patient. Some of the 'therapies' that we're seeing come out that are unregulated are concerning. We're in a very delicate stage with this work. Because of all the hype around stem cell research, and because of the real progress that we are making, you're starting to see groups capitalize on that offering stem cell treatments that really aren't stem cell treatments. Basically, one rouge group could mess up the field right now, or at least the perception of it. It's important to keep these groups in line, and the best option is the regulatory system. The regulatory agencies are very important, not only that they exist, but to ensure compliance. There's a push to be able to want to use some of these treatments before they've really been approved, because approval takes time and sometimes you're working with a ticking clock. Overall

"I've never spoken to an actual FDA regulator who didn't make a lot of sense and wasn't trying to be very helpful."

I think the FDA does a good job with what they're given. Clinical trials are expensive, but they ask for what they ask for, for a reason. All the interactions that we've had directly with the FDA have all been very positive. It's kind of interesting when I hear people say, 'The FDA will never let this happen,' and all I can think is, it's very clear to me you haven't talked to very many regulators because in my experience they never tell you never. They'll always say things like, 'Well, you know, it would be something for us to consider,' or, 'We'll have to see a risk benefit analysis on that.' Essentially, they will ask you to identify and mitigate risk, to provide a good analysis and back it up with data. My personal interactions are fairly limited, but I haven't met any of them that are unreasonable when you speak with them. I find that very helpful. I've never spoken to an actual FDA regulator or USDA regulator who didn't make a lot of sense and wasn't trying to be very helpful."

If you had unlimited amount of funding, like $100 million, how would you spend it?

"My answer probably won't surprise you. I happen to believe in resource cores. To see a centralized bank and a centralized core that could serve the international community would be how I would spend the money. A hundred million dollars is about the right amount of money to endow a centralized repository. There is no stem cell bank that makes money. We're a non-profit, and the stem cell bank operates at a loss. I think if you could use that money to endow a resource, that would help the entire community. You could get a variety of cell lines in, all sorts of wild type and controls, disease models, including orphan materials. You could bank them all correctly, you could characterize them all well, you could provide them to researchers and make sure that the materials that all the groups are starting with are clean and appropriate and get them off to a running start. You could give a real firm, solid foundation to the research, and ensure rigor and reproducibility. This would help everybody rather than put it in one particular indication or lineage. I think all the science is really important, but my whole focus is service-based. If you had that much money, you could make all the high quality starting materials free to everybody, routinely characterized."

"You know, we just submitted an abstract to the ISSCR meeting in LA. What it will show you is that thirty percent of the materials we receive from investigators, that they tell us that they have screened, that they believe is high quality material, has a significant problem. We see a wide variety of issues from failure to thaw to contamination, mis-identification, mycoplasma and other terrifying things."

"I'm very surprised by the amount of routine characterization that doesn't happen in many laboratories. Mycoplasma, karyotype, and STR screening, in my opinion, should be part of routine maintenance for lab cultures. This is one big advantage of freezing and characterizing materials early and being able to return to a tested, qualified bank regularly. You know what you're working with. Then just test at the end of the experiments to be sure you still have what you think you have, and it's not compromised. But it doesn't seem to happen in most laboratories. So, based on what we are seeing, even in good quality labs from well known institutions, if you're getting your materials from a colleague or researcher down the hall and not characterizing them before use, there is at least a 30% chance you don't really know what you're working with, and it could significantly impact your research or results. Yeah, it's scary. People need to know. So using the money to fund a global resource to solve this problem, for me it's a no-brainer."

I'm looking forward to seeing you at ISSCR in LA.

"Yes, ISSCR in LA! Lots to celebrate – it's going to be a big party, that's for sure. You guys are celebrating 10 years, and then we're doing 20 years of WiCell this year. I look forward to it. Looking forward to seeing your group! I've always loved the Swedish companies. Every interaction I've ever had from anybody from BioLamina or Cellartis/Takara has just always been really fantastic. I have always found all of the Swedish groups to be really collaborative and cordial and congenial. They'll tell you what they think right up front, in the best way. There are no games. Always as helpful as possible, and I have found that to be true across the board with the whole BioLamina staff, so I love you guys. I'm not a client yet. I've tested your products. I think they're fabulous and I recommend them regularly. I just don't use it a lot internally yet. At this point we would use it for cGMP work, but there isn't a project ongoing at the moment. One day..."

TENNEILLE LUDWIG ON BREAKTHROUGHS

? You've basically been along the ride for the last 20 years. What do you think has been the biggest breakthrough during these years?

! "I would love to say that it is the advent of xeno-free, feeder-independent media. It's not. I mean, I hope that that helped enable some of the other work, and I hope that it will make some difference to the work people do in the future. But it's got to be first iPSCs, right? It allows the development of disease models which may make a tremendous difference, and philosophically and politically it solves a potential ethical dilemma. Then behind that, the more recent development, CRISPR/Cas, to be able to edit and modify. Although all the little incremental steps that got to those places have to be recognized, but for major breakthroughs, it's got to be iPS and then new genetic technologies. "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