Key Considerations for Maximizing LV & AAV Production in Transient Transfection Workflows
Key considerations for maximizing LV and AAV production in transient transfection workflows

Leisha Kopp, Beth Larimore, Nolan Sutherland & Anindya Dasgupta

Recombinant adeno-associated virus (AAV) and lentivirus (LV) are essential components of gene and cell therapies, which show incredible promise for the treatment of genetic and acquired diseases. Accordingly, the need for large-scale manufacture of safe and effective viral vectors has never been greater. In this article, critical parameters for optimizing viral vector production are discussed, along with how TransIT-VirusGEN® Transfection Reagent and accompanying enhancer components can support manufacturers from research and development through commercial manufacturing.

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THE POTENTIAL OF CELL & GENE THERAPIES REQUIRES IMPROVED VECTOR PRODUCTION

Cell and gene therapies hold the potential to address diseases and indications that were previously considered challenging or even hopeless. These therapies commonly utilize AAV and LV vectors, and a critical consideration is the virus quantity required for creating effective therapeutics.

Gene therapies typically utilize AAV to deliver a corrected genetic sequence, and the average delivered dose is between $1.0 \times 10^{11}$ to $1.0 \times 10^{16}$ viral particles per patient. The treatment is administered in vivo and must impact the entire organ or system.
In addition, the treatment has to contend with the patient’s immune response. For cell therapies, where cells are modified outside of the body before re-administration to the patient, the required dosage is lower, i.e., between 1.0E+8 to 1.0E+10 viral particles per patient. Lentivirus is often used in cell therapies to transduce cells ex vivo which means that the virus does not have to contend with the patient’s immune response. However, a large amount of virus is still needed for a single patient dose.

Transient transfection is the critical first step in AAV and LV production and involves delivering a series of plasmid DNAs to HEK293 cells in culture. Transfection must be extremely efficient to enable cells to produce virus at the highest possible levels.

Within life science research, transfection is often considered a fundamental scientific tool. However, maximizing transfection efficiency for therapeutic viral vector production requires a deep understanding of all critical parameters – including cell density at the time of transfection, reagent-to-DNA ratio, plasmid ratios, total DNA amount, complexation dynamics and timing of transfection – as well as how these parameters interact in this intricate, complex process.

Many different components go into enabling successful transfection and subsequent gene expression. One of the most critical components is the transfection reagent used since transfection efficiency leads to higher virus titers and superior virus quality. However, with so many options available on the market, how can cell and gene therapy developers choose the best option for them?

**TransIT-VirusGEN® TRANSFECTION REAGENT FOR HIGH TITER VIRUS PRODUCTION IN MULTIPLE FORMATS**

The TransIT-VirusGEN Transfection Reagent is specifically designed by scientists at Mirus Bio for improved LV and AAV production.
over existing reagent formulations. During the development process, libraries of lipids and polymers were screened to find a formulation that effectively delivered multiple different plasmids to HEK 293 cells in a manner than maintained good cell health, so that the cells could produce high quantities of functional virus.

In addition to high transfection efficiency, VirusGEN also offers flexibility. Both LV and AAV can be produced using TransIT-VirusGEN in a variety of different adherent and suspension HEK 293 cell lines and cell culture formats, including T-flasks, HYPER-Stack® vessels, shake flasks and stirred-tank bioreactors.

Batch-to-batch reliability is also required with any commercial transfection reagent, and Mirus has demonstrated that similar performance can be expected with every VirusGEN lot and across reagent grades – from research grade to SELECT to the newly-released TransIT-VirusGEN GMP Transfection Reagent. No commercial license is required to use this product for research or further manufacturing, and it is chemically defined and animal-origin free.

Virus specific enhancer and complex formation solution components have also been developed to pair with TransIT-VirusGEN Transfection Reagent which further increase functional AAV and LV production over reagent-alone transfections.

TRANSFECTION OPTIMIZATION WITH THE VIRUSGEN AAV TRANSFECTION KIT

The following studies demonstrate the significant benefits TransIT-VirusGEN Transfection Reagent, with or without enhancer components, can bring to the transient transfection process.

FIGURE 2

Effect of cell density on transfection optimization.
Key consideration 1: choosing a reagent

As discussed above, one of the first variables to address when optimizing a transfection process is the reagent used. Here, AAV and LV was produced in suspension cell culture using TransIT-VirusGEN Transfection Reagent, with and without AAV enhancer, compared against two commonly used PEI-based formulations (Figure 1). TransIT-VirusGEN Transfection Reagent clearly outperforms PEI-based reagents in head-to-head comparisons for both recombinant AAV and LV manufacture.

Key consideration 2: cell density

Cell maintenance schedules and cell density at the time of transfection are additional...
critical parameters to consider for LV and AAV production, as robust cell health and division rate are paramount to a successful transient transfection.

This study involved lentivirus production in adherent HEK293T/17 cells. Cells were grown in DMEM + 10% FBS and transfected at densities ranging from 30–90% confluence at the time of transfection. Lentivirus production levels were measured 48 hours post-transfection for each condition. (Figure 2).

As the data indicates, there is a significant difference in virus production levels when transfecting cells that are too sparse, i.e., 30% confluent, versus cells that are 80–90% confluent at transfection. It is important to note that cells can be too confluent for transfection as well. If cells are 100% confluent, this may result in contact inhibition and cause cells to become senescent. Therefore, 80–90% confluence is a desirable target for transfection to maximize virus production levels.

Cell density is also an important parameter for virus production in suspension cells. In Figure 3, either AAV (top) or LV (bottom) was produced by transfecting cells at densities ranging from 1E6–4E6 cells/ml at the time of transfection. For AAV and LV produced using TransIT-VirusGEN® Reagent alone (i.e., no enhancers) highest titers are observed at 2 million cells per ml. However, when incorporating the enhancer components, maximum AAV and LV production levels were observed at 2 million cells per ml, and above.

It should be noted that the suspension cell type and the suspension culture media will also have an impact on growth rate and transfectability. Therefore it is important to evaluate for optimal density with each new cell type and media composition tested.

When working with suspension cells for AAV production, it is also important to passage the cells the day before transfection to ensure they are high viability and actively dividing at the time of transfection. Similar to adherent cells, suspension cells that have grown to a high density are less amenable to transfection and will underperform for virus
production until they return to log-phase growth.

Key consideration 3: cell culture media

Mirus tested a number of different serum-free suspension cell culture medias. Figure 4 compares Viral Production cells from Thermo Fisher Scientific adapted to four different media formulations: LV-MAX™ Production Medium, Expi293™ Expression Medium, and Freestyle™ F17 Expression Medium from ThermoFisher, and BalanCD HEK293 from Irvine Scientific. Higher overall functional titers were observed with the Irvine media, but it is important to note that the TransIT-VirusGEN Transfection Reagent performed well in all the media used, and the VirusGEN Complex Formation Solution and Enhancer increased titers over reagent alone in all four media. For lentivirus, similar results were seen again with a higher expression level using BalanCD media from Irvine.

Key consideration 4: reagent-to-DNA ratio & total DNA

Reagent-to-DNA ratio and total DNA per milliliter of culture are two additional parameters to consider, and both should be optimized for each unique recombinant virus and cell culture system. Figure 5 shows a comparison of AAV functional titers when virus was produced using 2:1 and 3:1 reagent-to-DNA ratios. Comparing both graphs, it is clear that the 3:1 ratio is inferior to the 2:1 ratio for AAV production.
For the 2:1 reagent-to-DNA ratio (Figure 5A), maximum performance is seen when delivering 2 micrograms of total DNA per ml of culture. For the 3:1 reagent-to-DNA ratio (Figure 5B), 1.5 micrograms total DNA appears to be the best choice.

Overall, it is key to find the conditions that provide a high virus titer. Fine tuning and testing different ratios with a specific vector set, cell type, and cell culture media is crucial in order to discover the truly optimal parameters for a specific viral vector expression process.

Considerations for harvest & analysis

When assessing viral vector titers, and especially when working with AAV, researchers will utilize either qPCR or ddPCR to measure the genome copies per ml of total culture (GC per ml). This is commonly referred to as the physical titer. In addition to measuring GC per ml, Mirus, routinely performs infectivity assays to determine the functional titer (TU per ml) of a virus prep since this measures the viral particles capable of transducing gene expression in target cells.

In Figure 6, physical and functional titers are assessed for four different transfection conditions and two different harvest points. Overall, similar trends are observed between the two titering methods (i.e., VirusGEN Reagent plus AAV Enhancer produces highest titers whereas competitor products produce lower titers); however, physical titer differences are less dramatic, as the genome copies measured via ddPCR are not necessarily capable of transducing target cells. For this reason, functional titer could be considered a more reliable measurement for the transduction potential of a virus population.

It is also important to consider harvest time points. With Mirus Bio’s lipid and polymer-based transfection reagent formulations, expression can occur sooner than what is typically observed with PEI-based reagents. Accordingly, we recommend testing several harvest time points (e.g., 48- and 72-hours post-transfection) to determine what is optimal for each culture situation.

**FIGURE 6**
Comparison of functional and genome AAV2 titers when transfecting with VirusGEN and VirusGEN plus enhancer.

Expi293 cells were transfected with TransIT-VirusGEN Reagent and TransIT-VirusGEN AAV Transfection Kit yield higher functional and physical titers than competitor reagents A1 and A3 (reagent for AAV production and PEI reagent, respectively) at 48 (red and green bars) and 72 hours (yellow and turquoise bars).
Q&A

Leisha Kopp
Applications Scientist, Mirus Bio LLC

Beth Larimore
Associate Director of Viral Vector Process Development, Bristol Myers Squibb

Nolan Sutherland
Scientist, Molecular and Vector Biology, bluebird bio

Anindya Dasgupta
Director Vector Development, Expression Therapeutics

TAKING A HOLISTIC APPROACH TO THE VIRAL VECTOR WORKFLOW

Mirus Bio supports customers throughout their viral vector manufacturing process. An optimized transfection workflow is not solely reliant on high performing reagents – it is important to understand how all the different aspects of the workflow perform together. Process development for each platform and/or target will empower manufacturers to truly get the most from their recombinant virus production which will in turn, lead to achieving higher viral vector titers results in fewer runs, cost and time savings, and ultimately, better and faster therapeutic development. In the following panel discussion, a group of industry experts listed above delves deeper into the challenges facing viral vector manufacture today, along with the potential solutions.

Q
Beth, throughout your career you have held viral vector process development roles of increasing responsibility at several prominent companies. What are some of the biggest challenges you faced early in your career, in terms of viral vector engineering and expression?

BL: I started my career in downstream purification and monoclonal antibodies. That experience gave me a good foundation on the principles of process development, and allowed me to see a very mature industry and platform.

When I transitioned into working on lentiviral process development, what stood out to me was how the industry was still developing. The clues to that were the viral process vendors, who didn’t have much, if any, experience to share with me. All of the tools were really designed for
protein purification applications. There were no GMP transfection reagents available at the time. We had tremendous challenges with the quality and supply chain for our large-scale adherent vessels. Lentivirus production in suspension was quite rare at the time. I would say the CMO landscape and the talent pool was pretty sparse. It was hard to find people with relevant experience.

Now, the industry is in an exponential growth phase, and I think most if not all of these bottlenecks have started loosening up. It is a really exciting time.

**Q** Nolan, maintaining high viral vector titers when moving from small-scale studies in shake flasks to large-scale production in bioreactors is notoriously difficult. In your experience, what steps can you take to ensure scalability, and what is the smallest scale and format you trust will translate to success at larger bioreactor scale?

**NS:** When moving from shake flasks to stirred tank bioreactors we definitely ran into a lot of challenges. The academic process we inherited out of the development groups just didn't translate. What we had going for us at the time was a rapidly maturing adherent process, which informed a lot of the bioreactor unit development.

Our main goals when developing a suspension process included increasing yield per batch compared to adherent, and an animal component free process. In addition, we wanted to adapt the unit operations of the adherent as closely as possible, based on the robustness of that process and any comparability required from regulators during a possible post-commercial change to suspension.

To assure a lot of our sustainability, we focused on breaking up the upstream process by those unit ops, and then evaluating and developing technologies which had scalability built in. We chose perfusion filters and other technologies from vendors who already had scaled products marketed. We also focused on off-the-shelf medias and reagents that would be easy to source. The transfection step was one of the most difficult to scale, and it required more creativity than leveraging available technologies.

When choosing a scale-down model, it is really a matter of where you are in your development cycle. We moved very quickly from scale up and tech transfer right into process characterization. As most people will know, process characterization has to be completed using a qualified scale-down model which is shown to be able to mimic the at-scale process at every unit op, either by an exact comparison or with a known offset in their outputs.

For LV processes, there are unit ops which can't be scaled down below the bench top stirred tank reactor systems, so that was the scaling cut-off for our model. Therefore, our qualified scale-down model is at the benchtop scale.

Finally, other development exercises that are maybe earlier in stage or more exploratory in nature could benefit from a higher throughput and lower cost system which could be a screening platform of some sort. There are a lot of these out there now. A lot of companies are coming out with scaled down systems, such as the Ambr® system from Sartorius, which are also stirred tank reactors.
For screening, a system can be chosen which doesn’t exactly mimic every unit of the qualified scale-down model, or the commercial scale, but if you can patch together those lapses and still produce vector with similar supernatant titers and other characteristics, then the model can be really informative for your process. Correlation to the bench scale is also key for your screening platform, where positive results from that screening model can be optimized in the qualified scale-down model.

I actually have an innovator insight in peer review on this topic, so stay tuned.

Q Anindya, in your recent role at Cincinnati Children’s Hospital you led a program to develop high titer lentiviral vectors. In your experience, what modifications to expression platforms tend to lead to the most significant improvements in expression levels?

AD: To echo Beth and Nolan’s points, you need to have a system that you can scale up. It is also advisable, although not always possible, to have a system that you can take to GMP manufacturing.

At Cincinnati Children’s, I was involved in GMP manufacturing, and I faced the situations and challenges that come in a clean room. In that process, we decided to first test a system that we never tested before – an electroporation-based device that has a GMP compliant version, so that you can integrate it into your manufacturing. You can scale it up from a mid-scale 20 billion up to 200 billion cells that you can electroporate in 30 minutes.

We optimized a lot of transfection protocols, and we got 95% or more efficiency. The most important modification that we did was the transfection method, which was electroporation. We found several factors that affect the optimization process. One is the type of vector producing cells you are using, whether it is adherent or suspension. Our legacy system is an adherent system, and you can go to an iCELLis® and other fixed bed bioreactors to do that. But there is a limit up to which you can scale up, so then the suspension system comes into being.

We tested both cell types in this transfection system. We found that cell density is very critical, and we had about 1 x 10^8 cells per ml. The ratio of the expression plasmid to the packaging construct is also critical, and if people are interested, there is a paper in molecular therapy [1] that talks about how that affects packaging of your lentiviral particles. It also talks about gag-pol mRNA as a surrogate marker that you can use to somewhat approximate your packaging efficiency, which is an interesting thought.

We found that the amount of DNA that you electroporate is crucial. Electroporation creates harsher conditions, so you are going to end up with more dead cells than you do in chemical-based transfection. Therefore, you need to have the right balance of the amount of DNA and of viability, and we found that about 200–400 micrograms per ml DNA is good.

We also found that the amount of DNA per amount of cells, meaning the number of micrograms of DNA, is also critical in the transfection process. Post-transfection, we found that the addition of DNase right after electroporation is extremely critical, especially in the survival of suspended cells. We found the effect to be minimal in adherent cell types.
We also tested transfection enhancers, particularly sodium butyrate, and we found it gives you about 1.5–2-fold more expression. But again, you need to consider the time of sodium butyrate addition, and the amount that you need. There is some optimization that needs to happen. Interestingly, we found that the enhancer had less enhancing effect on adherent cells, and it is more pronounced in suspension cells.

The way we cultured the cells was also critical. In adherent cells we stuck to our GMP media, which is basically your regular DNA with serum. But the situation changes when you start using suspension cells. We used a platform with serum-free media, and we found that some media was better than others. How you cultured those cells in suspension, and the type of vessel you use for cultures, is also important.

You can also think about changing your temperature. It has been suggested that 37°C is optimal, but if you go down to 32 °C you can have more pronounced expression of your LV.

The time of harvest was also critical in this system, and we found that 24–48 hours for transfection is ideal. 32 hours is also not bad.

So, we found that this electroporation-based transfection system, along with several modifications that we used with that, gave us a titer, and unconcentrated titer, from 5 x 10^7 to 5 x 10^8 infection units per ml. And again, the whole point was to have a scaled down version that we can use in a GMP process.

**Q** How would a two to 10-fold increase in viral vector titer change your production workflow? What aspect of the overall expression process would higher titers improve the most?

**BL:** The major impact would be that we can do fewer runs, and a lot of benefits would flow from that regarding cost of goods and supply availability. It would reduce the quality control tax on the lot and reduce the full-time equivalent hours per released vial. For those of us who are reliant on CMOs, we are really at the mercy of their calendars, so fewer lots would have lots of benefits.

**NS:** If you are looking more towards the oncology landscape, increases in productivity are absolutely required to meet the needs of that area. Comparatively, the severe genetic disorder landscape does not have the same scalability requirement, and is more focused on supply.

There are also considerations from an upstream perspective. We are constantly trying to increase our titer, but there are also downstream impacts to that. You need to be working very closely with your purification group to make sure that impurity profiles and capture steps are scaling as you are increasing those yields.

**AD:** Even a two-fold increase is a tremendous advantage. It also depends on your disease indication. Some indications may not need that number of particles, and some may. When you are developing your process, you need to be prepared to test or optimize two or three processes, and adapt that to your manufacturing needs.
Where do you anticipate seeing the greatest cost savings from significantly improved LV or AAV titers?

NS: In my opinion, the greatest savings will be reduced cost of overall manufacturing – both the vector process and the cell therapy process – due to higher yields producing more vials per patient.

Increasing those yields in a single batch will lower the demand of vials for your cell therapy process, and you will be able to treat more patients with a single batch of vector.

We have a lot of room to significantly impact those calculations, purely based on yield. Unfortunately, the consumables around the transfection process will probably remain high in cost, so there is a huge balance to be focused on when looking at cost of improvements versus impact per yield.

A lot of our focus is on developing a next-generation process which reduces deviations, is more robust, and overall increases yield from previous generations, whether through increases in productivity or through more reliably being able to consistently produce across batches. LV processes can be more variable in yield than other biologics, so increasing process robustness is also just as important as increasing your process yield.

Specifically to the vector process, we may actually be creating an overall more expensive process in terms of operation through extending it to run longer, but this again will be balanced with cost per titer unit, which we are really trying to decrease. Dollar per titer is really what we are trying to decrease at this point.

LK: Our focus has always been on optimization, creating higher titers and finding different solutions – whether that’s cells, media, or vector sets – that enable those higher titers. What we hope to see is a reduction in the number of runs required, and ultimately, enabling customers to produce more molecules faster, because this field moves at a lightning speed.

What methods or assays do you employ to determine upstream viral titers and virus quality?

AD: We are based on technology that was developed for particular use over the years. It is a functional titer, and at the moment there is no substitution for that. You can still use the physical titer, of course, that is p24, or you can do a genomic titer. You can also use something like product enhanced reverse transcription to measure the reverse transcriptive assay, but those are the most physical properties, physical ways, and functional categorization is through a functional titer infectious-based method.

I strongly believe that there should be more in-line processes to calculate your amount of genome particles. You will have empty particles, partially filled particles with spliced host DNA, and you will have full particles. There are things that are coming up now, and the FDA would probably be interested in having this data as well, as part of in-line processing that can be done quickly to see how your optimization is taking place.
**BL:** I agree with Anindya. One thing to add is that as our assays become more sophisticated, it is really clear that your process optimization is only as good as your assay. So if you have a new, more sophisticated assay, and you go back and look at your previous optimization, you may not have been optimizing the right things.

**NS:** For upstream titers, we typically have either a GFP titer, which is cell-based, or genomic titers that are qPCR-based. Like the other panelists, we also calculate our particle to infectivity (P to I) ratio. We do particle based on an ELISA against the P24 protein, and the LVV particle, which is a capsid protein, and we are looking to equate how many particles are actually infective in our prep.

On the upstream side that is something we are looking to optimize. We are looking to make a higher number of infectious particles; lowering that P to I ratio.

Something else we are looking into is trying to get away from these cell-based assays, and doing more instrument-based determination. What is coming up in the field now is a lot of nanoparticle flow cytometry. With this we can not only probe for envelope proteins, but probe for genomes. This means you can start to equate and determine empty versus full LVV particles as well as total nanoparticles, and the psuedotype-positive particles that have infectivity based on something like VSVG, versus a non-infective, non-psuedotype particle.

**Q** Leisha, have you characterized empty/full content with the Mirus transfection reagent, and how does that compare to empty/full content with competitor agents?

**LK:** This is something that everyone producing AAV is struggling with: how to generate more full particles versus empties, and how to characterize them.

Infectivity assays, which we often do at Mirus, are great because they will only measure functional viral particles – those that are completely full capsids. But it’s worth noting that there are caveats to that. It is a cell-based assay with some inherent variability. There is also serotype and cell type compatibility to consider. At Mirus, we often functionally titer by transducing HT-1080 cells with AAV2 and this works very well. AAV2 transduces a lot of different cell types well. However, if you switch to something more challenging like AAV5, which doesn’t transduce many cells well at all, that functional titering assay becomes much more of a challenge and perhaps less reliable, because not all AAVs that work well in vivo will work equally well in vitro.

This is why companies heavily rely on those GC/ml measurements, ELISAs, and the ratio of the two to determine percent empty/full capsids. We have all acknowledged on the panel here that this assessment method is not ideal. It is definitely not 100% clear what you are really reading there. We have started looking at analytical ultracentrifugation to try to get a better idea of how that all teases out in terms of percent full, or percent empty. The problem with AUC is that it is not necessarily a high throughput method, but I know there are companies working on that to address the need in cell and gene therapies.
The methods that everyone spoke to are also really valid. It is clear that we need to improve analysis methodologies. There are a lot of different teams working on that now that we are talking with.

But back to functional titering – the TU/ml measurement you obtain will be from full and functional capsids so if using as a relative measurement where you are comparing different expression systems, the data is likely still valid. This is also where VirusGEN tends to shine – the ability to produce fully functional viral particles compared to competitor reagents.

**Q** How would you approach optimization of viral vector expression at small scale, and does that change when shifting to larger expression formats such as the iCELLis bioreactor for adherent, or stirred tank bioreactors?

**BL:** Overall, I would say we are very cautious with scale up and scale down. We systematically check and double-check the reproducibility of our results at small-scale, pilot-scale, and full-scale. If we do an optimization experiment at a given scale, we are cautious to make only internal comparisons among those samples. But we also retain samples so that later we can make direct comparisons across scales in a single assay.

**NS:** As I mentioned before, we have utilized an Ambr® 15 system for a lot of small-scale studies, and that is a great system. A lot of the limitations are around the perfusion steps at a 15 ml bioreactor scale – with any experimentation that is looking at perfusion rates, you know they aren’t that translatable to the at-scale or the bench scale process. But for other aspects you are looking to screen in terms of titer, transfection enhancers, or media components and things like that, it is pretty indicative.

If you can match your cell growth kinetics, and you can match your transfection kinetics in these systems, they are typically quite indicative outside of that perfusion step.

**Q** Leisha, is the Mirus AAV enhancer available to purchase?

**LK:** Right now it is not available to purchase. We are beta testing the AAV Enhancer, which will be a component of a the VirusGEN AAV Transfection Kit launching in either July or August of this year. If someone is interested in evaluating the VirusGEN AAV Kit, we can certainly provide them with research grade material, so that they can test. We will also work together with them on optimization.

**Note:** The VirusGEN® AAV Transfection Kit and VirusGEN® GMP LV Transfection Kit are now commercially available.

**Q** ... and do you have any data regarding retroviral production?
LK: We have focused mostly on lentivirus production, and of course those are very similar. We have several customers that are using VirusGEN for retrovirus production, and it is definitely very compatible, but we don’t have that data internally.

Q Finally, what advice would you offer to a startup biotech or pharma company entering into the cell and gene therapy space?

BL: What I often see is a startup company that has a great idea, something that is super promising, and they then want to scale up. My advice is to get an experienced CMC person in early. A lot of decisions that are made early can continue to have ripple effects. For example, if you choose the wrong kind of serum or wrong raw materials. Going fast and trying to fix things later can often cause lots more headaches. I always advise to try and do things right the first time.

AD: I believe that how you prepare for your pre-IND application package will drive you towards fulfilment. As Beth alluded to, there are all these factors to scale up or out in later stages of production. If possible, use the scale-down version in your initial developmental studies as soon as you can, so that you can integrate that as you scale up, because often things don’t scale up as you desire.

NS: Bluebird can probably be seen as a case study for going from startup to commercialization, so anyone can look into our history and what works and what doesn’t work. We have such an immediate impact to patients with these therapies, and it is very easy to be patient-focused, and it is easy to lose sight of your people. A huge focus should also be on the people. It is a very competitive landscape, and it can be a very stressful landscape in doing some of this work. Foster a culture at the company that is open, honest, and transparent with the employees. Because there are just so many challenges, and also all these products are going at lightning speed. There is a lot to learn, there are a lot of inherent challenges, and at this point in time it is really hard to retain people if the culture is waning.

LK: Don’t skimp on the optimization steps. I know everyone is trying to move at a really fast pace, but it is definitely worth pausing and looking at all parameters to ensure your stealth isn’t tripped up by a lack of attention to details. Also, don’t be afraid to reach out to others that have experiences or expertise that you may lack. Scientists are generally pretty collaborative. If you find teams at pharma companies or in medical research organizations that are able to talk and share their experiences, utilize and learn from that.

REFERENCES

Leisha Kopp
Applications Scientist, Mirus Bio LLC

Leisha Kopp is an Applications Scientist at Mirus Bio LLC, a biotech company providing innovative transfection products to cell culture researchers worldwide. Leisha has over 15 years of molecular biology and mammalian cell culture experience in industrial labs, and her combined bench and business knowledge enables support of scientists in all stages of the drug discovery process – from R&D to commercial manufacturing. Leisha is a graduate of the University of Wisconsin-Madison, with key interests in biotherapeutic antibody discovery and gene therapy.

Beth Larimore
Associate Director of Viral Vector Process Development, Bristol Myers Squibb

Dr. Beth Larimore is Associate Director of Viral Vector Process Development at Bristol Myers Squibb and is based in Seattle. A Bay Area native, Beth earned a BA in Biological Sciences at Cornell University and a PhD in Molecular and Cellular Biology at the University of Washington, where her doctoral work focused on the basic biochemistry of a tumor suppressor protein frequently mutated in human cancers. Beth started her industry career in purification of antibody therapeutics, but has worked on viral vector process development since 2014. She has held process development roles of increasing responsibility at Amgen, Immune Design, Nohla Therapeutics, and Juno Therapeutics/BMS. She is motivated by challenging scientific and engineering problems and feels privileged to contribute to novel, life-changing therapies.

Nolan Sutherland
Scientist, Molecular and Vector Biology, bluebird bio

Nolan Sutherland graduated from the University of Massachusetts, Amherst with a BS in Biochemistry and Molecular Biology where his research areas of focus were on molecular cloning and RNAi. Over the past 10 years he has worked in both Upstream and Downstream Process Development groups, getting his start in the area of enzyme replacement therapies before entering the gene therapy field at bluebird bio. Since that time, the majority of Nolan’s work has been focused on developing suspension-based lentiviral vector production systems for commercial manufacturing. His areas of expertise include process establishment and improvement at the miniature and bench-scales, emerging technology evaluations, transient transfection optimization, nanoparticle analysis and characterization, as well as scale-up and technology transfer. With a new role in viral vector R&D, he aims to support the development of novel vectors for in vivo use.

Anindya Dasgupta
Director Vector Development, Expression Therapeutics

Anindya is the director of vector development at Expression Therapeutics. He obtained his PhD from University of South Carolina, USA. His post-doctoral training and research associateship at the school of medicine, Emory University, Atlanta, USA, were focussed on the evaluation of novel anti-cancer therapies and the development of strategies for expansion and lentivirus based bioengineering of γδ T cells in serum free media. Anindya is a co-inventor of a patent on anti-cancer strategy. At his recent role at Cincinnati Children’s Hospital Medical Centre he led vector development to manufacture high titer lentiviral vectors.
AUTHORSHIP & CONFLICT OF INTEREST

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With industry-leading performance in high-titer virus manufacturing, the VirusGEN® GMP Viral Vector Production Platform offers a simplified, cost-effective workflow, making it the superior choice for large-scale therapeutic lentivirus and adeno-associated virus (AAV) production.

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