Plasmid Pain Points In The Gene Therapy Pipeline

Gene therapy has had its share of setbacks, but finally gene therapies are proving successful at treating diseases like neuromuscular disorders, cancer and blindness. Adeno-associated virus (AAV) has emerged as a leading gene delivery vehicle because it is efficient and has demonstrated safety in humans. Although obstacles remain, optimism about AAV gene therapy has spurred an influx of potential gene therapies into the research pipeline. Biotage is helping to speed up the flow of research with automated systems such as their PhyPrep plasmid purification system.

Mark Champe manages a gene therapy research lab in South San Francisco, previously called Audentes, which is now part of the Japanese pharmaceutical company Astellas Pharma. Before being acquired last year, Audentes Therapeutics had developed a gene replacement therapy for X-linked myotubular myopathy. X-linked myotubular myopathy is a devastating disease, causing extreme muscle weakness and respiratory failure. It is lethal in half the boys who have it and the other half will require a ventilator to breath and a wheelchair for mobility.

In clinical trials the Astellas gene therapy for X-linked myotubular myopathy has brought marked improvements for some kids. “The kids can get off the ventilator, they can stand up, walk around, throw balls. That’s just a striking example of what gene therapy can do”, says Champe.

Since being acquired by Astellas, Champe says that his workload has increased. Their research pipeline includes gene replacements and splicing modifications for neuromuscular diseases such as Duchenne muscular dystrophy, Friedrich’s ataxia and myotonic dystrophy. The main challenge he faces is throughput. “We have lots and lots of requests for plasmids from different groups and we’re constantly doing plasmid preps which had been time consuming and tedious the way we had been doing them.”

Plasmids are the common starting point for many therapies including DNA vaccines, monoclonal antibodies, viral vectors and mRNA vaccines. For Champe’s lab research group, preparing AAV vectors for gene therapy involves constructing DNA plasmids with the desired gene flanked by nucleotide sequences that will later instruct that gene to be packaged into AAV vectors.

The plasmids prepared by Champe’s research team, which contain the DNA to be introduced into AAV, are passed on to another team which transfects them into mammalian cells along with the helper plasmid. The helper plasmid contains the genes that encode viral proteins that are the structural components of the virus as well as DNA polymerase that makes the single stranded DNA of interest that goes inside the virus. The cells then produce AAV virus carrying the gene of interest which is collected and used either for animal or in vitro studies.

> “PhyPrep was just extremely attractive for us for its walk away automation ability”

In order do the necessary research on gene therapy candidates Champe and his colleague need to prepare AAV plasmids in large quantities of around 10 milligrams (Giga scale), which is very labor intensive. “We were getting to the point where half the time was spent just doing plasmid preps”. Plasmid preps are a pain point many biomedical researchers can relate to.

To alleviate that pain in their research process Champe’s lab turned to Biotage which has an automated plasmid prep workflow solution. The Biotage automated PhyPrep system uses anion exchange columns, similar to the most commonly used manual plasmid purification kits, to capture negatively charged DNA. Both types of plasmid preps begin the same: growing the plasmids in bacteria, collecting bacteria cells by centrifugation, breaking those cells open by alkaline lysis and precipitating the proteins and chromosomal DNA.

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If you are using the PhyPrep system, this is where the manual work ends. You hand your samples off for automated column capture and washing, and collect your plasmid DNA about one-two hours later. “PhyPrep was just extremely attractive for us for its walk away automation ability”. Champe contrasts this to manually using drip columns where “you’re always going back to fill the column, so it interrupts other things that you’re doing.”

It only took a few tries before this benchtop robotic system was increasing productivity in Champe’s lab. “It just allows us to do more with the number of people we have”. Their lab’s plasmid production has gone up from 2 Gigapreps in a day to 8, allowing them to move more gene therapy candidates forward along the pipeline.

There have been no compromises in the quality and quantity of the DNA produced after switching to the PhyPrep system. One of the differences between Biotage PhyPrep and manual plasmid purification preps is that the former enables the elimination of the final alcohol precipitation step. This advance is enabled by the higher plasmid concentration and lower salt concentration in the eluted DNA solution. Champe likes this more streamlined process which has minimized yield losses without impacting the transfection result. Now there is no waiting for precipitated DNA to resuspend and the DNA filter sterilizes much better. Another time-saving aspect of the PhyPrep system is that reagents come premeasured. “The wash buffer, you have to measure with a graduated cylinder but that’s it. Everything else is just prepackaged in the right quantities. That definitely saves time.”

Automation in the laboratory is not a new thing for procedures that use very small volumes of fluid or industrial scale plasmid purification, but Champe says the PhyPrep automated plasmid prep system is the first time he has seen plasmid prep automation for the Maxi, Mega and Giga scales he needs in a preclinical research lab setting. “I’m surprised it took so long to get here. I’ve been doing this kind of stuff for over thirty years. Up until just recently we had been doing them very much like I did in graduate school in the early eighties, so not that much has changed in plasmid preps.”

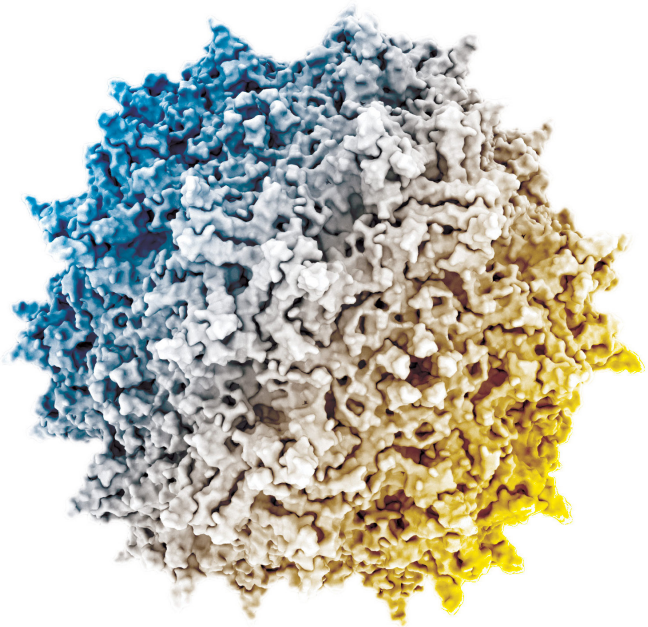
DNA is one of the biggest input costs in AAV gene therapy because cells are transiently transfected each time with two or three plasmids. “Doing the transient transfection each time, on the face of it, seems like a silly way to do it large scale,” says Champe. The cost of preparing large quantities of GMP grade plasmid material to make AAV for clinical trials and therapeutic product is a challenge yet to be overcome.

Other biotherapeutic products like monoclonal antibodies are produced using stable cell lines. Champe says his research group and others have tried to make stable cell lines for AAV gene therapy, but for now, transient transfections still achieve better virus yields.

If other biotherapeutics have overcome production costs, there is hope that AAV gene therapy will as well. Gene therapy has the potential to improve the lives of a lot of people. In the meantime, Biotage’s PhyPrep automated plasmid purification system is improving workflows for biomedical researchers and helping them move gene therapy research forward.

Learn more about the PhyPrep automated plasmid purification system here:

https://www.biotage.com/phyprep-maxi-mega-giga-product-page



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