

Q&A WITH AJI BIO-PHARMA: PLASMID MANUFACTURING FOR GENE THERAPIES

An increased demand for plasmids for targeted gene therapeutic delivery has made partnering with a CDMO equipped with the capacity, expertise, and equipment for plasmid DNA production critical to a stable therapeutic supply. Ajinomoto Bio-Pharma Services, an industry leader in microbial fermentation, recently hosted a webinar, Plasmid Manufacturing for Gene Therapies: An Outsourcing Partnership, which outlined both the company's capabilities in the space and some best practices for vetting CDMOs.

As part of the webinar, Dr. Katya McLane, director of process science, and Ron Chantung, director of drug substance for Ajinomoto Bio-Pharma Services, discussed why optimizing, scaling up, and manufacturing plasmids is necessary for clinical and commercial program success. The pair also highlighted how Aji Bio-Pharma's proven expertise and capabilities in cGMP production of biotherapeutics enable a strong, client-focused partnership that guarantees market success.

Q: What are the current challenges for potential clients?

Chantung: Some factors are out of our control, but certainly a relevant topic now, more so than ever, is supply chain lead times as a result of the pandemic. There's certainly been a tightening of the release of materials from manufacturers and from vendors for non-COVID-related projects. It's been a focal point for not only our ongoing clients, but our potential clients. At Aji, we'll end up using our qualified vendor list, which is pretty deep. Ever since the pandemic started, our supply chain group has been actively procuring and increasing our safety stock, so that we can keep production going for our clients.

Q: How is [Ajinomoto Bio-Pharma Services] harvesting cell paste?

Chantung: At the 100-liter scale we employ dead-end centrifuges and then freeze down cell pellets. At the 1,000-liter scale we use our stainless-steel continuous centrifuges.

Q: Does Ajinomoto produce plasmid DNA at the 1,000-liter scale, and what are the challenges with that production?

Chantung: Katya's team has conducted technical transfer at the 1,000-liter scale and has seen success at that scale. The challenges for us is our footprint and our capacity to down-process cell paste at the 1,000L scale. Our lysis and TFF-1 steps are limited or maxed out at 200 liters of cell paste per downstream drug substance batch.

Q: Do you also provide high-quality plasmid for use in phase one and two clinical trials in the cell and gene therapy field?

Chantung: We have experience doing that and we certainly partner up with the client and also our quality assurance folks to ensure that we set release specifications that are in line with early phase all the way through phase three clinical trials.

Q: What is the biggest batch size for plasmid DNA chemical lysis that you can achieve? Can you speak to the scalability of your lysis method?

Chantung: We're at that 200-liter max right now, but we would be advocates of increasing that capacity.

Q: Do you have any experience with linearizing of plasmid DNA for mRNA manufacturing?

McLane: That can be done. We've been asked to do that in the past, but people will typically do that on-site right before they're going to generate the mRNA, so it isn't typically something we would be asked to do.

Q: What are some of the bottlenecks to material sourcing supporting plasmid production that you're facing?

Chantung: In our production downstream suites, we have not only single-use purification skids, but also stainless-steel purification skids. We've seen a little bit of a stranglehold on single-use materials for our purification skids. That's certainly been a challenge for non-COVID-related projects.

Q: With regard to ABEC fermenters – do you have the option to use single-use fermenters for some applications?

Chantung: Not at this point. Single-use fermenters for microbial systems do present some challenges as you scale up in size. We have found a lot of success with our stainless-steel fermenters; there's heat transfer that is certainly more optimal in stainless vs. single-use culture systems. We have, in the past, looked at exploring single-use capabilities, and we will keep that on the table if we choose to expand.

Q: Do you also provide process development perfusion mode cell culture for biosimilars and with single-use bioreactors (SUBs)?

McLane: We do work with people who do biosimilars, and if it's a process they've developed, we will take it and directly tech transfer it. For a plasmid process, we will use our proprietary plasmid platform. This is partially because it yields consistent results for us from all different sizes of plasmids.

Chantung: At the moment we don't utilize single-use vessels, but it is certainly still an option to vet.

Q: What percent of supercoiled plasmid DNA do you guarantee for your drug substance?

Chantung: We have seen typically, over the years, at least 90 percent supercoiled. Depending on agency guidance for what the plasmid is used for, I want to say that for an injectable plasmid it's either 80 or 85 percent, but we have seen anywhere from 90 to the high 90s, percentage-wise.

Q: Do you offer different grades, like GMP for injectables for plasmid DNA therapeutics, versus non-GMP for transfection?

Chantung: We do what we call internal engineering runs, as opposed to GMP runs. They're void of the quality umbrella that you'll gain with a GMP run, and those non-GMP runs are typically used by clients for toxicology studies or reference material.

McLane: It really depends on how much material they need for non-GMP. We can generate one to two grams of very high-quality material in the process science lab. If they need more than that, they will typically need to do the fermentation in our GMP facility. Let's say they need 10 grams of material – it would be called a non-GMP run, but everything would be done in our manufacturing facility. The only difference is that it wouldn't go through the quality assurance review.

Q: Do you have or plan to work on end-to-end continuous plasmid production?

McLane: It's something we would be interested in if we had a client that needed it, but typically our clients only need a certain number of batches. For instance, for gene therapy clients, often they need several different plasmids produced at the same time, and so instituting a continuous program isn't something that really fits the needs of our clients right now.

Q: As a follow up to the plasmid DNA for mRNA question, what types of quantities are needed, and which grade of plasmid DNA?

McLane: For the template plasmid, people will typically use the same grade of DNA that would be used for vaccines that are directly injected. The reason they do so is related to the regulatory agencies, because it's the easiest way for them to ensure that the quality requirements are met. Even if it's just a critical reagent, if it's produced under our GMP conditions that's typically the route we see.

Q: Do you have a preferred bacterial cell line for plasmid manufacture and a platform for plasmid manufacture, or are all plasmid manufacturing campaigns specific to each plasmid?

McLane: It's very specific to the plasmid. We typically use commercially available host cell strains for host cell selections, and clients often decide what cell line they want to use. It goes either way – either we're looking to optimize both the yield and purity by doing host cell strain selection, or the client provides us the host cell strain we're going to use. We don't have one particular cell line that we see works well for each and every plasmid, so we often do host cell strain selection, which is very plasmid specific.

Q: Suppose you get a research cell bank (RCB) from a client – how long on average will it take to make one gram of supercoiled plasmid DNA?

McLane: That really depends on scheduling more than anything else. From the RCB, it can go directly to our process development group. We don't require testing of the RCB during our initial feasibility study while it goes out for phage testing, which takes about a month. It takes about two months before the RCB will be released for creation of the master cell bank. The master cell bank (MCB) process itself is only a week in the suite, but then all the testing and the release will take eight to ten weeks to do. During that time we're doing the process verification run with the MCB cell line that will be used in the manufacturing suite, so there are processes that are done in parallel. Let's say after that there's a slot open in the manufacturing suite. When the master cell bank is released it can go to the the manufacturing suite for what is typically three weeks of process, then it goes into testing and quality assurance release. Sometimes a client will want to test the plasmid that comes from the confirmation run. If they want to try a transfection or test a DNA or mRNA vaccine, that can add time to the process. That's up to the client; we're perfectly able to schedule that and wait for the gating decision.

Chantung: If we've got production capacity availability in our fermenters and downstream suites through final formulation, and if it's at a 100-liter scale batch where the ask was maybe one gram, we would hope that we're producing well over one gram for a 100-liter batch, and three to four weeks of operations is about right. Then release of a GMP batch is about 45 days after final formulation.

Q: Do you think the purification of plasmids with membrane absorbers is beneficial with plasmids as well as biosimilars?

McLane: For plasmids, we've seen that it's really the capacity of the column resin versus absorbers where the choice is made. It also depends on what the membrane absorber's purpose is: if it's removing contaminants and it's in flow-through mode, then the absorber works perfectly fine. If it's in capture mode, you're looking at the capacity, and typically the column capacity would be greater than the membrane.

Q: There was a slide on plasmid DNA yield of 25 grams per 1,000-liter fermentation. Is this number after the downstream processing (DSP) steps or before the DSP steps? What do recoveries look like during the DSP steps?

Chantung: We have seen with a case study in particular about 20 grams per 100-liter fermentation. That 20 grams is after purification, and that's final frozen bulk drug substance.

Q: Can you meet preclinical, clinical, and commercial manufacturing needs?

Chantung: We have experience with early-phase clinical all the way through phase three. Our manufacturing facilities in drug substance are not rated for commercial manufacturing, but we have produced critical raw materials that are used to produce commercial drugs.

Q: How can we get in touch with Aji with follow-up questions?

Chantung: Definitely reach out through our website. That's monitored very closely by our internal staff – <https://ajibio-pharma.com>.