

— PLASMID DNA cGMP PRODUCTION: — FINDING THE RIGHT MANUFACTURING PARTNER —

By Katya McLane, Ph.D., director of process science, and Ron Chantung, director of drug substance, Ajinomoto Bio-Pharma Services

The increased demand for plasmids for targeted gene therapeutic delivery will require creative solutions to maximize the commercial potential of these therapies. Partnering with a CDMO equipped with the quality oversight, capacity, expertise, and equipment for plasmid DNA production is critical to ensuring a stable therapeutic supply.

Finding a CDMO partner capable of meeting both pre-clinical and commercial manufacturing needs can be a difficult proposition. A dearth of suppliers and lack of external capacity, coupled with a growing need for gene therapy products and a burgeoning market, have served to limit the number of players with the track record for plasmid production.

Ajinomoto Bio-Pharma Services, with a global footprint and more than 40 years of CDMO experience, possesses the infrastructure to meet clients' plasmid production needs. In San Diego, Aji Bio-Pharma has facilities that possess capabilities in drug substance, drug product filling, and high-potency antibody drug conjugate services. Its sister locations, scattered across the world, boast unique services – at its Belgium site, the company produces small molecule products and API for both clinical and commercial partners. India also is home to a small molecule manufacturing site. At its two sites in Japan, Ajinomoto employs its AJIPHASE® technology to produce pure peptides and oligonucleotides – short, synthetic strands of DNA or RNA with a broad set of applications in pharmaceutical development. The AJIPHASE process uses fewer solvents and reagents, resulting in cost savings for clients.

In San Diego, Aji Bio-Pharma's drug substance business segment has two primary facilities, with dedicated utility yards that provide USP, water for injection (WFI), oxygen, clean compressed air, plant steam, chilled water, and nitrogen into its suites. Each suite also has a dedicated air handler unit,

which enables multi-campaign production activities. Its suites are ISO-7 and ISO-8 rated, sanitized on a weekly basis, and the campus' quality control team performs weekly environmental monitoring to ensure a state of control. The San Diego site is a microbial cell host production facility, specializing in producing biologics from E. coli and yeast, as well as employing its proprietary CORYNEX protein expression system.

The Cell Banking Process

In Aji Bio-Pharma's cell banking process, researchers either start with a client-provided plasmid, protein, or DNA sequence and synthesize the plasmid. After transforming different host cell strains, researchers screen clones cultured in shake flasks using varying electrophoresis techniques. Researchers select the cell strain that produces the highest yield and appropriate molecular weight protein or plasmid and begin developing pre-Research Cell Banks (pre-RCBs), the clones from which will be grown using a small-scale fermenter. The clones are again analyzed – with agarose gel electrophoresis for plasmids and polyacrylamide gel electrophoresis for proteins – and compared for yields. A clone is selected and expanded to produce the Research Cell Bank (RCB).

Clients may also come to the table with their own cell bank already developed. In either case, the cell bank undergoes restriction enzyme analysis and phage testing, which ensure the correct plasmid is

expressed and identify potentially dangerous phage in the cell bank. Once the cell bank has been tested and cleared by the quality assurance group, it goes to Aji Bio-Pharma's manufacturing facility, where researchers produce the Master Cell Bank (MCB) (see Fig. 1).

Ajinomoto Bio-Pharma Services has developed a proprietary CORYNEX® Protein Expression System, which uses *Corynebacterium glutamicum*, a gram-positive bacterium that produces protein and secretes it directly in the growth media. This technology simplifies downstream processing, reduces cost, and shortens a product's time to market through this direct secretion of biologically active, properly folded recombinant proteins. The CORYNEX® system has succeeded in replicating difficult-to-produce proteins not adequately manufactured using other production systems, and Aji Bio-Pharma offers scale-up and GMP manufacturing services for clients pursuing manufacturing of these more complex proteins.

Producing Plasmids

Seven years ago, Aji Bio-Pharma developed a proprietary plasmid platform, including culture media, allowing for fermentation at high-cell density. Fermentation cell paste undergoes chemical lysis, and then researchers concentrate the lysate and perform a diafiltration buffer exchange. In this part of the

process, a large percentage of RNA is removed. The clarified lysate is then purified using anion exchange chromatography, hydrophobic interaction chromatography, and a final tangential flow filtration (TFF) step to arrive at the final formulation.

For both protein and plasmid production, Aji Bio-Pharma employs integrated teams in upstream and downstream development; its upstream scientists are experts in fermentation and cell breakage, while its downstream scientists are focused on protein refolding, protein and plasmid chromatography, and the TFF stage. Aji Bio-Pharma also possesses a dedicated fermentation suite with two types of fermentation units. The first is its small-scale, 250-milliliter Sartorius System, which has eight different fermenters used to optimize the fermentation conditions. Once complete, the parameters used in the Sartorius System are transferred to Aji Bio-Pharma's larger five-liter fermenters, which possess the same aspect ratio and utilize the same software as its smaller units. The conditions inside the five-liter units can, in turn, be transferred to Aji Bio-Pharma's 100-liter and 1,000-liter units in its manufacturing facilities, ensuring successful scale-up at each phase of development.

For the purification of the plasmid from the clarified lysate, Aji Bio-Pharma uses anion exchange chromatography and hydrophobic interaction chromatography. Aji Bio-Pharma possesses several smaller-scale

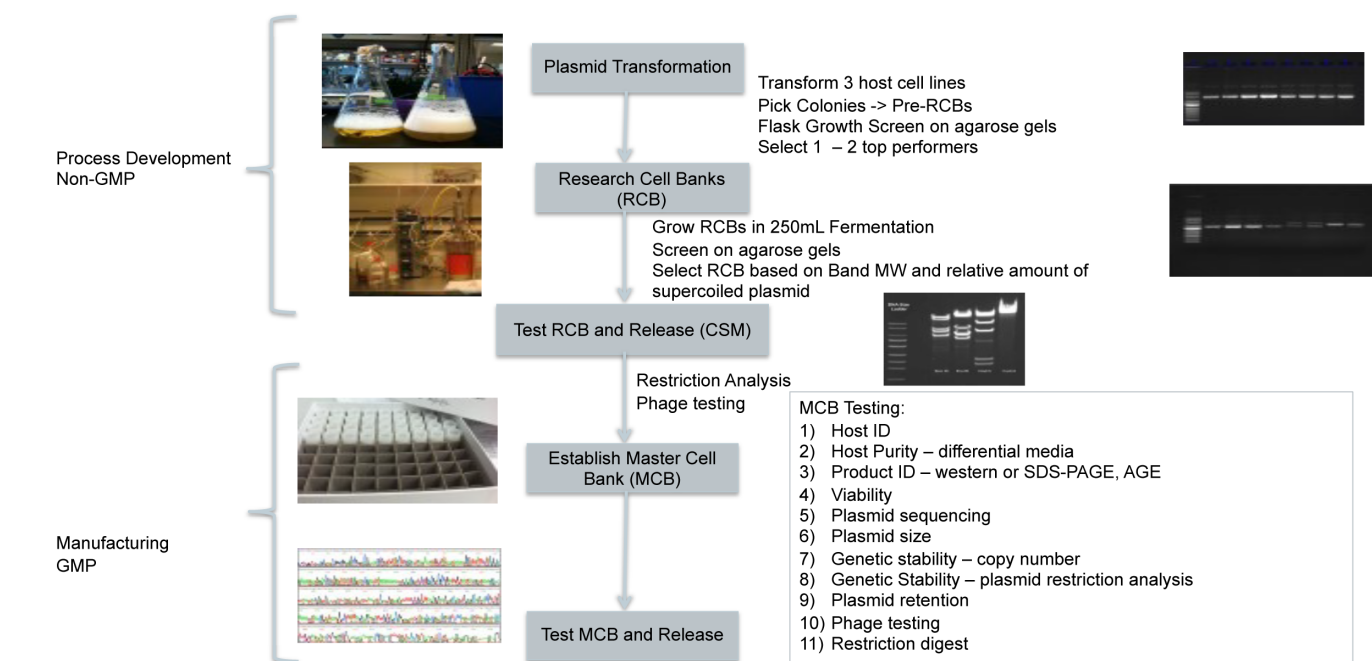


Fig. 1 – Ajinomoto Bio-Pharma Services' cell banking process

chromatography systems, but if clients request that one to two grams of plasmid go for testing, Aji Bio-Pharma can produce it on its pilot system before involving manufacturing. The equipment can accommodate different concentrations of plasmid and different buffers to meet client specifications.



Fig. 2 – An overview of Ajinomoto's plasmid platform

Within the process science group, Aji Bio-Pharma has its own analytical instrumentation it uses to assess yields and purity and perform endotoxin testing. It can also test, in process, material that originates from the cGMP suite, and it does so on a regular basis to monitor the process.

Scale Up and Technology Transfer

Once a process for a given project has been codified, Aji Bio-Pharma's upstream and downstream process development groups each develop process parameter reports for that project. These reports are utilized by Aji Bio-Pharma's technology transfer group, which trains others on the new process and develops a Critical Process Parameter sheet as a reference tool.

- 1) Technology Transfer is a Formal Process at Aji Bio-Pharma Services
- 2) Upstream and Downstream Process Development prepare Process Parameter Reports (PPRs).
- 3) Technology Transfer uses PPRs to Draft Critical Process Parameters (CPPs) for Scaled-up Unit Operations in Manufacturing.
- 4) The Batch Records are reviewed by the Technology Transfer Group to ensure that the CPPs are incorporated correctly.
- 5) The Technology Transfer Group monitors GMP campaigns for in-process and final plasmid yields and purity.

Fig. 3 – Aji Bio-Pharma's technology transfer process flow.

The process development group uses the Critical Process Parameter sheet to help generate batch records for the project, which are then cross-checked by the technology transfer group and others before going to manufacturing.

The Right Equipment for the Job

As touched on previously, Ajinomoto Bio-Pharma Services has a wide range of equipment and tools that support its cell banking and plasmid production

work. Some of its key equipment includes:

- Eight 250mL Sartorius Ambr250 Fermenters
 - Test different conditions simultaneously, including temperatures, pH, O₂, and media
- Four 5L Fermenters
 - Same aspect ratio as 100L and 1000L
 - Same Delta V software
 - Scale-up and confirmation of Fermentation Parameters
- Sartorius Sartoflow Advanced 10L Reservoir
 - 0.1 m² – 2.1 m²



The Sartorius Sartoflow Advanced 10L Reservoir

- Plasmid Lysate UF/DF
- Three AKTA PURE Chromatography Systems
 - 0.001-25 mL/min.
 - Test standard wash and elution conditions
 - Adjust conditions as necessary
 - Perform filtration studies
- AKTA Pilot
 - Chromatography Scale-up
 - Uses same Unicorn v7 software as on GMP systems
 - 1-2g non-GMP plasmid
- Sartorius Sartoflow Smart 1L Reservoir
 - 50 cm² – 0.14 m²
 - Final Formulation

On its drug substance manufacturing side, Ajinomoto Bio-Pharma Services has several large-scale pieces of equipment at its disposal, including:

- 100L, 120L, 1000L ABEC and New Brunswick Stainless Steel Fermenters
 - Equivalent aspect ratio as Process Science 5L Fermenters
 - Delta V software (same as Process Science)
 - Predictable Scale Up Parameters

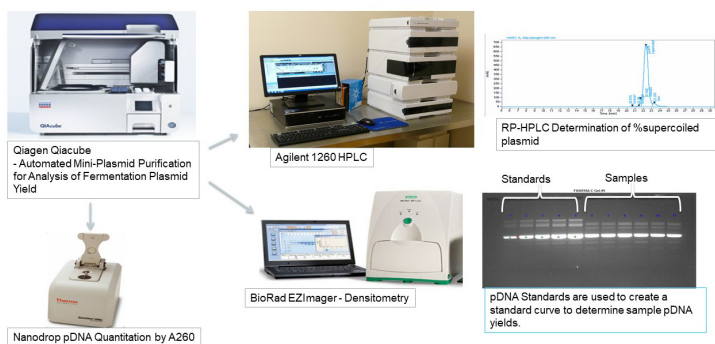


Fig. 4 – Aji Bio-Pharma also possesses advanced equipment and capabilities in plasmid analytics.

- Lysis, TFF1 UF/DF
 - o Controlled, Proprietary Resuspension, Alkaline Lysis, Depth Filtration, and Concentration
 - o 100L Cell Paste Per Cycle
- AKTA Ready and 10mm BioProcess Purification System
 - o Employs same Unicorn software as Process Science
 - o Pre-packed capture AEX and polishing HIC columns
 - o Single-Use TFF for Final Formulation

For all of its vessels and stainless steel equipment, Ajinomoto Bio-Pharma Services completes product changeovers in roughly one week. These changeovers include endotoxin, bioburden, conductivity, and total organic carbon (TOC) testing before equipment can be released for use on a new GMP project.

A Commitment to Quality Control

During the fermentation step, Aji Bio-Pharma's GMP production team works in staggered shifts to enable 24-hour monitoring, documenting various parameters during the process, such as feed temperature, pH balance, dissolved oxygen, agitation, and monitoring the acid-base and foam controls. Following cell harvest, its lysis and TFF 1 operations begin with

cell resuspension and the separation of the flocculent layer from the lysate. This is achieved through controlled mixing and depth filtration.

The scale for a TFF 1 step is 100 liters of cell paste per process day. TFF 1 places the plasmid in an appropriate buffer for chromatography loading. Up to two rounds of that cell paste processing can be combined into a single drug substance batch. These processes are performed at room temperature, and Aji Bio-Pharma has established with supporting data, that the resulting TFF 1 material can be safely stored for at least one month at 2-8 °C.

For a plasmid batch, Aji Bio-Pharma employs a two-step chromatography approach similar to the processes it applies to its smaller-scale work – first it utilizes an anionic exchange column, followed by a HIC (hydrophobic interaction) column. Operators take gating, supercoiled, and endotoxin samples after each chromatography step, and the final formulation places the plasmid in an appropriate final buffer state. The entire process, from cell thaw to final formulation, takes approximately six weeks. Once a batch is complete, Aji Bio-Pharma targets a full, quality-assured release within 45 days.

At Ajinomoto Bio-Pharma Services, a focus on operational excellence has equated to consistent and strong successes for its clients. About six years ago, a client approached Ajinomoto Bio-Pharma Services as a potential secondary production source for its plasmid needs. The company was in the process of vetting various CDMOs for capacity, pricing, yield, and batch release. Aji Bio-Pharma secured the client and became its primary production source; it has subsequently generated over 1000 g of GMP plasmid for them, ranging in plasmid size from six to 20 kilobases. By focusing on the fermentation step, executing small-scale growth studies, performing batch vs. fed batch media experiments, and analyzing metabolite data to support its conclusions, Aji Bio-Pharma was able to optimize the yields for each of this client's plasmids. The Process Science team then performed technology transfer into the GMP space and has continuously monitored output data. The consistent repeatability at the 100L GMP scale and six weeks or under batch release turnaround time has resulted in Aji Bio-Pharma transitioning to become the client's primary plasmid supplier.

Ultimately, choosing a CDMO as a partner for plasmid production requires a thorough evaluation of the capacity and capabilities of an organization. Ajinomoto Bio-Pharma Services has the expertise and capability in cGMP production of biotherapeutics to

We are committed to providing the highest quality products and services to our customers and supplying safe and effective medicines to our patients worldwide.



Fig. 5 – Ajinomoto Bio-Pharma Services' partnership principles.

distinguish it as an industry leader in microbial fermentation. Whether protein or plasmid production, Aji Bio-Pharma's experienced staff and proprietary processes can optimize a project end-to-end, from cell banking to final filled product.

About the Authors

Katya McLane, Ph.D., is the Director of the Process Science Department at Ajinomoto Bio-Pharma Services, which includes functional subgroups in Upstream and Downstream Process Development, Scale-up and Technology Transfer of Drug Substance unit operations. During her past six years at Aji Bio-Pharma, she has been responsible for implementing all of the protein and plasmid DNA Drug Sub-

stance projects and developing the company's proprietary plasmid manufacturing platform. She has over 25 years of experience in the biotechnology industry.

Ron Chantung is the Director of Drug Substance for Ajinomoto Bio-Pharma Services. Since joining Aji Bio-Pharma in June 2015, he has worked alongside a team of individuals dedicated to manufacturing protein and plasmid DNA drug substances to produce life-saving drugs. Ron has over 20 years of R&D and clinical and commercial manufacturing experience in the biotechnology industry. He holds a degree in Biomedical Engineering from the University of Southern California. When he is not working, he enjoys spending time with his family and surfing at his favorite reef break in Encinitas, CA.

About Ajinomoto Bio-Pharma Services

Ajinomoto Bio-Pharma Services is a fully integrated contract development and manufacturing organization, with sites in Belgium, United States, Japan, and India, providing comprehensive process development services, cGMP manufacturing and drug product fill finish services of small molecule and large molecule APIs and intermediates. Ajinomoto Bio-Pharma Services offers a broad range of innovative platforms and capabilities to rapidly scale from clinical and pilot programs to commercial quantities, including: Corynex technologies, oligonucleotide synthesis, antibody drug conjugations (ADC), high potency APIs (HPAPI), biocatalysis, continuous flow manufacturing, and more. Ajinomoto Bio-Pharma Services is dedicated to providing a high level of quality and service to meet our clients' needs.