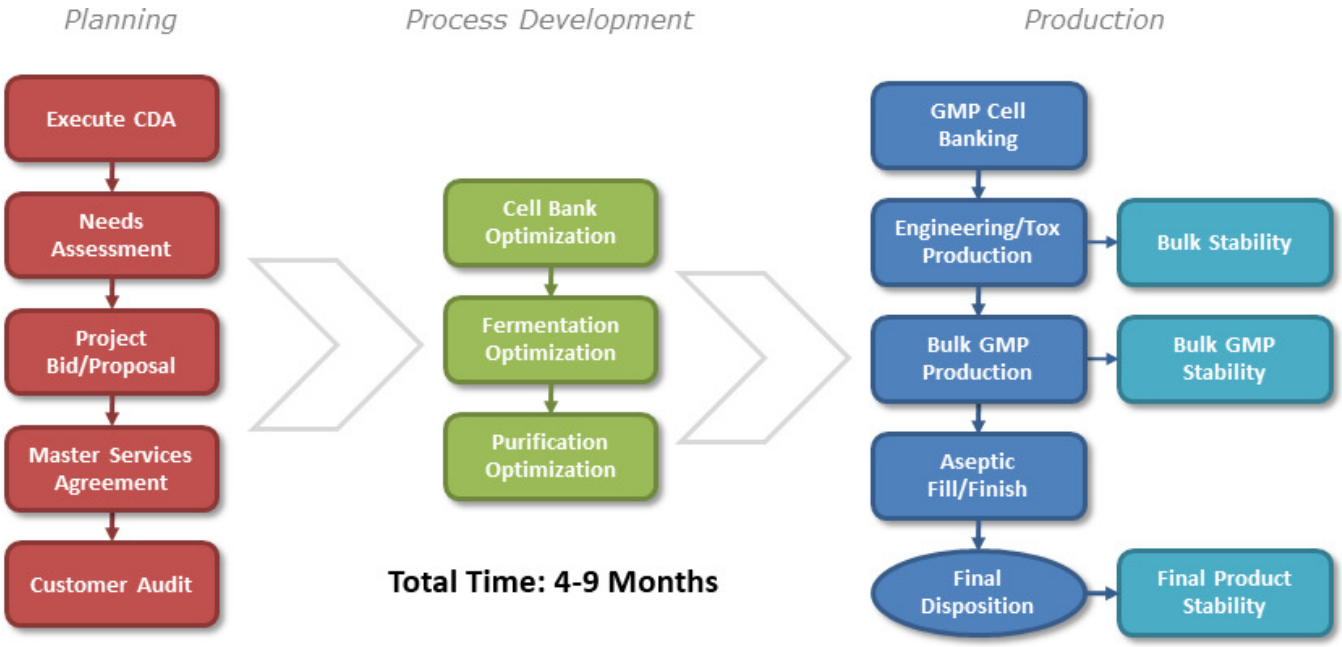
How Long Does it Take to Manufacture Plasmid under GMP?

Manufacturing plasmid under GMP generally takes four to nine months, depending upon your project's specific requirements. You may be wondering, "Why does it take so long to produce a plasmid under GMP when making a plasmid in a lab only takes a few weeks?" Considering that GMP plasmids are going to be injected into humans, an entirely different level of quality assurance and control is required. These additional requirements are the difference between a two week and four to nine month delivery time.

Regulatory agencies such as the FDA and EMA have strict guidelines that specify how products intended for use in humans must be manufactured and tested. These guidelines require extensive documentation, training, and validation for each step of the manufacturing process and quality control testing. The ultimate goal of these guidelines is to protect patients and to ensure the efficacy of the therapeutic.

GMP Plasmid Manufacturing Project

The typical contract plasmid manufacturing project consists of three phases: Planning, Process Development and Production.



Planning Phase (1-2 months)

The first phase of a plasmid manufacturing project consists of executing the necessary agreements and project discovery.

First, a two-way confidentiality agreement is executed. Although VGXI has a standard CDA template, we are happy to use your CDA template if required. After signing the CDA, we enter into a discovery phase which consists of filling out a plasmid questionnaire and conducting several teleconferences to discuss the details of your project. This process usually takes a couple of weeks and we use the information gathered to generate a detailed manufacturing proposal.

After reviewing our proposal and making the decision to hire VGXI to manufacture your plasmid, the next step is to execute a Manufacturing Services Agreement (MSA). The MSA spells out the terms and conditions of your relationship with VGXI. This step takes the most time because it usually requires both internal and legal review by you and your team.

After the MSA is executed, VGXI provides you with a purchase order (PO) that reflects the final specifications of the project. After signing the PO, you are invited to perform a quality audit to ensure that required regulatory requirements are being met.

Process Development Phase (1-2 months)

Process development (PD) is critical for the success of scale-up operations and the maximization of product quality and yield. Process Development is undertaken in a designated PD laboratory and consists of strain and clone selection, fermentation, and purification development. During process development, the ability to meet specific quality specifications is verified.

About one-third of the time in the process development stage is active bench time, while about two-thirds is comprised of waiting on test results to move on to the next step. All in all, process development takes approximately 1 to 2 months, depending largely upon the scale of the project and project specific requirements.

Production Phase (2-5 months)

Finally, production begins. Production includes all of the steps undertaken during the Process Development stage but at full-scale. Production occurs in six steps:

1. *Cell Banking:*

Master and working seed banks are produced using the bacterial strain selected during Process Development. Both the master and working seed banks are preserved in liquid nitrogen storage. The master seed bank serves as a repository for the cells while the working seed bank is actively used to produce the plasmids. A cell bank takes about 1 week to produce and 8 weeks for testing to be completed. Standard tests include:

- Plasmid sequencing;
- Host Cell Identification
- Cell viability;
- Plasmid retention;
- Phage Testing;
- Fungal Testing; and
- Bacterial Purity Testing.

2. *Engineering Production – Fermentation and Purification (Optional):*

The purpose of an engineering run is to verify your manufacturing process prior to large scale GMP manufacturing. Product produced during an engineering run is typically used for toxicology studies. Some clients also perform an engineering run to produce large quantities of HD (Highly Documented) plasmid for use as a raw material in cGMP manufacturing of viral vectors.

Keep in mind that an engineering run is optional and, if conducted, takes about three weeks to perform: one week of changeover followed by two weeks of production (one week for fermentation and one week for purification) and an additional eight weeks for optional release testing. Whether or not you perform an engineering run depends on how confident you are that your plasmid can be produced as is.

3. *cGMP Production – Fermentation and Purification:*

Fermentation of the plasmid can be performed in batch or fed batch mode. Bacteria containing the plasmid are grown from a master or working seed bank, fermented to high density, and harvested. The bacteria are then lysed using VGXI's patented AIRMIX™ technology to release their cellular contents, including the plasmid.

After lysis, the plasmid is purified using three purification steps: solid/liquid separation, ion exchange (IEX) chromatography and hydrophobic interaction chromatography (HIC). Subsequently, the purified plasmid is concentrated and desalted by ultrafiltration/diafiltration (UF/DF). The final step is aseptic filtration to provide the bulk drug substance. This step typically requires one week for changeover, one week for fermentation, and one week for purification.

4. *Bulk Drug Substance Release Testing:*

Following the production of the bulk drug substance, release testing is performed. Taking six to eight weeks to perform, typical release tests include:

- Endotoxin;
- Microbial Enumeration Testing;
- Plasmid Forms by HPLC;
- pH;
- Osmolality;
- Nucleic Acid Concentration;
- Visual Appearance;
- Percent Host Cell Genomic DNA;
- Percent Host Cell RNA; and
- Percent Host Cell Protein.

5. *Aseptic Fill/Finish:*

Once the bulk has been produced and dispositioned, the bulk product is filtered and filled into client specified vials. The fill is conducted by an automated system in a cleanroom environment. It typically takes about one week to complete a fill.

6. *Drug Product Release Testing:*

After the product is filled, it is delivered to Quality Control for final release testing. Like bulk testing, it typically takes six to eight weeks to perform these tests. Typical release testing includes:

- Endotoxin;
- Sterility Testing;
- Plasmid Forms by HPLC;
- pH;
- Osmolality;
- Nucleic Acid Concentration; and
- Visual Appearance.

Summary

If you've been keeping track, the total amount of time required to manufacture a plasmid under GMP is four to nine months. To illustrate the difference in the four to nine month time span, here is a summary of two production scenarios:

Client A has a cGMP master cell bank that is fully tested. They have made their plasmid under cGMP using this cell bank in the past and are confident of its manufacturability. Client A also needs their product filled into 1,000 vials.

Client B has a new plasmid and consequently does not have a master cell bank or experience manufacturing their particular plasmid. They also need their product filled into 1,000 vials.

cGMP Plasmid Production Time

Step	*Client A*	*Client B*
Process Development	n/a	6 weeks
Cell Banking	n/a	1 week
Cell Bank Release Testing	n/a	8 weeks
Engineering	n/a	3 weeks
Bulk Production	3 weeks	3 weeks
Bulk Release Testing	8 weeks	8 weeks
Fill/Finish	1 week	1 week
Final Release Testing	8 weeks	8 weeks
Total Weeks	**20 weeks**	**38 weeks**
Total Months	**4.5 months**	**8.6 months**

Whatever your project requirements may be, our experienced staff can design a project around your specific needs. Whether your project lasts 4 months or 9 months, we will work with you every step of the way to ensure the best possible chance of success with your clinical program.

About VGXI

VGXI is a contract manufacturing organization that specializes in the production of DNA plasmids under GMP for human clinical trials. With over 13 years' experience, we have produced products for numerous clinical trials in the U.S., Europe and Asia. Our patented AIRMIX® manufacturing technology produces the highest quality plasmids in the industry with plasmid supercoil percentages up to 99% and final product concentrations as high as 15 mg/ml. Our highly experienced team can work with your project's unique requirements to create a manufacturing solution that ensures the success of your clinical program.

VGXI®

Delivering Life's Potential®

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