C Squared Consulting LLP

May 12, 2022

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41 Cooper Square
New York, NY 10013

Subject: Letter of Transmittal

Dear Mr. Davis,

Attached is the report on the design and economics of the Pfizer-BioNTech SARS-CoV-2 mRNA vaccine plant in Las Palmas, Medellín, Colombia. This plant is expected to produce a total of 100M doses per year over six batches and help address vaccine inequality in South America and the Caribbean.

Please contact us if you have any additional questions.

Sincerely,

Group 3 Process Engineers

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ABSTRACT

To date, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been responsible for more than 6 million deaths worldwide. Vaccination have been proven to reduce risk of infection, but in the second year of the pandemic, vaccine accessibility remains a global issue. Low-income countries have been shown to have a significantly lower vaccination rate. In addition, although global vaccination rates are on the rise, it is likely that doses will continue to be needed yearly to maintain immunity and protect against the virus as advised by the World Health Organization (WHO). Therefore, this plan discusses the development of a manufacturing process for Pfizer-BioNTech's mRNA vaccine in Las Palmas, Medellín, Colombia. This plant will produce 100 million doses a year, over a 10-year plant lifetime and is expected to address vaccine inequality in South America and the Caribbean. This design covers everything from vial thaw to purification for final formulation and packaging. As illustrated in Figure 1, the process consists of four major systems: fermentation, DNA separation, DNA amplification and transcription and finally, mRNA purification and concentration. Six batches are produced every year with each batch cycle spanning 769 hours. Every step of the process will be sampled and tested to ensure compliance with standards and that no risks are posed to consumers. Each dose is priced at $12 dollars, significantly lower than other competitors. Nevertheless, he discounted cash flow rate of return (DCFROR) over the plant lifetime is 113%. Capital costs are estimated to reach $3 million with a yearly operating cost of $470 million. Because of the urgent need for vaccines and the profitability of this vaccine plant, it is recommended that this proposal proceed as planned.

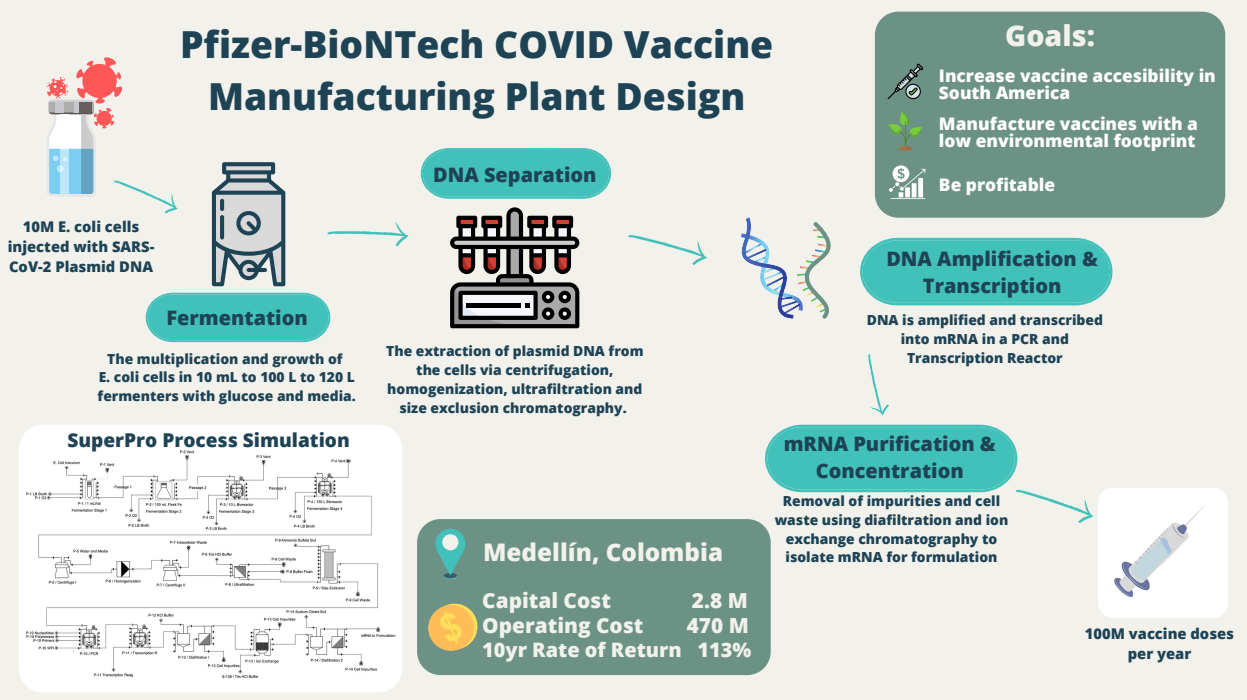


Figure 1. Project summary.

INTRODUCTION

The Covid-19 pandemic has become a global health concern, taking the lives of more than 6 million people worldwide. As of January, approximately 63.3% of South America has received a dose of a vaccine, however many neighboring countries in the Caribbean and Latin America have the second lowest domestic supply, only after other countries in Africa. In developing countries like Haiti, the vaccination rate is a mere 1.6%.[1] Vaccine inequality is a major issue as low-income countries have significantly lower vaccinations rates (Figure 2). Thus, the objective is to design a SARS-CoV-2 vaccine manufacturing facility for Pfizer-BioNTech to produce 100 million doses per year in Medellín, Colombia, while increasing vaccine accessibility and affordability to people worldwide. Although global vaccination rates are on the rise, it is likely that doses will continue to be needed yearly to maintain immunity and protect against the virus. This plan will follow current Good Manufacturing Protocols (cGMP), address sustainability and equity, the economics and encompass the production of these vaccines, from vial thaw to purification.

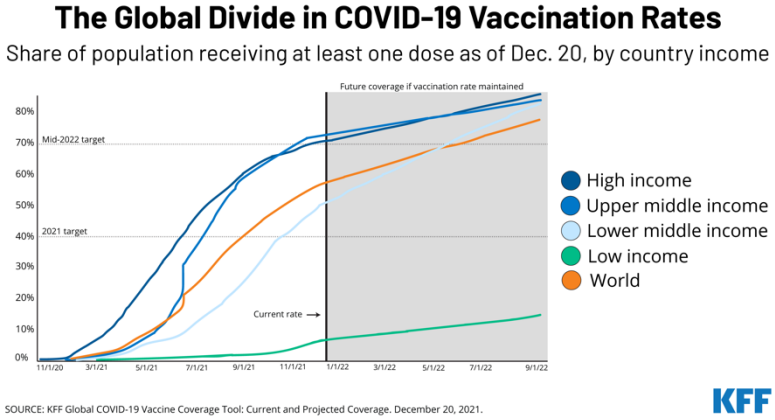


Figure 2. Vaccine inequality worldwide.[2]

Vaccinations are a simple and effective method to prevent and protect people from harmful diseases. They provide the body with the blueprints to be able to fight off a virus and can statistically cuts the risk of infection by more than 70% and death by even more (Figure 3). Currently, there are five Covid-19 vaccines widely available, which are made by Pfizer-BioNTech, Moderna, Oxford-AstraZeneca, Johnson & Johnson and Novavax. The three FDA approved vaccines are compared for their efficacy, price, and type in the table below. Pfizer's mRNA vaccine was the first to receive FDA approval and has been demonstrated to have an efficacy rate of 95% in preventing Covid-19. At the end of 2021, global vaccine production reached 12 billion, but this number is not enough to cover the global population (including boosters).[3] Therefore, reaching that demand remains an issue today.

Table 1. Comparison of the three FDA approved vaccines.

	Pfizer-BioNTech	Moderna	Johnson & Johnson
Type	mRNA	mRNA	Adenovirus
Efficacy	95%	94.1%	66.1%
Cost[4]	$12	$29.50	$10
FDA Age Approval	5+	18+	18+

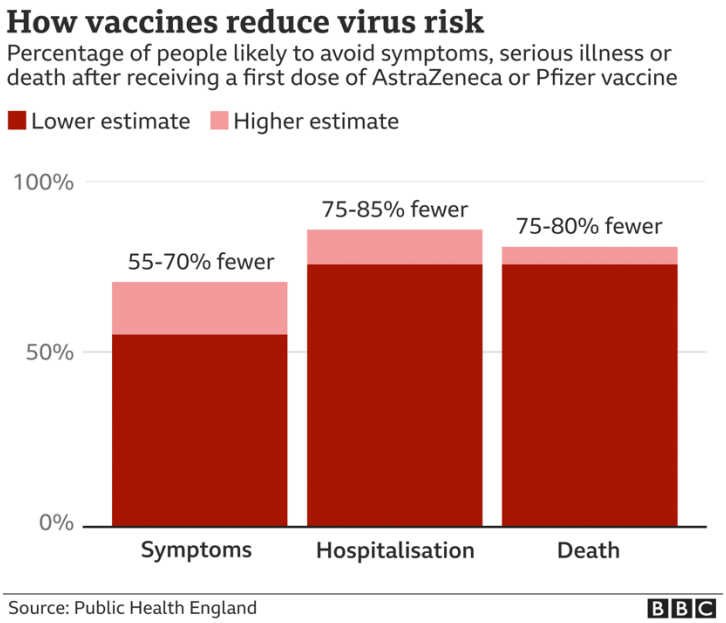


Figure 3. Reduction of risk of infection by vaccination.[5]

Of these, some are mRNA or adenovirus based. Most vaccines require a weakened or dead bacteria or virus to trigger an immune response. However, messenger RNA (mRNA) vaccines have been shown to be advantageous due to their versatility, efficient delivery, use of the protein translational machinery of the host, and short developmental time as compared to traditional vaccines.[6] As variants appear across the globe, mRNA facilities can easily adapt and be repurposed for new variants or other viruses as well, which makes them more economical and less risky than the manufacturing of other types of vaccines. Currently, only two mRNA vaccines have been approved by the FDA to provide protection against the coronavirus: Moderna's mRNA-1273 and Pfizer-BioNTech's BNT162b vaccine. Pfizer's vaccine is also cheaper and more effective than its other mRNA competitor, making it more attractive around the globe.

mRNA vaccines are relatively new, only discovered in the early 1960s.[7] The principle behind mRNA vaccines is to provide the human cells with the sequence to produce proteins to trigger an immune response. Figure 4 illustrates how mRNA vaccines work. Therefore, the goal of this process is to manufacture mRNA for inoculation.

Pfizer's manufacturing process for coronavirus vaccines has been previously analyzed and simulated. The New York Times published an article in April 2021 titled "How Pfizer makes it Covid-19 vaccines", giving a description of the steps required to produce vaccine doses.[8] Another article from Public Citizen outlined the steps and materials required to produce 8 billion vaccine doses, enough for the entire world.[9] The materials required for downstream processing including nucleotides and enzymes were scaled to 100 million doses to match the plant capacity of this process.

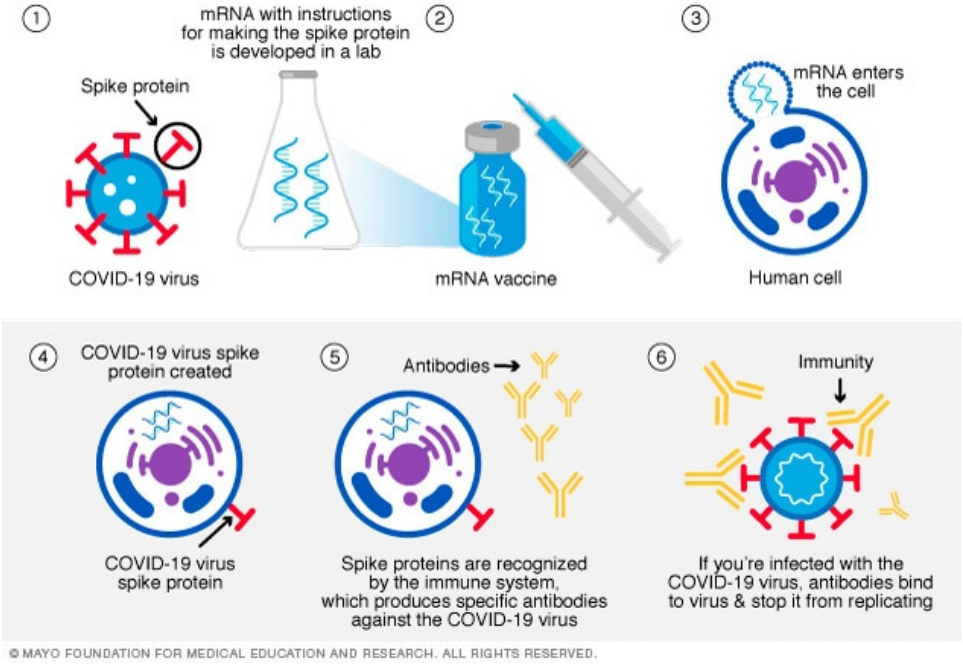


Figure 4. How mRNA vaccines prevent infection.[10]

Although details on Pfizer's process is limited, research on mRNA vaccine production is accessible. Upstream processing includes growth of E. coli cells in fermenters and a separation process to obtain the plasmid DNA. Large scale downstream production of mRNA vaccines is described to require "a 1 or 2-step in vitro reaction followed by a purification platform with multiple steps that can include Dnase digestion, precipitation, chromatography or tangential flow filtration".[11] Final purification typically includes a combination of tangential flow filtration and chromatography (ion pair reverse phase, anion exchange, or affinity). From this information, the following process illustrated in Figure 5 was designed.

This process includes six major steps including: a cell fermentation train, a DNA separation train, DNA amplification, DNA transcription, mRNA separation train and finally a formulation and packaging step. There are six batches a year, each producing 16.7 million doses. This process uses disposables in the manufacturing process, since steam in place (SIP) and clean in place (CIP) produces significantly more wastewater, and energy as well as chemical additives. SIP/CIP processes require higher initial capital investment, but lower operating costs over time. However, disposables are safer, less prone to issues, and more environmentally friendly as these wastes can be incinerated in waste-to-energy plants.

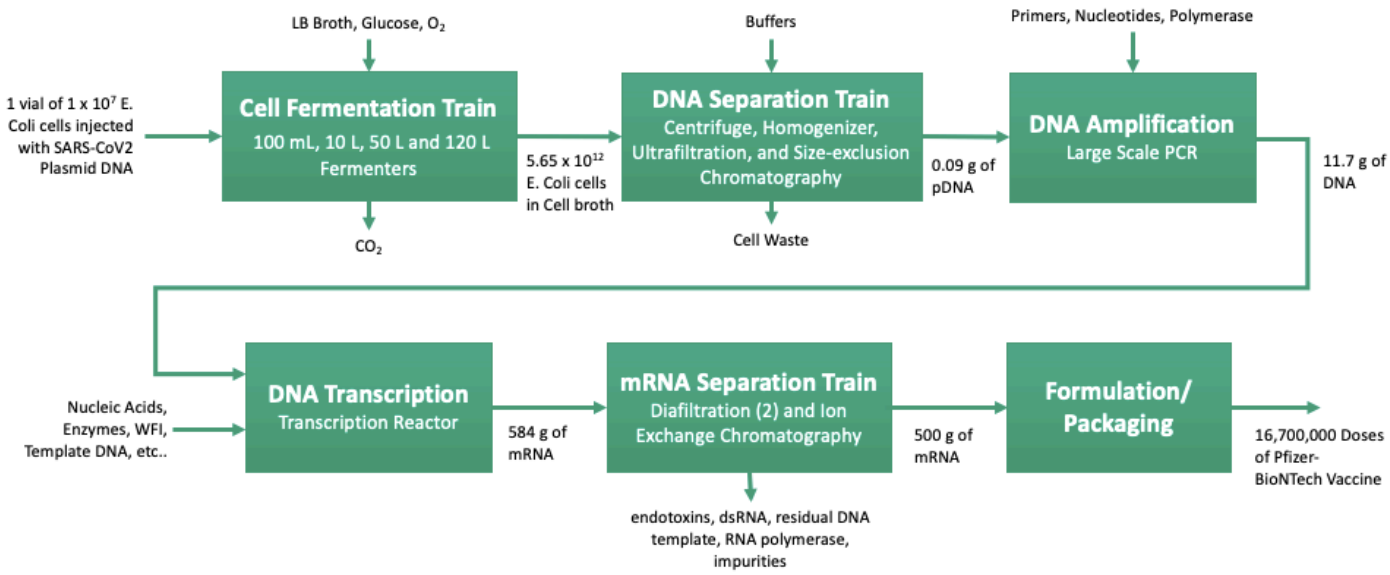


Figure 5. Block flow diagram of the manufacturing process.

The increasing prevalence of infectious diseases are a major threat to humans today. Today, in the second year of the pandemic, the demand for vaccination remains high. The global market for vaccine technologies is expected to grow from $38.2 billion in 2021 to 55.1 billion in 2026.[12] Pfizer is expected to top $32 billion dollars is Covid vaccine sales in 2022. Some South American countries are on the top 20 countries for vaccine manufacturing, with Colombia meeting 7.7% of their vaccine demand.[13] Pfizer's vaccine is priced at $12 a dose and 100 million doses are expected to generate an annual revenue of $12 billion a year. The economics of this plant is discussion in Section 10.

Another goal of this plant to be sustainable and equitable for customers, the community, workers, and the environment. For the customers, the manufacturing of this vaccine is expected to address vaccine equity for low-income areas and areas with low accessibility. This plant does not pose any health risks or major environmental risks to the surrounding community and proper regulations will be in place to ensure this (Section 8). In addition, there will be educational programs and tours provided to locals and institutions to educate the community on the various technologies in the plant and the biopharma industry. Workers are provided with training, support, and PPE to ensure a safe working environment. All other details including shift durations and salaries comply with Colombian and international laws.

Medellín is a major city in Colombia with a GDP of $43 million, the second largest, only to Bogota. Other major consumer goods manufacturers such as Mondelez and P&G have plants in Medellín as well. This ensures that Medellín has the proper infrastructure to facilitate the scale of this plant. This report discusses the process, equipment, economics, and safety of a vaccine manufacturing plant in Las Palmas, Medellín, Columbia.

PROCESS FLOW DIAGRAM & MATERIAL BALANCES

The overall goal of the process is to produce enough mRNA for 100 million doses of vaccine. To do so, enough DNA must first be produced to go through a transcription reaction, producing the mRNA. The cheapest method to produce a large amount of DNA is through a cell fermentation train, which comprises of shaker flasks and seed fermenters to grow E. Coli cells injected with the desired DNA plasmids. The DNA from these cells is then extracted and go through DNA amplification and transcription to produce mRNA. The formulation and packaging of the vaccine will be done in an offsite facility, but the costs of the materials will be included in this report.
This plant is expected to have an annual operating time of 4,369.09 hours with six batches a year, which have a batch time of 769.09 hours. Section 3.1 discusses the feed requirements for each stage of the manufacturing process in order achieve an outlet of 500 g of mRNA per batch.

SuperPro Designer was used to simulate the vaccine process from vial to final formulation and to obtain the material balances of this system. This process includes fourteen major unit operations including four fermentation steps, a DNA separation train, large scale PCR, a transcription reactor and a final mRNA separation train which are included in the SuperPro simulation. Auxiliary equipment for storage, media prep, decontamination and water for injection production are not included in the SuperPro simulation but considered in the economics. Figure 6 provides the process flow diagram and the stream tables are provided in Appendix 1. Descriptions of each stream are discussed in

Table 2.

3.1 Feed Requirements

To determine the required amount of mRNA in the process, it was assumed that each vaccine requires 3.00 x 10^{-5} g of mRNA. For a six-batch process producing 1.67 x 10^{7} doses, that would mean approximately 500 g of mRNA is required. Each step in the mRNA separation train is expected to have a 95% recovery rate of mRNA, therefore the mass of RNA leaving the transcription reactor was determined to be 584 g. This calculation is outlined below.

Equation 1. mRNA production requirement for a single batch.

$$3.00\ x\ 10^{5} g\ of\ mRNA\ \cdot 1.67\ x\ 10^{7} doses = 500\ g\ of\ mRNA$$

Equation 2. The amount of mRNA required to leave the transcription reactor.

$$\frac{500\ g\ of\ mRNA}{0.95^{3}} = 584\ g\ of\ mRNA$$

In the transcription reactor, the DNA is transcribed to produce mRNA. The RNA mass is expected to be 50 times the mass of the DNA. Therefore, the feed is required to have 11.7 g of DNA. Then, the PCR step doubles the amount of plasmid DNA each cycle. This step is expected to have seven cycles, meaning that the amount of pDNA entering the PCR unit must be 0.09 g/batch. Before PCR, the plasmid DNA must be separated and isolated from other intracellular components. The mass fraction of plasmid DNA in an E. coli cell was estimated to be 5.7%

(Equation 3). Hence the mass of E. coli leaving the fermenters was required to be 1.70 g/batch. The batch sizes and PCR cycles were optimized to lower costs and wastes produced.

Table 2. Stream descriptions.

Stream	Unit	Description
S-101	1 mL Vial	1.00 x 10^7 E. coli cells with the SARS-CoV-2 plasmid DNA
S-102	1 mL Vial	1 mL of Luria Bertani broth
S-103	1 mL Vial	Oxygen for fermentation
S-105	1 mL Vial	Vent of fermentation gases, mainly carbon dioxide and oxygen
S-104	Passage	Transfer of 5.00 x 10^7 E. coli cells (0.0003 g) to 100 mL Flask
S-106	100 mL Flask	99 mL of Luria Bertani broth
S-107	100 mL Flask	Oxygen for fermentation
S-108	100 mL Flask	Vent of fermentation gases, mainly carbon dioxide and oxygen
S-109	Passage	Transfer of 5.00 x 10^9 E. coli cells (0.00152 g) to 100 mL Flask
S-110	10 L Bioreactor	9.9 L of Luria Bertani broth
S-111	10 L Bioreactor	Oxygen for fermentation
S-112	10 L Bioreactor	Vent of fermentation gases, mainly carbon dioxide and oxygen
S-113	Passage	Transfer of 5.00 x 10^{11} E. coli cells (0.15203 g) to 100 mL Flask
S-114	120 L Bioreactor	103 L of Luria Bertani broth
S-115	120 L Bioreactor	Oxygen for fermentation
S-116	120 L Bioreactor	Vent of fermentation gases, mainly carbon dioxide and oxygen
S-117	Passage	Transfer of 5.65 x 10^{12} E. coli cells (1.70 g) to 100 mL Flask
S-123	Centrifuge I	68 L of cell media and water separated out
S-122	Passage	Transfer of E. coli cells (1.70 g) in 45 L of media and water to Homogenizer
S-124	Passage	Transfer of 0.10 g of pDNA to Centrifuge II
S-125	Centrifuge II	68 L of cell media and water
S-126	Passage	Transfer of 0.10 g of pDNA to UF
S-127	UF	Cell waste including RNA, RNA, gDNA, proteins, endotoxins and salts
S-129	UF	Tris HCl buffer at a flow rate of 1 mL/min
S-130	UF	Wash outlet of Tris HCl buffer
S-128	Passage	Transfer of 0.10 g of pDNA to SEC
S-131	SEC	Ammonia sulfate buffer at a flow rate of 1 mL/min
S-133	SEC	Cell waste and buffer solution
S-132	Passage	Transfer of 0.09 g of pDNA to PCR
S-134	PCR	Nucleotides for PCR (CTP, GTP, and mod-UTP solutions)
S-143	PCR	Polymerase for PCR (T7 RNA Polymerase)
S-144	PCR	Primers for PCR (RNase enzyme inhibitor)
S-145	PCR	WFI for PCR
S-135	Passage	Transfer of 11.7 g of DNA to Transcription Reactor
S-136	Transcription	Reagents and buffers including DNA template, CleanCap AG, DNase I, Sucrose, ATP solution, cholesterol, and Tris HCl
S-118	Passage	Transfer of 584 g of mRNA to Diafiltration I
S-119	Diafiltration I	KCl buffer
S-121	Diafiltration I	Cell impurities and buffer solution
S-120	Passage	Transfer of 555.39 g of mRNA to Ion Exchange
S-138	Ion Exchange	Tris HCl buffer

S-139	Ion Exchange	Cell impurities and buffer solution
S-137	Passage	Transfer of 528 g of mRNA to Diafiltration II
S-142	Diafiltration Ii	Sodium citrate buffer
S-141	Diafiltration Io	Cell impurities and buffer solution
S-140	Passage	Transfer of 501 g of mRNA to Packaging and Formulation

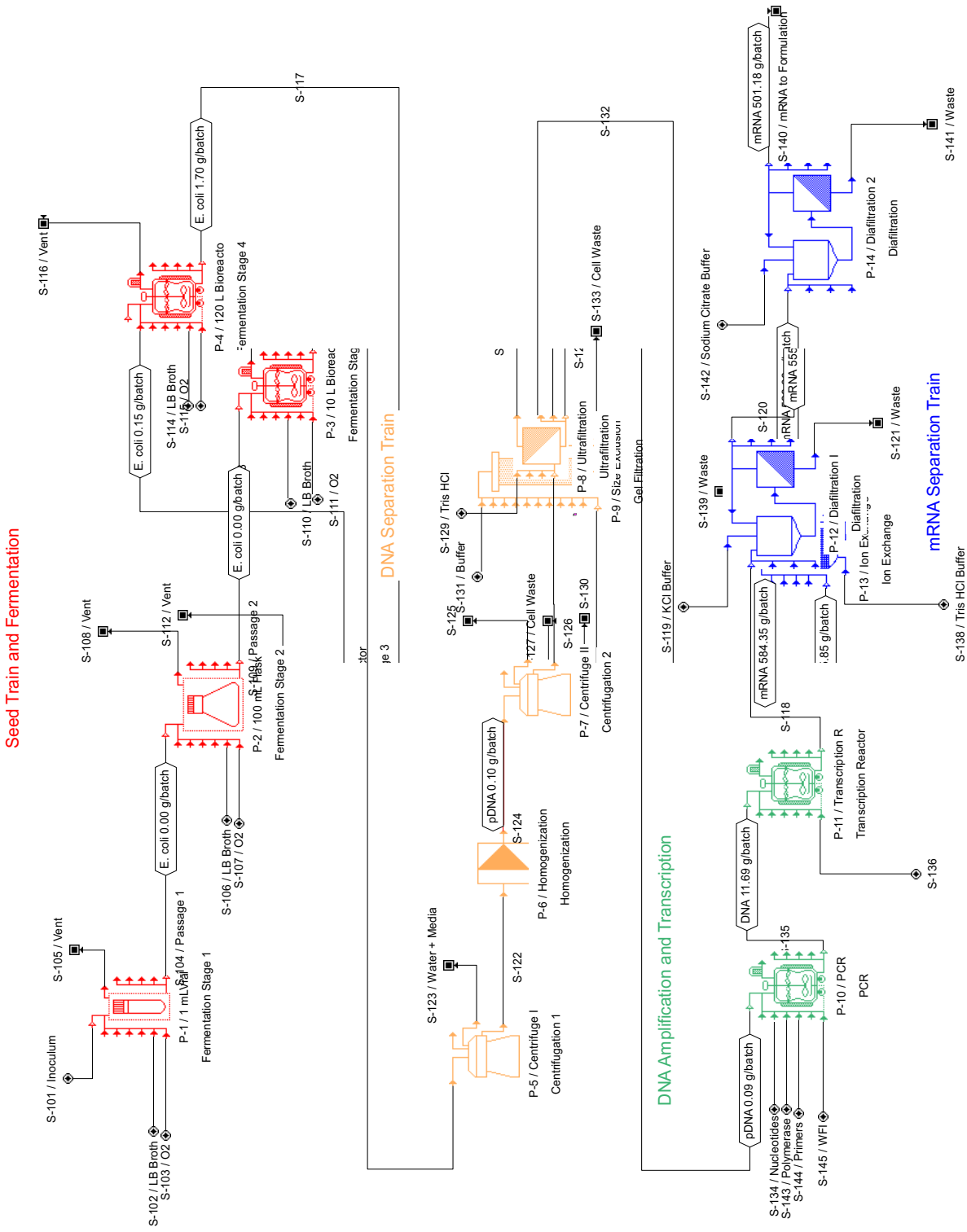


Figure 6. Process flow diagram of the manufacturing process via SuperPro.

3.1 Scheduling

Table 3 provides the equipment schedule for this process. The duration includes time for set up, transfer in, and transfer out of materials from one unit to another. The total operating time for one batch is 769.09 hours, with the ultrafiltration unit occupying the most time. The Gannt chart for two batches is shown in Figure 7. More details on the schedule of each unit operation are located in Appendix 2.

Table 3. Equipment scheduling.

Unit Operation	Duration (h)	Start Time (h)	End Time (h)
P-1 in 1 mL Vial	4.87	0.00	4.87
P-2 in 100 mL Flask	6.29	4.87	11.16
P-3 in 10 L Bioreactor	6.28	11.16	17.44
P-4 in 120 L Bioreactor	5.20	17.44	22.61
P-5 in Centrifuge I	7.17	22.61	29.78
P-6 in Homogenizer	1.08	29.78	30.86
P-7 in Centrifuge II	1.12	30.86	31.98
P-8 in Ultrafiltration	720.00	31.98	751.98
P-9 in Size Exclusion Chromatography	8.03	751.98	760.01
P-10 in PCR	0.34	760.01	760.35
P-11 in Transcription Reactor	1.40	760.35	761.75
P-12 in Diafiltration I	2.07	761.75	763.82
P-13 in Ion Exchange	2.68	763.82	766.50
P-14 in Diafiltration II	3.07	766.50	769.09
Total Operation	**769.09**	**0.00**	**769.09**

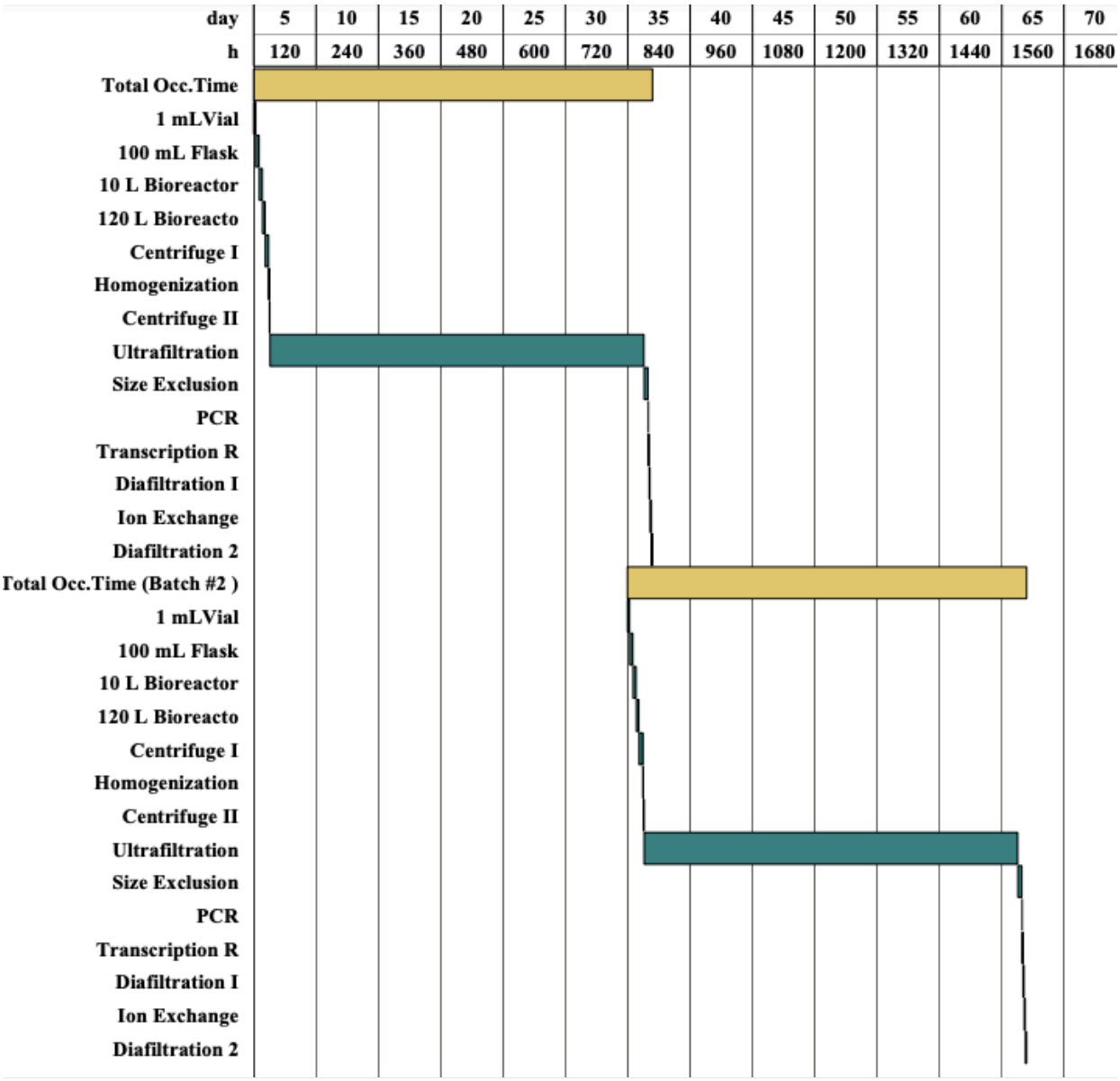


Figure 7. Gannt chart of the manufacturing process for two batches.

PROCESS DESCRIPTION

The process to obtain mRNA for formulation consists of four major steps. It begins with a vial of E. coli cells, where the cells ferment in a media solution of glucose and Luria Broth in multiple fermenters. Once the cells reach the desired population size, they are moved to DNA separation stage where the cells are lysed, and the plasmid DNA (pDNA) is extracted. From there, the pDNA is amplified and transcribed into mRNA. Finally, mRNA and other cell impurities go through multiple separation units to purify and concentrate the mRNA for the formulation and packaging step which occurs offsite.

4.1 Fermentation and Seed Train

The process begins with a 1 mL vial of 1×10^7 *E. coli* cells from Pfizer's master seed bank. With the correct conditions, the bacteria replicate approximately every 20 minutes in a Luria Bertani cell broth, which provides a pH of 7 and nutrients for the bacteria to grow. Details on this media are provided in Section 4.5.1. The following table illustrates the optimal conditions for *E. coli* growth. These conditions are applicable for all four fermentation steps in this process and will be monitored thoroughly to ensure proper conditions for the cells.

Table 4. E. coli growth requirements.[14]

Condition	
Temperature	37 °C
Pressure	1 atm
Agitation	High
Shear Sensitivity	Low
pH	6.5-7.5

Fermentation is a process of a chemical breakdown of a substance using microorganisms, such as bacteria, to generate energy for metabolism of a substrate to produce adenosine triphosphate (ATP).[15] E. coli is aerobic, requiring oxygen as well as sugar, such as glucose to generate energy. Background on the cell growth is provided in Appendix 3.

4.1.1 Cell Growth Background

Inoculating a sample of *E. coli* into a fresh medium for a batch process results in a growth that follows four distinct phases: lag phase, exponential phase, stationary phase, and finally cell death. Current fermentation and seed train processes start with a low initial inoculum (0.02 to 0.05 OD) and are followed by the addition of new medium to achieve a maximum growth rate. Understanding the rate of growth in the fermenters is important to utilize the cells before death. The figure below provides an overview of a typical cell growth cycle.[16]

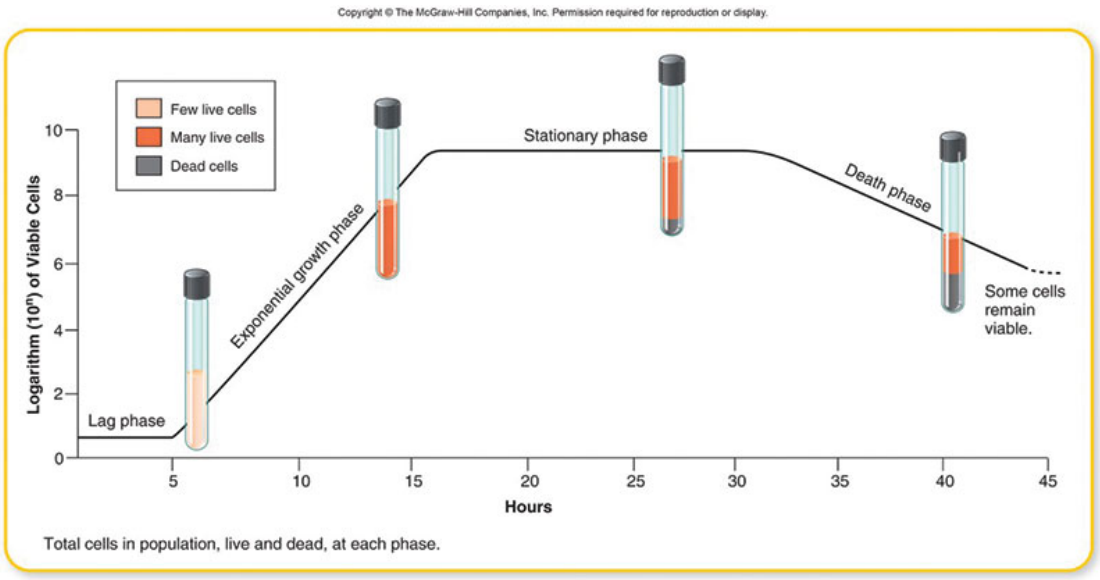


Figure 8. Typical cell growth curve.[16]

Lag Phase[17]

When cells are introduced to their growth media, they do not immediately start growing and multiplying. Instead, they enter the lag phase. During this period, cells are acclimating to their new environment. However, a growth lag phase only typically occurs when cells are "non growing" cells, which have been newly inoculated to fresh media. "Non growing" cells mean that they are in either the stationary or death phase. "Growing" cells are cells in the exponential phase and will pass over a lag phase. When "growing" cells are added to the last bioreactor, they will ideally enter the exponential phase, without any lag. This is also when the sample contains the greatest number of live cells (see Figure 8Figure 8). Thus, it is important to inoculate cells during the exponential growth phase to ensure the maximum growth of the colony.

Exponential Phase

The exponential phase is when most of the cell multiplication occurs. Cells double according to an exponential cycle, from 1 to 2 to 4 to 8, etc. For *E. coli* cells, the exponential phase ends at OD_{600} of 7 but cell growth begins to decline at 0.3.

Stationary Phase[18]

The stationary phase is marked by little cell growth due to a lack of nutrients, oxygen, pH change of the medium or even an accumulation of cell waste in the bioreactor. For *E. coli* cells in LB broth, this phase begins at OD_{600} of 7. Although this phase has the highest density of cells, the death rate exceeds the replication rate.

Death Phase

The final phase of the cell cycle is the death phase. During the death phase, cells begin to die due to a lack of nutrients and an accumulation of cell waste. It is important not to reach this point in the cell cycle during the fermentation process to ensure a viable number of cells to produce the vaccine.

4.1.2 Material Balance

To complete a material balance on the fermenters, the following data on E. coli was obtained.

Table 5. E. coli characteristics.

Characteristic	
Doubling Time	20 min
Lag Phase	4 hours
Mass of an E. coli cell	3.00×10^{-13}

Figure 9 and
Table 6 provide a PFD of the cell culture process to reach the required number of *E. coli* cells per batch and the cell counts for each fermenter, per batch, respectively. The mass of E. coli cells in each stage is provided in Appendix 1. The final cell count was determined by considering the end of the exponential growth phase when the OD_{600} reaches 0.03 (Figure 10).[19] Here, the cell concentration is approximately 5.00×10^7 cells/mL of broth. For each batch, approximately 5.65×10^{12} cells are produced from a starting vial of 1.00×10^7 cells.

Figure 9. PFD of the cell culture process on SuperPro.

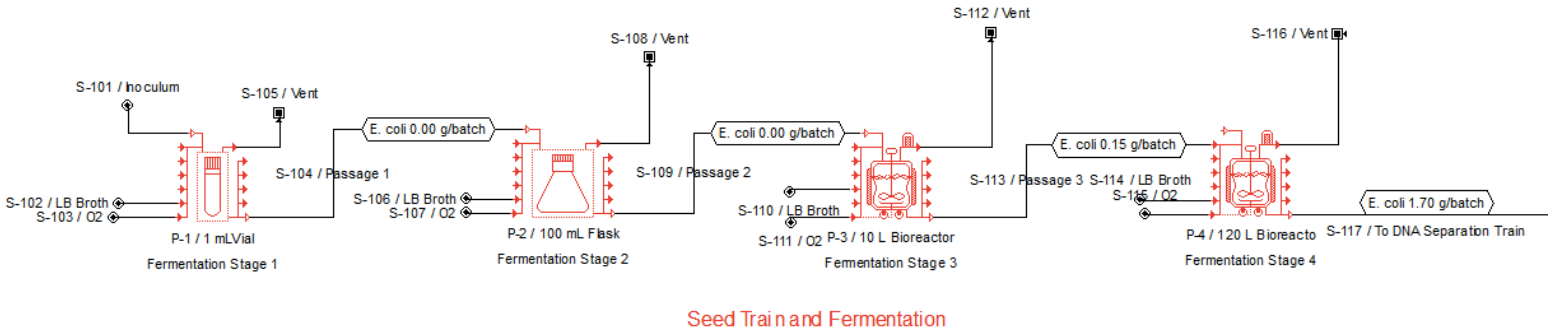


Table 6. Final cell counts in each fermenter, per batch.

Fermenter Size	Cell Culture Volume	Fermentation Time (h)	Final Cell Count (cells)
1 mL Vial	1 mL	4.77	5.00×10^9
100 mL Flask	100 mL	6.21	5.00×10^{10}
10 L Bioreactor	10 L	6.21	5.65×10^{11}
120 L Bioreactor	113 L	5.17	7.42×10^{12}

During fermentation, E. coli cells consume oxygen and glucose to form amino acids, carbon dioxide, hydrogen gas and water. The carbon dioxide and hydrogen gas are vented to the atmosphere. The following equation was used to represent the mixed-acid fermentation in SuperPro. The outlet stream compositions for each fermenter are provided in Appendix 1.

Equation 3. Estimated mass-based fermentation reaction.

$$40\ \text{Glucose} + 10\ \text{Oxygen} \rightarrow 20\ CO_2 + 10\ \text{E. coli} + 15\ \text{Hydrogen} + 5.00\ \text{Organic Acids}$$

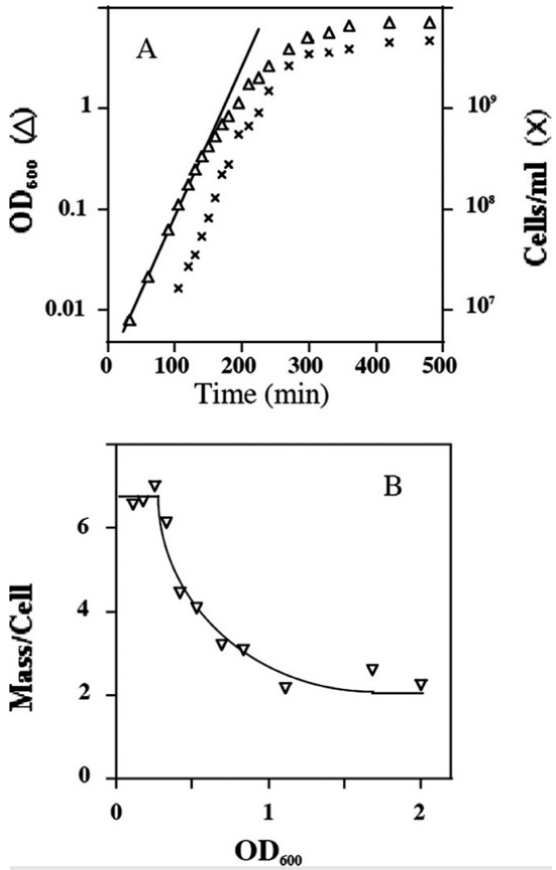


Figure 10. (A) Cell growth curve of *E. coli* and (B) the mass per cell at each cell concentration.

4.2 DNA Separation Train

The DNA Separation Train follows the fermentation step. The outlet of the fermenters consists of E. coli, water, and media. The first centrifuge is meant to separate out the solid cells from most of the water and media. After that step, the cells are homogenized at high pressure (800 bar) to break how the cell wall and obtain the cell lysate which contained the intracellular components, including the plasmid DNA. Again, the cell lysate is centrifuged to obtain the solid components. Ultrafiltration and size exclusion chromatography is then used to purify the pDNA to remove nucleotides, endotoxins, supercoiled and open-circle forms of pDNA. Any cell wastes from this process will be sent to kill tanks to be decontaminated (Section 4.5.2). Each step will be discussed in detail in the following sections.

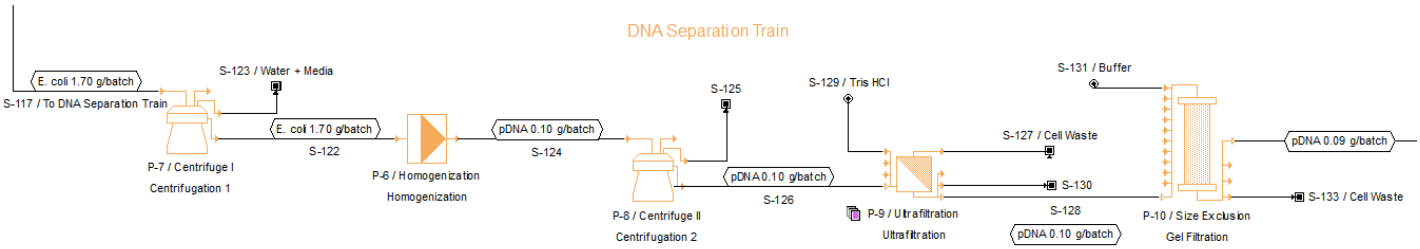


Figure 11. PFD of the DNA separation train.

4.2.1 Centrifuge

Centrifugation is a mechanical separation process used to separate biological solids from a liquid solution. It uses centrifugal force created by a constant angular velocity powered by an electric motor. The force creates a radius of rotation, where the distance along this radius relative to the center varies for particles depending on their densities.[20] The same centrifuge will be used for both centrifuge steps and be operated between 20°C and 37°C and atmospheric pressure. The outlet is expected to be 40% volume[21] of the solvent for the first centrifugation and 60% volume of the solvent after homogenization, which was specified in the SuperPro simulation. The free-standing centrifuge has a volume capacity of 4 liters per run[22].

4.2.2 Homogenizer

Homogenization is a method used to breakdown cell membranes and walls to release intracellular components, such as plasmid DNA. Common methods include mechanical homogenization or chemical lysis. Chemical lysis commonly utilizes enzymes and ethanol, however, to minimize flammables in the lab, high pressure homogenization was chosen. High pressure homogenization occurs at 800 bar and a temperature of 4°C. After homogenization, the cell lysate is transferred to another centrifuge step to extract the solid cellular components.
To simulate this step in SuperPro, the following reaction was used:

Equation 4. Estimated mass-based homogenization reaction.

$$100\ \text{E. coli} \rightarrow 5.7\ \text{pDNA} + 47.6\ \text{Cellular Waste} + 46.7\ \text{Intracellular Components}$$

This equation was determined using the fraction DNA in an E. coli cell:

Equation 5. Mass fraction of DNA in an E. coli cell.

$$\frac{mass\ of\ DNA}{mass\ of\ E.coli\ cell} = \frac{1.70\ x\ 10^{-14}}{3.00\ x\ 10^{-13}} = 5.7\%$$

This step is expected to produce 0.09 g of plasmid DNA from a feed that includes 1.7 g of E. coli cells.

4.2.3 Ultrafiltration

Ultrafiltration (UF) is a membrane separation unit used to remove impurities such as RNA, gDNA, proteins, and endotoxins, as well as other unnecessary salts such as sodium chloride.[23,24] This unit operation is a type of tangential flow filtration, where the solution is fed tangential to the membrane to improve efficiency. Pore diameters of 0.2 μm of 4.13 m^2 membrane surface is used. With a semi-permeable membrane and a pressure differential, the solution and plasmid DNA is allowed to travel through the membrane to the permeate side, while any impurities are trapped in the membrane or remain in the retentate side. It requires a continuous flow of a 50 mM Tris HCl buffer at a flow rate of 1 mL/min and is run at atmospheric temperature and 2 atm to drive the separation.

4.2.4 Size Exclusion Chromatography

Size Exclusion Chromatography (SEC) is used to remove impurities including nucleotides, supercoiled and linear forms of pDNA. SEC is a pressure driven method where the mixture flows through the pores of the gel packing to separate molecules by size. Smaller proteins remain the gel longer, while larger sized proteins flow through the column faster. This unit operates at a pressure of 2 atm to drive the elution. An elution buffer such as ammonium sulfate, which has a high salt content can be useful since it produces a better separation recovery.[25] The sample volume is required to be 0.5-2% of the total bed volume, therefore a 120 mL column was chosen.[26]

4.3 DNA Amplification and Transcription

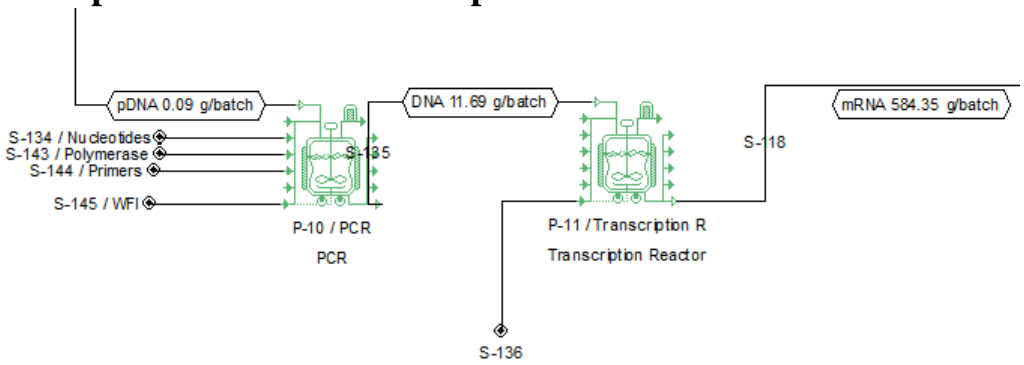


Figure 12. PFD of the DNA amplification and transcription step.

4.3.1 PCR

Polymerase Chain Reaction (PCR) is a technique that allows for millions of copies of a specific region of DNA to be created, in this case, the viral plasmid DNA that contains instructions for viral spike protein procreation. The DNA amplification reaction is the main reaction that occurs in this process with no other side reactions. All steps are conducted at atmospheric pressure and in a basic buffer solution at a pH of roughly 8 to 9.5 that supports the activity of the DNA polymerase enzyme.

The process is largely endothermic due to the amount of heat that is required for the first stage, DNA denaturing. During this phase the DNA is helix is separated by heating to 90-95°C. The next phase, annealing, is conducted at roughly 40-60°C, where primers, single strands of nucleic acids (CTP, GTP, and mod-UTP), that are complementary to the target DNA portion, are allowed to attach to the target DNA for amplification. Primers are chosen based on which segment of DNA is to be amplified. Lastly, the extension phase occurs at 70-75°C where DNA polymerase extends DNA from where primers are attached using dNTPs. The result is a new DNA helix with one old strand and one new strand of DNA. Due to the temperature changes involved in PCR, the reaction is adiabatic.

In a single cycle of PCR, the DNA is doubled. Seven cycles were chosen to optimize costs and batch times. To simulate this on SuperPro, the following reaction equation was used:

Equation 6. Estimated mass-based PCR amplification reaction.

$$0.74\ \text{Nucleotides} + 1.00\ \text{pDNA} + 0.16\ \text{Polymerase} + 0.10\ \text{Primer} \rightarrow 2.00\ \text{DNA}$$

This step is expected to output 11.7 g of DNA from a feed of 0.09 g of pDNA, nucleotides, polymerase, and primers.

4.3.2 Transcription Reactor

Transcription is the synthesis of RNA from DNA and enzymes. Linear DNA is split by an enzyme called DNase I, which is then read by RNA polymerase. As the RNA polymerase reads the DNA, it uses the nucleotides in the solution around it to construct the desired RNA. Transcription begins with initiation. This is where RNA polymerase binds to the promoter of the DNA sequence to be transcribed. During elongation the RNA polymerase adds the correct complementary base pair to the strand of DNA that the promoter has bonded to. RNA polymerase uses base pairs adenine,

guanine, and cytosine, but replaces thymine with uracil instead. Lastly, during termination the RNA polymerase releases the new mRNA strand that has been formed.

The feed for this step is composed of 11.7 g of pDNA. In the transcription reactor, this pDNA reacts with DNase 1, RNA polymerase, CleanCap AG and DNA template to produce 50 times more mRNA.[9] Therefore, the following reaction was determined based on estimating the raw materials provided by a Public Citizen report.[9]

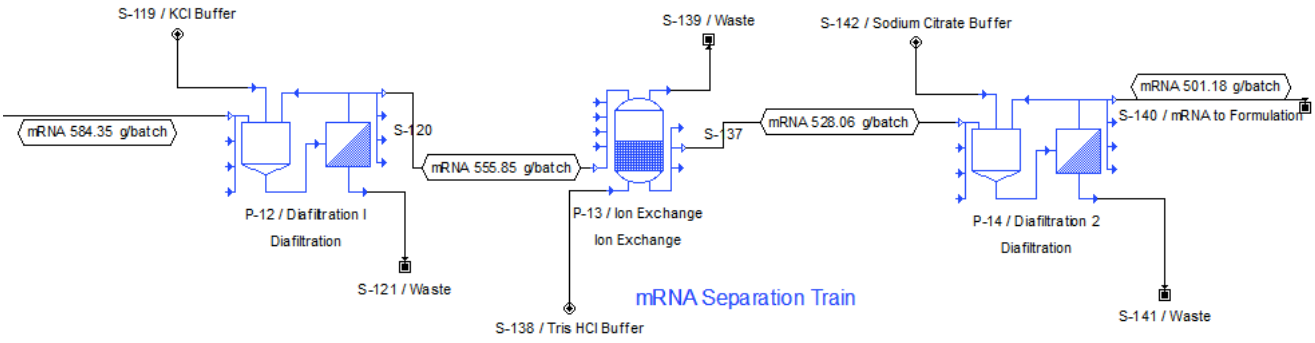
Equation 7. Estimated mass-based transcription reaction.

14.52 ATP Solution + 6.18 Cholesterol + 14.75 CleanCap AG + 1.00 DNA + 18.14 DNA Template + 0.36 DNase I + 25.04 Sucrose → 50.00 mRNA + 30.00 Cell Waste

4.4 mRNA Separation Train

The purpose of the mRNA separation train is to extract, concentrate and purify mRNA from any additional impurities before final formulation. This process consists of three separation operations, an ion exchange chromatography unit between two diafiltration steps. Each will remove any remaining endotoxins, dsRNA, residual DNA template, RNA polymerase as well any cellular components. These wastes will be sent to kill tanks to be decontaminated and sent into the local sewer system (Section 4.5.2).

Figure 13. PFD of the mRNA separation train.



4.4.1 Diafiltration

Diafiltration utilizes a permeable ultrafiltration membrane with a tangential flow system to separate out impurities. The membrane is typically a polymer, such as polyether sulfone and requires a support. The membrane traps impurities and unwanted components, while the desired components flow through as the permeate. A buffer solution is added to pump the fluid tangentially. This is typically operated at pressures between 2-4 atm and at room temperatures.

The first step uses a KCl buffer, while the second diafiltration unit uses a sodium citrate buffer. The second step is mainly to concentrate mRNA for final formulation, removing salts and any remaining solvents. Both steps each have a mRNA recovery rate of 95%.

4.4.2 Ion Exchange Chromatography

Ion exchange chromatography (IEC) is a technique used to remove impurities by binding to hydrophobic and positively charged ligands. The solution is added to the unit with a Tris HCl buffer which drives the elution. As the solution travels through a column, impurities bind with the bed, while mRNA is recovered. With a contact time of 2 hours, this process operates at 4°C and 2 atm and is estimated to recover 95% of the mRNA.[27]

4.5 Other Process Treatment Steps

4.5.1 Media Prep

Cell culture media is necessary to provide the appropriate amount of energy, nutrients, growth factors, and hormones for the cell to grow and multiply. It also helps to regulate the pH and osmotic pressure for the culture. Media is typically available in both a liquid and powdered form. Powdered media is used in this process. Therefore, a water for injection loop is required.

The table below provides the components required for the media for a fed-batch protocol.[28] Luria Broth (LB) is nutrient-rich and contains peptides, amino acids, and carbohydrates in a low-salt formulation. LB consists of 5 g/L NaCl, 10 g/L tryptone, and 5 g/L yeast extract and has a pH of 7.0. For each batch producing 16 million doses, approximately 113 L of Luria broth is required.

Table 7. Media composition for fermentation.

Component	Concentration Required (g/ L)	Amount Required (g/batch)
Lennox Luria Broth	20	2300 g
Glucose	2	230 g

Water for injection is fed to a sterile, single-use media bag fitted within a containment barrel and mixed with the media. Then it is sterilized using a 0.2 μm filter and stored in a storage tank until it is needed in the fermentation steps.

4.5.2 Kill Tanks

Biologically contaminated effluents can be treated by chemical or thermal means. Chemical methods include using sodium hypochlorite or peracetic acid. However, leaves harmful chemicals to leech into the wastewater supply. Thermal methods require a combination of heat and pressure conditions to ensure that all biological components are inactive. Continuous decontamination processes require high pressures (5 to 7 bar), high temperatures (130°C to 165°C), but shorter exposure times (3 to 10 seconds to a maximum of a few minutes).

The equipment required for this process include three heat exchangers- a pre heater to bring the wastewater up to 100°C, a sterilization heater at 140°C for 30 seconds and a cooling heat exchanger. In addition, three collection tanks will be required, one to collect the wastewater, as well as two holding tanks to sample the wastewater before releasing it into the sewer systems. Figure 14 shows the decontamination process for the vaccine plant.

4.5.2 Water for Injection Loop

Water for injection (WFI) can either be purchased or produced on site. The following table provides the specifications for WFI as per United States Pharmacopoeia (USP). For our plant which is estimated to require almost 2 million liters of WFI a year, the purchasing cost would reach $19 million dollars. Considering these costs, it was determined that producing this WFI onsite would be more economical. Producing WFI is a three-step process including 1) purified water formation, 2) WFI generation and finally 3) WFI storage. The major equipment required include a filtration unit, a reverse osmosis unit, multiple columns (6) and a WFI storage tank. The process flow diagram is provided in Figure 15.

Table 8. WFI standards.

Specification[29]	
Conductivity (at 25°C)	1.3 µS/cm
Total Organic Carbon	≤ 500 pb
Bacterial Endotoxin	≤ 0.25 IU/mL
Total Viable Aerobic Count (using R2A @ 30-35°C for 5 days)	≤ 10 cfu/100 mL

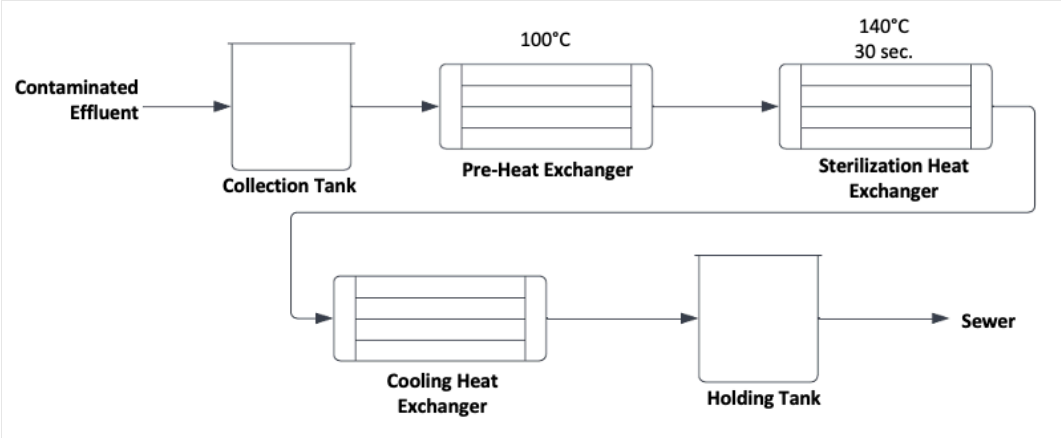


Figure 14. Decontamination process flow diagram.

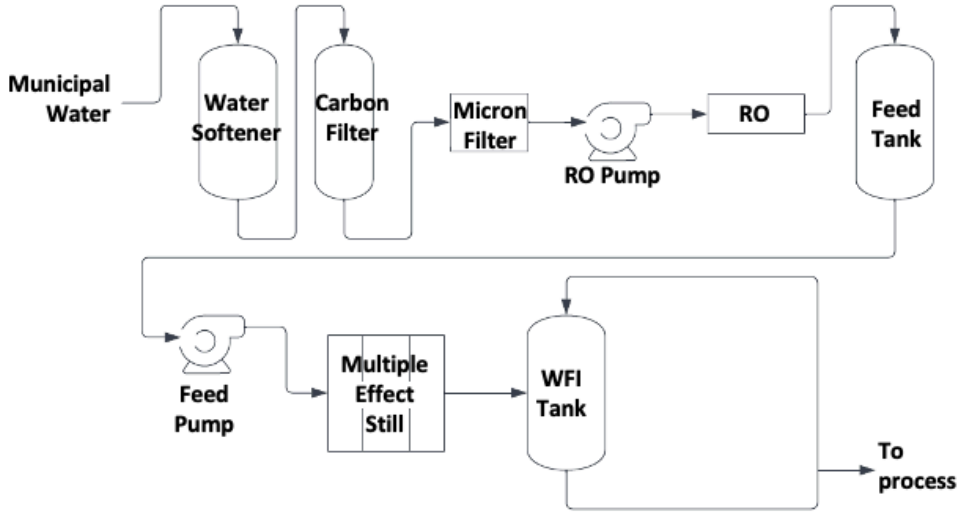


Figure 15. WFI production process.

Equipment Lists and Unit Descriptions

Below is the list of the major equipment that is required for the facility. Sections are separated by their functionality according to the block flow diagram proposed for the manufacturing design.

Table 9. Major equipment list

System	Equipment	Description
Fermentation Train	XCellerex XDR 10L Single Use Stirred Tank Bioreactor	To provide an environment for culturing E.coli cells containing SARS-CoV-2 plasmid
	XCellerex XDR 50L Single Use Stirred Tank Bioreactor	To provide an environment for culturing E.coli cells containing SARS-CoV-2 plasmid
	XCellerex XDR 120L Single Use Stirred Tank Bioreactor	To provide an environment for culturing E.coli cells containing SARS-CoV-2 plasmid
DNA Separation Train	Multifuge X4 Pro Series Centrifuge Packages by Thermo Fisher Scientific	To separate solid cell matter from liquid cell broth and waste
	GEA Ariete Homogenizer NS2006	To break the cell wall and release cell contents
	Ultipor N66 Sterilizing-Grade Filter Cartridges	To clarify pDNA by tangential flow filtration
	ÄKTAprocess™ Automated Liquid Chromatography System	To remove impurities including supercoiled and open-circles forms of pDNA by size
mRNA Separation Train	Repligen KrosFlow TFF System	To remove impurities and isolate mRNA using a permeable membrane
	Pall PKP Chromatography System	To remove impurities by binding to hydrophobic and positively charged ligands
Large Scale PCR	PCRMax Alpha Cycler 2 Thermal Cycler, dual 96-Well Capacity	To create more copies of separated pDNA
DNA Transcription	XCellerex XDR 120L Single Use Stirred Tank Bioreactor	To transcribe DNA to mRNA
Media Prep	XCellerex XDM QUAD Single-Use Mixing Systems	To prepare broth for fermenter system
Decontam-ination	Alfa Laval Pharma-line P Double-Tube Shell & Tube Heat Exchanger	To inactivate cell wastewater through thermal treatment
WFI Loop	Crystal Quest 300 GPD RO	To removes dissolved ions, bacteria, viruses and suspended solids
	MECO 6ME25 Six Unit Multiple Effect Still	To separate impurities and destroy microorganisms
	MECO WS18 Water Softener	For dechlorination of municipal water

EQUIPMENT SPECIFICATION SHEETS

The following sections provide equipment specifications and photos for each major unit operation.

6.1 Fermentation Train

XCellerex XDR 10L Single Use Stirred Tank Bioreactor Specifications	
	
Model No.	601C-K111-C4
Size	10 L
Manufacturer Description	The single-use Xcellerex XDR is a flexible, stirred-tank bioreactor system suitable for use in process development, small-scale production, and process troubleshooting. Allows smooth scale-up to full production. Sparging flexibility offers excellent adaptability to a broad range of cell lines. Low-shear, high-input power impeller for gentle, efficient mixing for sensitive cells or media. Optimized impeller position helps maximize mass transfer and turndown ratio. Smart automation ensures process performance and consistent data management.
XCellerex XDR 50L Single Use Stirred Tank Bioreactor Specifications	
	
Model No.	601E-F114-C4
Size	50 L
Manufacturer Description	The single-use Xcellerex XDR is a flexible, stirred-tank bioreactor system suitable for use in process development, small-scale production, and process troubleshooting. Allows smooth scale-up to full production. Sparging flexibility offers excellent adaptability to a broad range of cell

	lines. Low-shear, high-input power impeller for gentle, efficient mixing for sensitive cells or media. Optimized impeller position helps maximize mass transfer and turndown ratio. Smart automation ensures process performance and consistent data management.

XCellerex XDR 120L Single Use Stirred Tank Bioreactor Specifications	
	
Model No.	601G-F224-C4
Size	120L
Manufacturer Description	The single-use Xcellerex XDR is a flexible, stirred-tank bioreactor system suitable for use in process development, small-scale production, and process troubleshooting. Allows smooth scale-up to full production. Sparging flexibility offers excellent adaptability to a broad range of cell lines. Low-shear, high-input power impeller for gentle, efficient mixing for sensitive cells or media. Optimized impeller position helps maximize mass transfer and turndown ratio. Smart automation ensures process performance and consistent data management.

6.2 DNA Separation Train

Multifuge X4 Pro Series Centrifuge Packages by Thermo Fisher Scientific	
	
Model No.	75009500
Size	4L
Manufacturer Description	Pro series touch screen enables easier programming for quicker results--plus easily access and track all runs, rotor life and centrifuge health. With capacity up to 4L, including 196 blood tubes and 96 15mL conical tubes, AutoLock for fast rotor exchange.

GEA Ariete Homogenizer	
	
Model No.	NS2006
Size	900 L/h (maximum at 800 bar)
Operating Pressures	1-1500 bar
Manufacturer Description	Available up to 1500 bar, suitable for CIP and SIP, the Ariete machines can be supplied with all necessary customizations for easy integration in any process line, sanitary or aseptic. The wide range of available materials, linear plunger velocity allow Ariete machines to perform at their best also on abrasive and viscous product. High efficiency, excellent performance at very high pressures, high reliability in continuous processing (24/7), reduced operating costs (water, lubrication oil and power supply), suitable for abrasive and viscous products. equipped with complete documentation for the pharmaceutical sector, aseptic design, fully automated and remote control option, safe and easy to use, easy maintenance
Ultipor Sterilizing-Grade Filter Cartridges	
	
Model No.	N66
Size	Diameter 70 mm; Length 254 mm
Operating Pressures	1-1500 bar
Manufacturer Description	Pall Ultipor® N66 sterilizing-grade filter cartridges feature high-strength pure Nylon 6,6 membranes for higher sterility assurance. In wide use for almost 20 years, these filters have a proven record of performance in the production of sterile biologicals and pharmaceuticals. High-area pleated into single open-ended (SOE) AB sanitary style cartridges, Ultipor N66 sterilizing-grade filter cartridges are available with ratings from 0.45 μm for LVPs, reagents and viscous fluids, 0.2 μm for sterile products and intermediates, 0.1 μm for sterilization and mycoplasma removal from biologicals, to 0.04 μm for sterilization and virus reduction from biologicals.

ÄKTAprocess™ Automated Liquid Chromatography System	
	
Model No.	29692670
Size	1-2000 L/h
Max Operating Pressures	6 bar
Manufacturer Description	ÄKTA process™ DCS is a high-quality chromatography system delivered with a distributed control system designed for an integrated manufacturing environment. Standard platform available in three sizes with thousands of possible configurations together with a predefined automation solution, intelligent packing of AxiChrom™ columns, gradient mixing and inline dilution (ILD), post-purchase upgrades increase usability and lifespan, full regulatory documentation, service and support

6.3 DNA Amplification and Transcription

PCRMax Alpha Cycler 2 Thermal Cycler, dual 96-Well Capacity	
	
Model No.	EW-93945-22
Size	470 mm x 535 mm x 330 mm
Temperature Range	10-100°C
Manufacturer Description	Pro series touch screen enables easier programming for quicker results--plus easily access and track all runs, rotor life and centrifuge health. With capacity up to 4L, including 196 blood tubes and 96 15mL conical tubes, AutoLock for fast rotor exchange.

XCellerex XDR 120L Single Use Stirred Tank Bioreactor Specifications	
	
Model No.	601G-F224-C4
Size	120L
Manufacturer Description	The single-use Xcellerex XDR is a flexible, stirred-tank bioreactor system suitable for use in process development, small-scale production, and process troubleshooting. Allows smooth scale-up to full production. Sparging flexibility offers excellent adaptability to a broad range of cell lines. Low-shear, high-input power impeller for gentle, efficient mixing for sensitive cells or media. Optimized impeller position helps maximize mass transfer and turndown ratio. Smart automation ensures process performance and consistent data management.

6.4 mRNA Separation Train

Repligen KrosFlow TFF System	
	
Model No.	-
Size	1-500 L feed volume capacity
Membranes	Flat Sheet Casettes
Maximum Pressure	2.5 bar
Manufacturer Description	The KrosFlo® FS Systems achieve an unparalleled combination of configurability and automation and compliance for flat sheet TFF processes. All components, either directly or indirectly, connect to the main KrosFlo® FS diaphragm pump, which then communicates with the KF Comm 2 software for control and monitoring. Configure a minimal number of components for simple applications such as concentration (C) or diafiltration (D). Plug-and-play additional components for fractionation (F) and multi-step processes (i.e. CDC/D/C

Pall PKP Chromatography System	
	
Model No.	-
Size, Diameter	3/8”
Pump Flow Rate	1-150 L/h
Maximum Pressure	6 bar
Maximum Temperature	2-60°C
Manufacturer Description	Pall’s PKP chromatography products, a family of standard modular automated systems, are designed to perform all types of low pressure column biochromatography (e.g., ion exchange, affinity, mixed mode, hydrophobic interaction, size exclusion and hydroxyapatite) and membrane chromatography. These systems are suitable for small-scale production or process optimization, in addition to manufacturing biotherapeutic molecules according to GAMP guidelines. Includes a flow, air, pressure, temperature, conductivity, pH and UV sensor.

6.4 Other Process Treatment Steps

Alfa Laval Pharma-line P Double-Tube Shell & Tube Heat Exchanger	
	
Model No.	-
Heat Transfer Area	0.1 m2 – 8.5 m2
Operating Temperatures	-15°C to 150°C
Maximum Pressure	10 bar
Manufacturer Description	The Pharma-line shell and tube heat exchanger is designed to operate at pressures up to 10 bar and operating temperatures of 150°C. It is easy to drain and clean and features leakage detection. It is fully drainable on the product side, with no dead spots and has vent and drain possibilities on the utility side. The hygienic heat exchanger also comes with a high-quality documentation package as standard.

Crystal Quest 300 GPD RO	
	
Model No.	CQE-CO-02023
Size, capacity	300 GPD
Operating Pressure	150 psi
Maximum NaCl - % Rejection	98.5%
Maximum TDS	1000 ppm
Maximum Hardness (GPG)	15

MECO 6ME25 Six Unit Multiple Effect Still	
	
Model No.	6ME25
Distillate Capacity	341-382 GPH
Steam Capacity	670-800 lbs/hr
Manufacturer Description	Multiple effect distillation is the most commonly used method for the production of WFI throughout the world. MECO multiple effect distillers are designed to produce Water for Injection (WFI) in accordance with all recognized international pharmacopoeias. The ME stills are modular in design and include options for simultaneous and exclusive pure steam production. In addition, the MECO multiple effect stills meet or exceed the latest cGMP requirements, including sanitary construction features such as double tubesheets on the first effect, interstage and condenser exchangers, sloped piping and low point drains, minimum deadlegs, and sanitary connections. MECO ME stills come equipped with a dual condenser mounted above the unit for gravity flow into the WFI tank.

MECO WS18 Water Softener	
	
Model No.	WS18
Flow Rate Range	2-11 LPM
Manufacturer Description	MECO water softeners are designed for the removal of hardness, ammonia or both. The softeners are designed based on your effluent water quality required and the feedwater analysis. Softeners can be provided individually or as part of a total water treatment solution. The control system for the water softener can be dedicated to the softener or part of a total system. Softener systems are designed specifically for your application using either fiberglass, PVC or stainless steel in the materials of construction.

EQUIPMENT COST SUMMARY

The total cost of equipment required for the manufacturing plant was estimated to be $1,767,450. The cost of each unit is summarized below in Table 10.

Table 10. Equipment cost summary

System	Equipment	Quantity	Total Cost
Fermentation Train	XCellerex XDR 10L Single Use Stirred Tank Bioreactor	1	$65,000
	XCellerex XDR 50L Single Use Stirred Tank Bioreactor	1	$130,000
	XCellerex XDR 120L Single Use Stirred Tank Bioreactor	1	$220,000
DNA Separation Train	Multifuge X4 Pro Series Centrifuge Packages by Thermo Fisher Scientific	1	$14,000
	GEA Ariete Homogenizer NS2006	1	$95,000
	Ultipor N66 Sterilizing-Grade Filter Cartridges	2	$15,040
	Masterflex L/S Digital Miniflex Peristaltic Pump System	2	$4,310
	ÄKTAprocess™ Automated Liquid Chromatography System	1	$160,000
mRNA Separation Train	KrosFlow TFF System	2	$168,000
	PKP Chromatography System	1	$100,000
Large Scale PCR	PCRMax Alpha Cycler 2 Thermal Cycler, dual 96-Well Capacity	1	$9,100
DNA Transcription	XCellerex XDR 120L Single Use Stirred Tank Bioreactor	1	$220,000
Media Prep	XCellerex XDM QUAD Single-Use Mixing Systems	1	$39,000
Decontam-ination	Alfa Laval Pharma-line P Double-Tube Shell & Tube Heat Exchanger	3	$81,000
WFI Loop	Crystal Quest 300 GPD RO	1	$2,000
	MECO 6ME25 Six Unit Multiple Effect Still	1	$480,000
	MECO WS18 Water Softener	1	$4,000
		Total	**$1,767,450**

MANUFACTURING COSTS

The manufacturing costs of the plant were determined from the replaceable equipment, input costs, utilities, and operating labor. Unlike the fixed capital costs, the manufacturing costs begin during the first year of operation. The manufacturing costs represent the operating cost to produce the vaccines. Prior to producing vaccines, the plant is under construction; so, costs to produce the vaccines was not considered. The plant is in Las Palmas, Medellín, Colombia, which is where the raw materials are shipped to.

8.1 Material Costs

Equipment requiring regular replacements or recycling are shown in the table below.

Table 11. Equipment operating costs.

Equipment	Amount	Cost/year
Centrifuge Bottle	24	$900
Ultrafiltration Filter Membrane & Ultipor Biodyne Amphoteric Support Nylon	6	$18,000
Size Exclusion Chromatography HiPrep™ 16/60 Sephacryl® S-500 HR columns Column	48	$362
PCR Tubes	12	$12,000
Ion Exchange Capto Core 400 Resin	200 mL	$1,300
Diafiltration TangeX ProStream 300 kDA membrane (2 units)	4	$3,700
Reactor Bags	24	$12,000
Miscellaneous Disposables[9]	-	$1,800,000
	Total	**$1,850,000**

Inputs needed for the production cycle as well as their costs and where they will be purchased from can be seen in the table below.

Table 12. Input material costs.

Material	Amount (kg)	Cost/year	Source
Nucleotides	68	$27,000	Thermofisher (Bogota, Colombia)
Primer	0.02	$6,000	Thermo Fisher (Bogota, Colombia)
Deoxyribonuclease I (DNase I)	3	$1,600	Thermofisher (Bogota, Colombia)
RNAase Enzyme Inhibitor	5	$13,000	Thermofisher (Bogota, Colombia)
T7 RNA Polymerase	20	$2,700,000	Qiagen (Brazil)
Modified RNA Base	23,000	$18,000,000	
Phospholipid	10,200	$204,000,000	Thermofisher (Bogota, Colombia)
Cholesterol	26,000	$300,000	Thermofisher (Bogota, Colombia)
Ethanol	10,000,000	$2,800,000	Thermofisher (Bogota, Colombia)
Ionizable Lipid	47,000	$85,000,000	BroadPharm (USA)
PEG Lipid	8,000	$153,000,000	BroadPharm (USA)
DNA Template Solution	7	$7,000	BroadPharm (USA)
Tris HCl Buffer	1,283	$18,000	Thermofisher (Bogota, Colombia)
Sodium Citrate Buffer	5,000	$22,000	Thermofisher (Bogota, Colombia)
Ammonia Sulfate Buffer	5	$1,200	Thermofisher (Bogota, Colombia))
Luria-Bertani broth	2.3	$400	Sigma Aldrich (Bogota, Colombia)
	Total	**$468,000,000**	

mRNA ready and prepared for formulation and processing will be shipped to ___. Once this site completes the final steps of vaccine production, doses will be distributed across South America.

8.2 Labor Costs

Labor costs for operators, engineers, lab technicians, managers, facility personnel, and office/secretarial employees are included in operating costs. Operators, engineers, and facility personnel are present on the plant all day long, so 3 sets of 8-hour shifts throughout each day were determined. Lab technicians work on batch processes or operations that do not take long period of time, so they are expected to work daily 8-hour shifts. Office and secretarial employees are also expected to work 8-hour shift days. The operating time for 100 million doses of the vaccine per year do not include weekends because our shorter operating time allows for this. Salaries were determined for average annual salaries for each role in Colombia. The salaries for each employee

are much greater than the minimum wage salary in Colombia. Employee benefits were approximated to be 120% of each employee's salary.

Table 13. Labor Operating Costs

Type of Employee	Number of Employees Needed	Approximate Cost (USD$/year)
Operators	27	$450,000
Engineers	12	$300,000
Lab Technicians	5	$56,000
Managers and Facility Personnel	15	$104,000
Office and Secretarial Employees	5	$75,000
Employee Benefits		$1,182,000
Total	**64**	**$2,167,000**

FIXED CAPITAL INVESTMENT SUMMARY

9.1 Working Capital

An open plot of land in Las Palmas, Medellin, Colombia has been selected for the construction of the vaccine plant. Near this plant site location, there are many pharmaceutical manufacturing facilities available, which allows for better accessibility of transporting products and ensures technical capability. This site costs $204,490 USD for purchase and is 1000 m^2 in size. This land was desirable due to its location in Medellin and its proximity to the city's center and relevant facilities. Construction of the facility in the figure below, including HVAC, plumbing, and electricity, in Colombia is approximated to cost $900,000 USD. This value was determined by considering the average construction costs by a bioengineering manufacturing facility construction firm[30] and applying the correct area. The cost includes general construction of the building from ground up, HVAC, plumbing, electrical, and sitework. The plot of land and building construction are considered working capital because they are assets that are assumed to have minimal to negligible depreciation over the ten-year plant lifetime.

Table 14. Working Capital Assets

Asset	Area	Cost
Land	1000 m^2	$205,000
Building Construction (HVAC, Plumbing,…)	1000 m^2	$900,000

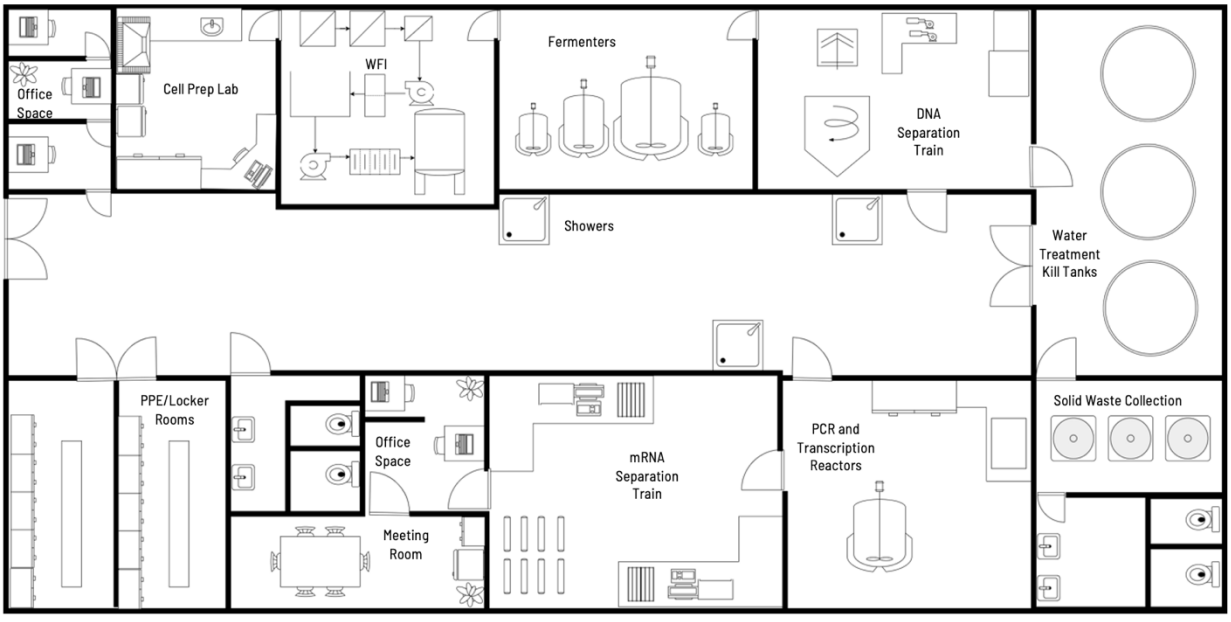


Figure 16. Manufacturing Plant Layout

9.2 Fixed Capital

The fixed capital includes the one-time purchase equipment, where 30% of the fixed capital is invested in the first year of construction and the rest is invested in the year after. For years during operations, which are the years the vaccines are produced, depreciation of the fixed cost was

dependent on the respective previous year. The fixed capital includes the one-time purchase equipment.

9.3 Fixed Capital Depreciation

The depreciation for the first six years, including the two years before operation, was found using the MACRS scheme. The MACRS method used a double declining balance method before switching to a straight-line method for the remaining lifetime of the processing plant. This uses a constant depreciation rate of 1/6 to have a total fixed capital cost of $0 by the end of the last year, which is 10 years of operations. The straight-line method for depreciation is found using the number of years of depreciation:

Equation 8. Straight-Line Depreciation Rate

$$Depreciation\ Rate_{straight-line} = \frac{1}{number\ of\ years}$$

The depreciation factors multiplied with fixed capital costs apply to years before operating as well, and are shown in the table below:

Table 15: Depreciation Rates by Year

Year	Depreciation Allowance (of Total Capital Cost)
Construction Year 1	20.00 %
Construction Year 2	32.00 %
Operating Year 1	19.20 %
Operating Year 2	11.52 %
Operating Year 3	11.52 %
Operating Year 4	5.76 %
Operating Year 5-10	$^1/_6$

The approximated depreciation is applied to the fixed capital at the end of the year. As shown below, the fixed capital from year -1 to year 0 increases because the remaining 70% of fixed capital is invested during plant construction. During years of operation, the fixed capital is seen depreciating at the rates shown in the above table. The end of year 10 shows that the depreciation is applied at the end of the year and the equipment considered in fixed capital are considered to have lost value.

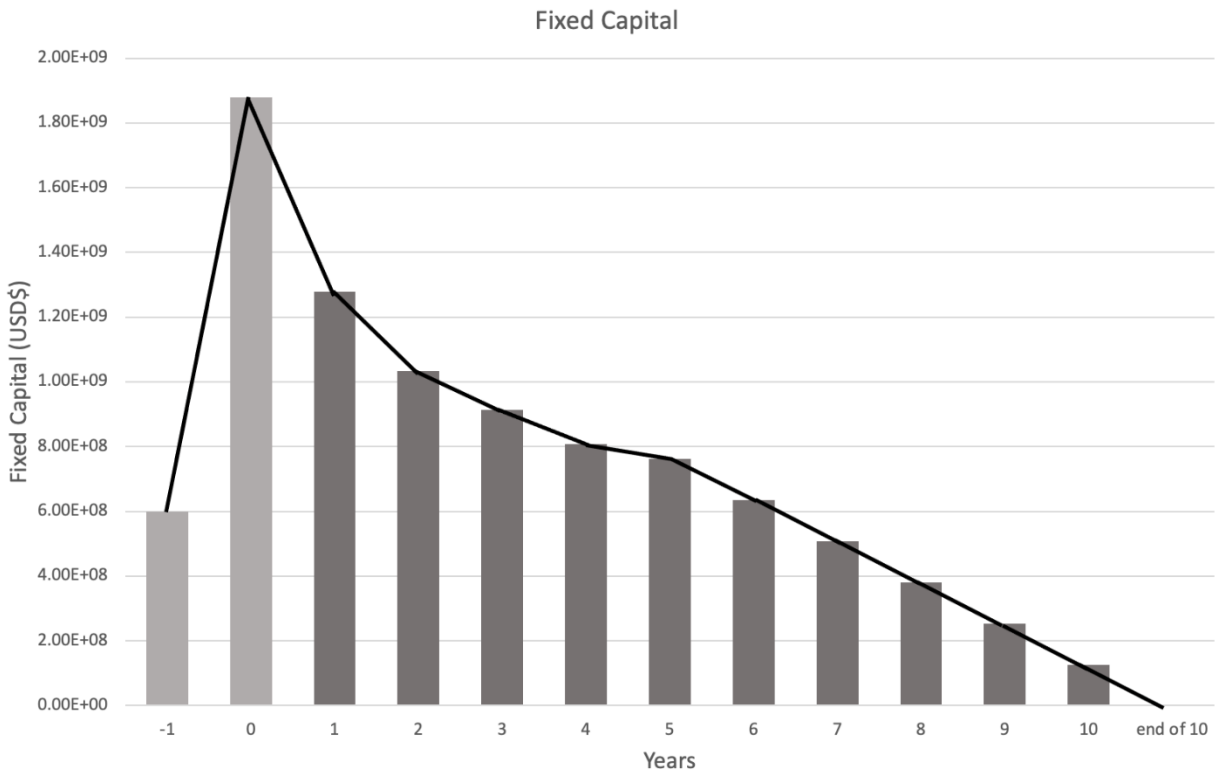


Figure 17. Depreciating Fixed Capital, where year -1 is the first year of construction, 0 is the second year of construction, and years 1-10 represent the years the plant is operating

ECONOMIC ANALYSIS

A cash flow position form is meant to accumulate data regarding a company's profits and liabilities to determine its overall cash flows from sales, investments, debts, etc. For these calculations, it was assumed that the first two years would be spent constructing the plant and setting up the manufacturing floor, and that the plant would be in operation for 10 years. The revenue from sales was assumed to be constant throughout the 10 years of the plant's operation. The depreciation signifies how the value of the company's assets decrease over time. The net profit was calculated by accounting for the manufacturing cost and depreciation with respect to the revenue. The tax rate was predetermined to 35% and was multiplied by the net profit. Lastly, the cumulative cash flow rate for each year was determined by subtracting the depreciation from the sum of the profit after tax and the cumulative cash flow from the previous year. The cumulative cash flow for the first year was determined by subtracting the depreciation from the net profit.

As of now, the Pfizer vaccine goes for $12 per dose in Columbia. Using this price, a cumulative cash flow sheet was made. The return on investment, assuming manufacturing costs predictions are correct, and all vaccines are sold for $12, is 209588.58%. For an ROI of 20%, our required price for one dose would be $5.55. For a fully vaccinated adult, including the booster shot, it would cost $16.65. A cash flow diagram was made, the cumulative cash flow becomes positive between 2-3 years of operation.

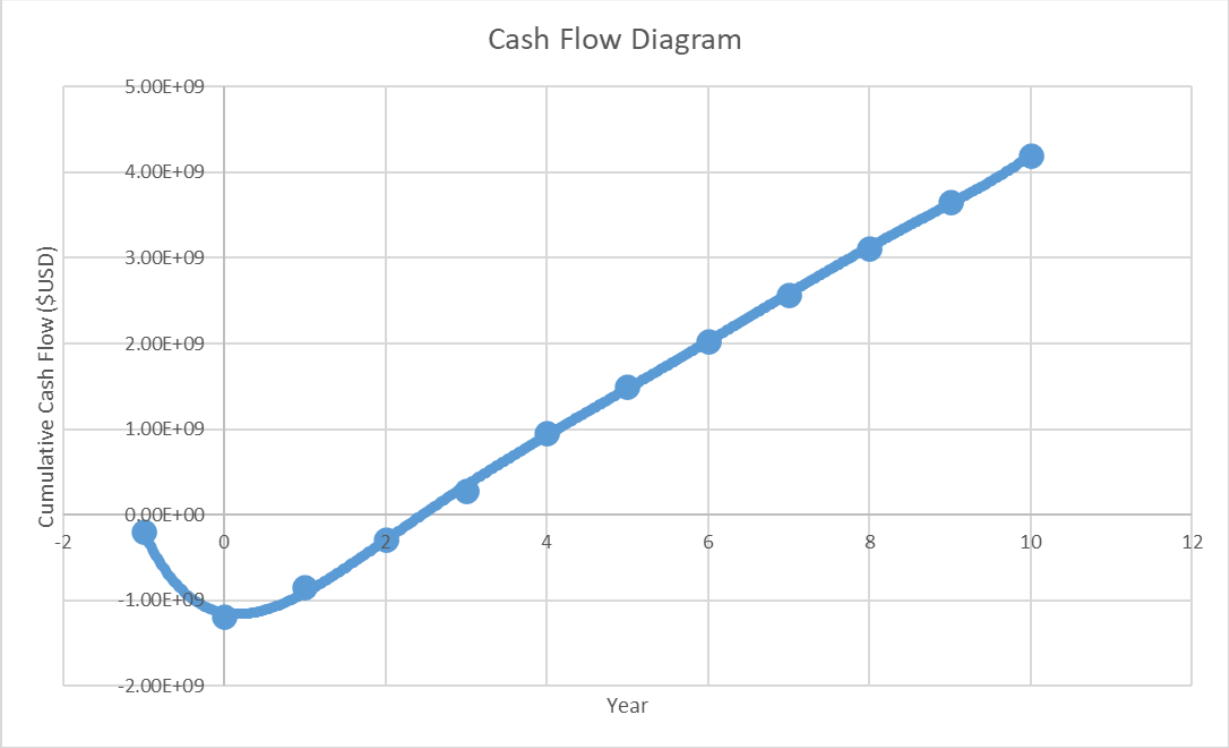


Figure 18. Cash Flow Diagram

The three variables that were thought to be the greatest risk to our profit were competition, price, and cost of the materials. Competition was studied by looking at the possibility of 85% to 100% of our product being sold, in increments of 5%. The variable costs were studied by looking at the

possibility of our material costing 90% to 110% of our initial estimation. Sensitivity analysis was done to determine the ideal price of the vaccine.

Payback period (PBP) is the length of time required for an investment to recover its initial investments. For the analysis of competition and cost of materials, the payback period was calculated for a combination of conditions. The table shown below shows the payback period in years for these conditions, at the price of $12 per vaccine. As shown, the percent of vaccines sold will have a greater effect on the profits than variable costs. The table below shows sensitivity of payback period dependent on number of vaccines sold by percent. This is assuming a possible decrease in demands as covid cases decrease.

Table 16. Payback Period for Varying Vaccines Sold and Costs

Payback Period		Vaccine Sold			
		85%	90%	95%	100%
Variable Costs	90%	3-4	2-3	2-3	2-3
	95%	3-4	2-3	2-3	2-3
	100%	3-4	2-3	2-3	2-3
	105%	3-4	2-3	2-3	2-3
	110%	3-4	2-3	2-3	2-3

In order to analyze the PBP of the plant for different prices, a cumulative cash flow diagram was created with an assumption of 100% sold vaccines. As shown in the figure below, at a selling price of $10, the PBP is between 3-4 years, and at a selling price of $8, the PBP is between 4-5 years.

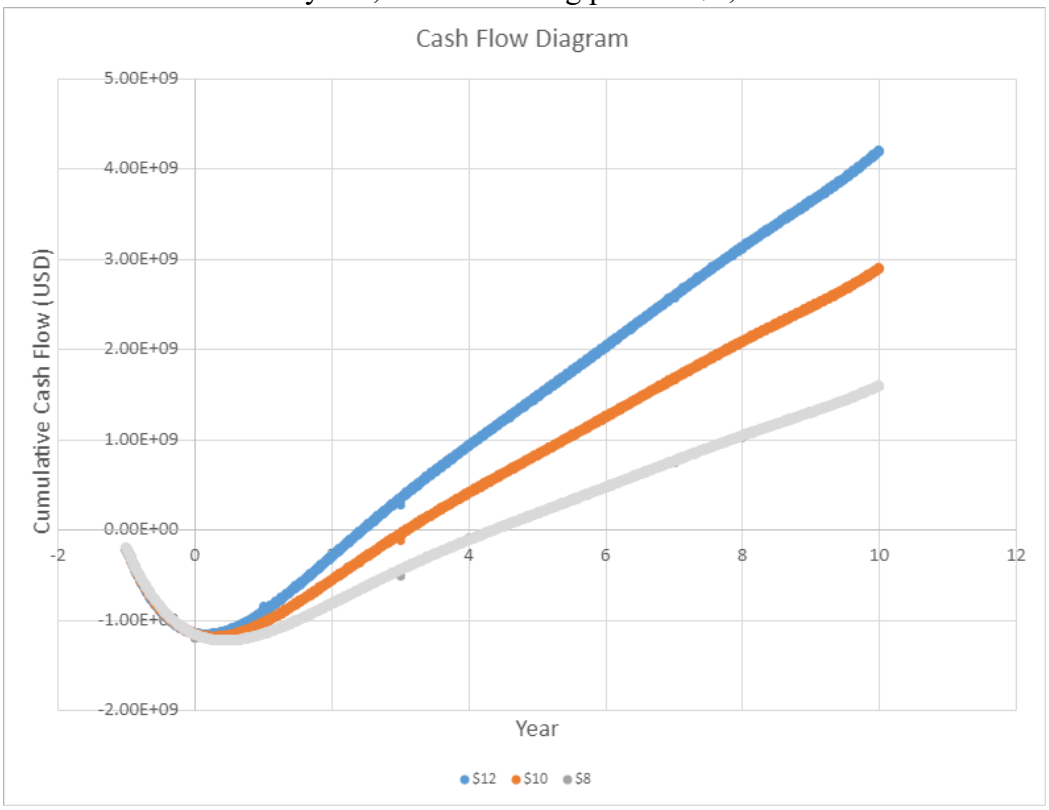


Figure 19: Cash flow diagram for different vaccine prices.

The Discounted Cash Flow Rate of Return (DCFROR) represents the highest after tax interest rate at which the project can break even. A good value for the DCFROR for investors is 50% and above. The DCFROR tables are colored with red being low and green being high. The greener values are therefore considered to be safer because the interest rate can be higher. Considering the possibility of fluctuating manufacturing costs and percent of vaccines sold, it would be safest for our investment if the vaccines are sold for at least $10.50.

Table 17: DCFROR for vaccines sold

DCFROR		Vaccines Sold			
		85%	90%	95%	100%
	5	-107.22%	-106.72%	-106.02	-105.19%
	5.5	-92.76%	-91.86%	-90.78%	-89.59%
	6	-78.29%	-77.00%	-75.54%	-73.99%
	6.5	-63.83%	-62.13%	-60.30%	-58.39%
	7	-49.36%	-47.27%	-45.06%	-42.79%
	7.5	-34.90%	-32.41%	-29.82%	-27.18%
	8	-20.43%	-17.55%	-14.59%	-11.58%
	8.5	-5.96%	-2.68%	0.65%	4.02%
	9	8.50%	12.18%	15.89%	19.62%
	9.5	22.97%	27.04%	31.13%	35.22%
	10	37.43%	41.90%	46.37%	50.82%
	10.5	51.90%	56.76%	61.61%	66.43%
	11	66.36%	71.63%	76.85%	82.03%
	11.5	80.83%	86.49%	92.09%	97.63%
Price ($)	12	95.29%	101.35%	107.33%	113.23%

Table 18. DCFROR for variable costs.

DCFROR		Variable Cost				
		90%	95%	100%	105%	110%
	5	-105.32%	-105.26%	-105.19	-105.13	-105.05
	5.5	-89.67%	-89.64%	-89.59%	-89.55%	-89.50%
	6	-74.03%	-74.01%	-73.99%	-73.97%	-73.94%
	6.5	-58.39%	-58.39%	-58.39%	-58.39%	-58.38%
	7	-42.74%	-42.77%	-42.79%	-42.81%	-42.82%
	7.5	-27.10%	-27.14%	-27.19%	-27.22%	-27.26%
	8	-11.46%	-11.52%	-11.58%	-11.64%	-11.70%
	8.5	4.19%	4.10%	4.02%	3.94%	3.86%
	9	19.83%	19.72%	19.62%	19.52%	19.41%
	9.5	35.48%	35.35%	35.22%	35.10%	34.97%
	10	51.12%	50.97%	50.82%	50.68%	50.53%
	10.5	66.76%	66.59%	66.42%	66.26%	66.09%
	11	82.41%	82.22%	82.03%	81.84%	81.65%
	11.5	98.05%	97.84%	97.63%	97.42%	97.21%
Price ($)	12	113.69%	113.46%	113.23%	113.00%	112.76%

SUSTAINABILITY, SAFETY, HEALTH, AND ENVIRONMENTAL CONSIDERATIONS

The safety and health of our customers, workers, community, and surrounding environment remain the forefront of the concern in the planning of this manufacturing plant. International human rights and environmental agreements as well as Colombian labor and environmental regulations were examined to ensure this plant follows and promotes a safe and productive workplace for all.

11.1 Promoting Sustainability and Equity

To promote sustainability and equity for our facility, policies and regulations will be well communicated with employees. This is not limited to:

- Nondiscrimination policies
- Compliance with national labor laws/regulations
- Compliance with international human rights and environmental agreements
- Compliance with national labor laws/regulations
- Avoidance of child labor

Colombian labor laws state that minimum wage is 1,000,000 pesos per month, which is equivalent to $250 per month. For the proposed plant, the lowest paid employees will receive $930 a month, almost $700 more than required by law. In addition, the plant shift structure makes it possible that employees are not over worked and fatigued. Workers have 6-hour shifts and ensure that employees are not working more than 48 hours a week. Employees will receive rigorous training on new technologies to promote education and education programs and sponsorships are possible for those who may qualify.

Outside the plant, this facility promotes equity by giving back to the surrounding community. Workers are expected to be hired directly from neighboring areas to provide jobs to residents. In Colombia, only 42% of 25 to 64 year-olds have at least gone through upper secondary education, while the average Organization for Economic Co-operation and Development (OECD) rate is 75%.[31] Therefore, this plant will contribute to educating the local community by providing resources and tours for people to learn about the vaccine process. This may include institutes of higher education and schools to provide students with exposure to STEM outside of the classroom.

In terms of the customers, the vaccine provides individuals with the antibodies to fight the virus, therefore it is expected to help any person who may be exposed to the virus. Vaccine equity is important is not only to reach herd immunity but also to prevent additional deaths. This may be especially important in lower income areas where social distancing and masking, a proven way to reduce risk, may be harder to achieve. This vaccine is expected to address vaccination disparities in countries like Haiti where vaccination rates are around 1.6% and poverty and quality of life is low. Haiti is ranked 170 out of 189 of countries on the UN's Human Development Index.[32]

For the surrounding community, the location of the plant will not affect people in the surrounding community. The plant is located in an area with other manufacturing industries and not expected to present any new issues. Additionally, wastewater from the facility is properly decontaminated via heat treatment and all other wastes are sent to waste-to-energy (WTE) plants, rather than landfills. These waste streams will not pose risks.

11.2 Risks

In the biopharmaceutical industry, risks and hazard are associated from the acquisition of raw materials to the delivery of the final product. The identification and analysis of risks at each step is important to the workers, customers, and communities that the manufacturing process impacts. Appropriate prevention and control measures that follow regulatory (cGMP) and ethical standards should be set in place and communicated with all workers to ensure a safe and productive work environment. Clean rooms will also be in place to reduce the risk of contamination.

The risks associated with each step of the vaccine manufacturing process is summarized in the table below.

Table 19. Risk Summary.[33,34]

Process Stage	Associated Risks
Raw Materials	Lot-to-Lot variability; vendor qualification; origins of raw material; contamination; stability
Cell Banking	Viral/TSE/BSE clearance; bacterial endotoxins; mycoplasma; facilities; equipment; personnel training; documentation; improper storage conditions
Fermentation/Cell Banking	Contamination; suitable environment; culture media; oxygen requirements; sterilization; facilities and equipment;
Downstream Processing	Inefficient harvest/recovery conditions; inefficient purification conditions; viral contamination; endotoxins (pyrogens)
Waste Treatment	Contamination
All	Extractables and leachables[35]; contamination during passages between unit operations;

Each risk can be evaluated by the severity and probability of each event. This is summarized in the tables below.

Table 20. Risk probability scales.[36]

Rank	Probability
Frequent (5)	Likely to occur
Probable (4)	Will occur several times
Occasional (3)	Likely to occur sometime
Remote (2)	Unlikely but possible to occur
Improbable (1)	So unlikely, it can be assumed an occurrence may not be experienced.

Table 21. Risk severity scales.[34,36]

Rank	Severity
Catastrophic (4)	Failure has a severe impact on product safety and efficacy. Impacts may lead to death thereby requiring immediate cessation of the unsafe activity or operation.
Critical (3)	Failure has a high impact on product safety and efficacy. Impacts may commonly cause severe injury or illness thereby requiring immediate corrective action.
Marginal (2)	Failure has a medium impact on product safety and efficacy. Impacts can be counteracted or controlled without injury or illness.
Negligible (1)	Failure has a low impact on product safety and efficacy. Impacts will result in no, or less than minor, illness, and/or injury.

From there, by using the equation:

Equation 9. Risk.

$$Risk = Severity \times Probability$$

the risk can be quantified and visualized on a matrix to determine if the level is high, medium, serious, or low (Figure 19).

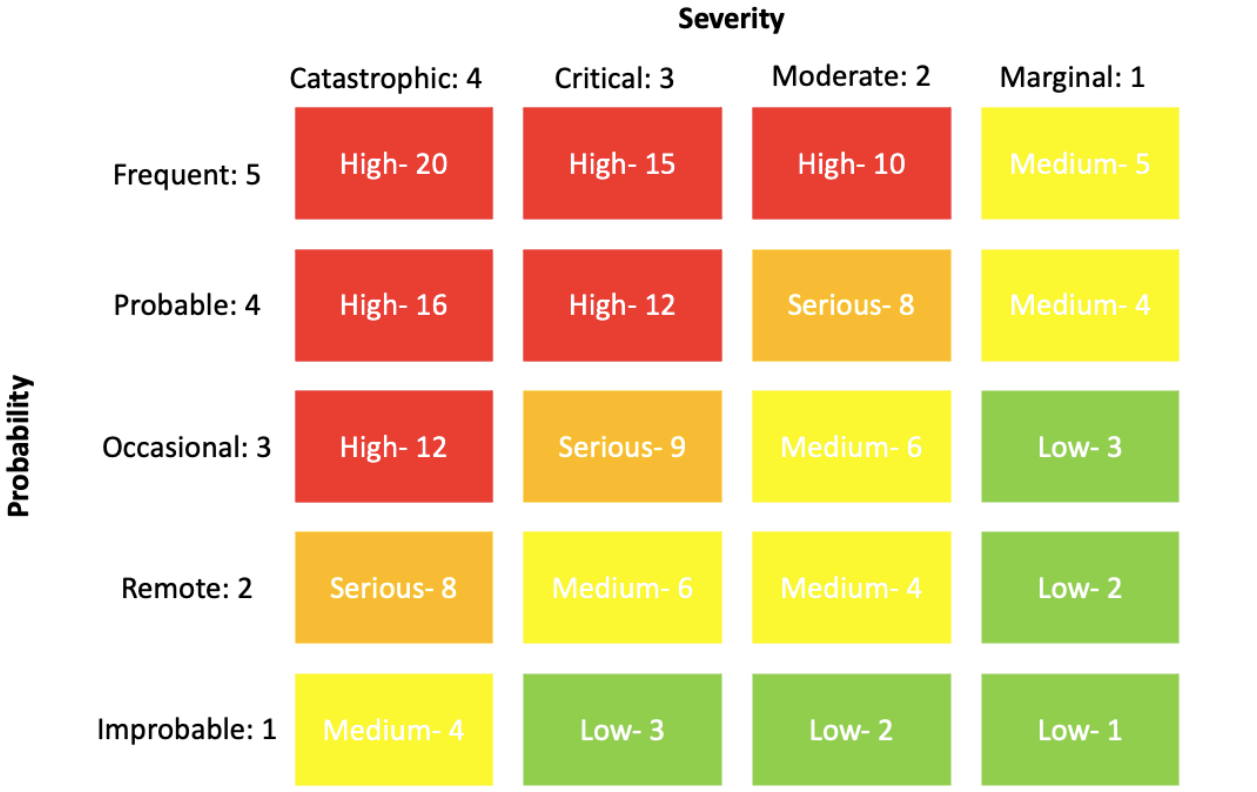


Figure 19. Risk Assessment Matrix.[36]

To determine ways to mitigate risks, questions that can be asked should include methods to minimize, substitute/eliminate, and simplify. These questions are not limited to:[37,38]

- How can we minimize the inventories of hazardous raw materials, intermediates and/or finished products?
- Is it possible to substitute less hazardous raw materials?
- Can procedures be designed so that it is difficult to create potentially hazardous situations due to an operating or maintenance error?
- Is it possible to design and construct vessels and piping to be strong enough to withstand the highest pressures to withstand even the "worst credible event"?

These issues can be addressed by implementing just-in-time deliveries, reducing the use of flammables such as ethanol (high pressure homogenization vs chemical lysis), use of automation and higher pressure vessels and tubing in the design.

There are many ways to reduce the overall risk of a hazard. Mitigation methods can be reduced by reducing the *probability* by making it less likely to occur, or by reducing the *severity* by making it less dangerous. For example, with Risk No. 2, *Contamination due to unwanted contaminants present in single use materials*, the probability can be reduced by using disposables, including single use reactors and columns. This type of equipment can be carefully selected to ensure the manufacturers follow proper sanitation protocols. Thus, the risks from improper cleaning for stainless steel equipment are reduced. In terms of severity, the severity of the issue cannot be practically reduced. If endotoxins are present in our inoculum, it may lead to death or aseptic shock. Thus, it is crucial that contaminants are properly separated out and the inoculum is rigorously tested so that these risks are minimized.

The calculation for the reduction in risk is provided below. Without these mitigation methods, the severity and likelihood of contamination due to equipment can be estimated to be *critical* and *probable*, making the risk level *High* (12). However, through the mitigation methods, the risk level can be reduced to *Medium* (6) by reducing the probability of the event, decreasing the risk level by 50%.

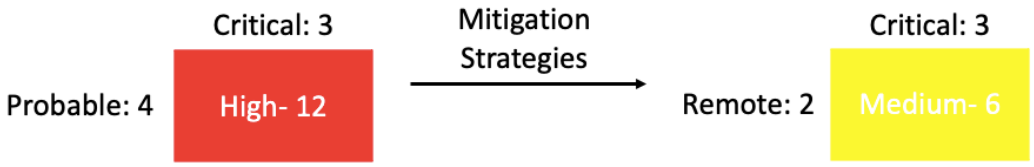


Figure 20. Risk reduction of Risk No.2 by reducing the probability of the event.

outlines the major risks that are posed to workers, customers, and the surrounding community. If the specified mitigation techniques are used, it is expected to reduce the overall risks by more than 50%.

No.	Hazard/Unwanted Event	Cause	Harm/ Consequences	To	Severity	Likelihood	Risk	Mitigations	% Risk Reduction
1	Contamination	Unwanted contaminants in raw materials	Death, Sickness	Customers	Critical	Remote	Medium (6)	careful selection and pre-treatment of raw materials to prevent viruses from entering upstream processes, rigorous testing, and appropriate purification and filtration technologies to remove contaminants downstream.	60%
2		Unwanted contaminants present in equipment	Death, Sickness	Customers	Critical	Remote	Medium (6)	careful selection of single use materials to ensure quality of reactor bags, tubing, and other associated disposables to ensure no reaction or leeching of the material occurs.	60%
3		Unwanted contaminants present in manufacturing space	Death, Sickness	Customers	Critical	Remote	Serious (6)	use of air locks, clean rooms and air filtration and proper aseptic training for employees on PPE, cGMP, regulatory compliance, equipment sterilization and maintenence.	50%
4		Inefficient Purification Conditions/Cell Harvest	Death, Sickness	Customers	Catastrophic	Remote	Serious (8)	Quality control measures to ensure proper purification and cell levels for formulation and packaging.	50%
5		Improper decontamination of cell waste	Death, Sickness	Local Community	Catastrophic	Remote	Serious (8)	proper thermal decontamination of effluent, sampling and testing of wastewater prior to release into local sewage.	60%
6	Fires	Improper equipment use or storage of flammables	Death, Injury	Workers	Moderate	Remote	Medium (4)	careful storage and documentation of flammable chemicals and proper training for workers, use of alarms and sensors to alert workers.	67%

Table 22. Risk Summary

11.3 Facility and Worker Risks

Hazard prevention and control in the workplace according to the Occupational Safety and Health administration (OSHA) guidelines include six levels of control methods to implement in the plant. This is called the hierarchy of controls, and from least effective to most effective it includes: PPE to protect the worker with personal protective equipment, administrative controls to change the way people work, engineering controls to isolate people from hazard, substitution to replace the hazard, and elimination to physically remove the hazard. Some of the hazards may include slips over spills, chemical spills, chemical contact with skin, and exposure to electrical outlets. Chemical spills and contact on skin are avoided using the PPE and using tubes or carrying over material in smaller volumes. This also lessens exposure of the solvents to the environment to prevent contamination. There are also showers on the ceilings of three different locations, as shown in the figure below, in case of contact with chemicals. To prevent the water from the shower impacting equipment or solutions, the showers are placed directly in front of doors. Slips caused by spills are prevented by including facility personnel for proper clean-up and maintenance over spills. PPE/locker rooms are also present for before and after a shift in the manufacturing plant to separate clean and used PPE wear.

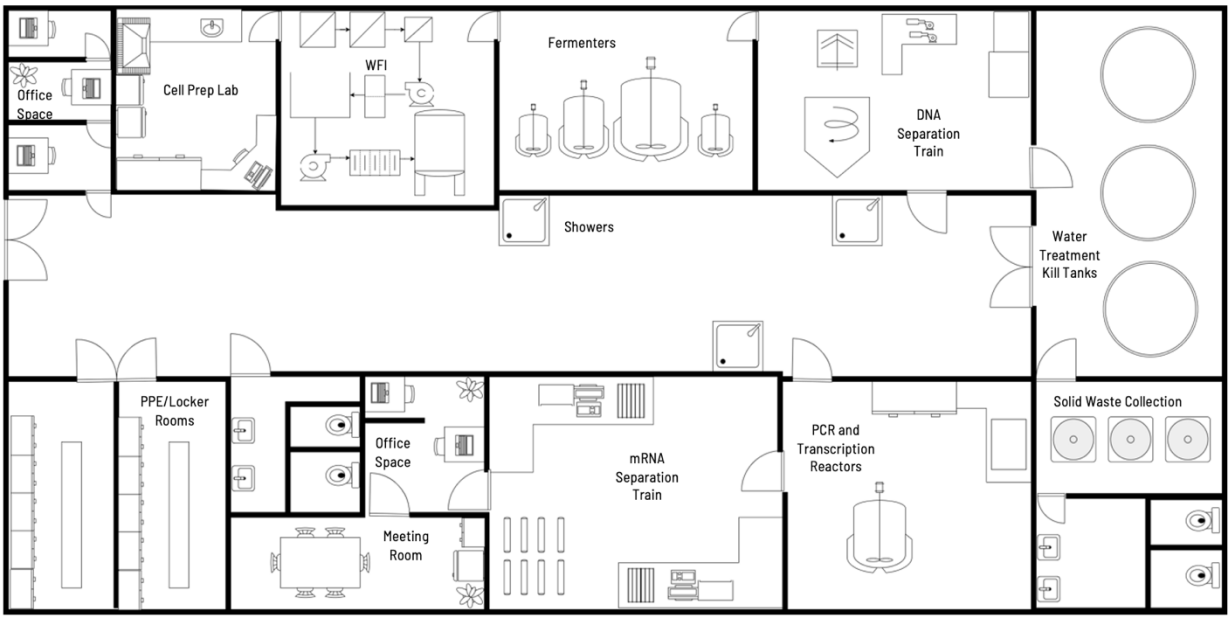


Figure 21. Plant Layout for Reference to Facility Safety

11.4 Emissions and Wastes

The major wastes and emissions associated with the vaccine manufacturing process are solid cellular waste and wastewater (from DNA separation), protein waste (from RNA separation), carbon dioxide from fermentation, plastic from single use equipment, and PPE waste. Cellular waste is produced as E. coli cells are fermented, homogenized, purified, and clarified. Carbon dioxide is produced during the fermentation process. Plastic waste includes the single-use equipment, such as the plastic bags used in the Xcellerex XDR bioreactors and nylon-6,6 membranes used in ultrafiltration. Two sets of PPE are used per day per worker which are discarded at the end of the day.

11.4.1 Cellular Waste/Cell Broth

Fermentation and DNA Separation Train

Cell waste and water are produced as pDNA is harvested from the E.coli cells that have finished multiplying in the fermenters. As sugars are metabolized during fermentation, succinate, ethanol, acetate, hydrogen gas, carbon dioxide gas, formate, and lactate are produced.[39] Among the products listed, all excluding hydrogen gas and carbon dioxide gas are liquid products. These liquid wastes pose safety concerns with risks of irritation of skin and eyes.[40,41,42,43] Ethanol, acetate, and hydrogen gas are highly flammable chemicals; so, proper closure of disposal containers is important. The liquid wastes are not generally harmful to the environment, but disposal of such wastes in environment are advised against due to possible risks such as oxygen depletion in soil.[44]

Additionally, cellular waste and cellular broth are produced in the homogenizer, ultrafiltration unit, and size exclusion chromatography unit. Once the cells have been broken in the homogenizer, they cannot be reused and must be discarded. Additionally, the cells cannot be recycled or composted, since they still pose a health hazard can carry the risk of infecting the food, water supply, or the environment if not properly disposed of. Bacterial cells, once dead, have little practical use and cannot be used for energy generation. According to the Environment, Health & Safety (EH&S) guidelines[45], bacterial waste must be segregated from regular trash and disinfected by autoclave or other method as its usage results in a non-exempt experiment. According to the CDC liquid wastes of Bio level Safety 2 and 3 require close care to prevent cross contamination, infection of workers, and environmental contamination.[46] In this case, E. coli cells and cell broth produced during fermentation and DNA separation must be discarded in a kill tank once they have been broken to harvest pDNA in the homogenizer. Wastes are continuously collected from the ultrafiltration unit and the SEC unit to the same tank. The cellular waste contained in wastewater can have environmental impact on freshwater usage, so wastewater is collected in a kill tank. When a certain volume is reached in the tank, it is sterilized by injection of hot high-pressure steam. After steam sterilization, the wastewater is cooled again and is tested to ensure that it meets the requirements for water to be discarded to the sewage system. Actini Biopharma offers a Micro Steam kill tank that cleans up to 1,000L of fluid a day.[47]

11.4.2 Recombinant DNA

mRNA Separation Train

Recombinant DNA waste is produced when viral DNA is inserted into E.coli plasmids. Once the DNA is amplified in the PCR and is transcribed into mRNA, the endotoxins, DNA, RNA polymerases, and remaining impurities becomes a waste that needs to be disposed. This DNA poses a risk to general health and safety and cannot be directly disposed of in a landfill since it is unclear how this matter will react with other organisms in the environment. For recombinant DNA that has been inserted into an organism with genes that cause viral resistance in an E.coli host, the EH&S suggests disinfection before disposal. This can be accomplished with a kill tank in a similar manner as the cellular waste and broth.

Table 23. Major wastes from the manufacturing process

Waste	Output/year	Disposal Method
Carbon dioxide Gas	11,463 L	Released to Atmosphere
Hydrogen Gas	4,452.9 L	Released to Atmosphere
Cell Broth	720 L	Decontaminated and released into the water supply or reused
Bioreactor Bags	24 bags	Incinerated for energy creation
UF Membranes	6 membranes	Incinerated for energy creation
Recombinant DNA Waste	720 grams	Decontaminated and released into the water supply or reused

11.4.3 Plastic and Paper Wastes

Single use bioreactor bags are generally not very recyclable. Due to the many layers of various plastics (nylon, polyethylene, polypropylene, ethylene vinyl alcohol, etc.), they typically require too much effort and money to be separated and recycled. As a result, these items are often disposed of in landfills. However, these plastics can also be used as a fuel for a waste to energy plant. Single use bioreactor bags will be sent to energy plants where they will be incinerated and used to generate heat, electricity, and hot water.[25] Burning ultimately reduces the volume of waste that is deposited in landfills and kills any contaminants that may be left on the bags[48].

UF membranes are typically composed of nylon. In order to dispose of these membranes, they must first be sanitized in an autoclave to rid of any cellular or DNA waste. Membranes should be sanitized at 121°C with injected steam at 1 bar for 15 minutes. Care should be taken not to exceed this temperature as the membranes are highly flammable[49]. The membrane should then be kept moist before being incinerated to create energy[50].

Six batches of 16,700,000 doses of the Pfizer BioNTech vaccine will be produced per year. PPE is measured in "units" where one "unit" represents one protective suit, head covering, set of gloves, and set of shoe coverings. Safety goggles are expected to be sanitized and reused every day. Each worker is expected to use approximately two units of PPE per day, totaling to 43,070 units a year. This waste is expected to be sent out for incineration in nearby waste-to-energy facilities. Each ton of waste has an average energy generation of 563 kWh. The total waste produced, and the estimated energy produced from WTE is outlined in **Error! Reference source not found.**.

Table 24. Summary of total weight of PPE waste.

PPE Waste	Approximate Weight per Unit	Total Weight per Year
Protective Suits[51]	152 grams	6,546 kg
Set of Gloves[52]	8 grams	344 kg
Head Coverings[53]	35 grams	1,507 kg
Set of Shoe Coverings[54]	40 grams	1,723 kg
	Total	10,120 kg
	Energy Generated	6280 kWh/yr

11.5 Construction Risks and Ethics

Due to increased demands and production of SARS-CoV2 vaccines globally, there are several construction risks, where some are caused by high demands and outsourcing of materials. This may include[55]:

- Incorrect budget, where final project cost exceeds project budget.
- Adverse weather to cause delay and damage existing completed work
- Shortage of resources
- A major contractor or supplier becoming bankrupt
- Poor public perception of the project
- Poor safety

To prevent exceeding expected budgets, working capital cost, fixed capital cost including equipment cost, and manufacturing costs were rounded up. Also, minimal changes in design during construction ensure fewer budget changes and variations.

Adverse weather effects on plant operations are avoided because the location chosen was determined using a decision matrix and the weather in Colombia was similar to that of currently existing Pfizer manufacturing facilities. So, similar conditions to currently productive facilities minimize expected risks from weather concerns.

Shortage of resources due to the increased demand and production of Covid vaccines are of concern. Contractors and suppliers becoming bankrupt due to decreased sales or not enough supply to maintain profitability is also of concern. This can be avoided by diversifying supplier sources in the near future.

Poor public perception of the project is a human impact concern but ensuring pay well over minimum wage and promoting vaccination rates in South America are positively perceived.

Safety risks have been discussed previously.

11.6 Violations

Vaccine manufacturing is highly regulated by FDA cGMP standards. The most common violations relate to the False Claims Act [31 U.S.C. §3729]. Each violation results in fines of $11,000 to $21,000.[56] This may include:

- "Adding, omitting, or altering raw data in control records,
- Allowing significant deviations in drug component manufacture and bulk drug substance
- Allowing untrained, unqualified personnel to aid in drug manufacture, processing, packing, or holding,
- Analytical errors (using an average of sample results that include out of spec data),
- Destroying batch records prior to required date,
- Failure to demonstrate consistent, reproducible product performance between batches,
- Failure to establish proper controls to ensure product strength, purity, and quality,
- Failure to examine batches / products related to other spec failures,
- Failure to investigate out of spec batches,
- Failure to investigate recurring contamination,
- Failure to maintain manufacturing equipment,
- Failure to maintain valid computer systems,
- Failure to prevent contamination,
- Failure to reject out of spec test batches,
- Failure to take corrective action and ensure no repeated incidents,
- Failure to validate manufacturing processes and equipment,
- Improper Quality Control protocols,
- Poor sanitization, sterilization, and cleaning practices,
- Preparing, packing, or holding products in unsanitary environments,
- Substandard management of electronic data,
- Using lots without prior testing and quality control approval,
- Using outdated, non-CGMP compliant or inaccessible SOPs".[56]

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, the proposed plant design should be executed as planned. The design sees profit in 2-3 years and is in line with all the recommendations set forth by the FDA and other relevant organizations. The plant achieves its goals to manufacture Pfizer BioNTech vaccines economically, in an environmentally neutral manner, and in such a way that distribution can be done with ease. There is great potential in the design to promote vaccine equality and accessibility in South America and can be repurposed in the event that Covid-19 vaccines are no longer needed.

The plant's location in Medellin, Colombia is a strategic location. Its proximity to city resources and other South American countries ensures that distribution and obtaining raw materials can be easily achieved. Essentially all the wastes created by the plant can be recycled, incinerated for energy, or safely released into the environment, contributing little to environmental damage. This plant can also serve to educate the local community and provide tours for people to learn about the vaccine process and pharmaceutical industry.

The design of this plant was economically evaluated using a tax rate of 35% and the MACRS method depreciation rate. Using the average price that the Pfizer vaccine goes for currently, $12, our return on investment would be 209000%. At this price, the plant is safe from sensitivity to competition and fluctuations in cost of raw materials.

Future improvements include shortening the time required to produce a single batch of vaccines. Improving the amount of time required to produce vaccines will greatly increase output per year and increase profits per year. Other recommendations involving the current design may include decreasing material requirements in PCR. This way the variation of number of PCR cycles and largest fermenter volume can allow for more vaccine produced annually.

Additionally, carbon capture methods could be incorporated into the plant to reduce carbon dioxide emissions. While the expenses involved with constructing such a system presently do not appear to be economically beneficial, it's possible that it will be viable in a few years. Lastly, in the future, the plant can possibly be expanded to contain formulation and packaging departments onsite in Colombia. This would reduce the transportation costs involved in shipping prepared mRNA offsite.

ACKNOWLEDGEMENTS

We would like to acknowledge our advisors of this project, Professor Benjamin Davis and Professor Charles Okorafor at The Cooper Union. Thank you for your support, guidance, and advice in completing this project and in completing our undergraduate degrees. This project would not be possible without them.

APPENDICES

Appendix 1: Stream Tables

Materials & Streams Report

for Group3VaccineProduction

May 12, 2022

1. OVERALL PROCESS DATA

Annual Operating Time	4,369.09 h
Recipe Batch Time	769.09 h
Recipe Cycle Time	720.00 h
Number of Batches per Year	6.00

MP = Undefined

2.1 STARTING MATERIAL REQUIREMENTS (per Section)

Section	Starting Material	Active Product	Amount Needed (g Sin/kg MP)	Molar Yield (%)	Mass Yield (%)	Gross Mass Yield (%)
Seed Train	(none)	(none)	Unknown	Unknown	Unknown	Unknown
DNA Separation Train	(none)	(none)	Unknown	Unknown	Unknown	Unknown
DNA Amplification and Transcrip	(none)	(none)	Unknown	Unknown	Unknown	Unknown
mRNA Separation Train	(none)	(none)	Unknown	Unknown	Unknown	Unknown

Sin = Section Starting Material, Aout = Section Active Product

2.2 BULK MATERIALS (Entire Process)

Material	g/yr	g/batch	g/kg MP
(NH4)2SO4 (10%)	1,519	253.20	
E. coli	0	0.00	
KCl	434,273	72,378.81	
LB Broth	679,619	113,269.85	
Nucleotides	16,560	2,760.00	
Oxygen	164,912	27,485.26	
Polymerase	2,300	383.33	
Primer	345	57.50	
Sodium Citrate	245,119	40,853.24	
Transcription R	42,060	7,010.04	
TRIS HCl	1,392,648	232,107.99	
Water	6	0.99	
WFI	166,514	27,752.25	
TOTAL	3,145,875	524,312.48	

2.3 BULK MATERIALS (per Section)

SECTIONS IN: Main Branch

Seed Train

Material	g/yr	g/batch	g/kg MP
E. coli	0	0.00	
LB Broth	679,619	113,269.85	
Oxygen	164,912	27,485.26	
Water	6	0.99	
TOTAL	844,537	140,756.11	

DNA Separation Train

Material	g/yr	g/batch	g/kg MP
(NH4)2SO4 (10%)	1,519	253.20	
TRIS HCl	1,121,818	186,969.60	
TOTAL	1,123,337	187,222.80	

DNA Amplification and Transcrip

Material	g/yr	g/batch	g/kg MP
Nucleotides	16,560	2,760.00	
Polymerase	2,300	383.33	
Primer	345	57.50	
Transcription R	42,060	7,010.04	
WFI	166,514	27,752.25	
TOTAL	227,779	37,963.13	

mRNA Separation Train

Material	g/yr	g/batch	g/kg MP
KCl	434,273	72,378.81	
Sodium Citrate	245,119	40,853.24	
TRIS HCl	270,830	45,138.39	
TOTAL	950,223	158,370.45	

2.4 BULK MATERIALS: SECTION TOTALS (g/batch)

Raw Material	Seed Train	DNA Separation Train	DNA Amplification and Transcrip	mRNA Separation Train
(NH4)2SO4 (10%)	0.00	253.20	0.00	0.00
E. coli	0.00	0.00	0.00	0.00
KCl	0.00	0.00	0.00	72,378.81
LB Broth	113,269.85	0.00	0.00	0.00
Nucleotides	0.00	0.00	2,760.00	0.00
Oxygen	27,485.26	0.00	0.00	0.00
Polymerase	0.00	0.00	383.33	0.00
Primer	0.00	0.00	57.50	0.00
Sodium Citrate	0.00	0.00	0.00	40,853.24
Transcription R	0.00	0.00	7,010.04	0.00
TRIS HCl	0.00	186,969.60	0.00	45,138.39
Water	0.99	0.00	0.00	0.00
WFI	0.00	0.00	27,752.25	0.00
TOTAL	140,756.11	187,222.80	37,963.13	158,370.45

2.5 BULK MATERIALS: SECTION TOTALS (g/yr)

Raw Material	Seed Train	DNA Separation Train	DNA Amplification and Transcrip	mRNA Separation Train
(NH4)2SO4 (10%)	0	1,519	0	0
E. coli	0	0	0	0
KCl	0	0	0	434,273
LB Broth	679,619	0	0	0
Nucleotides	0	0	16,560	0
Oxygen	164,912	0	0	0
Polymerase	0	0	2,300	0
Primer	0	0	345	0
Sodium Citrate	0	0	0	245,119
Transcription R	0	0	42,060	0
TRIS HCl	0	1,121,818	0	270,830
Water	6	0	0	0
WFI	0	0	166,514	0
TOTAL	844,537	1,123,337	227,779	950,223

2.6 BULK MATERIALS (per Material)

(NH4)2SO4 (10%)

Procedure	% Total	g/yr	g/batch	g/kg MP
DNA Separation Train (Main Branch)				
P-9	100.00	1,519	253.20	
TOTAL	100.00	1,519	253.20	

E. coli

Procedure	% Total	g/yr	g/batch	g/kg MP
Seed Train (Main Branch)				
P-1	100.00	0	0.00	
TOTAL	100.00	0	0.00	

KCl

Procedure	% Total	g/yr	g/batch	g/kg MP
mRNA Separation Train (Main Branch)				
P-12	100.00	434,273	72,378.81	
TOTAL	100.00	434,273	72,378.81	

LB Broth

Procedure	% Total	g/yr	g/batch	g/kg MP
Seed Train (Main Branch)				
P-1	0.00	6	1.00	
P-2	0.09	595	99.24	
P-3	8.76	59,542	9,923.64	
P-4	91.15	619,476	103,245.97	
TOTAL	100.00	679,619	113,269.85	

Nucleotides

Procedure	% Total	g/yr	g/batch	g/kg MP
DNA Amplification and Transcrip (Main Branch)				
P-10	100.00	16,560	2,760.00	
TOTAL	100.00	16,560	2,760.00	

Oxygen

Procedure	% Total	g/yr	g/batch	g/kg MP
Seed Train (Main Branch)				
P-1	0.00	2	0.40	
P-2	0.10	160	26.64	
P-3	9.60	15,831	2,638.50	
P-4	90.30	148,918	24,819.71	
TOTAL	100.00	164,912	27,485.26	

Polymerase

Procedure	% Total	g/yr	g/batch	g/kg MP
DNA Amplification and Transcrip (Main Branch)				
P-10	100.00	2,300	383.33	
TOTAL	100.00	2,300	383.33	

Primer

Procedure	% Total	g/yr	g/batch	g/kg MP
DNA Amplification and Transcrip (Main Branch)				
P-10	100.00	345	57.50	
TOTAL	100.00	345	57.50	

Sodium Citrate

Procedure	% Total	g/yr	g/batch	g/kg MP
mRNA Separation Train (Main Branch)				
P-14	100.00	245,119	40,853.24	
TOTAL	100.00	245,119	40,853.24	

Transcription R

Procedure	% Total	g/yr	g/batch	g/kg MP
DNA Amplification and Transcrip (Main Branch)				
P-11	100.00	42,060	7,010.04	
TOTAL	100.00	42,060	7,010.04	

TRIS HCl

Procedure	% Total	g/yr	g/batch	g/kg MP
DNA Separation Train (Main Branch)				
P-8	80.55	1,121,818	186,969.60	
mRNA Separation Train (Main Branch)				
P-13	19.45	270,830	45,138.39	
TOTAL	100.00	1,392,648	232,107.99	

Water

Procedure	% Total	g/yr	g/batch	g/kg MP
Seed Train (Main Branch)				
P-1	100.00	6	0.99	
TOTAL	100.00	6	0.99	

WFI

Procedure	% Total	g/yr	g/batch	g/kg MP
DNA Amplification and Transcrip (Main Branch)				
P-10	100.00	166,514	27,752.25	
TOTAL	100.00	166,514	27,752.25	

3. STREAM DETAILS

Stream Name	S-101	S-102	S-103	S-105
Source	**INPUT**	**INPUT**	**INPUT**	**P-1**
Destination	**P-1**	**P-1**	**P-1**	**OUTPUT**
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	37.00	25.00	37.00
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	994.71	997.32	1.31	1.25
Total Enthalpy (kW-h)	0.00	0.00	0.00	0.00
Specific Enthalpy (kcal/kg)	25.11	36.78	5.47	8.13
Heat Capacity (kcal/kg-°C)	1.00	0.99	0.22	0.22
Component Flowrates (g/batch)				
CO2	0.0000	0.0000	0.0000	0.0001
Glucose	0.0000	0.0019	0.0000	0.0000
Hydrogen	0.0000	0.0000	0.0000	0.0000
Nitrogen	0.0000	0.0000	0.0000	0.0027
Oxygen	0.0000	0.0000	0.4033	0.4028
Sodium Chloride	0.0000	0.0097	0.0000	0.0000
Tryptone	0.0000	0.0097	0.0000	0.0000
Water	0.9947	0.0000	0.0000	0.0000
WFI	0.0000	0.9711	0.0000	0.0000
Yeast Extract	0.0000	0.0049	0.0000	0.0000
TOTAL (g/batch)	0.9947	0.9973	0.4033	0.4056
TOTAL (L/batch)	0.0010	0.0010	0.3083	0.3234

Stream Name	S-104	S-106	S-107	S-108
Source	**P-1**	**INPUT**	**INPUT**	**P-2**
Destination	**P-2**	**P-2**	**P-2**	**OUTPUT**
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	37.00	37.00	25.00	37.00
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	993.81	1,002.39	1.31	1.25
Total Enthalpy (kW-h)	0.00	0.00	0.00	0.00
Specific Enthalpy (kcal/kg)	36.94	36.78	5.47	8.12
Heat Capacity (kcal/kg-°C)	0.99	0.99	0.22	0.22
Component Flowrates (g/batch)				
CO2	0.0000	0.0000	0.0000	0.0030
E. coli	0.0000	0.0000	0.0000	0.0000
Glucose	0.0018	0.1933	0.0000	0.0000
Hydrogen	0.0000	0.0000	0.0000	0.0022
Nitrogen	0.0000	0.0000	0.0000	0.2246
Organic Acids	0.0000	0.0000	0.0000	0.0000
Oxygen	0.0000	0.0000	26.6448	26.5273
Sodium Chloride	0.0097	0.9665	0.0000	0.0000
Tryptone	0.0097	0.9665	0.0000	0.0000
Water	0.9947	0.0000	0.0000	0.0000
WFI	0.9711	96.6269	0.0000	0.0000
Yeast Extract	0.0049	0.4833	0.0000	0.0000
TOTAL (g/batch)	1.9920	99.2364	26.6448	26.7571
TOTAL (L/batch)	0.0020	0.0990	20.3716	21.3319

Stream Name	S-109	S-110	S-111	S-112
Source	**P-2**	**INPUT**	**INPUT**	**P-3**
Destination	**P-3**	**P-3**	**P-3**	**OUTPUT**
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	37.00	37.00	25.00	37.00
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	997.24	1,002.39	1.31	1.25
Total Enthalpy (kW-h)	0.00	0.42	0.02	0.03
Specific Enthalpy (kcal/kg)	36.78	36.78	5.47	8.14
Heat Capacity (kcal/kg-°C)	0.99	0.99	0.22	0.22
Component Flowrates (g/batch)				
CO2	0.0000	0.0000	0.0000	0.2902
E. coli	0.0015	0.0000	0.0000	0.0000
Glucose	0.1892	19.3313	0.0000	0.0000
Hydrogen	0.0000	0.0000	0.0000	0.2176
Nitrogen	0.0000	0.0000	0.0000	61.5762
Organic Acids	0.0008	0.0000	0.0000	0.0000
Oxygen	0.0000	0.0000	2,638.5012	2,584.2985
Sodium Chloride	0.9762	96.6463	0.0000	0.0000
Tryptone	0.9762	96.6463	0.0000	0.0000
Water	0.9947	0.0000	0.0000	0.0000
WFI	97.5980	9,662.6900	0.0000	0.0000
Yeast Extract	0.4881	48.3281	0.0000	0.0000
TOTAL (g/batch)	101.2246	9,923.6421	2,638.5012	2,646.3825
TOTAL (L/batch)	0.1015	9.9000	2,017.2978	2,114.2387

Stream Name	S-113	S-114	S-115	S-116
Source	**P-3**	**INPUT**	**INPUT**	**P-4**
Destination	**P-4**	**P-4**	**P-4**	**OUTPUT**
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	37.00	37.00	25.00	37.00
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	997.31	1,002.39	1.31	1.25
Total Enthalpy (kW-h)	0.43	4.41	0.16	0.24
Specific Enthalpy (kcal/kg)	36.78	36.78	5.47	8.13
Heat Capacity (kcal/kg-°C)	0.99	0.99	0.22	0.22
Component Flowrates (g/batch)				
CO2	0.0000	0.0000	0.0000	3.0792
E. coli	0.1507	0.0000	0.0000	0.0000
Glucose	18.9236	201.1232	0.0000	0.0000
Hydrogen	0.0000	0.0000	0.0000	2.3094
Nitrogen	0.0000	0.0000	0.0000	251.1873
Organic Acids	0.0754	0.0000	0.0000	0.0000
Oxygen	0.0000	0.0000	24,819.7138	24,688.5247
Sodium Chloride	97.6225	1,005.5125	0.0000	0.0000
Tryptone	97.6225	1,005.5125	0.0000	0.0000
Water	0.9947	0.0000	0.0000	0.0000
WFI	9,760.2880	100,531.0168	0.0000	0.0000
Yeast Extract	48.8163	502.8079	0.0000	0.0000
TOTAL (g/batch)	10,024.4937	103,245.9729	24,819.7138	24,945.1006
TOTAL (L/batch)	10.0515	103.0000	18,976.2100	19,894.7681

Stream Name	S-117	S-123	S-122	S-124
Source	**P-4**	**P-5**	**P-5**	**P-6**
Destination	**P-5**	**OUTPUT**	**P-6**	**P-7**
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	37.00	20.00	20.00	20.00
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	997.31	1,003.47	1,003.47	1,003.47
Total Enthalpy (kW-h)	4.84	1.57	1.05	1.05
Specific Enthalpy (kcal/kg)	36.78	19.93	19.93	19.93
Heat Capacity (kcal/kg-°C)	0.99	0.99	0.99	0.99
Component Flowrates (g/batch)				
Cell Waste	0.0000	0.0000	0.0000	0.8107
E. coli	1.7032	0.0000	1.7032	0.0000
Glucose	213.8368	128.3021	85.5347	85.5347
Intracellular C	0.0000	0.0000	0.0000	0.7960
Organic Acids	0.8516	0.5110	0.3406	0.3406
pDNA	0.0000	0.0000	0.0000	0.0965
Sodium Chloride	1,103.1351	661.8810	441.2540	441.2540
Tryptone	1,103.1351	661.8810	441.2540	441.2540
Water	0.9947	0.5968	0.3979	0.3979
WFI	110,291.3048	66,174.7829	44,116.5219	44,116.5219
Yeast Extract	551.6242	330.9745	220.6497	220.6497
TOTAL (g/batch)	113,266.5853	67,958.9293	45,307.6561	45,307.6561
TOTAL (L/batch)	113.5717	67.7239	45.1509	45.1510

Stream Name	S-125	S-126	S-129	S-127
Source	**P-7**	**P-7**	**INPUT**	**P-8**
Destination	**OUTPUT**	**P-8**	**P-8**	**OUTPUT**
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	20.00	20.00	25.00	22.00
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	1,003.47	965.16	2,164.00	899.71
Total Enthalpy (kW-h)	1.05	0.00	0.62	0.00
Specific Enthalpy (kcal/kg)	19.93	15.24	2.85	11.04
Heat Capacity (kcal/kg-°C)	0.99	0.76	0.11	0.50
Component Flowrates (g/batch)				
Cell Waste	0.0405	0.7702	0.0000	0.0047
Glucose	85.5341	0.0006	0.0000	0.0000
Intracellular C	0.0000	0.7960	0.0000	0.0310
Organic Acids	0.3406	0.0000	0.0000	0.0000
pDNA	0.0000	0.0965	0.0000	0.0000
Sodium Chloride	441.2507	0.0033	0.0000	0.0001
TRIS HCl	0.0000	0.0000	186,969.6000	0.0000
Tryptone	441.2507	0.0033	0.0000	0.0001
Water	0.3979	0.0000	0.0000	0.0000
WFI	44,116.1882	0.3337	0.0000	0.0013
Yeast Extract	220.6480	0.0017	0.0000	0.0001
TOTAL (g/batch)	45,305.6507	2.0053	186,969.6000	0.0374
TOTAL (L/batch)	45.1489	0.0021	86.4000	0.0000

Stream Name	S-130	S-128	S-131	S-132
Source	**P-8**	**P-8**	**INPUT**	**P-9**
Destination	**OUTPUT**	**P-9**	**P-9**	**P-10**
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	22.00	25.00	24.99
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	2,164.00	966.38	1,055.00	1,040.16
Total Enthalpy (kW-h)	0.62	0.00	0.01	0.00
Specific Enthalpy (kcal/kg)	2.85	16.88	23.45	23.44
Heat Capacity (kcal/kg-°C)	0.11	0.77	0.93	0.93
Component Flowrates (g/batch)				
Amm. Sulfate	0.0000	0.0000	25.3200	8.5937
Cell Waste	0.0000	0.7655	0.0000	0.0000
Glucose	0.0000	0.0006	0.0000	0.0000
Intracellular C	0.0000	0.7650	0.0000	0.0000
pDNA	0.0000	0.0965	0.0000	0.0946
Sodium Chloride	0.0000	0.0032	0.0000	0.0000
TRIS HCl	186,969.6000	0.0000	0.0000	0.0000
Tryptone	0.0000	0.0032	0.0000	0.0000
Water	0.0000	0.0000	227.8800	77.3437
WFI	0.0000	0.3323	0.0000	0.1662
Yeast Extract	0.0000	0.0016	0.0000	0.0000
TOTAL (g/batch)	186,969.6000	1.9680	253.2000	86.1982
TOTAL (L/batch)	86.4000	0.0020	0.2400	0.0829

Stream Name	S-133	S-134	S-143	S-144
Source	P-9	INPUT	INPUT	INPUT
Destination	OUTPUT	P-10	P-10	P-10
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	24.98	25.00	25.00	25.00
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	1,039.36	1,050.00	1,050.00	1,050.00
Total Enthalpy (kW-h)	0.00	0.08	0.01	0.00
Specific Enthalpy (kcal/kg)	23.37	24.99	24.99	24.99
Heat Capacity (kcal/kg-°C)	0.93	1.00	1.00	1.00
Component Flowrates (g/batch)				
Amm. Sulfate	16.7263	0.0000	0.0000	0.0000
Cell Waste	0.7655	0.0000	0.0000	0.0000
Glucose	0.0006	0.0000	0.0000	0.0000
Intracellular C	0.7650	0.0000	0.0000	0.0000
Nucleotides	0.0000	2,760.0000	0.0000	0.0000
pDNA	0.0019	0.0000	0.0000	0.0000
Polymerase	0.0000	0.0000	383.3330	0.0000
Primer	0.0000	0.0000	0.0000	57.5000
Sodium Chloride	0.0032	0.0000	0.0000	0.0000
Tryptone	0.0032	0.0000	0.0000	0.0000
Water	150.5363	0.0000	0.0000	0.0000
WFI	0.1662	0.0000	0.0000	0.0000
Yeast Extract	0.0016	0.0000	0.0000	0.0000
TOTAL (g/batch)	168.9697	2,760.0000	383.3330	57.5000
TOTAL (L/batch)	0.1626	2.6286	0.3651	0.0548

Stream Name	S-145	S-135	S-136	S-118
Source	**INPUT**	**P-10**	**INPUT**	**P-11**
Destination	**P-10**	**P-11**	**P-11**	**P-12**
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	25.45	25.00	25.00
Pressure (bar)	1.01	1.82	1.01	1.04
Density (g/L)	994.70	1,000.11	1,463.20	1,049.76
Total Enthalpy (kW-h)	0.81	0.92	0.12	1.02
Specific Enthalpy (kcal/kg)	25.11	25.54	14.72	22.97
Heat Capacity (kcal/kg-°C)	1.00	1.00	0.59	0.92
Component Flowrates (g/batch)				
Amm. Sulfate	0.0000	8.5937	0.0000	8.5937
ATP Solution	0.0000	0.0000	920.7830	751.0310
Cell Waste	0.0000	0.0000	0.0000	350.6089
Cholestrol	0.0000	0.0000	391.6440	319.4420
CleanCap AG	0.0000	0.0000	935.3639	762.9239
DNA	0.0000	11.6870	0.0000	0.0000
DNA Template	0.0000	0.0000	1,149.9413	937.9398
DNase I	0.0000	0.0000	22.9158	18.6910
mRNA	0.0000	0.0000	0.0000	584.3481
Nucleotides	0.0000	2,751.3546	0.0000	2,751.3546
pDNA	0.0000	0.0590	0.0000	0.0590
Polymerase	0.0000	381.5329	0.0000	381.5329
Primer	0.0000	56.2999	0.0000	56.2999
Sucrose	0.0000	0.0000	1,587.4169	1,294.7672
TRIS HCl	0.0000	0.0000	2,001.9768	2,001.9768
Water	0.0000	77.3437	0.0000	77.3437
WFI	27,752.2507	27,752.4168	0.0000	27,752.4168
TOTAL (g/batch)	27,752.2507	31,039.2877	7,010.0417	38,049.3294
TOTAL (L/batch)	27.9000	31.0359	4.7909	36.2457

Stream Name	S-119	S-120	S-121	S-138
Source	**INPUT**	**P-12**	**P-12**	**INPUT**
Destination	**P-12**	**P-13**	**OUTPUT**	**P-13**
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	25.60	25.60	25.00
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	1,996.90	1,644.63	1,401.45	2,164.00
Total Enthalpy (kW-h)	0.49	0.70	0.85	0.07
Specific Enthalpy (kcal/kg)	5.88	10.11	14.33	2.85
Heat Capacity (kcal/kg-°C)	0.24	0.39	0.56	0.11
Component Flowrates (g/batch)				
Amm. Sulfate	0.0000	3.1615	5.4323	0.0000
ATP Solution	0.0000	276.2889	474.7422	0.0000
Cell Waste	0.0000	128.9818	221.6271	0.0000
Cholestrol	0.0000	117.5161	201.9258	0.0000
CleanCap AG	0.0000	280.6640	482.2599	0.0000
DNA Template	0.0000	345.0488	592.8910	0.0000
DNase I	0.0000	6.8760	11.8150	0.0000
KCl	72,378.8136	45,283.7610	27,095.0526	0.0000
mRNA	0.0000	555.8491	28.4990	0.0000
Nucleotides	0.0000	1,012.1668	1,739.1878	0.0000
pDNA	0.0000	0.0217	0.0373	0.0000
Polymerase	0.0000	140.3581	241.1748	0.0000
Primer	0.0000	20.7116	35.5883	0.0000
Sucrose	0.0000	476.3182	818.4490	0.0000
TRIS HCl	0.0000	736.4861	1,265.4907	22,569.1971
Water	0.0000	28.4532	48.8906	0.0000
WFI	0.0000	10,209.5436	17,542.8732	0.0000
TOTAL (g/batch)	72,378.8136	59,622.2065	50,805.9365	22,569.1971
TOTAL (L/batch)	36.2457	36.2527	36.2525	10.4294

Stream Name	S-139	S-137	S-142	S-140
Source	P-13	P-13	INPUT	P-14
Destination	OUTPUT	P-14	P-14	OUTPUT
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.45	25.60	25.00	27.04
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	1,976.20	1,518.90	1,857.00	1,769.24
Total Enthalpy (kW-h)	0.31	0.47	0.08	0.15
Specific Enthalpy (kcal/kg)	5.46	11.99	1.74	4.11
Heat Capacity (kcal/kg-°C)	0.21	0.47	0.07	0.15
Component Flowrates (g/batch)				
Amm. Sulfate	3.1298	0.0316	0.0000	0.0061
ATP Solution	273.5260	2.7629	0.0000	0.5311
Cell Waste	127.6920	1.2898	0.0000	0.2479
Cholestrol	116.3410	1.1752	0.0000	0.2259
CleanCap AG	277.8574	2.8066	0.0000	0.5395
DNA Template	341.5983	3.4505	0.0000	0.6632
DNase I	6.8073	0.0688	0.0000	0.0132
KCl	22,641.8805	22,641.8805	0.0000	4,352.1000
mRNA	27.7925	528.0567	0.0000	501.1834
Nucleotides	1,002.0451	10.1217	0.0000	1.9455
pDNA	0.0215	0.0002	0.0000	0.0000
Polymerase	138.9545	1.4036	0.0000	0.2698
Primer	20.5045	0.2071	0.0000	0.0398
Sodium Citrate	0.0000	0.0000	40,853.2434	24,320.5338
Sucrose	471.5551	4.7632	0.0000	0.9156
TRIS HCl	23,298.3184	7.3649	0.0000	1.4156
Water	28.1686	0.2845	0.0000	0.0547
WFI	0.0000	10,209.5436	0.0000	1,962.4233
TOTAL (g/batch)	48,776.1923	33,415.2113	40,853.2434	31,143.1085
TOTAL (L/batch)	24.6819	21.9996	21.9996	17.6026

Stream Name	**S-141**
Source	**P-14**
Destination	**OUTPUT**
Stream Properties	
Activity (U/ml)	0.00
Temperature (°C)	26.64
Pressure (bar)	1.01
Density (g/L)	1,633.22
Total Enthalpy (kW-h)	0.43
Specific Enthalpy (kcal/kg)	8.50
Heat Capacity (kcal/kg-°C)	0.32
Component Flowrates (g/batch)	
Amm. Sulfate	0.0255
ATP Solution	2.2318
Cell Waste	1.0419
Cholestrol	0.9493
CleanCap AG	2.2672
DNA Template	2.7873
DNase I	0.0555
KCl	18,289.7805
mRNA	26.8732
Nucleotides	8.1761
pDNA	0.0002
Polymerase	1.1338
Primer	0.1673
Sodium Citrate	16,532.7096
Sucrose	3.8476
TRIS HCl	5.9492
Water	0.2298
WFI	8,247.1203
TOTAL (g/batch)	43,125.3462
TOTAL (L/batch)	26.4051

4. OVERALL COMPONENT BALANCE (g/batch)

COMPONENT	INITIAL	INPUT	OUTPUT	FINAL	IN-OUT
Amm. Sulfate	0.00	25.32	23.93	0.00	1.39
ATP Solution	0.00	920.78	629.68	0.00	291.10
Cell Waste	0.00	0.00	294.77	0.00	- 294.77
Cholestrol	0.00	391.64	267.83	0.00	123.82
CleanCap AG	0.00	935.36	639.65	0.00	295.71
CO2	0.00	0.00	3.37	0.03	- 3.41
DNA Template	0.00	1,149.94	786.39	0.00	363.55
DNase I	0.00	22.92	15.67	0.00	7.24
E. coli	0.00	0.00	0.00	0.00	0.00
Glucose	0.00	220.65	213.84	0.00	6.81
Hydrogen	0.00	0.00	2.53	0.03	- 2.55
Intracellular C	0.00	0.00	0.80	0.00	- 0.80
KCl	0.00	72,378.81	62,333.54	0.00	10,045.28
mRNA	0.00	0.00	572.02	0.00	- 572.02
Nitrogen	1,556.14	0.00	312.99	1,243.15	- 0.00
Nucleotides	0.00	2,760.00	2,306.79	0.00	453.21
Organic Acids	0.00	0.00	0.85	0.00	- 0.85
Oxygen	472.42	27,485.26	27,299.75	656.22	1.70
pDNA	0.00	0.00	0.05	0.00	- 0.05
Polymerase	0.00	383.33	319.88	0.00	63.45
Primer	0.00	57.50	47.20	0.00	10.30
Sodium Chloride	0.00	1,103.14	1,103.14	0.00	0.00
Sodium Citrate	0.00	40,853.24	40,853.24	0.00	0.00
Sucrose	0.00	1,587.42	1,085.56	0.00	501.86
TRIS HCl	0.00	234,109.97	222,844.26	0.00	11,265.71
Tryptone	0.00	1,103.14	1,103.14	0.00	0.00
Water	0.00	228.87	216.38	0.00	12.50
WFI	0.00	138,043.56	138,043.56	0.00	0.00
Yeast Extract	0.00	551.62	551.62	0.00	0.00
TOTAL	2,028.56	524,312.48	501,872.42	1,899.44	22,569.19
				Overall Error:	4.288%

Appendix 2: Scheduling

5. EQUIPMENT CONTENTS

1 mLVial

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in g)
P-1	START	0.00	0.00	0.00
P-1	Add media (Charge)	0.03	0.00	0.00
P-1	Inoculate (Charge)	0.07	0.00	0.00
P-1	Ferment (Batch Stoich. Fermentation)	4.84	0.00	0.00(*)
P-1	Transfer-Out (Transfer Out)	4.87	0.00	0.00(*)

(*) Contains material in vapor phase other than Oxygen & Nitrogen

100 mL Flask

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in g)
P-2	START	4.87	0.00	0.29
P-2	Add Media (Pull In)	4.90	0.10	0.29
P-2	Innoculate (Transfer In)	4.92	0.10	0.29
P-2	Ferment (Batch Stoich. Fermentation)	11.13	0.10	0.19(*)
P-2	Transfer Out (Transfer Out)	11.16	0.00	0.19(*)

(*) Contains material in vapor phase other than Oxygen & Nitrogen

10 L Bioreactor

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in g)
P-3	START	11.16	0.00	82.55
P-3	Add Media (Pull In)	11.20	9.95	82.55
P-3	Inoculate (Transfer In)	11.48	10.05	82.55
P-3	Ferment (Batch Stoich. Fermentation)	17.41	10.05	75.04(*)
P-3	Transfer Out (Transfer Out)	17.44	0.00	75.04(*)

(*) Contains material in vapor phase other than Oxygen & Nitrogen

120 L Bioreacto

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in g)
P-4	START	17.41	0.00	330.18
P-4	Add Media (Pull In)	17.44	103.52	330.18
P-4	Inoculum (Transfer In)	17.69	113.57	330.18
P-4	Ferment (Batch Stoich. Fermentation)	22.58	113.57	208.68(*)
P-4	Transfer Out (Transfer Out)	22.61	0.00	208.68(*)

(*) Contains material in vapor phase other than Oxygen & Nitrogen

Size Exclusion

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in g)
P-9	START	751.98	0.00	0.00
	AFTER AUTO-INIT	751.98	0.00	0.00
P-9	Load Column (Gel Filtration Column Loading)	752.01	0.00	0.00
P-9	Elution (Column Elution (Simplified))	756.01	0.00	0.00
P-9	Wash (Column Wash (Simplified))	760.01	0.00	0.00

Transcription R

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in g)
P-11	START	760.35	0.00	1,532.99
P-11	Add Transcription Reagents (Pull In)	760.39	5.30	1,532.99
P-11	Transfer In (Transfer In)	760.42	36.33	1,532.99
P-11	DNA Transcription (Batch Stoich. Reaction)	761.72	36.25	1,532.99
P-11	Transfer Out (Transfer Out)	761.75	0.00	1,532.99

PCR

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in g)
P-10	START	760.01	0.00	82.55
P-10	Transfer In (Transfer In)	760.04	0.08	82.55
P-10	Add WFI (Pull In)	760.08	27.98	82.55
P-10	Add PCR Nucelotides (Pull In)	760.08	30.61	82.55
P-10	Add Primers (Pull In)	760.11	30.67	82.55
P-10	Add Polymerase (Pull In)	760.14	31.03	82.55
P-10	DNA Amplification (Batch Stoich. Fermentation)	760.32	31.04	82.55
P-10	Transfer Out (Transfer Out)	760.35	0.00	82.55

Diafiltration I

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in g)
P-12	START	761.75	0.00	0.00
P-12	Transfer In (Transfer In)	761.79	36.25	0.00
P-12	Diafiltration (Diafiltration)	763.79	36.25	0.00
P-12	Transfer Out (Transfer Out)	763.82	0.00	0.00

Diafiltration 2

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in g)
P-14	START	766.02	0.00	0.00
P-14	Transfer In (Transfer In)	766.05	22.00	0.00
P-14	Diafiltration (Diafiltration)	768.05	22.00	0.00
P-14	Concentrate mRNA (Batch Concentration)	769.05	17.60	0.00
P-14	Transfer Out (Transfer Out)	769.09	0.00	0.00

Ion Exchange

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in g)
P-13	START	763.82	0.00	0.00
P-13	Load Column (Ion Exchange Column Loading)	765.85	14.25	0.00
P-13	Wash (Ion Exchange Column Wash)	766.02	14.25	0.00
P-13	Regenerate (Ion Exchange Column Regeneration)	766.50	0.00	0.00

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