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### 3.2.S.2.3. CONTROL OF MATERIALS – SOURCE, HISTORY AND GENERATION OF PLASMIDS

#### 3.2.S.2.3.1. Plasmid Used for Production of the Linear DNA Template

Manufacture of the BNT162b2 drug substance is achieved using in vitro transcription that includes a linear DNA template as a starting material. The linear DNA template is produced via plasmid DNA from transformed DH10B *Escherichia coli* cells. The plasmid, pST4-1525, is a 7,824 base pair plasmid designed for the production of BNT162b2. In addition to the sequence coding for the transcribed regions, the plasmid DNA contains a promoter for the T7 RNA polymerase, the recognition sequence for the endonuclease used for linearization, the kanamycin resistance gene, and an origin of replication.

The plasmid map of pST4-1525 is provided in Figure 3.2.S.2.3-1. Functional elements of the plasmid are described in [Table 3.2.S.2.3-1](#), and the plasmid sequence is shown in [Figure 3.2.S.2.3-2](#).

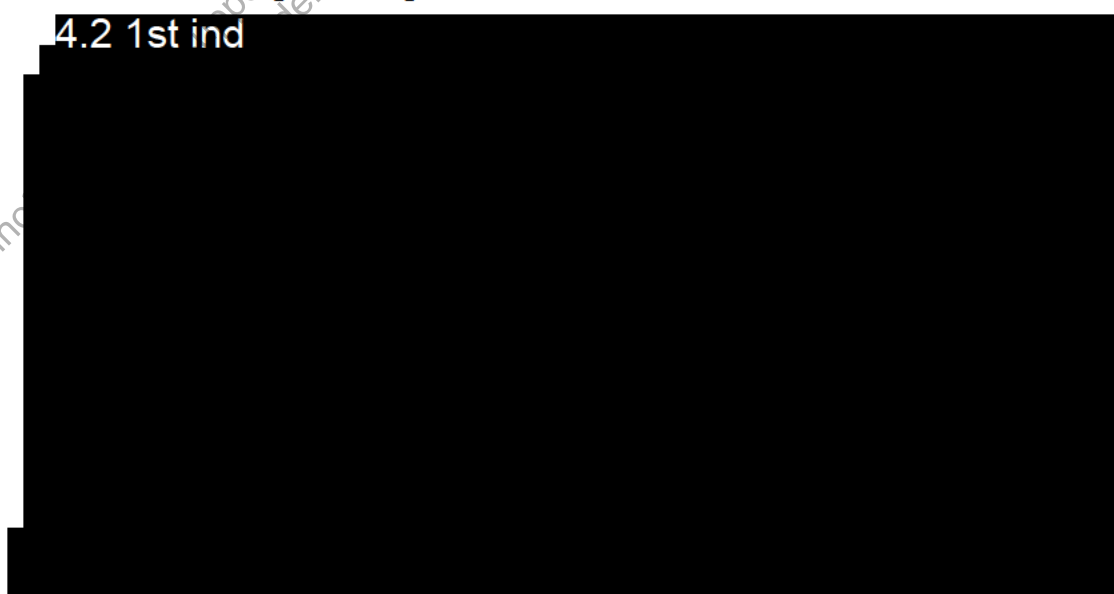
**Figure 3.2.S.2.3-1. pST4-1525 Plasmid Map**



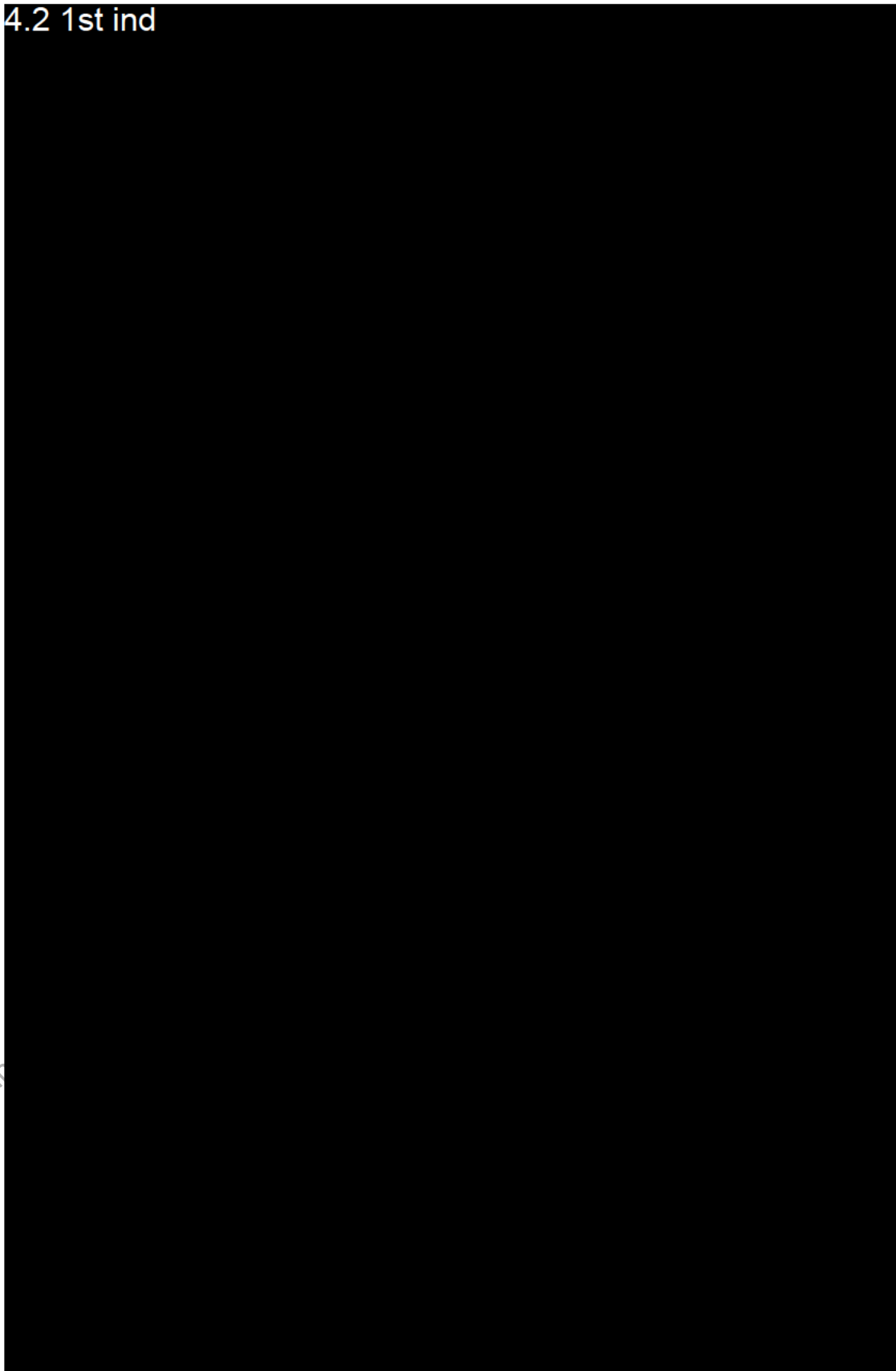
**Table 3.2.S.2.3-1. Functional Elements of pST4-1525**

Element	Origin	Start	End	Expected Function
Ori	<i>E. coli</i>	4.2 1st ind		Serves as the plasmid origin of replication
Kan <sup>R</sup>	<i>E. coli</i>	4.2 1st ind		This gene (aph(3')-II) encodes kanamycin resistance to bacterial host cells used for plasmid production
T7 promoter	Bacteriophage T7	4.2 1st ind		Initiation site for T7 RNA polymerase
5' UTR	<i>Homo sapiens</i>	4.2 1st ind		5' UTR of RNA
Kozak	n.a. (optimized)	4.2 1st ind		Ribosome binding site
mRNA initiation site		1		Transcription initiation <sup>a</sup>
S protein (modRNA-V09)	SARS-COV-2	4.2 1st ind		Signal peptide and spike (S) protein of SARS-COV-2 (S1S2) <sup>a</sup>
FI element	<i>Homo sapiens</i>	4.2 1st ind		3' UTR of RNA
polyA (A30L70)	artificial	4.2 1st ind		A poly(A)-tail measuring 110 nucleotides in length designed to enhance RNA stability
4.2 1st ind restriction site		4.2 1st ind		Enzyme recognition site

**Figure 3.2.S.2.3-2. Sequence of pST4-1525**



## 4.2 1st ind



## 4.2 1st ind



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#### 3.2.S.2.3.1.1. Plasmid Cell Bank and Linear DNA Template Manufacturer(s)

The plasmid cell bank manufacture, starting material (linear DNA template) manufacture and associated testing are performed in the Pfizer facility at 875 Chesterfield Parkway West, Chesterfield MO 63017. The plasmid cell banks inventory is stored in Pfizer's GMP storage facilities located at 875 Chesterfield Parkway West, Chesterfield MO 63017 and 1 Burtt Road, Andover MA 01810. The cell bank testing was performed at 4.2 1st ind [REDACTED] The cell bank sequence testing was performed at Genewiz, 115 Corporate Boulevard, South Plainfield, NJ 07080.

#### 3.2.S.2.3.1.2. Plasmid Cell Banking System, Characterization and Testing

Plasmid cell banks have been prepared in accordance with the following guidelines:

*ICH Q5D Derivation and characterization of cell substrates used for production of biotechnological/biological products*

Cell banking operations were performed in a controlled manufacturing area with appropriate precautions against adventitious contamination and cross-contamination from other cell lines.

All materials used in the establishment of the pST4-1525 master cell bank (MCB) and working cell bank (WCB) are sourced and manufactured consistent with the current industry guidelines including those from the European Medicines Agency (EMA), the Therapeutic Goods Administration (TGA), and the World Health Organization (WHO) for minimizing the risk of transmitting transmissible spongiform encephalopathies (TSE), including bovine spongiform encephalopathies (BSE).

The LB broth used in the establishment of the pST4-1525 MCB and WCB contains casein digest peptone derived from bovine milk. Additional information on the casein digest peptone is presented in [Table 3.2.S.2.3-2](#).

**Table 3.2.S.2.3-2. Material of Animal Origin Used in the Establishment of the pST4-1525 MCB and WCB**

Name of Material	Biological source	Country of origin of the source animals	Comments
Casein digest peptone (component of LB broth)	Bovine milk	New Zealand and Australia	Based on information provided by manufacturer 4.2 1st ind [REDACTED] casein digest peptone was derived from bovine milk fit for human consumption. The site of manufacture complies with the current USDA regulations which prohibit the importation of ruminant origin material from countries with reported cases of TSE or from countries which USDA feels are at risk for TSE, according to the guideline entitled "Note for Guidance on Minimizing the Risk of Transmitting Animal Spongiform Encephalopathy Agents Via Human and Veterinary Medicinal Products".

### 3.2.S.2.3.1.2.1. Preparation of pST4-1525 Master Cell Bank

The plasmid pST4-1525 pre-Master Cell Bank (pre-MCB) was generated by transforming *Escherichia coli* DH10B competent cells with pST4-1525. A pure culture of transformed cells was produced by growth on selective medium. A single colony isolate was then grown in liquid culture and aliquots were taken and frozen to generate pre-MCB pST4-1525\_preMCB\_DH10B\_20Apr2020.

A MCB was prepared at the Pfizer Chesterfield, MO, USA site on 05 May 2020. Vials from pre-MCB pST4-1525\_preMCB\_DH10B\_20Apr2020 were thawed to inoculate shake flasks containing LB broth, additional yeast extract and kanamycin to a final concentration of 50 µg/mL. The flasks were placed in a shaker incubator (200 rpm) and incubated at 32 ± 2°C, for a maximum of 10 hours. The cultures were stopped once the optical density (OD) at 600 nm (OD<sub>600</sub>) reached a value of ≥ 2.0. Sterile glycerol was added to the cell culture to a final concentration of 20% (v/v). Aliquots of the formulated cell culture were dispensed into screw-cap cryovials, each containing approximately 1.5 mL of cell suspension. The vials were frozen using a controlled rate freezer and then transferred to storage in the vapor phase of liquid nitrogen freezers.

The MCB vials were labeled with the batch number, DW8968, date of manufacture, 05 May 2020, and were individually numbered with a unique vial number. There were 423 vials prepared for MCB DW8968.

MCB DW8968 vials are stored at  $\leq -125^{\circ}\text{C}$ . Storage is in the vapor phase of liquid nitrogen in validated freezers, with temperature and alarm monitoring. The freezers are in controlled access storage areas at multiple sites as a precaution against loss due to catastrophic events.

### 3.2.S.2.3.1.2.2. Release testing of the Plasmid Master Cell Bank

Culture purity and identity testing performed on the plasmid MCB DW8968 provide confirmation that the cell bank is free from microbial and bacteriophage contamination and is of an *E. coli* lineage. The studies were designed in accordance with ICH Q5D guidelines.

Table 3.2.S.2.3-3 lists the release tests performed, acceptance criteria, and results for the MCB DW8968.

**Table 3.2.S.2.3-3. Release Testing of Plasmid MCB Batch DW8968**

Test	Description	Acceptance Criteria	Result
Culture purity	Demonstrate that the culture exhibits the appropriate colony morphology on selective and non-selective agar and shows no evidence of contaminating organisms.	Typical <sup>a</sup>	Typical
Bacteriophage – lytic	Detect the presence, if any, of lytic bacteriophages in the MCB.	Absent of lytic bacteriophage	Absent of lytic bacteriophage
Bacteriophage – lysogenic	Detect the presence, if any, of lysogenic bacteriophages in the MCB.	Absent of lysogenic bacteriophage	Absent of lysogenic bacteriophage
Host cell identity (Genotypic testing)	Ensure the host strain is identified as <i>E. coli</i>	Identifies organism as <i>E. coli</i>	Identifies organism as <i>E. coli</i>
Viability	Determines an estimated number of viable organisms present in the MCB by plating to enumerate the number of colony forming units present.	Report result (CFU <sup>b</sup> /mL)	$2.3 \times 10^8$
Plasmid retention	Demonstrates retention of the expression plasmid by evaluation of the kanamycin resistance marker.	4.2 1st ind	100%
Restriction map analysis	Confirms plasmid integrity through specific restriction enzyme digest and analysis by agarose gel electrophoresis.	Comparable to reference	Comparable to reference
Plasmid copy number	Provides assurance that the cells retain the plasmid.	Report result	173 copies
DNA sequencing	Demonstrate that the plasmid carrying the gene expression cassette remains unaltered during the cell bank manufacturing and preservation process.	Comparable to reference sequence	Comparable to reference sequence: 100% homology to the reference sequence for the coding region, 5' UTR and the poly A tail

a. Typical: Growth characteristics of *E. coli* species and no evidence of contaminating organisms.

b. CFU = colony forming unit

#### 3.2.S.2.3.1.2.3. Preparation of pST4-1525 Working Cell Bank

A WCB was prepared at the Pfizer Chesterfield, MO, USA site on 07 May 2020. Vials of MCB DW8968 were thawed to inoculate shake flasks containing LB broth, additional yeast extract and kanamycin to a final concentration of 50 µg/mL. The flasks were placed in a shaker incubator (200 rpm) and incubated at 32 ± 2°C, for a maximum of 10 hours. The cultures were stopped once the OD<sub>600</sub> reached a value of ≥ 2.0. Sterile glycerol was added to the cell culture to a final concentration of 20% (v/v). Aliquots of the formulated cell culture were dispensed into screw-cap cryovials, each containing approximately 1.5 mL of cell suspension. The vials were frozen using a controlled rate freezer and then transferred to storage in the vapor phase of liquid nitrogen freezers.

The WCB vials were labeled with the batch number, DW8970, date of manufacture, 07 May 2020, and were individually numbered with a unique vial number. There were 423 vials prepared for WCB DW8970.

WCB DW8970 vials are stored at ≤ -125°C. Storage is in the vapor phase of liquid nitrogen in validated freezers, with temperature and alarm monitoring. The freezers are in controlled access storage areas at multiple sites as a precaution against loss due to catastrophic events.

#### 3.2.S.2.3.1.2.4. Release testing of the Plasmid Working Cell Bank

Culture purity and identity testing performed on the plasmid WCB DW8970 provide confirmation that the cell bank is free from microbial and bacteriophage contamination and is of an *E. coli* lineage. The studies were designed in accordance with ICH Q5D guidelines.

[Table 3.2.S.2.3-4](#) lists the release tests performed, acceptance criteria, and results for the WCB DW8970.

**Table 3.2.S.2.3-4. Release Testing of Plasmid WCB Batch DW8970**

Test	Description	Acceptance Criteria	Result
Culture purity	Demonstrate that the culture exhibits the appropriate colony morphology on selective and non-selective agar and show no evidence of contaminating organisms.	Typical <sup>a</sup>	Typical
Bacteriophage - lytic	Detect the presence, if any, of lytic bacteriophages in the WCB.	Absent of lytic bacteriophage	Absent of lytic bacteriophage
Bacteriophage - lysogenic	Detect the presence, if any, of lysogenic bacteriophages in the WCB.	Absent of lysogenic bacteriophage	Absent of lysogenic bacteriophage
Host cell identity (Genotypic testing)	Ensure the host strain is identified as <i>E. coli</i>	Identifies organism as <i>E. coli</i>	Identifies organism as <i>E. coli</i>
Viability	Determines an estimated number of viable organisms, present in the WCB by plating to enumerate the number of colony forming units present.	Report result (CFU <sup>b</sup> /mL)	4.2 x 10 <sup>8</sup>
Plasmid retention	Demonstrates retention of the expression plasmid by evaluation of the kanamycin resistance marker.	4.2 1st ind	100%
Restriction map analysis	Confirms plasmid integrity through specific restriction enzyme digest and analysis by agarose gel electrophoresis.	Comparable to reference	Comparable to reference
Plasmid copy number	Provides assurance that the cells retain the plasmid.	Report result	220 copies
DNA sequencing	Demonstrate that the plasmid carrying the gene expression cassette remains unaltered during the cell bank manufacturing and preservation process.	Comparable to reference sequence	Comparable to reference sequence: 100% homology to the reference sequence for the coding region, 5' UTR and the poly A tail

a. Typical: Growth characteristics of *E. coli* species and no evidence of contaminating organisms.

b. CFU = colony forming unit

#### 3.2.S.2.3.1.2.5. Preparation, Qualification and Storage of Renewal Plasmid Working Cell Banks (WCBs)

Renewal plasmid working cell banks (WCBs) will be prepared by expanding cells thawed from the MCB DW8968. WCB manufacturing operations will be performed in a controlled manufacturing area with appropriate precautions against adventitious contamination and cross-contamination from other cell lines. The process steps for WCB preparation are described in Table 3.2.S.2.3-5. All steps of the WCB preparation process are documented in a manufacturing batch record.

**Table 3.2.S.2.3-5. Process Steps for the Preparation of Renewal Plasmid WCBs**

Process Step	Description
Thaw and expansion	MCB vials are thawed and cells are inoculated into shake flasks containing LB Broth, additional yeast extract and kanamycin to a final concentration of 50 µg/mL. The cultures are incubated at 32 ± 2 °C at 200 RPM until the culture reaches an OD <sub>600</sub> of 4.0 ± 2.0 within 6 – 10 hours.
Harvest	The cell culture is formulated with glycerol to a final concentration of 20% (v/v).
Vialing	Cell suspension is dispensed into screw-cap sterile cryogenic vials at a target of 1.5 mL per vial.
Freezing	Cell bank vials are frozen using a controlled rate freezing method. After freezing, vials are transferred to storage in the vapor phase of liquid nitrogen.
Storage	WCB vials will be stored at ≤ -125 °C. Storage is in the vapor phase of liquid nitrogen in qualified freezers. The WCB vials will be stored at multiple sites.

### 3.2.S.2.3.1.2.6. Qualification of Renewal Plasmid Working Cell Banks

Tests for identity, purity, and bacteriophage contamination will be performed to confirm the acceptability of renewal WCBs. In addition, future WCBs will be analyzed to demonstrate genotypic and plasmid integrity consistent with the MCB. The acceptance criteria for WCB qualification are summarized in Table 3.2.S.2.3-6.

**Table 3.2.S.2.3-6. Specification for Renewal Plasmid WCBs**

Test	Acceptance Criteria
Culture Purity	Typical <sup>a</sup>
Bacteriophage- Lytic	Absent of lytic bacteriophage
Bacteriophage-Lysogenic	Absent of lysogenic bacteriophage
Host cell identity	Identifies organism as <i>E.coli</i>
Viability	4.2 1st ind
Plasmid Retention	4.2 1st ind
Copy Number	Report results
Restriction Map Analysis	Comparable to reference
DNA Sequencing	Transcribed region: 100% homology with the DNA reference sequence in transcribed region excluding poly(dA:dT)-tract Poly A tail: Report results

a. Typical: Growth characteristics of *E.coli* species and no evidence of contaminating organisms.

b. CFU = colony forming unit

### 3.2.S.2.3.1.2.7. Plasmid Cell Bank Stability Testing

The plasmid MCB and WCBs are enrolled in a cell bank stability program and tested according to a pre-approved stability protocol. Vials are assessed for viability and plasmid retention at defined intervals beginning at the from the cell bank release date (time zero) and subsequently at 24 months, 48 months, 72 months, and then every 5 years until the cell bank is depleted or no longer used for manufacturing. The expected cell bank viability at thaw is

#### 4.2 1st ind

and plasmid retention is  
and plasmid retention of

An investigation is required for viability

#### 3.2.S.2.3.1.3. Linear DNA Template Manufacturing

The fermentation, purification, and linearization of the plasmid to manufacture the linear DNA template is described below in [Table 3.2.S.2.3-7](#). Cells from the WCB are thawed and the culture is expanded in shake flasks, which are then used to inoculate the fermenter. The culture medium used for expansion and fermentation is a minimal salts/glucose medium that is free of animal-derived components. Following fermentation, the cells are harvested and chemically lysed to recover the plasmid DNA. After this lysis step, the circular plasmid DNA is purified by ultrafiltration/diafiltration and chromatography.

Following purification, the circular plasmid DNA is incubated with a restriction enzyme, [4.2 1st ind](#) in order to linearize the plasmid followed by ultrafiltration/ diafiltration.

The linear DNA template is filtered and dispensed in a qualified biosafety cabinet in a qualified cleanroom in order to ensure the final linear DNA template is free of any adventitious agent.

The circular plasmid DNA and linear DNA template are tested to the specifications outlined in [Table 3.2.S.2.3-10](#) and the linear DNA template is used as a starting material in the drug substance manufacturing.



**Table 3.2.S.2.3-7. Process Flow for Linear DNA Template**

Process Step	Process Description
Shake Flask	One vial from the working cell bank is thawed and used to inoculate a shake flask.
Production Fermenter	The shake flask culture is transferred to a production fermenter. 4.2 1st ind [REDACTED] [REDACTED] At the end of the fermentation, a sample is collected to screen for contamination. <sup>a</sup>
Harvest	The production fermenter is harvested and the cell slurry is collected. 4.2 1st ind [REDACTED]
Lysis and Depth Filtration	After re-suspension with buffer, the circular plasmid DNA is recovered by chemical lysis of the cells and clarified by depth filtration. The depth filtered lysate is filtered through a 0.2 µm filter prior to the next step. The depth filtered lysate is sampled for bioburden. <sup>b</sup>
Ultrafiltration/ Diafiltration 1	The circular plasmid DNA is concentrated and diafiltered to prepare for the next step. The pool is filtered through a 0.2 µm filter prior to the next step. The pool is sampled for bioburden. <sup>b</sup>
Anion Exchange Chromatography	The anion exchange chromatography step is operated under bind-wash-elute mode. This step is used to reduce endotoxin impurities. The elution pool is filtered through a 0.2 µm filter prior to the next step. The elution pool is sampled for bioburden and endotoxin. <sup>b</sup>
Ultrafiltration/ Diafiltration 2	The circular plasmid DNA is diafiltered and concentrated. The pool is sampled for bioburden and endotoxin. <sup>b</sup>
Filtration	The circular plasmid DNA is filtered via 0.2 µm filtration and stored frozen at -90 to -60 °C. The filtrate is sampled for the circular plasmid DNA specification. <sup>c</sup>
Linearization	The circular plasmid DNA is thawed and linearized with a restriction enzyme. The linearization pool is sampled for bioburden and endotoxin.
Ultrafiltration/ Diafiltration 3	The linear DNA is concentrated and diafiltered into purified water. A concentrated HEPES solution is added to achieve a linear DNA template in HEPES buffer. The buffer exchanged pool is sampled for bioburden and endotoxin.
Filtration	The linear DNA template filtered via 0.2 µm filtration and stored frozen at -25 to -15 °C. The filtrate is sampled for the linear DNA template specification. <sup>c</sup>

a. A contamination screen is performed prior to the production fermenter harvest and contamination results in rejection of the batch

b. The noted samples for bioburden or endotoxin are taken prior to the described 0.2 µm filtration

c. [Section 3.2.S.2.3.1.4](#)

Purified water manufactured at the facility is used throughout the linear DNA template process and meets USP requirements. A list of the raw materials used in the manufacture of the linear DNA template is provided in [Table 3.2.S.2.3-8](#). All the materials used in the manufacture of the linear DNA template are animal origin free and sourced from approved suppliers. Inspection of materials received and examination of vendor certificate of analysis are performed for raw materials.



**Table 3.2.S.2.3-8. Raw Materials Used in the Manufacture of Linear DNA Template**

Raw Material	Supplier Grade <sup>a</sup>
Ammonium Hydroxide (28-30%)	NF <sup>b</sup>
Acetic Acid Glacial	USP <sup>b</sup>
Ammonium Sulfate	Non compendial <sup>c</sup>
Benzyl Alcohol	NF, Ph. Eur., JP <sup>c</sup>
Biotin	USP <sup>c</sup>
Boric Acid	NF, Ph. Eur. <sup>b</sup>
Calcium Chloride Dihydrate	USP, Ph. Eur., JP <sup>c</sup>
Calcium Pantothenate	Non compendial <sup>c</sup>
Cobalt Chloride Hexahydrate	Non compendial <sup>b</sup>
Copper (II) Chloride Dihydrate	Non compendial <sup>b</sup>
Cyanocobalamin	USP <sup>c</sup>
Dextrose, Anhydrous	USP, Ph. Eur., JP <sup>c</sup>
DL-Dithiothreitol (DTT)	Non compendial <sup>c</sup>
4.2 1st ind	Non compendial <sup>c</sup>
Edetate Disodium Dihydrate (EDTA)	USP, Ph. Eur., JP <sup>c</sup>
Ferrous Sulfate Heptahydrate	USP, Ph. Eur. <sup>c</sup>
Folic Acid	USP <sup>c</sup>
Glacial Acetic Acid	USP <sup>b</sup>
HEPES	Non compendial <sup>c</sup>
HEPES Sodium Salt	Non compendial <sup>c</sup>
Hydrochloric Acid	Non compendial <sup>b</sup>
Kanamycin Sulfate	USP <sup>b</sup>
L-Isoleucine	Ph. Eur., JP, USP <sup>c</sup>
L-Leucine	Ph. Eur., JP, USP <sup>c</sup>
L-Valine	Ph. Eur., JP, USP <sup>c</sup>
Magnesium Acetate Tetrahydrate	Non compendial <sup>c</sup>
Magnesium Sulfate Heptahydrate	USP, Ph. Eur. <sup>c</sup>
Manganese (II) Chloride Tetrahydrate	USP <sup>c</sup>
Niacinamide	USP <sup>c</sup>
P(4) - Aminobenzoic Acid	Non compendial <sup>c</sup>
Potassium Acetate	USP, Ph. Eur. <sup>c</sup>
Potassium Phosphate Dibasic	USP, Ph. Eur. <sup>c</sup>
Potassium Phosphate Monobasic	NF, Ph. Eur. <sup>c</sup>

**Table 3.2.S.2.3-8. Raw Materials Used in the Manufacture of Linear DNA Template**

Raw Material	Supplier Grade <sup>a</sup>
Pyridoxine HCl	Non compendial <sup>c</sup>
Riboflavin	Non compendial <sup>c</sup>
SDS	Non compendial <sup>c</sup>
Sodium Chloride	USP, Ph. Eur., JP <sup>c</sup>
Sodium Citrate Dihydrate	USP, Ph. Eur., JP <sup>c</sup>
Sodium Hydroxide, 50% w/w	Non compendial <sup>b</sup>
Sodium Molybdate Dihydrate	Non compendial <sup>b</sup>
Sodium Sulfate	USP <sup>c</sup>
Thiamine HCl	USP <sup>c</sup>
Tris Base	USP, Ph. Eur., JP <sup>c</sup>
Tris HCl	Non compendial <sup>c</sup>
Zinc Chloride	Non compendial <sup>b</sup>

a. Equivalent can be used. Supplier Grades of incoming materials listed

b. Gross visual and CoA check

c. Gross visual, CoA check and ID test

HEPES = 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; DTT = DL-dithiothreitol; EDTA = edetate disodium dihydrate or ethylenediaminetetraacetic acid; NF = National Formulary; USP = United States Pharmacopeia; JP= Japan Pharmacopeia; Ph. Eur. = European Pharmacopeia

The chromatography resins and filters used in linear DNA template manufacture are described in Table 3.2.S.2.3-9.

**Table 3.2.S.2.3-9. Chromatography Resins and Filters Used in Linear DNA Template Manufacture**

Fractogel 4.2 1st ind resin
100 kDa MWCO ultrafiltration membrane
30 kDa MWCO ultrafiltration membrane
Depth filters
0.2 µm filters

#### 3.2.S.2.3.1.4. Linear DNA Template Specifications

The release specifications for the linear DNA template are given in Table 3.2.S.2.3-10. The analytical control strategy includes sampling and testing of a selected number of attributes prior to linearization and the remainder of the attributes on the final linear DNA template. Those attributes analyzed prior to linearization are listed first (*CIRCULAR PLASMID DNA*) and those analyzed after linearization are listed second (*LINEAR DNA TEMPLATE*).

**Table 3.2.S.2.3-10. Linear DNA Template Specifications**

Analytical Procedure	Quality Attribute	Acceptance Criteria
CIRCULAR PLASMID DNA		
Characteristics		
UV260	DNA Concentration	4.2 1st ind
Identity		
Restriction map	Identity	Comparable to reference or Comparable to theoretical
Sanger sequencing	Identity of transcribed region	Homology to reference <sup>a</sup>
	Identity of poly A tail	Report results
Purity		
Agarose gel electrophoresis	Plasmid topology	Supercoiled form: 4.2 1st ind Linear form: 4.2 1st ind
Process-Related Impurities		
HPLC RNA analysis	Residual host cell RNA	4.2 1st ind
qPCR DNA analysis	Residual host cell DNA	4.2 1st ind
Residual kanamycin	Residual kanamycin	4.2 1st ind
LINEAR DNA TEMPLATE		
Characteristics		
Appearance (clarity)	Clarity	4.2 1st ind
Appearance (coloration)	Coloration	Not more intensely colored than level of the color standard
pH	pH	4.2 1st ind
UV260	DNA Concentration	4.2 1st ind
Restriction map	Poly A tail integrity	4.2 1st ind
Purity		
Agarose gel electrophoresis	Linearization Efficiency (Plasmid topology)	Linear form: 4.2 1st ind
Process-Related Impurities		
Total residual protein by µBCA	Residual protein	4.2 1st ind
Safety		
Bioburden	Bioburden	4.2 1st ind
Endotoxin	Endotoxin	4.2 1st ind

- a. Defined as 100% identity with DNA reference sequence in transcribed region excluding poly(dA:dT)-tract  
b. NTU = Nephelometric Turbidity Unit  
c. CFU = colony forming unit  
d. EU = endotoxin unit

#### 3.2.S.2.3.1.5. Linear DNA Template Method Descriptions

Specific analytical procedures, including compendial and non-compendial methods, were used to assess characteristics, identity, purity and safety of the *CIRCULAR PLASMID DNA* and *LINEAR DNA TEMPLATE*. Descriptions of the analytical procedures are provided below. The applicant will update future submission with information provided below.

##### 3.2.S.2.3.1.5.1. Appearance (Clarity and Coloration)

The *LINEAR DNA TEMPLATE* is assessed for clarity and coloration in accordance with the current European Pharmacopoeia procedure, Ph. Eur. 2.2.1 and Ph. Eur. 2.2.2, respectively.

##### 3.2.S.2.3.1.5.2. pH

The *LINEAR DNA TEMPLATE* is analyzed for pH in accordance with the current USP Procedure <791>.

##### 3.2.S.2.3.1.5.3. DNA Concentration by Spectroscopy

Using a spectrophotometer, the absorbance at 260 nm is used to determine the concentration of the *CIRCULAR PLASMID DNA* and *LINEAR DNA TEMPLATE*. The specific absorption coefficient or absorptivity ( $a_{260}$ ) for dsDNA (given below) is used for the concentration calculation.

$$a_{260} = 4.2 \text{ 1st ind}$$

##### 3.2.S.2.3.1.5.4. Identity by Restriction Mapping

A restriction endonuclease mapping method is used to determine *CIRCULAR PLASMID DNA* identity. The reference material and test samples are subjected to digestion using the following enzymes or groups of enzymes: 4.2 1st ind all of which cleave at specific sites. The enzymatic digest produces a set of nucleic acid fragments which are separated through sieving via agarose gel electrophoresis and visualized by addition of a detection reagent. The bands of test samples are compared to the reference material DNA\* band profile with regard to the number of DNA bands and band position to confirm identity.

\* To confirm identity in the absence of an appropriate reference standard, the bands of test samples are compared to the theoretical banding pattern using the DNA ladder as a size reference.

##### 3.2.S.2.3.1.5.5. Identity of the Transgene Region by DNA Sanger Sequencing

DNA sequencing of the transcribed region is performed on the *CIRCULAR PLASMID DNA* using the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). 4.2 1st ind

Sequencing reactions were purified using the BigDye XTerminator™ Purification kit (Applied Biosystems) and analyzed on an ABI 3500 Genetic Analyzer. Using SeqScape™ software version 4.0, the sequence data were assembled and aligned to the reference sequence. Assembly and sequence identification is manually confirmed.

#### 3.2.S.2.3.1.5.6. Poly A Tail Integrity by Restriction Digestion

A restriction endonuclease mapping method is used to determine poly A tail integrity of the *LINEAR DNA TEMPLATE*. The reference material and test samples are subjected to digestion using the following enzymes or groups of enzymes: 4.2 1st ind all of which cleave at specific sites. The enzymatic digest produces a set of nucleic acid fragments which are separated through sieving via agarose gel electrophoresis and visualized by addition of a detection reagent. The bands of test samples are compared to the expected band profile 4.2 1st ind to confirm integrity of the PolyA tail by ensuring that all appropriate bands are present.

#### 3.2.S.2.3.1.5.7. Plasmid Topology by Densitometry of AGE

The AGE method is used to determine the *CIRCULAR PLASMID DNA* and *LINEAR DNA TEMPLATE* purity. The test samples are diluted and mixed with the loading dye. The topoisomers are separated through sieving via agarose gel electrophoresis (AGE) and visualized by addition of a detection reagent. Topoisomers are quantified by densitometry upon illumination with UV light. The content of supercoiled, open circle, linear, and other forms is reported as the percent of the total area for all plasmid topoisomer peaks.

#### 3.2.S.2.3.1.5.8. Total Residual Protein by $\mu$ BCA

This assay quantitates host cell derived proteins and total residual protein from the linear DNA template with a commercially available micro bicinchoninic acid (BCA) protein assay kit. The principle of the BCA assay is that protein reduces  $\text{Cu}^{+2}$  to  $\text{Cu}^{+1}$  in an alkaline solution (the biuret reaction) and this results in a purple color formation by BCA. The colorimetric response is measured spectrophotometrically. The concentration of residual protein is calculated from the bovine serum albumin (BSA) standard curve plotted as absorbance versus BSA concentration. Sample results are reported as  $\mu\text{g/mL}$  and percent protein (w/w %) in the *LINEAR DNA TEMPLATE*.

#### 3.2.S.2.3.1.5.9. Host Cell DNA by qPCR

The host cell DNA is measured using a sensitive, quantitative PCR (qPCR)-based method. The assay was developed using standard qPCR technologies. The PCR target is a conserved region of the *E. coli* 16S rDNA sequence. The fluorescent signal generated is correlated to the amount of PCR product produced, which is proportional to the amount of *E. coli* DNA in the sample. The concentration of residual DNA in the samples is calculated from the standard curve. Sample results are reported as both ng *E.coli* DNA per mL (ng/mL) and percent residual DNA (w/w %) in the *CIRCULAR PLASMID DNA*.

#### 3.2.S.2.3.1.5.10. Host Cell RNA by RP-HPLC

The host cell RNA is measured using a reversed phase high performance liquid chromatography (RP-HPLC) method. In this method, *E. coli* total RNA standards and plasmid DNA samples are digested with RNase A. When an increasing gradient of acetonitrile is applied, the digested ribonucleic acids are eluted. The concentration of residual RNA in the plasmid samples is calculated from the RNA standard curve. Sample

results are expressed as both  $\mu\text{g}$  *E.coli* RNA per mL ( $\mu\text{g/mL}$ ) and percent host cell RNA (w/w %) in the *CIRCULAR PLASMID DNA*.

#### **3.2.S.2.3.1.5.11. Immunoassay for Residual Kanamycin**

This assay quantitates residual kanamycin in circular plasmid DNA using a commercially available kanamycin competitive enzyme-linked immunosorbent assay (ELISA) kit. The principle of the competitive ELISA is that kanamycin in samples prevents anti-kanamycin antibodies from binding to exogenous kanamycin pre-coated on the ELISA plate, resulting in a drop in colorimetric signal with increasing amounts of kanamycin that is quantified alongside a kanamycin standard curve plotted as absorbance versus kanamycin concentration. The amount of residual kanamycin is reported as ng kanamycin per mL sample (ng/mL) as well as a ratio (ng/mg) in the *CIRCULAR PLASMID DNA*.

#### **3.2.S.2.3.1.5.12. Bacterial Endotoxin Assay (Limulus Amebocyte Lysate [LAL])**

The endotoxin assay is performed in accordance with the current USP General Chapter <85>, the current European Pharmacopoeia procedure, Ph. Eur. 2.6.14, and the current Japanese Pharmacopoeia procedure, JP 4.01. Results are reported as endotoxin units (EU)/mL.

#### **3.2.S.2.3.1.5.13. Bioburden**

This is a general test method for enumerating viable mesophilic microorganisms. Bioburden monitoring is designed for the recovery of a broad range of microorganisms that are likely to be present in the material being processed. This method follows the principles as defined in USP General Chapter <61> as well as other pharmacopoeia. The method has demonstrated acceptable microbial recovery verifying its suitability.

### 3.2.S.2.3.1.6. Linear DNA Template Method Verification/Qualification Data

The plasmid-specific methods are listed in Table 3.2.S.2.3-11. The compendial methods were verified following applicable Pharmacopoeias. The non-compendial methods were qualified/verified (previously qualified plasmid platform methods) to ensure analytical methods are sound and suitable for their intended use. A gap analysis will be performed and documented to identify any supplemental qualification to align with ICH requirements. The gaps identified will be addressed either prior to transferring the methods to relevant sites or during the transfer activities. The qualification/verification results are summarized in Table 3.2.S.2.3-11.

**Table 3.2.S.2.3-11. Product Specific Qualification or Verification Summaries**

Attribute	Method	Qualification-Verification Summaries
<i>CIRCULAR PLASMID DNA</i>		
DNA Concentration <sup>a</sup>	UV 260	Precision – Repeatability: 4.2 1st ind Specificity: 4.2 1st ind
Identity <sup>b</sup>	Restriction digestion – AGE	Precision – Repeatability: 4.2 1st ind Specificity: 4.2 1st ind 4.2 1st ind
Identity of the Transgene Region <sup>b</sup>	Sanger Sequencing	Precision – Repeatability: 4.2 1st ind Specificity: 4.2 1st ind
Plasmid Topology <sup>a</sup>	Densitometry of AGE	Precision – Repeatability: 4.2 1st ind QL: 4.2 1st ind Specificity: 4.2 1st ind
Residual Host Cell DNA <sup>a</sup>	qPCR	Precision – Repeatability: 4.2 1st ind QL: 4.2 1st ind Accuracy: 4.2 1st ind Specificity: 4.2 1st ind



**Table 3.2.S.2.3-11. Product Specific Qualification or Verification Summaries**

Attribute	Method	Qualification-Verification Summaries
Residual Host Cell RNA <sup>a</sup>	RP-HPLC	Precision – Repeatability: 4.2 1st ind Linearity: 4.2 1st ind Accuracy: 4.2 1st ind Specificity: 4.2 1st ind
Residual Kanamycin <sup>a</sup>	ELISA	QL: 4.2 1st ind Accuracy: 4.2 1st ind Range: 4.2 1st ind Specificity: 4.2 1st ind
<i>LINEAR DNA TEMPLATE</i>		
Appearance <sup>c</sup>	Ph. Eur. 2.2.1 and Ph. Eur. 2.2.2	Compendial procedure: verified for use in accordance with local compendial requirements. Initial analyses were assessed and found to meet all system suitability and assay acceptance criteria.
pH <sup>c</sup>	USP <791>	Compendial procedure: verified for use in accordance with local compendial requirements. Initial analyses were assessed and found to meet all system suitability criteria. Precision was also assessed; Precision -Repeatability: 4.2 1st ind
DNA Concentration <sup>a</sup>	UV 260	Precision – Repeatability: 4.2 1st ind Specificity: 4.2 1st ind
Poly A Tail Integrity <sup>b</sup>	Restriction digest – AGE	Precision – Repeatability: 4.2 1st ind Specificity: 4.2 1st ind
Plasmid Topology <sup>a</sup>	Densitometry of AGE	Precision – Repeatability: 4.2 1st ind QL: 4.2 1st ind Specificity: 4.2 1st ind
Total Residual Protein <sup>a</sup>	μBCA	Precision – Repeatability: 4.2 1st ind QL: 4.2 1st ind Accuracy: 4.2 1st ind Specificity: 4.2 1st ind



**Table 3.2.S.2.3-11. Product Specific Qualification or Verification Summaries**

Attribute	Method	Qualification-Verification Summaries			
Endotoxin <sup>d</sup>	USP <85>; Ph Eur. 2.6.14 & JP 4.01	Compendial qualification performed per local compendia. The criteria of the standard curve was found to be valid (i.e. correlation coefficient (r) must be ≥   0.980   The sample solution must not interfere with the test (e.g. inhibition/enhancement) The sample must have a maximum valid dilution (MVD) established			
		Summary of Inhibition/Enhancement Data			
		Endotoxin Limit	λ (EU/mL)	Calculated MVD	Qualified Dilution
		4.2 1st ind	0.01	1:2000	1:20
		Inhibition/Enhancement Results			
		Sample Dilution	Spike Recovery (%)	Results (EU/mL)	
		1:10	86	<0.100	
		1:20	102	<0.200	
		1:100	107	<1.00	
		Bioburden <sup>d</sup>	USP <61>	Challenge Recovery Testing (based on Compendial guidance to ensure test articles are non-inhibitory to the recovery of inoculated organisms):	
Organism	%Recovery				
S. aureus	93				
P. aeruginosa	101				
B. subtilis	70				
C. albicans	79				
A. brasiliensis	106				

RSD = Relative Standard Deviation; QL = Quantitation Limit

- a. Platform Method Verification
- b. Product Specific Method Qualification
- c. Compendial Verified
- d. Compendial Qualified

**3.2.S.2.3.1.7. Linear DNA Template Batch Analysis**

The batch analysis data for representative lots of the linear DNA template are given in below (Table 3.2.S.2.3-12). At the time of manufacture of these batches, the commercial specifications (Table 3.2.S.2.3-10) had not yet been determined. These differences are footnoted in the tables.

**Table 3.2.S.2.3-12. Linear DNA Template Batch Analysis for Representative Batches**

Circular Batch Number:		PF-07305883-AUG20-D01	PF-07305883-AUG20-D02	CPF-D001
Linear Batch Number:		CPF-L001	CPF-L002	CPF-L003
Analytical Procedure	Acceptance Criteria	Batch Results	Batch Results	Batch Results
<b>CIRCULAR PLASMID DNA</b>				
<b>Characteristics</b>				
DNA Concentration (UV260)	4.2 1st ind			
<b>Identity</b>				
Restriction map	Comparable to reference or Comparable to theoretical	Comparable to theoretical	Comparable to theoretical	Comparable to theoretical
Sanger sequencing - identity of the transcribed region	Homology to reference <sup>a</sup>	Homology to reference	Homology to reference	Homology to reference
Sanger sequencing - identity of the poly A tail	Report results	4.2 1st ind		
<b>Purity</b>				
Agarose Gel Electrophoresis	Supercoiled form: 4.2 1st ind Linear form: 4.2 1st ind	Supercoiled: 4.2 1st ind Linear: 4.2 1st ind	Supercoiled: 4.2 1st ind Linear: 4.2 1st ind	Supercoiled: 4.2 1st ind Linear: 4.2 1st ind
<b>Process Related Impurities</b>				
Residual Host Cell RNA	4.2 1st ind			
Residual Host Cell DNA	4.2 1st ind			
Residual kanamycin	Report Results <sup>b</sup>	4.2 1st ind		
<b>LINEAR DNA TEMPLATE</b>				
<b>Characteristics</b>				
Appearance (clarity)	4.2 1st ind			
Appearance (coloration)	Not more intensely colored than level 4.2 1st ind of the color standard			
pH	Report Results			
DNA Concentration (UV260)	4.2 1st ind			
Poly A tail integrity	4.2 1st ind			
<b>Purity</b>				
Agarose Gel Electrophoresis	Linear form: 4.2 1st ind	Linear form: 4.2 1st ind	Linear: 4.2 1st ind	Linear: 4.2 1st ind

**Table 3.2.S.2.3-12. Linear DNA Template Batch Analysis for Representative Batches**

Circular Batch Number:	Acceptance Criteria	PF-07305883-AUG20-D01	PF-07305883-AUG20-D02	CPF-D001
Linear Batch Number:		CPF-L001	CPF-L002	CPF-L003
Analytical Procedure		Batch Results	Batch Results	Batch Results
<b>Process Related Impurities</b>				
Total residual protein	Report Results	4.2 1st ind		
<b>Safety</b>				
Bioburden	4.2 1st ind			
Endotoxin	4.2 1st ind			

- a. Defined as 100% identity with DNA reference sequence in transcribed region excluding poly(dA:dT)-tract  
b. Denotes specification at time of release. Specification was subsequently updated as shown in [Table 3.2.S.2.3-10](#).  
c. NTU = Nephelometric Turbidity Unit  
d. CFU = colony forming unit  
e. EU = endotoxin unit

### 3.2.S.2.3.1.8. Linear DNA Template Reference Standards

#### Preparation of *CIRCULAR PLASMID DNA* Reference Material

The current clinical circular plasmid DNA reference material in use is PF-07305883-JUN20-D01-RM. It was prepared from circular plasmid DNA batch PF-07305883-JUN20-D01, manufactured according to the commercial process detailed in Section 3.2.S.2.3 Control of Materials - Source, History and Generation of Plasmids (sequence 0001).

#### Storage of *CIRCULAR PLASMID DNA* Reference Material

The PF-07305883-JUN20-D01-RM is stored at -90 to -60°C (setpoint of -70°C) in 0.5 mL Matrix™ cryovials with a 0.2 mL fill.

#### Characterization of *CIRCULAR PLASMID DNA* Reference Material

The parent circular plasmid DNA batch PF-07305883-JUN20-D01 used to generate the reference material (PF-07305883-JUN20-D01-RM) was characterized per the release testing panel. The release data for the parent batch and subsequent analyses of reference material after vialing are given in Table 3.2.S.2.3-13.

**Table 3.2.S.2.3-13. Batch Analysis of *CIRCULAR PLASMID DNA* Reference Material**

Circular Batch Number	DV Data	PF-07305883-JUN20-D01-RM
Analytical Procedure	Acceptance Criteria	Batch Results
<i>CIRCULAR PLASMID DNA</i>		
<b>Characteristics</b>		
DNA Concentration (UV260)	4.2 1st ind	
<b>Identity</b>		
Restriction Map	Comparable to reference or Comparable to theoretical	Comparable to theoretical
Sanger Sequencing - Identity of the Transcribed Region	Homology to reference <sup>a</sup>	Homology to reference
Sanger Sequencing - Identity of the Poly A Tail	Report results	4.2 1st ind
<b>Purity</b>		
Agarose Gel Electrophoresis	Supercoiled form: 4.2 1st ind Linear form: 4.2 1st ind	4.2 1st ind
<b>Process Related Impurities</b>		
Residual Host Cell RNA	4.2 1st ind	
Residual Host Cell DNA	4.2 1st ind	
Residual Kanamycin	Report Results	4.2 1st ind

a. Defined as 100% identity with DNA reference sequence in transcribed region excluding poly(dA:dT)-tract

b. Concentration testing performed on n=20 reference material vials to ensure homogeneity

### Preparation of *LINEAR DNA TEMPLATE* Reference Material

The current clinical linear DNA template reference material in use is PF-07305883-JUL20-L03-RM. It was prepared from circular plasmid DNA batch PF-07305883-JUL20-D01 (Table 3.2.S.2.3-13) followed by linear DNA template batch PF-07305883-JUL20-L03, manufactured according to the commercial process detailed in Section 3.2.S.2.3 Control of Materials - Source, History and Generation of Plasmids (sequence 0001).

### Storage of *LINEAR DNA TEMPLATE* Reference Material

The reference material PF-07305883-JUL20-L03-RM is stored at -90 to -60°C (setpoint of -70°C) in 0.5 mL Matrix™ cryovials with a 0.2 mL fill.

### Characterization of *LINEAR DNA TEMPLATE* Reference Material

Parent linear DNA template batch PF-07305883-JUL20-L03 used to generate the reference material (PF-07305883-JUL20-L03-RM) was characterized per the release testing panel. The release data for the parent batch and subsequent analyses of reference standard after vialing are given in Table 3.2.S.2.3-14 and Table 3.2.S.2.3-15.

**Table 3.2.S.2.3-14. Batch Analysis of *CIRCULAR PLASMID DNA* Parent Batch**

Circular Batch Number:		PF-07305883-JUL20-D01
Analytical Procedure	Acceptance Criteria	
<i>CIRCULAR PLASMID DNA</i>		
<b>Characteristics</b>		
DNA Concentration (UV260)	4.2 1st ind	
<b>Identity</b>		
Restriction map	Comparable to reference or Comparable to theoretical	Comparable to theoretical
Sanger sequencing - identity of the transcribed region	Homology to reference <sup>a</sup>	Homology to reference
Sanger sequencing - identity of the poly A tail	Report results	4.2 1st ind
<b>Purity</b>		
Agarose Gel Electrophoresis	Supercoiled form: 4.2 1st ind Linear form: 4.2 1st ind	Supercoiled: 4.2 1st ind Linear: 4.2 1st ind
<b>Process Related Impurities</b>		
Residual host cell RNA	4.2 1st ind	
Residual host cell DNA	4.2 1st ind	
Residual kanamycin	Report Results	4.2 1st ind

a. Defined as 100% identity with DNA reference sequence in transcribed region excluding poly(dA:dT)-tract

**Table 3.2.S.2.3-15. Batch Analysis of *LINEAR DNA TEMPLATE* Reference Material**

Linear Batch Number: Analytical Procedure	Acceptance Criteria	PF-07305883-JUL20-L03-RM
<b>Characteristics</b>		
Appearance (clarity)	4.2 1st ind	4.2 1st ind
Appearance (coloration)	Not more intensely colored than level 4.2 of the color standard	4.2 1st ind
pH	Report Results	4.2 1st ind
DNA Concentration (UV260)	4.2 1st ind	4.2 1st ind
Poly A tail integrity	4.2 1st ind	4.2 1st ind
<b>Purity</b>		
Agarose Gel Electrophoresis	Linear form: 4.2 1st ind	Linear: 4.2 1st ind
<b>Process Related Impurities</b>		
Total residual protein	Report Results	4.2 1st ind

a. Concentration testing performed on n=20 reference material vials to ensure homogeneity

### Stability and Reassessment of the Clinical Reference Materials

Both the current clinical circular plasmid DNA reference material and the clinical linear DNA template reference material have been assigned an initial period of use of 12 months, when stored at the intended storage condition of -90 to -60 °C for circular plasmid DNA reference material batch PF-07305883-JUN20-D01-RM and -20 ± 5°C for linear DNA template reference material batch PF-07305883-JUL20-L03. This is based on Pfizer's significant experience with storage and handling of circular plasmid DNA intended for use as therapeutic compounds or for use in the manufacture of therapeutic compounds and a BioNTech 12M stability study on the linear DNA template at the intended storage condition of -20 ± 5°C (results are detailed in Table 3.2.S.2.3-16). Both reference materials have been enrolled in formal stability programs and protocols are detailed in Table 3.2.S.2.3-17 through Table 3.2.S.2.3-24. The periods of use for both the reference materials may be extended based on the data from the formal stability programs, provided that acceptable product quality and stability are demonstrated.

### 3.2.S.2.3.1.9. Linear DNA Template Stability and Period of Use

The batch analysis data for representative lots of the linear DNA template are given in below. An initial shelf life of 12 months has been established for both the circular plasmid DNA and the linearized DNA template. This is based on Pfizer's significant experience with storage and handling of circular plasmid DNA intended for use as therapeutic compounds or for use in the manufacture of therapeutic compounds and a BioNTech 12M stability study on the linear DNA template at the intended storage condition of  $-20 \pm 5^{\circ}\text{C}$ .

Reference [Table 3.2.S.2.3-16](#) for a summary of data from the BioNTech linear DNA template stability study. There were no significant changes seen in the linearized DNA template over the 12 month study. Of note, the linear DNA template used in this stability study was a platform plasmid and formulated in water.

The initial shelf life claims are supported by on-going stability studies that have been initiated for the circular plasmid DNA and linearized DNA template reference materials. See Tables below for information on on-going stability protocols ([Table 3.2.S.2.3-17](#) through [Table 3.2.S.2.3-24](#)).

Current available stability data is provided in [Table 3.2.S.2.3-25](#) through [Table 3.2.S.2.3-28](#) for the circular plasmid DNA reference material and in [Table 3.2.S.2.3-29](#) through [Table 3.2.S.2.3-32](#) for the linearized DNA template reference material. There are no significant changes seen when compared to release data through the 1 month time point at all conditions, for the linearized DNA template reference material, stored at the intended storage condition of  $-20 \pm 5^{\circ}\text{C}$ . There are no significant changes seen when compared to release data through the 1 month time point for the circular plasmid DNA reference material, stored at the intended storage condition of  $-90$  to  $-60^{\circ}\text{C}$ . The accelerated  $2$  to  $8^{\circ}\text{C}$  and stressed  $25 \pm 2^{\circ}\text{C}/60 \pm 5\% \text{ RH}$  conditions show a decrease in the percent supercoiled form when compared to the release data, with the circular plasmid DNA reference material result being out of specification at the 1 month timepoint for the stressed  $25 \pm 2^{\circ}\text{C}/60 \pm 5\% \text{ RH}$  condition. The  $-20 \pm 5^{\circ}\text{C}$  accelerated condition for the circular plasmid DNA reference material also show a decrease in the percent supercoiled form when compared to the release data. Stability trends can be expected at the accelerated and stressed stability conditions and do not impact the overall stability of the material when stored at the intended storage of  $-90$  to  $-60^{\circ}\text{C}$ .

Primary batches of both the circular plasmid DNA and the linearized DNA template will be enrolled in formal stability programs. The formal stability programs will also be established according to the protocols detailed in [Table 3.2.S.2.3-17](#) through [Table 3.2.S.2.3-24](#). In addition to DNA concentration and Topology, the Appearance and pH of linear DNA template will be monitored on stability. Batches are in the process of being identified for stability study set-up and initiation.

**Table 3.2.S.2.3-16. BioNTech Batch 130925VT\_293 Linearized DNA Template Stability Data at  $-20 \pm 5^{\circ}\text{C}$  Storage Condition**

Assay	Acceptance Criteria	Time Point					
		0	1	3	6	12	18
DNA Concentration	Report results (mg/mL)	4.2	4.2	4.2	4.2	4.2	4.2
DNA Integrity <sup>a</sup>	Single linearized DNA peak	Single linearized DNA peak	Single linearized DNA peak	Single linearized DNA peak	Single linearized DNA peak	Single linearized DNA peak	Single linearized DNA peak
Bioburden	Report Result	4.2	4.2	4.2	4.2	4.2	4.2

M = Month; NS = Not Scheduled

a. Synonymous with the “Topology” measurement described in subsequent stability protocols

**Table 3.2.S.2.3-17. Stability Protocol for CIRCULAR PLASMID DNA Reference Material Lot PF-07305883-JUN20-D01-RM Stored at  $-90$  to  $-60^{\circ}\text{C}$  (Long Term Storage Condition)**

Analytical Procedure	Test Intervals (Months) <sup>a</sup>
UV Spectroscopy (DNA concentration)	0, 1, 3, 6, 9, 12, 18, 24, 36, 48, 60
Agarose Gel electrophoresis (Topology)	0, 1, 3, 6, 9, 12, 18, 24, 36, 48, 60

a. Initial data (t0) are from release testing.

**Table 3.2.S.2.3-18. Stability Protocol for CIRCULAR PLASMID DNA Reference Material Lot PF-07305883-JUN20-D01-RM Stored at  $-20 \pm 5^{\circ}\text{C}$  (Alternate Long Term Storage Condition)**

Analytical Procedure	Test Intervals (Months) <sup>a</sup>
UV (DNA concentration)	0, 1, 3, 6, 9, 12, 18, 24, 36, 48, 60
Agarose Gel electrophoresis (Topology)	0, 1, 3, 6, 9, 12, 18, 24, 36, 48, 60

a. Initial data (t0) are from release testing.

**Table 3.2.S.2.3-19. Stability Protocol for CIRCULAR PLASMID DNA Reference Material Lot PF-07305883-JUN20-D01-RM Stored at  $5 \pm 3^{\circ}\text{C}$  (Accelerated Storage Condition)**

Analytical Procedure	Test Intervals (Months) <sup>a</sup>
UV (DNA concentration)	0, 1, 3, 6
Agarose Gel electrophoresis (Topology)	0, 1, 3, 6

a. Initial data (t0) are from release testing.



**Table 3.2.S.2.3-20. Stability Protocol for CIRCULAR PLASMID DNA Reference Material Lot PF-07305883-JUN20-D01-RM Stored at  $25 \pm 2^\circ\text{C}/60 \pm 5\%$  RH (Stressed Storage Condition)**

Analytical Procedure	Test Intervals (Months) <sup>a</sup>
UV (DNA concentration)	0, 1, 3
Agarose Gel electrophoresis (Topology)	0, 1, 3

a. Initial data (t0) are from release testing.

**Table 3.2.S.2.3-21. Stability Protocol for LINEAR DNA TEMPLATE Reference Material Lot PF-07305883-JUL20-L03-RM Stored at  $-20 \pm 5^\circ\text{C}$  (Long Term Storage Condition)**

Analytical Procedure	Test Intervals (Months) <sup>a</sup>
Appearance (Clarity)	0, 1, 3, 6, 9, 12, 18, 24, 36, 48, 60
Appearance (Coloration)	0, 1, 3, 6, 9, 12, 18, 24, 36, 48, 60
Appearance (Visible Particulates)	0, 1, 3, 6, 9, 12, 18, 24, 36, 48, 60
pH	0, 1, 3, 6, 9, 12, 18, 24, 36, 48, 60
UV (DNA concentration)	0, 1, 3, 6, 9, 12, 18, 24, 36, 48, 60
Agarose Gel electrophoresis (Topology)	0, 1, 3, 6, 9, 12, 18, 24, 36, 48, 60

a. Initial data (t0) are from release testing.

**Table 3.2.S.2.3-22. Stability Protocol for LINEAR DNA TEMPLATE Reference Material Lot PF-07305883-JUL20-L03-RM Stored at  $-90$  to  $-60^\circ\text{C}$  (Alternate Long Term Storage Condition)**

Analytical Procedure	Test Intervals (Months) <sup>a</sup>
Appearance (Clarity)	0, 1, 3, 6, 9, 12, 18, 24, 36, 48, 60
Appearance (Coloration)	0, 1, 3, 6, 9, 12, 18, 24, 36, 48, 60
Appearance (Visible Particulates)	0, 1, 3, 6, 9, 12, 18, 24, 36, 48, 60
pH	0, 1, 3, 6, 9, 12, 18, 24, 36, 48, 60
UV (DNA concentration)	0, 1, 3, 6, 9, 12, 18, 24, 36, 48, 60
Agarose Gel electrophoresis (Topology)	0, 1, 3, 6, 9, 12, 18, 24, 36, 48, 60

a. Initial data (t0) are from release testing.

**Table 3.2.S.2.3-23. Stability Protocol for LINEAR DNA TEMPLATE Reference Material Lot PF-07305883-JUL20-L03-RM Stored at  $5 \pm 3^\circ\text{C}$  (Accelerated Storage Condition)**

Analytical Procedure	Test Intervals (Months) <sup>a</sup>
Appearance (Clarity)	0, 1, 3, 6
Appearance (Coloration)	0, 1, 3, 6
Appearance (Visible Particulates)	0, 1, 3, 6
pH	0, 1, 3, 6
UV (DNA concentration)	0, 1, 3, 6
Agarose Gel electrophoresis (Topology)	0, 1, 3, 6

a. Initial data (t0) are from release testing.

**Table 3.2.S.2.3-24. Stability Protocol for LINEAR DNA TEMPLATE Reference  
Material Lot PF-07305883-JUL20-L03-RM Stored at  $25 \pm 2$  °C/ $60 \pm 5\%$   
RH (Stressed Storage Condition)**

Analytical Procedure	Test Intervals (Months) <sup>a</sup>
Appearance (Clarity)	0, 1, 3
Appearance (Coloration)	0, 1, 3
Appearance (Visible Particulates)	0, 1, 3
pH	0, 1, 3
UV (DNA concentration)	0, 1, 3
Agarose Gel electrophoresis (Topology)	0, 1, 3

a. Initial data (t0) are from release testing.

**Table 3.2.S.2.3-25. Stability Data for CIRCULAR PLASMID DNA Reference  
Material Lot PF-07305883-JUN20-D01-RM Stored at -90 to -60 °C (Long  
Term Storage Condition)**

Assays	DNA Concentration	Agarose Gel Electrophoresis	
		Supercoiled Form	Linear Form
Acceptance Criteria <sup>a</sup>	4.2 1st ind		
0	4.2 1st ind		
1 month	4.2 1st ind		
3 months	S	S	S
6 months	S	S	S
9 months	S	S	S
12 months	S	S	S
18 months	S	S	S
24 months	S	S	S
36 months	S	S	S
48 months	S	S	S
60 months	S	S	S

a. Acceptance criteria in place at time of testing.

S = Scheduled

**Table 3.2.S.2.3-26. Stability Data for CIRCULAR PLASMID DNA Reference  
Material Lot PF-07305883-JUN20-D01-RM Stored at  $-20 \pm 5^{\circ}\text{C}$**

Assays	DNA Concentration	Agarose Gel Electrophoresis	
		Supercoiled Form	Linear Form
Acceptance Criteria <sup>a</sup>	4.2 1st ind		
0	4.2 1st ind		
1 month	4.2 1st ind		
3 months	S	S	S
6 months	S	S	S
9 months	S	S	S
12 months	S	S	S
18 months	S	S	S
24 months	S	S	S
36 months	S	S	S
48 months	S	S	S
60 months	S	S	S

a. Acceptance criteria in place at time of testing.  
S = Scheduled

**Table 3.2.S.2.3-27. Stability Data for CIRCULAR PLASMID DNA Reference  
Material Lot PF-07305883-JUN20-D01-RM Stored at  $2$  to  $8^{\circ}\text{C}$**

Assays	DNA Concentration	Agarose Gel Electrophoresis	
		Supercoiled Form	Linear Form
Acceptance Criteria <sup>a</sup>	4.2 1st ind		
0	4.2 1st ind		
1 month	4.2 1st ind		
3 months	S	S	S
6 months	S	S	S

a. Acceptance criteria in place at time of testing.  
S = Scheduled

**Table 3.2.S.2.3-28. Stability Data for CIRCULAR PLASMID DNA Reference Material Lot PF-07305883-JUN20-D01-RM Stored at  $25 \pm 2^\circ\text{C}/60 \pm 5\%\text{RH}$**

Assays	DNA Concentration	Agarose Gel Electrophoresis	
		Supercoiled Form	Linear Form
Acceptance Criteria <sup>a</sup>	4.2 1st ind		
0	4.2 1st ind		
1 month	4.2 1st ind		
3 months	S	S	S
6 months	S	S	S

a. Acceptance criteria in place at time of testing.  
S = Scheduled

**Table 3.2.S.2.3-29. Stability Data for LINEAR DNA TEMPLATE Reference Material Lot PF-07305883-JUL20-L03-RM Stored at  $-20 \pm 5^\circ\text{C}$  (Long Term Storage Condition)**

Assays	Appearance (Clarity)	Appearance (Coloration)	Appearance (Visible Particulates)	pH	DNA Concentration	Plasmid Topology
Acceptance Criteria <sup>a</sup>	4.2 1st ind	Not more intensely colored than level 1 of the color standard	Report Results	Report Results	4.2 1st ind	
0	1	4.2 1st ind	EFVP	4.2 1st ind		
1 month	2	4.2 1st ind	EFVP	4.2 1st ind		
3 months	S	S	S	S	S	S
6 months	S	S	S	S	S	S
9 months	S	S	S	S	S	S
12 months	S	S	S	S	S	S
18 months	S	S	S	S	S	S
24 months	S	S	S	S	S	S
36 months	S	S	S	S	S	S
48 months	S	S	S	S	S	S
60 months	S	S	S	S	S	S

a. Acceptance criteria in place at time of testing.  
S = Scheduled, EFVP = Essentially Free from Visible Particulates

**Table 3.2.S.2.3-30. Stability Data for LINEAR DNA TEMPLATE Reference Material  
Lot PF-07305883-JUL20-L03-RM Stored at -90 to -60 °C**

Assays	Appearance (Clarity)	Appearance (Coloration)	Appearance (Visible Particulates)	pH	DNA Concentration	Plasmid Topology
Acceptance Criteria <sup>a</sup>	4.2 1st ind	Not more intensely colored than level 1 of the color standard	Report Results	Report Results	4.2 1st ind	
0	1	4.2 1st ind	EFVP	4.2 1st ind		
1 month	2	4.2 1st ind	EFVP	4.2 1st ind		
3 months	S	S	S	S	S	S
6 months	S	S	S	S	S	S
9 months	S	S	S	S	S	S
12 months	S	S	S	S	S	S
18 months	S	S	S	S	S	S
24 months	S	S	S	S	S	S
36 months	S	S	S	S	S	S
48 months	S	S	S	S	S	S
60 months	S	S	S	S	S	S

a. Acceptance criteria in place at time of testing.  
S = Scheduled, EFVP = Essentially Free from Visible Particulates

**Table 3.2.S.2.3-31. Stability Data for LINEAR DNA TEMPLATE Reference Material  
Lot PF-07305883-JUL20-L03-RM Stored at 2 to 8 °C**

Assays	Appearance (Clarity)	Appearance (Coloration)	Appearance (Visible Particulates)	pH	DNA Concentration	Plasmid Topology
Acceptance Criteria <sup>a</sup>	4.2 1st ind	Not more intensely colored than level 1 of the color standard	Report Results	Report Results	4.2 1st ind	
0	1	4.2 1st ind	EFVP	4.2 1st ind		
1 month	2	4.2 1st ind	EFVP	4.2 1st ind	4.2 1st ind	4.2 1st ind
3 months	S	S	S	S	S	S
6 months	S	S	S	S	S	S

a. Acceptance criteria in place at time of testing.  
S = Scheduled, EFVP = Essentially Free from Visible Particulates

**Table 3.2.S.2.3-32. Stability Data for LINEAR DNA TEMPLATE Reference Material  
Lot PF-07305883-JUL20-L03-RM Stored at 25 ± 2 °C/60 ± 5%RH**

Assays	Appearance (Clarity)	Appearance (Coloration)	Appearance (Visible Particulates)	pH	DNA Concentration	Plasmid Topology
Acceptance Criteria <sup>a</sup>	4.2 1st ind	Not more intensely colored than level 1 of the color standard	Report Results	Report Results	4.2 1st ind	
0	1	4.2 1st ind	EFVP	4.2 1st ind		
1 month	2	4.2 1st ind	EFVP	4.2 1st ind		
3 months	S	S	S	S	S	S

a. Acceptance criteria in place at time of testing.  
S = Scheduled, EFVP = Essentially Free from Visible Particulates