3.2.S.2.3. CONTROL OF MATERIALS – 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 (bp) plasmid containing functional elements essential for the manufacture of linear DNA template and BNT162b2 Drug Substance (DS) - sequence coding for the transcribed regions, a promoter for the T7 RNA polymerase, the recognition sequence for the endonuclease used for linearization, gene conferring kanamycin resistance, and an origin of replication.

Moreover, the plasmid DNA also contains sequence elements which are not utilized for the starting material and DS manufacture and originate from the backbone cloning vector sequence initially used for the generation of the DNA construct.

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, not utilized elements are presented in Table 3.2.S.2.3-2, and the plasmid sequence is shown in Figure 3.2.S.2.3-2.

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Figure 3.2.S.2.3-1. pST4-1525 Plasmid Map Detailed Map of the Circular Plasmid (pST4-1525) DNA used to Create the Linear DNA Template.



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Table 3.2.S.2.3-1. Functional Elements of Plasmid pST4-1525 essential and utilized for Plasmid and BNT162b2 mRNA production

Name of Element	Origin	Start	End	Expected Function
mRNA Initiation Site	N/A	CCI		Initiation of Transcription[a]
hAG	*Homo sapiens*	CCI		5' UTR of RNA
Kozak	N/A (optimized)	CCI		Ribosome binding site
S-protein	SARS-CoV-2 wild type spike protein	CCI		Signal peptide and spike protein of SARS CoV-2
FI-element	*Homo sapiens*	CCI		RNA stability motif, 3' UTR of mRNA
Poly(A) (A30LA70)	artificial	CCI		A poly(A)-tail plus linker, measuring 110 nucleotides in length designed to enhance RNA stability
Type II Restriction Site CCI	N/A	CCI		Enzyme Recognition Site
Amp^R Promoter	*E. coli*[b]	CCI		Promotes transcription of Kanamycin resistance gene (prokaryotic cells)
Kan^R	*E. coli*[b]	CCI		This gene (aph(3')-II) encodes the protein (aminoglycoside phosphotransferase), which confers kanamycin-resistance to bacterial host cells used for plasmid production
pUC Origin	*E. coli*[b]	CCI		Serves as the plasmid origin of replication
T7 Promoter	Bacteriophage T7[b]	CCI		Initiation site for T7 RNA polymerase

CCI

Abbreviation: N/A = Not Applicable

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Table 3.2.S.2.3-2. Plasmid Elements that are not utilized in BNT162b2 mRNA production

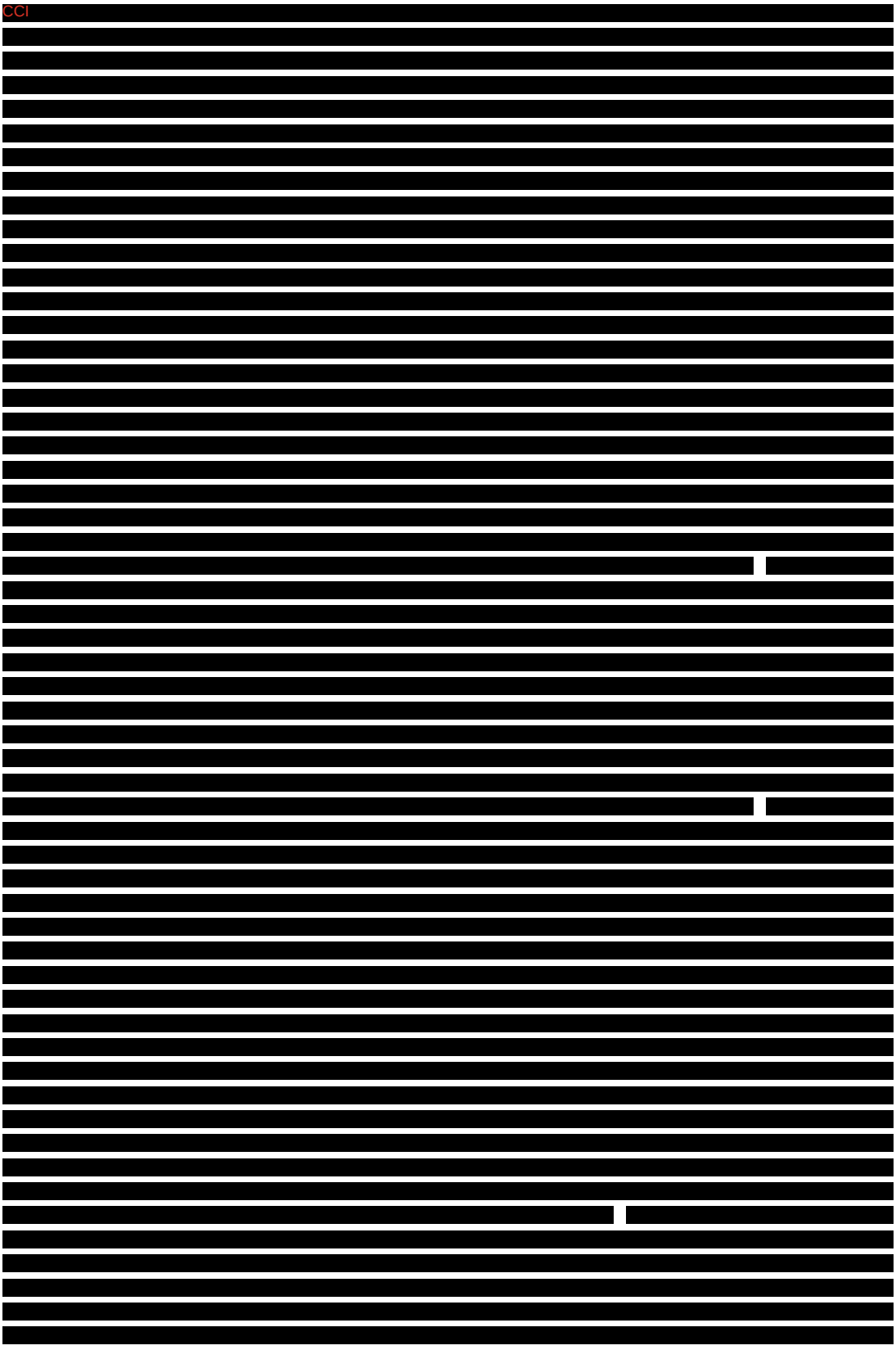
Name of Element	Origin/Source	Start	End	Hypothetical Function Not Utilized for BNT162b2 mRNA Production
CCI	From backbone Cloning Vector Sequence	CCI		Provides the potential for mRNA adenylation in eukaryotic cells
CCI	From backbone Cloning Vector Sequence	CCI		Can be used to generate single strand DNA for sequencing or to package DNA into phage in the presence of a helper phage
CCI	From backbone Cloning Vector Sequence	CCI		This multipartite element promotes and enhances Kan^R gene expression for neomycin resistance in susceptible eukaryotic cells
CCI	From backbone Cloning Vector Sequence	CCI		Provides the potential for mRNA adenylation in eukaryotic cells

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

CCI

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CCI



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CCI

[redacted]

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

The plasmid cell bank manufacture and associated testing is performed in the Pfizer facility at 875 Chesterfield Parkway West, Chesterfield MO 63017. Starting material (linear DNA template) is manufactured in CCI [redacted]

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