The chromatography resins and filters used in linear DNA template manufacture are described in Table 3.2.S.2.3-10.

Table 3.2.S.2.3-10. Chromatography Resins and Filters Used in Linear DNA Template Manufacture

Fractogel CCI Resin[c]
100 kDa MWCO Ultrafiltration Membrane
30 kDa MWCO Ultrafiltration Membrane
Depth Filters
0.2 µm Filters

CCI

Chromatography resin used is Fractogel CCI resin. CCI

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.2.3-11. 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*).

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Table 3.2.S.2.3-11. Linear DNA Template Specifications

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

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.

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