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1.0 PURPOSE

The purpose of this procedure is to confirm the identification of mRNA sequence by using Reverse Transcription-Polymerase Chain Reaction (RT-PCR) to create an approximate 200 base pair (bp) double-stranded DNA fragment for Sanger sequencing on an [redacted] genetic analyzer and using [redacted] software for data analysis.

2.0 SCOPE

This method applies to identify the first 200 base pairs (bp) of mRNA isolated from lipid nano particles (LNP) and drug product (DP).

3.0 REFERENCED DOCUMENTS

Document #	Title
FRM-0742	APW- SOP-1032 Identity Confirmation of mRNA in a Lipid Nanoparticle by Sequencing Analysis
SOP-0004	Operation and Maintenance of [redacted] Biological Safety Cabinets (BSC)
SOP-0017	Maintaining a RNase Free Work Environment
SOP-0033	Out of Specification (OOS)
SOP-0081	Preparation of Solutions and Samples in the GMP Quality Control Laboratory
SOP-0210	Assignment of Assay Reference Numbers and Use of QC Assay Performance Worksheets
SOP-0409	Quality Control Invalid Assay Procedure
SOP-0450	Operation and Maintenance of the [redacted] Sequencer
SOP-0452	Personnel Flow and Gowning in the QC Bioassay Laboratories
SOP-0454	Use and Maintenance of the [redacted] Gel Imager
SOP-0465	Use of the [redacted] Microcentrifuge and the [redacted] Centrifuge
SOP-0470	Operation and Maintenance of the [redacted] and [redacted]
SOP-1035	Operation and Maintenance of the [redacted] Sequencer

4.0 RESPONSIBILITIES

Department/ Functional Area	Title
Quality Control Laboratory Personnel	• Following all procedures outlined in this document, as applicable. • Maintaining a RNase free work environment per SOP-0017. • Following proper safety measures in the GMP laboratory. • Documenting sample information and preparation in the appropriate laboratory notebook or QC controlled document. • Data Review.
Quality Control Manager or Designee	• Ensuring that laboratory personnel are trained in this procedure. • Ensuring that all procedures in this document are followed when applicable. • Ensure that this procedure is revised as necessary. • Data Review

5.0 DEFINITIONS

Term	Definition
[redacted]	
°C	Degrees Celsius
DNA	Deoxyribonucleic acid
DP	Drug Product
GMP	Good Manufacturing Practices
LNP	Lipid Nanoparticle
mL	Milliliters
mM	Millimolar
Negative BSC	[redacted] and [redacted]
ng	Nanograms
NTC	No Template Control
PCR	Polymerase Chain Reaction
PCI	Phenol – Chloroform – Isoamyl alcohol 49.5:49.5:1
Positive BSC	[redacted] and [redacted]
PPE	Personal Protective Equipment
QC	Quality Control
RT	Reverse Transcription
µg	Micrograms
µL	Microliters

6.0 MATERIALS

6.1. Reagents

Item	Vendor	Item #
Phenol – Chloroform – Isoamyl alcohol (PCI) 49.5:49.5:1	[redacted]	
Nuclease Free Water (or equivalent)		
Tris-EDTA buffer (TE buffer, or equivalent)		
ExoSAP-IT		
E-Gel		
1 kb E-gel Express ladder		
5M betaine (or equivalent)		
SuperScript III		
2X SuperScript reaction mix		
[redacted] (do not substitute)		
Buffer (10x) with EDTA for [redacted] (do not substitute)		

Primer Name	Use	Vendor	5'-Sequence-3'
RT-PCR_Fw	RT-PCR	[redacted]	Specific primers will be found in the qualification protocol/report for each respective material.
Seq_Fw	Sequencing		
Sample_R1	Sequencing		
Sample_R2	RT-PCR & Sequencing		

NOTE: Assign the sequence specific primer located further within the ORF as the evenly numbered reverse primer to be used for both RT-PCR and sequencing.

6.2. Consumables

Item	Vendor	Item #
PCR Plate, [redacted] well, semi-skirted, flat deck (or equivalent)	[redacted]	
Adhesive PCR Plate Seals (or equivalent)		
[redacted]		
Retainer & Base Set (Standard) for [redacted] well		
[redacted]		
Plate Retainer a (septa seal), [redacted] well for [redacted]		
Microcentrifuge tubes (or equivalent)		
[redacted] Polypropylene Centrifuge Tubes		
Reagent Reservoirs		
[redacted] µL pipette tips		
[redacted] µL pipette tips		
[redacted] µL pipette tips		
[redacted] µL pipette tips		
[redacted] Bath Beads		
[redacted]		
[redacted] (do not substitute)		

6.3. Equipment

Item	Vendor	Model #
Micropipettes, Multichannel Pipettes	Various	[redacted]
[redacted]	[redacted]	

Item	Vendor	Model #
[redacted]	[redacted]	
E-Gel™ Simple Runner Electrophoresis Device		
Mini-centrifuge		
Microcentrifuge		
[redacted]		
[redacted] Gel Imager		
Biosafety Cabinet		
Fume Hood		
[redacted] Centrifuge		
Mini vortex		
Benchtop Coolers		

6.4. Controls

6.4.1. Positive Control

6.4.1.1. Positive Control Construct

Construct	Lot/Batch	RNA Concentration (mg/mL)	Parent Plasmid ID	Size of ORF (bp)	Expected Length of RT-PCR Product (bp)
[redacted]					

6.4.1.2. Positive Control Primers

RT-PCR Primers [redacted]	
Primer Name	5'-Sequence-3'
[redacted]	

Sequencing Primers [redacted]	
Primer Name	**5'-Sequence-3'**
[redacted]	

6.4.1.3. Positive Control ORF Reference Sequence [redacted]

[redacted]

NOTE: Additional constructs and lots may be used as the positive control. Additional positive controls must be validated.

6.4.2. Negative Control – Nuclease-Free Water

7.0 SAFETY

7.1. Wear proper PPE (lab coat, gloves, safety glasses). Use Moderna Safety Manual as a reference. Follow all safety information provided on material SDSs.

8.0 PROCEDURE

NOTE: "Negative BSC" refers to any of the following BSCs: [redacted] and [redacted]. "Positive BSC" refers to any of the following [redacted] and [redacted]. Refer to **SOP-0004** for BSC cleaning and operation. Refer to **SOP-0452** for personnel flow between the Bioassay labs.

NOTE: Record the following steps on **FRM-0742.** Assign an ARN per **SOP-0210**.

NOTE: Refer to Attachment 1 for BSC location map.

8.1. Sample mRNA Extraction

8.1.1. [redacted]

8.1.2. [redacted]

NOTE: [redacted]

[redacted] Proceed to **step 8.1.3.**

8.1.3. [redacted]

8.1.4. [redacted]

8.1.5. [redacted]

8.1.6. [redacted]

8.1.7. [redacted]

8.1.8. [redacted]

8.2. Positive Control Dilution

8.2.1. [redacted]

8.2.1.2. [redacted]

8.3. Preparation of primers in a negative BSC

NOTE: For sample specific primers, refer to the validation or qualification report.

NOTE: Assign the sequence specific primer located further within the ORF as the evenly numbered reverse primer to be used for both RT-PCR and PCR

8.3.1. [redacted]

8.3.2. Preparation of Stock Primers

8.3.2.1. [redacted] per **SOP-0081.**

8.3.2.2. [redacted]

8.3.2.3. [redacted]

8.3.3. Preparation of PCR Working Primers

8.3.3.1. [redacted]

8.3.3.2.

8.3.3.3.

8.3.3.4.

8.3.4. Preparation of Sequencing Working Primers

8.3.4.1. [redacted]

8.3.4.2.

8.3.4.3.

8.3.4.4. [redacted]

8.3.5. Preparation of the Sequencing Working Primers Plate

8.3.5.1. [redacted] per **Table 1**.

Table 1 Example Plate Map for Sequencing primers

	1	2	3	4	5	6	7	8	9	10	11	12
A	[redacted]											
B												
C												
D												
E												
F												
G												
H												

8.4. RT-PCR reaction Set-up.

8.4.1. [redacted]

8.4.2. [redacted]

8.4.2.1. [redacted]

8.4.2.2. [redacted] **Table 2**.

Table 2 Preparation of RT-PCR Master Mix

Component	A Per Reaction (µL)	B	Master Mix (Column A X Column B) (µL)
5M betaine		*TBD*	*TBD*
Nuclease Free Water			*TBD*
2X SS reaction mix			*TBD*
SuperScript III			*TBD*
Total			*TBD*

8.4.2.3.

8.4.2.4.

Table 3.

8.4.2.5.

8.4.2.6.

8.4.2.7.

8.4.2.8.

Table 3 Example Plate Map for RT-PCR reactions

	1	2	3	4	5	6
A						
B						
C						
D						
E						
F						
G						
H						

8.4.2.9.

8.4.3. Preparation of RT-PCR Reaction

8.4.3.1. [redacted]

[redacted] per Table 4.

Table 4: Preparation of RT-PCR reaction

Component	A Per Reaction (µL)	B [redacted]	RT-PCR Mix (Column A X Column B) (µL)
Master Mix	[redacted]	*TBD*	*TBD*
Sample			*TBD*
RT-PCR_Fw			*TBD*
Sample_R2			*TBD*
Total			*TBD*

8.4.3.2. [redacted]

8.4.3.3. [redacted] (Table 3).

8.4.3.4. [redacted]

8.4.3.5. [redacted] (SOP-0465).

8.4.3.6. [redacted] (SOP-0470).

8.4.3.7. [redacted] (Table 5) [redacted] Table 5.

[redacted]

Table 5 The Thermocycler Program of 200RT_PCR

Step	Temperature	Time
[redacted]		

8.4.3.8. [redacted]

8.4.3.9.

8.4.3.10

8.5. E-Gel analysis

8.5.1 [redacted]

8.5.2

8.5.3

8.5.4

8.5.5

8.5.6

8.5.7

8.5.8

8.5.9

8.5.10. [redacted] "SOP-1032 ORF" [redacted] (**SOP-0454**).

8.5.11. [redacted]

8.5.12. [redacted] the SOP-1032 [redacted]

8.5.13 [redacted]

NOTE: [redacted]

8.5.14. [redacted]

8.5.15. [redacted] (**SOP-0409**). [redacted]

(**SOP- 0409**).

8.6. ExoSAP-IT Clean-up (in a positive BSC)

8.6.1. [redacted]

8.6.2. [redacted]

(**Table 3**).

8.6.3. [redacted]

8.6.4.

8.6.5.

8.6.6.

(**Table 6**).

Table 6 ExoSAP Thermocyler program

Step	Temperature	Time
[redacted]		

8.6.7 [redacted]

8.6.8

8.6.9

8.7. Sequence reactions set-up

8.7.1. [redacted]

8.7.2.

8.8. Preparation of Sequencing Master Mixture in a negative BSC

8.8.1. [redacted]

Table 7.

Table 7. Preparation of Sequencing Reaction Master Mixture

Component	A Per Reaction (µL)	B Multiplier	Master Mix (Column A X Column B) (µL)
Nuclease Free Water	[redacted]	*TBD*	*TBD*
5X Buffer			*TBD*
[redacted]			*TBD*
Total			*TBD*

8.8.2. [redacted]

[redacted] (Table 8) [redacted]

Table 8. Plate Map for Sequencing Reaction

	1	2	3	4	5	6	7	8	9	10	11	12
A	[redacted]											
B												
C												
D												
E												
F												
G												
H												

8.8.3. [redacted]

8.8.4.

8.8.5.

8.8.6. [redacted]

8.9. Preparation of Positive Control Sequencing Reaction in a positive BSC

8.9.1. [redacted] per **Table 9**.

Table 9. Preparation of Positive control sequencing Reaction

Component	A Per Reaction (µL)	B Multiplier	Master Mix (Column A X Column B) (µL)
Master Mix	[redacted]		
Pooled Positive Control			
Total			

8.9.2. [redacted]

8.9.3. [redacted] per **Table 8**.

8.10. Preparation of Sample Sequencing Reaction in a positive BSC

8.10.1. [redacted] per **Table 10**.

Table 10. Preparation of Sequencing Reaction

Component	A Per Reaction (µL)	B [redacted]	Master Mix (Column A X Column B) (µL)
Master Mix	[redacted]	*TBD*	*TBD*
Pooled Sample			*TBD*
Total			*TBD*

8.10.2. [redacted]

8.10.3. [redacted] per **Table 8**.

8.11. Preparation of Sequencing Plate in a positive BSC

8.11.1. [redacted]

8.11.2. [redacted]

8.11.3. [redacted]

8.11.4. [redacted] (Table 11).

Table 11. [redacted]

Table 11 The 200BigDye Thermocycler program

Step	Temperature	Time
Initial denature		
Extension (Cycle [redacted] times)		
Hold		

8.12. [redacted] Sequencing Purification.

8.12.1 [redacted]

NOTE: [redacted]

8.12.2 [redacted]

8.12.3 [redacted]

8.12.4 [redacted]

8.12.5. [redacted] per **Table 12.**

Table 12 Preparation of [redacted] Solution Mix

Component	A Per Reaction (µL)	B Multiplier (Total Sequencing Reaction Number +20)	Master Mix (Column A X Column B) (µL)
SAM Solution	[redacted]	*TBD*	*TBD*
[redacted] Solution	[redacted]		*TBD*
Total	[redacted]		*TBD*

8.12.6. [redacted]

8.12.7. [redacted]

8.12.8. [redacted]

8.12.9. [redacted]

8.12.10. [redacted]

8.12.11. [redacted]

8.12.12. [redacted]

8.12.13. [redacted]

8.12.14. [redacted]

8.12.15. [redacted]

8.12.16. [redacted]

8.13. [redacted] **(SOP-0450/SOP-1035)**.

NOTE: [redacted] refer to SOP-1035.

8.13.1. [redacted]

8.13.2. [redacted]

8.13.3. [redacted]

8.13.4. [redacted]

[redacted]

8.13.5 [redacted]

8.13.6 [redacted]

8.13.7 [redacted]

8.13.8 [redacted]

8.13.8.1. [redacted]

8.13.8.2. [redacted]

8.13.9 [redacted]

8.13.10 [redacted]

8.13.11 [redacted]

8.13.12 [redacted]

8.13.13 [redacted]

8.13.14 [redacted]

8.13.15 [redacted]

8.13.16 [redacted]

8.13.17 [redacted]

8.13.18 [redacted]

8.13.19 [redacted]

8.13.20 [redacted]

8.14. Sequence Analysis using SeqScape Data

8.14.1. Analyzing Samples and Positive Control

8.14.1.1. [redacted]

8.14.1.2. [redacted]

8.14.1.3. [redacted]

NOTE: [redacted]

8.14.1.4. [redacted]

8.14.1.5. [redacted]

8.14.1.6. [redacted]

8.14.1.7. [redacted]

8.14.1.8. [redacted]

8.14.1.9. [redacted]

8.14.1.10 [redacted]

8.14.1.10.1. [redacted]

8.14.1.10.2. [redacted]

NOTE: [redacted]

8.14.1.10.3. [redacted]

8.14.1.10.4. [redacted]

8.14.1.10.5. [redacted]

8.14.1.10.6. [redacted]

8.15. System Suitability

8.15.1. [redacted]

8.15.2. [redacted]

8.15.3. [redacted]

8.15.4. [redacted]

8.15.5. [redacted] **SOP-0409.**

8.16. Sample Suitability

8.16.1. [redacted]

8.16.2. [redacted]

8.16.3. [redacted]

8.17. Results Reporting

8.17.1 [redacted]

8.17.2 [redacted]

[redacted], **SOP-0033.**

8.17.3. [redacted] **FRM-0742** [redacted] per SOP-0082 [redacted]

9.0 ATTACHMENTS

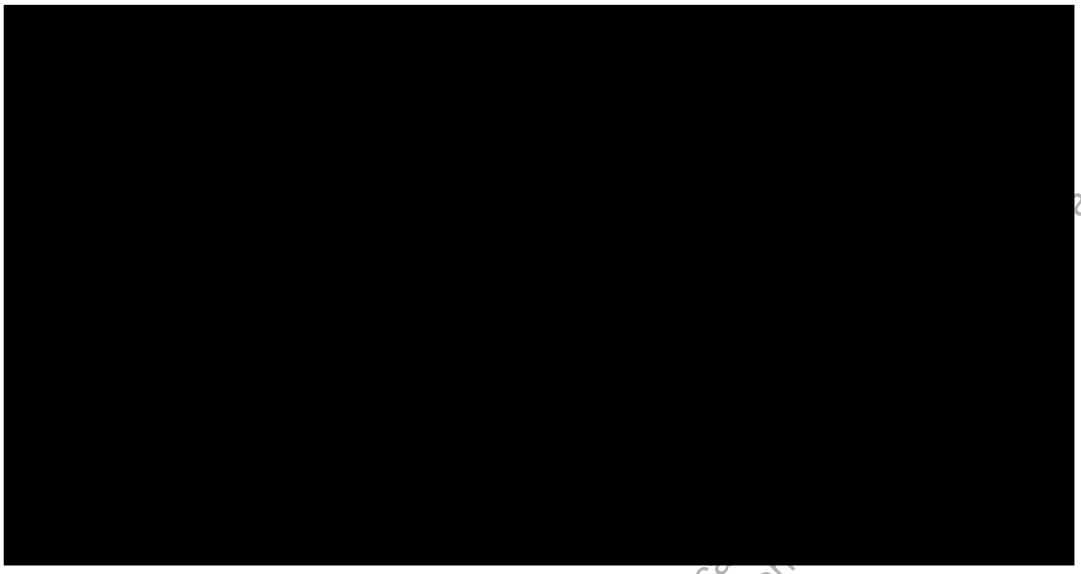
9.1. Attachment 1: BSC Placement

10.0 REVISION HISTORY

Revision #	Effective Date	Change Details	Author
1.0	Refer to Veeva Header for Effective Date	New Document	[redacted]

ATTACHMENT 1: BSC Placement

(Page 1 of 1)



Document Approvals

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