1.0 PURPOSE

The purpose of this procedure is to detect and quantify residual plasmid DNA in mRNA Drug Substance (DS) or mRNA Product intermediate (MPI) using a real time quantitative PCR (qPCR) assay designed to amplify the kanamycin resistance gene in the plasmid.

2.0 SCOPE

This procedure applies to detection of the residual plasmid DNA in mRNA DS or MPI samples for validated constructs.

3.0 REFERENCED DOCUMENTS

Document #	Title
FRM-0736	Assay Performance Worksheet: SOP-1020 Determination of Residual DNA by qPCR
FRM-0795	SOP-1020 Residual DNA Calculation Sheet
SOP-0017	Maintaining a RNase Free Work Environment
SOP-0004	Operation and Maintenance of CCI Biological Safety Cabinets (BSC)
SOP-0033	Out of Specification (OOS)
SOP-0081	Preparation of Solutions and Samples in the GMP-Quality Control Laboratory
SOP-0082	Data Review and Reporting 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-0451	Operation and Maintenance of the CCI PCR System
SOP-0452	Personnel Flow and Gowning in the QC Bioassay Laboratories
SOP-0465	Use of the CCI Microcentrifuge and the CCI Centrifuge

4.0 RESPONSIBILITIES

Department/ Functional Area	Responsibilities
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
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
ABI	Applied Biosystems Instruments
C_T	The PCR cycle at which an increase in reporter fluorescence above the baseline signal can first be detected
°C	Degrees Celsius
DS	Drug Substance
DNA	Deoxyribonucleic acid
FAM	Fluorescein
GMP	Good Manufacturing Practices
IPA	Isopropyl Alcohol
MPI	mRNA Product Intermediate
MW	Molecular Weight
mL	Milliliters
mM	Millimolar
ng	Nanograms
NTC	No Template Control
PPE	Personal Protective Equipment
qPCR	Quantitative Polymerase Chain Reaction
QC	Quality Control
R^2	Coefficient of Determination (square of correlation coefficient (R))
SDM	Second Derivative Maximum
TAM	Tetramethylrhodamine
µg	Micrograms
µL	Microliters

6.0 MATERIALS

NOTE: Alternative vendors or part numbers may be used, provided the reagent grade or classification is maintained.

6.1. Reagents

Item	Vendor	Catalog#
CCI		

	Primer Name	Vendor	5'-Sequence-3'
CCI			

6.2. Consumables

Item	Vendor	Catalog#
CCI		

6.3. Equipment

Item	Vendor	Model #
CCI		

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: Refer to **Attachment 2** for BSC location map.

8.1. Assay preparation: Perform the following preparation steps as needed.

8.1.1. Preparation of Primers and Probes (In a Negative BSC)

NOTE: Record preparation of stock primer tubes, probes and carrier RNA on **FRM-0180** in the solution preparation logbook per **SOP-0090**.

8.1.2. Prepare and Aliquot the Primers

8.1.2.1. The standard desalting purified forward and reverse primers are ordered as CCI .

8.1.2.2. Spin the primer tubes at CCI in the microcentrifuge before opening them to make sure all the lyophilized primer is at the bottom of the tubes.

8.1.2.3. Check the amount of primer synthesized in the tube, in CCI of TE buffer (For Example: add CCI primer to obtain the CCI stock).

8.1.2.4. Vortex for CCI to dissolve the lyophilized primer.

8.1.2.5. Spin the primer tubes at CCI in the microcentrifuge.

8.1.2.6. Aliquot CCI of each primer into CCI tubes.

8.1.2.7. Store the aliquots at CCI Assign an expiry date of 1 year from the date of receipt of the lyophilized primers. Place the aliquots in a box and label with the primer name, lot, and expiry.

8.1.3. Prepare and Aliquot the Probe

8.1.3.1. The HPLC purified probe is ordered as CCI .

8.1.3.1.1. CCI

8.1.3.1.2. CCI

8.1.3.1.3. CCI

8.1.3.2. Spin the primer tubes at CCI in the microcentrifuge before opening it to make sure all the lyophilized probe is at the bottom of the tube.

8.1.3.3. Check the amount of probe synthesized in the tube, in CCI of TE buffer (For Example: add CCI primer to obtain the CCI stock).

8.1.3.4. Vortex for CCI to dissolve the lyophilized pellet.

8.1.3.5. Spin the probe tubes at CCI in the microcentrifuge

8.1.3.6. Aliquot CCI of probe into CCI tubes.

8.1.3.7. Store the aliquots at CCI Assign an expiry date of 1 year from the date of receipt of the lyophilized probe. Place the aliquots in a box and label with the probe name, lot, and expiry.

8.1.4. Preparation of Carrier Poly A RNA Dilution Buffer (CCI) (In a Negative BSC: BSC-1138-01/BSC-1138-02)

8.1.4.1. Add CCI to the 1CCI .

8.1.4.2. Vortex for CCI .

8.1.4.3. Spin the tubes at CCI in the microcentrifuge.

8.1.4.4. Aliquot CCI tubes and store the aliquots atCCI . Assign an expiry date of 1 year from the dilution date.

8.1.5. Preparation of CCI Plasmid Standard (In a Positive BSC)

8.1.5.1. Clean the tube of standard linearized plasmid CCI by wiping with an IPA wipe.

8.1.5.2. Aliquot CCI into CCI tubes and store at CCI Place the aliquots in a box and label with the name, lot, and expiry.

NOTE: Record the following steps on **FRM-0736**. Assign **FRM-0736** an ARN number per **SOP-0210**.

8.2. Preparation of working stock solutions (BSC-1138-01).

NOTE: working stocks will be diluted at time of use and then discarded.

8.2.1. Thaw the stock primer, probe, dilution buffers and qPCR kit reagents on Aluminum Bead Bath (the basket of aluminum bead is stored at C before using) at least CCI.

8.2.2. Dilute CCI Primers (CCI to the CCI working concentration.

8.2.2.1. In a new CCI tube, dilute CCI Stock Primer into CCI CCI

8.2.2.2. Mix by pipetting up and down at least C times with half the total volume.

8.2.3. Dilute CCI Stock Probe (CCI) to the CCI working concentration

8.2.3.1. In a new CCI tube, dilute CCI of stock probe into CCI .

8.2.3.2. Mix by pipetting up and down at least C times with half the total volume.

8.2.4. Preparation of CCI Dilution buffers

8.2.4.1. Diluent 1 Preparation: CCI.

8.2.4.1.1. In a new CCI tube, dilute CCI of stock CCI CCI (**Table 1**).

8.2.4.1.2. Mix by pipetting up and down at least C times with half the total volume.

Table 1 Preparation of Diluent 1: CCI

Diluent	Volume of CCI	Volume of CCI
Diluent 1	C	

8.2.4.2. Diluent 2 Preparation: CCI

Diluent 2 is for the initial dilution of Standard and Sample.

8.2.4.2.1. In a new CCI tube, dilute CCI of Diluent 1 (CCI) into CCI of CCI (**Table 2**).

8.2.4.2.2. Mix by pipetting up and down at least CCtimes with half the total volume.

Table 2 Preparation of Diluent 2: CCI

Diluent	Volume of Diluent 1 (CCI)	Volume of CCI
Diluent 2	CCI	

8.2.4.3. Diluent 3 Preparation: CCI

Diluent 3 is used for the additional dilutions of Standard and Sample.

8.2.4.3.1. Using a CCI Serological Pipets, add CCI to a new CCI tube.

8.2.4.3.2. TransferCCI of Diluent 1 into the tube.

8.2.4.3.3. Mix by pipetting up and down with a serological pipette at least CCtimes.

Table 3 Preparation of Diluent 3: CCI

Diluent	Volume of Diluent 1 CCI	Volume of CCI
Diluent 3	CCI	

8.3. Preparation of qPCR Master Mix

8.3.1. Calculate the master mix multiplier using the equation below:

NOTE: Round up to the nearest whole number

$$Multiplier = 4.4 \times (8 + 6 \times Number\ of\ Samples)$$

Multiplier for 1 sample = 62

Multiplier for 2 Samples = 88

Multiplier for 3 Samples = 115

8.3.2. Using the multiplier from **Step 8.2.5.1,** prepare the qPCR Master Mix per **Table 4** into CCI conical tube.

Table 4: qPCR Master Mix Preparation

Reagent	A Volume for 1 well (µL)	B Multiplier	Actual Volume (µL) (Column A x Column B)
TaqMan Fast Advanced Master Mix	CCI	TBD	TBD
Forward Primer (CCI			TBD
Reverse Primer CCI			TBD
Probe CCI)			TBD
Exogenous IPC mix (CCI			TBD
Exogenous IPC DNACCI			TBD
CCI			TBD
Total Volume			TBD

8.3.3. Mix by pipetting up and down at least CCtimes with half the total volume.

8.3.4. Divide the master mix into tubes using 8-Tube Strips. 1 8-strip tube (8 tubes) will be needed for the standards and NTC. 1 8-tube strip with 6 tubes will be needed for each sample. Add CCI Master Mix to each tube per **Table 5.**

Table 5 The Master Mix for Controls and Samples

Tube Number	Standard & Control Tubes		Sample(s) Tubes		Volume of Master Mix (µL)
	Tube Name	Control	Tube Name	Sample	
1	STD3	Standard 3	Sample D1	Sample Dilution 1	CCI
2	STD4	Standard 4	Sample D2	Sample Dilution 2	
3	STD5	Standard 5	Sample D3	Sample Dilution 3	
4	STD6	Standard 6	Sample D4	Sample Dilution 4	
5	STD7	Standard 7	Sample D5	Sample Dilution 5	
6	STD8	Standard 8	Sample D6	Sample Dilution 6	
7	STD9	Standard 9			
8	NTC	No Template Control			

8.4. Prepare the No Template control (NTC)

8.4.1. Add CCI of Diluent 3 into the NTC Master Mix tube.

8.4.2. Mix by pipetting up and down at least C times with half the total volume.

8.4.3. Plate CCI of NTC Master Mix into the destination wells of CCI Plate. An example plate map is shown in **Table 6**.

Table 6 Example Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD3	STD3	STD3	Sample1 D1	Sample1 D1	Sample1 D1	Sample2 D1	Sample2 D1	Sample2 D1	Sample3 D1	Sample3 D1	Sample3 D1
B	STD4	STD4	STD4	Sample1 D2	Sample1 D2	Sample1 D2	Sample2 D2	Sample2 D2	Sample2 D2	Sample3 D2	Sample3 D2	Sample3 D2
C	STD5	STD5	STD5	Sample1 D3	Sample1 D3	Sample1 D3	Sample2 D3	Sample2 D3	Sample2 D3	Sample3 D3	Sample3 D3	Sample3 D3
D	STD6	STD6	STD6	Sample1 D4	Sample1 D4	Sample1 D4	Sample2 D4	Sample2 D4	Sample2 D4	Sample3 D4	Sample3 D4	Sample3 D4
E	STD7	STD7	STD7	Sample1 D5	Sample1 D5	Sample1 D5	Sample2 D5	Sample2 D5	Sample2 D5	Sample3 D5	Sample3 D5	Sample3 D5
F	STD8	STD8	STD8	Sample1 D6	Sample1 D6	Sample1 D6	Sample2 D6	Sample2 D6	Sample2 D6	Sample3 D6	Sample3 D6	Sample3 D6
G	STD9	STD9	STD9									
H	NTC	NTC	NTC									

8.4.4. Seal the CCI Plate with Adhesive PCR Film.

8.4.5. Cap the tubes containing master mix.

8.4.6. Move the CCI Plate and the tubes containing master mix into BSC-1138-02.

8.5. Preparation of the test samples (BSC-1138-02).

8.5.1. Thaw the sample on Aluminum Bead Bath (the basket of aluminum beads is stored at CCI before using) at least CCI .

8.5.2. For each sample, into the first tube of a new 8-tube strip, dilute CCI of sample into CCI of **Diluent 2** to make Sample Dilution 1.

8.5.3. Mix by pipetting up and down at least C times with half the total volume.

8.5.4. Serially dilute (CCI dilutions) Sample Dilution 1 with **Diluent 3** in the 8-Tube Strip with Attached Domed Caps per **Table 7**.

8.5.4.1. Add CCI of **Diluent 3** to tubes 2-6 within the 8-tube stip.

8.5.4.2. Add CCI of Sample Dilution 1 to tube 2 and mix by pipetting up and down with at least half the total volume of the tube.

8.5.4.3. Continue diluting CCI of the previous dilution tube to the next tube containing **Diluent 3** until all 6 CCI serial dilutions are complete.

8.5.4.4. When performing serial dilutions, mix by pipetting up and down at least CC times with at least half the total volume before transferring to the next tube.

Table 7: Sample Serial Dilutions

Dilution Name	Final Dilution	Volume of Previous Dilution (µL)	Volume of Diluent 3 (µL)
Sample Dilution 1	CCI		
Sample Dilution 2		CCI	
Sample Dilution 3			
Sample Dilution 4			
Sample Dilution 5			
Sample Dilution 6			

8.5.5. Add CCI of each Testing sample dilution into destination tube containing Master Mix from **Table 5**.

8.5.6. Mix by pipetting up and down at least C times with half the total volume.

8.5.7. Plate CCI of Sample Master Mix into the destination wells of the CCI plate (**Table 6**).

8.5.8. Seal the CCI plate with Adhesive PCR film. Move the CCI plate and the tubes containing the Standard Master Mix into BSC-1139-01/BSC-1139-02.

8.6. Preparation of the Standard Curve (BSC-1139-01/BSC-1139-02)

8.6.1. Remove one tube of aliquoted CCI standard from CCI

8.6.2. In a CCI tube, dilute CCI standard into CCI of **Diluent 2** to make **Stock 1** CCI).

8.6.3. Mix by pipetting up and down at least C times with half the total volume.

8.6.4. Dilution of Standard samples

8.6.4.1. Serially dilute the plasmid **Stock 1** (CCI) using Diluent 3 in a new 8-tube strip per **Table 8.**

NOTE: 2 8-tube strips will be needed as there are 9 total standard dilutions.

8.6.4.2. Prepare Standard 1 by diluting CCI of **Stock 1** into CCI of **Diluent 3**.

8.6.4.3. Mix by pipetting up and down at least C times with half the total volume.

8.6.4.4. Add CCI of **Diluent 3** to tubes 2-9 within the 8-tube stip.

8.6.4.5. Add CCI of Standard 1 to tube 2 and mix by pipetting up and down with at least half the total volume of the tube.

8.6.4.6. Continue diluting CCI of the previous dilution tube to the next tube containing **Diluent 3** until all 9 CCI serial dilutions are complete.

8.6.4.7. When performing serial dilutions, mix by pipetting up and down at least CC times with at least half the total volume before transferring to the next tube.

Table 8 Standard Dilutions

Dilution Name	Number of Copies/ µL	Volume of Previous Dilution (µL)	Volume of Diluent 3 (µL)
Standard 1	CCI		
Standard 2			
Standard 3			
Standard 4			
Standard 5			
Standard 6			
Standard 7			
Standard 8			
Standard 9			

8.6.5. Add CCI of Standard 3 – Standard 9 into their corresponding destination tubes containing Master Mix per **Table 5**.

8.6.6. Mix by pipetting up and down at least C times with half the total volume.

8.6.7. Plate CCI of Standard Master Mix (Standard 3 – Standard 9) into the destination wells per **Table 6**.

8.6.8. Seal the plate with CCI Optical Adhesive Film.

8.6.9. Centrifuge the plate at CCI Repeat as needed until all bubbles are gone and the contents are at the base of the wells.

8.7. Running the Assay

8.7.1. Start the QuantStudio™ 7 per **SOP-0451.**

8.7.2. Select Method "SOP-1020 Residual plasmid" from Run template.

8.7.3. Ensure all wells containing material are selected.

8.7.4. Ensure all wells are named according to plate map (**Table 6**).

8.7.5. Ensure standard curve concentrations are assigned appropriately.

8.7.6. Ensure replicates are assigned appropriately.

8.7.7. Verify the following parameters are displayed:

Step	Temperature	Time
CCI		

8.7.8. Load plate on to QuantStudio™ 7.

8.7.9. Start run.

8.8. Data Analysis

8.8.1. When the run is complete, go to the Analysis screen.

8.8.2. Select the Standard Curve.

8.8.3. Select the wells that include the samples, NTC wells, and standards 3-9 (CC).

8.8.4. Select "Target 1" to record the R^2, Slope and C_T.

NOTE: *Target 1 detects the residual DNA in the samples.*
The standard curve of Target 2 will generate a blank graph in the report because Target 2 is Internal Positive Control (IPC).

8.8.5. Save the PDF report to the SDMS folder.

8.8.6. Open the PDF report from the SDMS folder and print it out.

8.8.7. Using the excel version of **FRM-0795** "SOP-1020 Residual DNA Calculation Sheet", calculate the standard curve and sample concentrations. All excel formulas may be found on **Attachment 1**.

8.8.8. The copies/µL of each point of the standard curve and each sample dilution are calculated using the following equation: inputting the C_T, slope, and y-intercept of the standard curve.

$$\text{Copies}/\mu\text{L} = 10^{(Ct\ -\ y\text{-}intercept)/Slope}$$

8.8.9. The copies/µL of each sample dilution is then multiplied by the corresponding dilution factor to determine the neat copies/µL.

8.8.10. The % (g/L DNA)/(g/L RNA) (%w/w) of the sample is then calculated using the following equations:

NOTE: The Plasmid MW can be found within the Validation Report of the test construct.

$$(g/L\ DNA) = \frac{(\frac{copies}{\mu L})(Plasmid\ MW\ (Da))(1 \times 10^6(\frac{\mu L}{L}))}{(6.022 \times 10^{23}\ molecules/mole)}$$

$$\%\,{}^{w}/_{w} = \frac{\text{g/L DNA}}{\text{g/L}\left(\frac{\text{mg}}{\text{mL}}\right)\text{ RNA}}$$

8.8.11. The % (g/L DNA)/(g/L RNA) (%w/w) of the sample dilutions in which the C_T was within the C_T range of the standard curve are then averaged to determine the %w/w of the sample.

8.8.12. Save the calculation sheet **FRM-0795**, print the calculation sheet with and without formulas showing, and attach to the APW.

8.9. System and Sample Suitability Criteria

8.9.1. R^2 must be CCI

8.9.2. The slope of the standard curve must be between CCI inclusive.

8.9.3. Amplification must be observed in at least 2 of 3 replicates for 10^2 standard.

8.9.4. The Average C_T value observed in NTC must be above the average C_T of the lowest point of standard curve.

8.9.5. IPC must be amplified in NTC wells.

8.9.6. The %RSD of each sample dilution's software calculated concentration (copies/µL) for all standards and samples within the quantitative range of the standard curve must have a %RSD of [redacted] If the average C_T value of a sample is higher than the average C_T of the lowest standard (10^2) of the standard curve, the %RSD criteria does not apply.

8.9.7. If all system and sample suitability criteria are met, the sample is valid. If the criteria are not met the assay is invalid. Proceed with the invalid assay procedure, **SOP-0409**.

8.10. Results Reporting

8.10.1. Have FRM-0736 reviewed per SOP-0082 and report results. The reviewer must review the formulas of the attached calculation sheet to ensure correct calculations.

8.10.2. Report the result (% w/w). If the value is less than the LOQ %w/w then report <(LOQ %w/w).

8.10.3. Refer to the specification of the test sample to determine if the %w/w is within specification. If the residual plasmid %w/w is out of specification, refer to the OOS procedure, **SOP-0033**.

8.10.4. Have **FRM-0736** reviewed per **SOP-0082** and report results. The reviewer must review the formulas of the attached calculation sheet to ensure correct calculations.

9.0 ATTACHMENTS

9.1. Attachment 1: SOP-1020 Residual DNA Calculation – Formula Sheet (Electronically attached in Veeva)

9.2. Attachment 2: BSC Placement

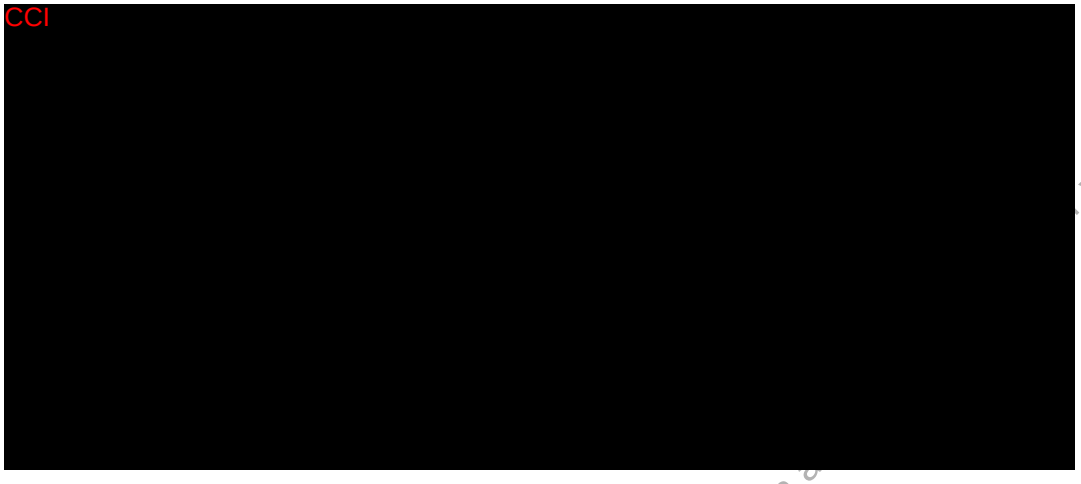
10.0 REVISION HISTORY

Revision #	Effective Date	Change Details	Author
1.0	Refer to Veeva Header for Effective Date	New Document	PPD

ATTACHMENT 2: BSC Placement

(Page 1 of 1)

CCI



Document Approvals

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