Frederick National Laboratory for Cancer Research

**BIOPHARMACEUTICAL DEVELOPMENT PROGRAM**

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| **SOP Title:** | **Quantitative PCR (qPCR) Method for Detection of Replication Competent Lentivirus** |
| **SOP Number:** | **22209** |
| **Revision:** | **01** |

## TABLE OF CONTENTS


1. PURPOSE ........ 1
2. SCOPE ........ 1
3. BACKGROUND ........ 1
4. RESPONSIBILITIES ........ 2
5. MATERIALS AND REAGENTS ........ 2
6. EQUIPMENT ........ 3
7. PROCEDURE ........ 3
8. VALIDITY OF THE ASSAY ........ 5
9. DATA ANALYSIS ........ 6
10. DOCUMENTATION AND RECORDS ........ 7
11. REFERENCES AND RELATED DOCUMENTS ........ 7


## 1. PURPOSE

This SOP describes the materials and methods needed to detect replication-competent lentivirus (RCL) in a sample by quantitative PCR (qPCR).

## 2. SCOPE

This method relies upon the ability of the primer/probe sequences to bind to VSV-G sequence that would be present if a replication-competent lentivirus were present in a sample. If the VSV-G plasmid used in the generation of the lentivirus vector possesses a VSV-G sequence that differs from the primer/probe sequences used in this method, this SOP would not be appropriate for detecting replication competent lentivirus in that sample.

## 3. BACKGROUND

Lentiviral vectors are engineered to be replication defective, however, replication competent lentiviruses could be generated through recombination events among the plasmids used in the lentivirus vector production. One of these plasmids used in lentivirus vector production is the envelope plasmid encoding VSV-G which is used for virus pseudotyping. The replication competent lentivirus qPCR assay targets the VSV-G sequence to determine whether replication competent lentivirus was generated.

## 4. RESPONSIBILITIES

4.1 Director / Process Analytics/Quality Control (PA/QC)

- Defines this procedure.

4.2 PA/QC Personnel

- Trains lab personnel.
- Performs the procedure.
- Records and reviews data.

4.3 Biopharmaceutical Quality Assurance (BQA)

- Provides quality oversight.

## 5. MATERIALS AND REAGENTS

| Part Number | Description | BDP Approved Substitution Permitted? |
|---|---|---|
| 31162 | Clinical Center VSVG Forward Primer<br>Sequence: 5’ – TGCAAGGAAAGCATTGAACAA – 3’ | ☐ YES ☒ NO |
| 31163 | Clinical Center VSVG Reverse Primer<br>Sequence: 5’ – GAGGAGTCACCTGGACAATCACT – 3’ | ☐ YES ☒ NO |
| 31164 | Clinical Center VSVG Probe<br>Sequence: 5’ - FAMAGGAACTTGGCTGAATCCAGGCTTCC-QSY – 3’ | ☐ YES ☒ NO |
| 30268 | TaqMan Universal PCR Master Mix (2X) | ☐ YES ☒ NO |
| 21141 | MicroAmp Optical 96 Well Reaction Plate | ☒ YES ☐ NO |
| 21142 | MicroAmp Optical Adhesive Film | ☒ YES ☐ NO |
| 10189 | Distilled Water, DNase Free RNase Free | ☒ YES ☐ NO |
| 30267 | 1X TE | ☒ YES ☐ NO |
| 30007 | 1X PBS | ☒ YES ☐ NO |
| 31238 | VSV-G DNA Control | ☐ YES ☒ NO |

## 6. EQUIPMENT

- qPCR Instrument – QuantStudio 6, SDS 7900HT or BDP-approved equivalent

## 7. PROCEDURE

7.1 Extract genomic DNA from each sample as described in **SOP 22212 Purification of DNA Using the DNeasy Blood and Tissue Kit** or **SOP 22972 Operation and Maintenance of the MagNA Pure 24 System for Nucleic Acid Extractions**.

7.1.1 Perform two or more replicate extractions for each transduced test sample.

7.1.2 If available, a non-transduced sample should be extracted in parallel with the transduced samples.

7.1.3 An Extraction Negative Control should be performed in which 200 µL of 1X PBS are extracted in parallel with the test samples.

7.1.4 An Extraction Positive Control should be performed in which 20 µL of VSV-G DNA Control at 1.00E+03 copies/µL is added to 180 µL of buffer blank or 1X PBS.

**NOTE:** The Extraction Positive Control should not be centrifuged prior to the addition of Proteinase K and Buffer AL.

7.2 Quantify the amount of DNA in each test sample using the NanoDrop ND1000 spectrophotometer according to SOP 22946 - The Operation of the NanoDrop ND1000 Spectrophotometer.

**NOTE:** It is not necessary to perform a spectrophotometry analysis of the Extraction Negative Control and Extraction Positive Control.

7.3 Dilute an aliquot of each sample to 8 ng/µL with 1X TE in a total volume of 100 µL (total DNA is 800 ng).

7.4 Replication Competent Lentivirus qPCR Master Mix Preparation

7.4.1 Record all reagent part numbers, lot numbers and expiration dates as well as any additional comments on Form 22209-01.

7.4.2 Vortex the reagents.

7.4.3 Additional steps Prepare a qPCR master mix as described below:

| Reagent | Volume for 1 Reaction | Final Concentration |
|---|---|---|
| TaqMan Universal PCR Master Mix (2X) | 25.0 | 1X |
| Clinical Center VSVG Forward Primer (10 µM) | 1.0 | 200 nM |
| Clinical Center VSVG Reverse Primer (10 µM) | 1.0 | 200 nM |
| Clinical Center VSVG Probe (FAM/QSY) (10 µM) | 0.5 | 100 nM |
| Water | 10 | N/A |

7.4.4 Prepare a sufficient volume of master mix to account for all test samples, standard curve points and controls with 10% excess to account for losses due to repeated pipetting.

7.4.5 Record the volumes used on **Form 22209-02**.

7.4.6 Mix the master mix by pipetting up and down.

7.4.7 Add 37.5 µL of master mix to each well of the 96-well PCR plate that will be used.

7.4.8 Add a 12.5 µL aliquot of 1X TE to each of three No Template Control (NTC) wells.

7.4.9 Seal the plate with adhesive film. The plate may be stored at 2-8°C for up to 1 hour while samples are prepared for addition to the plate.

7.5 Sample addition to the PCR plate

7.5.1 Test Sample: Add 12.5 µL of each extracted sample (5 replicate wells), Extraction Negative Control and Extraction Positive Control to the appropriate wells of the PCR plate.

7.5.2 Inhibition-control Reaction: Add 12.5 µL of non-transduced control sample + 2 µL of VSV-G DNA Control at 1.00E+03 copies/µL.

**NOTE:** If a non-transduced control sample is unavailable, add 2 µL of VSV-G DNA Control at 1.00E+03 copies/µL to 12.5 µL of transduced sample.

**NOTE:** The PCR inhibition spike control samples will contain a final volume greater than 50 µL due to the extra spike volume.

7.5.3 Dilute an aliquot of VSV-G DNA Control in 1X TE over a range of concentrations from 1.00E+06 to 1.00E+01 copies/µL. Add 12.5 µL of each VSV-G DNA Control standard curve point to the appropriate wells of the plate.

7.6 Seal the plate with adhesive film. The plate may be kept at room temperature for up to 30 minutes prior to amplification on a qPCR instrument.

7.7 Place the sealed plate into the qPCR instrument.

7.8 Program the qPCR instrument to detect a FAM reporter and Non-fluorescent quencher with the following amplification thermal profile:

| Temperature | Time (Min:Sec) | Repetition |
|---|---|---|
| 50°C | 02:00 | N/A |
| 95°C | 10:00 | N/A |
| 95°C | 00:15 | 40 |
| 60°C | 01:00 | |

7.9 Upon completion of the qPCR run, unload and discard the plate. Do not remove the adhesive cover to prevent laboratory contamination with the target amplicon. Retain and reuse the compression pad.

7.10 Save, analyze and print the run results using a manual Rn threshold of 0.2.

**NOTE:** Use of other threshold values may be reported with PA Supervisor approval.

## 8. VALIDITY OF THE ASSAY

8.1 Record the assay control results on **Form 22209-03**.

8.2 For the assay to be valid, the following criteria must be met:

8.2.1 The slope of the standard curve must be ≥ -3.7 and ≤ -3.1.

8.2.2 The R2 for the standard curve must be ≥ 0.990.

8.2.3 For the Extraction Positive Control, ≥10% of the spiked DNA must be recovered.

8.2.4 For the Inhibition Control, ≥10% of the inhibition spike must be detected.

8.3 If the run is valid, proceed to Step 8.1 to determine whether the sample is positive or negative for replication competent lentivirus.

8.4 If the run is invalid, the assay may be repeated with supervisor approval and indicate in the comments section of **Form 22209-03**.

## 9. DATA ANALYSIS

9.1 Assay results are reported as either “Positive” or “Negative” for the presence of replication competent lentivirus on **Form 22209-04**.

9.2 Record the qPCR data on **Form 22209-05**.

9.3 A sample replicate is reported RCL-positive if at least three out of five wells are positive; if less than three wells are positive, the sample is reported RCL-negative.

9.4 If both sample replicates are positive, the sample is reported RCL-positive.

9.5 If both sample replicates are negative, the sample is reported RCL-negative.

9.6 If one replicate is positive and one replicate is negative, the assay must be repeated (indicate in the comments section of **Form 22209-04**).

9.6.1 If both replicates in the repeat assay are negative, the sample is reported RCL-negative.

9.6.2 If one or both replicates in the repeat assay are positive, the sample is reported RCL-positive.

## 10. DOCUMENTATION AND RECORDS

Record all reagent part numbers, lot numbers and expiration dates on **Form 22209-01**. Record the 96-well plate layout and master mix preparation volumes on **Form 22209-02**. Record control results on **Form 22209-03.** Record sample analysis results on **Form 22209-04**. Record RCL-qPCR data on **Form 22209-05**.

## 11. REFERENCES AND RELATED DOCUMENTS

| Document Number | Title |
|---|---|
| 22212 | Purification of DNA Using the DNeasy Blood and Tissue Kit |
| 22972 | Operation and Maintenance of the MagNA Pure 24 System for Nucleic Acid Extractions |
| 22946 | The Operation of the NanoDrop ND1000 Spectrophotometer |
| 22209-01 | Quantitative PCR Reagents |
| 22209-02 | 96-Well qPCR Plate Key, Master Mix Preparation and Dilution of Standard |
| 22209-03 | RCL-qPCR Assay Validity Criteria Analysis |
| 22209-04 | RCL-qPCR Assay Sample Analysis |
| 22209-05 | RCL-qPCR Data |