| | |
|---|---|
| **SOP Title:** | **Quantitative PCR (qPCR) Methods for Detection and Quantification of Nucleic Acids** |
| **SOP Number:** | **22195** |
| **Revision:** | **09** |

## TABLE OF CONTENTS



## 1. PURPOSE

This procedure describes the general materials and methods for quantitative PCR (qPCR) amplification and detection of various nucleic acid sequences that may be present in a test sample

## 2. SCOPE

This Standard Operating Procedure (SOP) is intended for general qPCR assays that require the characterization and/or quantitation of specific nucleic acid target sequences in a test sample. Multiple qPCR reactions can be performed to elucidate more than one species or target sequences that may be present in a given sample. Specific reagents, primers, and probes necessary for each qPCR reaction are individually listed in the attachments associated with this SOP. Specific SOPs are available for some qPCR methods including Mycoplasma detection, viral contamination, and detection of RCA; the requirements of specific assay SOPs (if applicable) supersede those of this SOP.

## 3. RESPONSIBILITIES

### 3.1 Director, Process Analytics/Quality Control (PA/QC)

- Defines this procedure.
- Controls samples submitted for qPCR analysis.
- Reviews and approves result of test analyses.

3.2 PA/QC Laboratory Personnel / Analyst

- Implements this procedure.
- Follows this procedure.

3.3 PA/QC Supervisor

- Approves the use of alternate threshold values.

3.4 Quality Assurance

- Provides quality oversight.

## 4. MATERIALS AND REAGENTS

| Part Number | Description | BDP Approved Substitution Permitted? |
|---|---|---|
| **General qPCR Reagents, as required** | | |
| 21141 | Applied Biosystems, Inc. (ABI) 96-Well Plate | ☐ YES ☒ NO |
| 21142 | ABI Adhesive Optical Plate Cover | ☐ YES ☒ NO |
| 30268 | TaqMan Universal PCR Master Mix | ☐ YES ☒ NO |
| 30265 | Super Script III RT/Platinum 1-step kit | ☐ YES ☒ NO |
| 31244 | RNaseOUT | ☐ YES ☒ NO |
| 31129 | Microcentrifuge Tube, Low Binding (1.7 mL) | ☒ YES ☐ NO |
| 31130 | Microcentrifuge Tube, Low Binding (0.65 mL) | ☒ YES ☐ NO |
| 31486 | Eppendorf Protein Low Binding tubes (5.0 mL) | ☒ YES ☐ NO |
| **Buffer Reagents, as required** | | |
| 10189 | Nuclease-Free Water | ☒ YES ☐ NO |
| 30007 | PBS (pH 7.4) | ☒ YES ☐ NO |

| Part Number | Description | BDP Approved Substitution Permitted? |
|---|---|---|
| 30267 | TE (1X) | ☒ YES ☐ NO |
| 30295 | WFI, USP | ☒ YES ☐ NO |
| 30266 | DEPC Treated Water | ☒ YES ☐ NO |
| 10042 | Tris HCl (pH 7.4) | ☒ YES ☐ NO |
| **DNase Reagents as required** | | |
| 31243 | DNaseI (New England Biolabs) | ☒ YES ☐ NO |
| 10098 | EDTA (0.5M, pH 8.0) | ☒ YES ☐ NO |
| **DNA/RNA Extraction Reagents, as required** | | |
| 30442 | QIAGEN DNA Mini Kit | ☐ YES ☒ NO |
| 30444 | QIAGEN Blood Mini Kit | ☐ YES ☒ NO |
| 30450 | QIAGEN Viral RNA Mini Kit | ☐ YES ☒ NO |
| 30443 | QIAGEN DNeasy Blood & Tissue Kit | ☐ YES ☒ NO |
| 30446 | QIAGEN ATL lysis Buffer | ☐ YES ☒ NO |
| 30447 | QIAGEN AVL lysis Buffer | ☐ YES ☒ NO |
| 30445 | QIAGEN AL lysis Buffer | ☐ YES ☒ NO |
| 31236 | Roche MagNA Pure 24 Total NA Isolation Kit | ☐ YES ☒ NO |
| **qPCR Reaction Specific Reagent (see Attachments for PNR)** | | |
| Assay Specific | Nucleic Acid Standards | ☐ YES ☒ NO |

| Part Number | Description | BDP Approved Substitution Permitted? |
|---|---|---|
| Assay Specific | Forward Primer | ☐ YES ☒ NO |
| Assay Specific | TaqMan Probe | ☐ YES ☒ NO |
| Assay Specific | Reverse Primer | ☐ YES ☒ NO |
| Assay Specific | Positive Reference Control Reagents | ☐ YES ☒ NO |

## 5. EQUIPMENT

- QuantStudio™ 6 qPCR System
- Roche MagNA Pure 24

## 6. SAFETY

6.1 Treat all viral and biohazardous samples per **SOP 26101 - Labeling, Transport, Submission, Storage, and Handling of Biohazardous Materials within the BDP**.

6.2 All viral samples must be inactivated in QIAGEN buffer ATL or AL or by MagNA Pure extraction prior to use.

## 7. PROCEDURE

7.1 Determine the sample composition in order to determine whether a nucleic acid extraction is required. Refer to the assay-specific attachments for a list of the approved extraction kits to be used with each amplicon.

7.1.1 Previously purified DNA or RNA (such as a plasmid preparation) in low salt (< 100 mM) and cleaning swabs typically do not require extraction prior to amplification.

7.1.2 Samples containing concentrated protein, intact cells, and/or high salt or other PCR inhibitors (including some culture medias) will typically require extraction prior to amplification.

7.2 To control for potential loss of target DNA/RNA during the sample extraction step, perform an extraction spike control with either the control target DNA/RNA or a heterologous control target using a second qPCR amplicon.

7.2.1 To determine the extraction and DNA recovery efficiency, separately extract the appropriate requestor or PA/QC-provided buffer blank sample after adding a spike within the assay's standard control range to the buffer sample aliquot.

7.2.2 Record the amount (in the final reaction) and type of spiked DNA used on PCR Inhibition Spike Results or/and Sample Recovery Spike results on the Form 22195-03.

7.3 Determine the appropriate qPCR or RT-qPCR amplification reagent kit to use. Refer to the assay-specific attachments for the approved amplification reagent kits that are to be used with each amplicon.

7.4 Determine the appropriate amount of qPCR Master Mix or RT-qPCR Master Mix to prepare as follows.

7.4.1 Standard Curve reactions are performed in duplicate (R&D, cleaning, and in-process testing) or triplicate (product release testing only).

7.4.2 No Test Control (NTC) – performed in single, duplicate, or triplicate reactions with nuclease-free (NFW), Diethyl pyrocarbonate (DEPC) treated, or WFI water as the control sample. Perform triplicate NTC reactions for release testing.

7.4.3 Sentinel controls – optional water or buffer controls to monitor intra-assay and QC test contamination levels identical in setup to that of the NTC control and located near the corners of a 96-well reaction plate. Sentinel controls are most useful with samples known or expected to contain high target copy numbers.

7.4.4 Extraction efficiency (spike) control – performed in duplicate or triplicate reactions. Spike reactions can utilize either the standard DNA/RNA target or a heterologous amplicon using a secondary reporter dye / detector channel and a non-fluorescent quenching dye.

7.4.5 PCR Inhibition (post-extraction spike) control – performed in single or duplicate reactions with representative test sample(s) that may be expected to have the greatest level of PCR inhibition. For example, a column load can be expected to have a greater probability of exhibiting inhibition than a column eluate sample and samples containing high salt (≥ 0.25 M), Phosphate (≥ 50 mM) and/or Ethylenediaminetetraacetic acid (EDTA) (≥ 25 mM) concentrations may experience PCR inhibition even after extraction. Where feasible, test each submitted sample for the presence of PCR inhibition. Spike reactions can utilize either the standard DNA/RNA target or a heterologous amplicon using a secondary reporter dye / detector channel and a non-fluorescent quenching dye.

7.4.6 Test sample reactions – duplicate or triplicate reactions for each test sample and/or sample dilution to be assayed. Product release tests must utilize triplicate reactions, while cleaning, in-process, and intermediate release samples may utilize duplicate sample replicates.

7.4.7 It is advantageous to test the highest possible concentration of sample not exhibiting inhibition in order to obtain the optimal assay sensitivity. The expected target concentration must be no more than 3.33 Ct lower than the standard curve's upper limit in order to maintain quantitative accuracy. Ct scores < 12 are progressively less accurate and should be avoided. DNA concentrations > 100 ng/rxn may inhibit qPCR. Nucleic acid concentrations > 500 ng/rxn will inhibit qPCR.

7.4.8 Total the number of individual reactions necessary for the assay, multiply by the amount of Master Mix and other reagents required (see 7.5 below) per reaction and prepare at least 110% this amount for aliquoting. Record the number of reactions required on Form 22195-02.

7.5 Prepare the calculated amount of qPCR or RT-qPCR Master Mix in the "DNA free" PCR set-up area [redacted]) by adding sufficient reagents to generate the concentrations and volumes per individual reaction listed per the amplicon specific attachment. A general 50 µL reaction design for a qPCR is provided in the table below.

| REAGENT | VOLUME PER REACTION IN µL |
|---|---|
| Universal 2X Master Mix | 25 µL (1x final conc.) |
| DEPC-treated, Nuclease-free, or WFI Water | Assay Specific (Bring to a 30 µL volume after addition of primers / probe.) |
| Primers (Fwd and Rev) | Assay Specific (typically 5-20 pmol) |
| Probe | Assay Specific (typically 5-20 pmol) |
| Reagent Mix Volume | 30 µL |
| Volume of Sample to be added | 20 µL |
| **Total Reaction Volume** | **50 µL** |

**NOTE:** The PCR inhibition spike control samples will contain a final volume greater than 50 µL due to the extra spike volume unless the spike volume is substituted for water in the master mix.

7.6 Ensure that the proper final pmol concentration of primers and probe is added per reaction based on the assay-specific attachment. The amplicon-specific primer and probe pmol concentrations and other indicated reagent volumes take precedent over the volume figures above as necessary.

7.7 Record the reagent and material part numbers, lots, and expiration dates as well as any additional comments on **Form 22195-01**. Record the volumes used per reaction on **Form 22195-02**.

7.8 Load the 96-well plate with prepared Master Mix in a dedicated “DNA free” PCR set-up area (master mix setup room) according to the desired 96-well qPCR plate key (Form 22195-02). Complete the NTC controls by adding NFW or WFI to the NTC wells. Temporarily seal or cover the 96-well plate for transport to the “DNA allowed” final set-up area. Plate transport between laboratories must be between +4 °C (on ice) and room temperature.

7.9 In a dedicated PCR set-up area (PCR plate and DNA sample setup room), load the standard, test, sentinel (if needed), and spike control samples to their appropriate wells as designated on the 96-well qPCR plate key (**Form 22195-02**).

7.10 Seal the plate with an optical adhesive cover and ensure that all bubbles are removed from the samples in each well, by gently tapping the plate several times if necessary. The plate may be kept at room temperature for up to 30 minutes prior to amplification on the Applied Biosystems QuantStudio™ 6.

7.11 Place the sealed plate into the qPCR instrument, ensuring that the plate is appropriately placed in the carriage by lining up the notched corner of the plate to the notched corner of the plate holder.

7.12 Perform qPCR amplification according to **SOP 22973 – Operation and Maintenance of the QuantStudio™ 6 qPCR System** using the thermocycler parameters listed in the appendix for the specific qPCR assay to be used.

7.13 Refer to the amplicon-specific requirements (see assay specific attachment) for cycling profiles. A typical qPCR reaction profile is shown below.

| STAGE | TEMP | TIME (MIN:SEC) | REPETITION |
|---|---|---|---|
| 1 | 50°C | 2:00 | N/A |
| 2 | 95°C | 10:00 | N/A |
| 3 | 95°C<br>60°C | 0:15<br>1:00 | 40 cycles |

7.14 A typical RT-qPCR reaction profile is shown below.

| STAGE | TEMP | TIME (MIN:SEC) | REPETITION |
|---|---|---|---|
| 1 | 50°C | 15:00 | N/A |
| 2 | 95°C | 2:00 | N/A |
| 3 | 95°C<br>60°C | 0:15<br>1:00 | 40 cycles |

7.15 Since most qPCR reactions utilize the same reaction profile, more than one amplicon can be on a plate. This is especially useful when a test sample requires multiple qPCR assays for different nucleic acid targets.

7.16 Prior to starting the qPCR run, ensure that the assay file contains the appropriate Reporter dyes and cycle conditions for each assay performed on the plate.

7.17 Upon completion of the qPCR run, unload and discard the plate. For viral samples, treat the sealed plate as potentially infectious, biohazardous waste. Do not remove the adhesive cover to prevent laboratory contamination with the target amplicon!

7.18 Save, analyze, and print the run results according to **SOP 22973 – Operation and Maintenance of the QuantStudio™ 6 qPCR System**, using the Rn Threshold value suggested or indicated for the specific amplicon used (0.2 Rn is typical). Use of other threshold values, including software calculated threshold values, may be reported with PA/QC Supervisor approval. Software calculated Rn Threshold values are typically used with R&D samples and especially with samples containing large amounts of the target sequence.

## 8. VALIDITY OF RESULTS

8.1 Assay results are considered valid if:

8.1.1 The absolute standard curve $r^2$ value should be ≥ 0.945. The low copy (or low mass) point of the standard curve may be omitted from the best fit calculation at the PA/QC Supervisor's discretion. Document low copy (mass) point omission in the comments section of **Form 22195-03**.

8.1.2 For release tests using 3 reactions per standard point, no more than 2 other outliers on the standard curve may be dropped using the appropriate Dixon-Q outlier test (refer to Form 22195-04) with a one-sided alpha value of 5%. Only replicates that meet or exceed the appropriate Dixon-Q P-score (at alpha 0.05) may be considered outliers and be dropped. Putative outliers that do not meet or exceed the P-score value cannot be dropped from the standard curve. Only a single replicate Ct may be dropped from any given point on the standard curve. If more than 2 replicates must be dropped from the standard curve prior to the best fit calculation, excluding the low copy point replicates, the assay is considered invalid. Dixon-Q scoring is not required for R&D, FIO, cleaning, EM, and In-process testing, due to the increased sample replicate variability inherent in these samples and the use of two standard replicates (rather than 3) per point.

8.1.3 The Dixon-Q one-sided outlier test is performed as follows. For the affected standard point containing the putative outlier replicate, rank the point's replicate Ct values from lowest to highest. If the putative outlier Ct falls below the other replicate Ct values apply the Low Value Tau calculation (i.e., the outlier is the lowest ranked replicate), if the putative outlier falls above the other replicate Ct values apply the High Value Tau calculation (i.e., the outlier is the highest ranked replicate).
Low Value Tau calculation: (second lowest replicate Ct - outlier Ct)/(highest replicate Ct - outlier Ct).

High Value Tau calculation: (outlier Ct - second highest replicate Ct)/(outlier Ct - lowest replicate Ct).

The tau value obtained is compared to the P-score for the number of replicates tested at alpha = 0.05 (5%, one-sided test). Only tau values >= the P-score are considered genuine outliers and may be dropped from the standard curve.

For example, at 3 replicates per point, the one-sided P-score for alpha 0.05 (5%) is 0.941 (also see current USP). Only outliers confirmed to have tau-values >= 0.941 using the appropriate Dixon-Q calculation may be dropped from the standard curve best fit analysis.

Record the Dixon-Q analysis on **Form 22195-04**.

8.1.4 The average Ct score of the No Test Control(s) (NTC) must be ≥ 33 Ct (> 38 is typical). Values below 33 Ct indicate excessive cross-contamination on the plate or in the laboratory. Individual NTC Ct scores should not be dropped prior to averaging. Most amplicons will have NTC Ct scores < 33 Ct when a large amount (typically >1 x $10^9$ copies or > 100 ng per reaction) of target is present on the test plate, such low NTC Ct scores only invalidate a release assay. R&D, FIO, and in-process tests with NTC Ct scores < 33 Ct can be invalidated, or allowed with the QC Supervisor's discretion, when explained in the comments section of Form 22195-03 or elsewhere in the report.

8.1.5 The Extraction spike control (if required) indicates that ≥ 10% of the spiked DNA was recovered. Test samples that unexpectedly have large amounts of target DNA/RNA that substantially exceeds (≥ 10X) the amount of spiked control DNA will not be considered to invalidate the assay.

8.1.6 The PCR Inhibition control (if required) indicates that ≥ 10% of the inhibition spike DNA is recovered when compared to the otherwise identical but unspiked sample. For samples containing large amounts of target sequence (i.e., containing ≥ 10X the amount of DNA spiked), PCR inhibition will be considered to have occurred only if the spiked control Ct score is > 3 Ct greater than the unspiked sample Ct score.

8.2 All assay reactions will be reviewed for indications of false amplification, PCR inhibition, cross-plate contamination, or other unusual effects via the Amplification Plot report page.

## 9. ANALYSIS OF RESULTS

9.1 The final assay results in copies or mass per reaction (rxn) are typically converted to reflect the results in copies/mL or mass/mL.

**Calculation Method: ((([Average Sample copy/rxn based on Assay Standard Curve and averaged sample Ct] – [Average NTC copy number]) / [Sample Volume μL per rxn]) x 1000 μL/mL).**

**This calculation is performed by Quant Studio Software and the result can be exported as excel sheet.**

**NOTE:** This formula does not capture any relative difference in extraction to elution volume nor does it capture dilutions made to the sample by PA/QC or the test requestor prior to qPCR analysis. Any such dilutions to the sample performed by PA (such as on **SOP 22972 MagNAPure DNA extraction or SOP 23113 QIAamp DNA/RNA extraction** ) must be incorporated in the final result calculation as well. Use the **Form 22195-05** for quantitative PCR data.

9.1.1 The Average NTC copy number is subtracted from the average test sample copy number values.

9.1.2 The Extraction recovery efficiency (if determined) is set as 100% if the recovery is ≥ 10% with the corrected sample copy or mass/rxn value.

9.1.3 Any effective sample dilution or concentration made during the extraction (and subsequent elution) step are calculated and adjusted (i.e., if 400 μL of sample is extracted and a volume of 200 μL is eluted, the effective result is a 2X concentration of sample).

9.1.4 The volume per reaction is normalized to 1 mL by multiplying by 50 assuming 20 μL of test sample are used per reaction (20 μL sample per reaction x 50 = 1 mL).

9.1.5 Any sample dilutions made by PA/QC prior to amplification are corrected for errors.

9.1.6 The use of viral lysis buffers necessitates the appropriate correction factor to the final copy number/mL based on sample volume used and elution volume.

9.2 For product release tests, the 95% Confidence Interval (CI) for the test sample replicates is calculated using the formula:

95% CI = Average replicate value $\pm$ 1.96 ($\sigma$ of replicates / $\sqrt{}$ n)

Where “n” is the number of replicates per sample and the standard deviation ($\sigma$) of sample replicates is provided from QuantStudio Real time qPCR software.

9.3 Additional qPCR amplicons as appropriate may be used to clarify and further characterize the test sample as required.

9.4 Any additional analytical methods, such as melting curve analysis using SYBR Green dyes, TD-qPCR, etc., are performed according to this SOP or as directed by the Project Scientist, or QC requestor.

## 10. DOCUMENTATION AND RECORDS

10.1 Document the preparation of control/standard nucleic acids in the **Preparation of Control or Standard DNA/RNA Logbook (Form 22195-06**).

10.2 Document the resuspension of primers and probes in the **Primer Resuspension Logbook (Form 22195-07)**.

10.3 Document the use of the PCR Workstations for Master Mix preparation and sample set-up in the **PCR Workstation Logbook (Form 21531-05)**.

10.4 All documentation must be performed in accordance with **SOP 21409 – Good Documentation Practices.**

10.5 Record all reagent and material suppliers (indicate BDP, otherwise indicate the vendor), part numbers, lot numbers, and expiration dates on **Form 22195-01**. Record the 96-well plate layout, Master Mix preparation volumes and the volumes/concentrations of Standard used to construct the standard curve on **Form 22195-02**. Record assay parameters and control sample data on **Form 22195-03**. Record test sample data and post-instrument analysis results on **Form 22195-05**. Record Dixon-Q standard curve analysis results (if required) on Form 22195-04. Record DNA extraction procedure on **Form 22972-01, 22972-02.** Record viral RNA extraction procedure on **Form 23113-01**, **23113-03, 23113-04**. Refer to any amplicon, product, procedural, and instrument SOPs as necessary.

## 11. REFERENCES AND RELATED DOCUMENTS

| Document Number | Title |
|---|---|
| 21409 | Good Documentation Practices |
| 22195-01 | Quantitative PCR Reagents |
| 22195-02 | 96-Well qPCR Plate Key |
| 22195-03 | Quantitative PCR Analysis Report |
| 22195-04 | Two-Sided Dixon-Q Standard Outlier Test for qPCR Standard Curve Data |
| 22195-05 | Quantitative PCR Data |
| 22195-06 | Preparation of Control or Standard DNA/RNA |
| 22195-07 | Primer Working Stock Logbook |
| 22972 | MagNA Pure DNA Extraction |
| 22972-01 | Global Run Settings |
| 22972-02 | Magna Pure 24 Reagent and Sample Prep |
| 22973 | Operation and Maintenance of the QuantStudio™ 6 qPCR System |
| 23113 | QIAamp DNA RNA Extraction |

| Document Number | Title |
|---|---|
| 23113-01 | Sample Preparation Equipment, Reagents, Materials |
| 23113-03 | Virus RNA Extraction |
| 23113-04 | DNase treatment of Viral RNA |
| 26101 | Labeling, Transport, Submission, Storage, and Handling of Biohazardous Materials within the BDP |

## 12. ATTACHMENTS

Attachment 1-64 Assay-Specific Appendices

**Attachment 1**

**AMPLICON: *E. coli* genomic DNA**

**AMPLICON SEQUENCE:**

CGGGCTTTCTTTTGCTATCGACCAGGCGTAGCGGCATATATTCCGGTTGCTCATGATTATCTTGATGCGC
CCGATC

**AMPLICON REGION:** *E. coli* Polymerase I (polA) gene

**AMPLICON SIZE:** 77 bp

**COPY NUMBER / GENOME:** 1

**FORWARD PRIMER: EC1107F** 5' CGGGCTTTCTTTTGCTATCG 3'

**REVERSE PRIMER: EC1183R** 5' GATCGGGCGCATCAAGATAA 3'

**PROBE: EC1134P** 5' [6-FAM] CGTAGCGGCATATATTCCGGTTGCTCAT [Quencher] 3'

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**EC1107F Forward Primer / EC1183R Reverse Primer** BDP PN 30765

**EC1134P Probe** BDP PN 30761

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**CONTROL GENOMIC DNA:** *E. coli* total genomic DNA (ATCC 10798D)

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| **Universal Mastermix (BDP 30268)** | **25 (1x)** |
| **NFW or WFI Water (various)** | **2.5** |
| **Primers** | **1 each (5 pmol each)** |
| **Probe** | **0.5 (5 pmol)** |
| **Reaction Mix Volume** | **30** |
| **Sample Volume / rxn** | **20** |
| **Total Reaction Volume** | **50** |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| **1** | **50$^0$C** | **2:00** | **N/A** |
| **2** | **95$^0$C** | **10:00** | **N/A** |
| **3** | **95$^0$C**<br>**60$^0$C** | **:15**<br>**1:00** | **≥40** |

**RESULTS ANALYSIS:**

**Threshold:** 0.2 ΔRn

**Attachment 2**

**AMPLICON: Human genomic DNA**

**AMPLICON SEQUENCE:**

TGGTAGAGACAAAGGAGACACATTTTATCCATGGACCCAAAACTCGGCGCCGGTCACGGACTGGGAAG

| | |
|---|---|
| **AMPLICON REGION:** | Human Endogenous Retrovirus HERV-H2 LTR |
| **AMPLICON SIZE:** | 68 bp |
| **COPY NUMBER/GENOME:** | ~ 862 |
| **FORWARD PRIMER: HERV 712F** | 5' TGGTAGAGACAAAGGAGACACATTTT 3' |
| **REVERSE PRIMER: HERV 712R** | 5' CTTCCCAGTCCGTGACCG 3' |
| **PROBE: HERV712P** | 5' [6~FAM] CCATGGACCCAAAACTCCGGCG [Quencher] 3' |

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**HERV712F Forward Primer / HERV712R Reverse Primer** BDP PN 30764

**HERV712P Probe** BDP PN 30763

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**CONTROL GENOMIC DNA:** Total human genomic DNA (BDP PN 31222)

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| **Universal Mastermix (BDP 30268)** | **25 (1x)** |
| **NFW or WFI Water (various)** | **2.5** |
| **Primers** | **1 each (10 pmol each)** |
| **Probe** | **0.5 (5 pmol)** |
| **Reaction Mix Volume** | **30** |
| **Sample Volume / rxn** | **20** |
| **Total Reaction Volume** | **50** |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| **1** | **50$^0$C** | **2:00** | **N/A** |
| **2** | **95$^0$C** | **10:00** | **N/A** |
| **3** | **95$^0$C**<br>**60$^0$C** | **:15**<br>**1:00** | **40** |

**RESULTS ANALYSIS:**

**Threshold:** 0.2 ΔRn

**Attachment 3**

**AMPLICON: Murine genomic DNA**

**AMPLICON SEQUENCE:**

TACAGAAGTGGATGCTCACAGTCANCTATTGGATGGGTCACACAGGGCCCCCAATGGAGGAGCTAGAGA
AAGTGCCCAAGGA

| | |
|---|---|
| **AMPLICON REGION:** | SINE–R Murine retro-transposonic repeat sequence |
| **AMPLICON SIZE:** | 81 bp |
| **COPY NUMBER/GENOME:** | ~ 0.6% of murine genome |
| **FORWARD PRIMER: SHF** | 5' TACAGAAGTGGATGCTCACAGTCA 3' |
| **REVERSE PRIMER: SHR** | 5' TCCTTGGGCACTTTCTCTAGCT 3' |
| **PROBE: NOVAP** | 5' [6~FAM] CACAGGGCCCCCAATGGAGGA [Quencher] 3' |

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

| | |
|---|---|
| **SHF Forward Primer / SHR Reverse Primer** | BDP PN 30766 |
| **NOVAP Probe** | BDP PN 30759 |
| **RECOMMENDED EXTRACTION KIT:** | QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236) |

**CONTROL GENOMIC DNA:** Total murine genomic DNA (Clontech 6650-1)

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| **Universal Mastermix (BDP 30268)** | **25 (1x)** |
| **NFW or WFI Water (various)** | **2.5** |
| **Primers** | **1 each (10 pmol each)** |
| **Probe** | **0.5 (5 pmol)** |
| **Reaction Mix Volume** | **30** |
| **Sample Volume / rxn** | **20** |
| **Total Reaction Volume** | **50** |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| **1** | **50$^0$C** | **2:00** | **N/A** |
| **2** | **95$^0$C** | **10:00** | **N/A** |
| **3** | **95$^0$C**<br>**60$^0$C** | **:15**<br>**1:00** | **40** |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 4**

**AMPLICON: Tamarin (*sagninus oedipus*) genomic DNA**

**AMPLICON SEQUENCE:**

TCCAGCAGGAAAAACCTTGGTCCCAAGTACCCCTAGGCTCATATGGCTACCTCATTCAAGAAAAGGGCC
TTATG

**AMPLICON REGION:** mspE intronic sequence specific for Tamarin

**AMPLICON SIZE:** 74 bp

**COPY NUMBER/GENOME:** 3

**FORWARD PRIMER: TAMMSP1FWD** 5'TCCAGCAGGAAAAAACCTTGGT 3'

**REVERSE PRIMER: TAMMSP1REV** 5' CATAAGGCCCTTTTCTTGAATGA 3'

**PROBE: TAMMSP1PROBE** 5' [6~FAM] CCAAGTACCCCTAGGCTCATATGGC [Quencher] 3'

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**Tammarin Forward and Reverse PCR primers** BDP PN 30906
**Tammarin PCR Probe** BDP PN 30907

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**CONTROL GENOMIC DNA:** Total Tamarin genomic DNA (liver) (BDPQC)

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| **Universal Mastermix (BDP 30268)** | **25 (1x)** |
| **NFW or WFI Water (various)** | **2.5** |
| **Primers** | **1 each (10 pmol each)** |
| **Probe** | **0.5 (5 pmol)** |
| **Reaction Mix Volume** | **30** |
| **Sample Volume / rxn** | **20** |
| **Total Reaction Volume** | **50** |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| **1** | **50$^0$C** | **2:00** | **N/A** |
| **2** | **95$^0$C** | **10:00** | **N/A** |
| **3** | **95$^0$C**<br>**60$^0$C** | **:15**<br>**1:00** | **40** |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 5**

**AMPLICON: Human genomic DNA (β-Actin)**

**AMPLICON SEQUENCE:**

tcacccacactgtgcccatctacgaggggtatgccctcccccatgccatcctgcgtctggacctggctggccgggacctgactgactacctcatgaagatc
ctcaccgagcgcggctacagcttcaccaccacggccgagcgggaaatcgtgcgtgacattaaggagaagctgtgctacgtcgccctggacttcgagca
agagatggccacggctgcttccagctcctcccctggagaagagctacgagctgcctgacggccaggtcatcaccattggcaatgagcggttccgctg

| | |
|---|---|
| **AMPLICON REGION:** | Human Beta-Actin gene (ABI commercial kit) |
| **AMPLICON SIZE:** | 294 bp |
| **COPY NUMBER/GENOME:** | 6 |
| **FORWARD PRIMER: fwd** | 5' TCACCCACACTGTGCCCATCTACGA 3' |
| **REVERSE PRIMER: rev** | 5' CAGCGGAACCGCTCATTGCCAATTG 3' |
| **PROBE: probe** | 5' [6~FAM] ATGCCC(T)CCCCCATGCCATCCTGCG [TAMRA] 3' |

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**CONTROL GENOMIC DNA:** Total human genomic DNA (Invitrogen 14410-012)

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| **Universal Mastermix (BDP 30268)** | **25 (1x)** |
| **NFW or WFI Water (various)** | **N/A** |
| **Primers** | **5 each (15 pmol each)** |
| **Probe** | **5 (10 pmol)** |
| **Reaction Mix Volume** | **40** |
| **Sample Volume / rxn** | **10** |
| **Total Reaction Volume** | **50** |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| **1** | **50$^0$C** | **2:00** | **N/A** |
| **2** | **95$^0$C** | **10:00** | **N/A** |
| **3** | **95$^0$C**<br>**60$^0$C** | **:15**<br>**1:00** | **≥ 40** |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 6**

**AMPLICON: MuLV RNA (RT-qPCR)**

**AMPLICON SEQUENCE:**

AAAGGTGTCCTAACGCAAAAACTGGGACCTTGGCGTCGGCCGGTGGCCTACCTGTCCAAAAAGCTAGAC
CCAGTAGCA

| | |
|---|---|
| **AMPLICON REGION:** | Pol gene |
| **AMPLICON SIZE:** | 77 bp |
| **COPY NUMBER/GENOME:** | 1 |
| **FORWARD PRIMER: MU3465F** | 5' AAAGGTGTCCTAACGCAAAAACTG 3' |
| **REVERSE PRIMER: MU3542R** | 5' TGCTACTGGGTCTAGCTTTTTGG 3' |
| **PROBE: MU3498P** | 5' [6~FAM] CGTCGGCCGGTGGCCTACCT [Quencher] 3' |

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN Viral RNA Mini Kit (BDP 30450)

**CONTROL GENOMIC RNA:** X-MuLV pNFS Th-1 viral standard (BDP 30378)

| Reagent | Volume/reaction (µl) |
|---|---|
| One step rtqpcr kit (30265) 2x mix | 25 (1x) |
| Water | 15.4 |
| 20x primer/probe set<br>(or each primers and probe) | 2.5<br>(1, 1, 0.5) |
| ROX reference dye | 0.1 |
| RNAse out (31244) | 1 |
| Superscript/taq mix | 1 |
| Reaction mix volume | 45 |
| Sample volume / rxn | 5 |
| Total reaction volume | 50.0 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50°C | 15:00 | N/A |
| 2 | 95°C | 2:00 | N/A |
| 3 | 95°C | 00:15 | 40 |
| | 60°C | 00:30 | |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 7**

**AMPLICON: Adenovirus E1A region "Mark II" amplicon**

**AMPLICON SEQUENCE:**

GAACCACCTACCCTTCACGAACTGTATGATTTAGACGTGACGGCCCCCGAAGATCCCAACGAGGAGGC

**AMPLICON REGION:** Ad5 E1A locus (present in HEK293FT and 293Vec RD114)

**AMPLICON SIZE:** 68 bp

**COPY NUMBER/GENOME:** 1

**FORWARD PRIMER: E1A158F** 5' GAACCACCTACCCTTCACGAACT 3'

**REVERSE PRIMER: E1A225R** 5' GCCTCCTCGTTGGGATCTTC 3'

**PROBE: E1A182P** 5' [6~FAM] TATGATTTAGACGTGACGGCCCC [Quencher] 3'

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**E1A158F Forward Primer / E1A225R Reverse Primer** BDP PN 30769

**E1A182P Probe** BDP PN 30762

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**CONTROL GENOMIC DNA:** E1A control DNA (BDP 31456)

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| **Universal Mastermix (BDP 30268)** | **25 (1x)** |
| **NFW or WFI Water (various)** | **11** |
| **Primers** | **1 (10 pmol each)** |
| **Probe** | **0.5 (5 pmol)** |
| **Reaction Mix Volume** | **37.5** |
| **Sample Volume / rxn** | **12.5** |
| **Total Reaction Volume** | **50** |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| **1** | **50$^0$C** | **2:00** | **N/A** |
| **2** | **95$^0$C** | **10:00** | **N/A** |
| **3** | **95$^0$C**<br>**60$^0$C** | **:15**<br>**1:00** | **40** |

**RESULTS ANALYSIS:**

**Threshold:** 0.2 ΔRn or Automatic

Positive DNA control (E1A control, 107 bp, BDP 31456)

5'- CCTCCTAGCCATTTTGAACCACCTACCCTTCACGAACTGTATGATTTAGACGTGACGGCCCC CGAAGATCCCAACGAGGAGGCGGTTTCGCAGATTTTTCCCGACTC -3'

**Attachment 8**

**AMPLICON: Adenovirus E1B region**

**AMPLICON SEQUENCE:**

AGGTTTACTGGCCCCAATTTTAGCGGTACGGTTTTCCTGGCCAATACCAACCTTATCCTACACGGTGTAA GCTTCTATGG

| | |
|---|---|
| **AMPLICON REGION:** | Ad5 E1B locus |
| **AMPLICON SIZE:** | 80 bp |
| **COPY NUMBER/GENOME:** | 1 |
| **FORWARD PRIMER: E1B2736F** | 5' AGGTTTACTGGCCCCAATTTTAG 3' |
| **REVERSE PRIMER: E1B2791R** | 5'CCATAGAAGCTTACACCGTGTAGGA3' |
| **PROBE: E1B2764P** | 5' [6~FAM] CGGTTTTCCTGGCCAAYACCAACCTT [Quencher] 3' |

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**CONTROL GENOMIC DNA:** Adenoviral reference material DNA

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| **Universal Mastermix (BDP 30268)** | **25 (1x)** |
| **NFW or WFI Water (various)** | **2.5** |
| **Primers** | **1 each (10 pmol each)** |
| **Probe** | **0.5 (5 pmol)** |
| **Reaction Mix Volume** | **30** |
| **Sample Volume / rxn** | **20** |
| **Total Reaction Volume** | **50** |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| **1** | **50$^0$C** | **2:00** | **N/A** |
| **2** | **95$^0$C** | **10:00** | **N/A** |
| **3** | **95$^0$C**<br>**60$^0$C** | **:15**<br>**1:00** | **40** |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 9**

**AMPLICON: Adenovirus Hexon region**

**AMPLICON SEQUENCE:**

CCTACTCTGGCACTGCCTACAACGCCCTGGCTCCCAAGGGTGCCCCAAATCCTTGCGAATGGGATG

**AMPLICON REGION:** Ad5 Hexon gene

**AMPLICON SIZE:** 65 bp

**COPY NUMBER/GENOME:** 1

**FORWARD PRIMER: HEX568F** 5' CCTACTCTGGCACTGCCTACAA 3'

**REVERSE PRIMER: HEX633R** 5' CATCCCATTCGCAAGGATTT 3'

**PROBE: HEX593P** 5' [6~FAM] CCTGGCTCCCAAGGGTGCCC [Quencher] 3'

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**Hex568F Forward Primer / Hex633R Reverse Primer** BDP PN 30767

**Hex593P Probe** BDP PN 30757

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**CONTROL GENOMIC DNA:** Ad5 Hexon DNA control BDP PN 31420

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| **Universal Mastermix (BDP 30268)** | **25 (1x)** |
| **NFW or WFI Water (various)** | **2.5** |
| **Primers** | **1 each (10 pmol each)** |
| **Probe** | **0.5 (5 pmol)** |
| **Reaction Mix Volume** | **30** |
| **Sample Volume / rxn** | **20** |
| **Total Reaction Volume** | **50** |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| **1** | **50$^0$C** | **2:00** | **N/A** |
| **2** | **95$^0$C** | **10:00** | **N/A** |
| **3** | **95$^0$C**<br>**60$^0$C** | **:15**<br>**1:00** | **40** |

**RESULTS ANALYSIS:**

**Threshold:** 0.2 ΔRn

Positive control (Ad5 Hexon DNA, 110bp, BDP 31420)

5'- GGGGCCCTACTTTTAAGCCCTACTCTGGCACTGCCTACAACGCCCTGGCTCCCAAGGGTGC CCCAAATCCTTGCGAATGGGATGAAGCTGCTACTGCTCTTGAAATAAAC -3'

**Attachment 10**

**AMPLICON: HEK293 Adenovirus E1a Left Hand Junction**

**AMPLICON SEQUENCE:**

CACCATAACAACAGCTACTCAAACTGTAAACCAGGATAACAAGTTGATGACTT*GTC*CATCATCAATAATATACCTTATTTTGGATTGAAGCCAA

**AMPLICON REGION:** Left Hand Junction of HEK293-Ad5 E1 genomic DNA

**AMPLICON SIZE:** 109 bp

**13. COPY NUMBER/GENOME:** 1

**FORWARD PRIMER: LHF** 5' CACCATAACAACAGCTACTCAAACTG 3'

**REVERSE PRIMER: LHR** 5' TTGGCTTCAATCCAAAATAAGGTAT 3'

**PROBE: LHP** 5' [6~FAM] AACCAGGATAACAAGTTGATGACTTGTCCATCA 3' [Quencher] 3'

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**CONTROL GENOMIC DNA:** HEK293 genomic DNA (ATCC CRL-1573)

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| **Universal Mastermix (BDP 30268)** | **25 (1x)** |
| **NFW or WFI Water (various)** | **2.5** |
| **Primers** | **1 each (10 pmol each)** |
| **Probe** | **0.5 (5 pmol)** |
| **Reaction Mix Volume** | **30** |
| **Sample Volume / rxn** | **20** |
| **Total Reaction Volume** | **50** |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| **1** | **50$^0$C** | **2:00** | **N/A** |
| **2** | **95$^0$C** | **10:00** | **N/A** |
| **3** | **95$^0$C**<br>**60$^0$C** | **:15**<br>**1:00** | **40** |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 11**

**AMPLICON: HEK293 Adenovirus E1a Right Hand Junction**

**AMPLICON SEQUENCE**:

TGGTGTTGTAGATGATCCAGTCGTAGCAGGAGCGCTGGGCGTGGTGCCTAAAAATGTCTTTCAGTAGCA
AGCTGATTGCCAAAGATGTCAGAACAAGACTCCCCATCATGATAAGGCTCCCACCCCTCTTAACTGTCCT
TGCTCATGCCTG

**AMPLICON REGION:** Right Hand Junction of HEK293-Ad5 E1 genomic DNA

**AMPLICON SIZE:** 151 bp

**COPY NUMBER/GENOME:** 1

**FORWARD PRIMER: RHF** 5' TGGTGTTGTAGATGATCCAGTCGTA 3'

**REVERSE PRIMER: RHR** 5' CAGGCATGAGCAAGGACAGTT 3'

**PROBE: RHP** 5' [6~FAM] TGATTGCCAAAGATGTCAGAACAAGACTCC 3' [Quencher] 3'

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**CONTROL GENOMIC DNA:** HEK293 genomic DNA (ATCC CRL-1573)

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| **Universal Mastermix (BDP 30268)** | **25 (1x)** |
| **NFW or WFI Water (various)** | **2.5** |
| **Primers** | **1 each (10 pmol each)** |
| **Probe** | **0.5 (5 pmol)** |
| **Reaction Mix Volume** | **30** |
| **Sample Volume / rxn** | **20** |
| **Total Reaction Volume** | **50** |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| **1** | **50$^0$C** | **2:00** | **N/A** |
| **2** | **95$^0$C** | **10:00** | **N/A** |
| **3** | **95$^0$C**<br>**60$^0$C** | **:15**<br>**1:00** | **40** |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 12**

**AMPLICON: Adenovirus E1a Δ24 region (for detection of wild-type Ad5 E1a)**

**AMPLICON SEQUENCE:**

GTCCGGTTTCTATGCCAAACCTTGTACCGGAGGTGATCGAT*CTTACCTGCCAGGAGGCTGGCTTT*CCAC CCAGTGACGACGA

| | |
|---|---|
| **AMPLICON REGION:** | Ad5 E1a Δ24 region for detection of wild-type E1a sequence Refer to SOP 22172 |
| **AMPLICON SIZE:** | 79 bp |
| **COPY NUMBER/GENOME:** | 1 |
| **FORWARD PRIMER: RGDF** | 5' CGGTTTCTATGCCAAACCTTGT 3' |
| **REVERSE PRIMER: RGDR** | 5' TCGTCGTCACTGGGTGGAA 3' |
| **PROBE: RDGP** | 5' [6~FAM] ATCGATCTTACCTGCCAGGAGGCTG [Quencher] 3' |

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**CONTROL GENOMIC DNA:** Adenoviral reference material DNA

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| **Universal Mastermix (BDP 30268)** | **25 (1x)** |
| **NFW or WFI Water (various)** | **0.5** |
| **Primers** | **2 each (20 pmol each)** |
| **Probe** | **0.5 (5 pmol)** |
| **Reaction Mix Volume** | **30** |
| **Sample Volume / rxn** | **20** |
| **Total Reaction Volume** | **50** |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| **1** | **50$^0$C** | **2:00** | **N/A** |
| **2** | **95$^0$C** | **10:00** | **N/A** |
| **3** | **95$^0$C**<br>**60$^0$C** | **:15**<br>**1:00** | **40** |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 13**

**AMPLICON: Lambda phage DNA**

**AMPLICON SEQUENCE:**

GGTAAACATGGCGCTGTACGTTTCGCCGATTGTTTCCGGTGAGGTTATCCGTTCCCGTGGCG

| | |
|---|---|
| **AMPLICON REGION:** | λ capsid E gene |
| **AMPLICON SIZE:** | 60 bp |
| **COPY NUMBER/GENOME:** | 1 |
| **FORWARD PRIMER: LAMF** | 5' GGTAAACATGGCGCTGTACGT 3' |
| **REVERSE PRIMER: LAMR** | 5' CGCCACGGGAACGGATA 3' |
| **PROBE: LAMP** | 5' [6~FAM] TCGCCGATTGTTTCCGGTGAGG [Quencher] 3' |

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**CONTROL GENOMIC DNA:** lambda phage DNA [clind1ts857 Sam7]

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| **Universal Mastermix (BDP 30268)** | **25 (1x)** |
| **NFW or WFI Water (various)** | **2.5** |
| **Primers** | **1 each (10 pmol each)** |
| **Probe** | **0.5 (5 pmol)** |
| **Reaction Mix Volume** | **30** |
| **Sample Volume / rxn** | **20** |
| **Total Reaction Volume** | **50** |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| **1** | **50$^0$C** | **2:00** | **N/A** |
| **2** | **95$^0$C** | **10:00** | **N/A** |
| **3** | **95$^0$C**<br>**60$^0$C** | **:15**<br>**1:00** | **40** |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 14**

**AMPLICON: Polio wild type (Sabin-1 strain) virus**

**AMPLICON SEQUENCE:**

CGTGGCGGAACCGACTACTTTGGG*TGTCCGTGTTTCCTTTTATTTT*ATTGTGGCTGCTTATGGTGACA

| | |
|---|---|
| **AMPLICON REGION:** | 5' – UTR/IRES region Sabin-1 strain polio |
| **AMPLICON SIZE:** | 68 bp |
| **COPY NUMBER/GENOME:** | 1 |
| **FORWARD PRIMER: PVS2F** | 5' CGTGGCGGAACCGACTAC 3' |
| **REVERSE PRIMER: PVS2R** | 5' TGTCACCATAAGCAGCCACAA 3' |
| **PROBE: PVS1P** | 5' [6~FAM] TGTCCGTGTTTCCTTTTATTTT [Quencher] 3' |

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN Viral RNA Mini Kit (BDP 30450)
**CONTROL GENOMIC RNA:** Sabin strain 1 wild-type polio virus

| Reagent | Volume/reaction (µl) |
|---|---|
| One step RTqPCR kit (30265) 2x mix | 25 (1x) |
| Water | 15.4 |
| 20x primer/probe set (or each primers and probe) | 2.5 (1, 1, 0.5) |
| ROX reference dye | 0.1 |
| RNAse out (31244) | 1 |
| Superscript/taq mix | 1 |
| Reaction mix volume | 45 |
| Sample volume / rxn | 5 |
| Total reaction volume | 50.0 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50°C | 15:00 | N/A |
| 2 | 95°C | 2:00 | N/A |
| 3 | 95°C | 00:15 | 40 |
| | 60°C | 00:30 | |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 15**

**AMPLICON: PVS-RIPO Plasmid and virus (HRV-2 IRES)**

**AMPLICON SEQUENCE:**

AACCCAATGTGTATCTAGTCGTAATGAG*CAATTGCGGGATGGGACCAACT*ACTTTGGGTGTCCGTGTTTCA

| | |
|---|---|
| **AMPLICON REGION:** | PVS-RIPO HRV-2 IRES sequence |
| **AMPLICON SIZE:** | 71 bp |
| **COPY NUMBER/GENOME:** | 1 |
| **FORWARD PRIMER: PVS1F** | 5' AACCCAATGTGTATCTAGTCGTAATGA 3' |
| **REVERSE PRIMER: PVS1R** | 5' TGAAACACGGACACCCAAAG 3' |
| **PROBE: PVS2P** | 5' [6~FAM] CAATTGCGGGATGGGACCAACT [Quencher] 3' |

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN Viral RNA Mini Kit (BDP 30450)

**CONTROL GENOMIC DNA:** PVS-RIPO plasmid (BDP L0305006)

| Reagent | Volume/reaction (µl) |
|---|---|
| One step RTqPCR kit (30265) 2x mix | 25 (1x) |
| Water | 15.4 |
| 20x primer/probe set (or each primers and probe) | 2.5 (1, 1, 0.5) |
| ROX reference dye | 0.1 |
| RNAse out (31244) | 1 |
| Superscript/taq mix | 1 |
| Reaction mix volume | 45 |
| Sample volume / rxn | 5 |
| Total reaction volume | 50.0 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50°C | 15:00 | N/A |
| 2 | 95°C | 2:00 | N/A |
| 3 | 95°C | 00:15 | 40 |
| | 60°C | 00:30 | |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 16**

**AMPLICON: Wolinella Asparaginase AsnA**

**AMPLICON SEQUENCE:**

CACCCCGATGATACTGATGTTTTAGTCAATGCAGCCCTTCAGGCAGGAGCCAAAGGAATCATCCATGCA
G

**AMPLICON REGION:** Wolinella Asparaginase gene [AsnA] in plasmid rWS-Asp pET28a

**AMPLICON SIZE:** 70 bp

**COPY NUMBER/GENOME:** 1

**FORWARD PRIMER: WOLF** 5' CAC CCC GAT GAT ACT GAT GTT TT 3'

**REVERSE PRIMER: WOLR** 5' CTG CAT GGA TGA TTC CTT TGG 3'

**PROBE: WOLPR** 5' [6~FAM] TCA ATG CAG CCC TTC AGG CAG GA [Quencher] 3'

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**CONTROL GENOMIC DNA:** rWS-Asp/pET28a plasmid L0401018

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| Universal Mastermix (BDP 30268) | 25 (1x) |
| NFW or WFI Water (various) | 2.5 |
| Primers | 1 each (10 pmol each) |
| Probe | 0.5 (5 pmol) |
| Reaction Mix Volume | 30 |
| Sample Volume / rxn | 20 |
| Total Reaction Volume | 50 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | $50^0$C | 2:00 | N/A |
| 2 | $95^0$C | 10:00 | N/A |
| 3 | $95^0$C<br>$60^0$C | :15<br>1:00 | 40 |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 17**

**AMPLICON: *E. coli* Asparaginase gene AsnB**

**AMPLICON SEQUENCE:**

TACCCCGGC ACGTAAGCAT ACCAGCGACA CGCCATTCGA TGTCTCTAAG CTGAATGAAC TGCCGA

**AMPLICON REGION:** E. coli asparaginase AsnB gene (*E. coli* gene most homologous to Wolinella AsnA)

**AMPLICON SIZE:** 65 bp

**COPY NUMBER/GENOME:** 1

**FORWARD PRIMER: ECBF** 5' TAC CCC GGC ACG TAA GCA T 3'

**REVERSE PRIMER: ECBR** 5' - TCG GCA GTT CAT TCA GCT TAG A 3'

**PROBE: ECBPR** 5' [6~FAM] CCA GCG ACA CGC CAT TCG ATG T [Quencher] 3'

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**CONTROL GENOMIC DNA:** *E. coli* total genomic DNA (ATCC 10798D)

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| Universal Mastermix (BDP 30268) | 25 (1x) |
| NFW or WFI Water (various) | 2.5 |
| Primers | 1 each (10 pmol each) |
| Probe | 0.5 (5 pmol) |
| Reaction Mix Volume | 30 |
| Sample Volume / rxn | 20 |
| Total Reaction Volume | 50 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | $50^0$C | 2:00 | N/A |
| 2 | $95^0$C | 10:00 | N/A |
| 3 | $95^0$C<br>$60^0$C | :15<br>1:00 | 40 |

**RESULTS ANALYSIS:**

**Threshold:** 0.2 ΔRn

**Attachment 18**

**AMPLICON: *E. coli* asparaginase gene AsnA**

**AMPLICON SEQUENCE:**

TGACGTCGTGCGCAATTTTCTGCGCCAACCGGTGAAAGCATTGATTCTGCGCTCCTATGGC

| | |
|---|---|
| **AMPLICON REGION:** | *E. coli* asparaginase gene AsnA (low homology to Wol AsnA) |
| **AMPLICON SIZE:** | 61 bp |
| **COPY NUMBER/GENOME:** | 1 |
| **FORWARD PRIMER: ECAF** | 5' TGA CGT CGT GCG CAA TTT T 3' |
| **REVERSE PRIMER: ECAR** | 5' GCC ATA GGA GCG CAG AAT CA 3' |
| **PROBE: ECAPR** | 5' [6~FAM] TGC GCC AAC CGG TGA AAG CA [Quencher] 3' |

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

| | |
|---|---|
| **RECOMMENDED EXTRACTION KIT:** | QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236) |
| **CONTROL GENOMIC DNA:** | *E. coli* total genomic DNA (ATCC 10798D) |

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| **Universal Mastermix (BDP 30268)** | **25 (1x)** |
| **NFW or WFI Water (various)** | **2.5** |
| **Primers** | **1 each (10 pmol each)** |
| **Probe** | **0.5 (5 pmol)** |
| **Reaction Mix Volume** | **30** |
| **Sample Volume / rxn** | **20** |
| **Total Reaction Volume** | **50** |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| **1** | **50$^0$C** | **2:00** | **N/A** |
| **2** | **95$^0$C** | **10:00** | **N/A** |
| **3** | **95$^0$C**<br>**60$^0$C** | **:15**<br>**1:00** | **40** |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 19**

**AMPLICON: *E. coli* putative asparaginase [ybiK] gene**

**AMPLICON SEQUENCE:**
TGGATTACGGCGGATTAAGTCTCGCGGAAGCCTGCGAGCGGGTAGTAATGGAAAAACTCCCTGCG

| | |
|---|---|
| **AMPLICON REGION:** | *E. coli* putative asparaginase [ybiK] gene (low homology to Wol AsnA) |
| **AMPLICON SIZE:** | 65 bp |
| **COPY NUMBER/GENOME:** | 1 |
| **FORWARD PRIMER: YBIF** | 5' TGG ATT ACG GCG GAT TAA GTC T 3' |
| **REVERSE PRIMER: YBIR** | 5' CGC AGG GAG TTT TTC CAT TAC T 3' |
| **PROBE: YBIPR** | 5' [6~FAM] CGG AAG CCT GCG AGC GGG [Quencher] 3' |

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**CONTROL GENOMIC DNA:** *E. coli* total genomic DNA (ATCC 10798D)

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| **Universal Mastermix (BDP 30268)** | **25 (1x)** |
| **NFW or WFI Water (various)** | **2.5** |
| **Primers** | **1 each (10 pmol each)** |
| **Probe** | **0.5 (5 pmol)** |
| **Reaction Mix Volume** | **30** |
| **Sample Volume / rxn** | **20** |
| **Total Reaction Volume** | **50** |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| **1** | **50$^0$C** | **2:00** | **N/A** |
| **2** | **95$^0$C** | **10:00** | **N/A** |
| **3** | **95$^0$C**<br>**60$^0$C** | **:15**<br>**1:00** | **40** |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 20**

**AMPLICON: Herpes Simplex 1 (HSV-1) Major Capsid gene**

**AMPLICON SEQUENCE:**

AACAGC ACGTTCTCAG TCACAAAGCG GTCCTGTCGG ACGACGGTGA ACCCAAACCC GGGATGGAGG CCCGTCTTGA GCTGATGATG CAAGG

| | |
|---|---|
| **AMPLICON REGION:** | Universal HSV-1 major capsid gene UL36 |
| **AMPLICON SIZE:** | 91 bp |
| **COPY NUMBER/GENOME:** | 1 |
| **FORWARD PRIMER: HSVCAPF** | 5' AACAGCACGTTCTCAGTCACAAA 3' |
| **REVERSE PRIMER: HSVCAPR** | 5' CCTTGC ATCATCAGCTCAAGAC 3' |
| **PROBE: HSVCAPPR** | 5' [6~FAM] ACCCAAACCCGGGATGGAGGC [Quencher] 3' |

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

| | |
|---|---|
| **HSV CAP forward and reverse primers** | BDP PN 30944 |
| **HSV CAP probe** | BDP PN 30945 |
| **RECOMMENDED EXTRACTION KIT:** | QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236) |
| **CONTROL GENOMIC DNA:** | rHSV-1 DNA reference material ARR (BDP 02012004C |

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| **Universal Mastermix (BDP 30268)** | **25 (1x)** |
| **NFW or WFI Water (various)** | **2.5** |
| **Primers** | **1 each (10 pmol each)** |
| **Probe** | **0.5 (5 pmol)** |
| **Reaction Mix Volume** | **30** |
| **Sample Volume / rxn** | **20** |
| **Total Reaction Volume** | **50** |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| **1** | **50$^0$C** | **2:00** | **N/A** |
| **2** | **95$^0$C** | **10:00** | **N/A** |
| **3** | **95$^0$C**<br>**60$^0$C** | **:15**<br>**1:00** | **40** |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 21**

**AMPLICON: HSV-1 Ribonucleotide reductase gene (UL39 ICP6)**

**AMPLICON SEQUENCE:**

GCCTGTGTCTGGACGTTCCTCCGGTCCCGCCGAACGCATACATGCCCTATTATCTCAGGGAGTATGTGACG

| | |
|---|---|
| **AMPLICON REGION:** | HSV-1 RR large subunit gene UL39<br>Amplicon is not present in rRp450-HSV-1 |
| **AMPLICON SIZE:** | 71 bp |
| **COPY NUMBER/GENOME:** | 1 |
| **FORWARD PRIMER: HSVRRF** | 5' GCCTGTGTCTGGACGTTCCT 3' |
| **REVERSE PRIMER: HSVRRR** | 5' CGTCACATACTCCCTGAGATAATAGG 3' |
| **PROBE: HSVRRP** | 5' [6~FAM] CGGTCCCGCCGAACGCAT [Quencher] 3' |

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**CONTROL GENOMIC DNA:** wild-type HSV-1 DNA extract (ABi 08-705-000)

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| Universal Mastermix (BDP 30268) | 25 (1x) |
| NFW or WFI Water (various) | 2.5 |
| Primers | 1 each (10 pmol each) |
| Probe | 0.5 (5 pmol) |
| Reaction Mix Volume | 30 |
| Sample Volume / rxn | 20 |
| Total Reaction Volume | 50 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | $50^0$C | 2:00 | N/A |
| 2 | $95^0$C | 10:00 | N/A |
| 3 | $95^0$C<br>$60^0$C | :15<br>1:00 | 40 |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 22**

**AMPLICON: Acholeplasma 16S Ribosomal DNA**

**AMPLICON SEQUENCE:**

AACAAA GGGCACACAG TGGATGCCTT GGCACCAAGA GGCGATGAAG GACGGAACTA ACACCGAAAT GCTCGGG

| | |
|---|---|
| **AMPLICON REGION:** | Mollicute *Acholeplasma laidlawii* 16S rDNA |
| **AMPLICON SIZE:** | 73 bp |
| **COPY NUMBER/GENOME:** | 1 |
| **FORWARD PRIMER: ARF** | 5' AAC AAA GGG CAC ACA GTG GAT **3'** |
| **REVERSE PRIMER: ARR** | 5' CCC GAG CAT TTC GGT GTT AG 3' |
| **PROBE: ARPR** | 5' [6~FAM] CAC CAA GAG GCG ATG AAG GAC GGA [Quencher] 3' |

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**CONTROL GENOMIC DNA:** *A. laidlawii* gDNA (ATCC 23206D)

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| Universal Mastermix (BDP 30268) | 25 (1x) |
| NFW or WFI Water (various) | 2.5 |
| Primers | 1 each (10 pmol each) |
| Probe | 0.5 (5 pmol) |
| Reaction Mix Volume | 30 |
| Sample Volume / rxn | 20 |
| Total Reaction Volume | 50 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | $50^0$C | 2:00 | N/A |
| 2 | $95^0$C | 10:00 | N/A |
| 3 | $95^0$C<br>$60^0$C | :15<br>1:00 | 40 |

**RESULTS ANALYSIS:**
**Threshold:** Variable ΔRn

**Attachment 23**

**AMPLICON: Tn903 Kanamycin resistance gene [AphA]**

**AMPLICON SEQUENCE:**

GATGTTGGACGAGTCGGAATCGCAGACCGATACCAGGATCTTGCCATCCTATGGAACTGCCTCGGTG

| | |
|---|---|
| **AMPLICON REGION:** | Tn903 derived AphA kanamycin resistance gene Present in most KanR plasmids |
| **AMPLICON SIZE:** | 67 bp |
| **COPY NUMBER/GENOME:** | 1 |
| **FORWARD PRIMER: KAN689F** | 5' GATGTTGGACGAGTCGGAATC 3' |
| **REVERSE PRIMER: KAN755R** | 5' CACCGAGGCAGTTCCATAGG 3' |
| **PROBE: KAN711P** | 5' [6~FAM or VIC] CAGACCGATACCAGGATCTTGCCA [Quencher] 3' |

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

| | |
|---|---|
| **Kanamycin TN903 dPCR Primers/probes (FAM) set** | BDP PN 31227 |
| **Kanamycin TN903 dPCR Primer/probe set (VIC/HEX)** | BDP PN 31232 |

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**CONTROL GENOMIC DNA:** plasmid pNGVL4a-CRT/E7(detox) L0506020

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| **Universal Mastermix (BDP 30268)** | **25 (1x)** |
| **NFW or WFI Water (various)** | **2.5** |
| **Primers** | **1 each (10 pmol each)** |
| **Probe** | **0.5 (5 pmol)** |
| **Reaction Mix Volume** | **30** |
| **Sample Volume / rxn** | **20** |
| **Total Reaction Volume** | **50** |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| **1** | **50$^0$C** | **2:00** | **N/A** |
| **2** | **95$^0$C** | **10:00** | **N/A** |
| **3** | **95$^0$C**<br>**60$^0$C** | **:15**<br>**1:00** | **40** |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 24**

**AMPLICON: Chloramphenicol resistance gene [CAT]**

**AMPLICON SEQUENCE:**

AACGGCATGATGAACCTGAATCGCCAGCGGCATCAGCACCTTGTCGCCTTGCGTATAATATTTGCCCAT
GGTGAA

| | |
|---|---|
| **AMPLICON REGION:** | [CAT] chloramphenicol actyltransferase gene (CamR) |
| **AMPLICON SIZE:** | 75 bp |
| **COPY NUMBER/GENOME:** | 1 |
| **FORWARD PRIMER: CAMP147F** | 5' AACGGCATGATGAACCTGAATC 3' |
| **REVERSE PRIMER: CAMP221R** | 5' TTCACCATGGGCAAATATTATACG 3' |
| **PROBE: CAMP174P** | 5' [6~FAM] CGGCATCAGCACCTTGTCGCC [Quencher] 3' |

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**CONTROL GENOMIC DNA:** HA22 (plasmid pRB698 VL) lot L0212018

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| **Universal Mastermix (BDP 30268)** | **25 (1x)** |
| **NFW or WFI Water (various)** | **2.5** |
| **Primers** | **1 each (10 pmol each)** |
| **Probe** | **0.5 (5 pmol)** |
| **Reaction Mix Volume** | **30** |
| **Sample Volume / rxn** | **20** |
| **Total Reaction Volume** | **50** |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| **1** | **50$^0$C** | **2:00** | **N/A** |
| **2** | **95$^0$C** | **10:00** | **N/A** |
| **3** | **95$^0$C**<br>**60$^0$C** | **:15**<br>**1:00** | **40** |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 25**

**AMPLICON: Tn5 Neomycin/Kanamycin resistance gene**

**AMPLICON SEQUENCE:**
TGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACA

| | |
|---|---|
| **AMPLICON REGION:** | Neomycin / Kanamycin resistance gene from Tn5 |
| **AMPLICON SIZE:** | 62 bp |
| **COPY NUMBER/GENOME:** | 1 |
| **FORWARD PRIMER: NEOF** | 5' TGGATTGCACGCAGGTTCT 3' |
| **REVERSE PRIMER: NEOR** | 5' GTGCCCAGTCATAGCCGAAT 3' |
| **PROBE: NEOPR** | 5' [6~FAM] CGGCCGCTTGGGTGGAGAGG [Quencher] 3' |

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

| | |
|---|---|
| **NEOF Primer** | BDP PN 31154 |
| **NEOR Primer** | BDP PN 31155 |
| **NEOPR Probe** | BDP PN 31156 |

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**CONTROL GENOMIC DNA:** plasmid phGM-CSF L0312004

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| **Universal Mastermix (BDP 30268)** | **25 (1x)** |
| **NFW or WFI Water (various)** | **2.5** |
| **Primers** | **1 each (10 pmol each)** |
| **Probe** | **0.5 (5 pmol)** |
| **Reaction Mix Volume** | **30** |
| **Sample Volume / rxn** | **20** |
| **Total Reaction Volume** | **50** |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| **1** | **50$^0$C** | **2:00** | **N/A** |
| **2** | **95$^0$C** | **10:00** | **N/A** |
| **3** | **95$^0$C**<br>**60$^0$C** | **:15**<br>**1:00** | **40** |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 26**

**AMPLICON: VERO (and CV-1) cell line genomic DNA**

**AMPLICON SEQUENCE:**

cctctgcc cagcgtgaag atcacccagg tcacgtggca gaagatcacc caagccacca atggctccaa gcagaacgtg gccatctaca acccatccat gggcgtgtct gtg

**AMPLICON REGION:** *Cercopithecus aethiops* Nectin-1 gene

**AMPLICON SIZE:** 111 bp

**COPY NUMBER/GENOME:** 1

**FORWARD PRIMER: NECF-1** 5' CCTCTGCCCAGCGTGAAG 3'

**REVERSE PRIMER: NECR-1** 5' CACAGACACGCCCATGGAT 3'

**PROBE: NECPR-1** 5' [6~FAM] CACCCAAGCCACCAATGGCTCCAA [Quencher] 3'

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**NECF Forward Primer / NECR Reverse Primer** BDP PN 30770

**NECPr Probe** BDP PN 30760

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**CONTROL GENOMIC DNA:** CV-1 genomic DNA (WCB BDP 10205, EOP 10377)

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| **Universal Mastermix (BDP 30268)** | **25 (1x)** |
| **NFW or WFI Water (various)** | **2.5** |
| **Primers** | **1 each (10 pmol each)** |
| **Probe** | **0.5 (5 pmol)** |
| **Reaction Mix Volume** | **30** |
| **Sample Volume / rxn** | **20** |
| **Total Reaction Volume** | **50** |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| **1** | **50$^0$C** | **2:00** | **N/A** |
| **2** | **95$^0$C** | **10:00** | **N/A** |
| **3** | **95$^0$C**<br>**60$^0$C** | **:15**<br>**1:00** | **40** |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 27**

**AMPLICON**: Universal Polio virus – polyprotein 3C gene

**AMPLICON SEQUENCE:**

TTGGTGGGAACGGTTCACACGGGTTTGCAGCGGCCCTGAAGCGATCATACTTCACTCAGAGTCAAGGTG
A

**AMPLICON REGION:** Polio polyprotein 3C region in Sabin-1 strain and PVR-RIPO virus

**AMPLICON SIZE:** 70 bp

**COPY NUMBER/GENOME:** 1

**FORWARD PRIMER: PO1F** 5' TTG GTG GGA ACG GTT CAC A 3'

**REVERSE PRIMER: PO1R** 5' TCA CCT TGA CTC TGA GTG AAG TAT GA 3'

**PROBE: PO1PR** 5' [6~FAM] TTG CAG CGG CCC TGA AGC G [Quencher] 3'

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**Polio PPP primers PO1F and PO1R** BDP PN 30947

**Polio PPP PO1 Probe** BDP PN 30948

**RECOMMENDED EXTRACTION KIT:** QIAGEN Viral RNA Mini Kit (BDP 30450)

**CONTROL GENOMIC RNA:** Sabin strain 1 wild-type polio virus WHO std.

| Reagent | Volume/reaction (µl) |
|---|---|
| One step RTqPCR kit (30265) 2x mix | 25 (1x) |
| Water | 15.4 |
| 20x primer/probe set<br>(or each primers and probe) | 2.5<br>(1, 1, 0.5) |
| ROX reference dye | 0.1 |
| RNAse out (31244) | 1 |
| Superscript/taq mix | 1 |
| Reaction mix volume | 45 |
| Sample volume / rxn | 5 |
| Total reaction volume | 50.0 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50°C | 15:00 | N/A |
| 2 | 95°C | 2:00 | N/A |
| 3 | 95°C | 00:15 | 40 |
| | 60°C | 00:30 | |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 28**

**AMPLICON: Polio Type 1 and Type 2 specific IRES (Sabin and Mahoney)**

**AMPLICON SEQUENCE:**

TTGGCGG CCTACCTATG GCTAACGCCA TGGGACGCTA GTTGTGAACA AGGTGTGAAGAGCCTATTGA GCTACATAAG AATCCTCCGG CCCCTGAATG CGGCTAATCC CA

**AMPLICON REGION:** Polio type 1 and type 2 IRES, will not amplify PVS-RIPO

**AMPLICON SIZE:** 109 bp

**COPY NUMBER/GENOME:** 1

**FORWARD PRIMER: POSAF** 5' TTG GCG GCC TAC CTA TGG 3'

**REVERSE PRIMER: POSAR** 5' TGG GAT TAG CCG CAT TCA G 3'

**PROBE: POSAPR** 5' [6~FAM] AGC CTA TTG AGC TAC ATA AGA ATC CTC CGG C [Quencher] 3'

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN Viral RNA Mini Kit (BDP 30450)

**CONTROL GENOMIC RNA:** Sabin strain 1 wild-type polio virus WHO std.

| REAGENT | VOLUME/REACTION (ML) |
|---|---|
| ONE STEP RTQPCR KIT (30265) 2X MIX | 25 (1X) |
| WATER | 15.4 |
| 20X PRIMER/PROBE SET (OR EACH PRIMERS AND PROBE) | 2.5 (1, 1, 0.5) |
| ROX REFERENCE DYE | 0.1 |
| RNASE OUT (31244) | 1 |
| SUPERSCRIPT/TAQ MIX | 1 |
| REACTION MIX VOLUME | 45 |
| SAMPLE VOLUME / RXN | 5 |
| TOTAL REACTION VOLUME | 50.0 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50°C | 15:00 | N/A |
| 2 | 95°C | 2:00 | N/A |
| 3 | 95°C | 00:15 | 40 |
| | 60°C | 00:30 | |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 29**

**AMPLICON: CHO cell line genomic DNA - DHFR gene**

**AMPLICON SEQUENCE:**

CCGGTGAA GTGAGTGGAG AAAGGGGATA CGAAGACAGC ATCCCACATG ACTGCTCCCA GTAAAGGCAA GGTCTTCATC CAT

**AMPLICON REGION:** Chinese Hamster Ovary cell line K1 DHFR (MetR)

**AMPLICON SIZE:** 81 bp

**COPY NUMBER/GENOME:** 1 (for wild-type Chinese Hamster and non-K1 cell lines),
> 1000 copies (for K1 derived cell lines such as C400)

**FORWARD PRIMER: CHO5243F** 5' CCGGTGAAGTGAGTGGAGAAA 3'

**REVERSE PRIMER: CHO5300R** 5' ATGGATGAAGACCTTGCCTTTACT 3'

**PROBE: CHO5264P** 5' [6~FAM] TACGAAGACAGCATCCCACATGACTGCT [Quencher] 3

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**CHO5243F Forward Primer** **BDP PN 31276**

**CHO5300R Reverse Primer** **BDP PN 31277**

**CHO5264P Probe** **BDP PN 31278**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**CONTROL GENOMIC DNA:** CHO-CC49(dhfr-) – one copy/haploid genome (BDP 012203)

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| Universal Mastermix (BDP 30268) | 25 (1x) |
| NFW or WFI Water (various) | 2.5 |
| Primers | 1 each (10 pmol each) |
| Probe | 0.5 (5 pmol) |
| Reaction Mix Volume | 30 |
| Sample Volume / rxn | 20 |
| Total Reaction Volume | 50 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | $50^0$C | 2:00 | N/A |
| 2 | $95^0$C | 10:00 | N/A |
| 3 | $95^0$C<br>$60^0$C | :15<br>1:00 | 40 |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 30**

**AMPLICON: CHO cell line genomic DNA–Endogenous type-C retrovirus sequence**

**AMPLICON SEQUENCE:**

CCCG GACCCCTGAG TCACCGGACT GCATGGGTAC AGGGAGCTAC AGGCGGAAAG CAGTACCATT GGACTACAAA TCGGCAGCTC CAGCTCGCGA CTGGT

| | |
|---|---|
| **AMPLICON REGION:** | CHO cell type-C proviral (endogenous retroviral) pol-region |
| **AMPLICON SIZE:** | 98 bp |
| **COPY NUMBER/GENOME:** | ~ 100 – 300 copies |
| **FORWARD PRIMER: CHF** | 5' CCC GGA CCC CTG AGT CAC 3' |
| **REVERSE PRIMER: CHR** | 5' ACC AGT CGC GAG CTG GAG 3' |
| **PROBE: CHPR** | 5' [6~FAM] TGG GTA CAG GGA GCT ACA GGC GGA AA [Quencher] 3' |

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

| | |
|---|---|
| **CHF Forward Primer / CHR Reverse Primer** | BDP PN 30768 |
| **CHPr Probe** | BDP PN 30758 |
| **RECOMMENDED EXTRACTION KIT:** | QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)For proviral RNA use Viral Mini Kit (30450) |

**CONTROL GENOMIC DNA:** CHO-CC49(dhfr-) – one copy/haploid genome (BDP 012203)

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| **Universal Mastermix (BDP 30268)** | **25 (1x)** |
| **NFW or WFI Water (various)** | **2.5** |
| **Primers** | **1 each (10 pmol each)** |
| **Probe** | **0.5 (5 pmol)** |
| **Reaction Mix Volume** | **30** |
| **Sample Volume / rxn** | **20** |
| **Total Reaction Volume** | **50** |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| **1** | **50$^0$C** | **2:00** | **N/A** |
| **2** | **95$^0$C** | **10:00** | **N/A** |
| **3** | **95$^0$C**<br>**60$^0$C** | **:15**<br>**1:00** | **40** |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 31**

**AMPLICON: PER.C6 cell line genomic DNA HDEP Junction (Ad5 E1b-βlac)**

**AMPLICON SEQUENCE:**

CTGGCCAATACCAACCTTATCCTACACGGTGTAAGCTTCTAAGGCCGTATCGTAGTTATCTACACGACGG
GGAGTCAGG

**AMPLICON REGION:** Ad5 E1b – plasmid β-lac junction sequence from

**AMPLICON SIZE:** 79 bp

**COPY NUMBER/GENOME:** 1

**FORWARD PRIMER: HDEPF** 5' CTGGCCAATACCAACCTTATCC 3'

**REVERSE PRIMER: HDEPR** 5' CCTGACTCCCCGTCGTGTAG 3'

**PROBE: HDEPPR** 5' [6~FAM] ACACGGTGTAAGCTTCTAAGGCCGTATCGTAGT [Quencher] 3'

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**CONTROL GENOMIC DNA:** plasmid pTOPO211-213 (Crucell E273-087, QC026038)

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| Universal Mastermix (BDP 30268) | 25 (1x) |
| NFW or WFI Water (various) | 2.5 |
| Primers | 1 each (10 pmol each) |
| Probe | 0.5 (5 pmol) |
| Reaction Mix Volume | 30 |
| Sample Volume / rxn | 20 |
| Total Reaction Volume | 50 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50$^0$C | 2:00 | N/A |
| 2 | 95$^0$C | 10:00 | N/A |
| 3 | 95$^0$C<br>60$^0$C | :15<br>1:00 | 40 |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 32**

**AMPLICON: Adenovirus 5 E1a (Mark I) amplicon for KD3 E1a RCA testing**

**AMPLICON SEQUENCE:**

GACACCG GGACTGAAAA TGAGACATAT TATCTGCCAC GGAGGTGTTA TTACCGAAGA AATGGCCGC

| | |
|---|---|
| **AMPLICON REGION:** | Ad5 E1a region deleted in virus Ad-KD3, present in wtAd5 |
| **AMPLICON SIZE:** | 66 bp |
| **COPY NUMBER/GENOME:** | 1 |
| **FORWARD PRIMER: E1A20F** | 5' GAC ACC GGG ACT GAA AAT GAG 3' |
| **REVERSE PRIMER: E1A93R** | 5' GCG GCC ATT TCT TCG GTA A 3' |
| **PROBE: E1A50P** | 5' [6~FAM] ACA TAT TAT CTG CCA CGG AGG TGT T [Quencher] 3' |

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**CONTROL GENOMIC DNA:** Adenoviral reference material DNA

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| **Universal Mastermix (BDP 30268)** | **25 (1x)** |
| **NFW or WFI Water (various)** | **2.5** |
| **Primers** | **1 each (10 pmol each)** |
| **Probe** | **0.5 (5 pmol)** |
| **Reaction Mix Volume** | **30** |
| **Sample Volume / rxn** | **20** |
| **Total Reaction Volume** | **50** |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| **1** | **50$^0$C** | **2:00** | **N/A** |
| **2** | **95$^0$C** | **10:00** | **N/A** |
| **3** | **95$^0$C**<br>**60$^0$C** | **:15**<br>**1:00** | **40** |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 33**

**AMPLICON: Adenovirus 5 wild-type fiber tropism (non-RGD)**

**AMPLICON SEQUENCE:**

TTACACTAAACGGTACACAGGAAACAGGAGACACAAC*TC*CAAGTGCATACTCTATGTCATTTTCATGGGACTGGTCTGGC

**AMPLICON REGION:** Wild-type Ad5 wild-type fiber knob sequence (for non-RGD virus)

**AMPLICON SIZE:** 80 bp

**COPY NUMBER/GENOME:** 1

**FORWARD PRIMER: WTFIBF** 5' TTACACTAAACGGTACACAGGAAACAG 3'

**REVERSE PRIMER: WTFIBR** 5' GCC AGA CCA GTC CCA TGA AA 3'

**PROBE: WTFIBPR** 5' [6~FAM] CAC AAC TCC AAG TGC ATA CTC TAT GTC A [Quencher] 3'

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**CONTROL GENOMIC DNA:** Adenoviral reference material DNA

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| **Universal Mastermix (BDP 30268)** | **25 (1x)** |
| **NFW or WFI Water (various)** | **2.5** |
| **Primers** | **1 each (10 pmol each)** |
| **Probe** | **0.5 (5 pmol)** |
| **Reaction Mix Volume** | **30** |
| **Sample Volume / rxn** | **20** |
| **Total Reaction Volume** | **50** |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| **1** | **50$^0$C** | **2:00** | **N/A** |
| **2** | **95$^0$C** | **10:00** | **N/A** |
| **3** | **95$^0$C**<br>**60$^0$C** | **:15**<br>**1:00** | **40** |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 34**

**AMPLICON: Adenovirus 5 RGD modified fiber knob tropism**

**AMPLICON SEQUENCE:**

*C*ACACTAAACGGTACACAGGAAACAGGAGACACAACTTGTGACTGCCGCGGAGACTGTTTCTGCCCA*TC*
TGCATACTCTATGTCATTTTCATGGGACTGGTCTGGC

| | |
|---|---|
| **AMPLICON REGION:** | RGD tropism modified Ad5 fiber knob sequence<br>(does not amplify wild-type fiber sequence) |
| **AMPLICON SIZE:** | 105 bp |
| **COPY NUMBER/GENOME:** | 1 |
| **FORWARD PRIMER: LAMF** | 5' ACA CTA AAC GGT ACA CAG GAA ACA 3' |
| **REVERSE PRIMER: LAMR** | 5' GCC AGA CCA GTC CCA TGA AA 3' |
| **PROBE: LAMP** | 5' [6~FAM] CCG CGG AGA CTG TTT CTG CCC A [Quencher] 3' |

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**CONTROL GENOMIC DNA:** Ad5-D24-RGD virus L0405025

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| **Universal Mastermix (BDP 30268)** | **25 (1x)** |
| **NFW or WFI Water (various)** | **2.5** |
| **Primers** | **1 each (10 pmol each)** |
| **Probe** | **0.5 (5 pmol)** |
| **Reaction Mix Volume** | **30** |
| **Sample Volume / rxn** | **20** |
| **Total Reaction Volume** | **50** |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| **1** | **50°C** | **2:00** | **N/A** |
| **2** | **95°C** | **10:00** | **N/A** |
| **3** | **95°C**<br>**60°C** | **:15**<br>**1:00** | **40** |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 35**

**AMPLICON: AAV2**

**AMPLICON SEQUENCE:**

CACAAGTTTGGGATCTAATACAATGGCTTCAGGCGGTGGCGCACCAATGGCAGACAATAACGAGGGTGCCG

| | |
|---|---|
| **AMPLICON REGION:** | Rep 78 protein, protein VP1 (cap) genes |
| **AMPLICON SIZE:** | 71 bp |
| **COPY NUMBER / GENOME:** | 1 |
| **FORWARD PRIMER: AAV F** | 5' ACAAGTTTGGGATCTAATACAATGG 3' |
| **REVERSE PRIMER: AAV R** | 5' CGGCACCCTCGTTATTGTCT 3' |
| **PROBE: AAV P** | 5' [6-FAM] TTCAGGCGGTGGCGCACCA [Quencher] 3' |

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**(Note – Viral detection qPCR assays for cell line characterization require target input quantitation, e.g. total DNA mass, genomic copy number, cell count, or similar measure)**

**CONTROL GENOMIC DNA:** Plasmid Rep 78 protein / protein VP1 (capsid) genes, accession #AY695376.1

| REAGENT | VOLUME / REACTION (µL) |
|---|---|
| Universal Mastermix (BDP 30268) | 25 (1x) |
| NFW or WFI Water (various) | 2.0 |
| Primers | 1 each (5 pmol each) |
| Probe | 1 (5 pmol) |
| Reaction Mix Volume | 30 |
| Sample Volume / rxn | 20 |
| Total Reaction Volume | 50 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50 C | 2:00 | N/A |
| 2 | 95 C | 10:00 | N/A |
| 3 | 95 C<br>60 C | :15<br>1:00 | ≥ 40 |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 36**

**AMPLICON: B19**

**AMPLICON SEQUENCE:**

GGCCTGGCAATGAGCTACAAGCTGGGCCCCCGCAAAGTGCTGTTGACAGTGCTGCAAGGATTCATGA

| | |
|---|---|
| **AMPLICON REGION:** | VP1 gene |
| **AMPLICON SIZE:** | 67 bp |
| **COPY NUMBER / GENOME:** | 1 |
| **FORWARD PRIMER: B19 F** | 5' GGCCTGGCAATGAGCTACAA 3' |
| **REVERSE PRIMER: B19 R** | 5' TCATGAATCCTTGCAGCACTGT 3' |
| **PROBE: B19 P** | 5' [6-FAM] CTGGGCCCCCGCAAAGTGC [Quencher] 3' |

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**(Note – Viral detection qPCR assays for cell line characterization require target input quantitation, e.g. total DNA mass, genomic copy number, cell count, or similar measure)**

**CONTROL GENOMIC DNA:** Plasmid Capsid protein (VP), accession #Z68146

| REAGENT | VOLUME / REACTION (µL) |
|---|---|
| Universal Mastermix (BDP 30268) | 25 (1x) |
| NFW or WFI Water (various) | 2.0 |
| Primers | 1 each (5 pmol each) |
| Probe | 1 (5 pmol) |
| Reaction Mix Volume | 30 |
| Sample Volume / rxn | 20 |
| Total Reaction Volume | 50 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50 C | 2:00 | N/A |
| 2 | 95 C | 10:00 | N/A |
| 3 | 95 C<br>60 C | :15<br>1:00 | ≥ 40 |

**RESULTS ANALYSIS:**

**Threshold:** 0.2 ΔRn

**Attachment 37**

**AMPLICON: BK/JC**

**AMPLICON SEQUENCE:**

TCCTCAATGGATGTTGCCTTTACTTTTAGGGTTGTACGGGACTGTAACACCTGCTCTTGAAGCATATGAA GATGGCCC

**AMPLICON REGION:** VP2 gene

**AMPLICON SIZE:** 78 bp

**COPY NUMBER / GENOME:** 1

**FORWARD PRIMER: BKJC F** 5' TCCTCAATGGATGTTGCCTTT 3'

**REVERSE PRIMER: BKJC R** 5' GGGCCATCTTCATATGCTTCAA 3'

**PROBE: BKJC P** 5' [6-FAM] TTTTAGGGTTGTACGGGACTGTAACACCTGCT [Quencher] 3'

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**(Note – Viral detection qPCR assays for cell line characterization require target input quantitation, e.g. total DNA mass, genomic copy number, cell count, or similar measure)**

**CONTROL GENOMIC DNA**: Plasmid Capsid protein VP2 region, spans the sequence of JCV Isolate 330A, #AY382188,.and BKV isolate TW-2 #AB213487 VP2 region

| REAGENT | VOLUME / REACTION (µL) |
|---|---|
| **Universal Mastermix (BDP 30268)** | **25 (1x)** |
| **NFW or WFI Water (various)** | **2.0** |
| **Primers** | **1 each (5 pmol each)** |
| **Probe** | **1 (5 pmol)** |
| **Reaction Mix Volume** | **30** |
| **Sample Volume / rxn** | **20** |
| **Total Reaction Volume** | **50** |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| **1** | **50 C** | **2:00** | **N/A** |
| **2** | **95 C** | **10:00** | **N/A** |
| **3** | **95 C**<br>**60 C** | **:15**<br>**1:00** | **≥ 40** |

**RESULTS ANALYSIS:**

**Threshold:** 0.2 ΔRn

**Attachment 38**

**AMPLICON: CMV**

**AMPLICON SEQUENCE:**

TCCGCGGTTGTCTCTGTGTATAACGTTTTTATTTCGGGTTCCGCGTTTGGTCGCCTGCCT

| | |
|---|---|
| **AMPLICON REGION:** | UL38 gene |
| **AMPLICON SIZE:** | 60 bp |
| **COPY NUMBER / GENOME:** | 1 |
| **FORWARD PRIMER: CMV F** | 5' TCCGCGGTTGTCTCTGTGTA 3' |
| **REVERSE PRIMER: CMV R** | 5' AGGCAGGCGACCAAACG 3' |
| **PROBE: CMV P** | 5' [6-FAM] CGTTTTTATTTCGGGTTCCG [Quencher] 3' |

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**(Note – Viral detection qPCR assays for cell line characterization require target input quantitation, e.g. total DNA mass, genomic copy number, cell count, or similar measure)**

**CONTROL GENOMIC DNA:** Plasmid UL38 gene (pp65 lower matrix protein) from ~120,656 to 122,341 in #AY446894,Merlin strain

| REAGENT | VOLUME / REACTION (µL) |
|---|---|
| Universal Mastermix (BDP 30268) | 25 (1x) |
| NFW or WFI Water (various) | 2.0 |
| Primers | 1 each (5 pmol each) |
| Probe | 1 (5 pmol) |
| Reaction Mix Volume | 30 |
| Sample Volume / rxn | 20 |
| Total Reaction Volume | 50 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50 C | 2:00 | N/A |
| 2 | 95 C | 10:00 | N/A |
| 3 | 95 C<br>60 C | :15<br>1:00 | ≥ 40 |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 39**

**AMPLICON: EBV**

**AMPLICON SEQUENCE:**

CGGCCGTGATGGAGGCTATGACGGCGGCGAGTGACTACGCGCGTGGCCTGGGCGTGAAGCTGACCTT
TGGCTCGGCCTCCTGCCCCGAGACCGGCTCGTCCGCCTCCAACTTCATGACCGTGGTGGCCTCTGTCT

**AMPLICON REGION:** BNRF1 gene

**AMPLICON SIZE:** 134 bp

**COPY NUMBER / GENOME:** 1

**FORWARD PRIMER: EBV F** 5' CGGCCGTGATGGAGGCTATG 3'

**REVERSE PRIMER: EBV R** 5' AGACAGAGGCCACCACGG 3'

**PROBE: EBV P** 5' [6-FAM] TGACCTTTGGCTCGGCCTCCTGC [Quencher] 3'

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**(Note – Viral detection qPCR assays for cell line characterization require target input quantitation, e.g. total DNA mass, genomic copy number, cell count, or similar measure)**

**CONTROL GENOMIC DNA**: Plasmid BNRF1 gene for p140, accession #X67777.1

| REAGENT | VOLUME / REACTION (µL) |
|---|---|
| Universal Mastermix (BDP 30268) | 25 (1x) |
| NFW or WFI Water (various) | 2.0 |
| Primers | 1 each (5 pmol each) |
| Probe | 1 (5 pmol) |
| Reaction Mix Volume | 30 |
| Sample Volume / rxn | 20 |
| Total Reaction Volume | 50 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50 C | 2:00 | N/A |
| 2 | 95 C | 10:00 | N/A |
| 3 | 95 C<br>60 C | :15<br>1:00 | ≥ 40 |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 40**
**AMPLICON: HHV 6 A/B**

**AMPLICON SEQUENCE:**

GGCGCGACCTCTGACAGTGCTCACGGAGAGGACAGAGGACGTCTCTAGAAAACATCTCATCCACCTGCT
TAATATAGTCCGTATCTTGCGTAAAC

**AMPLICON REGION:** Immediate early gene

**AMPLICON SIZE:** 95 bp

**COPY NUMBER / GENOME:** 1

**FORWARD PRIMER: HHV6 F** 5' GGCGCGACCTCTGACAGT 3'

**REVERSE PRIMER: HHV6 R** 5' GTTTACGCAAGATACGGACTATATTAAGC 3'

**PROBE: HHV6 P** 5' [6-FAM] ACAGAGGACGTCTCTAGAAAACATCTCATCCACC [Quencher] 3'

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**(Note – Viral detection qPCR assays for cell line characterization require target input quantitation, e.g. total DNA mass, genomic copy number, cell count, or similar measure)**

**CONTROL GENOMIC DNA:** Plasmid U25 / EPLF3 gene, accession #L25528 and NC001664, and AF157706 B-strain Z29, and AB021506 B-strain HST

| REAGENT | VOLUME / REACTION (µL) |
|---|---|
| **Universal Mastermix (BDP 30268)** | **25 (1x)** |
| **NFW or WFI Water (various)** | **2.0** |
| **Primers** | **1 each (5 pmol each)** |
| **Probe** | **1 (5 pmol)** |
| **Reaction Mix Volume** | **30** |
| **Sample Volume / rxn** | **20** |
| **Total Reaction Volume** | **50** |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| **1** | **50 C** | **2:00** | **N/A** |
| **2** | **95 C** | **10:00** | **N/A** |
| **3** | **95 C**<br>**60 C** | **:15**<br>**1:00** | **≥ 40** |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 41**

**AMPLICON: HHV 7**

**AMPLICON SEQUENCE:**

CCGTAAAGCGTGTGGGATTGTCATCCAATGCTTTAACAAACAAATGGAAGACTCTTCATTCTGCACTTCC
CATGAAAAAA

**AMPLICON REGION:** Glycoprotein B gene

**AMPLICON SIZE:** 79 bp

**COPY NUMBER / GENOME:** 1

**FORWARD PRIMER: HHV7 F** 5' CCGTAAAGCGTGTGGGATTG 3'

**REVERSE PRIMER: HHV7 R** 5' TTTTTTCATGGGAAGTGCAGAA 3'

**PROBE: HHV7 P** 5' [6-FAM] CATCCAATGCTTTAACAAACAAATGGAAGACTCTTC [Quencher] 3'

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**(Note – Viral detection qPCR assays for cell line characterization require target input quantitation, e.g. total DNA mass, genomic copy number, cell count, or similar measure)**

**CONTROL GENOMIC DNA:** Plasmid UL39 envelope glycoprotein (g)B, accession #AY192554 and NC_001716

| REAGENT | VOLUME / REACTION (µL) |
|---|---|
| Universal Mastermix (BDP 30268) | 25 (1x) |
| NFW or WFI Water (various) | 2.0 |
| Primers | 1 each (5 pmol each) |
| Probe | 1 (5 pmol) |
| Reaction Mix Volume | 30 |
| Sample Volume / rxn | 20 |
| Total Reaction Volume | 50 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50 C | 2:00 | N/A |
| 2 | 95 C | 10:00 | N/A |
| 3 | 95 C<br>60 C | :15<br>1:00 | ≥ 40 |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 42**

**AMPLICON: HHV 8**

**AMPLICON SEQUENCE:**

CTCGAATCCAACGGATTTGACCCCGTGTTCCCCATGGTCGTGCCGCAGCAACTGGGGCACGCT

**AMPLICON REGION:** Glycoprotein B gene

**AMPLICON SIZE:** 63 bp

**COPY NUMBER / GENOME:** 1

**FORWARD PRIMER: HHV8 F** 5' CTCGAATCCAACGGATTTGAC 3'

**REVERSE PRIMER: HHV8 R** 5' AGCGTCCCCAGTTGCT 3'

**PROBE: HHV8 P** 5' [6-FAM] CGTGTTCCCCATGGTCGTGCC [Quencher] 3'

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**(Note – Viral detection qPCR assays for cell line characterization require target input quantitation, e.g. total DNA mass, genomic copy number, cell count, or similar measure)**

**CONTROL GENOMIC DNA:** Plasmid ORF 26, minor capsid protein gene, accession #AY219444

| REAGENT | VOLUME / REACTION (µL) |
|---|---|
| Universal Mastermix (BDP 30268) | 25 (1x) |
| NFW or WFI Water (various) | 2.0 |
| Primers | 1 each (5 pmol each) |
| Probe | 1 (5 pmol) |
| Reaction Mix Volume | 30 |
| Sample Volume / rxn | 20 |
| Total Reaction Volume | 50 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50 C | 2:00 | N/A |
| 2 | 95 C | 10:00 | N/A |
| 3 | 95 C<br>60 C | :15<br>1:00 | ≥ 40 |

**RESULTS ANALYSIS:**

**Threshold:** 0.2 ΔRn

**Attachment 43**

**AMPLICON: HIV 1**

**AMPLICON SEQUENCE:**

AGCAGCCATGCAAATGTTAAAAGAGACCATCAATGAGGAAGCTGCAGAATGGGATAGATTGCATCCAGT GCATGC

**AMPLICON REGION:** gag gene

**AMPLICON SIZE:** 74 bp

**COPY NUMBER / GENOME** 1

**FORWARD PRIMER: HIV1 F** 5' AGCAGCCATGCAAATGTTAAA 3'

**REVERSE PRIMER: HIV1 R** 5' GCATGCACTGGATGCACTCT 3'

**PROBE: HIV1 P** 5' [6-FAM] CATCAATGAGGAAGCTGCAGAATGGGA [Quencher] 3'

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**(Note – Viral detection qPCR assays for cell line characterization require target input quantitation, e.g. total DNA mass, genomic copy number, cell count, or similar measure)**

**CONTROL GENOMIC DNA:** Plasmid gag gene, accession #308760

| REAGENT | VOLUME / REACTION (µL) |
|---|---|
| Universal Mastermix (BDP 30268) | 25 (1x) |
| NFW or WFI Water (various) | 2.0 |
| Primers | 1 each (5 pmol each) |
| Probe | 1 (5 pmol) |
| Reaction Mix Volume | 30 |
| Sample Volume / rxn | 20 |
| Total Reaction Volume | 50 |

**REACTION CONDITIONS:** This method detects proviral DNA when used without an RT step.

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50 C | 2:00 | N/A |
| 2 | 95 C | 10:00 | N/A |
| 3 | 95 C<br>60 C | :15<br>1:00 | ≥ 40 |

**RESULTS ANALYSIS:**

**Threshold:** 0.2 ΔRn

**Attachment 44**

**AMPLICON: HIV 2**

**AMPLICON SEQUENCE:**

ATGGGCGCGAGAAACTCCGTCTTGAGAGGGAAAAAAGCAGATGAATTAGAAAAAATTAGGTTACGGCCC
GGCGGAAAGAAAAAGTACAAGTTAAAACATATTGTGTGGGCAGCGAATGAATTGGACAGATTCGGATTAG
CAAAGAGCCTGTTGGA

| | |
|---|---|
| **AMPLICON REGION:** | 3' LTR gene |
| **AMPLICON SIZE:** | 154 bp |
| **COPY NUMBER / GENOME:** | 1 |
| **FORWARD PRIMER: H2DG F** | 5' ATGGGCGCGAGAARCTCCG 3' |
| **REVERSE PRIMER: H2DG R** | 5' TCCAACAGGCTYTCTGCYAATCC 3' |
| **PROBE: H2DG P** | 5' [6-FAM] YAGGTTACGGCCCRRCGGRAA [Quencher] 3' |

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**(Note – Viral detection qPCR assays for cell line characterization require target input quantitation, e.g. total DNA mass, genomic copy number, cell count, or similar measure)**

**CONTROL GENOMIC DNA:** Plasmid gag gene, accession #HIV2CAM2

| REAGENT | VOLUME / REACTION (µL) |
|---|---|
| Universal Mastermix (BDP 30268) | 25 (1x) |
| NFW or WFI Water (various) | 2.0 |
| Primers | 1 each (5 pmol each) |
| Probe | 1 (5 pmol) |
| Reaction Mix Volume | 30 |
| Sample Volume / rxn | 20 |
| Total Reaction Volume | 50 |

**REACTION CONDITIONS:** This method detects proviral DNA when used without an RT step.

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50 C | 2:00 | N/A |
| 2 | 95 C | 10:00 | N/A |
| 3 | 95 C<br>60 C | :15<br>1:00 | ≥ 40 |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 45**

**AMPLICON: MMV**

**AMPLICON SEQUENCE:**

GCCCATGATTTGTGCTTGGTTGGTAAAGAATGGTTACCAATCTACCATGGCAAGCTACTGTGCTAAATGG
GGCAAAGTTCCT

**AMPLICON REGION:** NS1gene

**AMPLICON SIZE:** 82 bp

**COPY NUMBER / GENOME:** 1

**FORWARD PRIMER: MVM F** 5' GCCCATGATTTGTGCTTGGTT 3'

**REVERSE PRIMER: MVM R** 5' AGGAACTTTGCCCCATTTAGC 3'

**PROBE: MVM P** 5' [6-FAM] AAAGAATGGTTACCAATCTACCATGGCAAGCTACT [Quencher] 3'

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**MVM forward and reverse primers** BDP PN 30902

**MVM Probe** BDP PN 30903

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**(Note – Viral detection qPCR assays for cell line characterization require target input quantitation, e.g. total DNA mass, genomic copy number, cell count, or similar measure)**

**CONTROL GENOMIC DNA:** Plasmid Major non-structural protein gene, accession #FJ445512.1 and equivalent to most homologous mouse parvovirus strains' sequences.

| REAGENT | VOLUME / REACTION (µL) |
|---|---|
| Universal Mastermix (BDP 30268) | 25 (1x) |
| NFW or WFI Water (various) | 2.0 |
| Primers | 1 each (5 pmol each) |
| Probe | 1 (5 pmol) |
| Reaction Mix Volume | 30 |
| Sample Volume / rxn | 20 |
| Total Reaction Volume | 50 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50 C | 2:00 | N/A |
| 2 | 95 C | 10:00 | N/A |
| 3 | 95 C<br>60 C | :15<br>1:00 | ≥ 40 |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 46**

**AMPLICON: SMRV**

**AMPLICON SEQUENCE:**

GGCTACTACTTCCCTTGCAAAGATTAGACAAGGCCCCGATGAGTCATACAGTGATTTTGTAAGCCGCCTCCAGG

**AMPLICON REGION:** gag protein gene

**AMPLICON SIZE:** 74 bp

**COPY NUMBER / GENOME:** 1

**FORWARD PRIMER: SMRV F** 5' GGCTACTACTTCCCTTGCAAAGAT 3'

**REVERSE PRIMER: SMRV R** 5' CCTGGAGGCGGCTTACAAA 3'

**PROBE: SMRV P** 5' [6-FAM] ACAAGGCCCCGATGAGTCATACAGTGA [Quencher] 3'

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**(Note – Viral detection qPCR assays for cell line characterization require target input quantitation, e.g. total DNA mass, genomic copy number, cell count, or similar measure)**

**CONTROL GENOMIC DNA:** Plasmid gag protein gene, accession #U23805

| REAGENT | VOLUME / REACTION (µL) |
|---|---|
| Universal Mastermix (BDP 30268) | 25 (1x) |
| NFW or WFI Water (various) | 2.0 |
| Primers | 1 each (5 pmol each) |
| Probe | 1 (5 pmol) |
| Reaction Mix Volume | 30 |
| Sample Volume / rxn | 20 |
| Total Reaction Volume | 50 |

**REACTION CONDITIONS:** This method detects proviral DNA when used without an RT step.

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50 C | 2:00 | N/A |
| 2 | 95 C | 10:00 | N/A |
| 3 | 95 C<br>60 C | :15<br>1:00 | ≥ 40 |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 47**

**AMPLICON: SRV 1/2**

**AMPLICON SEQUENCE:**

AATGAATGCAAATCCAAAACTGATAGTCAAGGAAACCCACTACCACCCCATCAGGGAAACGGACTGAGG GGC

**AMPLICON REGION:** gag gene

**AMPLICON SIZE:** 71 bp

**COPY NUMBER / GENOME:** 1

**FORWARD PRIMER: SRV F** 5' AATGAATGCAAATCCAAAACTGATAG 3'

**REVERSE PRIMER: SRV R** 5' GCCCCTCAGTCCGTTTCC 3'

**PROBE: SRV P** 5' [6-FAM] CAAGGAAACCCACTACCACCCCATCA [Quencher] 3'

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**(Note – Viral detection qPCR assays for cell line characterization require target input quantitation, e.g. total DNA mass, genomic copy number, cell count, or similar measure)**

**CONTROL GENOMIC DNA:** Plasmid gag gene region, SRV-1 accession #M11841and SRV 2 accession #M16605.

| REAGENT | VOLUME / REACTION (µL) |
|---|---|
| **Universal Mastermix (BDP 30268)** | **25 (1x)** |
| **NFW or WFI Water (various)** | **2.0** |
| **Primers** | **1 each (5 pmol each)** |
| **Probe** | **1 (5 pmol)** |
| **Reaction Mix Volume** | **30** |
| **Sample Volume / rxn** | **20** |
| **Total Reaction Volume** | **50** |

**REACTION CONDITIONS:** This method detects proviral DNA when used without an RT step.

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| **1** | **50 C** | **2:00** | **N/A** |
| **2** | **95 C** | **10:00** | **N/A** |
| **3** | **95 C**<br>**60 C** | **:15**<br>**1:00** | **≥ 40** |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 48**

**AMPLICON: SV 40**

**AMPLICON SEQUENCE:**

CACAGGCCTATGCTGTGATATCTGGGGCTCCTGCTGCTATAGCTGGATTTGCAGCTTTACTGCAAACTGT
GACTGGTGTGAGCGCTGTTGCTCAAGTGGGGTATAGATTTTT

**AMPLICON REGION:** VP2 gene

**AMPLICON SIZE:** 112 bp

**COPY NUMBER / GENOME:** 1

**FORWARD PRIMER: SV40 F** 5' CACAGGCCTATGCTGTGATATCTG 3'

**REVERSE PRIMER: SV40 R** 5' AAAAATCTATACCCCACTTGAGCAA 3'

**PROBE: SV40 P** 5' [6-FAM] AGCTTTACTGCAAACTGTGACTGGTGTGAGC [Quencher] 3'

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**(Note – Viral detection qPCR assays for cell line characterization require target input quantitation, e.g. total DNA mass, genomic copy number, cell count, or similar measure)**

**CONTROL GENOMIC DNA:** Plasmid VP2 gene, accession #AF316141

| REAGENT | VOLUME / REACTION (µL) |
|---|---|
| Universal Mastermix (BDP 30268) | 25 (1x) |
| NFW or WFI Water (various) | 2.0 |
| Primers | 1 each (5 pmol each) |
| Probe | 1 (5 pmol) |
| Reaction Mix Volume | 30 |
| Sample Volume / rxn | 20 |
| Total Reaction Volume | 50 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50 C | 2:00 | N/A |
| 2 | 95 C | 10:00 | N/A |
| 3 | 95 C<br>60 C | :15<br>1:00 | ≥ 40 |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 49**

**AMPLICON: HTLV 1**

**AMPLICON SEQUENCE:**

GGACAAGCATATATTGTCACAAAGATCATTCCCCCTTCCGCCACCGCACAAGTCGGCCCAA
CGGGCCGAACTTCTCGGACTTTTGCATGG

**AMPLICON REGION:** gag pol gene

**AMPLICON SIZE:** 89 bp

**COPY NUMBER / GENOME:** 1

**FORWARD PRIMER: HTLV1 F** 5' CCATGCAAAAGTCCGAGAAGTT 3'

**REVERSE PRIMER: HTLV1 R** 5' GGACAAGCATATATTGTCACAAAGATC 3'

**PROBE: HTLV1 P** 5' [6-FAM] CGTTGGGCCGACTTGTGCGG [Quencher] 3'

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**(Note – Viral detection qPCR assays for cell line characterization require target input quantitation, e.g. total DNA mass, genomic copy number, cell count, or similar measure)**

**CONTROL GENOMIC DNA:** Plasmids cloned gag/pol genes for each strain, various accession #'s.

| REAGENT | VOLUME / REACTION (µL) |
|---|---|
| Universal Mastermix (BDP 30268) | 25 (1x) |
| NFW or WFI Water (various) | 2.0 |
| Primers | 1 each (5 pmol each) |
| Probe | 1 (5 pmol) |
| Reaction Mix Volume | 30 |
| Sample Volume / rxn | 20 |
| Total Reaction Volume | 50 |

**REACTION CONDITIONS:** This method detects proviral DNA when used without an RT step.

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50 C | 2:00 | N/A |
| 2 | 95 C | 10:00 | N/A |
| 3 | 95 C<br>60 C | :15<br>1:00 | ≥ 40 |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 50**

**AMPLICON: HTLV 2**

**AMPLICON SEQUENCE:**

TTGCGACTTCCTGAGGAACTCCCCTCATCCAAGTGTCGGCATCCTCATTCACCACATGGGTCGATTCCAT AACCTTGGCAGCCA

**AMPLICON REGION:** rex protein gene

**AMPLICON SIZE:** 83 bp

**COPY NUMBER / GENOME:** 1

**FORWARD PRIMER: HTLV2 F** 5' TGGCTGCCAAGGTTATGGA 3'

**REVERSE PRIMER: HTLV2 R** 5' TTGCGACTTCCTGAGGAACTC 3'

**PROBE: HTLV2 P** 5' [6-FAM] CGACCCATGTGGTGAATGAGGATGC [Quencher] 3'

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**(Note – Viral detection qPCR assays for cell line characterization require target input quantitation, e.g. total DNA mass, genomic copy number, cell count, or similar measure)**

**CONTROL GENOMIC DNA:** Plasmids cloned gag/pol genes for each strain, various accession #'s.

| REAGENT | VOLUME / REACTION (µL) |
|---|---|
| Universal Mastermix (BDP 30268) | 25 (1x) |
| NFW or WFI Water (various) | 2.0 |
| Primers | 1 each (5 pmol each) |
| Probe | 1 (5 pmol) |
| Reaction Mix Volume | 30 |
| Sample Volume / rxn | 20 |
| Total Reaction Volume | 50 |

**REACTION CONDITIONS:** This method detects proviral DNA when used without an RT step.

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50 C | 2:00 | N/A |
| 2 | 95 C | 10:00 | N/A |
| 3 | 95 C<br>60 C | :15<br>1:00 | ≥ 40 |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 51**

**AMPLICON: Human AADC Exon Junction Amplicon from rAAV2-CMV-hAADC**

**AMPLICON SEQUENCE:**
CTGGCCTGATTCCTTTCTTTATGGTTGCCACCCTGGGGACCACAACATGCTGCTCCTTTGACAATCTCTT
AGAAGTCGGTCCTATCTGCAACAAGGAAGACATATG

| | |
|---|---|
| **AMPLICON REGION:** | Human AADC from rAAV2-CMV-hAADC |
| **AMPLICON SIZE:** | 106 bp |
| **COPY NUMBER/GENOME:** | 1 |
| **FORWARD PRIMER:** AADC Exon Jxn F | 5' CTGGCCTGATTCCTTTCTTTATGG 3' |
| **REVERSE PRIMER:** AADC Exon Jxn R | 5' CATATGTCTTCCTTGTTGCAGATAGG 3' |
| **PROBE:** AADC Exon Jxn P | 5' GGGACCACAACATGCTGCTCCTTTGAC 3' |
| **REAGENTS (and associated BDP and/or manufacturer part numbers):** | |
| **AADC Exon Junction Forward Primer** | BDP PN 31120 |
| **AADC Exon Junction Reverse Primer** | BDP PN 31121 |
| **AADC Exon Junction Probe** | BDP PN 31122 |
| **RECOMMENDED DNaseI ENZYME:** | DNaseI, RNase Free, Thermo Scientific (BDP 30695) |
| **RECOMMENDED DNaseI METHOD:** | Combine 150 µL aliquot of sample with 20 µL 1xPBS, 20 µL 10x Reaction Buffer and 10 µL of DNaseI, RNase-free. Incubate at 37°C for 30 minutes, then 65°C for 10 minutes (to inactivate the DNaseI). Perform prior to QIAGEN extraction. |

**RECOMMENDED EXTRACTION KIT:** QIAGEN Blood Mini Kit (BDP 30444)

**CONTROL GENOMIC DNA:** pAAV2-CMV-hAADC-KanR

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| Universal Mastermix (BDP 30268) | 12.5 (1x) |
| NFW or WFI Water (various) | 6.25 |
| Primers | 0.5 each (10 pmol each) |
| Probe | 0.25 (5 pmol) |
| Reaction Mix Volume | 20 |
| Sample Volume / rxn | 5 |
| Total Reaction Volume | 25 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50°C | 2:00 | N/A |
| 2 | 95°C | 10:00 | N/A |
| 3 | 95°C | 00:15 | 40 |
| | 60°C | 00:30 | |

**RESULTS ANALYSIS:**
Threshold: 0.05 ΔRn

**Attachment 52**

**AMPLICON: Human CD33 CAR Amplicon from CD33.28CAR Vector**

**AMPLICON SEQUENCE:**
GCTCCGACATTCAGATGACCCAGAGCCCTAGCAGCCTGAGCGCTTCCGTGGGAGACAGGGTGACCATC
ACATGCAGGGCCTCCGAGAGCGTGGACAATTACGGCATCAGCTTCATGAACT

**AMPLICON SIZE:** 120 bases
**COPY NUMBER/VIRAL VECTOR GENOME:** 1
**FORWARD PRIMER:** 5' CGA CAT TCA GAT GAC CCA GAG 3'
**REVERSE PRIMER:** 5' CAT GAA GCT GAT GCC GTA ATT G 3'

**PROBE:** 5'/5HEX/AGACAGGGT/ZEN/GACCATCACATGCAG/Quencher 3'
**REAGENTS (and associated BDP and/or manufacturer part numbers):**
**CD33FC dPCR Primers/probe set (VIC/HEX) 20X** BDP PN 31224

**RECOMMENDED EXTRACTION KIT:** QIAGEN Viral RNA Mini Kit (BDP PN 30450)
**RECOMMENDED DNaseI ENZYME:** NEB (BDP PN 31243)
**RECOMMENDED DNaseI METHOD:** Elute RNA from Viral RNA Mini Kit in 60.0 µL of Buffer AVE. Combine 39.0 µL 1X PBS, 5.0 µL eluted RNA, 5.0 µL 10x DNase I Buffer and 1.0 µL of DNaseI. Incubate at 37°C for 15 minutes. Add 4.0 µL of RNase-free water and 6.0 µL of 50 mM EDTA and incubate at 75°C for 10 minutes (to inactivate the DNaseI).
**CONTROL GENOMIC DNA:** CD33Fc RNA Control (BDP PN 31234)

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| DEPC-Treated Water or RNase-Free Water | 16.0 |
| 2X Reaction Mix (BDP 30265) | 25.0 (1X Final Concentration) |
| CD33Fc-VIC | 1.0 (200 nM Final Concentration) |
| ROX Reference Dye | 1.0 |
| RNaseOUT | 1.0 |
| SuperScript III RT/Platinum Taq Mix | 1.0 |
| Reaction Mix Volume | 45.0 |
| Sample Volume / rxn | 5.0 |
| Total Reaction Volume | 50.0 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50°C | 15:00 | N/A |
| 2 | 95°C | 2:00 | N/A |
| 3 | 95°C | 00:15 | 40 |
| | 60°C | 01:00 | |

**RESULTS ANALYSIS:**
Threshold: 0.2 ΔRn

**Attachment 53**

**AMPLICON: GFP**

**AMPLICON SEQUENCE:**
AGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATC
GAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAA
CAGCCACAACGTCTATATCATGGCCGACAAGCAGAA

**AMPLICON REGION:** GFP gene

**AMPLICON SIZE:** 172 bp

**COPY NUMBER / GENOME:** 1

**FORWARD PRIMER: BDP GFP Forward** 5' AGGACGACGGCAACTACAAG 3'

**REVERSE PRIMER: BDP GFP Reverse** 5' TTCTGCTTGTCGGCCATGAT 3'

**PROBE: BDP GFP Probe** 5' [6-FAM] AGGACGGCAACATCCTGGGGCACAA [Quencher] 3'

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**BDP GFP Forward** BDP PN 31270

**BDP GFP Reverse** BDP PN 31271

**BDP GFP Probe** BDP PN 31272

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP 30442) or QIAGEN Blood Mini Kit (BDP 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP 31236)

**CONTROL GENOMIC DNA:** GFP Gene Block DNA Control (BDP PN 31273)

| REAGENT | VOLUME / REACTION (µL) |
|---|---|
| **Universal Mastermix (BDP 30268)** | **15 (1x)** |
| **NFW or WFI Water (various)** | **3.5** |
| **Primers** | **0.6 each (5 pmol each)** |
| **Probe** | **0.3 (5 pmol)** |
| **Reaction Mix Volume** | **20** |
| **Sample Volume / rxn** | **10** |
| **Total Reaction Volume** | **30** |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| **1** | **50 C** | **2:00** | **N/A** |
| **2** | **95 C** | **10:00** | **N/A** |
| **3** | **95 C**<br>**60 C** | **:15**<br>**1:00** | **≥ 40** |

**RESULTS ANALYSIS:**
**Threshold:** 0.2 ΔRn

**Attachment 54** **AMPLICON:** Human GD2 CAR Amplicon

**AMPLICON SEQUENCE:**

CTCTGTGATGATCTCCTGCAAGGCCAGCGGCAGCTCCTTCACCGGCTACAACATGAACTGGGTGCGCCA
GAACATCGGCAAGAGCCTGGAATGGATCG

**AMPLICON SIZE:** 98 bases

**COPY NUMBER/VIRAL VECTOR GENOME:** 1

**FORWARD PRIMER: GD2 Forward Primer** 5' CTCTGTGATGATCTCCTGCAA 3'

**REVERSE PRIMER: GD2 Reverse Primer** 5' CGATCCATTCCAGGCTCTT 3'

**PROBE: GD2 Probe** 5'/5HEX/TCATGTTGT/ZEN/AGCCGGTGAAGGAGC/3IABkFQ 3'

**REAGENTS (and associated BDP and/or manufacturer part numbers):**

**GD2 CAR Primer/Probe set (VIC/HEX) 20X** BDP PN 31260

**RECOMMENDED EXTRACTION KIT:** QIAGEN Viral RNA Mini Kit (BDP PN 30450)

**RECOMMENDED DNaseI ENZYME:** NEB (BDP PN 31243)

**RECOMMENDED DNaseI METHOD:** Elute RNA from Viral RNA Mini Kit in 60.0 µL of Buffer AVE. Combine 39.0 µL 1X PBS, 5.0 µL eluted RNA, 5.0 µL 10x DNase I Buffer and 1.0 µL of DNaseI. Incubate at 37°C for 15 minutes. Add 4.0 µL of RNase-free water and 6.0 µL of 50 mM EDTA and incubate at 75°C for 10 minutes (to inactivate the DNaseI).

**CONTROL GENOMIC DNA:** GD2 RNA Control

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| DEPC-Treated Water or RNase-Free Water | 16.0 |
| 2X Reaction Mix (BDP 30265) | 25.0 (1X Final Concentration) |
| GD2CAR-VIC | 1.0 (200 nM Final Concentration) |
| ROX Reference Dye | 1.0 |
| RNaseOUT | 1.0 |
| SuperScript III RT/Platinum Taq Mix | 1.0 |
| Reaction Mix Volume | 45.0 |
| Sample Volume / rxn | 5.0 |
| Total Reaction Volume | 50.0 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50°C | 15:00 | N/A |
| 2 | 95°C | 2:00 | N/A |
| 3 | 95°C | 00:15 | 40 |
| | 60°C | 01:00 | |

**RESULTS ANALYSIS:**
Threshold: 0.2 ΔRn

**Attachment 55** **AMPLICON:** SV40 Large T Antigen

**AMPLICON SEQUENCE:**

ATAGTGGCTGGGCTGTTCTTTTTTAATACATTTTAAACACATTTCAAAACTGTACTGAAATTCCAAGTACAT
CCCAAGCAATAACAACACATCATCACATTTTGTTTCCATTGCATACTCTGTTACAAGCTTCCAGGACACTT
GTTTAGTTTCCTCTGCTTCTTCTGGATTAAAATCATGCTCCTTTAACCCACCTG

**AMPLICON REGION:** SV40 Large T Antigen

**AMPLICON SIZE:** 197 bases

**SV40 TAg Primer/Probe (FAM) set** BDP PN 31313

**FORWARD PRIMER:** 5' ATAGTGGCTGGGCTGTTCTTT 3'

**REVERSE PRIMER:** 5' CAGGTGGGTTAAAGGAGCATGA 3'

**PROBE:** 5' [FAM] TGA TGA TGT GTT GTT ATT GCT TGG GAT GTA CTT GG [Quencher] 3'

**RECOMMENDED EXTRACTION KIT:** QIAGEN DNA Mini Kit (BDP PN 30442) or QIAGEN Blood Mini Kit (BDP PN 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP PN 31236)

**CONTROL DNA:** SV40 TAg Positive Control Gene Fragment (BDP PN 31314)

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| Universal Master Mix (BDP 30268) | 25.0 (1X Final Concentration) |
| Water | 10 |
| SV40 TAg Primer/Probe Set | 2.5 |
| Reaction Mix Volume | 37.5 |
| Sample Volume / rxn | 12.5 |
| Total Reaction Volume | 50.0 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50°C | 2:00 | N/A |
| 2 | 95°C | 10:00 | N/A |
| 3 | 95°C | 00:15 | 40 |
| | 60°C | 01:00 | |

**RESULTS ANALYSIS:**
Threshold: 0.05 ΔR or Automatic

**Attachment 56 Residual plasmid DNA assay**

**AMPLICON:** b-Lactamase (Ampicillin resistance gene)

**AMPLICON SEQUENCE:**

5'-TTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCC
ATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCC
CAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAA-3'

| | |
|---|---|
| **AMPLICON REGION:** | Ampicillin resistance gene |
| **AMPLICON SIZE:** | 165 bases |
| **COPY NUMBER/GENOME:** | 1 |
| **b-Lactamase Primer/Probe (FAM) set** | BDP PN 31357 |
| **FORWARD PRIMER:** | 5' - TTGTTGCCGGGAAGCTAGAG -3' |
| **REVERSE PRIMER:** | 5'- TTGCACAACATGGGGGATCA - 3' |
| **PROBE:** | 5'- /56- FAM/TCCGGTTCC /ZEN/ CAACGATCAAGGCGA/3IIABkFQ/ -3' |
| **RECOMMENDED EXTRACTION KIT:** | MagNA Pure 24 Total NA Isolation Kit (BDP PN 31236) |
| **CONTROL DNA:** | bLactam_control (BDP PN 31368) |

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| Universal Master Mix (BDP 30268) | 25 (1X) |
| Water | 10 |
| 20x primer/probe set | 2.5 |
| Reaction Mix Volume | 37.5 |
| Sample Volume / rxn | 12.5 |
| Total Reaction Volume | 50.0 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50°C | 2:00 | N/A |
| 2 | 95°C | 10:00 | N/A |
| 3 | 95°C | 00:15 | 40 |
| | 60°C | 01:00 | |

**RESULTS ANALYSIS:**
Threshold: 0.2 or automatic

Positive fragment (bLactam_control) 184bp (BDP PN 31368)
CAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTG
CCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGGTATGGCTTCATTCAGCTCCGGTTCCCAAC
GATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGG

**Attachment 57**

**Virus genome copy assay (RTqPCR)**

**AMPLICON**: hYP218_VH (hYP218 CAR lentivirus VH_CDR3)

**AMPLICON SEQUENCE**: (114 bp)

5'-GCCGCGATAACTCCAAGAACACGCTCTACTTGCAAATGAATAGCCTTAGGGCAGAGGACAC

AGCAGTATACTACTGCGCGCGGAGTACGGCGAACACCAGGTCCACATACTATC-3'

**AMPLICON REGION**: hYP218_VH (CAR lentivirus)

**AMPLICON SIZE**: 114 bases

**COPY NUMBER/GENOME**: 1

**YP218_VH Primer/Probe (HEX(VIC)) set** BDP PN 31342

**FORWARD PRIMER**: 5' – GCCGCGATAACTCCAAGAA-3'

**REVERSE PRIMER:** 5'- GATAGTATGTGGACCTGGTGTTC -3'

**PROBE**: 5'- /5HEX/TTAGGGCAG/Zen/AGGACACAGCAGTAT/3IABkFQ/-3'

**RECOMMENDED EXTRACTION KIT:** **QIAmp Viral RNA kit (BDP 30450)**

**CONTROL RNA**: YP218_RNA control (BDP 31344)

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| One Step RTqPCR kit (30265) 2x mix | 25 (1X) |
| Water | 15.4 |
| 20x primer/probe set | 2.5 |
| ROX reference dye | 0.1 |
| RNAse out (31244) | 1 |
| SuperScript/Taq mix | 1 |
| Reaction Mix Volume | 45 |
| Sample Volume / rxn | 5 |
| Total Reaction Volume | 50.0 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50°C | 15:00 | N/A |
| 2 | 95°C | 2:00 | N/A |
| 3 | 95°C | 00:15 | 40 |
| | 60°C | 00:30 | |

**RESULTS ANALYSIS:**

Threshold: 0.2 or automatic

Positive fragment (YP218_RNA control, BDP PN 31344) 120 bp

TTAGCCGCGATAACTCCAAGAACACGCTCTACTTGCAAATGAATAGCCTTAGGGCAGAGGACACAGCAG

TATACTACTGCGCGCGGAGTACGGCGAACACCAGGTCCACATACTATCTCA

**Attachment 58**

**Virus genome copy assay (RTqPCR)**

**AMPLICON:** STEAP1_VH (STEAP1 CAR lentivirus VH_CDR3)

**AMPLICON SEQUENCE: (92bp)**

5'- CAGTCTGAGAGCGGAAGATACAGCAGTGTATTATTGTGCGCGCGAAAGGAACTATGATTA TGACGAC TATTATTATGCCATGGATTACTGGG-3'

**AMPLICON REGION:** STEAP1_VH (CAR lentivirus)

**AMPLICON SIZE:** 92 bases

**COPY NUMBER/GENOME:** 1

**STEAP1_VH Primer/Probe (HEX(VIC)) set** BDP PN 31350

**FORWARD PRIMER:** 5' – CAGTCTGAGAGCGGAAGATAC-3'

**REVERSE PRIMER:** 5'- CCCAGTAATCCATGGCATAATAATAG -3'

**PROBE:** 5'- /5HEX/TTCGCGCGC/Zen/ACAATAATACACTGC/3IABkFQ/-3'

**RECOMMENDED EXTRACTION KIT:** QIAmp Viral RNA kit (BDP 30450)

**CONTROL RNA:** STEAP1_RNA control(BDP PN 31366)

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| One Step RTqPCR kit (30265) 2x mix | 25 (1X) |
| Water | 15.4 |
| 20x primer/probe set | 2.5 |
| ROX reference dye | 0.1 |
| RNAse out (31244) | 1 |
| SuperScript/Taq mix | 1 |
| Reaction Mix Volume | 45 |
| Sample Volume / rxn | 5 |
| Total Reaction Volume | 50.0 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50°C | 15:00 | N/A |
| 2 | 95°C | 2:00 | N/A |
| 3 | 95°C | 00:15 | 40 |
| | 60°C | 00:30 | |

**RESULTS ANALYSIS:**
Threshold: 0.2 or automatic
Positive fragment (STEAP1_RNA control, BDP PN 31366) 120 bp
GTACCTCCAGATGAACAGTCTGAGAGCGGAAGATACAGCAGTGTATTATTGTGCGCGCGAAAGGAACTA TGATTATGACGACTATTATTATGCCATGGATTACTGGGGTCAGGGCACCCT

**Attachment 59**

**Virus genome copy assay (RTqPCR)**

**AMPLICON:** CD123 VL (CD123xCD3 BiTE retrovirus VL)

**AMPLICON SEQUENCE: (91bp)**

5'-CGAGGACTTTGCCATGTACTATTGCCAGCAGCACAACAAGTACCCTTACACCTTCGGCGGAGGCA CCAAGCTGGAAATCAAGAGCGGAGGG -3'

**AMPLICON REGION:** CD123 VL (CD123xCD3 BiTE retrovirus)

**AMPLICON SIZE:** 91 bases

**COPY NUMBER/GENOME:** 1

**CD123_VL Primer/Probe (HEX(VIC)) set** BDP PN 31369

**FORWARD PRIMER:** 5' – CGAGGACTTTGCCATGTACTATT-3'

**REVERSE PRIMER:** 5'- CCCTCCGCTCTTGATTTCC -3'

**PROBE:** 5'- /5HEX/AACAAGTAC/ZEN/CCTTACACCTTCGGCG/3IABkFQ/-3'

**RECOMMENDED EXTRACTION KIT:** QIAmp Viral RNA kit (BDP 30450)

**CONTROL RNA:** CD123_VL_RNA control (BDP PN 31370)

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| One Step RTqPCR kit (30265) 2x mix | 25 (1X) |
| Water | 15.4 |
| 20x primer/probe set | 2.5 |
| ROX reference dye | 0.1 |
| RNAse out (31244) | 1 |
| SuperScript/Taq mix | 1 |
| Reaction Mix Volume | 45 |
| Sample Volume / rxn | 5 |
| Total Reaction Volume | 50.0 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50°C | 15:00 | N/A |
| 2 | 95°C | 2:00 | N/A |
| 3 | 95°C | 00:15 | 40 |
| | 60°C | 00:30 | |

**RESULTS ANALYSIS:**
Threshold: 0.2 or automatic

Positive fragment (CD123_VL_RNA control, BDP PN 31370) 120 bp
TCAGCTCCCTGGAACCCGAGGACTTTGCCATGTACTATTGCCAGCAGCACAACAAGTACCCTTACACCTT CGGCGGAGGCACCAAGCTGGAAATCAAGAGCGGAGGGGGCGGATCCGACA

**Attachment 60**

**Virus genome copy assay (RTqPCR)**

**AMPLICON:** GPC2Re_VL (GPC2 retrovirus CDR VL)

**AMPLICON SEQUENCE: (88bp)**

5'- CCTCCAGCCTGAAGATGTTGCAACATATTACTGTCAACAGTATGATAATCTCCCGATCACCT

TCGGCCAAGGGACCAAGCTGGAAATC -3'

**AMPLICON REGION:** GPC2 Retrovirus CDR3 VL
**AMPLICON SIZE:** 88 bases
**COPY NUMBER/GENOME:** 1
**GPC2Re_VL Primer/Probe (HEX(VIC)) set** BDP PN 31355

**FORWARD PRIMER:** 5' - CCTCCAGCCTGAAGATGTTG -3'
**REVERSE PRIMER:** 5'- GATTTCCAGCTTGGTCCCTT - 3'
**PROBE:** 5'- /5HEX/ATAATCTCC/ZEN/ CGATCACCTTCGGCC/ 3IABkFQ/-3'

**RECOMMENDED EXTRACTION KIT:** QIAmp Viral RNA kit (BDP 30450)

**CONTROL RNA:** GPC2Re_VL_RNA control (BDP PN 31356)

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| One Step RTqPCR kit (30265) 2x mix | 25 (1X) |
| Water | 15.4 |
| 20x primer/probe set | 2.5 |
| ROX reference dye | 0.1 |
| RNAse out (31244) | 1 |
| SuperScript/Taq mix | 1 |
| Reaction Mix Volume | 45 |
| Sample Volume / rxn | 5 |
| Total Reaction Volume | 50.0 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50°C | 15:00 | N/A |
| 2 | 95°C | 2:00 | N/A |
| 3 | 95°C | 00:15 | 40 |
| | 60°C | 00:30 | |

**RESULTS ANALYSIS:**
Threshold: 0.2 or automatic

Positive fragment (GPC2Re_VL_RNA control, BDP PN 31356) 120 bp
CTTTCACCATCAGCAGCCTCCAGCCTGAAGATGTTGCAACATATTACTGTCAACAGTATGATAATCTCCC
GATCACCTTCGGCCAAGGGACCAAGCTGGAAATCAAACGTGGAGGTGGCG

**Attachment 61**

**Virus genome copy assay (RTqPCR)**

**AMPLICON:** GPC2Lenti_VL (GPC2Lenti CAR lentivirus VL_CDR3)

**AMPLICON SEQUENCE: (102bp)**

5'- TTCTCTCACAATCAGCAGCATGGAGGGTGAAGATGCTGCCACTTATTACTGCCAGCAGTTTT

CTAGTTCCCCATCCACGTTCGGTACTGGGACCAAGCTGGA-3'

| | |
|---|---|
| **AMPLICON REGION:** | GPC2Lenti_VL (CAR lentivirus) |
| **AMPLICON SIZE:** | 102 bases |
| **COPY NUMBER/GENOME:** | 1 |
| **GPC2Lenti_VL Primer/Probe (HEX(VIC)) set** | BDP PN 31378 |

| | |
|---|---|
| **FORWARD PRIMER:** | 5' – TTCTCTCACAATCAGCAGCAT-3' |
| **REVERSE PRIMER:** | 5'- TCCAGCTTGGTCCCAGTA -3' |
| **PROBE:** | 5'- /5HEX/TAAGTGGCA/ZEN/GCATCTTCACCCTCC/3IABkFQ/-3' |

**RECOMMENDED EXTRACTION KIT:** QIAmp Viral RNA kit (BDP 30450)

**CONTROL RNA:** GPC2Lenti_VL_RNA control

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| One Step RTqPCR kit (30265) 2x mix | 25 (1X) |
| Water | 15.4 |
| 20x primer/probe set | 2.5 |
| ROX reference dye | 0.1 |
| RNAse out (31244) | 1 |
| SuperScript/Taq mix | 1 |
| Reaction Mix Volume | 45 |
| Sample Volume / rxn | 5 |
| Total Reaction Volume | 50.0 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50°C | 15:00 | N/A |
| 2 | 95°C | 2:00 | N/A |
| 3 | 95°C | 00:15 | 40 |
| | 60°C | 00:30 | |

**RESULTS ANALYSIS:**
Threshold: 0.2 or automatic

Positive fragment (GPC2Lenti_RNA control, BDP P/N 31379) 120 bp
ACTCTTATTCTCTCACAATCAGCAGCATGGAGGGTGAAGATGCTGCCACTTATTACTGCCAGCAGTTTTC
TAGTTCCCCATCCACGTTCGGTACTGGGACCAAGCTGGAGCTGAAAACTA

**Attachment 62**

**Virus genome copy assay (RTqPCR)**

**AMPLICON:** mTRAC (CD22TCR retrovirus)

**AMPLICON SEQUENCE: (83 bp)**

5'-
ACAAGTGCGTGCTGGATATGAAGGCTATGGACAGCAAGTCCAACGGCGCCATCGCCTGGTCTAATCAGA
CCAGCTTCACATGC -3'

**AMPLICON REGION:** CD22TCR mTRAC (retrovirus)
**AMPLICON SIZE:** 83 bases
**COPY NUMBER/GENOME:** 1
**mTRAC Primer/Probe (HEX(VIC)) set** BDP PN 31459

**FORWARD PRIMER:** 5' – ACAAGTGCGTGCTGGATATG -3'
**REVERSE PRIMER:** 5'- GCATGTGAAGCTGGTCTGATTA -3'
**PROBE:** 5'- /5HEX/ AGGCTATGG/ZEN/ACAGCAAGTCCAACG /3IABkFQ/-3'

**RECOMMENDED EXTRACTION KIT:** QIAmp Viral RNA kit (BDP 30450)

**CONTROL RNA:** mTRAC_RNA control (BDP 31460)

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| One Step RTqPCR kit (30265) 2x mix | 25 (1X) |
| Water | 15.4 |
| 20x primer/probe set | 2.5 |
| ROX reference dye | 0.1 |
| RNAse out (31244) | 1 |
| SuperScript/Taq mix | 1 |
| Reaction Mix Volume | 45 |
| Sample Volume / rxn | 5 |
| Total Reaction Volume | 50.0 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50°C | 15:00 | N/A |
| 2 | 95°C | 2:00 | N/A |
| 3 | 95°C | 00:15 | 40 |
| | 60°C | 00:30 | |

**RESULTS ANALYSIS:**
Threshold: 0.2 or automatic
Positive fragment (mTRAC_RNA control, BDP P/N 31460) 120 bp
CTGGCACCTTTATCACAGACAAGTGCGTGCTGGATATGAAGGCTATGGACAGCAAGTCCAACGGCGC
CATCGCCTGGTCTAATCAGACCAGCTTCACATGCCAGGATATCTTTAAGGAGA

**Attachment 63**

**Virus genome copy assay (RTqPCR)**

**AMPLICON:** BCMA_VL (PRODH2 CAR lentivirus, targeting BCMA VL CDR3)

**AMPLICON SEQUENCE: (81 bp)**

5'- AGGACTTCGCCACCTACTACTGCCAGCAGTACAGGAAGCTCCCCTGGACTTTCGGCCAGG GCACCAAACTGGAGATCAAGC -3'

**AMPLICON REGION:** BCMA_VL
**AMPLICON SIZE:** 81 bases
**COPY NUMBER/GENOME:** 1
**BCMA_VL Primer/Probe (HEX(VIC)) set** BDP PN 31462

**FORWARD PRIMER:** 5' – AGGACTTCGCCACCTACTA -3'
**REVERSE PRIMER:** 5'- GCTTGATCTCCAGTTTGGTG -3'
**PROBE:** 5'- /5HEX/ TGCCAGCAG/ZEN/TACAGGAAGCTCC /3IABkFQ/-3'

**RECOMMENDED EXTRACTION KIT:** QIAmp Viral RNA kit (BDP 30450)

**CONTROL RNA:** BCMA VL RNA control (BDP 31463)

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| One Step RTqPCR kit (30265) 2x mix | 25 (1X) |
| Water | 15.4 |
| 20x primer/probe set | 2.5 |
| ROX reference dye | 0.1 |
| RNAse out (31244) | 1 |
| SuperScript/Taq mix | 1 |
| Reaction Mix Volume | 45 |
| Sample Volume / rxn | 5 |
| Total Reaction Volume | 50.0 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50°C | 15:00 | N/A |
| 2 | 95°C | 2:00 | N/A |
| 3 | 95°C | 00:15 | 40 |
| | 60°C | 00:30 | |

**RESULTS ANALYSIS:**
Threshold: 0.2 or automatic

Positive fragment (BCMA_VL_RNA control, BDP 31463) 119 bp
CCAGCCTGCAGCCCGAGGACTTCGCCACCTACTACTGCCAGCAGTACAGGAAGCTCCCCTGGACTTTC GGCCAGGGCACCAAACTGGAGATCAAGCGTGGTGGAGGAGGTAGCGGAGGA

**Attachment 64**
**AMPLICON:** SV40 gp6 (Large T Antigen)

**AMPLICON SEQUENCE:**

CGTTCCAGGCAATGCTTTAAATAATCTTTGGGCCTAAAATCTATTTGTTTTACAAATCTGGCCTGCAGTGT
TTTAGGCACACTGTACTCATTCATGGTGACTATTCCAGGG

| | |
|---|---|
| **AMPLICON REGION:** | SV40 gp6 gene (Large T Antigen) |
| **AMPLICON SIZE:** | 111 bases |
| **SV40 gp6 Primer/Probe (FAM) set** | BDP PN 31489 |
| **FORWARD PRIMER:** | 5' CGTTCCAGGCAATGCTTTAAATA 3' |
| **REVERSE PRIMER:** | 5' CCCTGGAATAGTCACCATGAAT 3' |
| **PROBE:** | 5' [FAM] TTACAAATC/ZEN/TGGCCTGCAGTGTTT [Quencher] 3' |
| **RECOMMENDED EXTRACTION KIT:** | QIAGEN DNA Mini Kit (BDP PN 30442) or QIAGEN Blood Mini Kit (BDP PN 30444) or MagNA Pure 24 Total NA Isolation Kit (BDP PN 31236) |
| **CONTROL DNA:** | SV40gp6 DNA Control (BDP PN 31490) |

| REAGENT | VOLUME/REACTION (µL) |
|---|---|
| Universal Master Mix (BDP 30268) | 25.0 (1X Final Concentration) |
| Water | 10 |
| SV40 gp6 Primer/Probe Set | 2.5 |
| Reaction Mix Volume | 37.5 |
| Sample Volume / rxn | 12.5 |
| Total Reaction Volume | 50.0 |

**REACTION CONDITIONS:**

| STAGE | TEMP | TIME | REPEATS |
|---|---|---|---|
| 1 | 50°C | 2:00 | N/A |
| 2 | 95°C | 10:00 | N/A |
| 3 | 95°C | 00:15 | 40 |
| | 60°C | 01:00 | |

**RESULTS ANALYSIS:**
Threshold: 0.05 ΔR or Automatic

Positive fragment (SV40gp6 DNA control, BDP PN 31490)

CTCACTGCGTTCCAGGCAATGCTTTAAATAATCTTTGGGCCTAAAATCTATTTGTTTTACAAATCTGGCCT
GCAGTGTTTTAGGCACACTGTACTCATTCATGGTGACTATTCCAGGGGGAAATATTTGAGTTC