TOOLS Epstein-Barr Virus (EBV) miRNA Multiplex RT-qPCR Assay

User Guide

Contents

Introduction

Epstein-Barr virus (EBV) is a γ -herpesvirus involved in human cancers, especially nasopharyngeal carcinoma. The biological processes regulated by EBV miRNA are key to cancer development. EBV BART and BHRF microRNAs may regulate key carcinogenic processes[1,2]. EBV has been identified to contain 44 mature miRNAs of 18-25 nucleotides in length, including 40 EBV BART miRNAs and 4 EBV BHRF1 miRNAs. BART miRNAs are more strongly expressed in EBV-associated epithelial cells than in B lymphocytes. BHRF1 miRNAs ware expressed in all latency types, especially expressed in the type III latency. During latent infection, miRNAs maintain latent infection by expressing a limited number of viral genes, enabling viruses to evade host immune surveillance. Abnormal expression of EBV miRNA may lead to metabolic abnormalities and parthenogenesis of EBV-infected cells through unbalanced signaling pathways[3].

TOOLS EBV miRNA Multiplex RT-qPCR Assay includes designed and validated RT-qPCR primer / probe set targeting 44 respect EBV mature microRNAs (Table 1) that are suggested of crucial and potential roles in EBV related disease[1-5]. This assay system utilizes stem-looped primer to specifically extend the cDNA of target miRNA in reverse transcription (RT) and exclusive designed set for the probe-based qPCR assay to increase detection accuracy. Combined with TOOLSQuant II Fast RT kit and TOOLS Easy 2X probe qPCR mix, TOOLS EBV miRNA Multiplex RT-qPCR Assay System allows rapid, sensitive, and specific detection and quantification of 48 microRNAs in a total RNA sample respectively. TOOLS EBV miRNA Multiplex RT-qPCR Assay also includes the primer/probe sets for U6 and SNORD48 as the normalization controls and two exogenous miRNAs (cel-miR-39-3p, and cel-miR 238-3p) as spike-in controls.

Table 1, EBV MicroRNA Array Arrangement

	1	2	3	4	5	6
A	ebv-miR-BHRF1-1-5p	ebv-miR-BART3-5p	ebv-miR-BART7-5p	ebv-miR-BART11-5p	ebv-miR-BART16-5p	ebv-miR-BART20-3p
B	ebv-miR-BHRF1-2-5p	ebv-miR-BART3-3p	ebv-miR-BART7-3p	ebv-miR-BART11-3p	ebv-miR-BART17-5p	ebv-miR-BART21-5p
C	ebv-miR-BHRF1-2-3p	ebv-miR-BART4-5p	ebv-miR-BART8-5p	ebv-miR-BART12-3p	ebv-miR-BART17-3p	ebv-miR-BART21-3p
D	ebv-miR-BHRF1-3-5p	ebv-miR-BART4-3p	ebv-miR-BART8-3p	ebv-miR-BART13-5p	ebv-miR-BART18-5p	ebv-miR-BART22-3p
E	ebv-miR-BART1-5p	ebv-miR-BART5-5p	ebv-miR-BART9-5p	ebv-miR-BART13-3p	ebv-miR-BART18-3p	cel-miR-39-3p
F	ebv-miR-BART1-3p	ebv-miR-BART5-3p	ebv-miR-BART9-3p	ebv-miR-BART14-5p	ebv-miR-BART19-5p	cel-miR-238-3p
G	ebv-miR-BART2-5p	ebv-miR-BART6-5p	ebv-miR-BART10-5p	ebv-miR-BART14-3p	ebv-miR-BART19-3p	SNORD48
H	ebv-miR-BART2-3p	ebv-miR-BART6-3p	ebv-miR-BART10-3p	ebv-miR-BART15-3p	ebv-miR-BART20-5p	RNU6-1

To ensure optimal results, please read the entire manual before using the reagents and material supplied with this kit.

Component

TOOLS EBV miRNA Multiplex RT-qPCR primer / probe set

Contents	TTH-EBV (10 RT rxn / 20 qPCR rxn)
#48 EBV RT primer mix	20 µl
# 48 qPCR primer/probe plate	60µl/per well X 48 well
Storage: All components of the Kit should be stored at -20°C.	

Recommended relative product

TOOLSQuant II Fast RT Kit

- ToolsQuant II Fast RT Kit is designed for the first strand cDNA synthesis from very tiny amount of total RNA.
- ToolsQuant II Fast RT Kit is optimized for use with 50ng to 2µg of RNA.

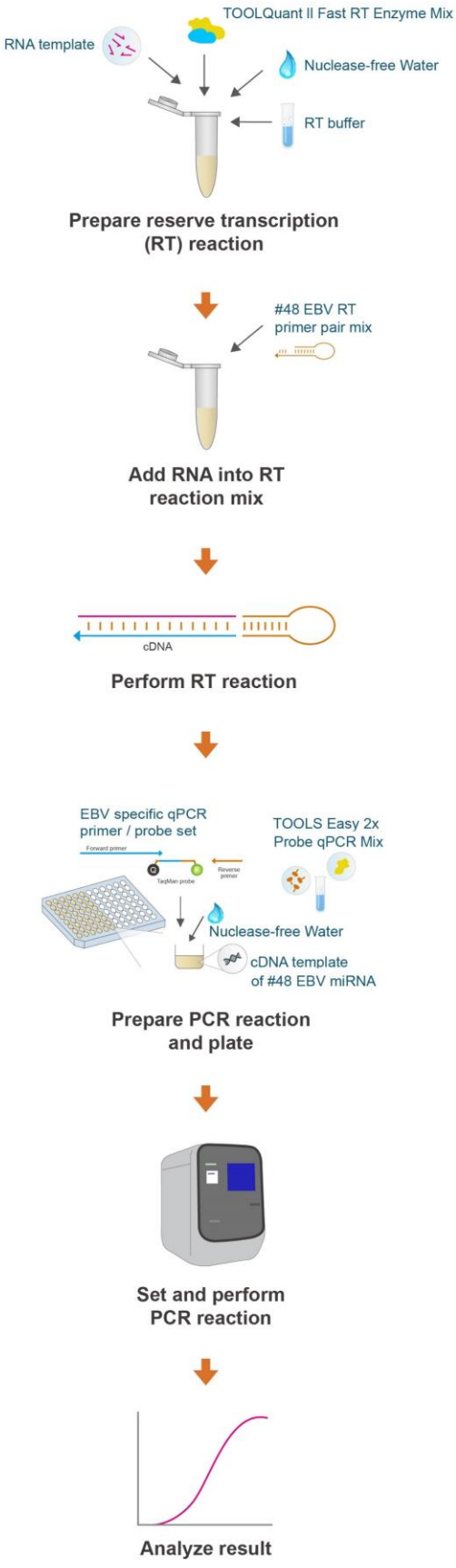
Contents	KRT-BA06 (25 rxn)
5×gDNA Eraser	50 µl
RT Primer Mix	50µl
RT Enzyme Mix	25µl
10×Fast RT Buffer	50µl
RNase- Free ddH2O	1 ml
Storage: All components of the Kit should be stored at -20°C.	

TOOLS Easy 2xProbe qPCR Mix

- The ready-to-load mix contains dNTP/dUTP mix, Mg2+, DNA polymerase, dUTP/UDG anticontamination system, specific ROX Reference Dye, and all components necessary to perform qPCR
- High efficiency and stability

Contents	TTC-QE13 (125 rxn) * 7
TOOLS Easy 2×Probe qPCR Mix	1.25ml
Storage: All components of the Kit should be stored at -20°C.	

Workflow


TOOLQuant II Fast RT Enzyme Mix
RNA template
Nuclease-free Water
RT buffer
Prepare reserve transcription (RT) reaction
#48 EBV RT primer pair mix
Add RNA into RT reaction mix
cDNA
Perform RT reaction
EBV specific qPCR primer / probe set
TOOLS Easy 2x Probe qPCR Mix
Nuclease-free Water
cDNA template of #48 EBV miRNA
Prepare PCR reaction and plate
Set and perform PCR reaction
Analyze result

Protocol

Perform Reverse Transcription

Guidelines for RNA input

- When handling RNA, it is important to use RNase-free plastic ware and reagents.
- Prepare samples using a method that preserves miRNAs
- Aliquot RNAs to minimize freeze-thaw and reduce accidental RNase contamination.
- This protocol is optimized for use with 100ng to 2ug RNA.
- For optimal reverse transcription, input RNA should have the following characteristics:
  - Free of inhibitors of reverse transcription (RT) and PCR.
  - Dissolved in PCR-compatible buffer.
  - Free of RNase activity.

Before you begin

- Prepare pipette, aerosol-resistant pipette tip, cold blocks and ice.
- Prepare RNase-free EP tube and thermal cycler.
- Thaw template RNA on ice. Thaw RT Primer Mix, 10×Fast RT Buffer and RNase-Free ddH2O at room temperature and then place on ice immediately. Vortex and centrifuge all solution briefly to collect residual liquid from the sides of the tubes before use.
- To ensure the exactness of the preparation of the RT reaction, make a master mix and then aliquot it for each reaction tube.

A. Prepare the genomic DNA (gDNA) removal reaction mix

Note: If you want to avoid the contamination of gDNA , please follow the step below of this section. Or skip to the " Prepare the RT Reaction mix" section.

1. Thaw 5×gDNA Eraser, RNase-Free ddH2O at room temperature and then place on ice immediately. Vortex and centrifuge all solution briefly to collect residual liquid from the sides of the tubes before use.
2. Prepare gDNA removal reaction on ice according to table below.

Component	Volume	final conc.
5×gDNA Eraser	2µl	-
Total RNA	Variable	X ul
RNase-Free ddH2O	Variable	Up to 10µl
Total Volume	**10µl**	

3. Mix thoroughly and carefully by vortexing and centrifuge briefly. Incubate for 3 min at 42°C and then store on ice.

B. Prepare the RT Reaction mix

4. Assemble your reaction as follows on ice.
5. Prepare the RT Reaction Mix according to the following components in a sterile RNase-free microfuge tube.

Component	Volume	final conc.
10X Fast RT Buffer	2 µl	2X
RT Enzyme Mix	1 ul	-
#48 EBV RT primer pair mix	2 µl	-
RNA	Variable	X ul
Nuclease-free H2O	Variable	Up to 10µl
Total Volume	**10µl**	

6. Mix the RT Reaction Mix by pipetting gently and spin down briefly. Place on ice.

C. Perform RT experiment

1. Incubate in the thermal cycler:

Step		Temperature	Time	cycle
Reverse Transcription	1	16℃	30 mins	1X
	2	25℃	30 sec	1X
	3	42℃	30 sec	1X
	4	50℃	1 sec	1X
	5	Go back to step 2	-	49X
	6	70℃ ,	10 mins	1X
Stop reaction	7	95°C	5mins	1X
Hold	8	4°C	Hold	-

2. Add 90 µl nuclease-free water into 10µl RT Reaction product for PCR amplification directly.
3. Store reaction product at 4°C within 7 days.

Perform PCR amplification

Guidelines for real-time PCR

- Follow best practices when preparing or performing PCR and prepare the PCR reactions in a clean area for avoiding any artificial templates or high-copy-number templates caused contamination of the

real-time PCR reactions.

- Protect the assays from light and store in a freezer. Excessive exposure to light might reduce efficiency of the fluorescent probes.
- Perform two replicates of each cDNA reaction.

Before you begin

- Prepare pipette, aerosol-resistant pipette tip, optical-grade qPCR tubes, plates, sealing films.
- Thaw 5X qPCR primer/probe plate, vortex briefly, then spin briefly to collect the contents at the bottom of the plate.
- Fμlly thaw the TOOLS Easy 2×Probe qPCR Mix before use, mix well by gentle pipette and avoid direct light.

A. Prepare the Real-time PCR Reaction Mix

1. Prepare the reaction mix and assemble reactions according to the following table:

Component	Volume	final conc.
TOOLS Easy 2×Probe qPCR Mix	7.5 μl	1X
cDNA	1 μl	-
Nuclease-free H2O	3.5 μl	-
Total Volume	**12 μl**	

2. Prepare reaction mixtures 12 μl per well in your qPCR plate. Gently vortex, spin down briefly to collect the contents at the bottom.

B. Prepare the Real-time PCR Reaction

1. Add 3μl of the # 48 5X QPCR primer/probe to each well of a reaction plate.
2. Seal the reaction plate. Mix gently and spin down briefly. Place on ice.

C. Set up and run the real–time PCR program

EBV MicroRNA qPCR, plate Arrangement

	1	2	3	4	5	6
A	ebv-miR-BHRF1-1-5p	ebv-miR-BART3-5p	ebv-miR-BART7-5p	ebv-miR-BART11-5p	ebv-miR-BART16-5p	ebv-miR-BART20-3p
B	ebv-miR-BHRF1-2-5p	ebv-miR-BART3-3p	ebv-miR-BART7-3p	ebv-miR-BART11-3p	ebv-miR-BART17-5p	ebv-miR-BART21-5p
C	ebv-miR-BHRF1-2-3p	ebv-miR-BART4-5p	ebv-miR-BART8-5p	ebv-miR-BART12-3p	ebv-miR-BART17-3p	ebv-miR-BART21-3p
D	ebv-miR-BHRF1-3-5p	ebv-miR-BART4-3p	ebv-miR-BART8-3p	ebv-miR-BART13-5p	ebv-miR-BART18-5p	ebv-miR-BART22-3p
E	ebv-miR-BART1-5p	ebv-miR-BART5-5p	ebv-miR-BART9-5p	ebv-miR-BART13-3p	ebv-miR-BART18-3p	cel-miR-39-3p
F	ebv-miR-BART1-3p	ebv-miR-BART5-3p	ebv-miR-BART9-3p	ebv-miR-BART14-5p	ebv-miR-BART19-5p	cel-miR-238-3p
G	ebv-miR-BART2-5p	ebv-miR-BART6-5p	ebv-miR-BART10-5p	ebv-miR-BART14-3p	ebv-miR-BART19-3p	SNORD48
H	ebv-miR-BART2-3p	ebv-miR-BART6-3p	ebv-miR-BART10-3p	ebv-miR-BART15-3p	ebv-miR-BART20-5p	RNU6-1

Compatible devices:

Applied Biosystems 5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast; StepOne™, StepOnePlus

™ 7500, 7500 Fast, ViiATM7;

Qiagen/Corbett Rotor-Gene® Q, Rotor-Gene® 3000, Rotor-Gene® 6000;

Stratagene MX4000™, MX3005P™, MX3000P™;

Eppendorf Mastercycler® ep realplex, realplex 2 s;

Roche Applied Science LightCycler™ 480 and other instruments

1. Set up the thermal Cycling Parameters according to the following table:

Parameter	Temperature	Time	cycle
Initial denaturation*	95°C	10 min	1x
Denature	95°C	15 sec	45 x
Annealing and extension	60°C	1min	

*The polymerase included in this mix is a hot-start Taq DNA polymerase, and thus, the pre-denaturation stage should be set at 95°C for at least 5 minutes

2. Set the appropriate reaction volume.
3. Load the plate into the real–time PCR instrument.
4. Start the run.

D. Analyze the results

The two strategies for analyzing qPCR data are absolute and relative quantification with respect to the ΔΔCT of the $2^{-\Delta\Delta CT}$ method. Relative quantification uses an internal control (reference gene) and/or a control group (reference group) to quantify the miRNA of interest relative to these references. At a certain threshold during the linear portion of the PCR reaction, the amount of the gene of the interest and the control double each cycle. For detailed information about data analysis, please see the appropriate documentation for your instrument to the crucial steps in qPCR data analysis.

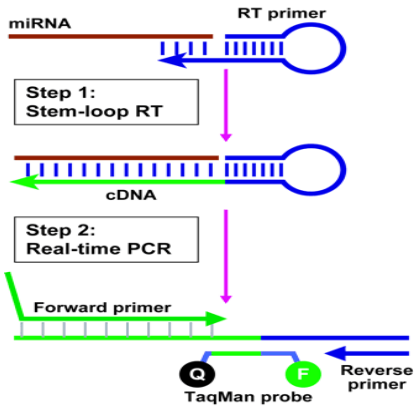
The general guidelines for analysis include:

- Adjusting your Software Output.
- View the amplification plot: if needed: adjust the baseline/threshold (recommended 0.2 threshold value as a starting point) and removing outliers on the estimated quantification.
- Comparing the Ct values (ΔCt) of all the samples replicates between each replicate group.

Supplemental information

About the Methodology of TOOLS miRNA RT-qPCR Assay System

The Tools RT-qPCR Assay System approaches based on stem-loop primer design for the analysis of mature miRNAs, and a miRNA-specific TaqMan probe is employed and the resulting fluorescence is detected. It included two steps: RT reaction and real-time PCR. First, each stem-loop primer specifically binds to an individual mature miRNA target. Then reverse transcribed to cDNA template by reverse transcriptase. Finally, the cDNA templates are quantified by probe-based qPCR.



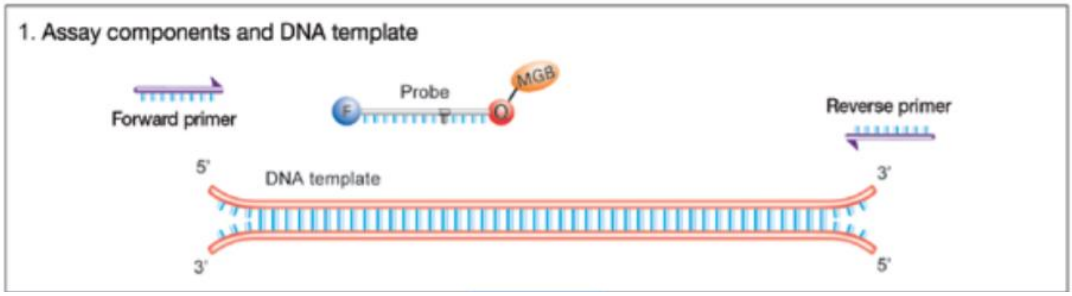
Nucleic acid ds research 33.20 (2005): e179-e179.

TaqMan MGB probes design

- The fluorescent probe was labeled at 5' with FAM (6-carboxyfluorescein) dye as a reporter dye.
- Labelled at the 3' end of the probe with non-fluorescent quencher (NFQ) as a quencher dye enhances spectral performance and maximizes sensitivity.
- Whereas at the 3' end of the probe with a minor groove binder (MGB) moiety at that increase of probe melting temperature (Tm) and stabilizes probe-target hybridization.

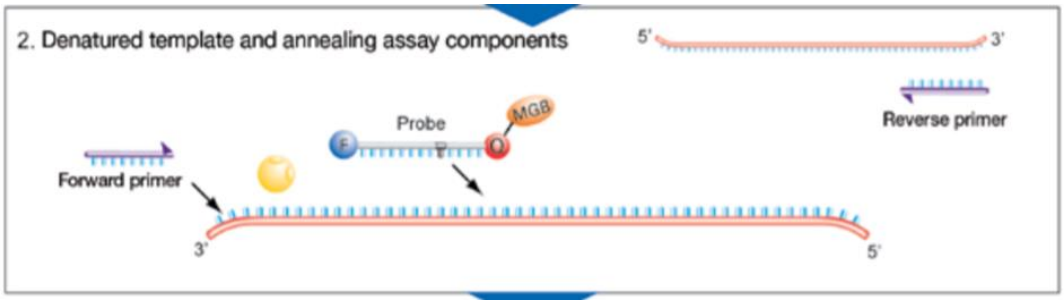
About the 5'-nuclease (TaqMan) assay

The 5'nuclease assay is convenient. Self-contained amplification process. This assay for routine PCR amplification of cDNA uses temperature-dependent Taq DNA polymerases process utilize the 5' → 3' exonuclease activity.



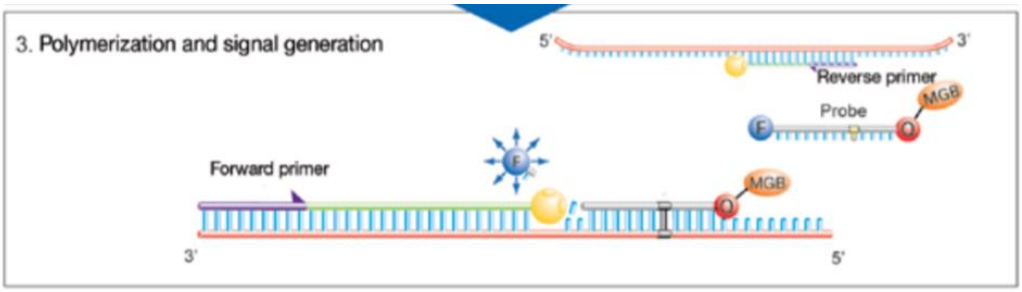
- Initial Denaturation/ annealing step:

- Initial Denaturation: The temperature is raised to denature the double-stranded cDNA are separated into single strands cDNA pool.
- Annealing: The next step is to lower the reaction temperature so that the primer and probe are annealed to a specific target sequence.



- Extension/ amplification step:

  -Extension: The DNA Taq polymerase synthesizes a new complementary DNA strand and a new DNA strand from the existing strand by adding dNTPs to the growing DNA.
- During the step of the amplification reaction, when the probe is hybridized with the target, the probe is cleaved by endogenous 5′ nuclease activity, the reporter gene is separated from the quencher, and the resulting FAM fluorescence signal is proportional to the number of amplified products in the sample.



https://www.thermofisher.com/content/dam/LifeTech/Documents/PDFs/PG1842-PJT3187-COL32661-TaqMan-GeneExpressionAssaySolutions-Brochure-Global-FLRspreads.pdf

Quality Control and Sample Data

A. Real-time qPCR Validation

The EBV qPCR Array plate was tested using a Nasopharyngeal carcinoma cell line (C666-1) RNA samples converted to cDNA using the ToolsQuant II Fast RT Kit The resulting cDNA was tested using 0.5 ul per well. Shown at left is the resulting Real-time amplification plot for the entire plate. The Ct value ranged from 18.22 to 33.07, The experiment was performed as detailed in protocol sections.

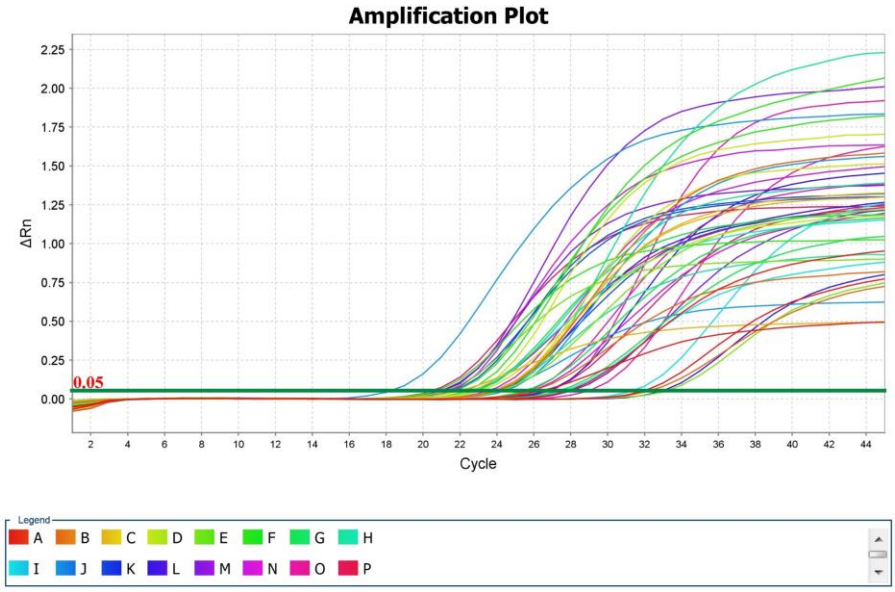


Figure X. Sensitivity of real-time PCR to miRNA copy number.

Using a synthetic RNAs in reverse transcription (RT) reactions as experiment group with a no-template control consisting only of the PCR reaction reagents and nuclease free water (TE) instead of template DNA. followed by qPCR experiment for assessing the usability of an environmental positive and an environmental negative control on the reaction optimization and threshold determination.

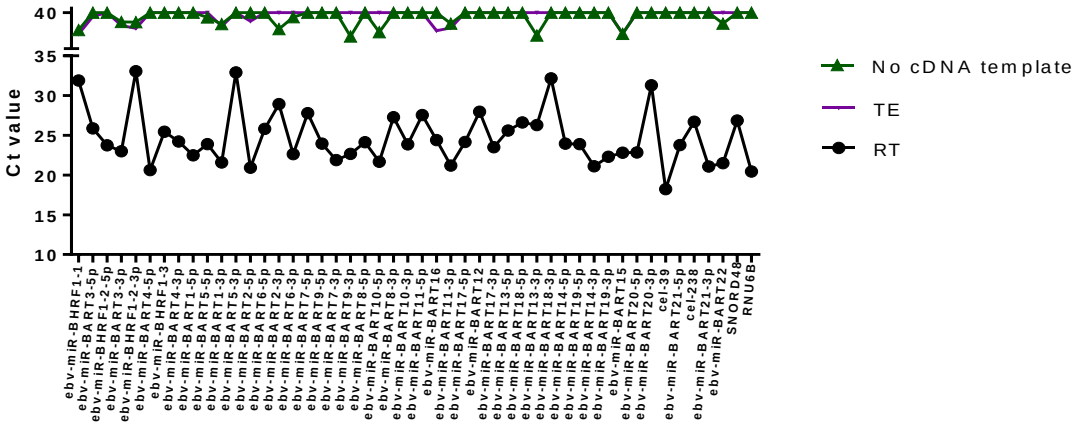


Figure X. No Template Control (NTC) qPCRs include all PCR reagents without the template.

B. Sensitivity Tests

We using a serial dilution of synthetic cDNAs as templates in qPCR experiment to examined the #48 EBV primer/probe mix specificity. The synthetic cDNA templates ($1X10^7$ -$1X10^1$copies) were detected by qPCR reaction. The linear Range and sensitivity fell within the expected range.

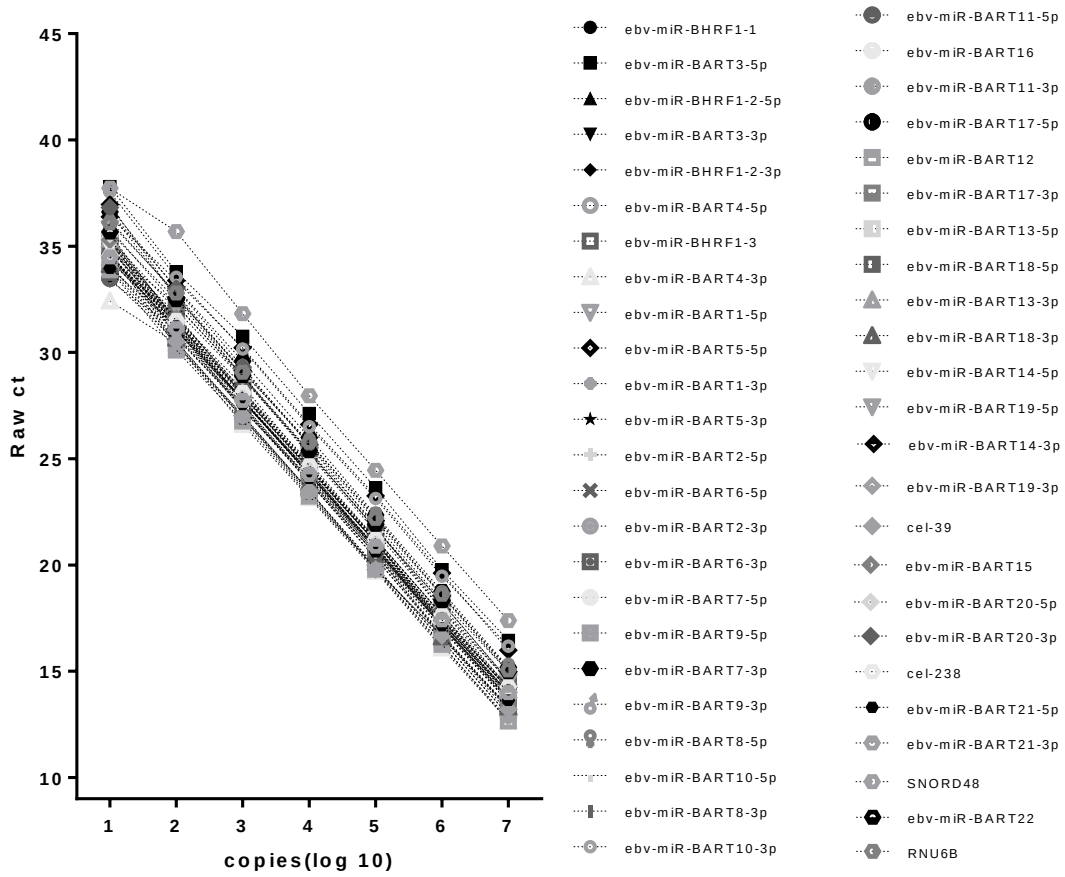


Figure X. High sensitivity with a range of synthetic cDNAs

C. Specificity Tests

We examined the #48 EBV primer Specificity using cross-hybridization of assay, using synthetic cDNAs as templates in qPCR experiment for assessing the corresponding qPCR primer/probe mix. Ct values (the threshold set as 0.2) are relevant to the Control Lot. The target signals fell within the expected range.

The final assay conditions demonstrated no cross-hybridization of each primer.

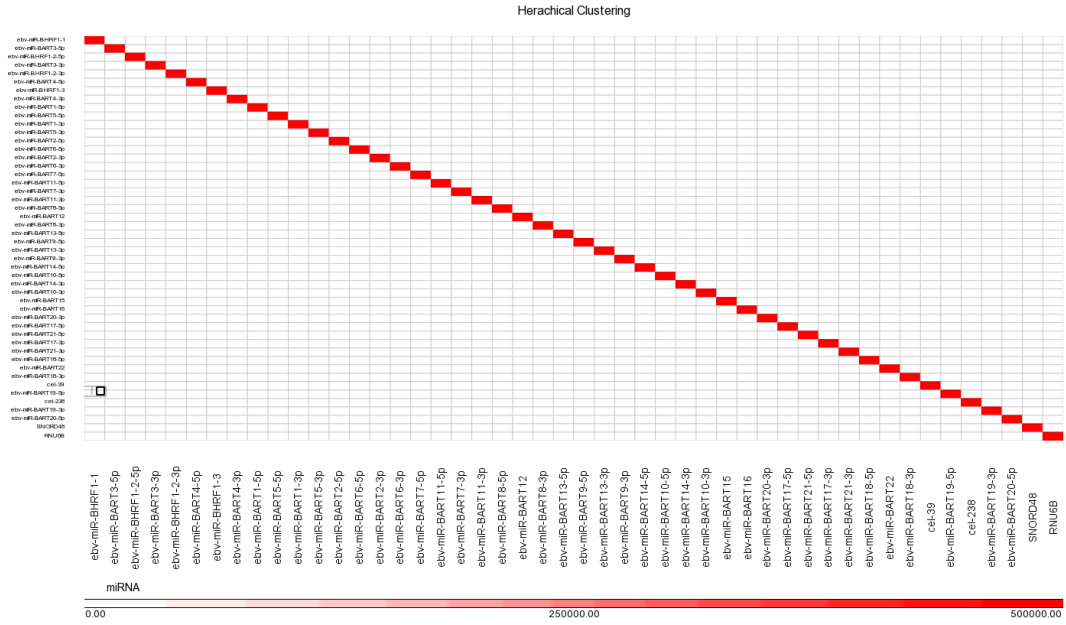


Figure X. Broad linear range and high sensitivity

D. Reproducibility Tests

Two EBV qPCR gene array replicates (plate A and B) were analyzed using C666.1 cell line RNA as start material. The Ct values of the replicate plates were plotted against each other. R square = 0.99 was observed for high inter-array reproducibility.

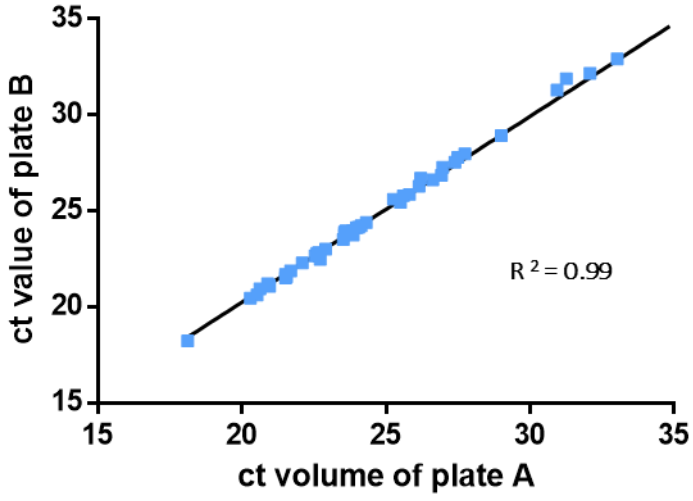


Figure X. High inter-array reproducibility

Troubleshooting & FAQs

Symptoms	Possible cause	Solutions
No signal or no amplification product	Template was degraded.	Verify template integrity by electrophoresis
	Inhibitor was present in sample.	Perform an ethanol precipitation to remove inhibitors.
	Insufficient number of cycles.	Return reactions to thermal cycler for 5 more cycles.
	One or more of the reaction components was not added.	Make sure all the required components have been added.
The amplification curve showed that the sample was not successfully amplified (Ct=40)	Too much or too little template was used.	Verify template concentration by comparing the staining intensity of the template after gel electrophoresis and increase sample input
	The template is degraded.	-Determine the quality of the template. -Rerun the assay with fresh template. -Use RNase-free reagents. -Use an RNase inhibitor.
	There are inhibitors in the reaction.	Ensure the presence of an inhibitor or return the assay with purified template.
	The baseline and/or threshold was improperly set.	Refer to your real-time PCR system's user guide for baseline setting procedures and thresholds.
	The reverse transcription step failed.	-Check for RNA integrity and concentration. -Follow recommended thermal configuration conditions. -Reverse transcription was repeated with the new reagent.
The amplification curves of the target samples with the same detection method show different results	The baseline was set improperly.	Please refer to your real-time PCR system's user guide for baseline setup.
	The reagents or equipment are contaminated.	Be sure that your workspace and equipment are cleaned properly.
	The sample quality was poor.	Check the quality of samples.

Reference

1. Caetano, B.F.R., et al., Epstein-Barr virus microRNAs in the pathogenesis of human cancers. 2021. 499: p. 14-23.
2. Serrano-Solis, Victor, et al. "Prediction of MicroRNAs in the Epstein–Barr Virus Reveals Potential Targets for the Viral Self-Regulation." Indian journal of microbiology 59.1 (2019): 73-80.
3. Kim, Hyoji, et al. "Herpesviral microRNAs in cellular metabolism and immune responses." Frontiers in microbiology 8 (2017): 1318.
4. Tian, Yutong, et al. "miRNAs in radiotherapy resistance of nasopharyngeal carcinoma." Journal of Cancer 11.13 (2020): 3976.
5. Gao, Wei, et al. "Detection of Epstein–Barr virus (EBV)-encoded microRNAs in plasma of patients with nasopharyngeal carcinoma." Head & neck 41.3 (2019): 780-792.

Other information

Table 2. List of EBV mature miRNA RT-qPCR assay

No	cat no	Product name	miRNA ACC	No	cat no	Product name	miRNA ACC
1	TTH-EBV-BHRF1-1	ebv-miR-BHRF1-1-5p	MIMAT0000995	25	TTH-EBV-BART11-5p	ebv-miR-BART11-5p	MIMAT0003421
2	TTH-EBV-BHRF1-2-5p	ebv-miR-BHRF1-2-5p	MIMAT0000996	26	TTH-EBV-BART11-3p	ebv-miR-BART11-3p	MIMAT0003422
3	TTH-EBV-BHRF1-2-3p	ebv-miR-BHRF1-2-3p	MIMAT0000997	27	TTH-EBV-BART12	ebv-miR-BART12-3p	MIMAT0003423
4	TTH-EBV-BHRF1-3	ebv-miR-BHRF1-3-5p	MIMAT0000998	28	TTH-EBV-BART13-5p	ebv-miR-BART13-5p	MIMAT0004818
5	TTH-EBV-BART1-5p	ebv-miR-BART1-5p	MIMAT0000999	29	TTH-EBV-BART13-3p	ebv-miR-BART13-3p	MIMAT0003424
6	TTH-EBV-BART1-3p	ebv-miR-BART1-3p	MIMAT0003390	30	TTH-EBV-BART14-5p	ebv-miR-BART14-5p	MIMAT0003425
7	TTH-EBV-BART2-5p	ebv-miR-BART2-5p	MIMAT0001000	31	TTH-EBV-BART14-3p	ebv-miR-BART14-3p	MIMAT0003426
8	TTH-EBV-BART2-3p	ebv-miR-BART2-3p	MIMAT0004744	32	TTH-EBV-BART15	ebv-miR-BART15-3p	MIMAT0003713
9	TTH-EBV-BART3-5p	ebv-miR-BART3-5p	MIMAT0003410	33	TTH-EBV-BART16	ebv-miR-BART16-5p	MIMAT0003714
10	TTH-EBV-BART3-3p	ebv-miR-BART3-3p	MIMAT0003411	34	TTH-EBV-BART17-5p	ebv-miR-BART17-5p	MIMAT0003715
11	TTH-EBV-BART4-5p	ebv-miR-BART4-5p	MIMAT0003412	35	TTH-EBV-BART17-3p	ebv-miR-BART17-3p	MIMAT0003716
12	TTH-EBV-BART4-3p	ebv-miR-BART4-3p	MIMAT0009204	36	TTH-EBV-BART18-5p	ebv-miR-BART18-5p	MIMAT0003717
13	TTH-EBV-BART5-5p	ebv-miR-BART5-5p	MIMAT0003413	37	TTH-EBV-BART18-3p	ebv-miR-BART18-3p	MIMAT0004835
14	TTH-EBV-BART5-3p	ebv-miR-BART5-3p	MIMAT0009205	38	TTH-EBV-BART19-5p	ebv-miR-BART19-5p	MIMAT0004836
15	TTH-EBV-BART6-5p	ebv-miR-BART6-5p	MIMAT0003414	39	TTH-EBV-BART19-3p	ebv-miR-BART19-3p	MIMAT0003718
16	TTH-EBV-BART6-3p	ebv-miR-BART6-3p	MIMAT0003415	40	TTH-EBV-BART20-5p	ebv-miR-BART20-5p	MIMAT0003719
17	TTH-EBV-BART7-5p	ebv-miR-BART7-5p	MIMAT0004815	41	TTH-EBV-BART20-3p	ebv-miR-BART20-3p	MIMAT0003720

18	TTH-EBV-BART7-3p	ebv-miR-BART7-3p	MIMAT0003416	42	TTH-EBV-BART21-5p	ebv-miR-BART21-5p	MIMAT0010130
19	TTH-EBV-BART8-5p	ebv-miR-BART8-5p	MIMAT0003417	43	TTH-EBV-BART21-3p	ebv-miR-BART21-3p	MIMAT0010131
20	TTH-EBV-BART8-3p	ebv-miR-BART8-3p	MIMAT0003418	44	TTH-EBV-BART22	ebv-miR-BART22-3p	MIMAT0010132
21	TTH-EBV-BART9-5p	ebv-miR-BART9-5p	MIMAT0004816	57	TTH-C39	cel-miR-39-3p	MIMAT0000010
22	TTH-EBV-BART9-3p	ebv-miR-BART9-3p	MIMAT0003419	58	TTH-C238-3p	cel-miR-238-3p	MIMAT0000293
23	TTH-EBV-BART10-5p	ebv-miR-BART10-5p	MIMAT0004817	70	TTH-SNORD48	SNORD48	NR_002745.1
24	TTH-EBV-BART10-3p	ebv-miR-BART10-3p	MIMAT0003420	147	TTH-RNU6-1	RNU6-1	NR_004394.1

*All 48 miRNA can also be ordered individually

TOOLS miRNA RT-qPCR primer / probe set

Contents	TTH-mi50 (50 rxn)	TTH-mi250 (250 rxn)
10X RT primer pair	100 µl	500 µl
10X qPCR primer/probe	300 µl	1500 µl
Storage: All components of the Kit should be stored at -20°C.		