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Japanese encephalitis virus real-time RT-PCR V.1

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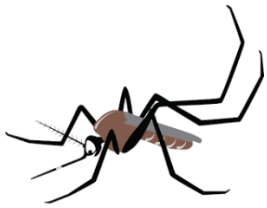

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NOTE: The reverse primer described in this protocol has undergone modification and additional validation since this assay was published in 2014.

Protocol status: Working
We use this protocol and it's working

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Protocol Integer ID: 8594

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Abstract

A real-time RT-PCR using an MGB probe, this assay detects Japanese encephalitis virus (JEV) from human and mosquito samples.
The assay targets the 3'UTR region of known JEV strains.

Guidelines

The concentration of ROX in this method is for the ABI7500 real-time thermocycler. It is not necessary for a Rotor-gene, however we run the assay with it in the recipe on a Rotor-gene machine. Concentration should be adjusted if using a different machine. Check your manufacturers' manual for guidance.

Materials

STEP MATERIALS

SuperScript™ III Platinum™ One-Step qRT-PCR Kit **Life Technologies Catalog #**11732088

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Protocol materials

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Before start

If using a different brand or model of real-time thermocycler, check that the concentration of ROX is adequate.
Method assumes the user is familar with the thermocycler and software used to run the protocol.

Oligonucleotides

1

	Name	5′-3′
	JEVMGBTAQ_For_10486	GTGCTGYCTGCGTCTCAGT
	JEVMGB-Rev2017	GAGACGGTTYTGAGGGCTTTC
	JEVMGB-PROBE_10514	6FAM- ACTGGGTTAACAAATCTGACA-MGB

Reagents

2

SuperScript™ III Platinum™ One-Step qRT-PCR Kit **Life Technologies Catalog #**11732088

Reaction Set-up

3 Assay has been used on both a Rotor-Gene 6000 and a Rotor-Gene Q real-time thermocycler

Prepare sufficient for number of reactions plus a 'dead volume' usually 2 extra. Adjust as necessary if using a robotic dispenser.

	Reagent	Vol (µL) X1	Final reaction concentration
	Nuclease-free water	4.38	
	JEVMGBTAQ_For_10486 (200pmol/µL)	0.06	600nM
	JEVMGB-Rev2017 (200pmol/µL)	0.06	600nM
	JEVMGB-Probe_10514 (100pmol/µL)	0.06	300nM
	2X Reaction Mix [1]	10.0	1X
	ROX reference Dye (25µM) [1,2]	0.04	50nM
	SuperScript™ III/Platinum™ Taq Mix [1]	0.4	
	TOTAL	**15**	

1

Superscript

TM

III Platinum

TM

One-step qRT-PCR kit;

2

See Guidelines

Dispense 15µL to each reaction well.
Add 5µL of template, extracted RNA, controls or NTC (nuclease-free water).
Total reaction volume is 20µL

Amplification

4 RT-PCR

50°C	5min	
95°C	2min	
95°C	3s	40X
60°C	30s*	

*Florescence acquisition step

Result analysis

5 The definition used for a satisfactory positive result from a real-time fluorogenic PCR should include each of the following:
A **sigmoidal curve** – the trace travels horizontally, curves upward, continues in an exponential rise and followed by a curve towards a horizontal plateau phase
A **suitable level of fluorescence** intensity as measured in comparison to a positive control (y-axis)
A defined threshold (C_T) value which the fluorescent curve has clearly exceeded (Fig.1 arrow) and which sits early in the log-linear phase and is <40 cycles
A flat or non-sigmoidal curve or a curve that crosses the threshold with a C_T value >40 cycles is considered a negative result
NTCs should not produce a curve

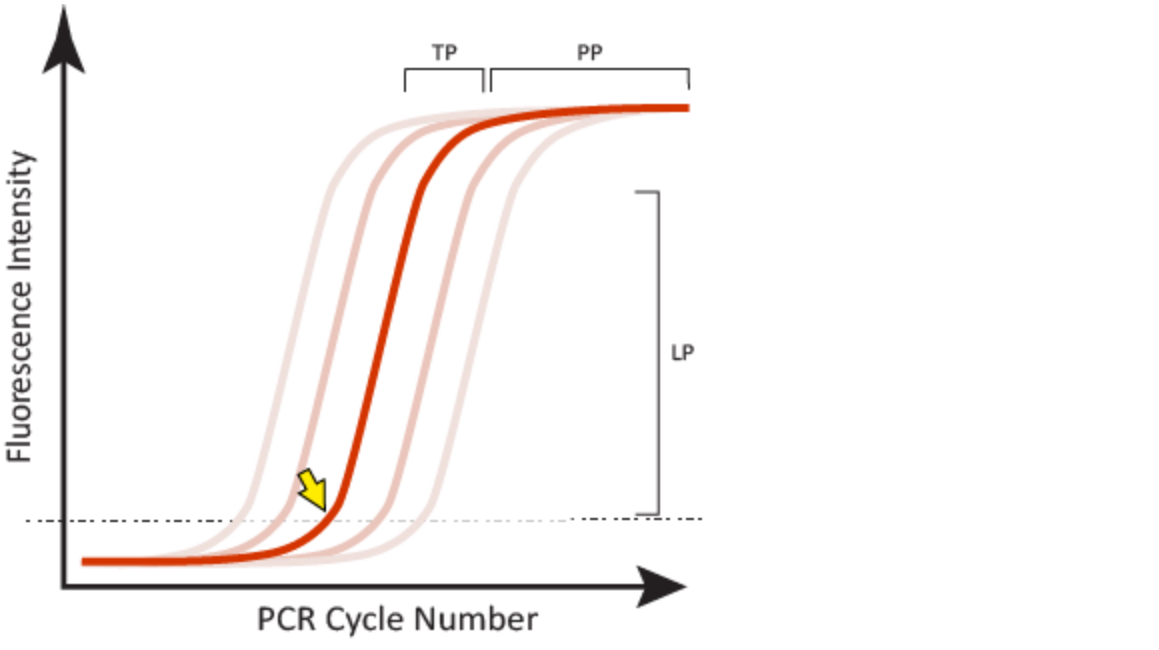

Figure 1

. Examples of satisfactory sigmoidal amplification curve shape when considering an assay's fluorescent signal output. The crossing point or threshold cycle (C

T

) is indicated (yellow arrow); it is the value at which fluorescence levels surpass a predefined (usually set during validation, or arbitrary) threshold level as shown in this normalized linear scale depiction. LP-log-linear phase of signal generated during the exponential part of the PCR amplification; TP-a slowing of the amplification and accompanying fluorescence signal marks the transition phase; PP-the plateau phase is reached when there is little or no increase in fluorescent signal despite continued cycling.