Real time PCR of mouse liver tissue

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Abstract

A real-time PCR to detect RNA in mouse liver tissue. This method has been adapted from a publication by Liu XJ et al 2014, and the oligonucleotides have been modified and a different PCR kit used.

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Before start

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

Protocol

Oligonucleotide sequence of primers for real-time RT-PCR

Step 1.

Genes	Forward (5'-3')	Reverse (5'-3')
18s	CGGCTACCACATCCAAGGAA	GCTGGAATTACCGCGGCT
Acc1	CCGTTGGCCAAAACTCTGGAGCTA A	GAGCTGACGGAGGCTGGTGACA
Fasn	CGCTCGGCTCGATGGCTCAG	CCAGCACCACGGCAT GCTCA
Pparγ	GGGCTGAGGAGAAGTCACAC	TCAGTGGTTCACCGCTTCTT
Cd36	CACAGCTGCCTTCTGAAATGTGTGG	TTTCTACGTGGCCCGGTTCTAATTC
ApoB	AGAGGCCAGTCAAGCTGT TC	GCGTTGGAGTAAGCTCCTGT

Reaction set-up

Step 2.

Assay has been used on
a LightCycler 480 instrument (Roche Diagnostics) using 96-place rotor discs.
Total reaction volume is 20µL.
Prepare sufficient for number of reaction plus a 'dead volume' usually 2 extra. Adjust as necessary if using a robotic dispenser.

Reaction set-up

Step 3.

Reagent	Vol (µL) x1 Final
Green I master	10
Primer (F)	1
Primer (R)	1
Nuclease free water	7
cDNA	1

Reaction set-up

Step 4.

95°C	5min	
95°C	15s	42×
60°C	15s	
72°C	30s	

Reaction set-up

Step 5.

The definition used for a satisfactory positive result from a realtime fluorogenic PCR should include each of the following:

Reaction set-up

Step 6.

A sigmoidal curve – the trace travels horizontally, curves upward, continues in an exponential rise and followed by a curve towards a horizontal plateau phase

Reaction set-up

Step 7.

A suitable level of fluorescence intensity as measured in comparison to a positive control (y-axis)

Reaction set-up

Step 8.

A defined threshold (CT) value which the fluorescent curve has clearly exceeded (Fig.1 arrow) and which sits early in the log-linear phase and is <40 cycles

Reaction set-up

Step 9.

A flat or non-sigmoidal curve or a curve that crosses the threshold with a CT value >40 cycles is considered a negative result

Reaction set-up

Step 10.

NTCs should not produce a curve

Warnings

Don't contaminate cDNA