qPCR (Bio-Rad)

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Abstract

For RNA extraction, the tissues were ground and the total RNA was isolated using NucleoSpin® RNA columns (Macherey-Nagel; Düren, Germany) according to the manufacturer's instructions. RNA was quantified using NanoDrop® spectrophotometer ND-1000 (Thermo Scientific Fisher; Waltham, MA, USA). Total RNA was reverse-transcribed into cDNA with a High Capacity Reverse-Transcription kit (iScript™ cDNA synthesis KIT; Bio-Rad, Hercules, CA, USA). Real-time PCR detection was performed with a SYBR Green supermix (Bio-Rad, Hercules, CA, USA). Gene specific primers were designed and checked using the BLAST algorithm. Gene expression was normalized using the expression of GAPDH, β-Actin and RPS13.

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Materials

NanoDrop spectrophotometer ND-1000 by Thermo Fisher Scientific

NucleoSpin RNA columns 740.955 by Macherey and Nagel

iScriptTM cDNA synthesis kit 170-8841 by BIO-RAD

Protocol

Step 1.

We have to prepare the reagents of the qPCR in the following proportion:

Step 2.

1 ul cDNA

10 ul SuperMix 2x

0.4 ul primer F (20 pmol / ul)

0.4 ul primer R (20 pmol / ul)

8.2 ul H2 O mcl autoclaved

Step 3.

We prepare in a sterile eppendorf tube a MasterMix containing SuperMix, primers and water mQ. We

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make the calculations for 1 more well than we are going to really put.

Step 4.
With 57 ul of the previous MasterMix + 3 ul of cDNA, we prepared a secondary MasterMix for each point of the PCR. Thus the triplicates will be in the same conditions.

Step 5.
Of the 60 ul of the secondary MasterMix, we pipetted 19 ul into each well in triplicate. We must be careful not to leave bubbles that may interfere with the optical measurement of fluorescence.

Step 6.
2 ul H 2 O mQ19ul per well57ul + 3ul of cDNATriplicates...Operating the BioRad iQ5 softwareBefore opening the program, we have to turn on the thermal cycler and the image module. If not, the program will give us an error message. If we are not going to put any PCR and we are only going to work with the software, it is not necessary to start the thermocycler.To insert a PCR

Step 7.
Open the program and select user.

Step 8.
Enter the "Workshop" tab [1] (it is usually activated by default).

Step 9.
Click on the "Protocol" tab [2] and select one of the existing ones [2a] or create a new one [2b] (see below)

Step 10.
Click on the "Plate" tab [3] and select a plate configuration (Plate Setup) already created [3a] or create a [3b] (see below)

Step 11.
When we have selected, both the protocol and the plate, we click on "Run" [4] and opens a new screen.

Step 12.
We have to make sure that this marked "Collect Well Factors ..." [5]

Step 13.
We click on "Begin Run" [6]

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