Protocol for Detection and Analysis of Cyclic RNA Using RT-PCR

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

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ABSTRACT

This protocol describes the design of divergent primers which face away from each other on the linear RNA, so that they can only amplify the circRNAs, and not the linear RNAs with the same sequence.

MATERIALS

1. Standard pipette tips with a volume capacity of 10 µl, 20 µl, 200 µl, and 1 ml
2. Nuclease-free 1.7-ml microcentrifuge tubes
3. Rigid strip 0.2-ml PCR tubes
4. Optical 384-well reaction plate
5. Optical adhesive film
6. Phosphate-buffered saline (DPBS)
7. Total RNA isolation-miRNeasy Mini Kit
8. (Optional) TRIzol reagent
9. Nuclease-free water
10. RNase inhibitor (40 U/µl)
11. RNase R
12. dNTP mix (10 mM each)
13. Random primers (150 ng/µl)
14. Maxima reverse transcriptase
15. FAST ABI prism 2x qPCR master mix
16. Quick Gel Extraction Kit
17. TBE Buffer, 10x, Molecular Biology Grade
18. 1 Kb Plus DNA Ladder
19. Agarose
20. Ethidium bromide solution
21. 2% agarose gel

Experiment Summary

1 This protocol describes the design of divergent primers which face away from each other on the

linear RNA, so that they can only amplify the circRNAs, and not the linear RNAs with the same sequence. The PCR amplicon for the detection of circRNAs using divergent primers spans the backsplice junction of circRNAs. This method has been successfully used in several studies for the detection and quantification of circRNAs.

Equipment

2 Manual pipettes (set of 2 µl, 20 µl, 200 µl and 1,000 µl), cell scraper, vortex mixer, UV transilluminator, microvolume UV-Vis spectrophotometer, PCR strip tube rotor, mini centrifuge C1201, 96-well thermal cycler, OwlTM EasyCastTM B1 mini gel electrophoresis systems, gel imaging system, MPS 1000 mini plate spinner, QuantStudio 5 eal-Time PCR system.

Procedure

3 A. Divergent primer design

1. Get the mature sequence of circular RNA (https://genome.ucsc.edu/) using the genomic coordinates.

2. As shown in Figure 1, make the PCR amplicon template by joining the 100 nt sequence from the 3' end to 100 nt sequence at the 5' end of the circRNA.