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The Education, Scholarships, Apprenticeships and Youth Entrepreneurship Programme – EEA Grants 2014-2021

#8 cDNA SYNTESIS AND GENE EXPRESSION ANALYSIS THROUGH RT-PCR

Research Center for Advanced Medicine – Medfuture (UMFIH) can perform molecular assays based on RNA, DNA or proteins in order to evaluate the expression and the modification of these molecules after different treatments or stimuli or as potential biomarkers for diseases. Specifically, gene expression at RNA level is analysis through RT-PCR, method that quantifies the expression of a specific mRNA based on selected primers. More info can be found at: https://medfuture.umfcluj.ro/.

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Synthesis of complementary DNA (cDNA) and RT-PCR are the standard protocol in evaluating gene expression at RNA level by using specific primers for the gene of interest. The expression is normalized to housekeeping genes and is compared between various samples depending on the specifics of the experiment (e.g. TP53 expression before and after cancer cell treatment).

IMPORTANT!

- Both cDNA synthesis and RT-PCR are done with commercial kits and the protocol can vary depending on the manufacturer's instructions.

1.1. Processing of RNA samples

a. Bring the RNA samples to the same concentration
b. Prepare the DNAse treatment mix in equal volumes for each of the RNA samples (master mix for all the samples that will be divided between all of them equally; calculate the volumes for three extra samples to cover the mix that is lost through repeated pipeting)
c. DNAse treatemt mix: 1 uL DNase Buffer, 0.5 uL Turbo Dnase, 0.25 uL RNAse inhibitor
d. In 0.2 ml tubes add 10ul of RNA and 1.75 ul Dnase treatment mix
e. Incubate the samples at 37°C for 30 minute
f. Add 2 ul of DNAse inhibitor and vortex
g. Incubate the samples at room temperature for 5 minutes
h. Centrifuge the samples at 14.000xg for 2 minutes, at room temperature
i. Transfer 11ul of supernatant in a 0.2 ml sterile tube.

1.2. Synthesis of cDNA

a. 10 uL of RNA from the previous step is mixed with 10 uL of the reaction mix from the reverse transcription kit that is prepared as follows: 2 uL RT Buffer + 0.8 uL dNTP (20X) + 2 uL Random primers (10X) + 1 uL Multiscribe RT + 0.25 uL RNAse inhibitor + 3.95 uL ultrapure water
b. The sample mixture + reaction mix is incubated in the thermocycler using the following program: 10 minutes at 25°C, followed by 120 minutes at 37°C, 5 minutes at 85°C and finally 4°C set for indefinitely time until the samples are collected from the equipment.

1.3. Preparation of primers for gene expression analysis

For a stock concentration of 100 µM, add 10 µL of ultrapure water for every 1 nmol of material (eg 19.4 nmol = 194 uL ultrapure water). For working solutions of 10 µM use a 10X dilution for the above primers.

a. Centrifuge the tube of lyophilized primers at 8000xg for 10 seconds
b. Add ultrapure water (as mentioned above)
c. Incubate at room temperature for 2 minutes
d. Incubate for 10 minutes at 60°C
e. Centrifuge for 30 seconds at 8000xg
f. Store the primers at 100 uM and/or prepare the working solution.

1.4. Analysis of gene expression through RT-PCR

IMPORTANT!

- The following protocol is based on SyBr green reaction mixture

a. Prepare the master mix as follows: 10 uL SyBr Green Master Mix + 0.1 uL Primer Left (100 uM) + 0.1 uL Primer Right (100 uM) + 8.8 uL H2O
b. For each determination, mix 19 µL of Master Mix with 1 µL of previously obtained cDNA and run the samples using the folowing RT-PCR program

50°C 2 minutes	Hold
95°C 2 minutes	
95°C 1 second	X 40 cycles
60°C 30 seconds	

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