University of Al-Maarif

Medical Laboratory Technique Department.

2nd. Stage

Practical Molecular Biology

cDNA Synthesis

The cDNA was created by cDNA ready to use kit (Bioneer, Korea) by

1. Eighteen microliter of RNA extract was added to microfuge tube
2. Two microliters of hexamer primer (for prokaryotic cells) or oligo dt (for eukaryotic cells) was added and then mixed well
3. the mixture was incubated in PCR with the following circumstance , 37 ºC for 10 min followed 42 ºC for 1hr and 95 ºC for 5-10 min in one cycle .
4. Synthesized cDNA was immediately used as template for qRT-PCR or for long-term storage at -20°C.

Quantitative reverse transcription-PCR (qRT-PCR)

Real time PCR amplification as follows :

1- two microliter of cDNA was added to PCR tube.

2- One microliter of each primers was added .

3- The volume was completed to 20µl with DNase free distilled water.

4- The mixture was mixed well and put in the qPCR machine. Table below reveals the PCR condition

Step	Temperature (°C)	Duration	Cycles
Initial	95	3min	1

Denaturation			
Denaturation	95	15sec	40
Annealing	55	45sec	
Extension	72	60sec	

Calculation the fold of gene expression

Levak equation was used to evaluate the fold expression against house keeping gene and control by the following steps

Ct Control – Ct house keeping control = ΔCt control

Ct sample – Ct house keeping sample =ΔCt sample

Δ Ct sample – Δ Ct control = ΔΔ Ct

Fold of gene expression = $(2^{-}{}_{\Delta\Delta Ct})$