

# Real-time PCR

---

## Objective:

To relatively quantitate gene A expression in 30 cell lines.

## Starting Materials

30 different cell pellets.

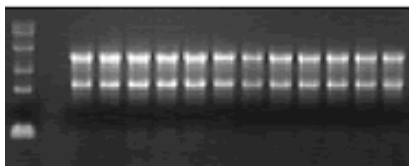
## Methods:

1. Total RNA extraction
2. RNeasy Mini Kit (Qiagen, Cat. No.: 74104) was used to purify total RNA from 30 cell lines.
3. Reverse Transcription
4. 2µg of total RNA was subject to reverse transcription per instruction of Taqman Reverse Transcription Reagents (Appliedbiosystems, Part No.: N808-0234).
5. Cycle program information: 25°C 10min, 48°C 30min, 95°C 5min, then kept at 4°C.
6. Real-time PCR
7. 2 Taqman(R) Gene Expression Assays mixes (Part No.: 4331182) labeled with FAM were ordered from Appliedbiosystems for targeting GAPDH (Assay ID: Hs00266705\_g1) and gene A (Assay ID: xxxxx), respectively.
8. 2.5µl of reverse transcription product was used for real-time PCR setup per instruction of Taqman Universal PCR Mastermix, 2X (Appliedbiosystems, Part No.: 4324018).
9. Cycle program information: 50°C 2min; 95°C 10min; (95°C 15sec, 60°C 1min) X 40
10. Data analysis
11. All real-time data was analyzed by comparative CT method and normalized to sample 1.

## Results:

1. Total RNA extraction  
All RNA samples showed good quality on 1%, 1X TBE agarose gel (Figure 1).

**Figure 1.** Total RNA isolation from 30 cell line samples (Partial)



2. Relative Quatitation (Table I, Partial)

Sample	Ct/gapdh	Ct/sept9	RQ
1	19.92242	21.17042	1
	19.89442	21.20604	
	19.87039	21.22282	
	19.83984	21.18611	
	19.83572	21.19677	
	19.81612	21.20374	

800.557.ACGT (2248)  
847.520.9162 *phone*  
847.520.9163 *fax*  
heron\_yu@acgtinc.com

35 Waltz Drive  
Wheeling, Illinois 60090  
dnaseq@acgtinc.com  
www.acgtinc.com

Sample	Ct/gapdh	Ct/sept9	RQ
2	20.7133	21.29042	1.53817644
	20.60758	21.35357	
	20.71035	21.35973	
	20.61213	21.40312	
	20.65307	21.39596	
	20.65166	21.42495	

**Table I.** Relative quantitation of gene A in different cell lines (partial)

**Conclusions**

All 30 cell pellet samples submitted for Real-time PCR were successfully analyzed. Results of graphics illustrating copy number detection per cycle of the PCR reaction, fluorescence values and calculated copy number values in spreadsheet format have been submitted to the client.