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1400 Page Mill Road
Palo Alto, CA 94304

Quantitative PCR Analysis Report

To: Customer

Date

Sample: A, B, E, F, L, K RNA

Target Gene: Gene1, GENE2, Gene3, GENE4

Materials and methods:

Oligonucleotide Primers Design

The DNA sequence was evaluated using the Primer Express software.

Primer sequences:

Homo GENE2-F	CCTTTGATGGACCAATTACCATAAC
Homo GENE2-R	AGCCGCTGCTCACGTCAT
Homo GENE1-F	AGCCCCTTCAGGATTTCCA
Homo GENE1-R	CAAAAGGATGTATTGACCCAAATTC
Homo GENE4 F	TGGGAAGGTCGCTGCTATTG
Homo GENE4 R	ACTCGTCCATCACCTCAAAGC
Homo GENE3-F	CCGCACAGACAAGCCTTACTC
Homo GENE4-R	GAAGAACAAAGCAGAGAGGAACAGA

Two-step RT-PCR

RNA samples were reverse-transcribed for 120 min at 37°C with High Capacity cDNA Reverse Transcription Kit according to the standard protocol of the supplier (Applied Biosystems). Quantitative PCR was performed by the condition: 10 min at 95°C, and 40 cycles of 15 sec at 95°C, 1 min at 60°C using 2X Power SYBR Green PCR Master Mix (Applied Biosystems) and 200 nM of forward and reverse primers.

Each assay was run on an Applied Biosystems 7300 Real-Time PCR system in triplicates and expression fold-changes were derived using the comparative C_T method.

Relative Quantification: using **GAPDH** as endogenous control

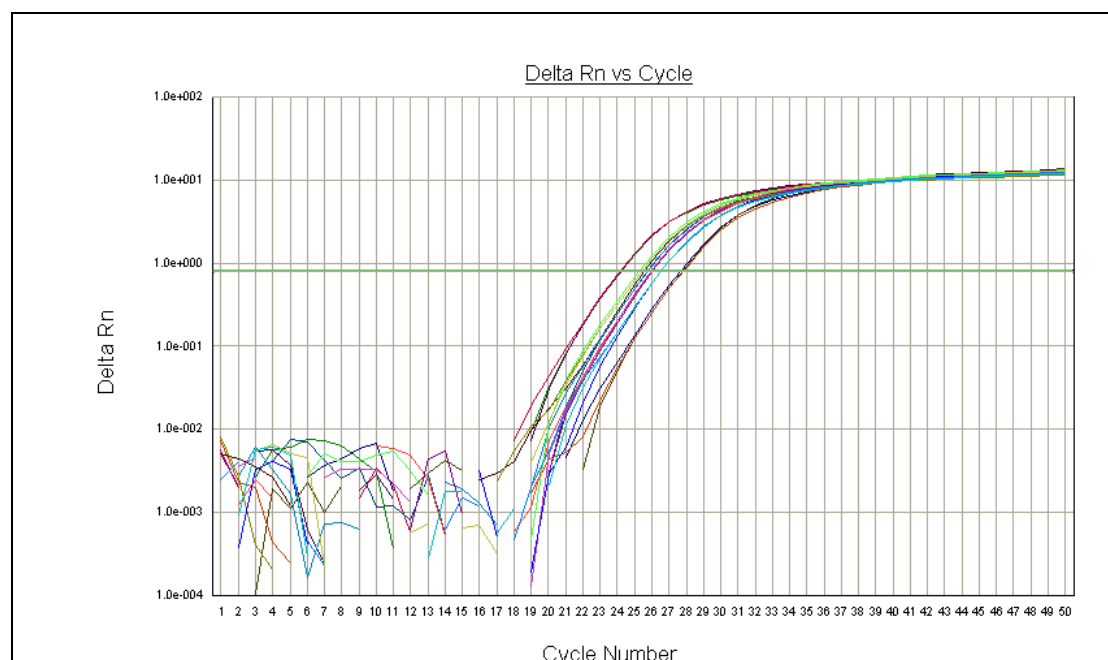
Result:

Relative quantitation using the comparative C_T method

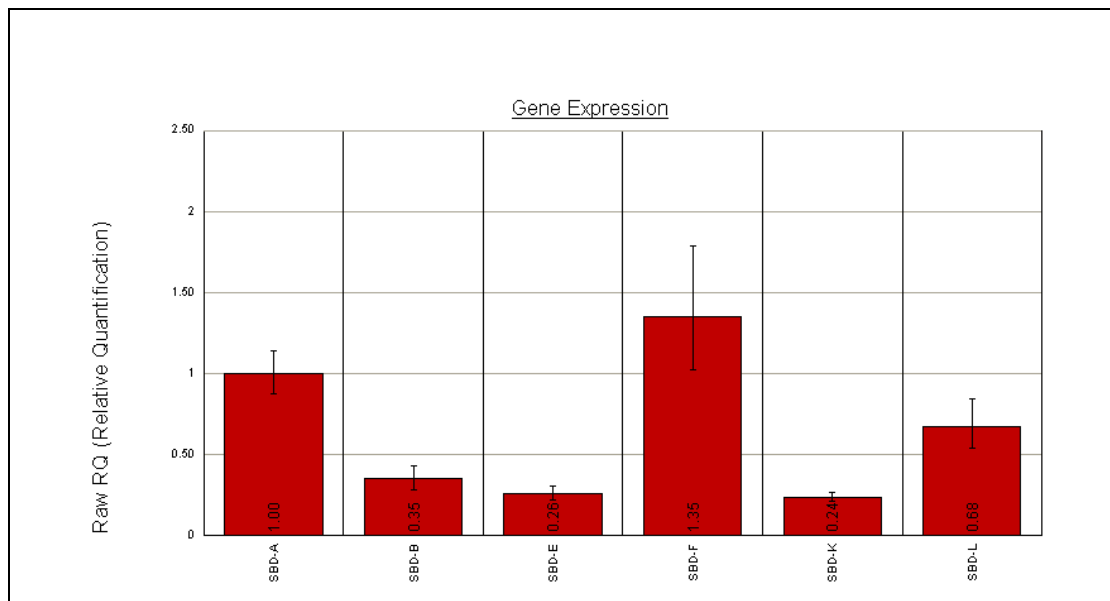
1. Gene1

Sample	Target Average C_T	GAPDH Average C_T	ΔC_T Target - GAPDH	$\Delta \Delta C_T$ $\Delta C_T - \Delta C_{T,-A}$	Rel. to -A
-A	23.73±0.01	19.86±0.06	3.87±0.06	0.00±0.06	1.00
-B	25.55±0.02	20.18±0.10	5.37±0.10	1.50±0.10	0.35
-E	27.27±0.04	21.47±0.07	5.80±0.08	1.93±0.08	0.26
-F	24.94±0.06	21.51±0.13	3.43±0.14	-0.44±0.14	1.35
-L	25.24±0.04	20.82±0.10	4.42±0.11	0.55±0.11	0.67
-K	26.11±0.01	20.19±0.05	5.92±0.05	2.05±0.05	0.24

Gene1 Amplification plot:



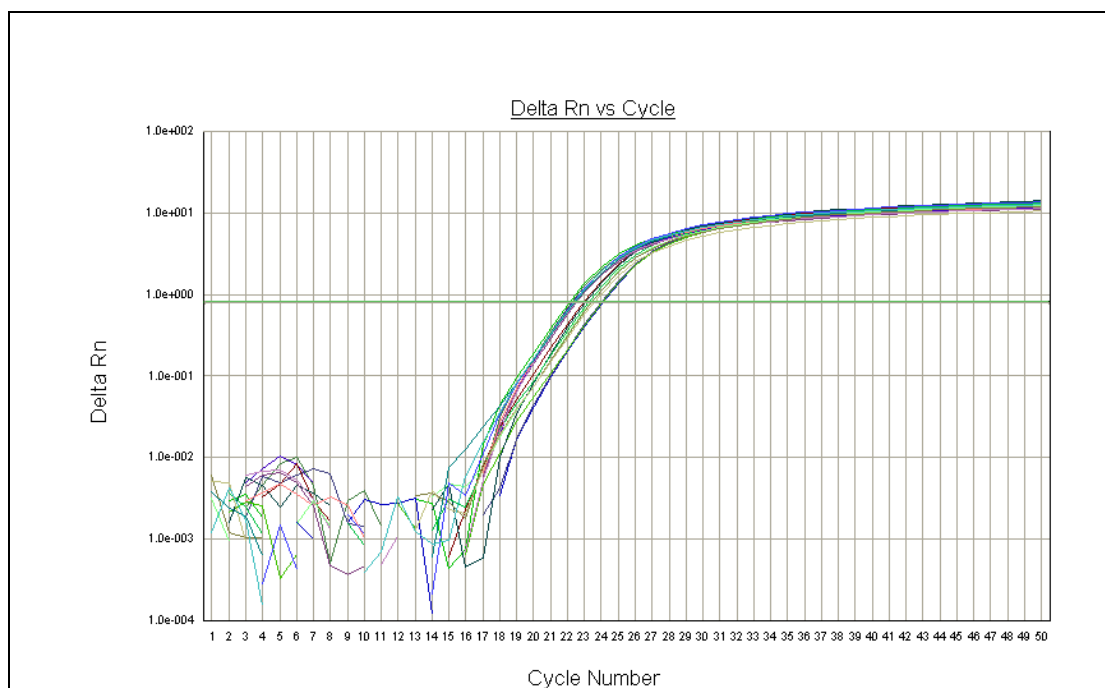
Gene1 Gene expression plot:



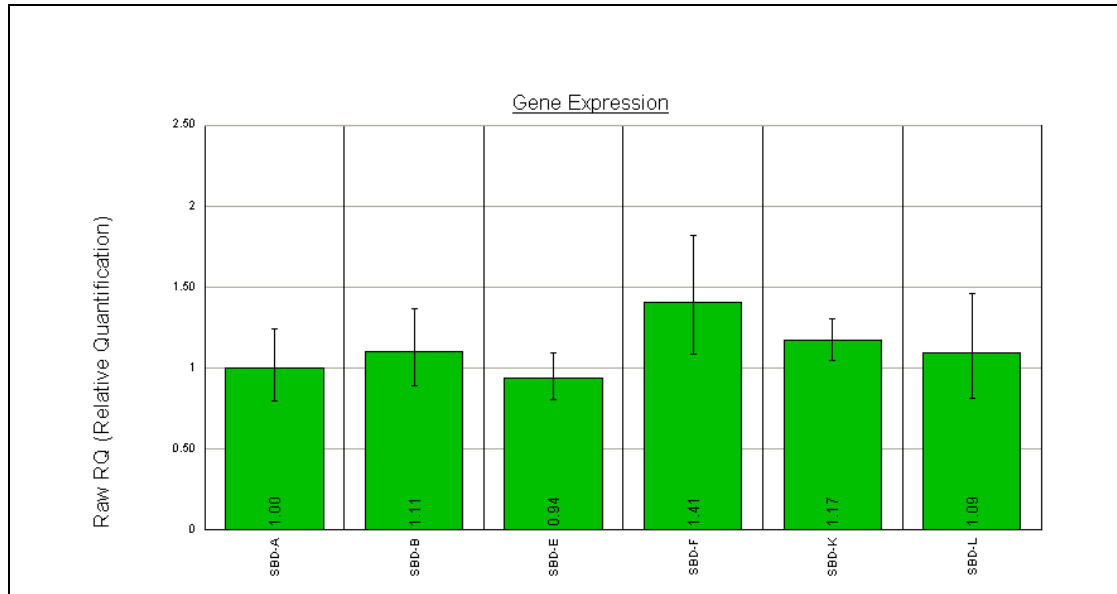
2. GENE2

Sample	Target Average C_T	GAPDH Average C_T	ΔC_T Target - GAPDH	$\Delta \Delta C_T$ $\Delta C_T - \Delta C_{T,-A}$	Rel. to -A
-A	19.97±0.09	19.86±0.06	0.11±0.11	0.00±0.11	1.00
-B	20.14±0.03	20.18±0.10	-0.04±0.11	-0.15±0.11	1.10
-E	21.67±0.02	21.47±0.07	0.20±0.08	0.09±0.08	0.94
-F	21.12±0.02	21.51±0.13	-0.39±0.13	-0.50±0.13	1.41
-L	20.79±0.10	20.82±0.10	-0.03±0.15	-0.14±0.05	1.09
-K	20.06±0.01	20.19±0.05	-0.13±0.05	-0.24±0.05	1.17

GENE2 Amplification plot:



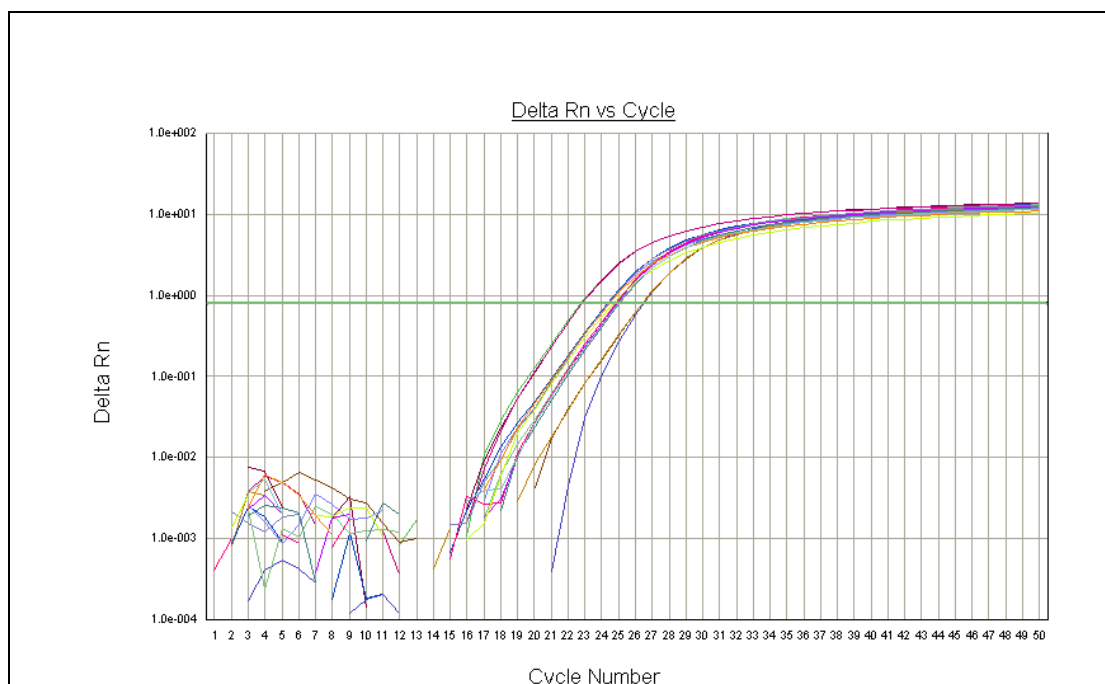
GENE2 Gene expression plot:



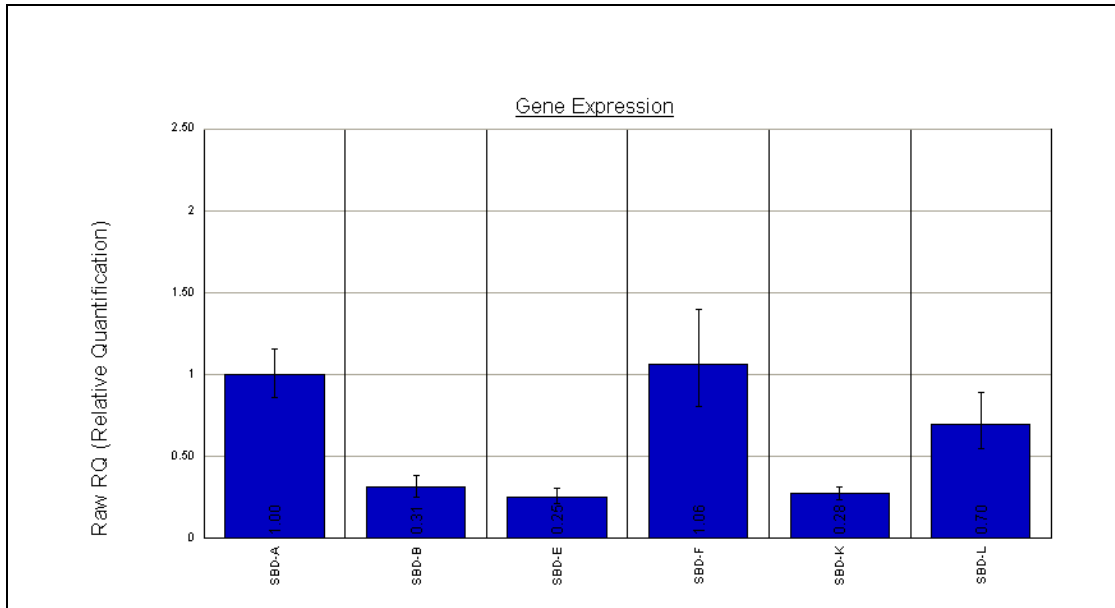
3. Gene3

Sample	Target Average C _T	GAPDH Average C _T	Δ C _T Target - GAPDH	Δ Δ C _T Δ C _T Δ C _T -A	Rel. to -A
-A	21.86±0.03	19.86±0.06	2.00±0.07	0.00±0.07	1.00
-B	23.85±0.04	20.18±0.10	3.67±0.11	1.67±0.11	0.31
-E	25.44±0.06	21.47±0.07	3.97±0.09	1.97±0.09	0.25
-F	23.42±0.05	21.51±0.13	1.91±0.14	-0.09±0.14	1.06
-L	23.33±0.03	20.82±0.10	2.51±0.11	0.51±0.11	0.70
-K	24.04±0.03	20.19±0.05	3.85±0.06	1.85±0.06	0.27

Gene3 Amplification plot:



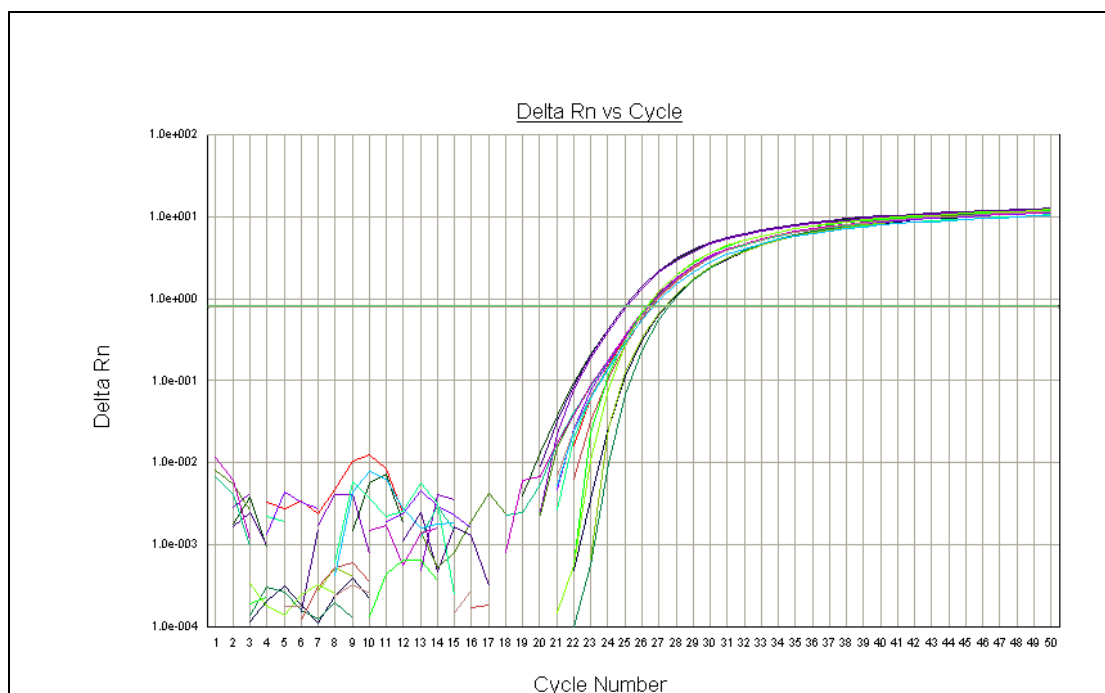
Gene3 Gene expression plot:



4. GENE4

Sample	Target Average C _T	GAPDH Average C _T	Δ C _T Target - GAPDH	Δ Δ C _T Δ C _T Δ C _T -A	Rel. to -A
-A	23.72±0.04	19.86±0.06	3.86±0.08	0.00±0.08	1.00
-B	25.03±0.03	20.18±0.10	4.85±0.10	0.99±0.10	0.50
-E	26.20±0.08	21.47±0.07	4.73±0.11	0.87±0.11	0.54
-F	25.15±0.06	21.51±0.13	3.64±0.14	-0.22±0.14	1.16
-L	25.12±0.08	20.82±0.10	4.30±0.13	0.44±0.13	0.73
-K	25.22±0.03	20.19±0.05	5.03±0.06	1.17±0.06	0.44

GENE4 Amplification plot:



GENE4 Gene expression plot:

