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Supplementary data

Identification of stable reference genes for quantitative PCR in jute under different experimental conditions: an essential assessment for gene expression analysis

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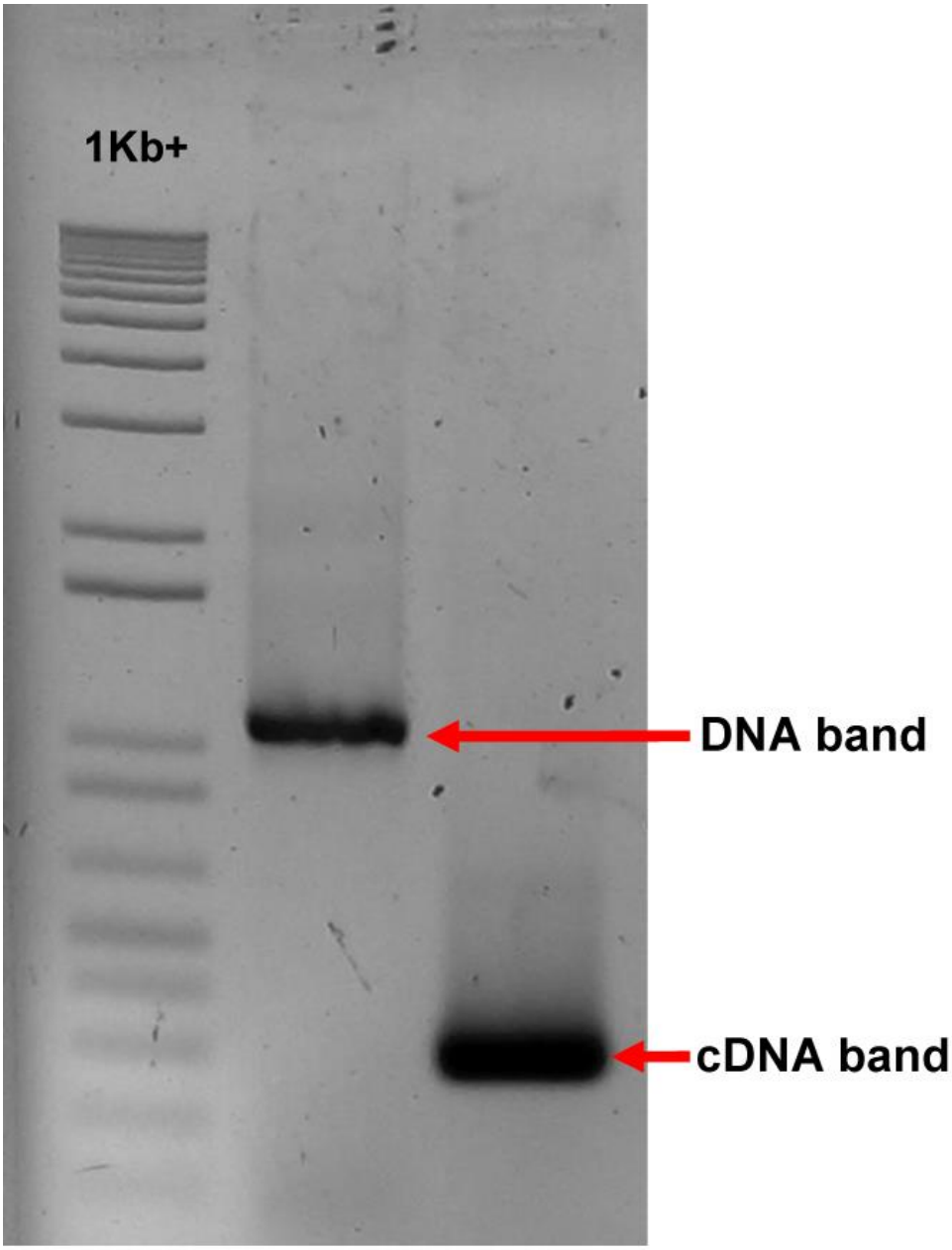
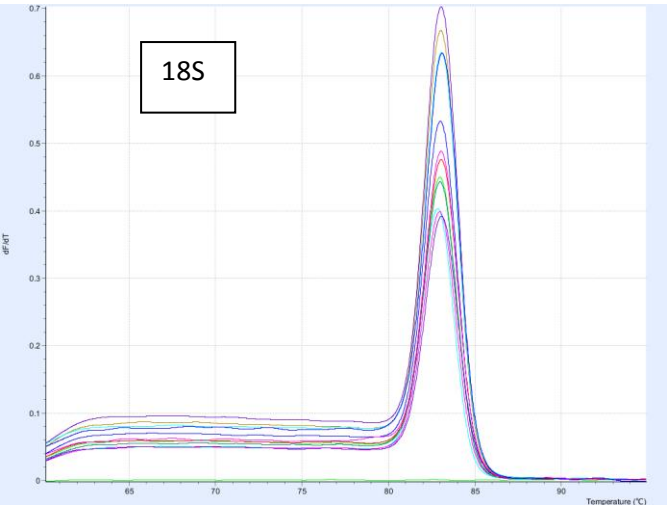
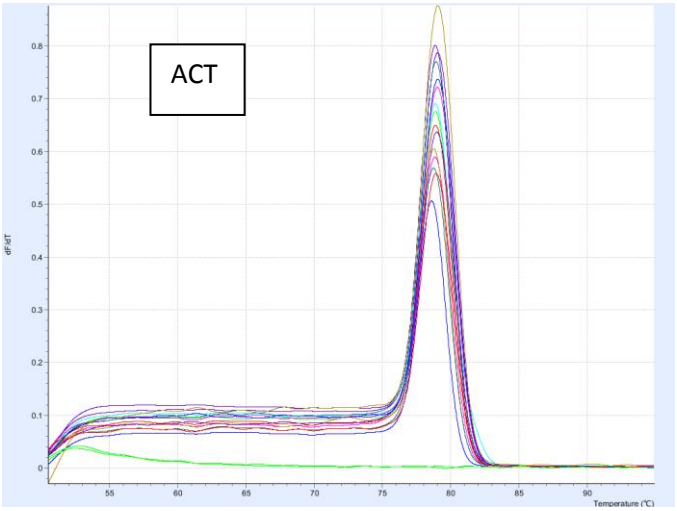
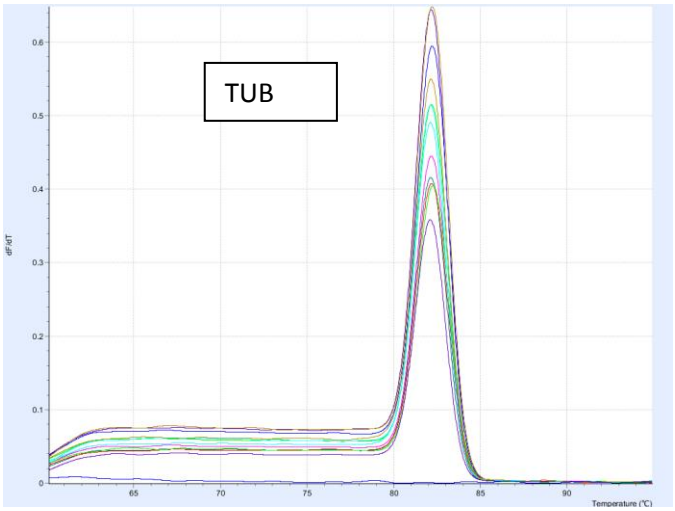


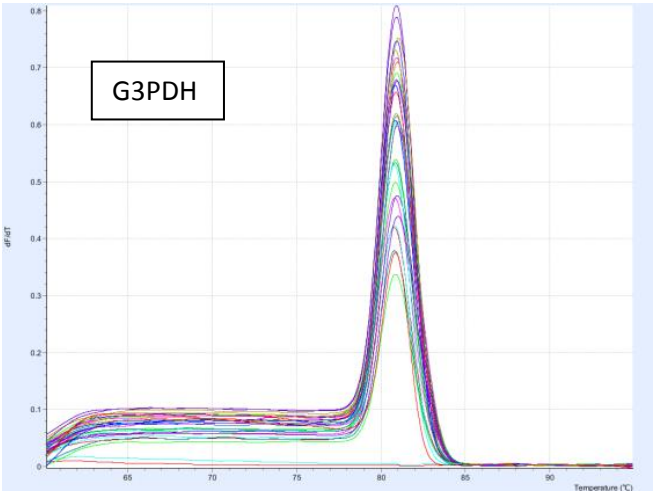
S-1: Checking for DNA contamination in cDNA samples: by designing primers to amplify specific portion that will have different amplicon from DNA and cDNA. A set of primers (Actin) was prepared by keeping an intron between the coding sequences so when amplified from DNA it will give larger amplicon than cDNA. The primer pair was used for checking DNA contaminations in all samples prior to use them for real time PCR. These primers are different from those used for quantitative assay for Actin (primer data not shown).

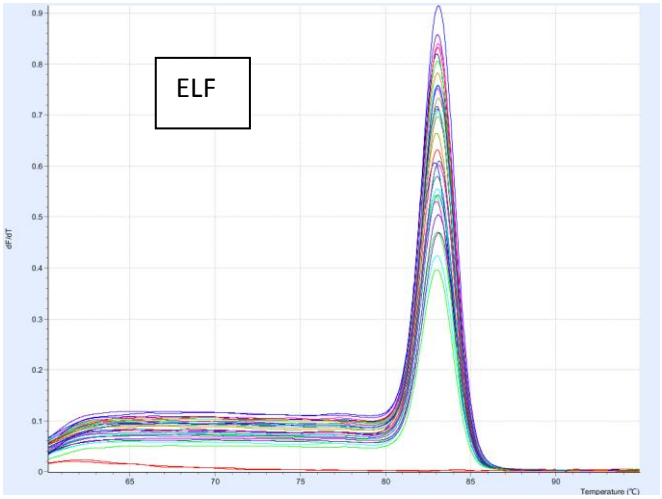
S-2: Melting curves of different primer pairs

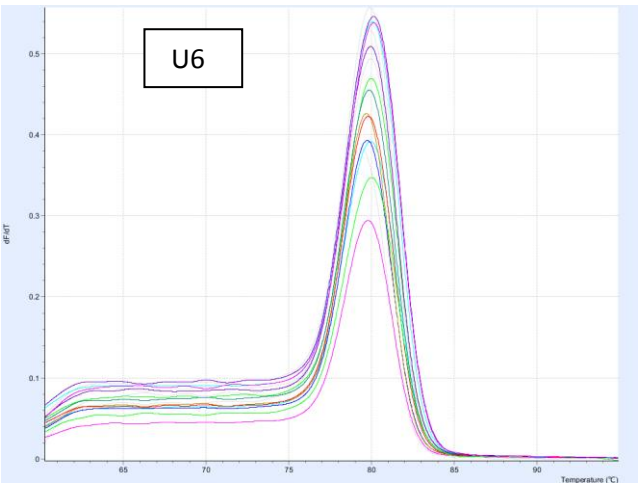


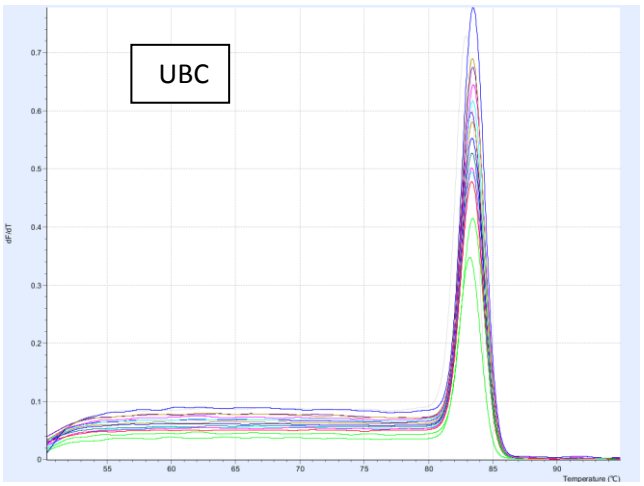


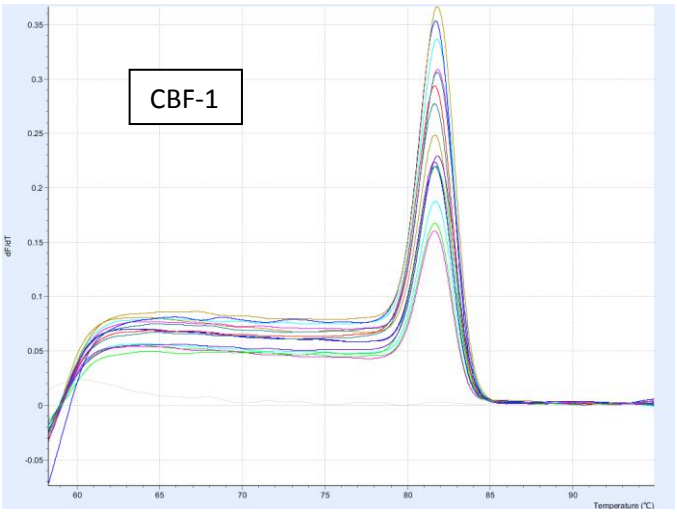




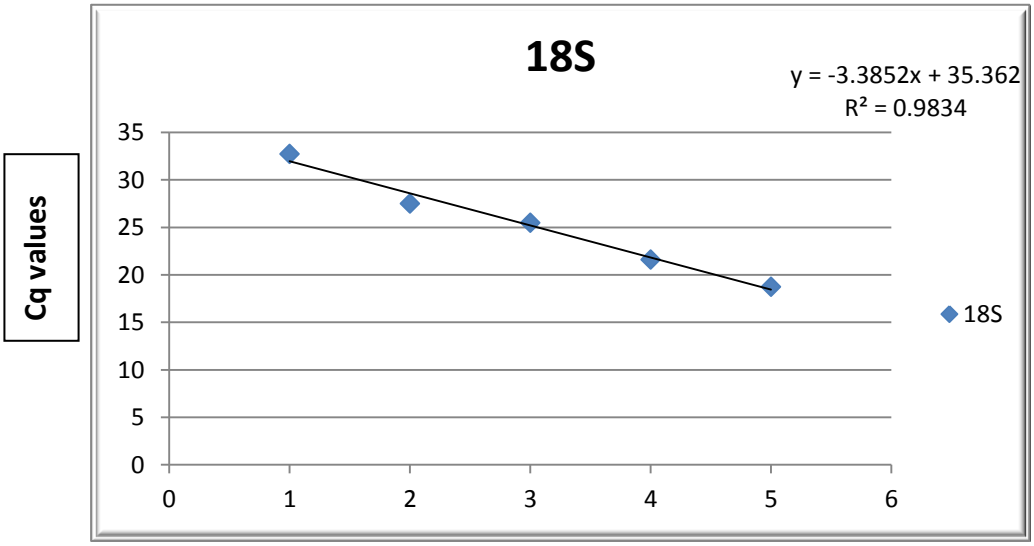


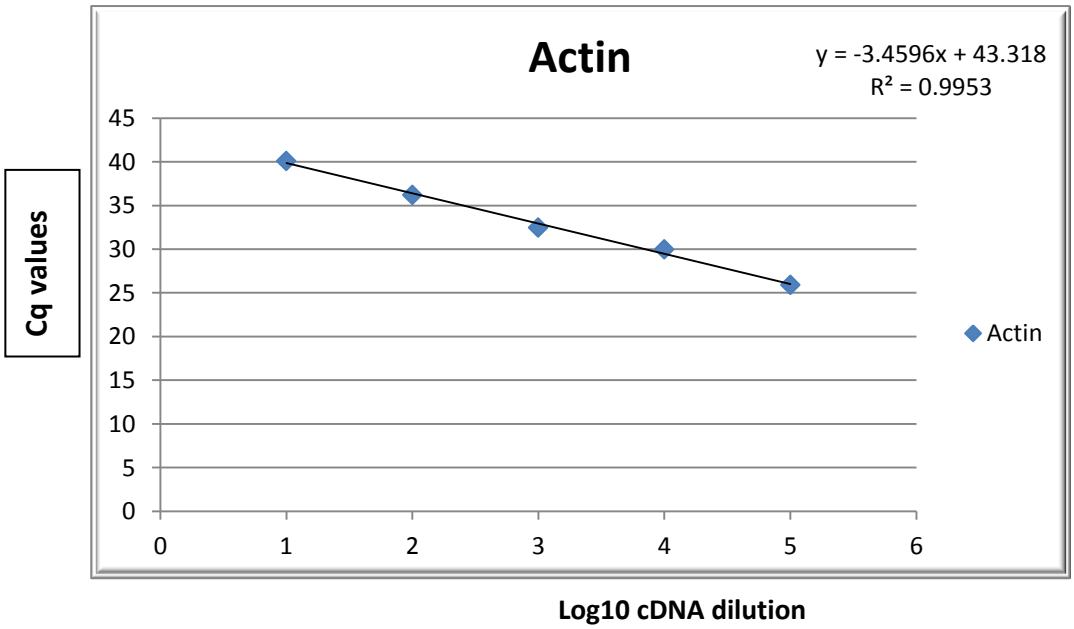

U6
dF/dT
Temperature (°C)

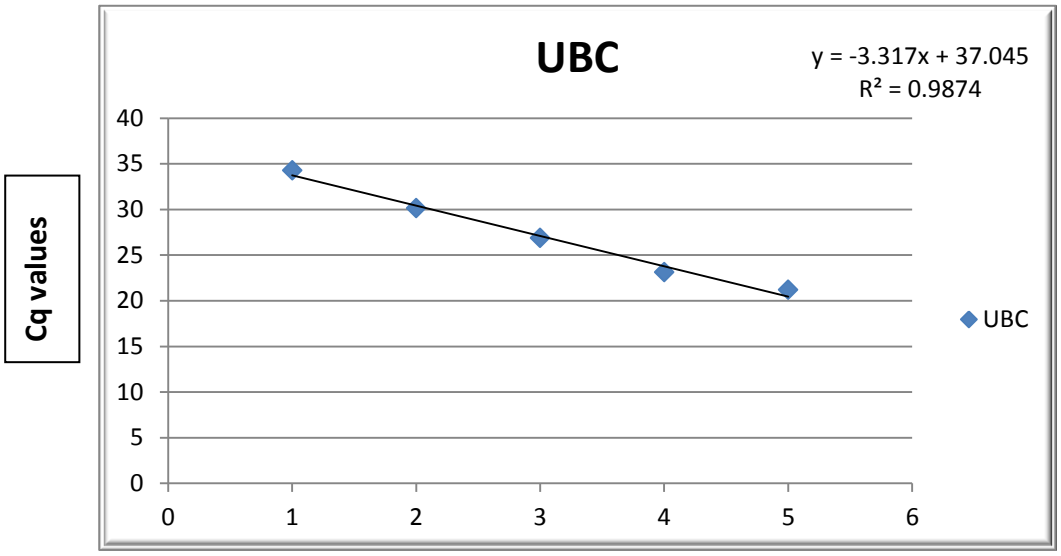

UBC
dF/dT
Temperature (°C)

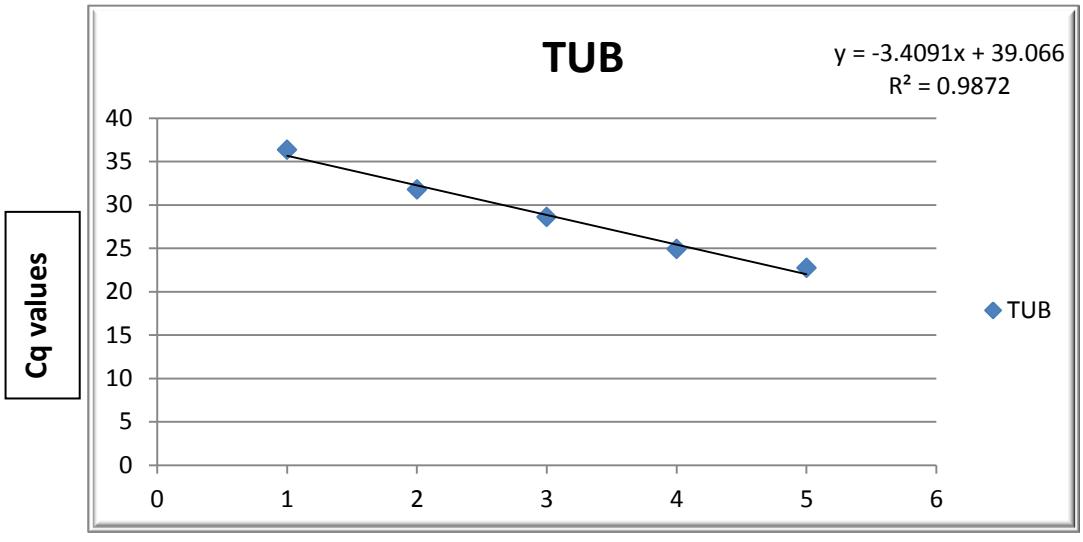

CBF-1
dF/dT
Temperature (°C)

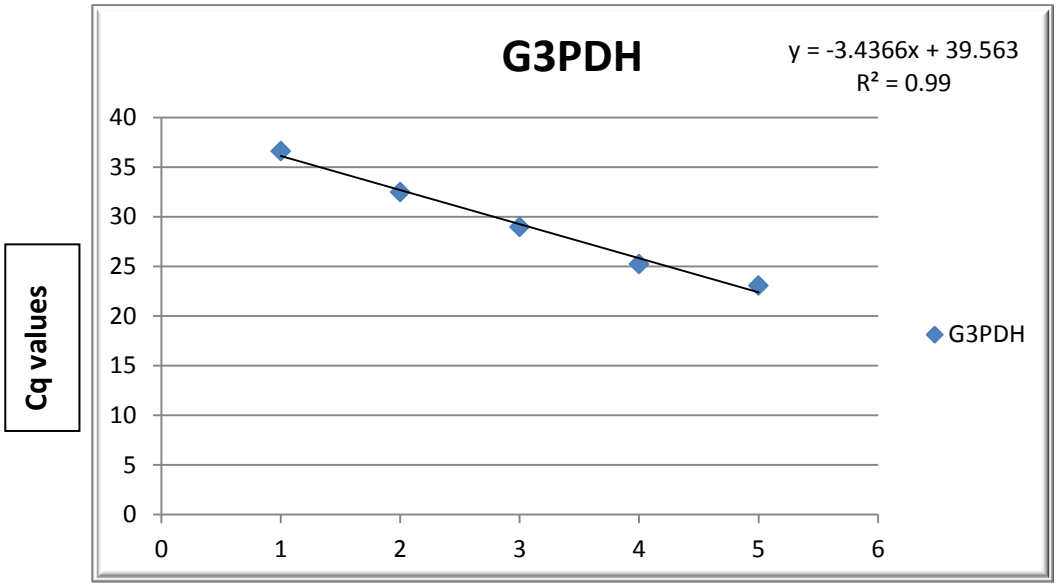
S-3: Primer efficiency calculation: (Linear regression)

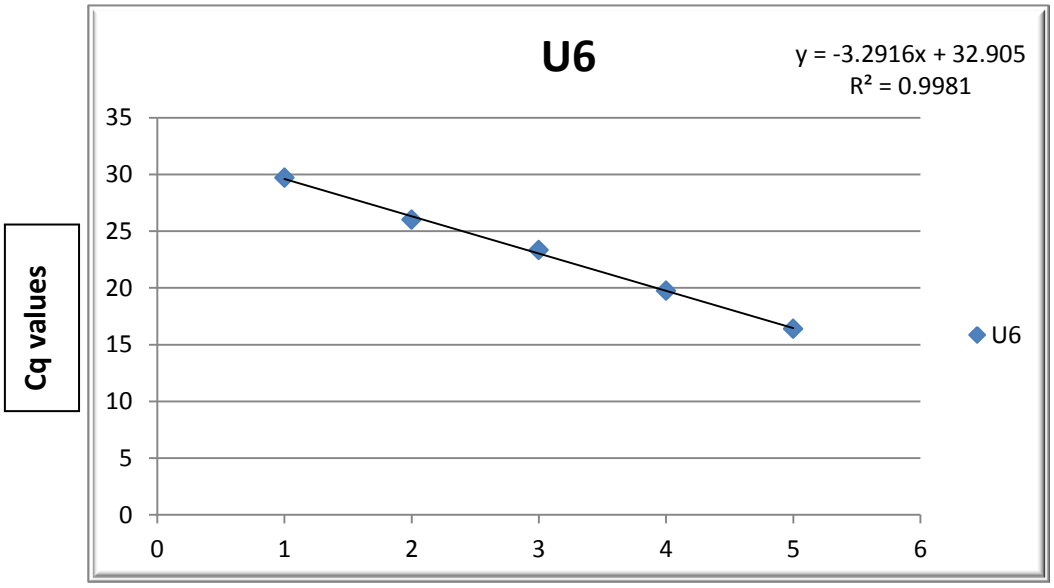


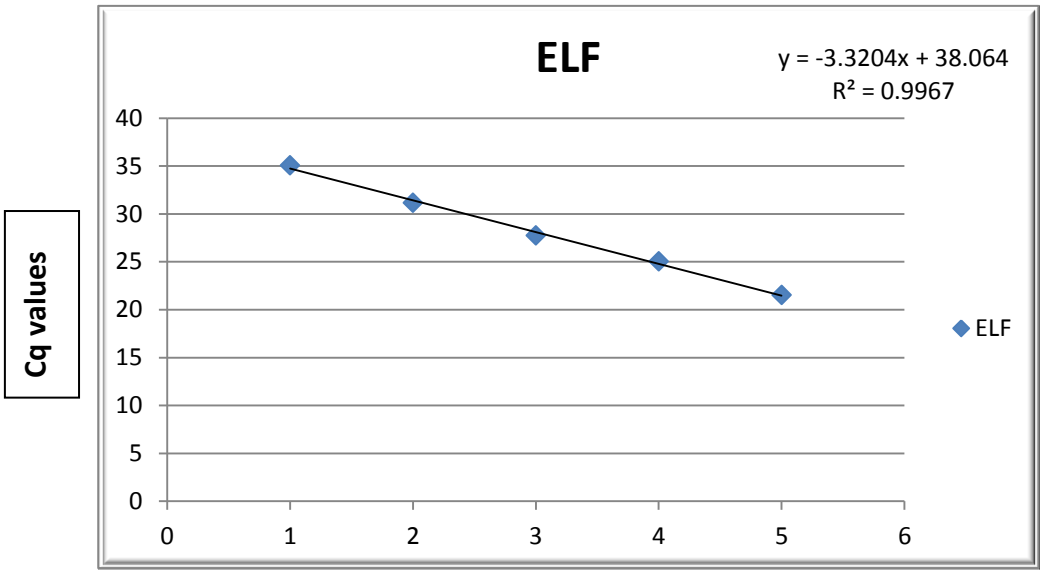


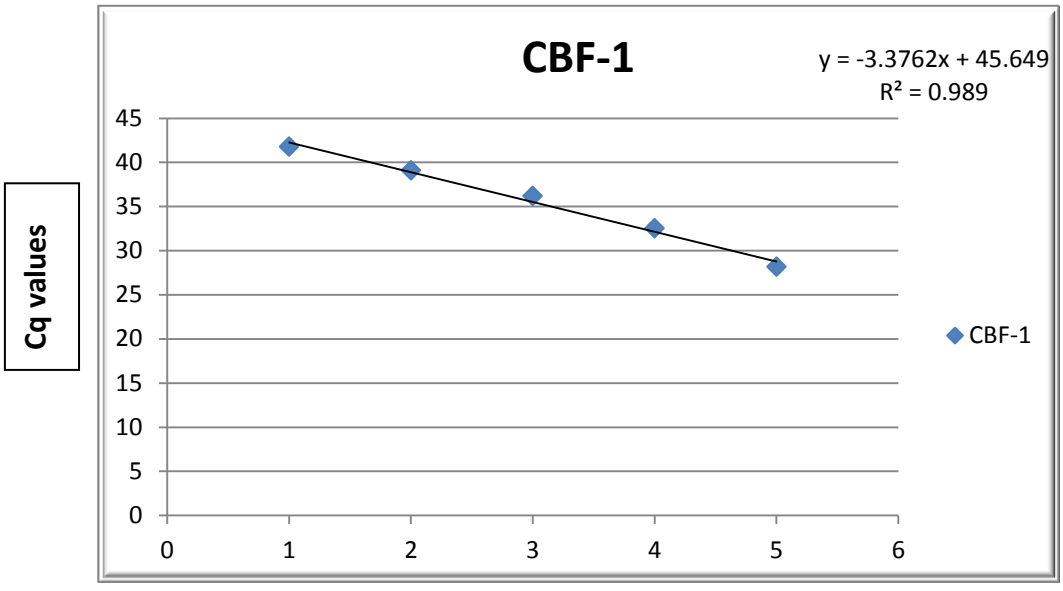

UBC
y = -3.317x + 37.045
R² = 0.9874
Cq values
40
35
30
25
20
15
10
5
0
0
1
2
3
4
5
6
UBC
Log10 cDNA dilution


TUB
y = -3.4091x + 39.066
R² = 0.9872
Cq values
40
35
30
25
20
15
10
5
0
0
1
2
3
4
5
6
TUB
Log10 cDNA dilution


G3PDH
y = -3.4366x + 39.563
R² = 0.99
Cq values
40
35
30
25
20
15
10
5
0
0
1
2
3
4
5
6
G3PDH
Log10 cDNA dilution


U6
y = -3.2916x + 32.905
R² = 0.9981
Cq values
35
30
25
20
15
10
5
0
0
1
2
3
4
5
6
U6
Log10 cDNA dilution


ELF
y = -3.3204x + 38.064
R² = 0.9967
Cq values
40
35
30
25
20
15
10
5
0
0
1
2
3
4
5
6
ELF
Log10 cDNA dilution


CBF-1
y = -3.3762x + 45.649
R² = 0.989
Cq values
45
40
35
30
25
20
15
10
5
0
0
1
2
3
4
5
6
CBF-1
Log10 cDNA dilution