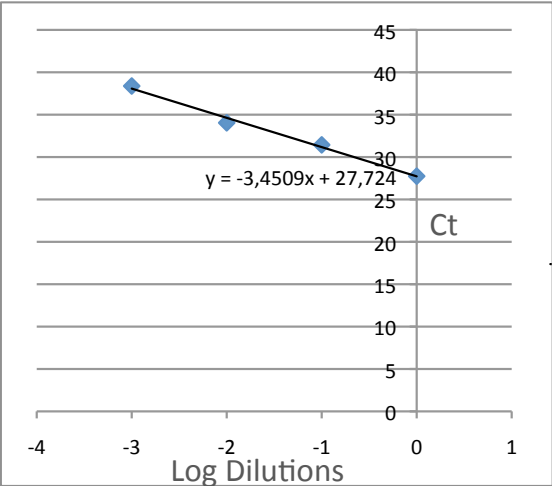


Supplementary Figure S2: Amplification Efficiency

Gene of interest: *Zim1*

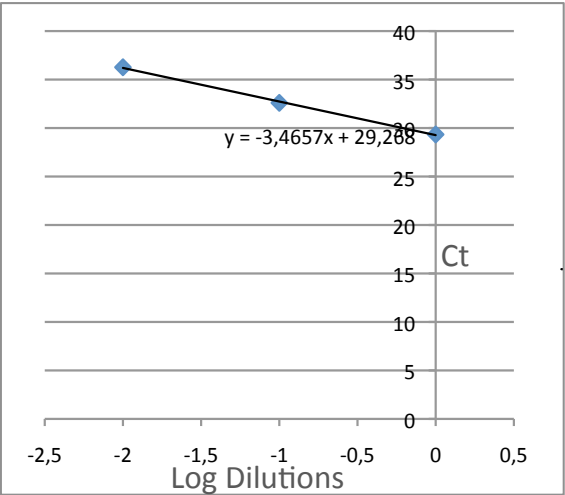
| GOI: <i>Zim1</i> | Replicates (Ct) |       |       |       | Ct Average | sd   | Dilution | Log Dilution | Ct Average |
|------------------|-----------------|-------|-------|-------|------------|------|----------|--------------|------------|
| Zim1 1:1         | 28,07           | 27,86 | 27,27 | 27,77 | 27,74      | 0,34 | 1,00     | 0,00         | 27,74      |
| Zim1 1:10        | 31,17           | 31,55 | 31,15 | 31,87 | 31,44      | 0,34 | 0,10     | -1,00        | 31,44      |
| Zim1 1:100       | 33,79           | 34,93 | 33,74 | 33,71 | 34,04      | 0,59 | 0,01     | -2,00        | 34,04      |
| Zim1 1:1000      | 38,42           | 38,16 | 38,57 | nd    | 38,38      | 0,21 | 0,00     | -3,00        | 38,38      |



|                      |  |       |
|----------------------|--|-------|
| Slope                |  | -3.45 |
| Amplification factor |  | 1.95  |
| Efficiency           |  | 95%   |

Gene of interest: *Chst2*

| GOI: <i>Chst2</i> | Replicates (Ct) |       |       |       | Ct Average | sd   | Dilution | Log Dilution | Ct Average |
|-------------------|-----------------|-------|-------|-------|------------|------|----------|--------------|------------|
| Chst2 1:1         | 29,55           | 29,00 | 29,48 | 29,31 | 29,34      | 0,25 | 1,00     | 0,00         | 29,34      |
| Chst2 1:10        | 32,09           | 32,22 | 32,15 | 33,92 | 32,60      | 0,89 | 0,10     | -1,00        | 32,60      |
| Chst2 1:100       | nd              | 36,64 | 35,73 | 36,43 | 36,27      | 0,47 | 0,01     | -2,00        | 36,27      |
| Chst2 1:1000      | nd              | 35,83 |       |       |            |      |          |              |            |

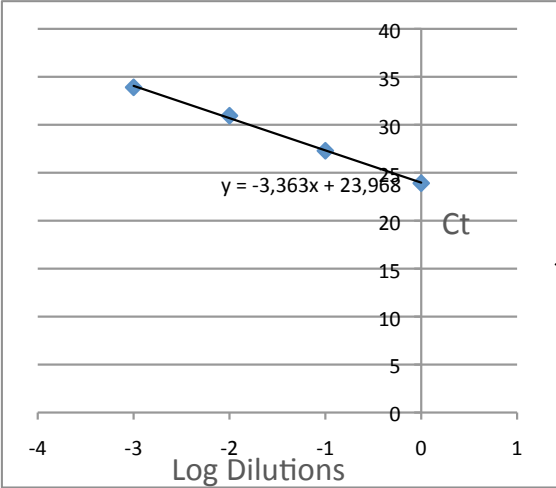


|                      |  |       |
|----------------------|--|-------|
| Slope                |  | -3.46 |
| Amplification factor |  | 1,95  |
| Efficiency           |  | 95%   |

# Amplification Efficiency

## Gene of interest: *Vcan*

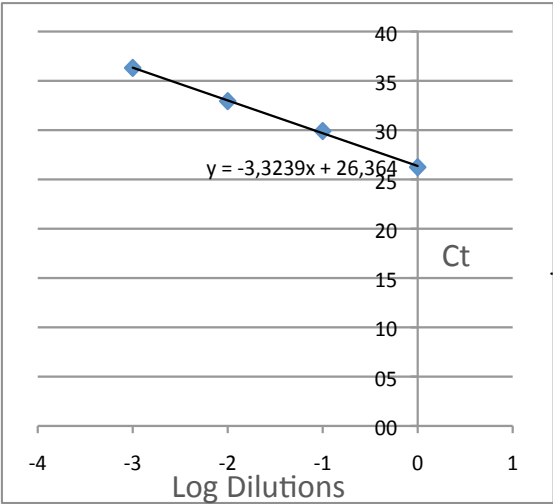
| GOI: <i>Vcan</i> | Replicates (Ct) |       |       |       | Ct Average | sd   | Dilution | Log Dilution | Ct Average |
|------------------|-----------------|-------|-------|-------|------------|------|----------|--------------|------------|
| Vcan 1:1         | 24,08           | 23,50 | 24,14 | 23,91 | 23,91      | 0,29 | 1,00     | 0,00         | 23,91      |
| Vcan 1:10        | 27,26           | 27,16 | 27,07 | 27,68 | 27,29      | 0,27 | 0,10     | -1,00        | 27,29      |
| Vcan 1:100       | 30,60           | 30,84 | 31,46 | 30,88 | 30,94      | 0,36 | 0,01     | -2,00        | 30,94      |
| Vcan 1:1000      | 34,34           | 33,79 | 33,92 | 33,57 | 33,90      | 0,32 | 0,00     | -3,00        | 33,90      |



|                      |       |
|----------------------|-------|
| Slope                | -3.36 |
| Amplification factor | 1.98  |
| Efficiency           | 98%   |

## Gene of interest: *Sdc4*

| GOI: <i>Sdc4</i> | Replicates (Ct) |       |       |       | Ct Average | sd   | Dilution | Log Dilution | Ct Average |
|------------------|-----------------|-------|-------|-------|------------|------|----------|--------------|------------|
| Sdc4 1:1         | 26,56           | 25,95 | 26,48 | 25,98 | 26,24      | 0,32 | 1,00     | 0,00         | 26,24      |
| Sdc4 1:10        | 30,83           | 28,95 | 30,23 | 29,62 | 29,91      | 0,81 | 0,10     | -1,00        | 29,91      |
| Sdc4 1:100       | 32,72           | 32,28 | 33,22 | 33,55 | 32,94      | 0,56 | 0,01     | -2,00        | 32,94      |
| Sdc4 1:1000      | 35,70           | 36,29 | nd    | 36,94 | 36,31      | 0,62 | 0,00     | -3,00        | 36,31      |

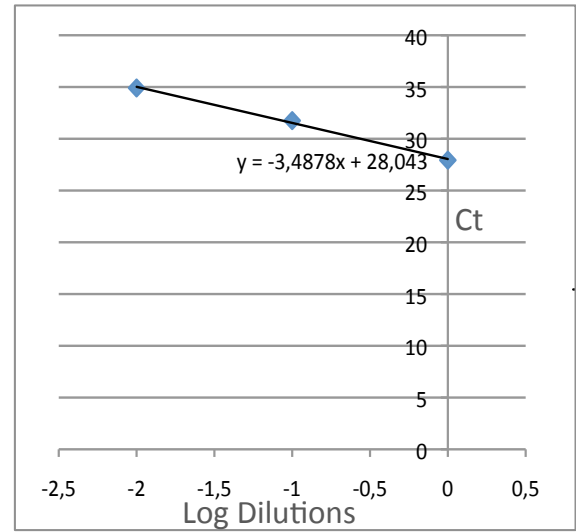


|                      |       |
|----------------------|-------|
| Slope                | -3.32 |
| Amplification factor | 2     |
| Efficiency           | 100%  |

# Amplification Efficiency

## Gene of interest: *Tnc*

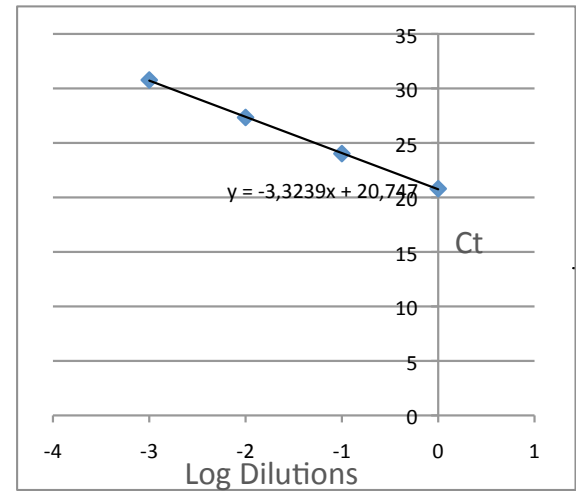
| GOI: <i>Tnc</i> | Replicates (Ct) |       |       |       | Ct Average | sd   | Dilution | Log Dilution | Ct Average |
|-----------------|-----------------|-------|-------|-------|------------|------|----------|--------------|------------|
| Tnc 1:1         | 28,37           | 27,80 | 27,84 | 27,72 | 27,93      | 0,30 | 1,00     | 0,00         | 27,93      |
| Tnc 1:10        | 32,34           | 31,36 | 31,25 | 32,06 | 31,75      | 0,53 | 0,10     | -1,00        | 31,75      |
| Tnc 1:100       | 35,68           | 34,68 | 35,10 | 34,17 | 34,91      | 0,64 | 0,01     | -2,00        | 34,91      |
| Tnc 1:1000      |                 |       |       |       |            |      |          |              |            |



|                      |  |       |
|----------------------|--|-------|
| Slope                |  | -3.48 |
| Amplification factor |  | 1.94  |
| Efficiency           |  | 94%   |

## Reference Gene: *Gapdh*

| RG: <i>Gapdh</i> | Replicates (Ct) |       |       |       | Ct Average | sd   | Dilution | Log Dilution | Ct Average |
|------------------|-----------------|-------|-------|-------|------------|------|----------|--------------|------------|
| Gapdh 1:1        | 20,75           | 20,55 | 21,47 | 20,42 | 20,80      | 0,47 | 1,00     | 0,00         | 20,80      |
| Gapdh 1:10       | 23,75           | 23,95 | 24,16 | 24,22 | 24,02      | 0,22 | 0,10     | -1,00        | 24,02      |
| Gapdh 1:100      | 27,45           | 27,09 | 27,31 | 27,51 | 27,34      | 0,19 | 0,01     | -2,00        | 27,34      |
| Gapdh:1000       | 30,92           | 30,09 | 31,54 | 30,53 | 30,77      | 0,62 | 0,00     | -3,00        | 30,77      |



|                      |  |       |
|----------------------|--|-------|
| Slope                |  | -3.32 |
| Amplification factor |  | 2     |
| Efficiency           |  | 100%  |

### **Supplementary Figure S2: PCR Amplification Efficiency.**

Serial dilutions of cDNA (1:1; 1:10; 1:100 and 1:1000) were used to performed amplification efficiency for *Zim1*, *Chst2*, *Vcan*, *Sdc4*, *Tnc* genes and for the reference gene *Gapdh*. Ct values were plotted on the y-axis along with corresponding logarithmic dilutions (x-axis). A linear regression curve and slope was determined for each genes. Amplification efficiency was calculated using the equation:  $E = -1 + 10^{(-1/\text{slope})}$ . Each point was analyzed in duplicate two times on two different 96-well plates.