

PDF S2

Some figures showing the results of the experiments performed:

Sample of some electrophoresis performed:

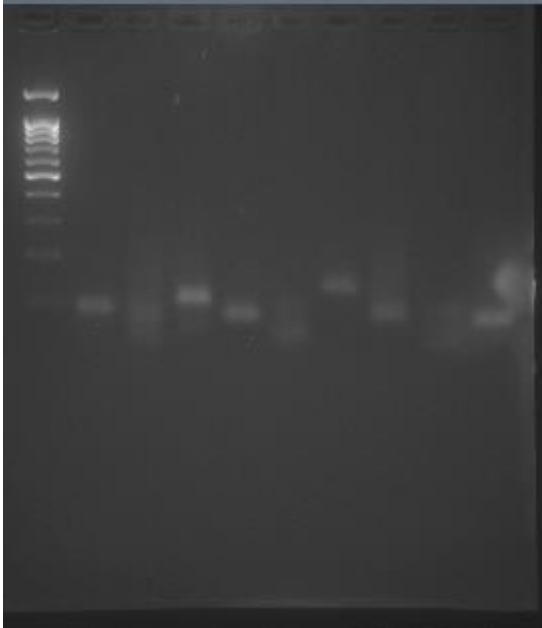


Figure 1. Electrophoresis 2% agarose, 100V, 70 min. Lane 1: DNA ladder (100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500 bp). Lane 2: Gsk3beta gene. Amplicon: 95 bp. Lane 3: Dkk1 gene. Amplicon: 80 bp. Lane 4: APC gene. Amplicon 114 bp. Lane 5: IL-33 gene. Amplicon 84 bp. Lane 6: AURKA gene. Amplicon 98 bp. Lane 7: VEGF alpha gene. Amplicon 141 bp. Lane 8: CDNK gene. Amplicon 90 bp. Lane 9: Ccnd1 gene. Amplicon 94 bp. Lane 10: ACTB gene. Amplicon 80 bp.

The lanes highlighted with red are not specific.

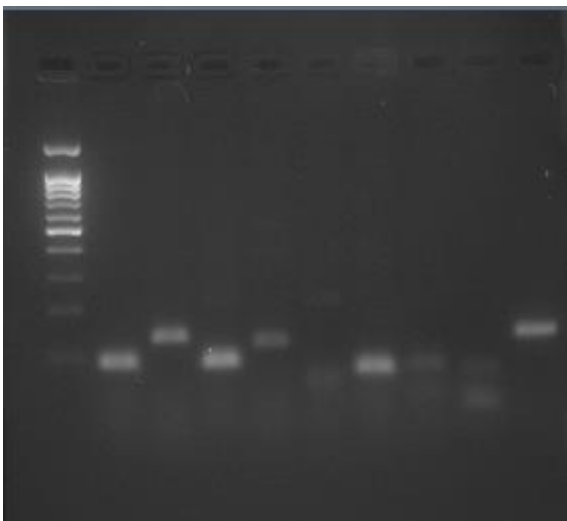


Figure 2. Electrophoresis 2% agarose, 100V, 70 min. Lane 1: DNA ladder (as previous image). Lane 2: BAX gene. Amplicon 93 bp. Lane 3: p53 gene. Amplicon 138 bp. Lane 4: Beta catenin gene. Amplicon 93 bp. Lane 5: COX2 gene. Amplicon 127 bp. Lane 6: iNOs gene. Amplicon 220 bp. Lane 7: RelA gene. Amplicon 81 bp. Lane 8: Tnf alpha gene. Amplicon 89 bp. Lane 9: Bcl2 gene. Amplicon 81 bp. Lane 10: B2M gene. Amplicon 157 bp.

The lanes highlighted with red are not specific.

For some genes an annealing gradient PCR was performed in order to choose the best T_a . An example for β -catenin and *Smad4* gene are showed.



Figure 3. Electrophoresis 2% agarose, 100V, 70 min. β -catenin gene presented unspecific bands when we working with annealing temperatures of 51 – 52°C.

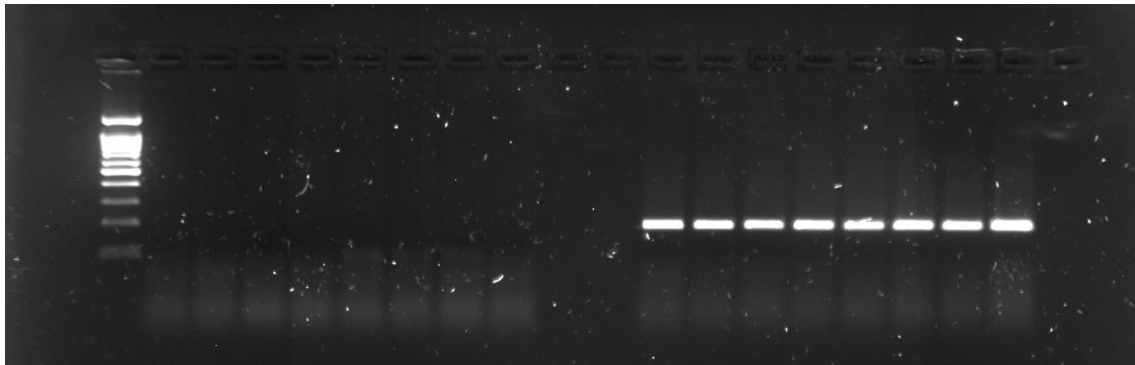


Figure 4. Electrophoresis 2% agarose, 100V, 70 min. On the left side there is an annealing temperature gradient for *Dkk1* gene (from PMID27468227) where no band is detected and on the right for *Smad 4* (amplicon size of 191 bp). The order of annealing temperatures in the lanes of the agarose gel are the same as **Figure 3**. In this case, *Smad 4* works well in any of the annealing temperatures within the range of 51°C to 61°C.

Sample of some efficiency qPCR assays output:

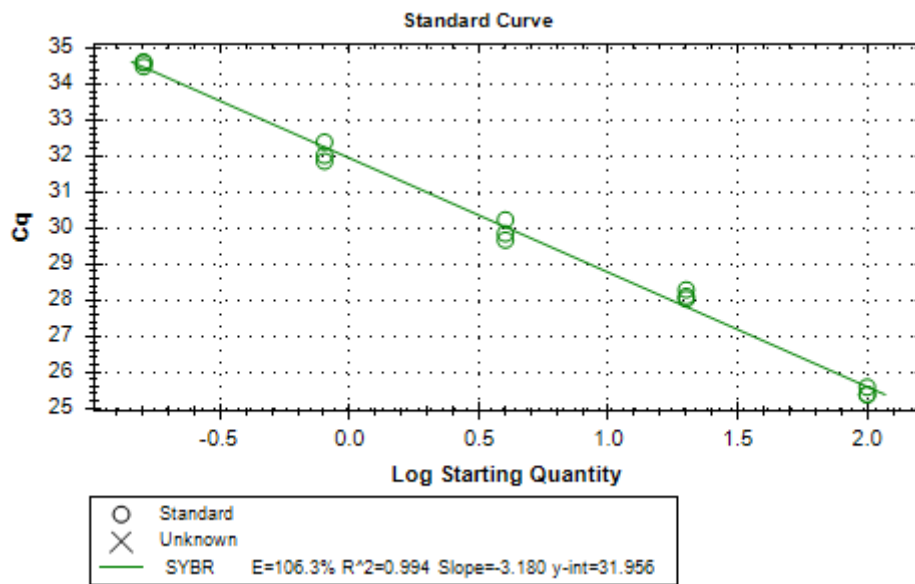


Figure 5. *BAX* gene linearity range and their % efficiency value (E).

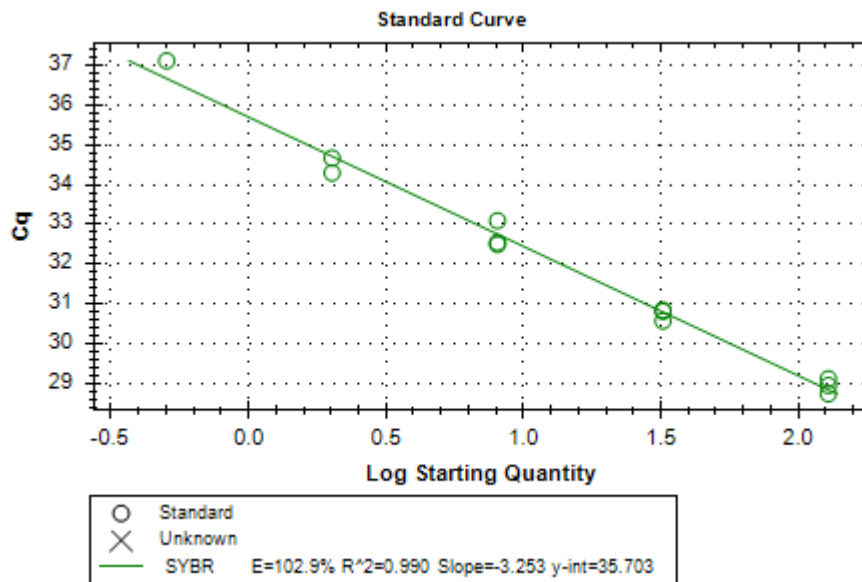


Figure 6. *Bcl2* gene linearity range and their % efficiency value (E).

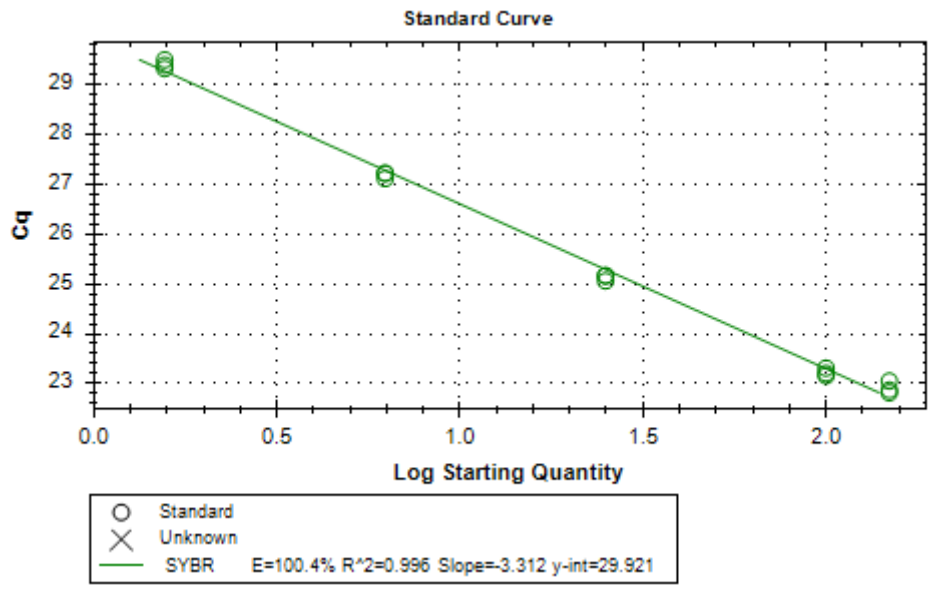


Figure 7. *B2m* gene (reference gene) linearity range and their % efficiency value (E).

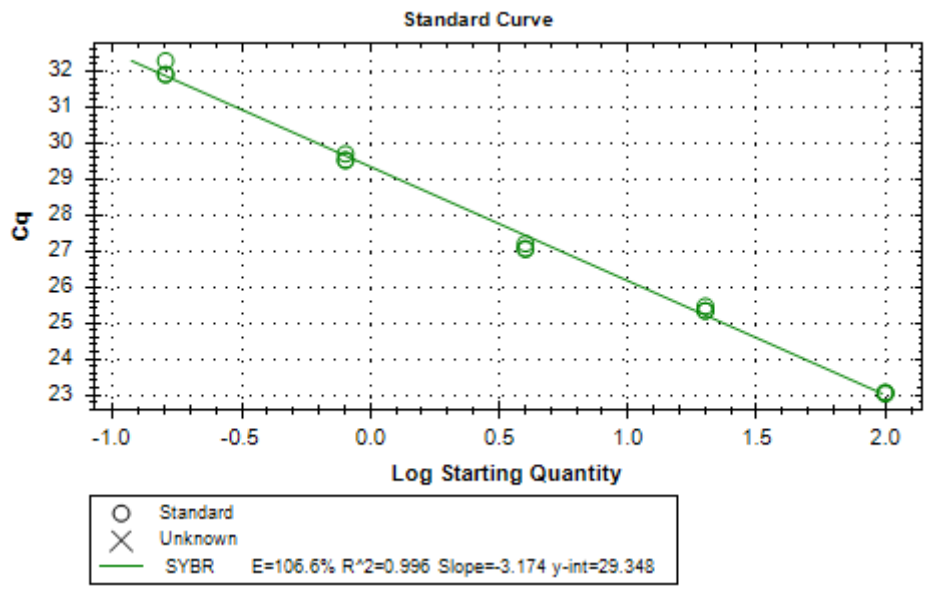


Figure 8. *Nrf2* gene linearity range and their % efficiency value (E).