Supplementary Material (ESI) for Photochemical & Photobiological Sciences
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Electronic supplementary information for:

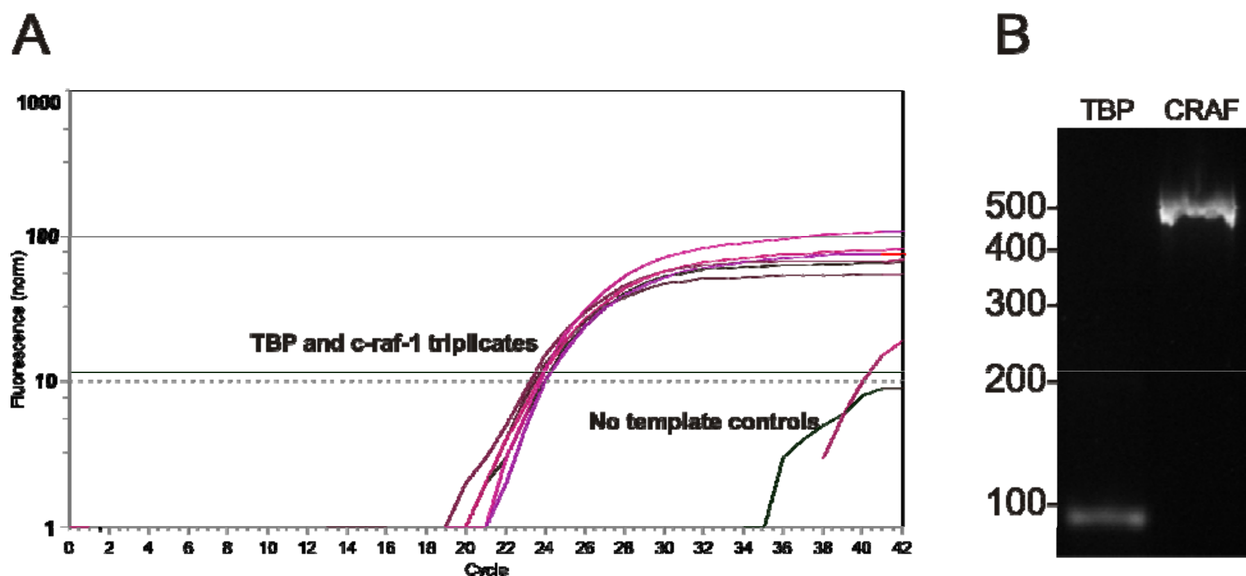
A tumor mRNA-triggered photodynamic molecular beacon based on oligonucleotide hairpin control of singlet oxygen production

Materials and Methods:

RNA was isolated from MDA-MB-231 cells by the acidic guanidine phenol-chloroform method (Chomczynski and Sacchi, 1987) with isopropanal precipitation. RNA was treated with DNAse I (Fermentas, Lithuania) and then with M-MuLV reverse transcriptase (Fermentas) with dT18 primers according to the manufacturer protocol. 20 or 200 ng of cDNA was then subjected to qPCR on an Eppendorf (Germany) Mastercycler Realplex instrument using Maxima SYBR Green qPCR Master Mix (Fermentas) according to the manufacturer protocol. The primers used for amplifying CRAF1 were: Forward-5'TCAGAGAAGCTCTGCTAAG and Reverse-5'CAATGCACTGGACACCTTA (Rudin et al., 2004); and for amplifying the Tata Box Protein (TBP): Forward-5'CACGAACCACGGCACTGATT and Reverse-5'TTTTCTTGCTGCCAGTCTGGAC (Latil et al., 2000). After an initial activation of 10 minutes at 95 C, the cycling parameters were 30 seconds at 95C, 45 seconds at 52C, 30 seconds at 72C. PCR products were subjected to 2% agarose gel electrophoresis using a 100 bp O'RangeRuler ladder (Fermentas).

Results:

cDNA from MDA-MB-231 cells was generated and used to amplify the CRAF1 and TBPs genes by qPCR. TBP has been used previously as a reference to quantify gene copies based on 1000 TBP transcripts per cell (Tian et al., 2004). Figure 1A shows the amplification curves of the qPCR reaction using 200 ng of template cDNA. CRAF1 and TBP show similar amplification profiles. Based on triplicate samples, the Ct value for TBP was 24.1 with standard deviation of 0.2, while the Ct value for CRAF1 was 23.8 with standard deviation of 0.4. When the starting template amount was diluted 10 fold, the obtained Ct value for CRAF1 was 26.5 with standard deviation of 0.5 and the Ct value for TBP was 26.6 with standard deviation 0.04, demonstrating the assay was in linear range. To ensure the correct products were formed, 5 uL of PCR product was subjected to agarose gel electrophoresis. Figure 1B shows the PCR products of the reactions are the expected sizes (89 bp for TBP and 494 bp for CRAF1).



Supplementary figure 1: mRNA Expression of TBP and CRAF1 in MDA-MB-231 cells. A) qPCR results showing amplification of the two genes. The two genes had similar amplification curves. B) 2% agarose gel showing expected size of PCR products

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