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Design, optimization and validation of genes commonly used in expression studies on DMH/AOM rat colon carcinogenesis model

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Colorectal cancer (CRC), also known as colon cancer, is the third most common form of cancer worldwide in men and the second in women and is characterized by several genetic alterations, among them the expression of several genes. 1, 2-dimethylhydrazine (DMH) and its metabolite azoxymethane (AOM) are procarcinogens commonly used to induce colon cancer in rats (DMH/AOM rat model). This rat model has been used to study changes in mRNA expression in genes involved in this pathological condition. However, a lack of proper detailed PCR primer design in the literature limits the reproducibility of the published data. The present study aims to design, optimize and validate the qPCR, in accordance with the MIQE (Minimum Information for Publication of Quantitative Real-Time PCR Experiments) guidelines, for seventeen genes commonly used in the DMH/AOM rat model of CRC (Apc, Aurka, Bax, Bcl2, β-catenin, Ccnd1, Cdnk-1A, Cox2, Gsk3beta, IL-33, iNOs, Nrf2, p53, RelA, Smad4, Tnfα and Vegfa) and two reference genes (Actb or β-actin and B2m). The specificity of all primer pairs was empirically validated on agarose gel, and furthermore, the melting curve inspection was checked as was their efficiency ranging from 90 to 110 with a correlation coefficient of r2>0.980. Finally, a pilot study was performed to compare the robustness of two candidates.

Recent Publications

1. Jacob F, Guertler R, Naim S, Nixdorf S, Fedier A, Hacker NF, et al. (2013) Careful selection of reference genes is required for reliable performance of RT-qPCR in human normal and cancer cell lines. PLoS One 8(3):e59180.
2. Kensara O A, El-Shemi A G, Mohamed A M, Refaat B, Idris S and Ahmad J (2016) Thymoquinone subdues tumor growth and potentiates the chemopreventive effect of 5-fluorouracil on the early stages of colorectal carcinogenesis in rats. Drug Des Devel Ther 10: 2239– 53.
3. Bi W, et al. (2017) Chemopreventive effects of Ku-Jin tea against AOM-induced precancerous colorectal lesions in rats and metabolomics analysis. Scientific Reports 7(1):15893.

Biography

David Bars-Cortina has completed his Degree in Biology at Universitat Autònoma de Barcelona (UAB), Bellaterra, Spain in 2011, awarded with an extraordinary prize of Degree in Biology for their academic merit. In addition, he has completed Master of Science in Nutrition and Health at Universitat Oberta de Catalunya (UOC), Barcelona, Spain in 2015, with his master's degree Final Project rewarded by the Scientific Academy of Catalonia (Institut d'Estudis Catalans, Barcelona, Spain). He is currently working as PhD student in Food Technology Department of University of Lleida with the olive oil metabolites and its bioavailability but mainly focused on natural crossbred red-fleshed apples collected from Spanish experimental orchard. These kinds of apples are commercialized in some European countries (e.g. Switzerland and Italy) under the brand name Redlove Era.

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