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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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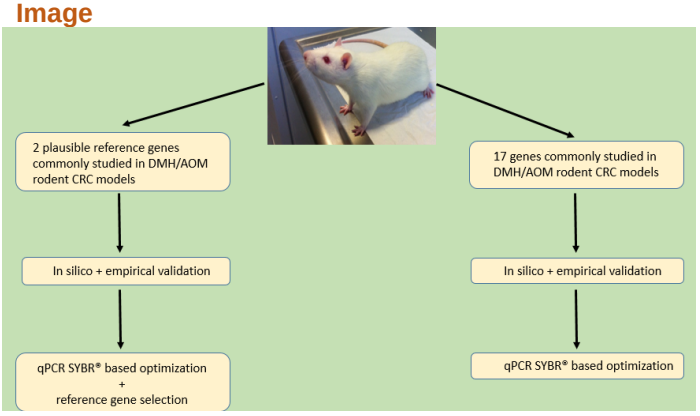
Abstract (300-word limit)

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 candidate reference genes.

Image



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: e59180.
2. Kensara OA, El-Shemi AG, Mohamed AM, Refaat B, Idris S, 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. Girardi R. et al. (2018) Chemoprevention of Inflammation-Related Colorectal Cancer by Silymarin, acetyl-11-keto-beta-boswellic acid, curcumin and maltodestrin enriched dietetic formulation in animal model. Carcinogenesis, doi: 10.1093/carcin/bgy104.
4. 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.



Universitat de Lleida

Biography (150-word limit)

Msc. David Bars-Cortina has a Degree in Biology Science obtained in 2011 at the Universitat Autònoma de Barcelona. After he has finished his degree studies, he was working during four years in Quality Control Laboratory and Research & Development Department rolled in different kind of projects (e.g ITCE 160.182 project certified by European Quality Assurance (EQA); implementing foodstuff with phytochemical compounds in cattle farms…) in a Spanish leading company in the agrifood sector (Vall Companys company).
After their experience in a company, he decided to start a PhD (current job situation) in order to study the possible relationship of some plant products in some pathology state, fundamentally colorectal cancer (CRC). My PhD studies are mainly focused in assessing the possible positive effect of the ingestion of red-fleshed apples towards to the first stages of CRC.

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Notes/Comments:

A preprint of this article is available on bioRxiv: https://www.biorxiv.org/content/early/2018/07/09/365353

The present work submitted in the 3rd Edition of International Colorectal Cancer is the previous work done previously to the performance of the **ongoing experiment** with 60 Wistar rats with the aim of addressing the possible effect of dietary supplementation with a particular fruit (white- and red-fleshed apples) and cyanidin galactoside (the main anthocyanin in red-fleshed apple) on AOM rat colon carcinogenesis model. The experiment flowchart will be showed also in the poster submitted if the abstract is accepted.