Quantitative Real-time Polymerase Chain Reaction (qRT-PCR)

Fatemeh Mehrpooyan*

MSc., Genetic and Molecular Biology, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia.

*sahar_mehr8261@yahoo.com

- ***What is Real Time PCR?***

In molecular biology, real-time polymerase chain reaction, also called quantitative real time polymerase chain reaction (qPCR) is a laboratory technique based on the PCR, which is used to amplify and quantify a targeted DNA molecule at the same time. For one or more specific sequences in a DNA sample, real-time PCR enables both detection and quantification. The quantity can be either an absolute number of copies or a relative amount when normalized to additional normalizing endogenous controls. Also, real-time PCR is combined with reverse transcription to quantify mRNA and non-coding RNA in cells or tissues.

- ***How does it work?***

Reverse transcription is done using the High Capacity cDNA Reverse transcription kit (Applied Biosystems, USA). Each 20 µl reaction mixture contains 2 µg of total RNA, 4 mM of 25X dNTP mix (100 mM), 2 µL (1X) of 10X RT Random primers, 2 µL (1X) of 10X RT buffer, 1U/µl of 40 U/ml Ambion RNase Inhibitor, 1 µl (50 U) of 50 U/ml MultiScribeTM Reverse transcriptase topped up with DEPC treated water. The thermal cycling profile is as follows: 25 °C 10 min 37 °C 120 min 85 °C 5 seconds and hold 4 °C.

Quantitative PCR amplification will be carried out using Power SYBR- Green PCR Master Mix kit (Applied Biosystems, USA). For each PCR, 12.5 µl (1X) 2XSYBR Green Power Master Mix will be mixed with 500 nM of each forward and reverse primer solutions (10 µM), 100 ng cDNA template topped up with autoclaved dH_2O to a final volume of 25 µl. The PCR Master mixes will be prepared using both β-actin endogenous control strips (the most stable control gene) as well as desired target RNA primers for the sample strips. The master mix containing endogenous primers will be added into the endogenous control strips while the master mix containing the main sample primers will be added into the sample strips. cDNA and nuclease free water will then be added to the specific strips. For each sample at least three replicates should be performed. No Template Control (NTC) tubes also will be prepared using both endogenous and sample primers to ensure that there will not be any master mix or/and primer contamination. Then the optical 8 tubes strips will be loaded into the Applied Biosystem 7500 Real-Time PCR system and the SDS 1.3.1 (Sequence Detection Software) is used to create a relative quantification (ddCt) plate. The run then will be started. The amplification program is: 95 °C for 10 minutes, 40 cycles of 95 °C for 15 seconds and finally 60 °C for 1 minute.

After the running of the program, the SDS software will be again used to generate a dissociation curve and later a relative quantification (ddCt) study will be created to analyze the results. The SDS software

on the Applied Biosystem 7500 Real-Time PCR system automatically uses the equation 2-UUCT to calculate the expression level of the pre-miRNA relative to the endogenous control.

- ***What is it used for?***

There are several applications for real-time PCR in laboratories. It is usually used for both diagnostic and basic research. Real-time PCR is mostly used to provide quantitative measurements of gene transcription. It may be used in recognizing how the expression of a particular gene changes over the time, e.g., in the response of tissue and cell cultures to an administration of a pharmacological agent, progression of cell differentiation, or in response to alterations in environmental conditions.

- ***Contact us***

For more information or troubleshooting on your Transformation, please do not hesitate to contact us at ijpp@iau-saveh.ac.ir. You can simply mention your problem by attaching your results. We look forward to hearing from you soon.

- ***Read more on***

http://www3.appliedbiosystems.com/sup/URLRedirect/index.htm?xDoD=4367218

http://www.appliedbiosystems.com/absite/us/en/home/support/tutorials/realtime-pcr-trouble-shooting-guide.html

IJPP
Iranian Journal of Plant Physiology

IJPP
Iranian Journal of Plant Physiology

Iranian Journal of Plant Physiology is a quarterly journal published by Islamic Azad University Saveh Branch in English. Manuscripts may be submitted in English. Tables of contents and other useful information, including these instructions for contributors, are available at the websites of the Islamic Azad University Saveh Branch and the Editorial Office (Department of Biology, Faculty of Agriculture, and Islamic Azad University Saveh Branch).

Aims and Scope

This journal publishes the new results of completed, original studies on any aspect of plant physiology based also on approaches and methods of biochemistry, biophysics, genetics, molecular biology, genetic engineering, applied plant physiology, and other related fields. We also accept descriptions of original methods and instruments opening novel possibilities for obtaining and analyzing experimental results. Papers outlining trends and hypotheses are accepted as well. Brief communications are not accepted. However, in some cases, the editors may suggest that authors shorten a manuscript to the size of a brief communication (no more than 10 pages of text and 4 figures and / or tables in all). Manuscript submission implies that the material has not been published before, and is not under consideration for publication anywhere else.

Manuscript Requirements

Manuscript length should not exceed 10 printed pages (reviews not more than 20 pages), including references, tables, and figure captions; it should contain no more than 7 figures. The manuscript must be typed (Times New Roman font, 12 pt, 1.5 spacing throughout) in a single column on one side of white paper (A4, 210 × 297 mm) with left and top margins of 2.5 cm and a right margin of 1.5 cm. All pages, including references, tables, and figure captions, should be numbered consecutively in the top right-hand corner. All lines should be enumerated throughout the entire text.

Please arrange your manuscript as follows: Title, author(s), affiliation(s), abstract, keywords, abbreviation (optional), introduction, materials and methods, results, discussion, acknowledgements (optional), references, tables, and figures.

The title must be concise (no more than 10 words) but informative. Capitalize the first letters in all nouns, pronouns, adjectives, verbs, adverbs, and subordinate conjunctions. Avoid nonstandard abbreviations.

Authors' initials and surnames should be written with one space between the initials and between the initials and an author's surname. Author affiliations should be marked as *1*, 2 etc. On a separate page, provide the full names of all authors, their postal addresses and telephone and fax numbers, as well as e-mail addresses, and indicate the corresponding author.

Author affiliations include the department, institution, and complete address of each author. The fax number and e-mail address of the corresponding author should be indicated after his or her postal address.

Abstract

All papers, including brief communications, should be preceded by a concise (of no more than 250 words) but informative abstract, in which the plant material (binomial, including authority) is given. The abstract should explain to the general reader the major contributions of the article. The abstract is typed as a single paragraph. Citing and discussing literature are not recommended.

Keywords. No more than seven items are listed beginning with the Latin name(s) of the organism(s) studied without author's name and arranged as follows***:***
Keywords: Lycopersicon esculentum; transgenic tomato plant; ethylene

Abbreviations. The abbreviation of the expressions used in the manuscript may be listed in alphabetical order and arranged as follows:

BA: benzyladenine; PSI: photosystem I; WT: wild type

Define nonstandard abbreviations when they are first mentioned in the text and abstract.

Main Headings

The main headings within the text (Introduction, Materials and Methods, etc.) should be placed on separate lines with the first letters capitalized. First-level subheadings should follow title capitalization (example: *Cytokinin, Dependent Signal Transduction*) and be placed on separate lines. Second-level subheadings (i.e., headings running into a paragraph) should follow sentence capitalization (example: *Plant material.*).

Introduction

The introductory part of the article should explain its objective and cite relevant articles published previously.

Materials and Methods

This section should include complete botanical names (genus, species, authority for the binomial, and, when appropriate, cultivar) for all plants studied. Following first mentions, generic names should be abbreviated to the initial except when confusion could arise by reference to genera with the same initial. Growth conditions must be described. Also new procedures should be described in sufficient detail to be repeated. A short description of other procedures should also be given. This section should also contain the names of the manufacturers (including country name) of materials and reagents. Statistical analysis of the results should be described. Identify the number of replications and the number of times individual experiments were duplicated. It should be clearly stated whether the standard deviation or the standard error is used.

Results

The result section should be presented mainly in figures and tables without their detailed discussion. Double documentation of the same points in figures and tables is not acceptable.

Discussion

This section should contain an interpretation but not a recapitulation of the results. The Results and Discussion sections may be combined if a description of experimental results is brief or when the interpretation of the previous experiment is required for the logical substantiation of the next one.

Acknowledgements

List dedications, acknowledgments, and funding sources if any, under the heading 'Acknowledgements'.

References

Cite published papers and books; citing the abstracts of meetings is not recommended. References at the end of the paper should be arranged alphabetically (by authors' names) in the reference list, all authors should be named unless there are 10 or more. For titles in English, including titles of books, journals, articles, chapters, and dissertations and names of conferences, use title capitalization. For titles given in a foreign language, follow the rules of capitalization for that language.

Journal articles:

Ouyang, D., J. Bartholic and **J. Selegean,** 2005. 'Assessing sediment loading from agricultural croplands in the great lakes basin'. *Journal of American Science*, 1 (2): 14-21.

Books:

Durbin, R., S. R. Eddy, A. Krogh and **G. Mitchison.** 1999. *Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids*. Cambridge: University Press.

A chapter in a book:

Leach, J. 1993. 'Impacts of the zebra mussel (Dreissena polymorpha) on water quality and fish spawning reefs of Western Lake Erie'. In *Zebra Mussels: biology, impacts and control*. Nalepa, T. and D. Schloesser (Eds.). Ann Arbor, MI: Lewis Publishers, pp: 381-397.

A Report:

Makarewicz, J. C., T. Lewis and **P. Bertram.** 1995. *Epilimnetic phytoplankton and zooplankton biomass and species composition in Lake Michigan 1983-1992.* U.S. EPA Great Lakes National Program, Chicago, IL. EPA 905-R-95-009.

Conference proceedings:

Stock, A. 2004. 'Signal transduction in bacteria'. Proceedings of the 2004 Markey Scholars Conference, pp: 80-89.

A thesis:

Strunk, J. L. 1991. *The extraction of mercury from sediment and the geochemical partitioning of mercury in sediments from Lake Superior.* M. Sc. thesis, Michigan State Univ., East Lansing, MI.

For correct abbreviations of journal titles, refer to Chemical Abstracts Service Source Index (CASSI).

Tables

Each table should have a brief title, be on a separate page, and be 1.5-spaced. Each column should have a heading; units should appear under the column heading(s). Some remarks may be written below the table, but they should not repeat details given in the Materials and Methods section.

Figure Captions

These must be a brief self-sufficient explanation of the illustrations. Provide them separately from figures.

Figures

All figures (photographs, graphs, and diagrams) should be cited in the text and numbered consecutively throughout. Figures should provide enough information to easily understand them. Figure parts should be identified by lowercase roman letters (I, II, etc.) in parentheses. The axes of each graph should have the numerical scale and the measured quantity with units (for example, CO_2 absorbance, µ $molm^{-2}s^{-1}$), but not photosynthesis, $\mu mol/m^{-2}s^{-1}$)). The curves should be defined by italic numbers, and their explanation should be provided in the caption. Submit all figures on separate pages. Supply figures at final size widths: 80 mm (single column) or 160 mm (double column). Maximum depth is 230 mm. Figure number, author's name, and manuscript title should be written in the bottom left-hand corner.

The manuscript should be signed by all authors. The *electronic version* is formed as a complete manuscript file, without figures. Text files should be submitted in Microsoft Word 6.0 or a later version, using Times New Roman font of 12 point size. Submit figures as separate files. The preferred figure format is TIFF, but JPEG and GIF are also permitted. Load your figures at 600 dpi (dots per inch) for linear and no less than 300 dpi for halftones and photos. Try to keep files under 5 MB.

Editorial Processing (Reviewing, Editing, and Proofs)

The Editorial Office informs authors by e-mail that a manuscript is received. Manuscripts prepared incorrectly or in poor English are not considered. All manuscripts submitted will be reviewed. The reviewer evaluates the manuscript, suggests improvements, and recommends accepting or rejecting the paper. Manuscripts and reviewer's comments are e-mailed to the authors. Revised manuscripts (two copies and the initial version, along with point-by-point responses to the referee) should be returned within 40 days; otherwise, they will be treated as new submissions. If the revised manuscript is not received within four months, it is rejected. The manuscript is then subjected to scientific editing. Accepted manuscripts are published in correspondence with the date of their receiving. Papers containing new information of exceptional significance may be, on the proposal of the Editor in Chief, published first in the shortest possible time. Manuscripts sent to the Editorial Office are not returned to the authors. The Publishing House will deliver the page proofs to authors electronically only to a single address indicated in the affiliation section.

Manuscript Submission

An electronic version should be sent as an attachment to the following e-mail address:

IJPP@iau-saveh.ac.ir
Website: www.ijpp.iau-saveh.ac.ir

Islamic Azad University Saveh Branch Publisher

Copyright Transfer Agreement and Ethical Requirements for the Submitted Paper

Copyright

The copyright of this article is transferred to the Islamic Azad University Saveh Branch Publisher effective if and when the article is accepted for publication. The copyright transfer covers the exclusive right to reproduce and distribute the article, including reprints, translations, photographic reproductions, microform, electronic form or any other reproductions of similar nature. The author warrants that this contribution is original and that he/she has full power to make this grant. The *corresponding author* signs for and accepts responsibility for releasing this material on behalf of any and all co-authors. The authors and their employers retain full rights to reuse their material for their own purposes, with acknowledgement of its original publication in the journal.

Ethical Requirements for the Submitted Paper

• All research or methodologies identified as being conducted or developed by the authors or institutions will in fact have been so conducted or developed.

• Relevant prior and existing research and methodologies will be properly identified and referenced using the standard bibliographic and scientific conventions.

• All the content of the submitted paper shall be the original work of the authors and shall not plagiarize the work of others. Short quotes from the work of others should be properly referenced with full bibliographic details of the quoted work. To quote or copy text or illustrations beyond a “short quote” will require the author to obtain permission from the rights holder.

• Duplicate submission of the same paper to more than one scholarly journal while the decision from another journal on that same paper is still pending, as well as reporting the same results in somewhat different form, is prohibited.

• Authors should take care not to defame other researchers in a personal sense.

• Co-authors should be properly and appropriately identified. To be identified as a co-author, the participant in the research project should have contributed to the conception and design of the project, drafted substantive portions of the paper and taken responsibility for the analysis and conclusions of the paper. Other participants with less responsibility should be identified and acknowledged for their contributions.

Title of article:

Author(s):

Author’s signature:

Author’s email:

Date: