COVER LETTER

April 14, 2016

Dear Professor Roberto Gambari Chief Editor of the Journal Minerva Biotecnologica Via Spallanzani 9 - 00161 ROMA - ITALY

My name is, Muhamad Ali, graduated from Laboratory of Molecular Biotechnology Nagoya University Japan in 2006 and followed Postdoctoral Program at Laboratory of Cellular and Molecular Biotechnology Toyama University Japan in 2012. Now, I am researcher at Laboratory of Microbiology and Biotechnology Mataram University Indonesia with research focusses on the monoclonal antibody engineering. I would like to submit the attached research paper to the Journal Minerva Biotecnologica.

Tittle of the research paper is "Comparison between Mammalian Cell and Bacterial Cell-free System for High-throughput Expression of Linear PCR-amplified Immunoglobulin Genes". Cell-free expression system is the most rapid and robust method for screening of immunoglobulin gene library as shown in the most recent article entitled "Ultra-high-throughput screening of an in vitro-synthesized horseradish peroxidase displayed on microbeads using cell sorter. (Zhu B, Mizoguchi T, Kojima T, Nakano H. in PLoS One. 2015). A mammalian cell-based expression have been developed for the same purpose (Rapid production of antigen-specific monoclonal antibodies from a variety of animals. Kurosawa N, Yoshioka M, Fujimoto R, Yamagishi F, Isobe M. in BMC Biol. 2012). However, the use of both methods to express the same linear Polymerase Chain Reaction (PCR)-amplified immunoglobulin genes (Ig-genes) for generation of a functional monoclonal antibody fragment (Fab) has not been compared yet.

This manuscript is the first report describes the comparison of mammalian cell and bacterial cell-free system for high-throughput expression of linear PCR-amplified immunoglobulin genes, which strongly necessary to reduce the burden of a novel antibody discovery.

I deeply hope that this article is suitable to publish in the Journal of Minerva Biotecnologica. I looking forward your responses.

Sincerely yours.

Muhamad Ali, Ph.D

Laboratory of Microbiology and Biotechnology

Faculty of Animal Sciences, Mataram University, Indonesia

E-mail: ali.molbiotech@gmail.com

AUTHOR STATEMENT FORM

Dear editorial board of the journal Minerva Biotecnologica

I, undersigned

Name : Muhamad Ali

Position : author of manuscript entitled "Comparison between Mammalian

Cell and Bacterial Cell-free System for High-throughput Expression

of Linear PCR-amplified Immunoglobulin Genes"

Declare that there are no competing interests with any financial organization regarding the material discussed in the manuscript.

Mataram, March 25 2016 Sincerely yours,

Muhamad Ali, Ph.D

Lab. Microbiology and Biotechnology Faculty of Animal Sciences, Mataram University Jl. Majapahit No. 62 Mataram, West Nusa Tenggara, Indonesia

AUTHORS' STATEMENT

JOURNAL TITLE: the Minerva of Biotecnologica

Manuscript title: Comparison between Mammalian Cell and Bacterial Cell-free System for High-throughput Expression of Linear PCR-amplified Immunoglobulin Genes

The undersigned authors transfer the ownership of copyright to the journal of Minerva Biotecnologica should their work be published in this journal. They state that the article is original, has not been submitted for publication in other journals and has not yet been published either wholly or in part. They state that they are responsible for the research that they have designed and carried out; that they have participated in drafting and revising the manuscript submitted, whose contents they approve.

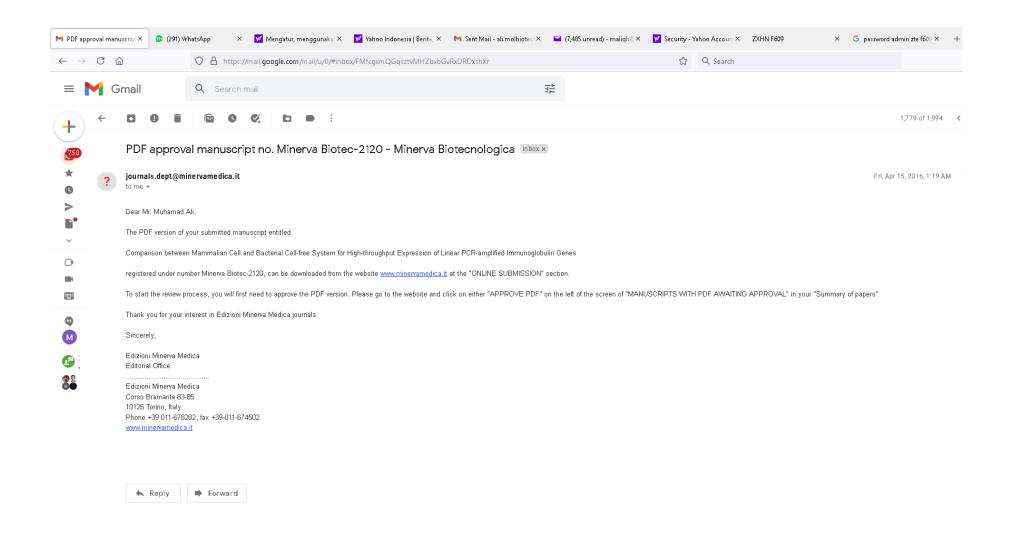
In the case of studies carried out on human beings, the authors confirm that the study was approved by the ethics committee and that the patients gave their informed consent.

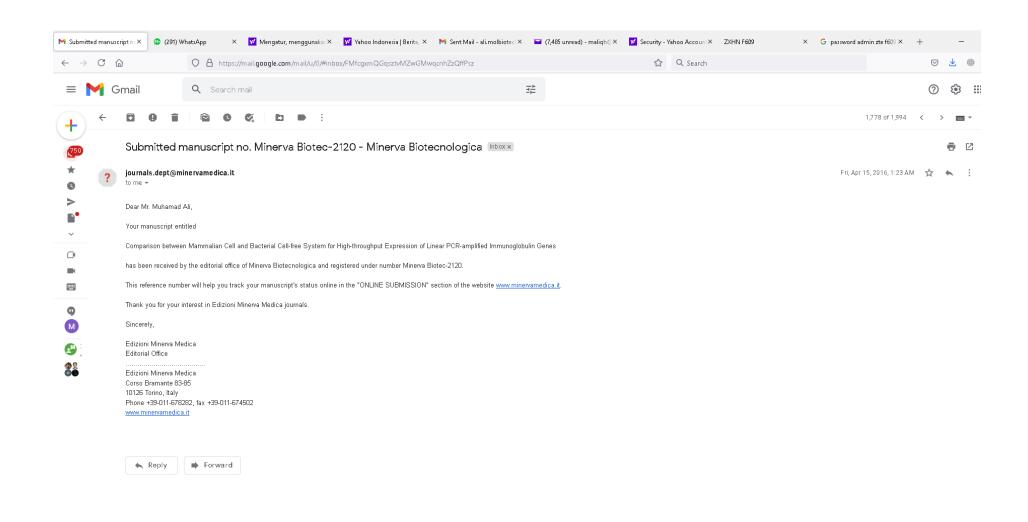
They also state that the research reported in the paper was undertaken in compliance with the Helsinki Declaration and the International Principles governing research on animals.

They agree to inform Edizioni Minerva Medica in the form below of any conflict of interest that might arise, particularly any financial agreements they may have with pharmaceutical or biomedical firms whose products are pertinent to the subject matter dealt with in the manuscript.

The authors agree to bear responsibility for payment of applicable publication charges, as specified in the journal's instructions to authors.

SURNAME AND NAME (in capital letters)	SIGNATURE
MUHAMAD ALI	4





© 2016 EDIZIONI MINERVA MEDICA

Online version at http://www.minervamedica.it

Minerva Biotecnologica 2016

ORIGINAL ARTICLE

Comparison between mammalian cell and bacterial cell-free system for highthroughput expression of linear PCR-amplified immunoglobulin genes

SHORT TITLE: LINEAR PCR-AMPLIFIED IMMUNOGLOBULIN GENES

Muhamad ALI

Laboratory of Microbiology and Biotechnology, Faculty of Animal Sciences, Mataram University

Mataram, Indonesia

Corresponding author: Muhamad Ali, Laboratory of Microbiology and Biotechnology, Faculty of Animal Sciences, Mataram University.

Animal Sciences, Mataram University. Jl. Majapahit No. 62 Mataram, 83125, Indonesia, 93125, E-maîl: all molbiotech@gmail.comali molbiotech@gmail.com

ABSTRACT

BACKGROUND: Monoclonal antibodies have become the most crucial and fastest growing group of protein therapeutics dedicated by modern biotechnology. These molecules represent a powerful reagent not only as a weapon to fight against lethal pathogens, but also as tools for many molecular immunology investigations. Therefore, the development of a high-throughput procedure to generate the antibodies in quite an amount is highly required. Two rapid methods, mammalian cells-based expression and bacterial cell-free transcription/translation system have been developed for the rapid generation of a functional monoclonal antibody. However, the use of both methods to express the same linear polymerase chain reaction (PCR)-amplified immunoglobulin genes (Ig-enes) for generation of a functional monoclonal antibody fragment (Eah) has not been compared yet.

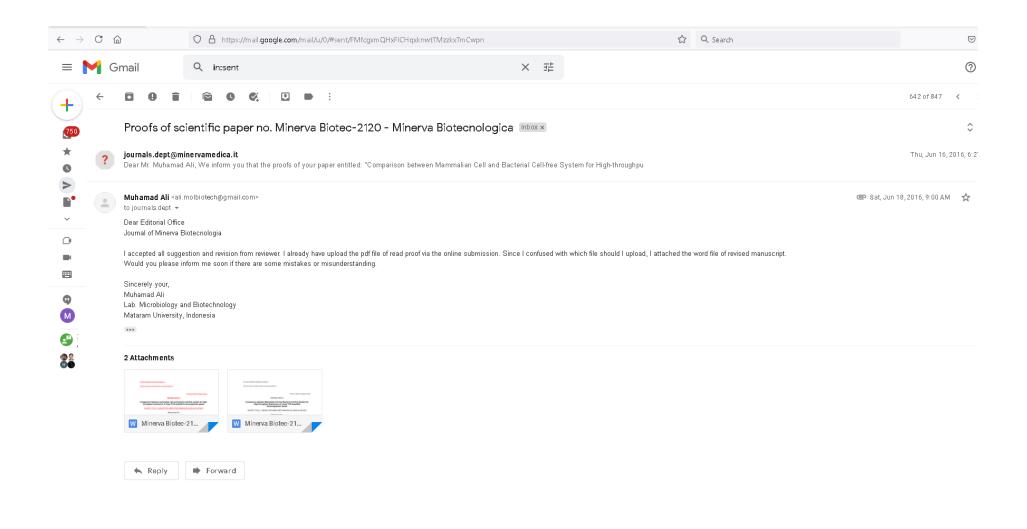
Formatted: Font: Not Bold

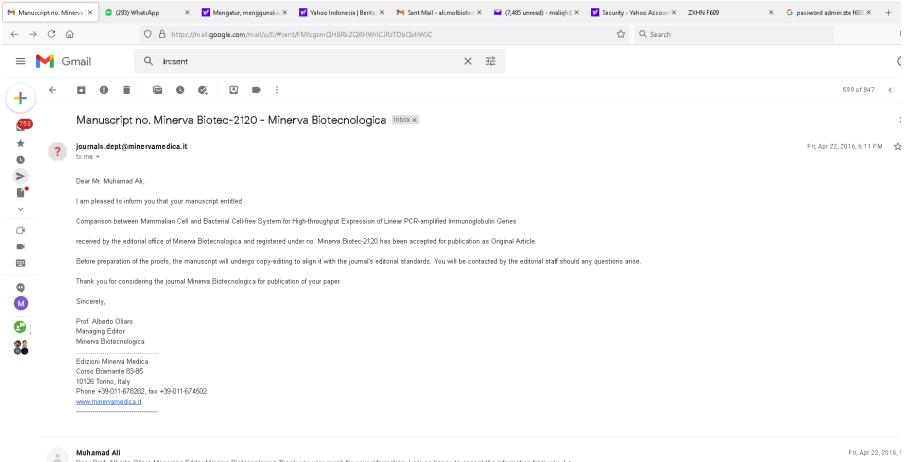
Formatted: Font: Not Italic

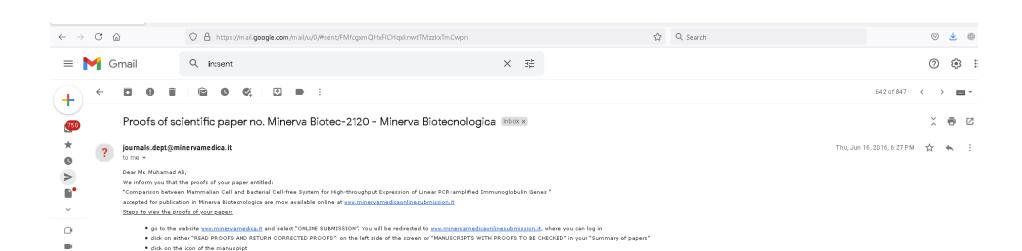
Formatted: Default ParagraphFont

Formatted: Default ParagraphFont

Field CodeChanged







Steps to send the corrected proofs of your paper:

=

•

STEP 1: go to the FILES AREA and choose one of the following options:

• go to the FILES area, download the proofs in pdf on which your corrections should be made.

- after viewing the proofs in pdf and making your corrections, go back to the UPLOADED FILE section and replace the old version of the proofs in pdf with the new version including your corrections
- o after viewing the proofs in pdf, send your comments as a list of corrections by using the AUTHORS' PROOF CORRECTION FORM which can be downloaded in the UPLOAD FILE section. Complete the form in every part, indicating for each correction the incorrect text and the correct text.

You can choose either option or both.

. STEP 2: go to the WORK AREA and click on SUBMIT to forward your corrections to the Editorial Office.

Step 2 is required even if no corrections on the proofs have been made.

Please return the corrected proofs to us within 3 days. Prompt return of proofs is essential for regular publication of the journal.

Proof corrections should be limited to typographical errors. Substantial changes in content (changes of title and authorship, new results and corrected values) will be subject to a completely new peer-review process. Changes that do not conform to the journal's style will not be accepted.

Please read the terms for publication and applicable fees to authors. You will shortly receive payment advice and/or instructions on how to order reprints and a copy of the manuscript in PDF. Your paper will be published pending payment of publication fees as applicable.

Thanking you very much indeed for your cooperation, we send you our very best regards.

Sincerely,

Other than the proofs, you will also find the submitted manuscript (paper files) and the manuscript with the changes made, if needed, by the copyeditors and language reviewers on the final version accepted by the reviewers (edited files). These files are for consultation only.

Edizioni Minerva Medica Editorial Office

Edizioni Minerva Medica Corso Bramante 83-85 10126 Torino, Italy Phone +39 011-678282, fax +39-011-674502

