Manuscript ID : 00001-02701

Source ID : 00000048

Jurnal Veterinar Malaysia

Volume 30, Issue 2, December 2018, Pages 1-7, Page Count - 7

CONSTRUCTION OF IRES-INCORPORATED pMG36e LACTOCOCCAL VECTOR AND ITS SEGREGATIONAL INSTABILITY IN Escherichia coli

Y.Y. Lim [1] I. Noor Hidayah [2] M. Nurul Asyifah [3] Nurulfiza Mat Isa [4]*

[1] Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Seri Kembangan, Malaysia.
[2] Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Seri Kembangan, Malaysia.
[3] Graduate School of Life Science and Systems Engineering, Kyushu Institute Of Technology, Wakamatsu, Kitakyushu 808-0196, Japan.
[4] Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Seri Kembangan, Malaysia.

Abstract

The increasing demand of Lactococcus lactis (L. lactis) beyond application in food industry raises the need to develop lactococcal bicistronic vector with target gene expression in a eukaryotic system for vaccination purposes. Despite the importance of functional studies of the expression vector in eukaryotic system, the stable and efficient host for vector propagation should be tackled. Therefore, the objectives of this study were to construct a pMG36e lactococcal vector containing modified eukaryotic expression cassette, with internal ribosome entry site (IRES) inserted between CMV promoter and Poly A signal to allow transcription and translation of two proteins in a single cassette and, study its stability in Escherichia coli (E. coli) TOP 10 as a propagation host. IRES sequence was PCR-amplified from pRetroX-IRES-ZsGreen1 vector, digested with EcoRV and XhoI, and ligated into eukaryotic expression cassette from pcDNA 3.1 HisA plasmid/vector. Constructed vector was transformed into E. coli TOP 10 and the orientation of IRES fragment in the modified eukaryotic expression cassette was confirmed by DNA sequencing. The modified eukaryotic expression cassette was PCR-amplified from pcDNA3.1HisA/IRES before sub-cloning into lactococcal vector pMG36e and transformed into E. coli TOP 10. pMG36e harbouring modified eukaryotic expression cassette was successfully constructed and transformed into E. coli TOP 10. However, the segregational instability of constructed vector in E. coli Top10 host was detected, which may have been caused by production of single-stranded intermediates and high-molecular weight DNA due to rolling-cycle replication of the pMG36e vector.

Author Keywords

E. coli Top10, eukaryotic expression cassette, IRES, Lactococcus lactis, pMG36e

Funding Details

This work was supported by the Sciencefund research grant 02-01-04-SF2033 and 02-01-04-SF1045 from the Ministry of Science, Technology and Innovation of Malaysia (MOSTI).

Acknowledgement

This work was supported by the Sciencefund research grant 02-01-04-SF2033 and 02-01-04-SF1045 from the Ministry of Science, Technology and Innovation of Malaysia (MOSTI). The authors would also like to thank Prof C.J. Leenhouts from University of Gronigen, Holland for the kind gifts of the lactococcal vector pMG36e and the host used in this study and, also acknowledge the valuable proof reading by Professor Tan Soon Guan, Associate Editor of Elsevier Editorial System, Gene.

ISSN Print: 9128-2506
ISSN Online: 2682-9339
Source Type: Journals
Document Type: Journal Article
Publication Language: English
DOI:
Abbreviated Journal Title:
Access Type: Open Access

Publisher Name: Veterinary Association Malaysia
Major Subject: Health Sciences
Subject area: Veterinary Sciences

Resource Licence: CC BY-NC
Subject Area classification: Veterinary
Source: SCOPEDATABASE

Reference

References (19)

1. Celie, P.H., Parret, A.H. and Perrakis, A
Recombinant cloning strategies for protein expression

(2016) Current Opinion in Structural Biology, Volume 38, Page No 145-154,

2. Dabert, P., Ehrlich, S.D. and Gruss, A
High-molecular-weight linear multimer formation by single-stranded DNA plasmids in Escherichia coli

(1992) Journal of Bacteriology, Volume 174, Issue 1, Page No 173-178,

3. De Ruyter, P.G., Kuipers, O.P. and De Vos, W.M
Controlled gene expression systems for Lactococcus lactis with the food-grade inducer nisin

(1996) Applied and Environmental Microbiology, Volume 62, Issue 10, Page No 3662-3667,

4. Glenting, J., Madsen, S.M., Vrang, A., Fomsgaard, A. and Israelsen, H
A plasmid selection system in Lactococcus lactis and its use for gene expression in L. lactis and human kidney fibroblasts

(2002) Applied and Environmental Microbiology, Volume 68, Issue 10, Page No 5051-5056,

5. Gruss, A. and Ehrlich, S.D
Insertion of foreign DNA into plasmids from gram-positive bacteria induces formation of highmolecular-weight plasmid multimers

(1988) Journal of Bacteriology, Volume 170, Issue 3, Page No 1183-1190,

6. Hennecke, M., Kwissa, M., Metzger, K., Oumard, A., Kröger, A., Schirmbeck, R., Reimann, J. and Hauser, H
Composition and arrangement of genes define the strength of IRES-driven translation in bicistronic mRNAs

(2001) Nucleic Acids Research, Volume 29, Issue 16, Page No 3327-3334,

7. Hongying, F., Xianbo, W., Fang, Y., Yang, B. and Beiguo, L
Oral immunization with recombinant Lactobacillus acidophilus expressing the adhesin Hp0410 of Helicobacter pylori induces mucosal and systemic immune responses

(2014) Clinical and Vaccine Immunology, Volume 21, Issue 2, Page No 126-132,

8. Hunt, I
From gene to protein: a review of new and enabling technologies for multi-parallel protein expression

(2005) Protein Expression and Purification, Volume 40, Issue 1, Page No 1-22,

9. Kiewiet, R., Kok, J., Seegers, J.F., Venema, G. and Bron, S
The mode of replication is a major factor in segregational plasmid instability in Lactococcus lactis

(1993) Applied and Environmental Microbiology, Volume 59, Issue 2, Page No 358-364,

10. Kusano, K. and Nakayama, H
Plasmid-mediated lethality and plasmid multimer formation in an Escherichia coli recBC sbcBC mutant. Involvement of RecF recombination pathway genes

(1989) Journal of Molecular Biology, Volume 209, Issue 4, Page No 623-634,

11. Mutalib, N.E.A., Isa, N.M., Alitheen, N.B., Song, A.A.L. and Rahim, R. A.
IRES-incorporated lactococcal bicistronic vector for target gene expression in a eukaryotic system

(2014) Plasmid, Volume 73, Page No 26-33,

12. Nanyan N,, Hooi, W.Y., Baradaran, A., Mohamad, R., Chin Chin, S., Md Illias, R., Yusoff, K. and Abdul Rahim, R
Lactococcus lactis M4, a potential host for the expression of heterologous proteins

(2011) Microbial Cell Factories, Volume 10, Issue 28,

13. Pontes, D.S., De Azevedo, M.S.P., Chatel, J.M., Langella, P., Azevedo, V. and Miyoshi, A
Lactococcus lactis as a live vector: heterologous protein production and DNA delivery systems

(2011) Protein Expression and Purification, Volume 79, Issue 2, Page No 165-175,

14. Renaud-Gabardos, E., Hantelys, F., Morfoisse, F., Chaufour, X., GarmySusini, B. and Prats, A. C
Internal ribosome entry site-based vectors for combined gene therapy

(2015) World Journal of Experimental Medicine, Volume 5, Issue 1, Page No 11,

15. Sambrook, J., Fritsch, E.F. and Maniatis, T
Molecular cloning

(1989) Volume 2, Page No 14-9,

16. Suarez, D.L. and Schultz-Cherry, S
The effect of eukaryotic expression vectors and adjuvants on DNA vaccines in chickens using an avian influenza model

(2000) Avian Diseases, Page No 861-868,

17. Tupperwar, N., Tiwari, A.K., Kataria, R.S., Kumar, S., Khurana, S.K. and Rai, A
Expression of IBD virus VP2 gene in eukaryotic expression system for use as DNA vaccine

(2010) Journal of Immunology and Immunopathology, Volume 12, Issue 1, Page No 52-58,

18. Vagner, S., Galy, B. and Pyronnet, S
Irresistible IRES

(2001) EMBO Reports, Volume 2, Issue 10, Page No 893-898,

19. Wang, C., Zhang, C. W., Du, J. and Lü, X. Y
Study of the genetic stability of expression plasmid vector pMG36e in host bacte ria

(2005) Journal of Hygiene Research, Volume 34, Issue 2, Page No 214-216,

What is Scope Database
Content Coverage Guide
Scope Database Blog
Content Coverage API
Scope Database App
Scope Database Key Persons
Contact us