# Isolation of Escherichia Coli Flag Gene for Transformation

**[1]M. Sidratullah, [2]Khairil Pahmi**
[1]Pharmacy (DIII) Study Program, Faculty of Health Science, Nahdlatul Wathan Mataram University
[2]Medical Education Study Program, Faculty of Medicine, Mataram University
**Corresponding author**: khairil_pahmi@staff.unram.ac.id

**Abstract**
The term "FLAG tag" refers to a hydrophilic peptide (DYKDDDDK) made up of eight residues that has an enterokinase cleavage site and is intended for the immunoaffinity purification and identification of recombinant proteins and strong specificity for monoclonal antibodies. Its hydrophilicity and tiny size help with efficient surface localization, which reduces disruption to protein activity. The method of this research is experimental. The experiment result will be described. Some processes will be done by polymerase chain reaction (PCR) to amplify the gene and then it will be extracted to pure the targeted gene. This research was successful to isolate Escherichia coli FLAG gene for transformation. This gene has important role to encode the flagella in the bacteria.
**Keywords**: *Isolation, Escherichia Coli Flag Gene, Transformation*

## 1. INTRODUCTION

The eight amino acid water-loving peptides that make up the "FLAG tag" are DYKDDDDK. It is intended to recognize and purify modified proteins, has a strong affinity for monoclonal antibodies, and has a site that enterokinase can cut. Its compact size and water-absorbing ability facilitate effective surface positioning and reduce interference with protein activity. Escherichia coli K-12 has two distinct, linked flagellar genes called fhiA-mbhA that do not have promoters, in contrast to *Salmonella enterica*. These genes are remnants of an ancient group of 44 genes that produced a novel flagellar system known as Flag-2, according to computer analysis. The enteroaggregative E contains the lfgC gene. This system has probably been disrupted by changes made to coli strain 042. 15 of the 72 well-researched ECOR strains have the Flag-2 gene family, according to research using tiling route PCR. The Flag-2 system is similar to the lateral flagellar systems.

## 2. METHODS

This study employs an experimental methodology. The experiment's results will be described in detail. In some processes, the targeted gene will be isolated for purity after the polymerase chain reaction (PCR) is used to increase the number of gene copies.

**Materials**

**Polymerase Chain Reaction (PCR) Components**

This kit contains the following components for 200 reactions:

KOD -Plus- (1.0 U/μL) * 200 μL × 1

10× Buffer for KOD -Plus- 1.0 mL × 1

25 mM MgSO4 1.0 mL × 1

2 mM dNTPs 1.0 mL × 1

*The enzyme solution contains anti-KOD DNA polymerase antibodies that neutralize polymerase and 3'→5' exonuclease activity.

Standard PCR setup

The following procedure is designed for use with the components included in this kit. Before preparing the mixture, all components except the enzyme solution must be completely thawed.

| Component | Volume<br>5 μL | Final Concentration 10x Buffer for KOD -Plus-<br>1x |
|---|---|---|
| 2mM dNTPs* | 5 μL | 0.2 mM each |
| 25mM MgSO4 | 2 μL | 1.0 mM |
| 10pmol/μL Primer #1 | 1.5 μL | 0.3 μM |
| 10pmol/μL Primer #2 | 1.5 μL | 0.3 μM |

| | | |
|---|---|---|
| Genomic DNA 10-200 ng/50 µL | | |
| Template DNA X µL | Plasmid DNA 1-50 ng/50 µL | |
| cDNA ≤ 100 ng | RNA equiv.)/50 µL | |
| PCR grade water | Y µL | |
| KOD-Plus- (1.0 U/µL) | 1 µL | 1.0 U / 50 µL |
| Total reaction volume | 50 µL | |

PCR Cycling conditions

The following cycling steps are recommended.

| | | |
|---|---|---|
| < 2-step cycle > | | |
| Pre-denaturation: | 94 °C , 2 min. | |
| Denaturation: | 94 °C, 15 sec. | 25-35 cycles |
| Extension: | 68 °C, 1 min./kb | |
| < 3-step cycle > | | |
| Pre-denaturation: | 94 °C , 2 min. | |
| Denaturation: | 94 °C, 15 sec. | 25-35 cycles |
| Annealing: | Tm-[5-10] oC*, 30 sec. | |
| Extension: | 68 °C, 1 min./kb | |

Miniprep procedure

1. Centrifuge 1-5mL of bacterial culture at maximum speed in a 1.5mL microcentrifuge tube. Because the 1.5mL tubes can only hold ~1.4mL at a time, repeat as necessary to pellet all of the culture. Discard the supernatant after each spin.
2. Add 600uL water or Zymo Resuspension buffer to the bacterial cell pellet and resuspend completely.
3. Add 100ul of 7X Zymo Lysis buffer (blue) and mix by inverting the tube 4-6 times. Proceed to step 4 within 2 minutes. After addition of the lysis buffer, the solution should change from opaque to clear blue, indicating complete lysis.
4. Add 350uL of cold Zymo Neutralization buffer (yellow) and mix thoroughly by inverting the tube 4-6 times. The sample will turn yellow when the neutralization is complete. Invert sample an additional 2-3 times to ensure complete neutralization.
5. Centrifuge at maximum speed for 2-4 minutes.
6. Transfer the supernatant (~900uL) to a provided Zymo-Spin IIN column in a collection tube. Avoid disturbing the cell debris pellet. This can be done by pipetting or careful pouring.

7. Centrifuge 15 seconds and discard the flow-through. Place the column back in the collection tube.
8. Add 200uL of Zymo Endo-Wash buffer to the column. Centrifuge for 30 seconds. It is not necessary to empty the collection tube after this spin.
9. Add 400uL Zyppy Wash buffer to the column. Centrifuge 2 minutes.
10. Transfer the column into a clean 1.5mL tube labeled "Plasmid miniprep." Add 30uL water directly to the column matrix and incubate 1 minute at room temperature.
11. Centrifuge 30 seconds to elute plasmid DNA.
12. Quantify DNA concentration by Nanodrop. Record the concentration on the side of the tube.

Store DNA at 4C until ready to use.

## 3. RESULTS AND DISCUSSION

This method was successful in isolating the Escherichia coli FLAG gene in order to prepare it for transformation (Figure 1). The extraintestinal pathogenic Escherichia coli (ExPEC) can infect and thrive in a variety of places outside the intestines, resulting in a wide spectrum of disorders, while the processes underlying its ability to cause various diseases are yet understood. By enabling them to adhere and invade, flagella help E. Coli strains spread to other people. A prior bioinformatics investigation of an ExPEC sample designated PCN033 revealed two groups of flagella genes. The first group is in charge of the traditional flagellum system (Flag-1), while the second group is linked to the Flag-2 system, whose purpose is yet unclear. According to earlier studies, the Flag-1 system controls bacterial swimming and swarming as well as the development and enhancement of the flagellum structure; the Flag-2 system is not involved in these processes. Furthermore, because it affects the production of FliC (flagellin), the flgD gene, which codes for a protein that supports the flagellar hook in the Flag-1 system, is crucial for flagellum formation. The deletion of flgD likely reduced the ability of the ExPEC strain PCN033 to colonize and invade live creatures due to a reduction in its capacity to adhere and invade, as well as its ability to resist being engulfed by circulating monocytes.

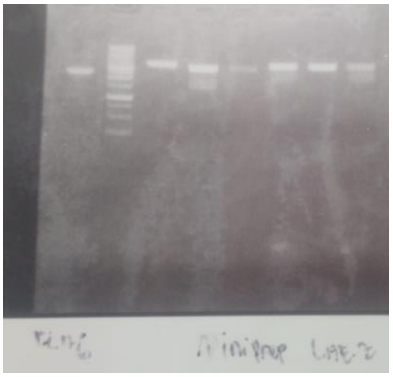

Figure 1. FLAG gene with the other gene isolation

All of the genes needed for a flagellar motility system may be found in the 44-gene region. All potential orthologs of the Vibrio parahaemolyticus lateral system are found in the Flag-2 locus, with the exception of the motY gene, which codes for a motor element. The Flag-2 system is most similar to the lateral system of V. parahaemolyticus, aside from similarities in gene products and possible operon structure [6]. Additionally, similar to the V. parahaemolyticus system and numerous other nonenteric flagellar systems, such as those in Caulobacter crescentus, Campylobacter jejuni, Legionella pneumophila, Pseudomonas aeruginosa, and other Vibrio species, the Flag-2 system appears to be controlled by RpoN (7, 8, 9, 10, 11, 12, 13, 14, 15, 16). The location encodes a potential σ54-dependent flagellar regulator, similar to the LafKvp regulator [15], and several of the potential operons are upstream of the consensus σ54 promoter areas [15].

## 4. CONCLUSION

FLAG gene of E. coli was isolated successfully for transformation. This gene has important role to encode the flagella in the bacteria.

## REFERENCES

Ren CP, Beatson SA, Parkhill J, Pallen MJ. The Flag-2 locus, an ancestral gene cluster, is potentially associated with a novel flagellar system from Escherichia coli. *J Bacteriol.* 2005 Feb;187(4):1430-40. doi: 10.1128/JB.187.4.1430-1440.2005. PMID: 15687208; PMCID: PMC545627.

Liu R, Ochman H. Origins of flagellar gene operons and secondary flagellar systems. *J Bacteriol.* 2007 Oct;189(19):7098-104. doi: 10.1128/JB.00643-07. Epub 2007 Jul 20. PMID: 17644605; PMCID: PMC2045201.

De Maayer, P., Pillay, T. & Coutinho, T.A. Flagella by numbers: comparative genomic analysis of the supernumerary flagellar systems among the Enterobacterales. *BMC Genomics* 21, 670 (2020). https://doi.org/10.1186/s12864-020-07085-w

Feng Liu, Jiyang Fu, Canying Liu, Jing Chen, Minhua Sun, Huanchun Chen, Chen Tan, Xiangru Wang, Characterization and distinction of two flagellar systems in extraintestinal pathogenic Escherichia coli PCN033, Microbiological Research, Volume 196, 2017, Pages 69-79, ISSN 0944-5013, https://doi.org/10.1016/j.micres.2016.11.013. (https://www.sciencedirect.com/science/article/pii/S0944501316304803)

Ren, C.-P., S. A. Beatson, J. Parkhill, and M. J. Pallen. 2004. The Flag-2 locus, an ancestral gene cluster, is potentially associated with a novel flagellar system from Escherichia coli. J. Bacteriol.187:1207-1209.

McCarter, L. L. 2004. Dual flagellar systems enable motility under different circumstances. J. Mol. Microbiol. Biotechnol.7:18-29.

Berg, H. C. 2003. The rotary motor of bacterial flagella. Annu. Rev. Biochem.72:19-54.

Dasgupta, N., M. C. Wolfgang, A. L. Goodman, S. K. Arora, J. Jyot, S. Lory, and R. Ramphal. 2003. A four-tiered transcriptional regulatory circuit controls flagellar biogenesis in Pseudomonas aeruginosa. Mol. Microbiol.50:809-824.

Jacobi, S., R. Schade, and K. Heuner. 2004. Characterization of the alternative sigma factor sigma54 and the transcriptional regulator FleQ of Legionella pneumophila, which are both involved in the regulation cascade of flagellar gene expression. J. Bacteriol.186:2540-2547.

Jagannathan, A., C. Constantinidou, and C. W. Penn. 2001. Roles of rpoN, fliA, and flgR in expression of flagella in Campylobacter jejuni. J. Bacteriol.183:2937-2942.

Kawagishi, I., M. Nakada, N. Nishioka, and M. Homma. 1997. Cloning of a Vibrio alginolyticus rpoN gene that is required for polar flagellar formation. J. Bacteriol.179:6851-6854.

Kim, Y. K., and L. L. McCarter. 2000. Analysis of the polar flagellar gene system of Vibrio parahaemolyticus. J. Bacteriol.182:3693-3704.

Millikan, D. S., and E. G. Ruby. 2003. FlrA, a σ54-dependent transcriptional activator in Vibrio fischeri, is required for motility and symbiotic light-organ colonization. J. Bacteriol.185:3547-3557.

Prouty, M., N. Correa, and K. Klose. 2001. The novel σ54- and σ28-dependent flagellar gene transcription hierarchy of Vibrio cholerae. Mol. Microbiol.39:1595-1609.

Stewart, B. J., and L. L. McCarter. 2003. Lateral flagellar gene system of Vibrio parahaemolyticus. J. Bacteriol.185:4508-4518.

Wu, J., and, N. A. 1997. Regulation of the Caulobacter flagellar gene hierarchy; not just for motility. Mol. Microbiol.24:233-239.