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Abstract. This research aimed to express F recombinant protein that is cloned from genes F of local isolate ND virus which can be used as vaccine candidate in order to improve the effectiveness of ND virus vaccination. Confirmation of NDV pBT7-N-His-Fusion plasmid on C1a clone is done by gel agarose 1% electrophoresis with staining by using fluorosafe DNA stain. To separate plasmid and insert that contain genes F, cutting is done with EcoRI restriction enzyme. EcoRI enzyme is able to cut NDV pBT7-N-His-Fusion plasmid through 37℃ incubation process during three hours. DNA cutting visualization is done by gel agarose 1% electrophoresis by using fluorosafe DNA stain. NDV pBT7-N-His-Fusion plasmid is express by E. coli extract in order to gain F protein. The product of protein expression is visualized by SDS – PAGE and western blot. NDV pBT7-N-His-Fusion plasmid visualization by gel agarose electrophoresis results 4643 bp band. Moreover, from the visualization of Eco RI enzyme cutting on gel agarose electrophoresis result, the researcher found two bands with different size, 4001 bp and 642 bp. After protein expression process 25,6 kDa band is seen both in the result of SDS – PAGE and western blot.

1. Introduction
Newcastle Disease is a very contagious disease that occurs to most avian in Indonesia [1]. It is caused by virulent strain of avian paramyxovirus type I (APMV – I) from Avulavirus genus Paramyxovirinae subfamily, Paramyxoviridae family [2]. Currently, the disease is being the main problem of poultry industry. It results a great number of economic loss since the mortality rate increases up to 100% [3]. Newcastle Disease virus has negative-stranded RNA and six proteins that is fusion protein, hemagglutinin-neuraminidase, nucleoprotein, phosphoprotein, matrix, and RNA polymerase [4]. Hemagglutinin-neuraminidase (HN) protein and fusion protein (F) have a very significant contribution in the virulence and spread of ND viruses in host [5]. Antibodies for HN and F neutralize and represent the main protective component induced by the ND virus vaccine [6].

In 2010, there was an outbreak of a highly pathogenic ND virus and a Ban / 010 strain of ND virus was isolated from chickens affected by the disease. Sequencing results from F and HN genes showed that homology in amino acid sequence F proteins ranged from 89%, and HN proteins ranged from 87%
between Ban / 010 strains and B1 and Lasota vaccine strains [7]. This shows an antigenic difference between NDV strains in farm and the ND virus vaccine strains in farm, causing poor post-vaccination protection. To prevent Newcastle Disease, vaccination in farm has been done. However, the failure of this prevention still can be found in several farm. It is shown by the finding of disease and the decrease of antibody titer after the vaccination. The failure might be caused by to possibilities, incompatibility between the vaccine and the virus which exists in most farm and virus mutation [8]. Thus, this research aimed to express F recombinant protein that is clon from genes F of local isolate ND virus which can be used as vaccine candidate in order to improve the effectiveness of ND virus vaccination.

2. Methodology

This research was conducted at Laboratorium Biokimia and Biologi Molekuler Fakultas Kedokteran Hewan Universitas Gadjah Mada and at Laboratorium Bioteknologi Pusat Studi Bioteknologi Universitas Gadjah Mada since July 2017 until February 2018. This study used C1a plasmid containing pBT7-N-His. In the plasmid gene F has been inserted from the Newcastle Disease virus isolate (ND) originating from the fowl line Kulon Progo, Daerah Istimewa Yogyakarta.

The agarose gel is used as a medium in the electrophoresis process to see DNA in the C1a plasmid with fluorosafe staining. The gel concentration used is 1%. Electrophoresis runs for 45 minutes with a voltage of 80 volts. Electrophoresis results were visualized with the help of UV Transiluminator (λ = 260 nm). Positive results are indicated by the formation of fluorescent DNA bands. Next, C1a plasmid DNA was cut using the EcoR1 restriction enzyme, this process requires incubation at 37 °C for three hours. The agarose gel is used as a medium in the electrophoresis process to see the results of restriction with fluorosafe staining and the gel concentration used is 1%. The electrophoresis process runs for 60 minutes with a voltage of 80 volts. DNA cutting electrophoresis results with restriction enzymes were visualized with the help of UV Transiluminator. Positive results are indicated by the formation of fluorescent DNA bands measuring 4001 bp and 642 bp. Then protein expression was performed using the AccuRapidTM Protein Expression Kit of Bioneer production consisting of Master mix, E. coli extract, DNA template, and DEPC DW. Incubation using Hot Dry Bath is done for three hours with a temperature of 30 °C.

Electrophoresis is done to see the results of protein expression. The technique of separating these protein expression products using the SDS-PAGE method (Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis). SDS - PAGE method consists of four stages, namely sample loading, Running, Staining, and Destaining [9]. The gel used in this method is a polyacrylamide gel consisting of two layers where the first layer is a stacking gel with a concentration of 3% and the second layer is a separating / gradient gel with a concentration of 12.5%. The results of SDS-PAGE will then be transferred to the PVDF membrane. Then the western blot method is done to see protein F. To see results from western blot, add a substrate. Products produced from enzyme and substrate bond reactions are in the form of a blue ribbon that shows positive results.

3. Result and Discussion

The sample used in this study was pBT7-N-His-Fusion plasmid. This plasmid is a recombinant plasmid that contains the desired insert gene for gene expression [10]. The insert gene inserted into the expression vector is the F gene from the Newcastle Disease virus from a local isolate. In this study, the clones used were C1a clones containing pBT7-N-His-Fusion plasmid. Plasmid pBT7-N-His is an expression vector measuring 4001 bp. This plasmid is inserted by gene F which is one of the genes that encode the ND virus. PBT7 plasmid-N-His-Fusion Newcastle Disease virus consists of several components, namely PUC Origin, LacI, Ampicillin, and cloning areas and expressions where in this area there are restriction enzymes namely Eco RI.
First confirmation was made to find out that in the clones used as samples there were pBT7-N-His-Fusion plasmid Newcastle Disease virus using DNA electrophoresis method. DNA electrophoresis uses agarose gel with a concentration of 1%. The first well filled DNA ladder measuring 100 bp and the second well filled with plasmid DNA. The running process is carried out for 45 minutes with an 80 Volt power supply. Electrophoresis results are shown in Figure 1.

![Figure 1](image)

**Figure 1.** PBT7-N-His-Fusion NDV plasmid DNA electrophoresis uses 1% agarose gel with 80 Volt voltage. [M]: DNA ladder 100 markers bp; [K]: C1a clone

There are glowing bands. These bands are plasmid DNA. DNA bands can be detected because previously DNA bands have been stained by fluorosafe DNA stain which will be seen when visualized under UV light [11]; [12]. Running plasmids on agarose gel electrophoresis have three DNA bands that appear, because they have three forms of conformation namely supercoiled, linear, and nicked circular [13].

Confirmation of the F gene in the pBT7-N-His-Fusion plasmid sample Newcastle Disease virus was carried out by DNA cutting method using enzyme retention endonuclease. In this method, the pBT7-N-His-Fusion plasmid Newcastle Disease virus was cut using the Eco RI enzyme where the enzyme was isolated from the Escherichia coli bacteria strain RY 13 which had the GGATCC identification sequence [14]. The Eco RI enzyme was chosen because in the pBT7-N-His-Fusion plasmid virus the Newcastle Disease which is to be cut there is an Eco RI enzyme restriction enzyme component.

Restriction endonuclease results can be separated and visualized by medium gel electrophoresis [15]. Visualization using 1% agarose gel where the first well was filled with 100 bp DNA ladder, the second well contained intact pBT7-N-His plasmid, and the third well filled with plasmid pBT7-N-His-Fusion Newcastle Disease virus which had been cut with Eco RI restriction enzyme. Running is carried out with an 80 Volt voltage for 1 hour. The results of cutting product electrophoresis with Eco RI enzymes are shown in Figure 2.
Figure 2. The cutting product electrophoresis results with Eco RI enzyme using 1% agarose gel with 80 Volt voltage. [M]: DNA ladder 100 markers bp; [U]: pBT7-N-His-Fusion-NDV intact; [P]: pBT7-N-His-Fusion NDV which has been cut by the Eco RI enzyme

The cutting process with the Eco RI enzyme produces two bands of different sizes in the third well, which are indicated by the luminous band with a size of 4001 bp and 642 bp. While in the second well, only one band was formed because it was not cut using Eco RI restriction enzymes. The 4001 bp DNA band is a pBT7-N-His plasmid and a 642 bp band is the F gene from the ND virus inserted in the pBT7-N-His-Fusion plasmid Newcastle Disease virus. This shows that the F gene has been successfully inserted in the pBT7-N-His plasmid so that the plasmid can then be used for the expression of recombinant proteins.

The expression of the F gene protein that has been inserted in the pBT7-N-His-Fusion plasmid Newcastle Disease virus was carried out in order to translate the protein from the F gene to be identified later. Protein expression was carried out using the cell free method using extract from Escherichia coli bacteria. The expression of proteins using the cell free method is a method of protein synthesis in vitro by involving whole cell extracts that are suitable for the protein expression process [16].

To find out the results of protein expression, a polyacrylamide gel electrophoresis (PAGE) was confirmed. Electrophoresis results of protein expression products are shown in Figure 3. Proteins separate into bands according to their molecular weight. Smaller molecular weight proteins will move faster and be able to pass through the gel pores, whereas larger molecular weight proteins will move more slowly and some will be left on top of the gel [15]. Positive and negative controls are used as a determinant of the presence or absence of protein F which is a sign of success of the protein expression process.
Figure 3. Electrophoresis results of protein expression products using polyacrilamide gel with a voltage of 100 volts and Comassie blue coloring 0.1%. [M]: Protein ladder marker; [(+)]: Positive controls; [(-)]: Negative controls; [C1a]: C1a clone that has expression.

The visualization of polyacrylamide gel with coomassie brilliant blue (CBB) staining in Figure 3 shows that in the C1a well there was a protein band of 25.6 kDa after being compared with protein markers and positive control showed a 28 kDa protein band. The protein markers used were 10 kDa - 180 kDa so that it was possible to identify recombinant protein F sizes of 25.6 kDa.

Based on the results of the SDS-PAGE, it can be assumed that the protein sample in C1a clone contains recombinant F protein. The results were further confirmed by the Histaq antibody (6x His Epitop Taq Antibody) with Western blot to determine the suitability of the target protein with the antibody. The western blot method is used to identify proteins using the principle of antigen-antibody reaction [17]. The antigen-antibody reaction complex that is formed is then compared to a standard protein (marker) that has known molecular weight. Antibodies that are specific to antigens, bind only to proteins that are expressed and visualized as single bands [18].

Primary antibodies and secondary antibodies are used where the primary antibodies are Histaq Antibodies (6x hisc epilopc Taq Antibody) and secondary antibodies used are IgG Anti-Mouse Goat. Western blot results of protein expression products are shown in Figure 4.
Figure 4. The Western blot product is a protein expression that is transferred to the PVDF (polyvinyl diflouride) membrane with a voltage of 500 mA for 1 hour. [M]: Protein ladder marker; [(-)]: Negative controls; [(+)]: Positive controls; [C1a]: Plasmid clone C1a

For visualization of the presence or absence of protein F, after adding primary antibodies, the addition of secondary antibodies, AntiHistaq Antibodies in the Goat Anti-Mouse IgG, is attached to the enzyme alkaline phosphatase where these secondary antibodies recognize and form antigen-antibody bonds with primary antibodies. Tape visualization is done by adding a substrate wherein the substrate will bind to the alkaline phosphatase enzyme and produce a blue color product on the PVDF membrane (polyvinyl diflouride).

Positive results are indicated by the presence of a band as seen on the PVDF (polyvinyl diflouride) membrane. Antibodies that are specific to the antigen will bind to proteins that are expressed and visualized as bands. Visualization with Western blot in Figure 16. shows the presence of one specific protein band with a molecular weight of 25.6 kDa. This shows that recombinant F protein is able to react positively with the Histaq Antibody (6x hisc epitope Taq Antibody) at a molecular weight of 25.6 kDa which means the expression process works well with protein F [19].

4. Conclusion
Protein expression techniques were carried out in vitro using E. coli extract. This technique can express the F gene in pBT7-N-His-Fusion plasmid from the C1a Newcastle Disease virus clone properly and visualize it using the SDS-PAGE and western blot methods. The results of the plasmid pBT7-N-His-Fusion virus Newcastle Disease analysis after going through several research methods showed that there was protein F with a molecular weight of 25.6 kDa which could later proceed to the purification process to become a vaccine candidate in farm.

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