ANALYZING TRANSFORMANTS OF GP198 AND GP270 OF JUMBO BACTERIOPHAGE RELATED TAIL FIBER GENES AND ITS UTILIZATION AS A SERIAL POSTER

THESIS

By:
Afifatur Rofiqoh
NIM 160210103090

Supervisor : Erlia Narulita S.Pd., M.Si., Ph.D.

Co supervisor : Mochammad Iqbal S.Pd., M.Pd.

STUDY PROGRAM OF BIOLOGY EDUCATION
DEPARTMENT OF MATHEMATICS AND SCIENCE EDUCATION
FACULTY OF TEACHER TRAINING AND EDUCATION
UNIVERSITY OF JEMBER
2020

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Compose to Fulfill One of the Requirements to Obtain the Degree of S1 at the Biology Education Program, Mathematics And Science Department, The Faculty of Teacher Training and Education, Jember University

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ACKNOWLEDGMENT

Praise the researchers for the presence of Allah SWT, who always gives grace, guidance, and His gifts. Prayers and greetings may ever say to the great prophet Muhammad SAW who became a role model for all Muslims. I present this thesis to:

1. Beloved parents May all the sacrifices and labors of father and mother be blessed;
2. My dear brother M.Irfan Fauzi who always taught me about how to live qona'ah and ta'dim, gave me enthusiasm in helping life and never succeeding in overcoming problems.
3. Thesis supervisor, Erlia Narulita S.Pd., M.Sc., Ph.D., and Mochammad Iqbal S.Pd., M.Pd. Who always guides, directs, and facilitates wholeheartedly in completing this thesis;
4. Friends of the "Bacteriophage 2016 Team" Riska, Wida, Zidna, Tania, Fitri, Icha, and Brevy who have helped struggle in grief during the thesis research process and research;
5. Friends of boarding Java 4c no.8 virtual, lilik, Salma, anastasya, and mirza who always makes me smile in every situation and provide enthusiasm and cheerfulness in every conversation;
6. Almamater of the Teacher Training and Education Faculty that I am still proud of, notably the Biology Education Study Program, Jember University.

MOTTO

Whoever does the slightest good, surely he will see his reply

(Translate Q.S Al-Zalzalah: 7)[1)]

We will never know the real answer before we try[2)]

We will never know the actual results before we decide. Never be bored to keep on trying and don't give up despite finding failure on your way before achieving perfect results

[1)] Kementrian Agama RI.2020. Al-Quran dan Terjemahannya
[2)] www.brilio.net

STATEMENT OF THESIS AUTHENTICITY

Full name : Afifatur Rofiqoh

Identity Number : 160210103090

With the identity written above, certify that this thesis is an original and authentic piece of work by the author. All materials incorporated from secondary sources have been fully acknowledged and referenced.

I am stating indeed that the thesis entitled "Analyzing Transformants of GP198 and GP270 of Jumbo Bacteriophage Related Tail Fiber Genes and Its Utilization as a Serial Poster". The argument is the work itself, the substance mentioned in the citation of the source, and this thesis has never been submitted to any institution and is not plagiarize work. I am responsible for the errors and correctness of their contents by the scientific attitude that must uphold.

Thus I make this statement in truth without any pressure and coercion from any party and is willing to get academic sanctions if it turns out later this statement is not valid.

Jember 28th April 2020

The writer

Afifatur Rofiqoh
NIM. 160210103090

THESIS

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By:

Afifatur Rofiqoh
NIM 160210103036

Supervisor : Erlia Narulita S.Pd., M.Si., Ph.D.
Co supervisor : Mochammad Iqbal S.Pd., M.Pd.

SUPERVISOR'S APPROVAL

ANALYZING TRANSFORMANTS OF GP198 AND GP270 OF JUMBO BACTERIOPHAGE RELATED TAIL FIBER GENES AND ITS UTILIZATION AS A SERIAL POSTER

THESIS

Compose to Fulfill One of the Requirements to Obtain the Degree of S1 at the
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The Faculty of Teacher Training and Education,
Jember University

Name	: Afifatur Rofiqoh
Identification Number	: 160210103090
Department	: Mathematics And Science Department
Study Program	: Biology Education Program
Generation	: 2016
Place and Date of Birth	: Banyuwangi, August 15, 1998

Approved by:

Supervisor	Co-Supervisor
Erlia Narulita S.Pd.,M.Si.,Ph.D.	Mochammad Iqbal, S.Pd.,M.Pd
NIP.198007052006042004	NIP. 19880120 201212 1 001

APPROVAL OF THE EXAMINATION COMMITTEE

The thesis entitled "Analyzing Transformants of GP198 and GP270 of Jumbo Bacteriophage Related Tail Fiber Genes and Its Utilization as a Serial Poster" is approved and accepted by the Faculty of Teacher Training and Education, University of Jember on:

Day : Monday

Date : 28th April 2019

Place : by the Faculty of Teacher Training and Education, University of Jember

The Examiner Team:

The Chairperson,

Erlia Narulita S.Pd., M.Si., Ph.D.
NIP.198007052006042004

The Secretary,

Mochammad Iqbal, S.Pd.,M.Pd
NIP. 19880120 201212 1 001

Member I

Dr. Slamet Hariyadi, M.Si.
NIP. 19680101 199203 1 007

Member II

Siti Murdiyah, S.Pd., M.Pd
NIP. 19790503 200604 2 001

The Dean of Faculty of Teacher Training and Education, Jember University

Prof. Drs. Dafik, M.Sc., Ph.D.
NIP. 19680802199303100

SUMMARY

Analyzing Transformants of GP198 and GP270 of Jumbo Bacteriophage Related Tail Fiber Genes and Its Utilization as a Serial Poster; Afifatur Rofiqoh, 160210103090; 2020; 75 of the pages; Biology Education Study Program; Department of Mathematics and Natural Sciences, Faculty of Teacher Training and Education, University of Jember.

Bacteriophage based therapy is an effective solution to overcome multidrug resistance. However, specific Bacteriophage can only infect one type of bacteria. Bacteria also can increase self-defense against bacteriophage infections. To overcome this problem, it needs to use jumbo bacteriophage. Jumbo Bacteriophage can reduce dependence on host bacteria, thereby reducing the chance of bacteriophage resistance. Besides, jumbo Bacteriophage has a wide range of hosts, so it is an excellent candidate to be used as a biocontrol pathogenic bacterial. In the process of bacteriophage infection to bacterial cells, the first step is attachment to recognize receptors on the bacterial cell membrane and adsorption to start the injection. In this process, the tail fiber of Bacteriophage has a significant role in the efficiency of the infection process. Jumbo Bacteriophage has many structural genes with unknown functions. The purpose of this research is to know the first cloning result to insert the tail fiber gene into the plasmid, the second cloning result to replace the tail fiber gene with the GFP gene, and to know the role of both gene GP198 and GP270 in adsorption process to the bacteria cell.

The research method was carried out by PCR, electrophoresis, and adsorbant assay. The samples used were the first cloned plasmid to pass the tail fiber gene insert, the second cloned plasmid to replace the tile fiber gene with the GFP gene, Ecs1 Bacteriophage (wild type), and mutants from the Ecs1 Bacteriophage. PCR performed with DNA polymerase in the form of KODone, using primary primers, secondary primers, and primary GFP. With a pre-denaturation stage of 94°C 2 minutes, denaturation of 98°C 10 seconds, annealing 60°C 30 seconds, extension 68°C 30 seconds and final extension 12°C with a cycle

of 30 replications. Analysis of PCR results using electrophoresis with agarose gel 1%, λ Stay 1 as a marker with a voltage of 100 V for 60 minutes. Adsorption assay and bacteriophage propagation are using LB double layer media with a concentration of 0.35% top agar. The results of PCR showed that the first cloning process to insert tail fiber gene in bacteriophages and the second cloning to replace the position of the tail fiber gene with the GFP gene is a success for GP198 and not a success for GP270 proven by the size of the band that appeared. The adsorbent assay test results indicated that mutants (without tail fiber gene) had lower adsorption ability than Ecs1 (wild type) bacteriophages. In summary, GP198 (short tail fiber) and GP2270 (the proximal part of the long tail fiber) have an essential role in the adsorption process in jumbo bacteriophage Ecs1.

ACKNOWLEDGMENT

Praise the author's pray to Allah SWT for His favor so that the author can complete a thesis entitled " Analyzing Transformants of GP198 and GP270 of Jumbo Bacteriophage Related Tail Fiber Genes and Its Utilization as a Serial Poster ". This thesis was prepared to complete the final project and fulfill one of the requirements to complete education and achieve a Bachelor of Education (S1) degree in the Biology Education Study Program, Department of Mathematics and Natural Sciences, Faculty of Teacher Training and Education, University of Jember.

The preparation of this thesis is inseparable from the help of various parties. Therefore, the author would like to express thanks to:

1. Prof. Drs. Dafik, M.Sc., Ph.D. as Dean of the Teaching and Education Faculty of Jember University;
2. Dr. Hj. Dwi Wahyuni, M.Kes., as Chairperson of the Department of Mathematics and Natural Sciences Faculty of Teacher Training and Education, University of Jember;
3. Dr. Iis Nur Asyiah, S.P., M.P., as Coordinator of Study Program of Biology Education, Faculty of Teacher Training and Education, University of Jember,
4. Erlia Narulita S.Pd., M.Si., Ph.D. and Mochammad Iqbal, S.Pd., M.Pd. As the supervisor who has been willing to provide advice, care, and motivation in writing this thesis;
5. Dr. Slamet Hariyadi, M.Si. And Siti Murdiyah, S.Pd., M.Pd. As Examiner who give criticism and suggestions for improvement of this thesis;
6. Aisyah and Mukhson, as parents who always provide prayer, motivation, and support in the form of time, energy, thoughts, materials and morals;
7. Makoto Fujie dan Takeru Kawasaki, as a supervisor at the Graduate School of Integrated Sciences for Life, Hiroshima University Japan, who has guided and facilitated this thesis research.

8. Alaaeldin Mohamed Saad, as a mentor at the Graduate School of Integrated Sciences for Life, Hiroshima University Japan, who patiently guides during the research process.
9. Zulhaj, Harino, Hida sensei, Tajima sensei, Mujaard, Sugisaki, Hayama, Yoneda, Nakayama, Nakano, Ojiro, Yoshioka, Kita, Nagata, Disa, Miho, Kamogi, Hori, Kawasaki, Luo, and all laboratory member who always provide support, throughout the research process.
10. All Biology Education lecturers, for all the knowledge that given while being a Biology Education student;
11. Mahbubatur Rohmah, Enki Dani Nugroho, Fendi and all laboratory technicians in the Biology Education Study Program who have helped in learning laboratory techniques;
12. Large families that always offer support and encouragement;
13. Fiqih Ramadhan and Ria Yulian who have provided support, accompanied and provided much assistance in laboratory research;
14. BEE SQUAD and Bacteriophage Team 2016 (Riska Ayu Febrianti, Widayanti Risqiyah, Tania Puji Anggraini, Zidna Amalia Firdausy, Icha Khairunnisa, Fitri Nur Amaliyah, and Brevy Qonita) who have provided support, accompanied and provided much assistance.
15. Boarding-mates of Jawa 4C no. 8 Nur Ummayatul Choiroh, Lilik Indrayani, Salma Khatami Alhadi, Anastasya Febrina Enggarwati, and Mirza Ayu Rosada thank you for your help and assistance for the completion of this thesis;
16. Friends of Biology in 2016 who have provided spirit and memories that are very memorable and unforgettable;
17. All parties cannot mention one by one.

The writer also accepts all criticism and suggestions from all parties for the perfection of this thesis. The author hopes that this thesis can be useful.

Jember March 2020

The writer

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CHAPTER 1. INTRODUCTION

1.1 Background of Study

Infectious diseases are count as the primary conditions that cause morbidity and mortality in the world (Konoralma, 2019). Based on the WHO (World Health Organization), one-third of the 25 million deaths in the world, caused by infectious diseases (Radji, 2011). The most common bacteria group cause of contagious diseases are *Enterobacteriaceae*, such as *Escherichia coli*, *Salmonella* sp., and *Shigella* sp. (Arens, 2018). The example of infectious diseases is Salmonellosis and Shigellosis. Based on WHO data in 2018, *Salmonella* was the highest cause of food poisoning in the world. *Salmonella* generally infects humans through the consumption of contaminated food mainly from meat, eggs, poultry, and milk. *Salmonella* infection is a very significant health problem (Nurhamidah, 2018). *Salmonella* sp. infects humans by salmonellosis disease. Some kind of salmonellosis diseases such as typhoid fever, paratyphoid, and non-typhoid or gastroenteritis (Velina, 2019). Symptoms caused by salmonellosis are gastroenteritis, diarrhea, abdominal pain, fever, septicemia, and total infection (Velina, 2019).

Outbreaks of food poisoning in Indonesia in 2000-2015 were 61,119 cases out of 715,579 populations at risk (8.5% attack rate) and case fatality rate of 0.4% (291 people). *Shigellosis* is an infectious disease caused by enteric bacteria, especially *Shigella* sp. This bacterium is considered a fairly serious health problem in the world. Based on the results of the World Health Organization report, *Shigella* spp. Cause of about 165 million shigellosis and 1 million deaths per year (Tang *et al.*, 2019). Infectious diseases are increasingly difficult to overcome because of the presence of multidrug-resistant (MDR) (Shahin *et al.*, 2019). The other solution to overcome MDR is to use a virus that can infect infectious diseases bacteria specifically, namely Bacteriophage. It is a good solution for a candidate as a biocontrol to overcome the multidrug-resistant (Santos *et al*., 2018).

In the last few decades, bacteriophage therapy is increasing. Such as bacteriophage use to kill *Pseudomonas aeruginosa* in the Murine Lung and on Cystic Fibrosis Lung Airway Cells (Alemayehu, 2012). Elbreki *et al*. (2014) used Bacteriophage as biotherapeutic agents in disease prevention and treatment. *Buttimer et al*. (2017) applied Bacteriophage to control bacterial plant diseases. Some bacteriophage is isolated and characterized by Narulita *et al*. (2018) to control *Escherichia coli* in Jember. Most bacteriophages have specific hosts and are limited to strains within the same species. Thus, to fight against various bacteria, several Bacteriophage types called 'cocktail Bacteriophage' are combined. However, the Bacteriophage cocktail application has some disadvantages, such as cannot use for medical and commercial formulations on an ongoing basis. Also, manufacturing cocktails with many Bacteriophage-type components require high production costs (Saad *et al.,* 2018).

To deal with these problems, some researchers suggested using jumbo Bacteriophages. Jumbo Bacteriophage has been applied, such as Bacteriophage, to control phytopathogen (Yamada *et al.,* 2010). The positive and negative gram bacterio-phage has isolated, the *Bacillus* -jumbo bacteriophages isolated by Yuan, *et al.,* (2016). The *Escherichia* jumbo bacteriophage ΦEcs1 isolated by Saad *et al.*, (2018). Jumbo Bacteriophages are tailed bacteriophages that have a genome $\geq$ 200 kbp. It is not like the common Bacteriophage that specific on one kind of bacteria and sometimes can be resistant; jumbo bacteriophages can infect a variety of bacteria. They can sustain infections in the long run, and their putative genes can reduce the dependence of bacteriophage jumbo on its host (Saad *et al.,* 2018). The wide range of hosts makes jumbo Bacteriophage as the right candidate for biocontrol of pathogenic bacteria (Saad *et al.,* 2018). These structural genes of jumbo bacteriophages are similar to common-size Bacteriophages. But there are more other less conserved genes inserted in the structural genes of jumbo Bacteriophage. The additional genes have no match with any other genes or match only with putative genes in the database of GeneBank. The unknown gene of jumbo Bacteriophage are supporting jumbo Bacteriophage to make infections more efficiently (Hendrix 2009).

The first step of bacteriophage infection to bacteria is attachment and adsorption. Tail fibers of Bacteriophage are vital organs that support the adsorption process. Tail in T-even bacteriophages is the organ that directly involved in the adsorption process. Tail constitutes a very highly complex structure (Anderson, 1953). The previous experiment showed that the long tail fibers of Bacteriophage are not only had an important role in adsorption organelle, but they also have an essential part in process control of tail contraction. There is a reversible and irreversible binding process between Bacteriophage and bacteria. For T4-like Bacteriophages, the reversible step is attachments of long-tail fibers to specific receptors. The baseplate changes its shape. Then the six short fibers extend then irreversibly bind to the lipopolysaccharides (LPS) core (Le, 2013). Saad (2018) inserted the tail fiber gene GP 270, which is responsible for the distal subunit of long-tail fiber and GP 198, which is responsible for short tail fibers of jumbo ΦEcs1 and cloned the mutant by primary plasmid (7D and 2C).

It's crucial to analyze the transformant of both GP198 and GP270 tail fiber-related genes, to know the successful removal and to examine the function of these genes**.** In this research, we continued to insert GFP (Green Fluorosense Protein) gene to the primary plasmid to replace the tail fiber gene by infusion cloning and confirm by PCR (*Polymerase Chain Reaction*), electrophoresis, digest by a restriction enzyme, adsorption assay, and EM observation. Both clones and confirmation were to prove the function/role of the tail fiber-related gene of jumbo Bacteriophage.

Many media can use to publish the results of this research. One of them is a scientific poster. Scientific posters are efficient and effective media for disseminating and communicating research results to the public. The poster can be seen at any time and a long time so that it can be read often and can be seen by audiences in different fields of research (Darmalaksana, 2017). The printed posters or those made of paper have the disadvantages of not being durable if not correctly stored (Nursalam, 2017). *The Global Education Census* concluded that Indonesian students are the most up-to-date technology users in the world. Digital technology is becoming more integrated and becomes part of modern society

(Sumardani *et al*., 2019). Thus the authors use posters in an electronic form called e-posters. E-poster will be more durable and have a broader and faster publication capacity through the internet.

1.2 Problems

1.2.1 What is the first cloning result in the insert of the tail fiber GP270 and GP198?

1.2.2 What is the second cloning result in the replacement of the tail fiber GP270 and GP198 with the GFP gene?

1.2.3 How is the adsorption ability of ΦEcs1 ΦMutant Ecs1?

1.2.4 How is the feasibility of the serial poster in the study of analyzing transformants of GP198 and GP270 tail fiber-related genes of jumbo Bacteriophage?

1.3 Scope Of Problems

1.3.1 Sample of *Escherichia coli* that used in this research was BL21 that comes from the previous experiment by Saad (2018).

1.3.2 Sample of Bacteriophage that used are ΦEcs1 and ΦMutant Ecs1 that come from the previous experiment by Saad (2018) that can infect *Escherichia coli, Salmonella spp.,*dan *Shigella spp.*

1.3.3 Sample of the vector that used are primary plasmid (7D dan 2C) and Secondary Plasmid (7E dan 2B) from the previous experiment by Saad (2018)

1.3.4 Restriction Enzyme that applies in this research are Eco Rv, Bgl II, dan Hind III

1.3.5 The success of cloning process observed by appearing or disappear the band

1.4 Purpose

1.4.1 To know the first cloning result in the inserting of the tail fiber GP270 and GP198

1.4.2 To understand the second cloning result in the replacement of the fiber tail GP270 and GP198 with the GFP gene.

1.4.3 To know the adsorption ability of ΦMutant Ecs1

1.4.4 To measure the validity of a serial poster as a result of the study on analyzing transformants of GP198 and GP270 tail fiber-related genes of jumbo Bacteriophage

1.5 Benefits of research

1.4.1 For researchers, to get more knowledge and experiences regarding the analysis of the tail fiber gene of jumbo Bacteriophage

1.4.2 For other researchers, provide sources of information regarding the study of the tail fiber gene of jumbo Bacteriophage

1.4.3 For the community, giving insight as a useful alternative for handling infectious diseases.

CHAPTER 2. LITERATURE REVIEW

2.1 Bacteriophage

2.1.1 Bacteriophage Therapy

Therapy using bacteriophages is a treatment using viruses that infect bacteria that act as antimicrobials; this alternative treatment is more promising than treatment with conventional methods of antibiotics because the continuous treatment with antibiotics can cause resistance to bacteria. However, the use of a single bacteriophage and bacteriophage-cocktail also has the potential for resistance. Therefore, one way is to use a single phytic lytic specific for pathogens that cause infection. Compared to other viruses, Bacteriophage has a more complex structure and has several different parts that are complete. All viruses have nucleic acids, the carriers of the genes needed to collect copies of the virus in living cells. Also, lytic bacteria and viruses have shown to accelerate the rate of molecular evolution of species that live together even under laboratory conditions (Ormala and Jalasvuori, 2015).

Bacteriophage therapy is an effective solution to overcome multidrug-resistant. The success of Bacteriophage therapy methods requires an appropriate framework or regulation and an effective strategy of choosing the right Bacteriophage and the proper protocol. It aims to overcome the Shigella spp. Resistant to various drugs and antibiotics (Tang *et al*., 2019). Bacteriophage therapy has applied in several Eastern regions, European countries with proper safety without causing significant side effects (Kutter, 2009). Bacteriophage therapy was successful in treating Russian soldiers during and after World War II (Kutter *et al*., 2010). Based on Slopek *et al.* (1987), the success of bacteriophage therapy methods reaches 91-100% in the case of treating diseases of the digestive system in Poland (Tang *et al.*, 2019). The results of a clinical trial called 'Phagoburn' show Bacteriophage therapy can treat burns as well as in patients infected with *Pseudomonas aeruginosa* and *Escherichia coli* This clinical trial based on Good Manufacturing and Good Clinical practices. As for bacteriophage therapy, conducted at the University of California San Diego, Faculty of Medicine, the US Naval Medical Research Center and Texas A&M

University successfully treat patients infected with Acinetobacter who are resistant to several types of drugs that deal with Acinetobacter baumannii infection (LaFee & Buschman, 2017) (Tang *et al.*, 2019).

2.1.2 Jumbo Bacteriophage

Bacteriophages are a virus, especially that infect bacteria. The lysis of bacteria's cell because of Bacteriophage gives impacts for environments by the release of dissolved organic carbon, micronutrients directly and also indirectly by modulation of microbial communities (Srinivasiah et al., 2008). Bacteriophages are the primary opponent of bacteria. Coevolution by bacteria-Bacteriophage has significantly increased diversity and create an evolution (Koskella and Brockhurst, 2014). Bacteria and Bacteriophages genes are essential to thrive in their environments. The variety of Bacteriophage genes coupled and unknown protein's function (Hatfull, 2015). Bacteriophage-derived enzyme has reemerged (Santos *et al*., 2018).

Bacteriophages that infect the Enterobacteriaceae are interested in analyzing because this bacterial family contains a lot of pathogenic bacteria. Frederick Twort and Felix d'Herelle isolate bacteriophages since 1915. Nowadays, bacteriophages are the biggest population on earth with significant contributions to infect different bacterial strains and transfer their genetic materials effectively. Bacteriophages already a success in infecting several pathogenic bacterial strains, such as *E. coli, C. diphtheriae,* and *V. cholerae* strains. Because of the ability to infect specific bacteria, it used as a diagnostic and also therapeutic agent (Arens, 2018).

When the process of infection, Bacteriophages can transfer foreign DNA to their host includes virulence factors. It will be integrated with the host's genome, or also kill and make the cell lysis (Chen and Novick, 2009). A large number of bacteriophages make them be an essential thing for understanding the evolution and ecology of bacteria, including pathogenic bacteria (Bollback and Huelsenbeck, 2009; Boyd, 2012). their specificity, rapid multiplication, and genomic plasticity make them be the potential one to treat the bacterial infection (Sharma,2019). Caudovirales is an order of tailed Bacteriophage that consists of 3

families (Buttimer, 2018). 25% of Caudovirales are Myoviridae Bacteriophages (Ackermann, 2007) and have a contractile tail that similar to a syringe to infect the bacteria (Browning *et al.,* 2012). a new class of Myoviridae viruses that carrying a large size of the genome (more than 200 kbp) called as "jumbo Bacteriophages" (Effantin, 2013).

Jumbo Bacteriophages are Bacteriophage, which has genome sizes for about from 208 to 497 kb. They have complex morphology and also complex virion structure (Yuan and Gao, 2017). They encode more than 60 structural proteins and with more than five protein complex head structure composed (Effantin et al., 2013) or sometimes they have a long, curly, and wavy tail fibers (Yuan and Gao, 2016). Jumbo bacteriophages have many unique putative hypothetical proteins with a significance function. It makes jumbo bacteriophages have an evolutionary advantage in a specific ecological niche (Attai, 2018). Jumbo Bacteriophages found with more than 11 clusters and 5 singletons from 52 complete jumbo Bacteriophage genomes analyzed. Many proteins of jumbo Bacteriophage uncharacterized (Yuan and Gao, 2017). Jumbo Bacteriophage families that already characterized, sequence analysis, and also EM observation (Sharma,2019).

Jumbo Bacteriophages, or sometimes named giant Bacteriophages. They isolated from environments that are water, plants, soil, and animal tissues (Yuan et al., 2017). A subset of 120 of them already sequenced to date (May 2019). 95,4% of jumbo bacteriophages are infecting Gram-negative bacteria and 11 jumbo bacteriophages infecting Gram-positive bacteria. The gram-positive Bacteriophage is mostly from the Bacillus strain. The characteristic plaque of jumbo Bacteriophage is tiny plaques, and they typically are smaller than 0.5 mm on 0.7% soft agar. It easily overlooks when standard propagation procedures. Their large virion also can be lead to their loss when filtration procedures that standard in a lot of Bacteriophage isolation procedure (Sewer et al., 2007). Jumbo bacteriophages are very interesting to study because of the structure and have something special during infection processes (Fokine *et al.,* 2007; 2005; Wu *et al.*, 2012). The understanding of their function of genetics and genome organization

are o remained limited to their results of in silico prediction genomes assembled from short-read sequencers, for example, Illumina machines (Lood, 2019).

Most jumbo Bacteriophages are from the family Myoviridae also have many genes that contain DNA polymerase and RNA polymerase. Jumbo bacteriophages can infect a variety of bacteria and can support long-term infections. Jumbo Bacteriophage gene expression does not depend on RNAP and can be activated only by the RNAP Phase. In addition, the jumbo Bacteriophage is easier to infect its host because of the help of several enzymes such as chitinase, endolysin, and glycoside hydrolase. Many other genes from jumbo Bacteriophages work to reduce bacteriophage dependence on their host. Because one of the characteristics of jumbo, bacteriophages have a wide variety of hosts. Since the first discovery at the beginning of the 20th century, bacteriophages use for clinical applications. An important factor that supports the effectiveness of bacteriophages as biocontrol agents are their host range, as well as the ability to install over a long period (Saad, 2018).

2.1.3 Φ EcS1

Φ EcS1 or "Escherichia Bacteriophage EcS1" is one lytic Bacteriophage that has a wide range isolated by (Saad. *et al.*, 2018). (Saad, 2018) isolated this lytic Bacteriophage from waste samples collected from the sewage treatment plant in Higashi-Hiroshima, Japan, using the host bacterium *E.coli* BL21. Bacteriophages can infect a variety of bacterial strains, including *Shigella sonnei* SH05001, *Shigella boydii* SH00007, *S. flexneri* SH00006, and *Salmonella enterica* serovar Enteritidis (SAL 01078). The lithic nature of EcS1 can see from the stable plaque consisting of each host strain. 4 nm, n = 10) and tails with a length of 110 ± 5.5 nm, n = 10 (Supplementary Image. S1) This morphological characteristic shows that EcS1 belongs to the T4 Bacteriophage (genus T4virus) in the Myoviridae family (Saad, 2018).

There is 11 *Escherichia* Bacteriophage isolated by (Saad 2018). that has successfully infected 11 germ bacteria, named E1-E11. Escherichia bacteriophages have a large capsid and long contractile tails. They include the myoviridae family, wich. The results showed genome sizes of around 200 kbp for phases E1, E2, E5, E6, E7, E9 and E10, and about 450 kbp for stages of E3, E4, E8, and E11. Bacteriophages that contain a wide variety of hosts, E9 is effective against the bacteria Shigella sonnei SH05001, Shigella by SH00007, Shigella flexneri SH00006, Salmonella enterica serovar Enteritidis SAL01078 and Escherichia coli C3000 (K-12 tour), and in Korean. The wide range of hosts makes jumbo bacteriophages transplanted to be the right candidate for use as a biocontrol of pathogenic bacteria (Saad, 2018).

2.2 Tail Fiber of Bacteriofag

Bacteriophage T4 will extend its tail fibers for adsorption. In this condition, Bacteriophage needs the tryptophan to continue its tail fiber. Without tryptophan, Bacteriophage can not extend its tail fiber, although in pH 7. Bacteriophage with a mutation in gene 37 results is only having a few tail fibers, and the ability of Bacteriophage to absorb decreased. Tail in T-even bacteriophages is the organ that directly involved in the adsorption process. The result of the investigation shows

that the tail constitutes a very highly complex structure (Anderson, 1953). Composed with a central tube surrounded by a contractile sheath and also a complex of the base plate, which tail fibers are attached (Kellenberger, 1965).

The ability of tail fibers' role in adsorption was first alluded to by Williams and Fraser (1956), who observed that the free tail fiber could adsorb specifically into host or bacteria. Wildy and Anderson (1964) also show that tail fibers are capable of agglutinating the bacteria. Based on the experiment of Brenner et al. (1962) show that the release of tail fibers is significant for adsorption, and the adsorption of T2 Bacteriophage to Escherichia coli depends on pH (Puck and Tolmaeh, 1954). Lauffer and Bender (1962) suggest that the change of structure is responsible for sedimentation and extension, which has coloration with retraction and extension of tail fibers. The extension of tail fibers with slow sedimentation is a necessary condition to do adsorption. The tail fibers of Bacteriophage will bound back, and will not be able to do adsorption to the host (bacteria). It happens when the sedimentation process (Kellenberger, 1965). Adsorption of Bacteriophage is dependent on either tryptophan or pH, proven by tail fibers found extended in all cases where the Bacteriophage will adsorb in pH 7 and or presence of tryptophan for cofactor dependent T4, 38. And the tail Fibers are can't be seen under the condition which the virus can't adsorb in low pH or absence of tryptophan. Mutants with lacking fibers can't do the adsorption. Tail fibers can agglutinate the bacteria (Wildy and Anderson,1964) (Kellenberger,1965).

Level, the adsorption of fix Bacteriophages, is biphasic, with the bipartite population. Tryptophan makes slow sedimentation of T4 Bacteriophage. For bacteriophage T2, where the pH 7 sedimentation is slow, but at pH 5 it is faster. Furthermore, in the absence of tryptophan, the sedimentation velocity of the tryptophan requiring T4 is independent of pH (Kellenberger, 1965). T4 Bacteriophage contracts their directly after attaching to bacteria cell and inject the DNA into the bacterial cell. The final step was a contraction of the tail sheath, which is pull up the baseplate along with the central tail core. At this time, short tail fibers connect the baseplate to the host's cell wall. The long tail fibers also have a participate in the mechanism of contraction. The previous experiment shows the result that the long tail fibers of Bacteriophage are not only had an

important role in adsorption organelle, but they also have an essential part in process control of tail contraction.

Six genes require for assembly of tail fiber. Four of this gene are 34, 35,36, and 37 (Revel., *et al.,* 1967). Short tail fibers are encoded by gene 12 (Kells & Haselkorn, 1974). It has a fibrous that are very thin. Based on the previous experiment, tell us that heat denaturation P12's carboxyl terminus of strongly inhibit the tail contraction, and the facts of some researches show that the translocation of P12 within the tail initiate the process of contraction. The denaturation of P12's carboxyl-terminal region blocks the translocation (Yamamoto, 1975). There is independent evidence of the importance of tail fibers in the adsorption process that is Tryptophan activation is need by T4.38 to be associated with tail-fiber extension (Franklin, 1961).

2.3 Conceptual Framework

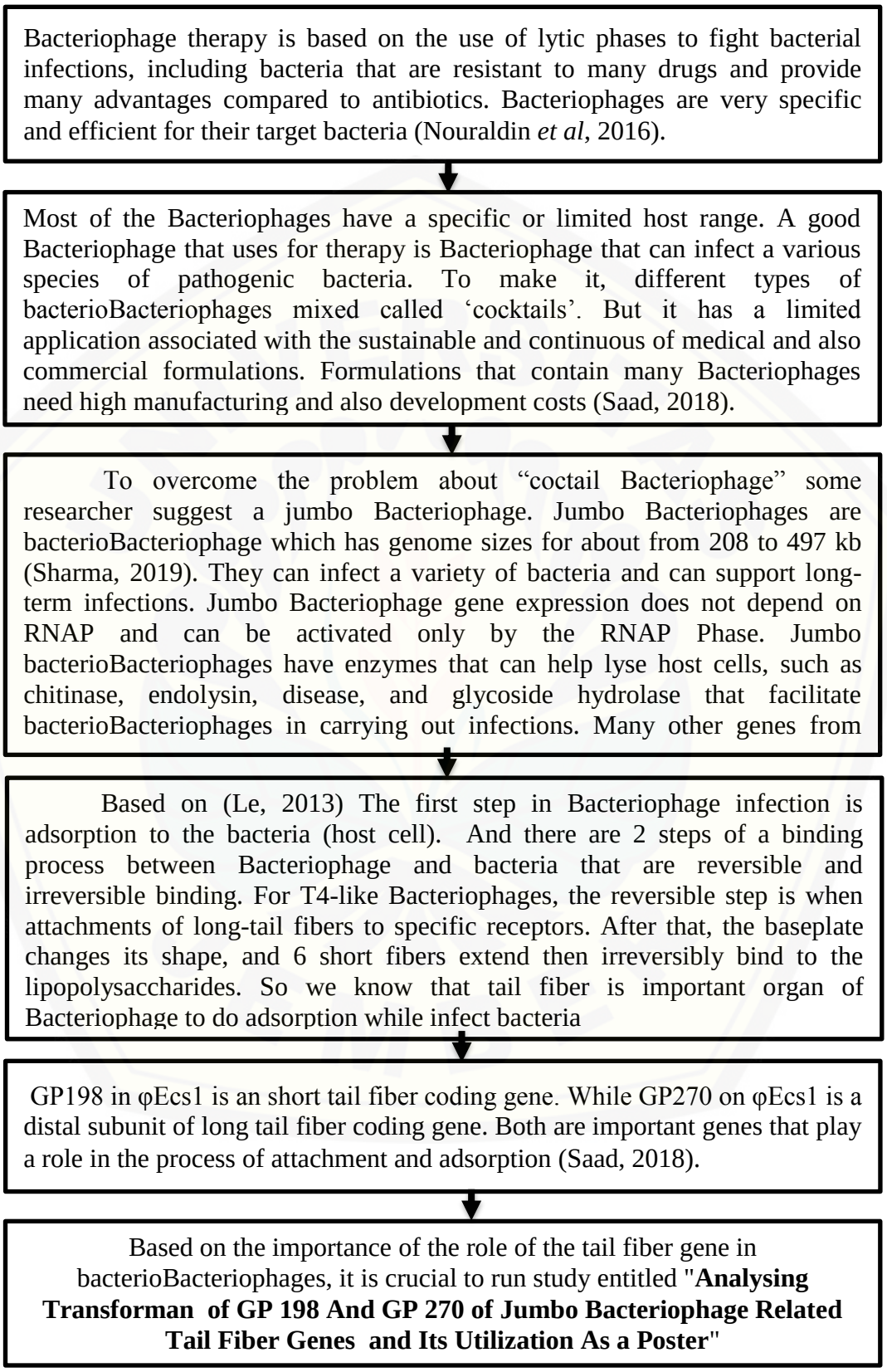

Figure 2.4 Chart of Conceptual Framework

CHAPTER 3. RESEARCH METHODOLOGY

3.1 Types of Research

This research is an experimental study conducted to confirm the success of cloning carried out after mutations in the GP198 fand GP 270 of tail fiber. The data generated in the form of a description of the success of the mutation and cloning process carried out previously

3.2 Research Place and Time

The study conducted at the Graduate School of Integrated Sciences for Life, Hiroshima University, Japan, in September 2019- March 2020.

3.3 Operational Definitions

a. Transformant is living creatures as a result of the transformation process (a movement of foreign genes isolated from plants, animals, viruses, or bacteria into the genomes of other living things). Transforman that will use in this study is cloned plasmids that are primary plasmid (7D and 2C) and secondary plasmid (7E and 2B).
b. Jumbo Bacteriophages are bacterial infectious viruses that have a size of genome >/= 200 kb with a broad host range. Bacteriophage isolates that will use bacteriophages that infect *Salmonella* spp., *Shigella* spp., And *Escherichia coli* including φEcs1 and Ecs1 Mutant
c. The tail fiber gene of Bacteriophage is a gene that encodes the formation of the tail fiber of bacteriophages. The tail fiber gene used in this study is tail fiber GP198 (short tail fiber encoder) and GP270 (encoding the proximal part of the long tail fiber) of φEcs1.
d. GFP (*Green Fluorescent Protein*) is a gene from jellyfish (*Aequoreavictoria*), which is as a reporter gene in genetic transformation, by the luminescence with luminous green color.
e. Adsorption is the process of imbibing Bacteriophage to bacterial cells during the infection process

3.4 Research Tools and Materials

3.4.1 Tools

The tools used in this study are Petri dishes, ose, bunsen, incubator, autoclave, oven, stove, spectrophotometer, electrophoresis, Centrifuge, Laminar Air Flow (LAF), thermometer, stir bar, refrigerator, micropipette, measuring cup 1000 ml, 500 ml measuring cup, UV transilluminator, doc gel, Erlenmeyer 1000 ml, Erlenmeyer 500 ml shaker, thermal cycler, tweezers, microwave, ultracentrifuge, shiring, filtration, 500 ml glass beaker, 1000 ml glass beaker, aret, analytical balance, pans, term shaker, stopwatch, 70% alcohol, glove.

3.4.2 Ingredients

The materials used in this study are LB (Hypopolypeptone, bacto yeast extract, sodium chloride, agar), *Escherichia coli bacteria* isolate, bacteriophage φEcs1, Mutant of φEcs1, primary plasmid (7D AND 2C), secondary plasmid (2B and 7E), TE (Tris-EDTA) buffer, 1X TAE buffer, loading dye, KOD one, restriction enzyme (Bgl II, Eco RV, Hind III), parafilm paper, SM buffer, λ stay 1 marker, aqua dest, ddh2o, agarose gel, seal, marker.

3.5 Research Procedure

The researcher does this study by five steps of the research's procedure. The first step is to prepare the media to propagate the bacteria and Bacteriophage. The second is cloning to insert the GFP gene into the plasmid. The third is confirmation of the presence of a gene with PCR (*Polymerase Chain Reaction*) and Electrophoresis. The fouth is an adsorbant assay to prove the function of the tail fiber gene, and the last step is to design the poster and poster validation analysis:

3.5.1 Preparation of LB medium

Preparation of LB medium used for propagating the bacteriophage φEcs1, and Mutant of φEcs1, and for the cloning media. LB (Luria Bertani) medium prepared by mix homogenously hypopolypeptone 35 gr, sodium chloride 35 gr, bacto yeast extract 17.5 gr and 3500 ml of distilled water in an Erlenmeyer glass.

Set the Ph to 7 by added NaOH. A 3000 ml medium will combine to make LB plate agar. 3000 ml medium divided into 2 of 1000 ml Erlenmeyer tubes and 2 of 500 ml Erlenmeyer tubes. Poured out 800 ml of media into every 1000 ml Erlenmeyer tube and added by 12 grams of agar powder. 400 ml into each 500 ml Erlenmeyer and added by 6 grams of agar powder. It covered by a sponge and aluminum foil. While the 500 ml one without agar powder to make LB broth. 500 ml of the medium was divided into 4.5 ml each test tube and covered it with a sponge. I placed the tube in the tube rack and covered it with aluminum foil. Cooked and sterilized all media into the autoclave for 20 minutes at 121 atm. Then be awaited until the slight temperature decrease, after that poured out into the petri dish and wait until solid. After it is solid, stored in a plastic bag and placed in a 4°C cooling room for stock. Wait for the media to cool and ready for use (Saad, 2018).

3.5.2 Preparation of 0.35% TOP agar

Preparation of 0.35% TOP agar used for top layer LB media for propagating φEcs1, and Mutant of φEcs1. 0.35% TOP agar prepared by mix homogenously 1.6 grams of hypopolipeptone, 1.6 grams of sodium chloride, 1.6 grams of agar, and 0.8 grams of bacto yeast extract with 500 ml of distilled water. Adjust the pH to 7 by added NaOH. Boil it in the microwave and poured out into each 5 ml canister. Covered the tube and wrapped it in aluminum foil, then autoclave for 20 minutes at 121 atm after the autoclave top was ready to use. Whereas for the next use TOP so that those who have compacted boiling first (Saad, 2018).

3.5.3 Preparation of agarose gel

Preparation 1% agarose gel used for electrophoresis φEcs1, Mutant of φEcs1, and plasmid. 1% agarose gel prepared by mix homogenously 2 grams of agarose powder and dissolved it in 200 ml of TAX buffer 1 X. Then boiled in the microwave, be awaited until the temperature decreased slightly then poured out it into the agar mold and place the comb in the pattern. Wait for it to be solid, and agarose gel, 1% was ready to use (Saad, 2018).

3.5.4 Propagation of ϕEcs1 and ϕEcs1 Mutant

Propagation of ϕEcs1 and ϕEcs1 Mutant used for work culture Performed inoculation of Escherichia coli (BL21) as the primary host of ϕEcs1 into 4.5 ml LB broth and incubated in a 37 °C shaker incubator for about 5 hours. Next, calculate the bacterial OD (Optical Density) and set it to reach the standard OD for the plaque assay that is equal to 0.1. Then prepared a 250 µl mixture plaque assay to consist of bacterial suspension and LB broth with OD 0.1 to reach 240 µl volume and 10 µl bacteriophage to be propagated (ϕEcs1 or utanEcs1 Mutant) with a concentration of 10^6 for ϕEcs1 and 10^4 for Ecs1 Mutant. Prepared 12 LB plates, 6 for ϕEcs1, and 6 for ϕEcs1 Mutant. Boil 12 TOP agar tubes. Wait for TOP so that the temperature dropped slightly (warm), then mix it with 250 µl mixture and poured out it into the LB plate. Wait until it's solid and incubate it in an incubator of 28°C for 1 night. The next day continues to add 5 ml of SM buffer to each plate, seal with parafilm paper. Put in into the shaker 4°C cooling room for 1 night. The next day harvest (plate lysate) by taking SM buffer from all plates with 5 ml syringe and filled in into a 5 ml tube then centrifuged with a speed of 15000 g or 10,200 rpm for 1 hour (1 to 3 times). After that, throw the supernatant and added 500 µl BC buffer then mixed well with the pellet. Moved into the Eppendorf tube, and the bacteriophage stock was ready (Saad, 2018).

3.5.5 Insert the GFP gene to Primary Plasmid by infusion Cloning

The cloning process is done by infusion cloning to insert the GFP gene in the plasmid. Before cloning, it is necessary to prepare LB media with a composition of 1% hypopolypeptone, 0.5% yeast extract, 1% NaCl, and 1% agar. Before pouring the medium into the plate, the LB medium was waited until warm, then added X-gal 1.25 ml, IPTG 0.75 ml, and Ampicillin 90 µl in every 400 ml LB. It also needs to be prepared 2XYT media for the transformation process with a component of 1.6% hypopolypeptone, 1% yeast extract, 0.5% NaCl. Next, make for the manufacture of mixture infusion cloning with ingredients including 5x HD E.premix infusion, linearized vector, purified PCR fragment, and ddh2o. Furthermore, the transformation process does by using a competent cell in the

form of Escherichia coli Xl10 gold bacteria 100 µl. Put the competent cells in the icebox until liquid. Mix the 100 µl competent cell with infusion HD en.mixture 5 µl. Put in the icebox wait until 15 minutes. Set in a water bath 47°C for 30 seconds. Mix with 2XYT 900 µl. Then incubate at 37°C for 1 hour. Centrifuge 6000 g for 5 minutes. Remove 970 µl supernatant. Mix well the pellet with 30 µl of 2XYT media broth. The last step, pour in the LBAX media plate and incubate in incubator 37°C. wait 24 hours, then observe the colonies that appear. Breed successful colonies into LB media then do a plasmid extraction with a plasmid extraction kit (Saad, 2018).

3.5.6 Check the presence of the tail fiber gene in the transforman of primary plasmid

This PCR (*Polymerase Chain Reaction*) used for amplifying the tail fiber gene (GP198 and GP270). The first confirmation did by PCR using primary plasmids. The primary plasmid is plasmid inserted by the tail fiber gene (plasmid 7D and 2C). Plasmid 7D is a plasmid that added with tail fiber gene 198. Plasmid 2C is a plasmid that has inserted with tail fiber gene 270. The PCR runs with primary primers that designed to detect the presence of inserted fiber tails). The primary primers number 2 is a primer to detect the tail fiber gene 270. The primary primers number 7 is a primer for detected the tail fiber gene 198 and checked by secondary primers (primers that detect the reverse part of plasmids). As an alternative control, a GFP gene PCR performers with a primary tail fiber gene detection. Furthermore, electrophoresis with a DNA polymerase enzyme that used was KODone (Saad, 2018).

The first is to prepare an icebox to place the Eppendorf containing KODone until KODone becomes liquid. Furthermore, made a PCR mixture with a formulation of 12.5 µl KODone, 9.5 µl ddH2O, 1 µl plasmid sample, 1 µl forward primer, and 1 µl reverse primer. Resuspend at the end of making the mixture and given by a name to the Eppendorf PCR. Inserted mixture into thermal cycler with a pre-denaturation temperature of 94°C for 2 minutes, denaturation with a temperature of 98°C for 10 seconds, annealing 60°C for 30 seconds, extension with the heat of 68°C for 30 seconds, and final extension with temperature 12°C.

cycles performed as many as 30 times. After completed PCR, the electrophoresis was tested by λ stay 1 as a 6 µl marker, and 1 µl sample mixed with 1 µl loading dye. The running of electrophoresis for 60 minutes with 100 V of power. The staining process using EtBr (*Ethidium Bromide*) and the visualization of the band by UV transilluminator (Saad, 2018).

3.5.7 Digest with retraction enzymes

Digest with retraction enzymes used to prove that the plasmid and ϕEcs1 have the same sequence and to confirm the cloning result. The first step did by prepared an icebox for the buffer and the enzyme retention used. It started by mix homogenously 23 µl ddH2O components, 3 µl buffer retraction enzymes, 3 µl DNA samples (original 2C plasmids and Ecs1), 1 µl retraction enzymes (Eco Rv, Hind III, Bgl II). After the mixture finish, it is resuspended and incubated at 37 °C for 3 hours. Furthermore, electrophoresis carries out using the λ stay marker. Run of electrophoresis was carried out for 60 minutes with a power of 100 V. Furthermore, stain the gel was did by used EtBr for 30 minutes and observed the band with a UV transilluminator. Checked is did together with sample (2C and Ecs1 Ori) without digest as a negative control (Saad, 2018).

3.5.8 Checking the presence of the GFP gene in the secondary plasmid

PCR (*Polymerase Chain Reaction*) test used to amplify the reverse plasmid portion. This PCR prepare by an took an icebox to put KODone, then amplified by PCR secondary plasmid (7E and 2B), which is a primary plasmid that has been removed from its tail fiber gene and then replaced with the GFP gene. The PCR process carries out using secondary primers (primers that detect the reverse plasmid portion) and primary GFP (primers that detect the GFP gene). The mixture formulations used were 12.5 µl KODone, 9.5 µl ddH2O, 1 µl forward primer, 1 µl reverse primer, and 1 µl DNA sample (7E and 2B). PCR carries out with a pre-denaturation temperature of 94°C for 2 minutes, denaturation with a temperature of 98°C for 30 seconds, annealing with a temperature of 60°C for 30 seconds, extension with a temperature of 68°C for 30 seconds and final extension with a heat of 12°C. Repeat the cycle 30 times. Furthermore, I checked the band

by electrophoresis with the λ stay 1 as a marker for 60 minutes, with a power of 100 V. The staining process is by soaking the agarose gel in EtBr liquid for 30 minutes. Followed by observation of the band with a UV transilluminator (Saad, 2018).

3.5.9 Electron Microscopy (EM) observation of ϕEcs1 and ϕEcs1 Mutant

Electron Microscopy (EM) observation used to know the morphology of ϕEcs1 and ϕEcs1 was carried out by preparing bacteriophage samples with high concentrations then negative staining with 1% of PTA.

3.5.10 Adsorption assay of ϕEcs1 and ϕEcs1 Mutant by Plaque Assay

Plaque assay based adsorption is to know the adsorption ability of each Bacteriophage. The Escherichia coli BL21 bacterial isolate was grown in a 4.5 ml LB broth tube, incubated at 37°C. After 5 to 7 hours, OD600 is calculating with a spectrophotometer. BL21 bacterial culture dilutes until it reached OD600 = 1 in 4.5 ml LB volume into a 5 ml Eppendorf tube. (diluted into 2 Eppendorf tubes measuring 5 ml). Then the ϕEcs1 10 µl bacteriophage was added to the Eppendorf 1 and ϕEcs1 Mutant 10 µl tubes into the Eppendorf 2 tube. Then every 1 minute, 5 minutes, 10 minutes, 20 minutes, and 30 minutes a 500 µl mixture of BL21 and take the Bacteriophage, then put into a mixture of Eppendorf 2. in a 1.5 ml Eppendorf tube. Then centrifuged for 1 minute at 15000 rpm. The supernatant is taken by 0.5 ml of the syringe and filtered by a membrane filter; the filtration results put into a 1.5 ml Eppendorf tube. Followed by a plaque assay. Making a mixture of LB broth media and BL21 culture with OD600 = 1 in a 240 µl volume and 10 µl bacteriophage suspension add beforehand. Mix the solution mixture with 5 ml of TOP so that 0.35%, which has boiled before. Wait until the temperature decreases slightly. Then poured the final mixture into the LB plate, wait until it is solid and incubated at 28°C overnight. Next, count the number of plaques that appear. Adsorbent assay and plaque assay perform several times, and data on the amount of plaque obtained processed in the form of curves (Saad, 2018).

3.5.11 Poster Preparation

The researcher designed a poster by the steps based on Darmalaksana, (2017) with a little modification as follows:

Poster made by graphics processing applications, such as Corel Draw, in printed form totaling 1 (one) sheet of height × width are 70 cm × 70 cm mounted vertically. The poster layout design paid attention to the principle of formal and informal balance, which includes symmetrical and asymmetrical terms, the principle of unity of arrangement of pictures, color, background and motion elements, and able to direct the reader's eyes to the entire poster area. The poster contains the top part considered of the title, the name of the implementer, and the university logo. The middle section (the content part) included the background (introduction or abstract), Methods, Main Research Results (text and pictures or photography or schematics), Conclusions, and References (additional); and at the bottom, inserted the sponsor or agency logo, contact details, date and time of research.

3.5.12 Poster Validation Analysis

Poster validation analysis obtained from the validator is in the form of quantitative data that is processed with a formula as follow

N (Score) = $\frac{obtained\ score}{maximum\ score}\ x\ 100\%$

Table 3.5 Poster Validation Criteria

Based on (Budiaji, 2013) is as follows:

Score	Criteria	Information
81-100	Very decent	Posters have complete components, correct contents and are worthy publication
61-80	Worthy	Posters have incomplete components, correct contents and are suitable for publication
41-60	Enough	Posters have incomplete components, inaccurate content and are worthy of publication with little revision
21-40	Inadequate	Posters have incomplete components, inaccurate content and are worthy of publication with a lot of revision
≤21	Not feasible	Posters have incomplete components, inaccurate content and are appropriate for a publication with significant revisions

3.6 Research Flow

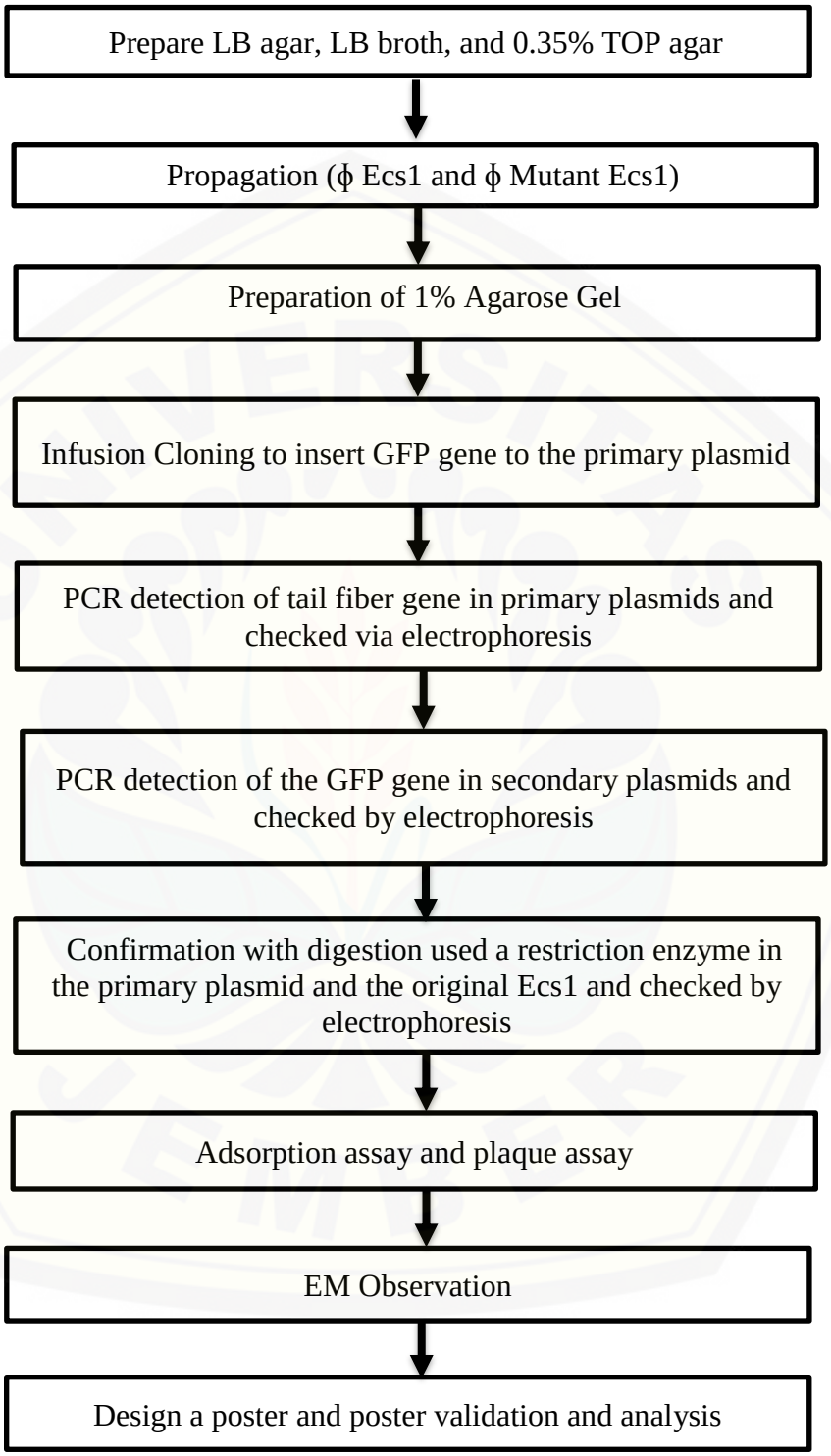

Figure 3.6 Chart of Research Flow

CHAPTER 5 CONCLUSIONS AND SUGGESTIONS

5.3 Conclusion

5.3.1 The first cloning process of inserting the tail fiber gene on the primary plasmid is success indicate by plasmid 7D with primary primer number 7 (PP7) appears a band with the size around three kbp and with secondary primer number 7 appears 5 Kbp of the band. Primary plasmid 2C with primary primer number 2 shows 5 Kbp and with secondary primer show 5 Kbp. That all confirmed with a restriction enzyme.

5.3.2 The second cloning process of replacement the tail fiber GP198 with the GFP gene is a success. It is proof with appearing 1 kbp band electrophoresis secondary plasmid 7E using GFP primer, 5 Kbp band with secondary primer, and EM observation of mutant without short tail fiber. The second cloning process of replacement the tail fiber GP270 with GFP gene was not successful proven by appearing 1 kbp band electrophoresis secondary plasmid 2B using GFP primer and show band less than 5 Kbps with secondary primer. Also, EM observation shows the long tail fiber still present on mutant Bacteriophage.

5.3.3 The adsorption ability of wild type and Ecs1 Bacteriophage is higher than the mutant indicate by the number of plaque. That was because the gene that encodes tail fiber GP198 of Ecs1 Bacteriophage was removed.

5.3.4 The validation of the poster eligibility criteria showed average eligibility of 86,36% which was included in the category of very feasible to be published

5.2 Suggestions

Most of the genes in jumbo Bacteriophage are unknown. Further research needs to analyze the function of these genes to support the optimal application of Bacteriophage.

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APPENDIX A. RESEARCH MATRIX

Title	Problems	Variable	Indicator	Reference	Research Methodology
Analyzing Transforman of GP198 and GP270 of Jumbo Bacteriophage Related Tail Fiber Genes and The Utilization as a Poster	1. What is the first cloning result in the removal of the tail fiber GP270 and GP198? 2. What is the result of the second clone in the replacement of the tail fiber GP270 and GP198 with the GFP gene? 3. How is the adsorption ability of ϕEcs1 and ϕEcs1 Mutant? 4. How is the feasibility of the poster in the study of analyzing transformants of GP198 and GP270 tail fiber-related genes of jumbo Bacteriophage?	1 **Independent Variable**, The independent variable in this study is the volume of bacteriophage and plasmid samples used in the electrophoresis test. 2.**Bound Variables** The dependent variable in this study is the size of the emerging band, the host range curve, 3. **Control Variables** The control variables in this study were the type of Bacteriophage used, incubator temperature, medium volume, medium type, and bacterial OD, the	1. The appearance of the band in the electrophoresis test with a specific size following the target	The subject of research: *Escherichia coli* bacteriophage (ΦEcs1), (ΦEcs1 mutant), Primary plasmid (2C and 7D), Secondary plasmid (2B and 7E) Reference: a. Saad, Alaaeldin Mohamed., Soliman, Ahmed Mahrous., Kawasaki, Takeru., Fujie, Makoto., Nariya, Hirofumi., Shimamoto, Tadashi, and Yamada, Takashi. 2018. Systemic method to isolate large bacteriophages for use in biocontrol of a wide range of	1. Types of Research: an experimental study 2. Data Acquisition Instrument: laboratory testing and poster validation test 3. Data Acquisition Method: a. Propagation of ϕEcs1 and ϕEcs1 Mutant b. Checking the presence of tail fiber gene in the transforman of primary plasmid c. Digest with retraction enzymes d. Checking the presence of the GFP gene in the secondary

		kind of restriction enzyme and PCR master mix		pathogenic bacteria. *Elsevier*: 1-6 b. Saad, Alaaeldin Mohamed., Askora, Ahmed., Kawasaki, Takeru., Fujie, Makoto., Yamada, Takashi. 2018. Full genome sequence of a polyvalent bacteriophage infecting strains of *Shigella*, *Salmonella*, and *Escherichia*. *Archives of Virology*: 2	plasmid e. Confirmation the presence of tail fiber gene of transforman f. Electron Microscopy (EM) observation of ϕEcs1 and ϕEcs1 Mutant g. Host Range Test of ϕEcs1 and ϕEcs1 Mutant h. Poster Preparation i. Poster Validation Analysis

APPENDIX B. RESEARCH DOCUMENTATION

Figure B.1 LB broth Media preparation

B.2 LB plate media preparation

Figure B.3 PCR (A) Running Electrophoresis (B)

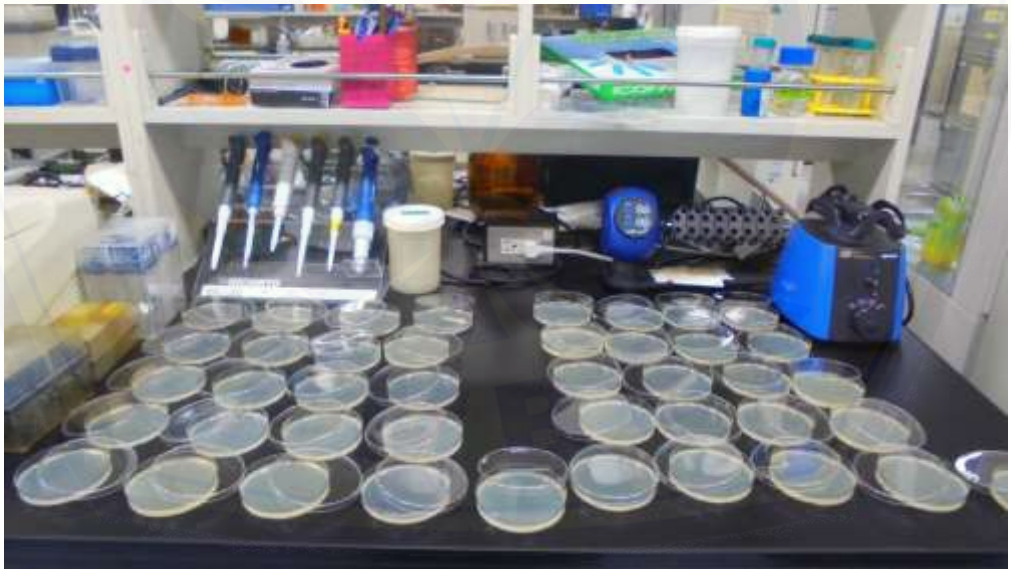
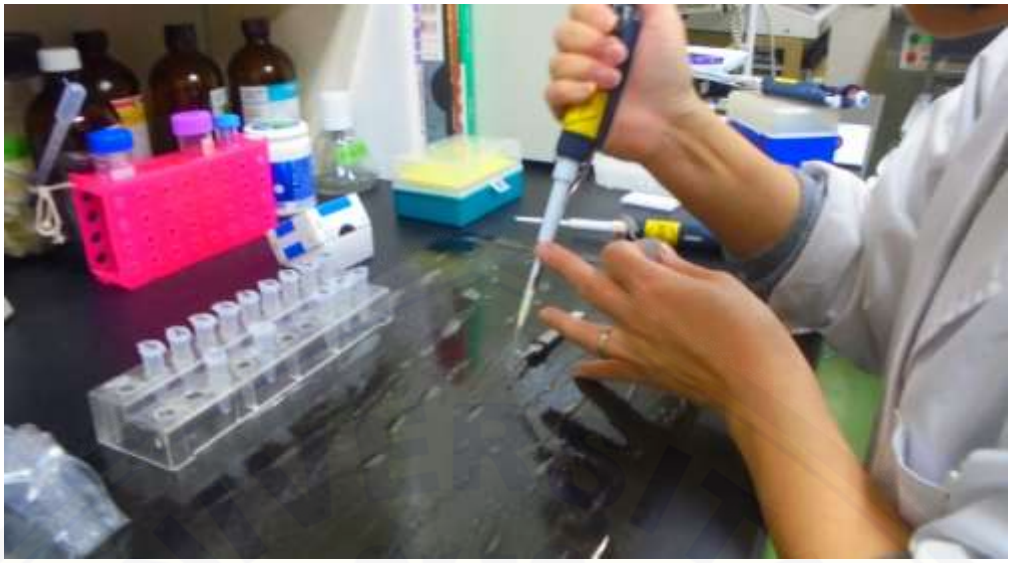


Figure B.4 Adsorption Assay



B.4 EM Observation Preparation

APPENDIX C. POSTER VALIDATION QUESTIONNAIRE SHEET

C.1 VALIDATION OF POSTER MEDIA EXPERT

QUESTIONNAIRE SHEET

POSTER PRODUCT TEST

I. Researcher's Identity

Name	: Afifatur Rofiqoh
NIM	: 160210103090
Department /Study Program	: Mathematics and Natural Sciences / Biology Education Faculty of Teacher Training and

II. Introduction

The undersigned, a college student of Bachelor of Biology Education Study Program, the Faculty of Education and Science University of Jember, is currently processing a thesis for entitled "**ANALYSING TRANSFORMAN OF GP198 AND GP270 OF JUMBO BACTERIOPHAGE RELATED TAIL FIBER GENES AND ITS UTILIZATION AS A SERIAL POSTER**."

In connection with this, the researcher needs assistance concerning the validation of her questionnaires necessary for the study. Knowing that you are most fit and capable of providing such, the undersigned would like to ask your approval to be the evaluator.

I am profoundly grateful for your kind approval.

Thank you very much, and may Allah bless you.

Sincerely yours,

AFIFATUR ROFIQOH

Student – Researcher

III. Validator Identity

Name	: Ika Lia Novenda, S.Pd., M.Pd
Home address	: Puri Bunga Nirwana Housing Blok Jimbaran B-16
No. Telephone	: 086749800388
Gender	: Female
Age	: 31 Years Old
Profession	: Lecturer

IV. Assessment Instrument

Hint:

5. Please assess each component by putting a checkmark (√) in the score column provided.
6. If a revision needs to do, please correct it at the suggestion point.
7. Please respond to the conclusion points by circling one of the choices available for the sustainability of the poster product.
8. Description of assessment:

1 = very bad

2 = not good

3 = good enough

4 = good

5 = very good

Sub Component	Assessment criteria	Score					Final score
		1	2	3	4	5	
1. Components of the Eligibility of Graphics							
A. Artistic and Aesthetic	1. The layout of poster components				√		24
	2. Background color selection				√		
	3. Font type selection				√		
	4. Color harmony on the poster				√		
	5. Image compatibility with font types				√		
	6. Accuracy inflow and overall harmony of poster components				√		
B.Poster Function	7. Readability (visible) provides clarity of information for the reader				√		15
	8. Posters possible enhanced the reader's knowledge of the material contained			√			
	9. Poster raises the attraction for readers to know the contents of the poster				√		
	10. the ability to influence the reader to act (to act)				√		
2. Presentation Components							
A. Presentati-on Techniques	11. The flow of the poster presentation is adjusted					√	32
	12. *Structured* of the poster's contents				√		
	13. Complete sentences presented				√		

	14. Continuity of the substance/aspect of the poster				√		
	15. Harmony and accuracy of the use of picture illustrations with poster material			√			
	16. Kesesuaian pemilihan gam bar dengan penjelasan			√			
	17. Continuity of content between posters				√		
	18. Derived from a definite source					√	
Total score : 71/90 x 100 = 78,8%							**71**

(Adapted from Puskurbuk, 2018; Sumartono and Astuti, 2018)

Comment:

Some things that need to fix:

1. Some of the pictures in poster three are missing, while in the picture on the right there is a description of Figure 4.5
2. There is no description of the picture in poster 2, only the source.
3. The shape of the poster one frame doesn't seem right.
4. Reference writing, too small even very small
5. Layout phage image on the bottom poster 3, do not just appear in the head only. Just show the whole even though later it is behind the reference writing that's okay.
6. Layout poster three don't choose black; it feels dark because in poster three, the majority of the images where the background is already dark.

Suggestion:

Correct according to records

Conclusion:

Based on the above assessment, then this poster product:

a. It cannot be used yet

b. It can be used with major revision

c. It can be used with minor revision

d. Can be used without revision

Jember, 19 April 2020

Validator

Ika Lia Novenda, S.Pd., M.Pd

C.2 VALIDATION POSTER OF MATTER EXPERTS

QUESTIONNAIRE SHEET
POSTER PRODUCT TEST

I. Researcher's Identity

Name	: Afifatur Rofiqoh
NIM	: 160210103090
Department /Study Program	: Mathematics and Natural Sciences / Biology Education Faculty of Teacher Training and

II. Introduction

The undersigned, a college student, taking up Bachelor of Primary Education in the Biology Education Study Program of the Faculty of Education and Science University of Jember, is currently processing a thesis for this semester entitled "**ANALYSING TRANSFORMAN OF GP198 AND GP270 OF JUMBO BACTERIOPHAGE RELATED TAIL FIBER GENES AND ITS UTILIZATION AS A SERIAL POSTER**."

In connection with this, the researcher will need assistance concerning the validation of his questionnaires necessary for the study. Knowing that you are most fit and capable of providing such, the undersigned has chosen and would like to ask approval from your right office to be the evaluator.

I am highly anticipating your kind approval regarding this matter.
Thank you very much, and God bless you.

Sincerely yours,

AFIFATUR ROFIQOH
Student – Researcher

III. Validator Identity

Name	: Aditya Kurniawan, S.Si., M.Biomed
Home address	: Semeru Utama A4 Jember
No. Telephone	: 081 334 155 366
Gender	: Male
Age	: 27
Profession	: Lecturer

IV. Assessment Instrument

Hint:

1. Please assess each component by putting a checkmark (√) in the score column provided.
2. If a revision needs to do, please correct it at the suggestion point.
3. Please respond to the conclusion points by circling one of the choices available for the sustainability of the poster product.
4. Description of assessment:

   1 = very bad

   2 = not good

   3 = good enough

   4 = good

   5 = very good

Assessment Criteria	Score					Final score
	1	2	3	4	5	
1. Conformity of the contents based on the purpose to prepare the poster				√		15
2. Complete information presented			√			
3. The depth of the content of the material presented				√		
4. Clarity of the language used				√		
5. Accuracy of images with illustrations					√	19
6. The accuracy of the theory with the concept of matter					√	
7. Accuracy of data according to research results				√		
8. Conformity with the development of science and technology, especially in the field of biotechnology					√	
9. The ability to influence the reader to act			√			10
10. Continuity of the substance between the poster parts			√			
11. Continuity of content between posters				√		
Total Score : 44/55 x 100% = 80%						**44**

(Adapted from: Puskurbuk, 2018; Sumartono and Astuti, 2018)

Comment:

1. Please sync your citations and references list
2. Your storyline still needs to improve

Suggestion:

I suggest you make a new poster to complete your serial poster named Poster 4. The new poster will cause your poster 3 less cramp and make your whole storyline improved. Please consider putting all your references at the end of your poster series.

Conclusion:

Based on the above assessment, then this poster product:

a. It cannot be used yet

b. It can be used with major revision

c. It can be used with minor revision

d. It can be used without revision

Jember, April 17, 2020

Validator

Aditya Kurniawan, S.Si., M.Biomed

C.3 POSTER USER RESPONSE

QUESTIONNAIRE SHEET
POSTER PRODUCT TEST

I. Researcher's Identity

Name : Afifatur Rofiqoh
NIM : 160210103090
Department /Study Program : Mathematics and Natural Sciences / Biology Education Faculty of Teacher Training and

II. Introduction

The undersigned, a college student, taking up Bachelor of Primary Education in the Biology Education Study Program of the Faculty of Education and Science University of Jember, is currently processing a thesis for this semester entitled "**ANALYSING TRANSFORMAN OF GP198 AND GP270 OF JUMBO BACTERIOPHAGE RELATED TAIL FIBER GENES AND ITS UTILIZATION AS A SERIAL POSTER**."

In connection with this, the researcher will need assistance concerning the validation of his questionnaires necessary for the study. Knowing that you are most fit and capable of providing such, the undersigned has chosen and would like to ask approval from your right office to be the evaluator.

I am highly anticipating your kind approval regarding this matter.
Thank you very much, and God bless you.

Sincerely yours,
AFIFATUR ROFIQOH
Student – Researcher

III. Validator Identity

Name	: Ria Yulian S.Pd
Home address	: Probolinggo
No. Telephone	: 082132315469
Gender	: Female
Age	: 23 years old
Profession	: Lecture assistant

IV. Assessment Instrument

Hint:

1. Please assess each component by putting a checkmark (√) in the score column provided.
2. If a revision needs to do, please correct it at the suggestion point.
3. Please respond to the conclusion points by circling one of the choices available for the sustainability of the poster product.
4. Description of assessment:

1 = very bad

2 = not good

3 = good enough

4 = good

5 = very good

Sub Component	Assessment criteria	Score					Final score
		1	2	3	4	5	
A. Format of the poster	1. Color harmony on the poster				✓		19
	2. The layout of the poster					✓	
	3. Accuracy in choosing the size and type of font					✓	
	4. Accuracy inflow and overall harmony of poster components					✓	
II. content	5. Appropriate selection of display pictures and writing according to the concept of material					✓	20
	6. Clarity of information presented					✓	
	7. The language easy to understand					✓	
	8. The emphasis of information on the poster					✓	
C. Presentation Techniques	9. The effectiveness of the presentation style					✓	13
	10. The flow of the poster presentation is adjusted				✓		
	11. Continuity of content between posters				✓		
Total Score : 52/55 x 100 = 94%							**52**

(Adapted from: Puskurbuk, 2018; Sumartono and Astuti, 2018)

Comment:

Overall it's okay, but it's better to change the color, it is based on the purpose of the poster to publish for general people, and I think pink is indicate a girl only.

Suggestion:

If it is possible to change the color and rearrange the color composition

Conclusion:

Based on the above assessment, then this poster product:

a. It cannot be used yet

b. It can be used with major revision

c. ***It can be used with minor revision***

d. It can be used without revision

Jember, April 16 2020

Validator

(Ria Yulian)

QUESTIONNAIRE SHEET

POSTER PRODUCT TEST

I. Researcher's Identity

Name : Afifatur Rofiqoh

NIM : 160210103090

Department /Study Program : Mathematics and Natural Sciences / Biology Education Faculty of Teacher Training and

II. Introduction

The undersigned, a college student, taking up Bachelor of Primary Education in the Biology Education Study Program of the Faculty of Education and Science University of Jember, is currently processing a thesis for this semester entitled "**ANALYSING TRANSFORMAN OF GP198 AND GP270 OF JUMBO BACTERIOPHAGE RELATED TAIL FIBER GENES AND ITS UTILIZATION AS A SERIAL POSTER**."

In connection with this, the researcher will need assistance concerning the validation of his questionnaires necessary for the study. Knowing that you are most fit and capable of providing such, the undersigned has chosen and would like to ask approval from your right office to be the evaluator.

I am highly anticipating your kind approval regarding this matter.

Thank you very much, and God bless you.

Sincerely yours,

AFIFATUR ROFIQOH

Student – Researche

III. Validator Identity

Name	: Qonita Nafilah
Home address	: Perum. Taman Kampus blok C3 no. 2 , Jember
No. Telephone	: 082331517914
Gender	: Female
Age	: 22 years old
Profession	: Dentistry student, University of Jember

IV. Assessment Instrument

Hint:

1. Please assess each component by putting a checkmark (√) in the score column provided.
2. If a revision needs to do, please correct it at the suggestion point.
3. Please respond to the conclusion points by circling one of the choices available for the sustainability of the poster product.
4. Description of assessment:

1 = very bad

2 = not good

3 = good enough

4 = good

5 = very good

Sub Component	Assessment criteria	Score					Final score
		1	2	3	4	5	
A. Format of the poster	1. Color harmony on the poster					√	17
	2. The layout of the poster				√		
	3. Accuracy in choosing the size and type of font				√		
	4. Accuracy inflow and overall harmony of poster components				√		
J. content	5. Appropriate selection of display pictures and writing according to the concept of material				√		16
	6. Clarity of information presented				√		
	7. The language easy to understand				√		
	8. The emphasis of information on the poster				√		
C. Presentation Techniques	9. The effectiveness of the presentation style					√	15
	10. The flow of the poster presentation is adjusted					√	
	11. Continuity of content between posters					√	
Total Score 48/55 x 100% = 87%							**48**

(Adapted from: Puskurbuk, 2018; Sumartono and Astuti, 2018)

Comment:

Overall, a well-written poster. It's describing the detail of Bacteriophage as therapy as the central theme of the project for people who is not familiar with this field very well.

Suggestion:

It is better to describe more about Bacteriophage

Conclusion:

Based on the above assessment, then this poster product:

a. It cannot be used yet

b. It can be used with major revision

c. It can be used with minor revision

d. It can be used without revision

Jember, April 16, 2020

Validator

Qonita Nafilah Febi

NIM. 161610101067

QUESTIONNAIRE SHEET

POSTER PRODUCT TEST

I. Researcher's Identity

Name : Afifatur Rofiqoh

NIM : 160210103090

Department /Study Program : Mathematics and Natural Sciences / Biology Education Faculty of Teacher Training and

II. Introduction

The undersigned, a college student, taking up Bachelor of Primary Education in the Biology Education Study Program of the Faculty of Education and Science University of Jember, is currently processing a thesis for this semester entitled "**ANALYSING TRANSFORMAN OF GP198 AND GP270 OF JUMBO BACTERIOPHAGE RELATED TAIL FIBER GENES AND ITS UTILIZATION AS A SERIAL POSTER**."

In connection with this, the researcher will need assistance concerning the validation of his questionnaires necessary for the study. Knowing that you are most fit and capable of providing such, the undersigned has chosen and would like to ask approval from your right office to be the evaluator.

I am highly anticipating your kind approval regarding this matter.

Thank you very much, and God bless you.

Sincerely yours,

AFIFATUR ROFIQOH

Student – Researcher

III. Validator Identity

Name	: Fifi Lanna Fauziah
Home address	: Banyuwangi
No. Telephone	: 082132315469
Gender	: Female
Age	: 22 years old
Profession	: Nursing student, University of Jember

IV. Assessment Instrument

Hint:

5. Please assess each component by putting a checkmark (√) in the score column provided.
6. If a revision needs to do, please correct it at the suggestion point.
7. Please respond to the conclusion points by circling one of the choices available for the sustainability of the poster product.
8. Description of assessment:

1 = very bad

2 = not good

3 = good enough

4 = good

5 = very good

Sub Component	Assessment criteria	Score					Final score
		1	2	3	4	5	
A. Format of the poster	1. Color harmony on the poster				✓		17
	2. The layout of the poster				✓		
	3. Accuracy in choosing the size and type of font					✓	
	4. Accuracy inflow and overall harmony of poster components				✓		
III. content	5. Appropriate selection of display pictures and writing according to the concept of material					✓	19
	6. Clarity of information presented					✓	
	7. The language easy to understand				✓		
	8. The emphasis of information on the poster					✓	
C. Presentation Techniques	9. The effectiveness of the presentation style					✓	15
	10. The flow of the poster presentation is adjusted					✓	
	11. Continuity of content between posters					✓	
Total Score : 51/55 x 100% = 92%							**51**

(Adapted from: Puskurbuk, 2018; Sumartono and Astuti, 2018)

Comment:

Poster 1:

- The title of the poster should be capital at the beginning
- Figures 3 and 4 labels as Figures 1 and 2

Poster 2:

- There is excess space after the range brackets.
- The picture is a bit unclear

Poster 3:

- The graphic color in figure 4.5 whose number of mutants must be clarified

Suggestion:

It's excellent overall, from the color selection, is cute. Tips for considering my comments before. Hopefully, it will be more comfortable in the future. I think your research is marvelous

Conclusion:

Based on the above assessment, then this poster product:

a. It cannot be used yet

b. It can be used with major revision

c. ***It can be used with minor revision***

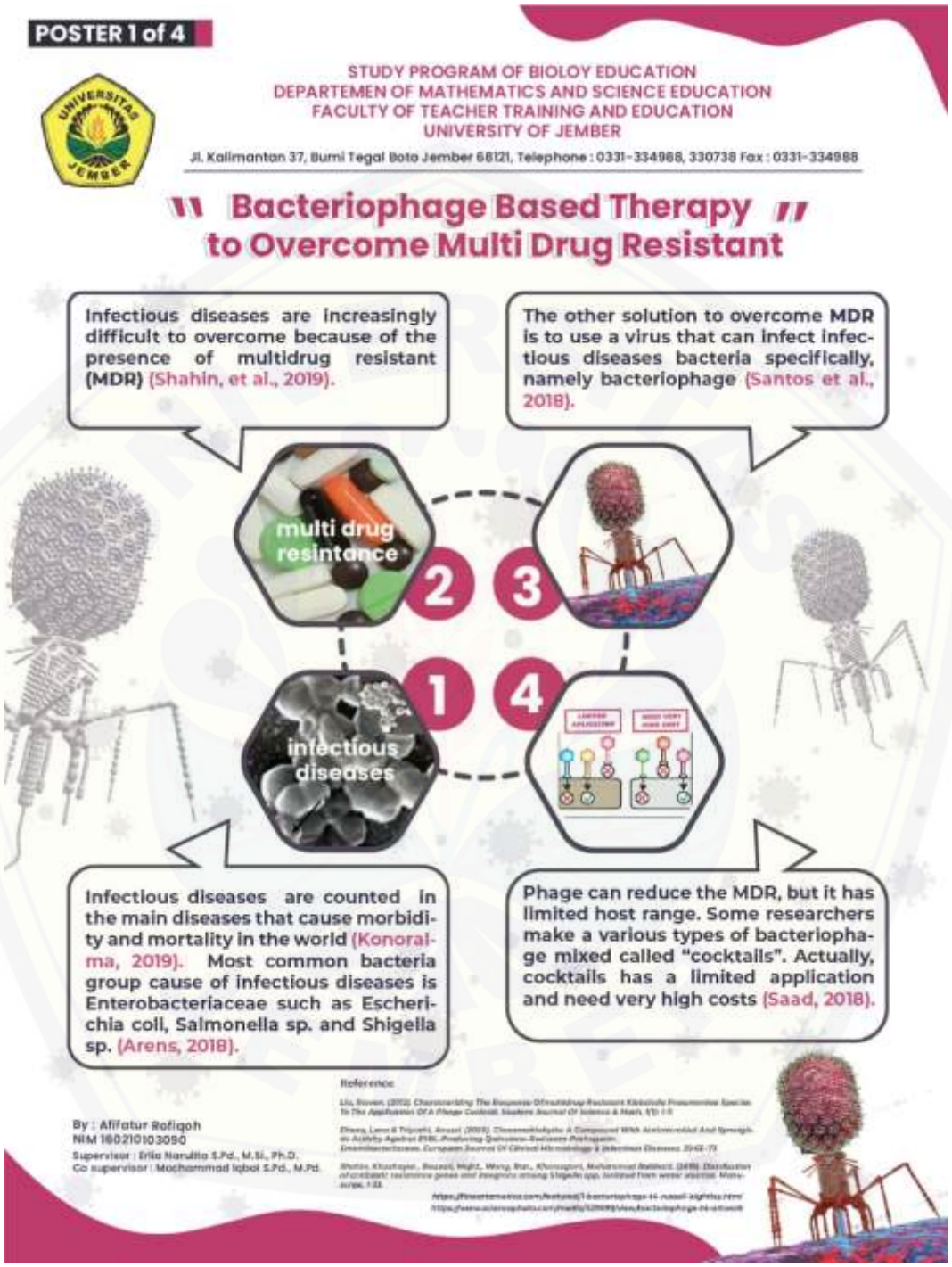
d. It can be used without revision

Jember, April 16, 2020

Validator

(Fifi Lanna Fauziah)
NIM. 162310101265

APPENDIX D. POSTER DESIGN



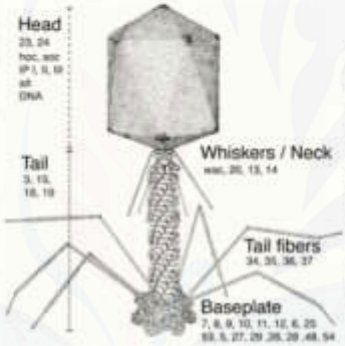
POSTER 2 of 4

STUDY PROGRAM OF BIOLOY EDUCATION
DEPARTEMEN OF MATHEMATICS AND SCIENCE EDUCATION
FACULTY OF TEACHER TRAINING AND EDUCATION
UNIVERSITY OF JEMBER
Jl. Kalimantan 37, Bumi Tegal Boto Jember 68121, Telephone : 0331-334988, 330738 Fax : 0331-334988

Jumbo Bacteriophage

Jumbo phage is phage that have genome size more than 200 kbp. It can infect a variety of bacteria (large of host range) and can support long-term infections. Jumbo phage gene expression does not depend on RNAP and can be activated only by the RNAP Phase. Many other genes from jumbo phages work to reduce bacteriophage dependence on their host. It make them to be a good candidate for use as a biocontrol of pathogenic bacteria (Saad et al, 2018).

Fiber and the tail has a ability as a cell recognition sensor by attaching phage to the surface of bacterial cell (Leiman et al, 2003).



Particle Structure of T4 Bacteriophage (Miller, 2003)

GP198 in φEcs1 is an extra tail fiber coding gene. While GP270 on φEcs1 is a distal subunit of long tail fiber coding gene (Saad, 2018).

Reference:

Miller, Eric S., Kutter, Elizabeth., Mosig, Gisela., Arisaka, Fumio., Kunisawa, Takashi., And Ruger, Wolfgang. (2003). Bacteriophage T4 Genome. Microbiology And Molecular Biology Reviews. 67(1). 86-156

Saad, Alzaedin Mohamed., Askora, Ahmed., Kawasaki, Takahiro., Fujie, Makoto., Yamada, Takashi. (2018). Full genome sequence of a polyvalent bacteriophage infecting strains of Shigella, Salmonella, and Escherichia. Archives of Virology. 2.

Hendrix, R.W. (2009). Jumbo Bacteriophages. Microbiology and Immunology. Pittsburgh Bacteriophage Institute and Department of Biological Sciences, University of Pittsburgh. Springer-Verlag Berlin Heidelberg

By : Afifatur Rofiqoh
NIM 160210103090
Supervisor : Erlia Narulita S.Pd., M.Si., Ph.D.
Co supervisor : Mochammad Iqbal S.Pd., M.Pd.

POSTER 3 of 4

STUDY PROGRAM OF BIOLOY EDUCATION
DEPARTEMEN OF MATHEMATICS AND SCIENCE EDUCATION
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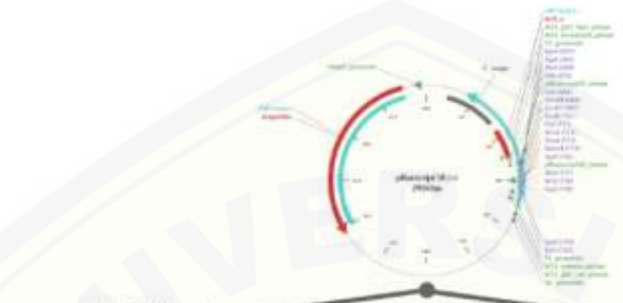
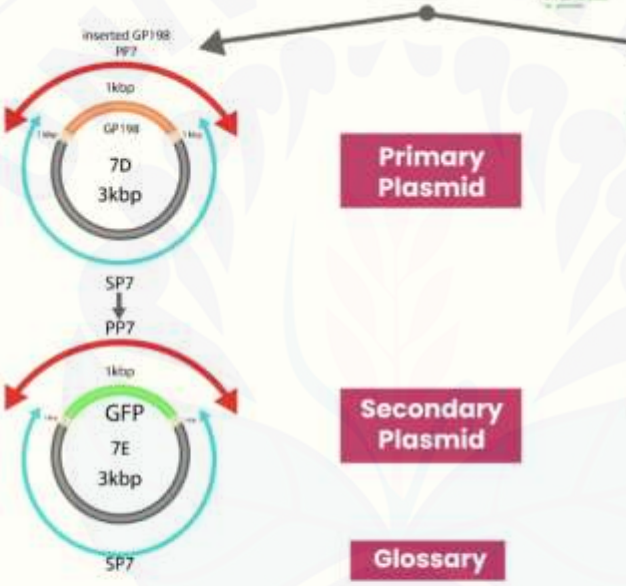
UNIVERSITY OF JEMBER
Jl. Kalimantan 37, Bumi Tegal Boto Jember 68121, Telephone : 0331-334988, 330738 Fax : 0331-334988

"Genetic Modification of GP198 and GP270"





Primary Plasmid

Secondary Plasmid

Glossary

GP198	= Encode Extra tail fiber	PP2	= Primary Primer 2
GP270	= Encode Proximal part of Long tail fiber	SP2	= Secondary Primer 2
GFP	= Green Fudrescent Protein Gene	PP7	= Primary Primer 7
		SP7	= Secondary Primer 7

Reference

Isnat, Alsaddin Mohamed, Sulistyo, Ahmed Makmun, Kawasaki, Takeru, Fujie, Manabu, Yamada, Tomoya, Hidefumi, Shimamoto, Tadashi and Yamada, Takashi. (2018). Systematic method to isolate large bacteriophages for use in biocontrol of a wide range of pathogenic bacteria. Zbornik, 1-6.

Isnat, Alsaddin Mohamed, Adkins, Ahmed, Kawasaki Takeru, Fujie, Manabu, Yamada, Takashi. (2018). Full genome sequence of a polyvalent bacteriophage infecting strains of Shigella, Salmonella, and Escherichia. Archives of Virology, 2.

By : Afifatur Rofiqoh
NIM 160210103060

POSTER 4 of 4

STUDY PROGRAM OF BIOLOY EDUCATION
DEPARTEMEN OF MATHEMATICS AND SCIENCE EDUCATION
FACULTY OF TEACHER TRAINING AND EDUCATION
UNIVERSITY OF JEMBER
Jl. Kalimantan 37, Bumi Tegal Boto Jember 68121, Telephone : 0331-334988, 330738 Fax : 0331-334988

"Analysing Transformant of GP198 and GP270 Tailfiber Related Genes of Jumbo Phage and The Utilization as a Poster"

A. Purposes

- To know the first cloning result in the inserting of the tail fiber GP270 and GP198
- To know the second cloning result in the replacement of the fiber tail GP270 and GP198 with GFP gene.
- To know the absorption ability of ΦEcsl and ΦMutant Ecsl

B. Methode

PCR-Digest with restriction enzyme-Electrophoresis-adsorban assay-ploque assay-EM Observation

C. Result

The presence of tail fiber gene

The presence of the GFP gene

M 3Sep 3Sgfp 3exp 3egfp

EM Observation

"Curve of Adsorption Assay"

Digest with Restriction enzymes

D. Conclusions

- The process of inserting tail fiber gene was succesed indicate by appears a band with size around 3 Kbp and 5 Kbp for plasmid 7B and 5 Kbp for plasmid 2C with. That's all confirmed with restriction enzyme.
- The process of replacement GP198 with GFP gene was succesed by appear 1 Kbp band of plasmid 7E but not succesed for GP270 show by band less than 5 Kbp for plasmid 2B. That's confirm by EM observation show the long tail fiber still presence on Δmutan.
- The adsortion ability of wild type ΦEcsl is higher than Δmutan that lost some tail fiber gene.

E. Reference

By : Afifatur Rofiqoh
NIM 160210103090
Supervisor : Erlia Narulita S.Pd., M.Si., Ph.D.
Co supervisor : Mochammad Iqbal S.Pd., M.Pd.

APPENDIX E. LETTER OF RESEARCH

HIROSHIMA UNIVERSITY

Graduate School of Integrated Sciences for Life

LETTER OF ACCEPTANCE

April 15, 2019

Dr. Erlia Narulita
Study Program of Biology Education
Faculty of Teacher Training and Education
University of Jember
Jl. Kalimantan 37 Jember
68121, Indonesia

Dear Dr. Erlia Narulita,

I would like to accept your students, (1) Zidna Amalia Firdausy and (2) Afifatur Rofiqoh to join our laboratory as research students at Division of Biological and Life Sciences, Graduate School of Integrated Sciences for Life from September 1, 2019 to November 30, 2019. During this period, they will participate in a collaboration work on "Molecular analysis of bacteriophages". This activity is under the MoU between Graduate School of Integrated Sciences for Life, Hiroshima University and CDAST, University of Jember. According to the MoU, your accommodation, research materials and expense will be covered by Graduate School of Integrated Sciences for Life.

Sincerely yours,

Makoto Fujie

Makoto Fujie Ph. D.
Associate Professor
Division of Biological and Life Sciences
Graduate School of Integrated Sciences for Life
Hiroshima University
Phone / FAX: 81-82-424-7750
e-mail: mfujie@hiroshima-u.ac.jp

APPENDIX F. CONSULTATION SHEET FOR THESIS PREPARATION

KEMENTERIAN PENDIDIKAN DAN KEBUDAYAAN
UNIVERSITAS JEMBER
FAKULTAS KEGURUAN DAN ILMU PENDIDIKAN
Jalan Kalimantan Nomor 37 Kampus BumiTegalbotoJember 68121
Telepon: 0331-334988, 330738 Fax: 0331-334988
Laman: www.fkip.unej.ac.id

LEMBAR KONSULTASI PENYUSUNAN SKRIPSI
Dosen Pembimbing Utama

Nama : Afifatur Rofiqoh
NIM : 160210103090
Jurusan / Program Studi : Pendidikan MIPA / Pendidikan Biologi
Judul : "Analysing Transformants of GP198 and GP270 of Jumbo Bacteriophage Related Tail Fiber Genes and Its Utilization as a Serial Poster"

Pembimbing Utama : Erlia Narulita S.Pd., M.Si., Ph.D.
Kegiatan Konsultasi

No.	Hari/ Tanggal	Materi Konsultasi	Tanda Tangan Pembimbing
1.	28 Agustus 2019	Penentuan Judul	
2.	1 Desember 2019	Pengajuan BAB 1, 2, dan 3	
3.	15 Januari 2020	Revisi BAB 1, 2, 3	
4.	20 Januari 2020	Revisi Bab 1, 2 , 3 dan Lampiran	
5.	27 Januari 2020	ACC Seminar Proposal	
6.	10 Maret 2020	Seminar Proposal	
7.	11 Maret 2020	Konsultasi Penelitian	
8.	20 Maret 2020	Penyerahan hasil penelitian dan pengajuan BAB 1, 2, 3, dan 4	
9.	24 Maret 2020	Revisi BAB 1, 2, 3, 4, 5 dan lampiran serta penyerahan artikel	
10.	22 April 2020	ACC ujian skripsi	

Catatan:
1. Lembar ini harus dibawa dan diisi setiap melakukan konsultasi
2. Lembar ini harus dibawa sewaktu seminar proposal skripsi dan ujian skripsi

KEMENTERIAN PENDIDIKAN DAN KEBUDAYAAN
UNIVERSITAS JEMBER
FAKULTAS KEGURUAN DAN ILMU PENDIDIKAN
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Laman: www.fkip.unej.ac.id

LEMBAR KONSULTASI PENYUSUNAN SKRIPSI

Dosen Pembimbing Anggota

Nama : Afifatur Rofiqoh
NIM : 160210103090
Jurusan / Program Studi : Pendidikan MIPA / Pendidikan Biologi
Judul : "Analysing Transformants of GP198 and GP270 of Jumbo Bacteriophage Related Tail Fiber Genes and Its Utilization as a Serial Poster"

Pembimbing Anggota : Mochammad Iqbal S.Pd., M.Pd.

Kegiatan Konsultasi

No.	Hari/ Tanggal	Materi Konsultasi	Tanda Tangan Pembimbing
1.	30 Agustus 2019	Penentuan Judul	
2.	24 Desember 2019	Pengajuan BAB 1, 2, dan 3	
3.	26 Januari 2020	Revisi BAB 1, 2,dan 3	
4.	21 Januari 2020	Revisi BAB 1, 2, 3 dan Lampiran	
5.	27 Januari 2020	ACC Seminar Proposal	
6.	10 Maret 2020	Seminar Proposal	
7.	13 Maret 2020	Konsultasi Penelitian	
8.	30 Maret 2020	Penyerahan hasil penelitian dan pengajuan BAB 1, 2, 3, dan 4	
9.	20 April 2020	Revisi BAB 1, 2, 3, 4, 5 dan lampiran serta penyerahan artikel	
10.	24 April 2020	ACC ujian skripsi	

Catatan:
1. Lembar ini harus dibawa dan diisi setiap melakukan konsultasi
2. Lembar ini harus dibawa sewaktu seminar proposal skripsi dan ujian skripsi