CHAPTER I

INTRODUCTION

1.1 Background

The primary cilia is a non-motile type of cilia, extruding from most of the mammalian cells. The structure consists of three main division, the basal body, the transition zone, and the axoneme. The basal body and the transition zone are surrounded by the pericentriolar matrix with more than 250 proteins which have been suspected to be involved in the formation of primary cilia. The axoneme, which form the framework of primary cilia, is composed of microtubules growing from the base of primary cilia. This unit is responsible for various motor protein pathways, such as kinesin and dynein to transport proteins in cells (Lu QL, 2015).

Primary cilia has been found to be a key organelle for various cell signal transduction pathway, such as Hedgehog, wnt, and Notch pathway; which are very important in the development of the cells (Pedersen, Veland, Schrøder, & Christensen, 2008; Wheway et al., 2018). Due to this reason, the research interest of primary cilia has gone up. The defects of primary cilia are usually referred to as ciliopathy, which will result in the disorder in the development of the cell (Fliegauf, Benzing, & Omran 2007; Waters & Beales, 2011). The most severe clinical manifestation of this condition is known as Meckel's Syndrome (MKS) and Jouberts syndrome (JBTS). Most fetus born with MKS would most likely die after birth (Alexiev, 2006).

This syndrome is caused by the mutation of Retinitis Pigmentosa GTPase Interacting Protein-1 Like Protein, also known Rpgrip1l (Wiliantara, 2018). It is a protein found in the transition zone of primary cilia. This protein has 1,315 amino acid residues, and consist of three domains, which includes, the CC domain, C2 domain, and the RIDL domain. It has been

found that this protein plays a crucial role to the formation of the structure of primary cilia, especially in the transition zone assembly (Arts, et al., 2007). This finding is supported by previous research that shows the silencing of this protein will result in the decreasing number of cilia, also the reduction of RPGRIP1L causes failure of localization of other transition zone protein on the base on primary cilia (Wiliantara, 2018). However, further research on the exact role and interaction of Rpgrip1l with other ciliary proteins, and which domain are responsible for the activity and function of the protein is still required.

It has been suspected that the transition zone acts as a gateway for proteins involved in protein transport in primary cilia. Failure of the transition zone formation will result in the failure of ciliogenesis, which result with the interference of mentioned signal transduction pathways. Leading to what is called ciliopathy (Wiliantara, 2018).

Due to the reasons mentioned above, it is important to identify which domain of the Rpgrip1l that actually plays a role in the localization of the proteins, as this will open a new means of curative therapies for various ciliopathy, especially Joubert and Meckel's Syndrome. From the three domains, this research will focus on the RIDL domain. This domain is responsible for the Rpgrip1l interaction with other protein called Rpgr, which is a ciliary protein that interacts with other protein, among them is the CEP290, which has been implicated in several ciliopathies, including Joubert and Meckel's Syndrome. There are various ways to confirm the role of the domain, one of them is to knockdown the target domain and observe the effects to the formation of transition zone of primary cilia. One of the cost-effective methods is to utilize the gene cloning method. Where the truncated gene is inserted into a plasmid, so that the expression of the gene may be observed in mammalian cell. This study aims to create the plasmid containing truncated RIDL domain of Rpgrip1l.

1.2 Hypothesis

The hypothesis of this research project is: A pEGFP-N1 plasmid containing *RPGRIP1L* gene with truncated RIDL domain be prepared and confirmed with colony PCR and Enzyme digestion

1.3 Research Objectives

The primary objectives of the research is to:

- Creating a pEGFP-N1 containing truncated RIDL Domain

While, the secondary objectives of the research are to:

- Optimize the PCR condition for Colony PCR
- Prepare glycerol stock containing the plasmid with the desired insert
- Prepare a competent cell