DEVELOPMENT OF CRISPR/CAS9 PLASMID FOR GENE EDITING OF EGFRvIII GENE OF GLIOBLASTOMA MULTIFORME

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Abstract

This study is to develop CRISPR/Cas9 plasmid for gene editing in glioblastoma multiforme (GBM). GGM is the most lethal type of cancer. There is no effective treatment for GBM. So far, treatment for GBM are radiotherapy, chemotherapy, and surgery. However, those treatment do not provide any effective result, while recurrence rate are still high. The CRISPR/Cas9 gene editing may provide treatment for patients of glioblastoma multiforme, by knocking off EGFRvIII gene, an oncogene for this cancer. In this study, we design, isolate, and verify CRISPR. We achieved a purity level of 1.8 and concentration of 38,1 ug/mL. However, we need to do further verification, prior to do the next steps, which are insertion and ligation of sgRNA into the plasmid.
Keywords: CRISPR/Cas9, plasmid, glioblastoma multiforme, EGFRvIII cancer.

1 INTRODUCTION

Glioblastoma multiforme (GBM) is one of the most difficult cancer to treat. Its survivability rate is low, as most patients can only live for 15-18 months after diagnosed with GBM. Meanwhile, its recurrence rate is high (Prieto & de La Fuente, 2021; Sepulveda et. al, 2017).

EGFR gene is the firstly found GBM oncogene (Prieto & de La Fuente, 2021). This gene produces epidermal growth factor (EGF) protein. It is also a transmembran glycoprotein. EGFRvIII is the most prominent mutation of EGFR in GBM (Sepulveda et. al, 2017).

Clustered regularly interspaced short palindromic repeats (CRISPR) is one of the promising technique for editing gene. The technique include CRISPR-associated proteins (Cas) systems.

The aim of this study is to develop a plasmid containing sgRNA and Cas9 to knock out EGFRvIII gene responsible for GBM. This is the beginning of those steps, which covers the design, isolation and optimization of plasmid.

2 METHODOLOGY

The first step is designing sgRNA, using NCBI website to search the mRNA. In online CRISPR design tools, we copy the sequence number to improve efficiency and reducing off-target sequence. We took first three sequence as candidates of sgRNA. The researchers purchased the designed plasmid *pSpCas9n(BB)-2A-Puro* from Addgene. This plasmid is sent as bacterial agar stab. This bacteria is cultured in Luria Bertani Broth (LBB) medium. This bacteria is cultured in agar media. Then, the plasmid is incubated in temperature of 37^0 C for 24-48 hours until a single colony appears. We incubate the single colony into lactose broth media that is added by 100 ug/mL of ampicillin. The incubation last for 2 days in 37^oC temperature. The isolation process uses miniprep plasmid isolation kit.

3 RESULTS

In the first plasmid incubation, there was no growth of bacteria in the culture (Figure 1). Therefore, there researcher repurchase the plasmid.

In the second trial, there was a purity level of 1.8 and concentration of 38,1 ug/mL.

Figure 1. Bacterial Culture contain pSpCas9n (BB)-2A-Puro and Cas-9 Recombinant

In this step, the researchers did not verify the plasmid yet. There is only one band during electrophoresis (Figure 2). There should be more optimation of plasmid restriction using FastDigestBbcI kit. There should also be optimation in electrophoresis. So far, there are 4 iteration of electrophoresis. Next step is bacterial culture in gliserol stock and another plasmid isolation.

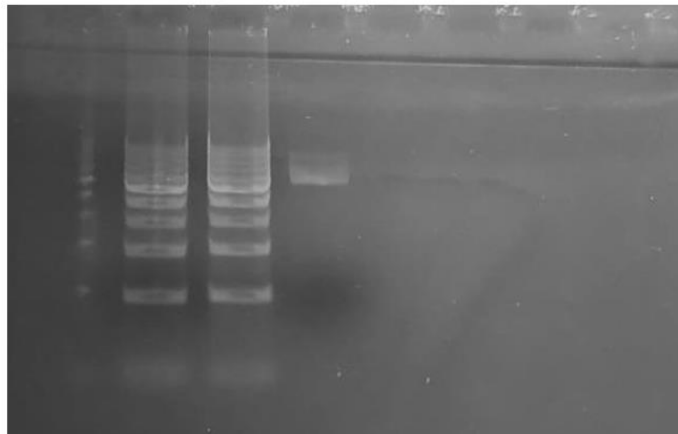

Figure 2. Gel Electrophoresis Result for Confirming the Plasmid Recombinant

4 CONCLUSIONS

This study achieved growth of plasmid for CRISPR/Cas9 with 1.8 level of purity and concentration of 38,1 ug/mL However, the verification were still unsuccessful. Therefore, there should be more focus on the verification, prior to moving into ligation step.

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