Creating E.coli Library of Diagnostic Gene for Influenza B Virus Using Molecular Cloning Technique

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Abstract

Influenza viruses cause seasonal epidemics associated with high morbidity, especially in immunocompromised and extreme age group individuals. Many strategies for combating these viruses are in practice; however, developing and maintaining sensitive and specific diagnostic method is essential to the success of strategies used. The purpose of this project is to clone a diagnostic gene for Influenza B virus and create *e.coli* colony type that we can propagate as needed for future test validations at the State Virology Laboratory in Fairbanks, AK.

Background

Influenza B is a linear single-stranded negative sense RNA virus with a total genome length of 13.5kb. It causes disease only in humans and seals. Its virions are enveloped and can either be quasi-spherical or filamentous. It has 8 gene segments that codes for 11 viral protein. Envelope is derived from host cell plasma membrane and it has a compact helical nucleocapsid. The virus attaches to sialic acid receptor through HA (hemagglutinin) protein and fuses with the host cell membrane via clathrin dependent endocytosis(1). Molecular diagnostic assays using PCR method most often targets regions of the genome that are highly conserved. In this project, we targeted the nuclear export protein(NEP) and non-structural protein 1(NSP1) in gene segment 8. This target of 103 bp region is found among all influenza B viral strains that are known to have infected human(2).

Methods

The project consisted of sequence of steps and the following flow chart outlines the complete process.

Extraction of viral RNA ➔PCR ➔Gel Electrophoresis ➔ Clone into vector ➔ M13 PCR & Patch Plate ➔Gel Electrophoresis➔ Culture transformants in LB broth➔ Extract DNA from transformants➔ Concentration Measurement (Nanodrop)➔ Send for sequencing

Methods (cont.d)

Two patients samples were used (B1 and B2). The viral RNA from the patients samples were extracted by RNAeasy microkit made by QIAGEN following the manufacturer's protocol. PCR reaction was set up to amplify the target regions (NEP and NS1 protein) of the viral genome using the designed primers and gel electrophoresis was done to confirm that we have the desired target region of 103 bp size fragment successfully amplified. Using pCR 4-TOPO cloning kit from Invitrogen®, the target fragment was cloned into the vector and incorporated into competent cells and plated on ampicillin coated platform. Next, M13 PCR and patch plate was to be performed the to determine which colony had the right size fragment inserted in it and followed by gel electrophoresis as confirmation. Based on the gel electrophoresis result, transformants are selected and cultured in LB broth and the genetic material is then extracted from the transformants. Nanodrop measurement is performed in order to measure the concentration of genetic material in the extraction. Finally the extraction containing the target genome sequence is sent off for sequencing to a lab in San Francisco.

Results

Gel electrophoresis result showed that viral RNA extracted from patient 2 (designated as B2) was successfully amplified by PCR. However, the PCR reaction did not work for patient 1 sample.

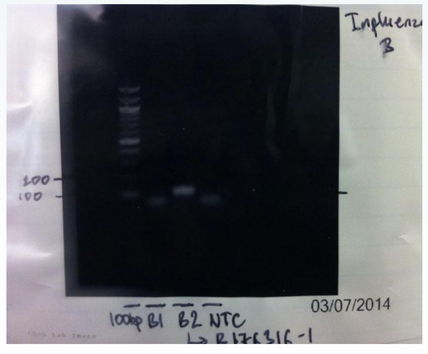

Figure 1. Result from gel electrophoresis on PCR amplified product of target sequence coding for proteins NEP and NS1. Target fragment size is 103 bp.

Results (cont.d)

Once the PCR product containing the right target sequence was obtained, here had been multiple attempts at cloning the desired the target genes. However, due to several technical issues we have encountered, none of the cloning attempts were successful. Therefore, the rest of the steps were halted, which are M13 PCR and patch plate, selection and culturing of transformants, extraction and sequencing of genetic materials from the bacterial colony.

Conclusion

Developing and replicating a reliable molecular diagnostic assay is not an easy task. There are various number of things that could go wrong at every stage of the process and yet there are many areas where it also needs to be optimized through trial and error. Cloning of the target genes involves number of steps. First step is a ligation reaction of PCR product with the TOPO vector plasmid bathed in a salt solution. The usage of salt solution was experimentally shown to increase the yield of ligated product. However, using highly concentrated salt solution would jeopardize the next step of the process, which is electroporation of the competent cells. After the ligation reaction, the ligated vector containing target gene is mixed with competent cells and the vectors are inserted through holes made in cell membrane by treating them electrically using an electroporator. One of the possible explanations for our failed cloning experiment could be non-optimized concentration of salt solution used.

References

(1) Acheson NH. Fundamentals of Molecular Virology. Wiley; 2011.

(2) Weinberg A, Walker ML. Evaluation of three immunoassay kits for rapid detection of influenza virus A and B. Clin Diagn Lab Immunol. 2005;12(3):367-70.