Genome engineering using CRISPR/Cas9-edited HEK293 cell lines

Abstract

PCR amplification of SCRIB sequences from CRISPR/Cas9 modified gene fragment of HEK293 cell line that has been done in vitro. Sequence analysis of cloned PCR product was done by electrophoresis through agarose gel and DNA band was visualized by gel red binding and gel documentation equipment. Lastly, TA cloning of the SCRIB PCR product from the mutated HEK293 Cells was performed into the plasmid Pcr2.1 vector and confirmed the mutation introduced by CRISPR/Cas9.

Introduction

The CRISPR-Cas9 system is the most remarkable breakthrough in genome editing technology and it has the ability to engineer biological systems and organism holds enormous potential for applications across basic science, medicine and biotechnology. The CRISPR-Cas9 genome editing system consists of two components: a “guide” RNA (gRNA) and a non-specific CRISPR-associated endonuclease Cas9 (Urnov et al., 2005, Bedell et al., 2012). Cas9 is a nuclease guided by small RNAs through Watson-Crick base pairing with target DNA representing a system that is markedly easier to design, highly specific and well-suited for high throughput and multiplexed gene editing for a variety of cell types and organisms.

SCRIB is a polarity regulator known to be abnormally expressed in cancer at the protein level. In epithelial cells planar polarity is maintained by proteins that are encoded by tumor suppressor genes such as SCRIB (Bilder and Perrimon, 2000). Scribble, the protein product of SCRIB, is crucial for the proper maintenance of epithelial cell integrity and function (Zhan et al., 2008).

TA cloning is one of the simplest and most efficient methods for the cloning of PCR amplified product. The procedure exploits the terminal transferase activity of certain thermophilic DNA polymerases, including (Taq).

Materials and Methods

1. PCR reaction

Reagents- Autoclaved milli Q Water 4 µl, 10 x PCR buffer 2 µl, 25mM MgCl2 2 µl, dNTPs 2 µl, Primer FP 2 µl, Primer RP 2 µl, Taq polymerase 1 µl.

The reagents were added to each thin-walled PCR tube then it was mixed thoroughly to make sure all the liquid was in the bottom. The PCR conditions which was follows: 95ºC for 4 min then (95ºC for 15 s; 55ºC for 30 s; 72ºC for 60 s) x 30 times, then 72ºC for 5 min, then hold at 4ºC.

2. Agarose gel electrophoresis of the SCRIB amplicon

3 µl of DNA loading buffer (10mM Tris-HCl pH 7.6, 60 mM EDTA, 0.03% bromophenol blue, 60% glycerol) and 5 µl of gel red were added in a 1.5ml microfuge tubes and then 5 µl of each PCR reactions from tubes 1 and 2 were mixed together. Spining of microfuge tube was done briefly to get the contents down to the bottom of the tube. 5 µl of the DNA size marker and 5 µl gel red were added. 10 µl of the DNA size marker (plus 5 µl gel red and 1 µl loading buffer) in the first well were loaded and in the next two wells 10 µl of prepared PCR DNA samples were loaded. The samples were analyzed by electrophoresis at ca. 100 V (which should give about 60 mA) for 1 hour. At the end of the electrophoresis the gel were placed on a UV-light gel transilluminator in the dark room and a digital image was taken to record the result from the PCR experiment.

3. Cloning of the SCRIB amplicon into the plasmid pCR2.

Reagent- 2µl Fresh PCR product, 2µl Open pCR2.1 vector (25 ng µl-1), 2 µl 5X ligation buffer, 1µl 21 T4 DNA ligase, 2µl Sterile milli-Q H2O were added to a total volume of 10µl.

The contents were mixed gently by pipetting up and down twice. Incubation was done at room temperature for 1 hour and transferred to E. coli cells. 2 µl of the ligation reaction from stage 1 were added into a microfuge tube containing 25 µl competent E. coli which was just thawed out and kept on ice and mixed gently by flicking the tube. The cells were kept in cold so that it would not get denature. Incubation was done on ice for 15 minutes then the Heat-shock method was done and kept the cells in the water-bath for 30 seconds at 42°C without shaking. Immediately the tube was transferred to ice. It was then removed from ice and then 250 µl of SOC medium was added. The tube was capped tightly and was transferred on a shaker platform at 37°C for 30 min. Spreading was done of 50 µl from the transformation onto each of two pre-warmed selective plates (Luria Agar containing 100 µgml-1 ampicillin and 80 µg ml-1 X-gal) by using clean spreader for each plate and left the plates for overnight Incubation at 37°C.

4. Results

After the PCR amplification was successfully performed the DNA fragment were analyzed by agarose gel electrophoresis was visible with the help of Gel Red binding and gel documentation equipment where I can get to see the bands of 674bp which was placed on a UV-Light gel transilluminator in dark room of the laboratory and a digital image was taken to record the image.

5. Result Figure

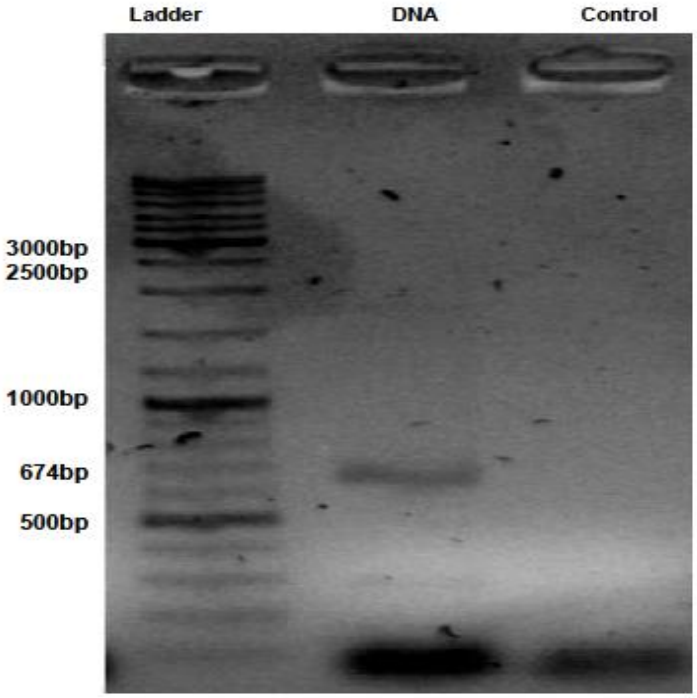

Fig:- DNA separation by Agarose Gel Electrophoresis

6. Discussion

As my experiment was successful and I got to see the result that I was expecting, after that I did the transformation method to grow the colonies and left it for overnight incubation into the incubator and on the next day those plates were put into the cold storage to avoid contamination and on the plates one white (lac-) and one blue(lac+) colony was appeared. Now for further processing both the colony will be picked and grown in time.

7. References

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