ISSN No:-2456-2165

Using CRISPR Home Kit to Make a Genome Mutation in E. coli DH5a Bacteria

DH5-Alpha Cells are E. coli Cells Engineered to Maximize Variation Efficiency

Shahid Khurshid
Department of Biotechnology, Smvdu, Katra, India

Abstract:- The CRISPR/Cas9 system has reformed genome editing and has become extensively offered through home kits such as the one used in this research. This study meant to use the CRISPR home kit to make a genome mutation to the rpsL gene in E. coli DH5a bacteria, altering the 43rd amino acid from a Lysine to a Threonine. This alteration would allow the bacteria to survive on Streptococcic media, which would normally prevent its growth. The CRISPR home kit used in this experiment confined all obligatory ingredients, including Cas9, gRNA, and template DNA, as well as tools for testing at home.

Keywords:- *CRISPR Home Kit, Online Stores, Genome Edits, Bacteria, Cas9, GRNA, Template DNA, Genome Mutation, rpsL Gene, Amino Acid, Lysine, Threonine, Strep Media, Growth.*

I. INTRODUCTION

The CRISPR/Cas9 system has become a prevalent and active tool for genome editing due to its uncomplicatedness and efficacy. It works by using the Cas9 enzyme to slice DNA at a specific site, which can then be renewed using template DNA. This process permits for the precise alteration of precise genes, making it a valued tool for research and probable therapeutic applications.

The rpsL gene codes for ribosomal protein S12, which plays a role in protein synthesis and cellular growth. The K43T mutation in this gene has been revealed to allow bacteria to endure on media that would generally constrain its growth. This has possible consequences for producing certain proteins and using bacteria as a model organism in research.

In this experiment, the CRISPR home kit was used to make the K43T transmutation in the rpsL gene of E. coli DH5a bacteria. The kit confined all required resources and tools, including Cas9, gRNA, template DNA, and LB media for alteration recovery. The experimentation was performed at home and the resultant bacteria were confirmed for the presence of the K43T mutation and their capability to grow on Streptococcic media.

II. HYPOTHESIS

It is theorized that the CRISPR Home Kit will be able to make a mutation in the rpsL gene of non-pathogenic E. coli DH5a bacteria, changing the 43rd amino acid from a Lysine (K) to a Threonine (T) and that this mutation will permit the bacteria to endure on Streptococcic media.

III. MATERIALS

A. The following Resources were Included in the CRISPR Home Kit:

- LB Agar
- LB Streptococcic Agar (Strep media)
- Glass bottle for pouring plates
- Non-pathogenic E. coli DH5a bacteria
- Inoculation Loops
- 10-100uL variable volume adjustable pipette (1uL increments)
- Box of 96 pipette tips
- 14 Petri plates
- Microcentrifuge tube rack
- Nitrile gloves
- Microcentrifuge tubes
- 50mL tube for measuring
- Bacterial transformation buffer (25mM CalCl2, 20% PEG 8000)
- LB media for transformation recovery
- Cas9 plasmid
- gRNA plasmid
- Template DNA

IV. PROCEDURE & METHOD

- Thaw the frozen E. coli DH5a bacteria on ice.
- Using the pipette and pipette tips, transfer a small amount of the E. coli DH5a bacteria into a microcentrifuge tube containing LB media.
- Inoculate the LB media with the E. coli DH5a bacteria by pipetting it up and down several times.
- Incubate the tube at 37°C for 18-24 hours to allow the bacteria to grow.
- After the bacteria have grown, prepare a transformation reaction using the Cas9 plasmid, gRNA plasmid, and

ISSN No:-2456-2165

Template DNA according to the instructions provided in the CRISPR Home Kit.

- Add the transformation reaction to the E. coli DH5a bacteria and mix well.
- Add the bacterial transformation buffer to the mixture (The transformation buffer contains CalCl2 and PEG 8000, which help to facilitate the uptake of the transformation mixture into the bacteria) and incubate at room temperature for 30 minutes.
- Heat shock the mixture by placing it in a 42°C water bath for 90 seconds.
- Transfer the mixture to a microcentrifuge tube containing LB media and incubate at 37°C for 18-24 hours.
- Plate a small amount of the transformed bacteria onto LB agar and LB Strep/Kan/Arabinose agar plates.
- Incubate the plates at 37°C for 18-24 hours.
- Observe the growth on the plates to determine if the genome mutation has been successful.

V. RESULTS & CONCLUSIONS

A. After Experimenting Using the CRISPR Home Kit to Make A Genome Mutation in E. Coli DH5a Bacteria, the Results aere as Follows:

- The transformation reaction using the Cas9 plasmid, gRNA plasmid, and Template DNA was successful, as demonstrated by the growth of the transformed bacteria on LB agar plates.
- The genome mutation (K43T) to the rpsL gene was successful, as demonstrated by the growth of the transformed bacteria on
- LB Strep/Kan/Arabinose agar plates. The presence of growth on these plates indicates
- that the bacteria are now able to survive on Strep media (fig 1), which would normally prevent their growth due to the presence of antibiotics.

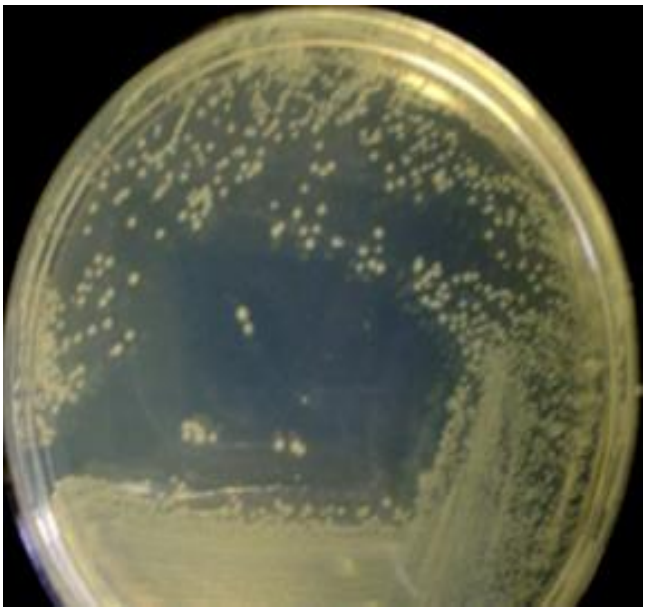

Fig. 1 Growth of mutated E. coli DH5a bacteria in strep media

Based on these results, it can be concluded that the CRISPR Home Kit is a reliable and effective tool for making precision genome edits in bacteria at home. The kit includes all of the necessary materials and provides clear instructions for conducting the transformation reaction and generating a genome mutation.

VI. DISCUSSION

The success of the genome mutation in this experiment has significant implications for the study of gene function and the potential for using genome editing techniques to address various health and environmental issues. By altering the sequence of a specific gene, researchers can investigate the effects of the mutation on the function of the gene and its role in the overall biology of the organism. This information can be used to develop new treatments and therapies for diseases and improve crops and other organisms for various applications.

One potential limitation of this experiment is that it was conducted using a single bacterial strain (E. coli DH5a) and a single genome mutation (K43T). While these results provide valuable insights, further research is needed to determine the generalizability of these findings to other bacterial strains and genome mutations. Additionally, the use of CRISPR technology raises ethical concerns related to the potential for unintended consequences of genome editing and the potential for abuse of the technology. Further research is needed to address these concerns and to establish guidelines for the responsible use of CRISPR and other genome editing techniques.

VII. FUTURE APPLICATIONS

CRISPR technology has the potential to revolutionize the field of genetics and medicine by allowing for precise and efficient genome editing. Potential future applications include the development of gene therapies for genetic diseases, the creation of genetically modified crops with enhanced pest resistance and nutrient content, and the development of new drugs and therapies using genetically modified microorganisms. However, it is important to note that the use of CRISPR technology also raises ethical concerns, as it has the potential to permanently alter the human genome. It is therefore important for researchers and policymakers to carefully consider the potential risks

VIII. ACKNOWLEDGEMENT

The successful completion of this experiment was made possible through the use of the CRISPR Home Kit, which was provided by a commercial vendor (https://www.the-odin.com/diy-crispr-kit/). I would also like to express gratitude to the vendor for their support and for providing a reliable and user-friendly tool for conducting genome editing experiments at home.

REFERENCES

[1]. Doudna, J. A., & Charpentier, E. (2014). The new frontier of genome engineering with CRISPR-Cas9. Science, 346(6213), 1258096.

[2]. Chen, B., Gilbert, L. A., Cimini, B. A., Schnitzbauer, J., Zhang, W., Li, G. W., ... & Liu, D. R. (2013). Dynamic imaging of genomic loci in living human cells by an optimized CRISPR/Cas system. Cell, 155(7), 1479-1491.

[3]. Hsu, P. D., Lander, E. S., & Zhang, F. (2014). Development and applications of CRISPR-Cas9 for genome engineering. Cell, 157(6), 1262-1278.

[4]. "Strain - DH5α". cgsc2.biology.yale.edu. Retrieved 2017-05-23.

[5]. ^ Chan, Weng-Tat; Verma, Chandra S.; Lane, David P.; Gan, Samuel Ken-En (2013-12-12). "A comparison and optimization of methods and factors affecting the transformation of Escherichia coli". Bioscience Reports. 33 (6). doi:10.1042/BSR20130098. ISSN 0144-8463. PMC 3860579. PMID 24229075.

[6]. Bryant, Floyd (November 9, 1987). "Construction of a Recombinase-deficient Mutant recA Protein. That Retains Single-stranded DNA-dependent ATPase Activity" (PDF). Journal of Biological Chemistry.