REPORT
WINTER INTERNSHIP

(1 December 2022-31 December 2022)

UNDER: DR. KUNDAN KUMAR, DEPARTMENT OF BIOLOGICAL SCIENCES, BITS PILANI, K.K. BIRLA GOA CAMPUS

SUBMITTED BY:
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CERTIFICATE OF COMPLETION

THIS CERTIFICATE IS PROUDLY PRESENTED TO

AAYUSHI JAIN

For having actively completed one month internship at BITS Pilani, KK Birla Goa Campus from 1-12-2022 to 31-12-2022

20/1/23

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INFORMATION ABOUT THE INTERNSHIP

INTRODUCTION: Cloning and expression of WNK proteins play a vital role in abiotic stress response in *Oryza sativa* (Rice). WNK proteins are lysine deficient protein kinases and are serine-threonine protein kinases. Rice is susceptible to various abiotic stresses like heat, cold, drought, osmotic , salinity, and heavy metal toxicity. WNK proteins are known to respond differentially under different abiotic stress conditions and are thus believed to provide tolerance which is still being studied during this project.

METHODOLOGY: 1. Glycerol stock preparation:
50% of glycerol solution in autoclaved distilled water + 50% overnight grown culture (*E. coli*) , then stored at -80 degrees.

2. Plasmid Isolation:
Plasmid was isolated from *E. coli* DH5 alpha strain using commercially available plasmid isolation kit “Nucleospin” Protocol mentioned in the manual was followed.

3. Restriction Digestion:

Both vector Pet28A(T) and gene was restriction digested using restriction enzymes BamH1 and HindIII and HindIII and XHO1 for given genes 1 and 6 respectively. Protocol has been followed from promega.

4. Colony PCR:

Added a small quantitity (colony) from bacterial plate to the PCR master mix and proceeded to thermocycling.

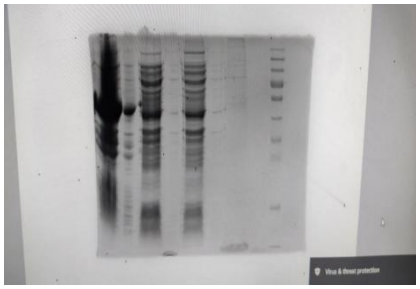
Gel image after colony pcr

5. Ligation:

Vector and insert were ligated in 1:1 molar ratio at 16 degrees for 16 hours incubation. Appropriate ligation buffer was used and T4 DNA ligase was used.

6. Transformation:

Competent *E. coli* cells were thawed , ligation mixture was added and again thawed on ice then heat shock was given at 42 degrees, again placed on ice then after addition of mrdia , kept for incubation at 37 degrees for 2 hours. Later, spread plated on LB agar+ kanamycin to check transformation.



Gel image after transformation

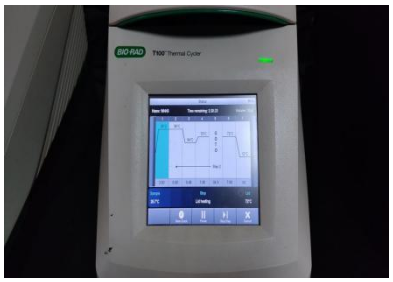
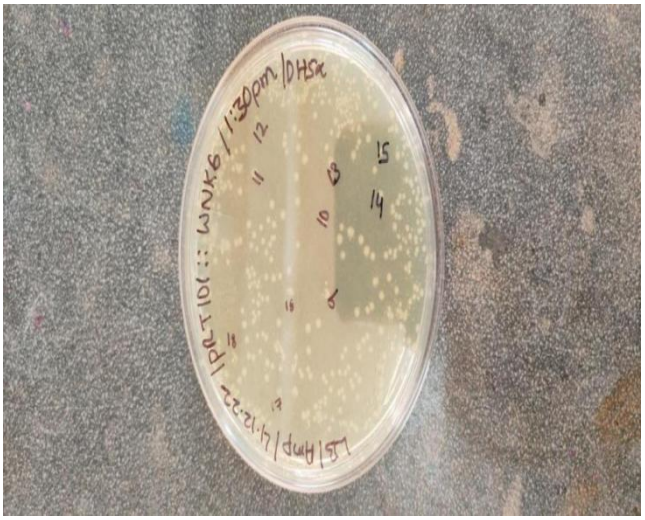


Image of thermal cycler performing colony pcr



LB agr plate showing transformed *E. coli* colonies with WNK6 protein (PRT101 vector)

7. Gel elution:

This was performed using commerically available kit.

8. Preparation for SDS PAGE:

Running buffer, Acylamide solution, Resolving Gel, Stacking Gel , Loading Dye, Staining and Destaining solution were prepared.

9. Performing SDS PAGE:

SDS PAGE was performed and gel was visualised under UV transilluminator for results.

10. Other techniques: A)Nanodrop Spectrophotometer - to check DNA and protein concentration

B) Sonication- to prepare lysed protein product

ACKNOWLEDGEMENT

- I would like to thank Department of Biotechnology, Government of India for giving me this opportunity to carry out internship at one of the premier institutes of India, BITS.

- I would like to thank my faculty members Prof. Savita Kerkar Maʾam (Dean, SBSB) , Prof. Sanjeev C. Ghadi Sir (Vice Dean, SBSB; program coordinator, DBT supported Marine Biotechnology), Dr. Samantha Fernandes Dʾmello maʾam, Dr. Meghanath Prabhu sir and Dr. Dhermendra K. Tiwari sir for their constant support and guidance.

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- I also thank Mr. Yogesh Negi Sir (Research fellow, plant biotechnology) for explaining both theory and practical work in detail with patience.

ABOUT BITS

BITS Pilani K.K Birla Goa Campus is a private deemed university campus located in Goa, India , established in 2004.

It is one of the four BITS campuses and is one of the finest institutes in India.

BITS Pilani works with and provides consultancy to Government and as well as industry through its many R and D centres.

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Results and Conclusion:

- From my internship at BITS, I gained hands-on-experience in molecular biology, microbiology and genetic engineering techniques.
- As I was assisting the pHD scholars in the lab, I gained practical exposure to various instruments used in research laboratories.
- I got direct understanding of what being in a research field is.
- After completing my internship, I feel very lucky and motivated to pursue research in future.
- Overall experience of the internship was positive and I am sure I'll be able to use the skills I learnt at BITS to practice while doing my dissertation work and in further research.

References:

- **Green**, Michael R. (Michael Richard), 1954-. **Molecular cloning** : a laboratory manual / Michael R. **Green**,. Joseph **Sambrook**. – 4th ed.

- Machery- Nagel NucleoSpin plasmid quikpure

- Brown, T. A. (2006). Genomes (3rd ed.). New York: Garland Science Pub

- https://www.chem.ufl.edu/wp-content/uploads/sites/17/2014/05/SDS-PAGE-Stain-Protocol.pdf