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Hide & Cru-Seq: Investigating Potential Crucivirus Host With Fluorescently Labeled Protein

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Portland State UNIVERSITY

Introduction

What are Cruciviruses?

Cruciviruses are DNA viruses that contain a capsid protein that shares striking similarities to capsid proteins from RNA viruses. Formerly known as “RNA-DNA hybrid viruses”, this novel type of viruses suggest gene exchange between unrelated RNA and DNA viruses. However, the hosts of cruciviruses remain unknown.

What are the steps toward host/virus elucidation?

Using cruciviruses found in soil samples on the PSU campus, along with crucivirus genomes found in the protist *Carpediemonas membranifera*, fluorescence microscopy will be used to determine the binding of these crucivirus capsid P-domains to Carpediemonas and PSU soil microorganisms in order to identify potential crucivirus host organisms. The capsid proteins of PSU crucivirus genomes node-34 and node 209, and the well-characterized tomato bushy stunt virus (TBSV), related to cruciviruses, were fused to the green fluorescent protein gene using molecular cloning techniques, expressed and purified recombinantly in *E.coli*.

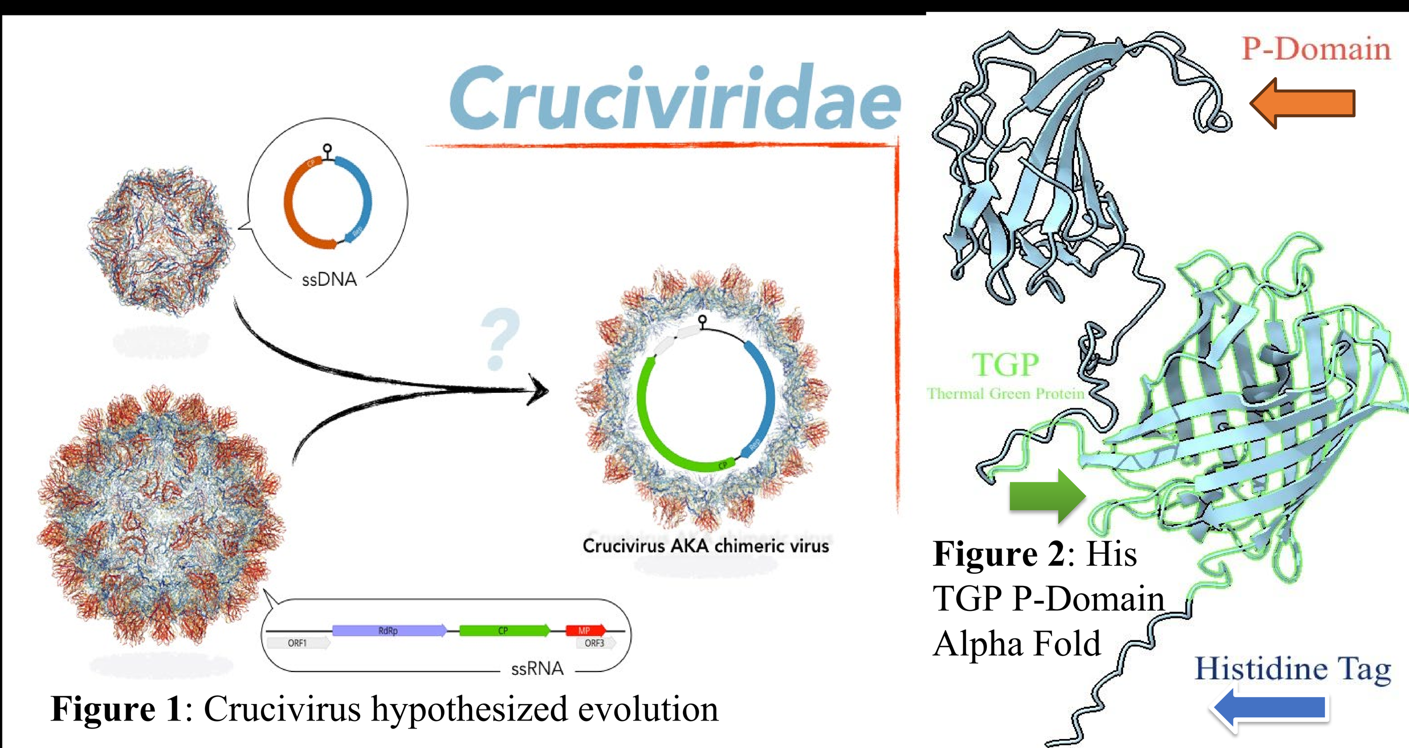


Figure 1: Crucivirus hypothesized evolution

Figure 2: His TGP P-Domain Alpha Fold

Objectives

1) Clone and sequence Crucivirus and TBSV His-TGP P-Domain plasmids using Gibson Assembly.
2) Produce fluorescently tagged P-domain recognition proteins for Crucivirus, TBSV, & *Carpediemonoas membranifera*.
3) Test if specific or not specific binding occurs with Crucivirus, TBSV, or *Carpediemonoas membranifera* and an environmental sample.

Results

Step 1: Cloning Plasmid Transformation in Bacteria

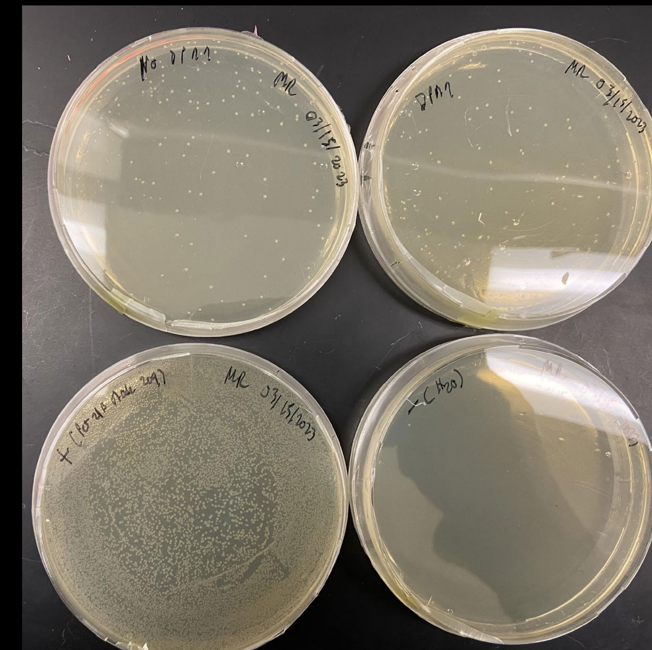


Figure 6: Results of Gibson cloning and transformation of His-TGP P-domain plasmid DNA into *E.coli* cells.

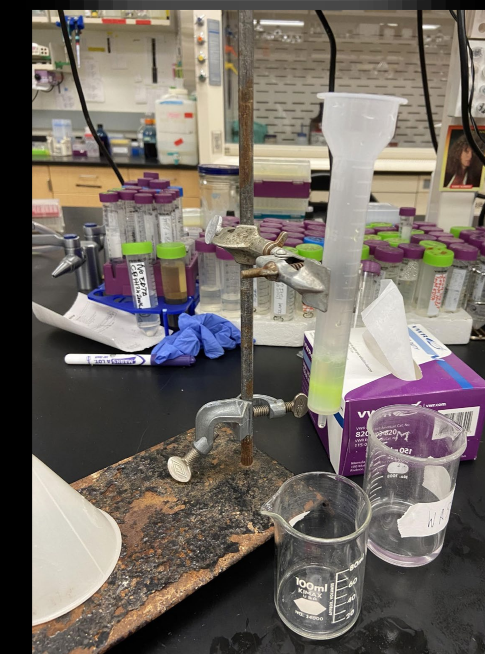


Figure 7: Nickel affinity chromatography to purify proteins via histidine tag.

Step 2: Protein Expression & Chromatography

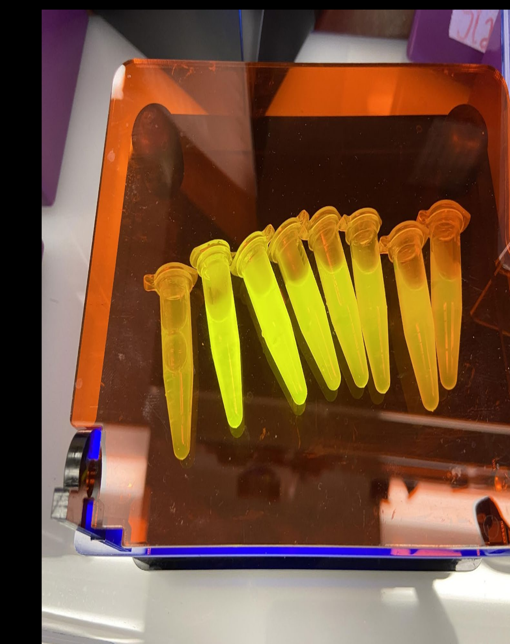
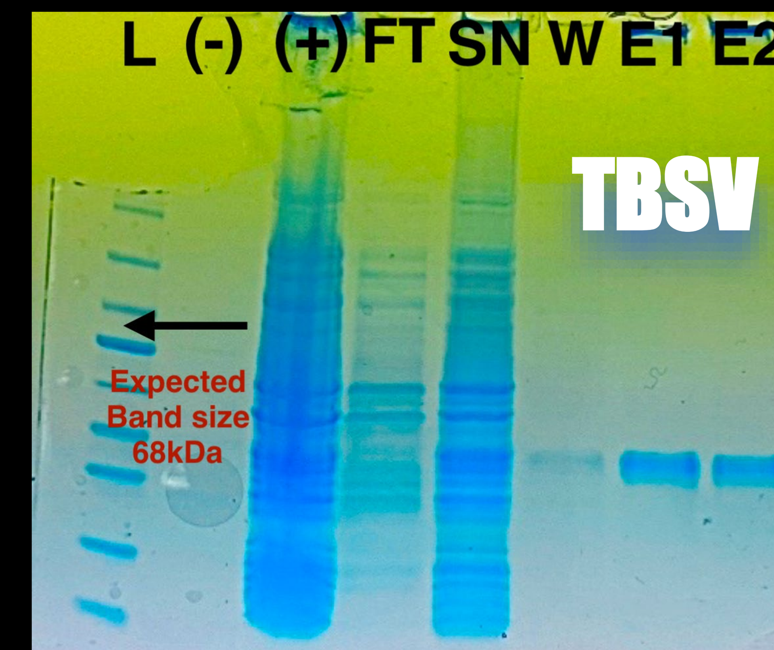
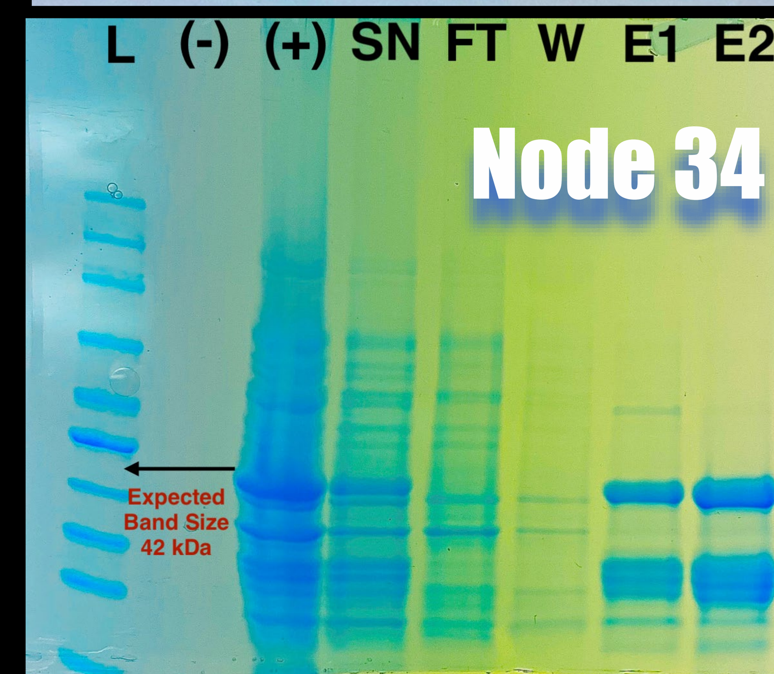


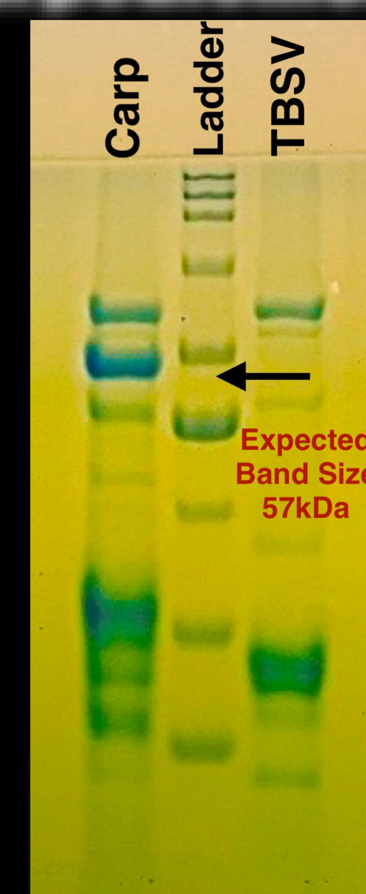
Figure 8: Fluorescence post Ni-affinity chromatography

Step 2 : Protein Analysis Via SDS-Page

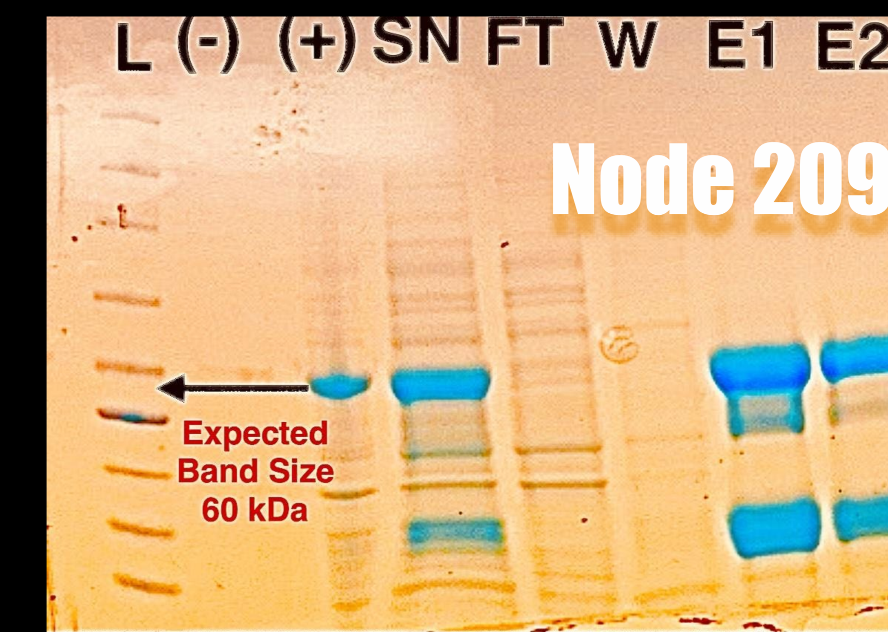




Protein gel key
L = Ladder
(-) & (+) = IPTG
SN= Supernatant
FT= Flow Through
W = Wash
P = Pellet
E = Elution



Figures 9-12: TBSV, Node 34, Node 209, and *carpediemonas* protein sizes and their expected band sizes post expression & chromatography

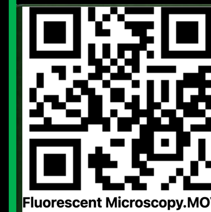


Conclusion

- Successful cloning and expression of Crucivirus Node 34 & 209 P-domain recognition proteins.
- Fluorescent proteins ready to be tested with environmental samples for host identification.

Future Aims

- Objective 3: Continue host elucidation with the assistance of fluorescent microscopy & environmental samples.
- Optimizing protein expression and purification methods for *carpediemonas membranifera* & TBSV.



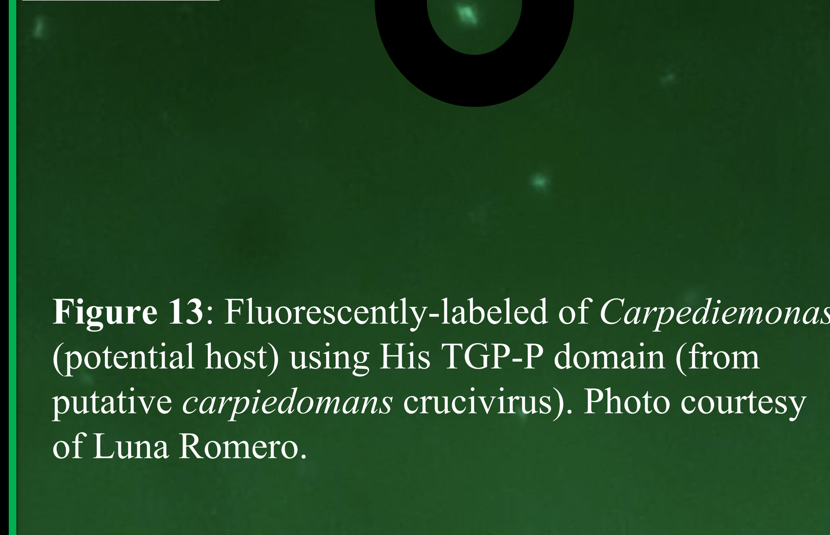


Figure 13: Fluorescently-labeled of *Carpediemonas* (potential host) using His TGP-P domain (from putative *carpiedomans* crucivirus). Photo courtesy of Luna Romero.

References

BioEssays . (2012, August 7). *Recombination between RNA viruses and plasmids might have played a central role in the origin and evolution of small DNA viruses Wiley* . Wiley Online Library . https://onlinelibrary.wiley.com/doi/full/10.1002/bies.201200083

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Jumper, J. et al. (2021, July 15). *Highly accurate protein structure prediction with alphafold*. Nature News. https://www.nature.com/articles/s41586-021-03819-2

Stedman , K. (2018). “*Cruciviridae projected evolution*” photograph, Portland Oregon, United States.

Figures 3: *Carpediemonas membranifera*

Methods

Step 1: Cloning, Transformation, & Sequencing

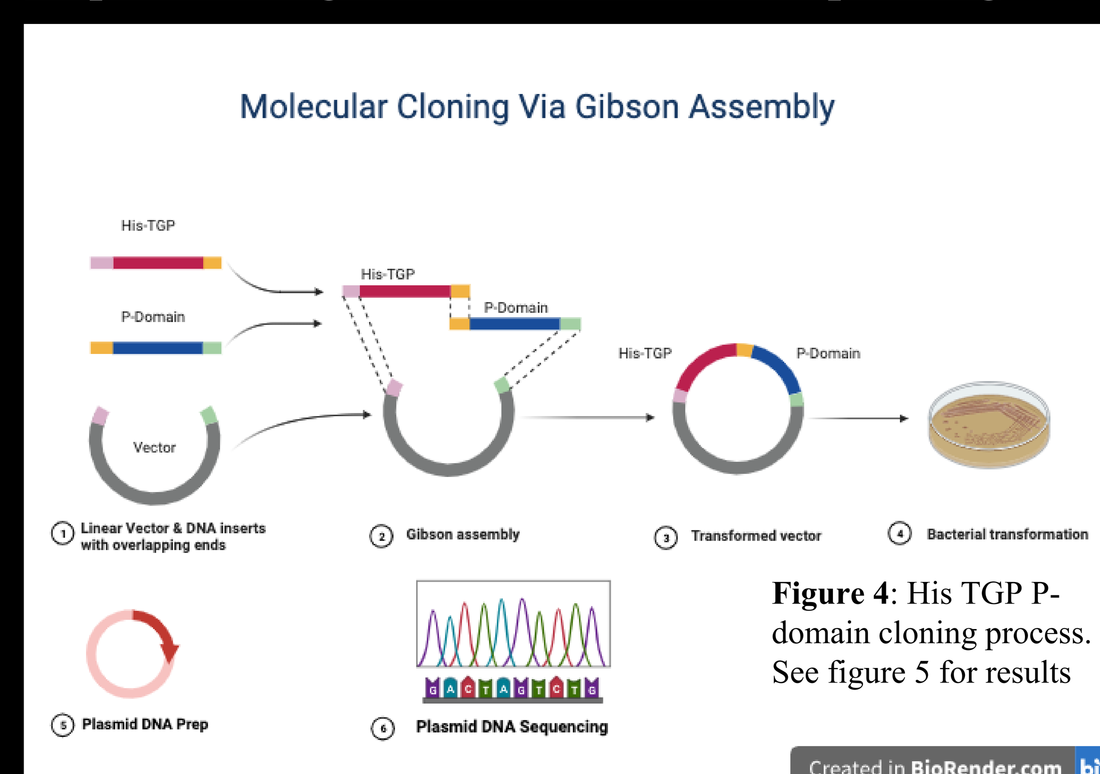

Figure 4: His TGP P-domain cloning process. See figure 5 for results

Step 2: Transformation & Expression

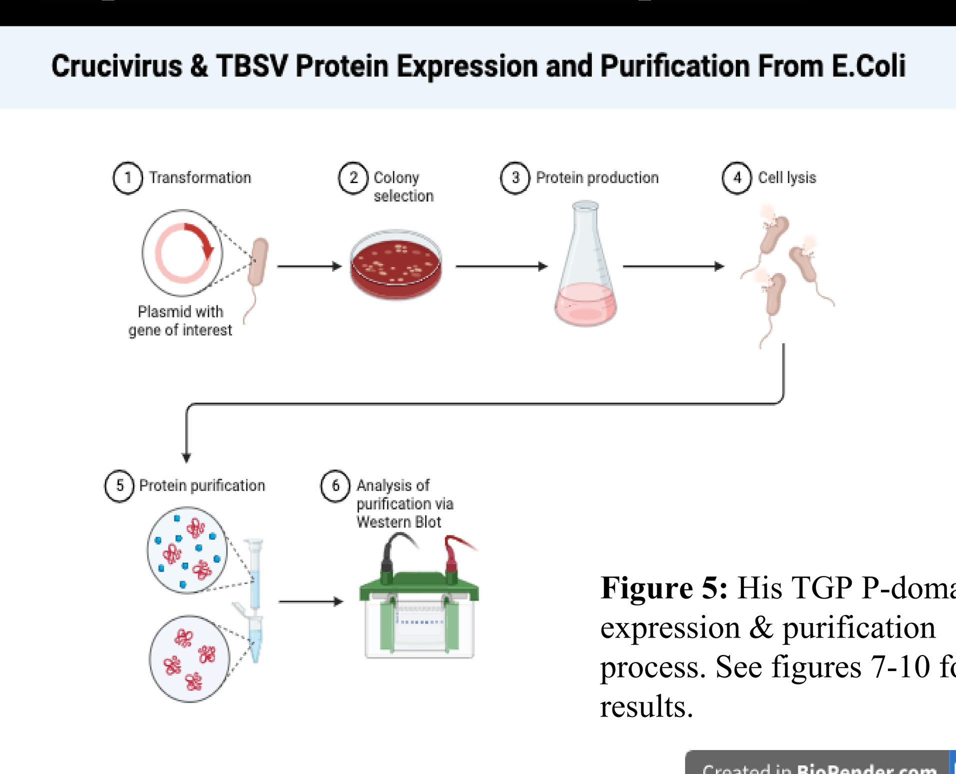

Figure 5: His TGP P-domain expression & purification process. See figures 7-10 for results.

Acknowledgements

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The XVL lab. Pictured left to right: Marcell Richard, Tyrone, Luna Romero Jenni Tran, Jane Arterberry, Lou-Ann O’Conner, Marisa Gonzalez, Miguel Dr. Ken Stedman, Helena Stedman, Dr. Nacho De La Higuera, Jono Abshier, and David Noeckel.

Figure 14: XVL Lab at Bumpass Hell Sampling site, Mineral California.

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