Optimizing Viral Detection: Creating Fluorescent Chikungunya Virus Infectious Clones

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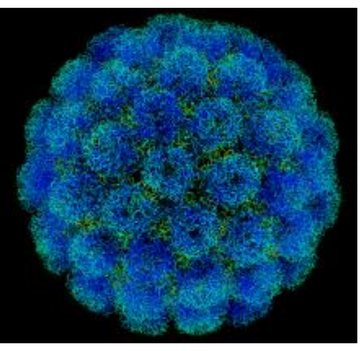

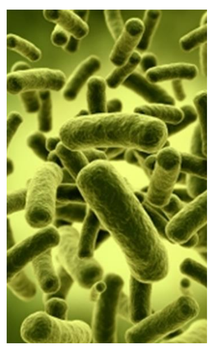

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Abstract

Human infection with Chikungunya virus (CHIKV) results in debilitating joint pain and arthritis that can persist for months or even years. CHIKV is a re-emerging virus that causes large epidemics in Asia, Africa, Europe, and most recently south and central America. Yet, Chikungunya has no specific treatment or vaccine. Therefore, steps towards eradicating the virus are important to public safety. Tools to facilitate drug screens are especially important when searching for viable treatments. Therefore, our lab sought to create reporter CHIKV encoding fluorescent markers, to detect infection in cells. Cloning was used to develop a CHIKV infectious clone, encoding GFP. Creation of this tool required use of PCR, Gibson assembly, and the CRISPR Cas9 system. Fluorescent viral strain cuts down on time and cost spent using other methods to find viable treatments. We are currently repeating this process to develop CHIKV-mCherry. Further directions for research include determining the efficiency of the reporter viruses compared to wildtype virus, and using these viruses in drug screen studies. Overall, progress has been made in responding to the outbreak of CHIKV.

Introduction

- Chikungunya virus (CHIKV) is a positive sense RNA alphavirus
- Spread by mosquitoes
- Considered a class C priority virus for national security risk
- More than 1.7 million suspected cases in the Americas since 2013
- Drug screens with antibody stains are time consuming, costly, lead to cell loss
- Tagged viral protein nsP3 with GFP allows for direct method of detecting virus

Figure 1. Map of American Countries with Reported CHIKV Infections

Methods

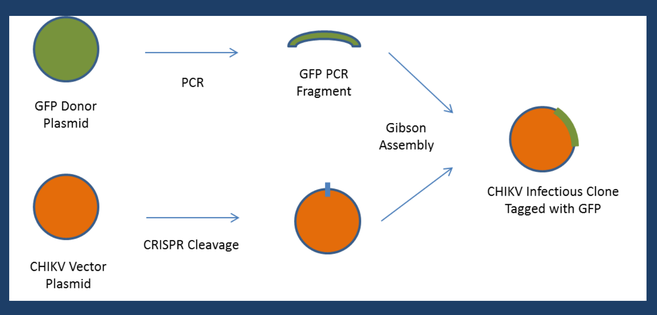

Figure 2. Simplified Schematic for Cloning Strategy

- GFP was PCR-amplified from a donor plasmid
- Designed and synthesized target guide RNA
- Cas9 proteins were complexed with guide RNA, and used to cut the CHIKV vector
- Gibson Assembly joined the GFP fragment with the target vector backbone
- The CHIKV-GFP infectious clone was transformed into *E. coli*
- Plasmid DNA was isolated from the bacterial genome
- Restriction digest check confirmed CHIKV-GFP infectious clone
- Sent for sequencing.
- RNA was transcribed from CHIKV-GFP and transfected into cells.

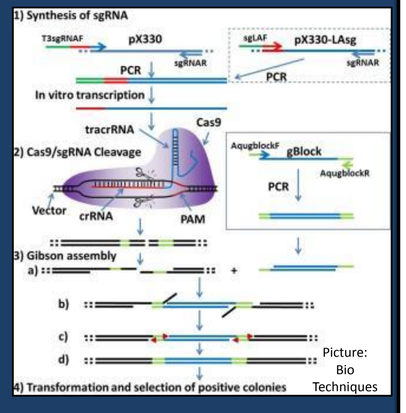

Figure 3. Outline of CRISPR Cas9 Cleavage and Gibson Assembly

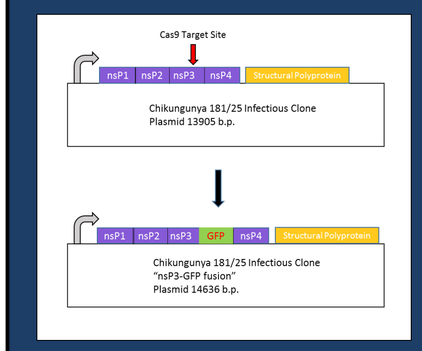

Figure 4. Plasmid Map of CHIKV-GFP

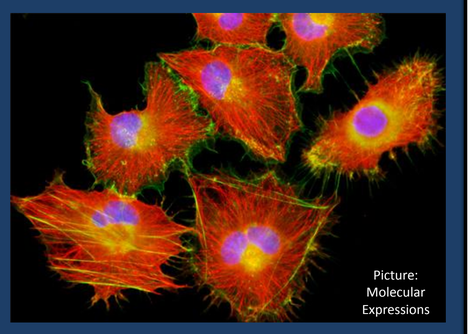

Figure 5. BHK-21 cells are used for transfection and viral production

Results

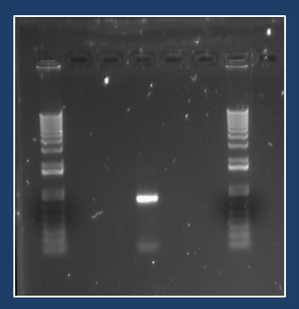

Figure 6. Representative DNA gel
DNA gels were used to verify amplification of PCR product, successful CRISPR cleavage, and restriction digests.

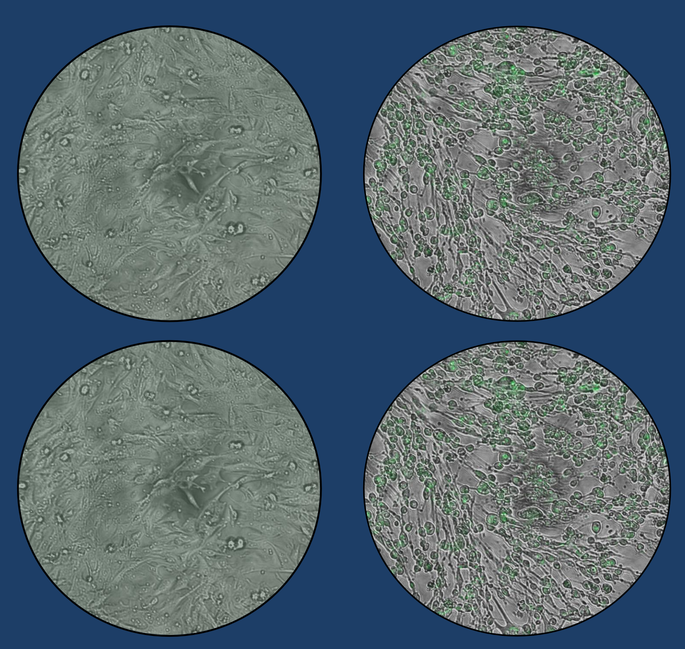

Figure 7. Left: Uninfected cells. Healthy, no green fluorescence with UV light
Right: Infected cells. Dying, green fluorescence with UV light

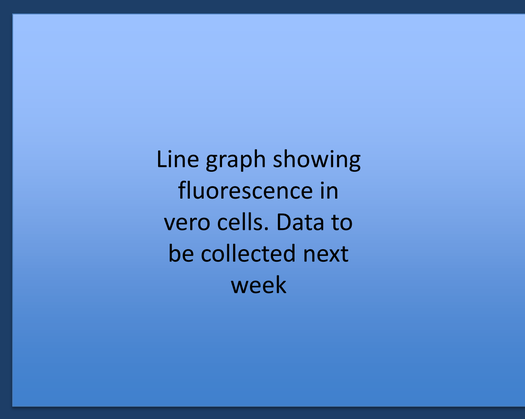

Figure 8. Level GFP fluorescence of virus after at 16, 24, 40 , and 48 hours.

Conclusions and Further Directions

- CRISPR based cloning is successful
- Generated stable CHIKV-GFP virus clone
- Virus is lethal to cells and fluoresces within 24 hours
- Comparison tests between CHIKV with GFP and the wild type virus. Can it be compared to wild type virus and infection?
- Additional infectious clones including CHIKV-mCherry
- Application to ongoing studies and immediate use in drug screens

References

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Acknowledgements

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