Knockout of the US29 gene of Human Cytomegalovirus using BAC Recombineering

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Abstract

The purpose of our research is to determine the function of the US29 gene in Human Cytomegalovirus (HCMV) by knocking it out using Bacterial Artificial Chromosome (BAC) technology. We began with a BAC that contained the entire HCMV genome as well as the chloramphenicol (Cam^r) antibiotic resistance gene (ADCRE) in DH10β *Escherichia coli (E.coli)*. We isolated the ADCRE from the DH10β cells and then electroporated it into the recombineering *E.coli* strain SW102 GalK$^-$ containing an excision defective λ prophage. Following electroporation, 800 Cam^r colonies were obtained, of these, 1 out of 21 screened had the entire viral genome based on a DNA fingerprint match between the SW102 and DH10β strains. The rest had large deletions in the HCMV sequence. A galactokinase positive, kanamycin resistant (GalK$^+$/Kan^r) cassette was amplified to contain flanking US29 regions using the polymerase chain reaction (PCR). This DNA was electroporated into the SW102 with the intact ADCRE to replace part of the US29 sequence with *galK*/Kan^r genes through homologous recombination (allelic exchange). Sixteen Cam^r/GalK$^+$/Kan^r colonies were obtained, indicating exchange of 956 base pairs in US29 with 2.3kb *galK*/Kan^r.

Introduction

HCMV is a ubiquitous human pathogen that by age forty, 90% of us will harbor in a latent form in our body. It remains hidden until immunosuppressive events cause it to be reactivated and spread throughout the body to cause a multitude of problems including pneumonia, retinitis, and multi-organ infections. Conditions such as AIDS, organ transplantation, or cancer almost always lead to reactivation and infection. How it remains hidden in the body and later reactivates to cause such widespread infection remains a mystery. Furthermore, it is one of the most complicated viruses in terms of its genetic makeup. The function of an HCMV gene designated **US29** is unknown. Its amino acid sequence shows no significant resemblance to any known proteins in the databanks, although a number of possibilities including immune response receptors have been postulated[1]. We do not know how important this gene is for infection and growth of the virus. In order to test a number of hypotheses, we plan to 'knockout' this gene by replacing it with the ***galK*/Kan^r reporter genes** using Bacterial Artificial Chromosome (BAC) recombineering technology. Once the gene is no longer expressed during infection, we can determine if it is essential for a productive infection or what host immune functions might be disrupted by comparing knocked-out and wild type versions of the virus.

In order to 'knockout' US29, BAC recombineering techniques were employed using BACs which are based on the F plasmid in *E. coli*[2]. They contain *Par* genes which limit the plasmid to 1-2 copies per cell, an origin of replication (ORI), and a Cam^r gene while all conjugative genes are removed. **The entire HCMV genome is available as a circular BAC plasmid DNA of 230KB (ADCRE)[3] (Figure 1)**.

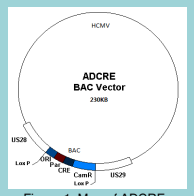
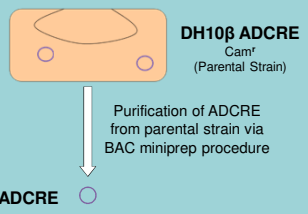


Figure 1. Map of ADCRE.

Materials, Methods and Results



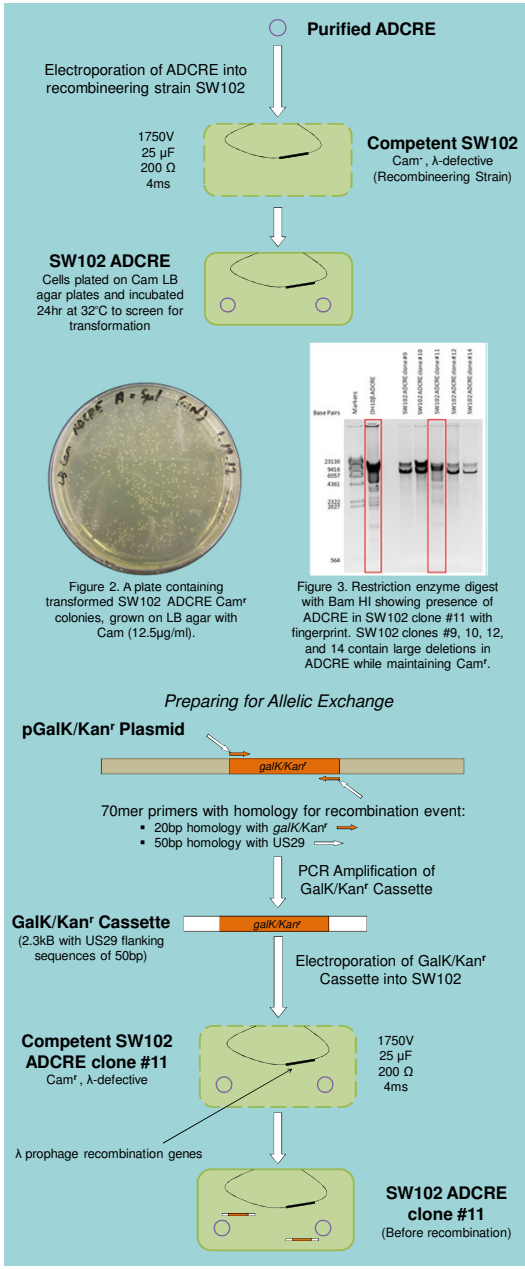
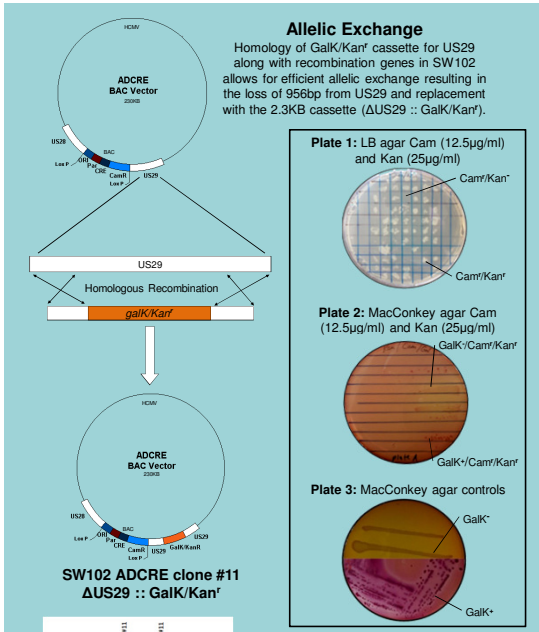


Figure 2. A plate containing transformed SW102 ADCRE Cam^r colonies, grown on LB agar with Cam (12.5µg/ml).

Figure 3. Restriction enzyme digest with Bam HI showing presence of ADCRE in SW102 clone #11 with fingerprint. SW102 clones #9, 10, 12, and 14 contain large deletions in ADCRE while maintaining Cam^r.



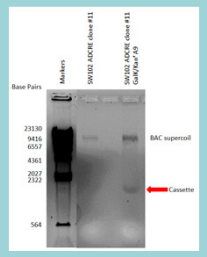


Figure 4. Gel of PCR product SW102 ADCRE clone #11 GalK/Kan^r A9 showing probable amplification of the GalK/Kan^r cassette indicating allelic exchange.

Figure 5. Cells that underwent recombination were grown on **Plate 1**, indicating the presence of the cassette. Cam^r/Kan^r colonies were transferred to **Plate 2** to further indicate allelic exchange of the full cassette. SW102 ADCRE clone #11 GalK/Kan^r A9 was isolated for PCR to show the presence of the cassette in US29. **Plate 3** shows growth of controls for GalK on MacConkey.

	DH10β ADCRE	SW102	SW102 ADCRE clone #11	SW102 ADCRE GalK/Kan^r A9	SW102 ADCRE clone #11 GalK/Kan^r C12	SW102 ADCRE clone #11 GalK/Kan^r C19
Cam^r	+	-	+	+	+	+
Kan^r	-	-	-	+	+	+
GalK$^-$	-	-	-	+	+	+

Table 1. Phenotypic traits of isolates.

Summary and Conclusions

- Transferred ADCRE BAC from DH10β to SW102 and obtained over 800 Cam^r colonies.
- Used PCR to amplify *galK*/Kan^r cassette from plasmid pGalK/Kan^r.
- Transfection of SW102 ADCRE clone #11 with *galK*/Kan^r cassette resulted in possible allelic exchange of US29 producing 16 GalK$^+$/Kan^r/Cam^r isolates.
- PCR of SW102 ADCRE clone #11GalK$^+$/Kan^r A9 isolate showing probable amplification of cassette within US29.

Next Steps

- Southern Blot probing for *galK/Kan^r* genes in SW102 ADCRE clone #11 GalK/Kan^r A9 to confirm recombination.
- Infect HFF cells with wild-type and ΔUS29 ADCRE to compare infection and growth of the virus to determine gene function.

References

1. Zuzek, K. University of Wisconsin-Eau Claire Master Thesis. 1999.
2. Yu, D., et al. *Journal of Virology*. 76: 5, 2002.
3. Warming, S., et al. *Nucleic Acids Research*. 33: 4, 2005.

Funding provided by Faculty Student Research Collaboration ORSP Grants.