Determining KSHV Copy Number Using Quantitative PCR (qPCR) with ERV-3 Sequence Embedded in Genomic DNA as a Reference

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Kaposi sarcoma-associated herpesvirus (KSHV) is an oncogenic herpesvirus and causative agent of Kaposi's sarcoma, primary effusion lymphoma, and Multicentric Castleman disease (Figure 1A)[1]. KSHV displays two phases of infection cycle. During latency, the viral DNA persist as an episome attached to the host chromatin and displays limited expression of viral genes. During the lytic phase, the full repertoire of genes is expressed, and viral progeny are produced. Latent viral replication of herpesviruses differs from lytic viral replication in at least three distinct ways: (1) latent replication initiates at ori-P and proceeds bidirectionally, while lytic replication originates from a distinct origin, referred to as ori-Lyt, and proceeds through a rolling circle mechanism; (2) latent viral replication depends on host cellular DNA polymerase and accessory factors, while viral lytic replication utilizes its own DNA polymerase and other factors; and (3) latent DNA replication occurs in synchrony with host DNA replication, maintaining a stable number of viral episomal DNA in each cell, while lytic replication amplifies DNA 100 to 1,000 fold. The goal of my project is to set up a real-time quantitative PCR (qPCR) assay aimed at measuring the KSHV episomal DNA load in naturally infected and recombinant cell lines.

The model system is KSHV positive body cavity-based lymphoma cells (BCBL-1), which allows the controlled chemical manipulation of viral infectivity cycle [2]. The cells are maintained in RPMI-1640 medium (ThermoFisher, 11875085) supplemented with 10% fetal bovine serum (FBS), 1% Penicillin/Streptomycin, 1% L-Glutamine at 37 °C in 5% CO2. The treatment with histone deacetylase inhibitor, sodium butyrate, stimulates the viral latent-to-lytic switch by uncoiling of DNA leading to transcriptional activation of viral lytic genes. Carefully timed collection of cells post sodium butyrate induction, allows examining the KSHV episomal DNA copy number at immediate early (8 h post-induction, pi), early (24 h pi), and late (48-72 h pi) stages of lytic, as well as before the treatment, during latency (0 h pi).

Two reference plasmids encoding K6 and ERV-3 genes were used to create standard curves that allow quantifying the viral episome and genomic DNA copy number, respectively. The K6 gene codes for viral macrophage inflammatory protein 1 and is present in each viral episome at a single copy. The ERV-3 is endogenous retrovirus that integrated into the human genome 40-100 million years ago and is also at a genomic copy number of one per human cell [3]. Both plasmids were first cloned into Escherichia coli DH5 alpha bacterial cells, purified with the Qiagen plasmid Midi kit, quantified, and used to create serial dilutions that are directed to qPCR analysis. Specific primers were used to amplify both products (Table 1).

Two standard curves of the cycle threshold values (Ct) plotted against the logarithm of the copy number were used to deduce the total episomal and genomic DNA copy number in each experiment (Figure 2). Having been established, this assay will be used in future work to (1) estimate the viral DNA copy number at different time-points of KSHV infectivity cycle in BCBL-1 as well as other patient-derived primary effusion lymphoma cell lines, and (2) address how specific mutations introduced within KSHV locus encoding important long noncoding RNA, i.e., polyadenylated nuclear (PAN) RNA, affect viral replication. The work is significant in that reproducible, sensitive, and specific quantitative techniques are needed to assess various hypotheses regarding the viral load and its correlation with different experimental conditions and clinical samples.

Statement of Research Advisor

Jake Tatum has established two standard curves for K6 and ERV-3 genes that are essential for estimating the KSHV episomal DNA copy number in BCBL-1 and patient-derived primary effusion lymphoma

cell lines. This assay will be also applied for studying specific mutations within KSHV locus encoding polyadenylated nuclear (PAN) RNA, a key regulator of KSHV infectivity cycle. His work largely contributes to the understanding of KSHV molecular biology and pathogenicity.

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References

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[3] Chiu Chin Yuan, Wendell Miley, David Waters A quantification of human cells using an ERV-3 real time PCR assay. *Journal of Virological Methods* 91, 2001,109–117.

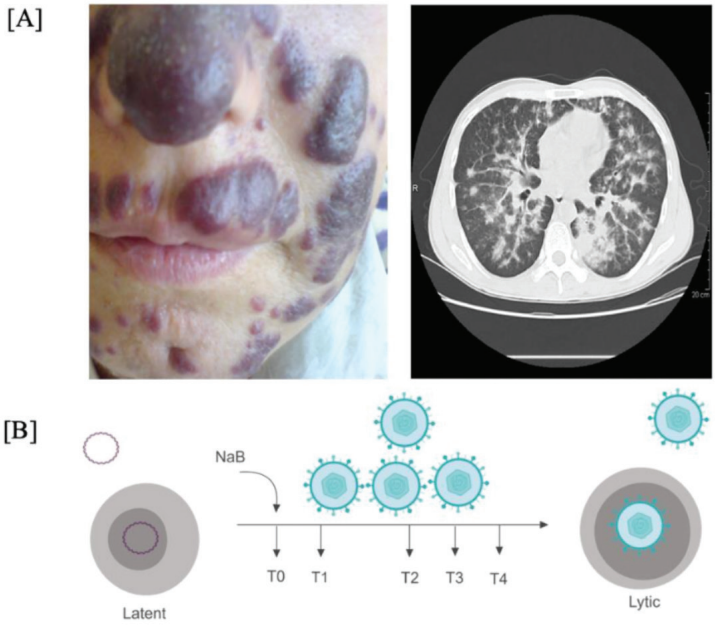

Figure 1. [A] Symptoms of Kaposi's sarcoma related to KSHV infection. [B] Experimental set-up used for manipulating KSHV latent (T0) and immediate early (T1), early (T2) and late lytic (T3-T4) lytic stages of KSHV replication.

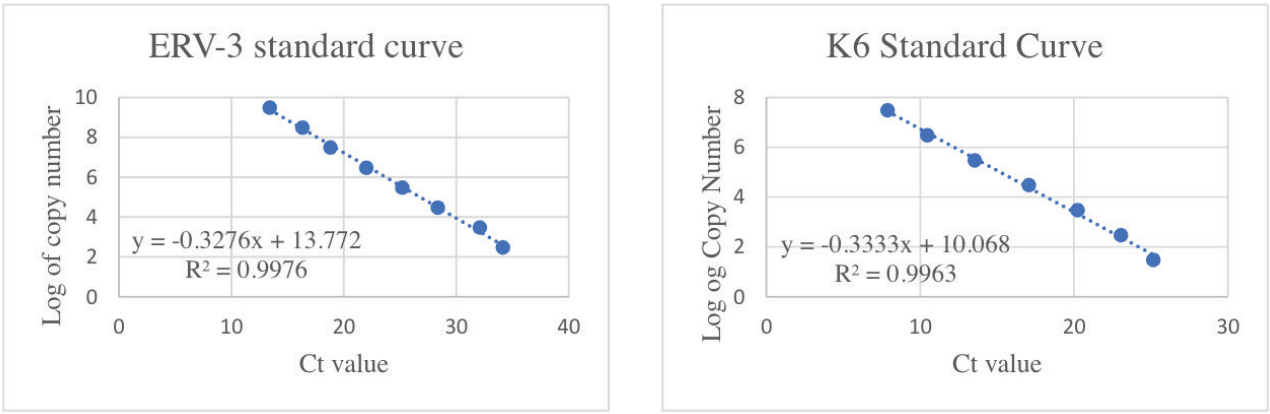

Figure 2: Two standard curves established with control plasmids ERV-3 and K6.

Name	5’ -3’ sequence	Purpose
K6_Forward	CGC CTA ATA GCT GCT GCT ACG G	Amplification of K6 specific viral locus
K6_Reverse	TGC ATC AGC TGC CTA ACC CAG	
ERV3_Forward	CAT GGG AAG CAA GGG AAC TAA TG	Amplification of ERV-3 genomic product
ERV3_Reverse	CCC AGC GAG CAA TAC AGA ATT T	

Table 1. Oligonucleotides used in the following study.