Published in final edited form as:

J Clin Virol. 2007 September ; 40(1): 19–25.

Evaluation of Global Clustering Patterns and Strain Variation over an Extended ORF26 Gene Locus from Kaposi's Sarcoma Herpesvirus

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Abstract

Background—Small 233-bp or 330-bp DNA fragments of the ORF26 gene of human Kaposi's sarcoma herpesvirus (KSHV) have been used extensively to identify KSHV by PCR in clinical samples; to associate KSHV with novel diseases; and to correlate KSHV strain differences with pathogenicity.

Objectives—We evaluated the nature, extent and source of nucleotide sequence variability among a large and diverse set of known KSHV-positive DNA samples.

Study Design—Direct DNA PCR sequencing was carried out on 136 distinct Kaposi's sarcoma and primary effusion lymphoma-related samples from different geographic locations.

Results—The presence of 26 diagnostic nucleotide polymorphisms across an expanded 965-bp PCR locus define eight distinct ORF26E genotypes, three being of Eurasian origin, one from the Pacific Rim, and five from Sub-Saharan Africa. Previous ambiguities between some genotype patterns in the 330-bp locus data are fully resolved.

Conclusions[U1]—This analysis provides an expanded database for understanding and evaluating ORF26 polymorphisms. In particular, the eight genotype clusters correlated with specific ethnic and geographic origins of the patients. Furthermore, the very low level of additional sporadic nucleotide variation found permits detection of spurious sequence errors or contamination present in some published data.

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Keywords

Subtype discrimination; Direct PCR sequencing; Multiple KSHV genotypes

INTRODUCTION

The most common DNA sequence used for detection of Kaposi's sarcoma herpes virus (KSHV) in clinical samples by PCR is the small ORF26 locus described in 1994 by Chang et al (Chang, 1994). ORF26 PCR gives positive results with most samples that come from the acknowledged KSHV-associated disease conditions (Moore and Chang, 1998), including all forms of Kaposi's sarcoma (KS) plus a subset of cases of both Primary Effusion Lymphoma (PEL) (Cesarman, 1995) and Multicentric Castleman's Disease (MCD) (Soulier, 1995). It was also used to help determine the very limited distribution of KSHV in most human populations, as well as in other lymphoma and leukemia clinical samples (Moore and Chang, 1995; Huang, 1995; Marchioli, 1996; Whitby, 1995; Su, 1995). However, ORF26 PCR data may have been used erroneously to support some controversial studies implicating KSHV in several other diseases as reviewed in Zong et al (2007).

All previous DNA sequence analysis with the ORF26 locus included data encompassing blocks of either 330-bp or a nested sub-segment of only 233-bp. This region contains several polymorphisms that cluster into a very limited number of distinctive patterns (referred to as subtypes or genotypes). Therefore, the data has potential to distinguish between samples on the basis of both their geographic and epidemiologic origin. Subtyping at other variable regions of KSHV correlates strongly with ethnicity and geography, rather than with particular disease associations (Zong, 1997; Zong, 1999; Poole, 1999; Zong, 2002).

To improve the interpretative value of data from the ORF26 locus, we undertook a thorough analysis of the polymorphic cluster patterns commonly found in KS and PEL cell DNA samples from human populations around the world, as well as an assessment of the levels of spontaneous variation outside of that represented by subtype-specific polymorphisms.

MATERIALS and METHODS

Source of DNA samples

The majority of the coded clinical samples from the USA, Europe, Israel, Middle East, South Korea, Taiwan and New Zealand sources and from Sub-Saharan African sources other than Uganda and South Africa were described previously (Zong, et al., 1999; Poole, et al., 1999; Davidovici, 2001; Zong, et al., 2002). Additional peripheral blood mononuclear cell (PBMC) samples collected anonymously with consent for diagnostic and research analysis from KS patients at the University of Kampala in Uganda (HKS6 to HKS67 set) were also reported on previously for ORF-K1 (Kajumbula, 2006). A series of KS biopsy DNA samples from the Republic of South Africa (SAKS21 to SAKS35 set) were provided by Lee Alagiozoglou, Lynn Morris and Fred Sitas (National Virology Institute, Johannesburg). Additional PMBC or lymph node samples from South Africa (SAPB3, SAPB5, SALN6, SALN7) were analyzed previously for ORF75 and ORF-K1 (Alagiozoglou, 2000). South African KS DNA sample (FTKS8) was provided by Flourette Treurnicht (Stellenbosch University, Capetown) (Treurnicht, 2002). VG1 DNA was from the Haitian PEL cell line described by Brander et al (2000). A new Taiwan Hwalein KS DNA sample (TKS13) was provided by Ih-Jin Su (National Cheng Kung University Hospital, Taiwan) (Su, et al., 1995). Samples of 021K and 391K DNA were from a KS biopsy DNA collection from Zaire (Republic of Congo) provided by Robert Biggar (NCI)

ORF26 Primers, PCR and DNA Sequencing Procedures

Table 1A lists all ORF26E region primers employed. Assigned ORF26 position numbers are derived from Chang et al (Chang, et al., 1994). The standard 330-bp block represents genomic coordinates 47,193 to 47,522, and our extended ORF26E locus represents genomic coordinates 47,193 to 48,157 (Russo, 1996) for the prototype KSHV(BC1) genome. The majority of the samples were also sequenced in the human mitochondrial HVS-1 control region (760-bp). Direct cycle sequencing on an ABI 310 DNA sequencer was carried out on both strands of purified agarose gel electrophoresis-separated PCR DNA products as described previously (Zong, et al., 1999). The sequence data for the 84 samples in the ORF26E set (965-bp) have Genbank accession numbers DQ984828 to DQ984739 and those for the 52 samples in the standard ORF26 set (330-bp) are DQ984740 to DQ984725.

RESULTS

Eight Distinct Genotype Clusters within the Expanded ORF26 Locus

To further investigate variability and subgroup clustering within the KSHV ORF26 locus, we extended our reported 330-bp nucleotide sequence data (Poole, et al., 1999) to encompass an enlarged ORF26E locus of 965-bp. Three additional PCR primers were generated (Table 1) and used to sequence both selected previously analyzed KS and PEL samples that were still available, and many additional new samples, particularly from Uganda and South Africa.

The results from 136 KSHV-positive samples from Europe, USA, Asia, the Middle East, North Africa, Sub-Saharan Africa and several aboriginal samples from the Pacific Rim are summarized in the form of difference charts in Figures 1A and 1B. The figures combine data for the standard 330-bp ORF26 locus for 52 strains with data for the 965-bp ORF26E locus for an additional 84 samples, but are separated into two distinct sets: 67 Eurasian, American and Pacific Rim patients (Fig 1A) compared to 69 patients of Sub-Saharan African origin (Fig 1B). Two reference samples representing the original RDA PCR sample of Chang et al (Chang, et al., 1994) and the KSF genome sequenced by Neipel et al (Neipel, 1997) are included. Within Fig 1A the samples are classified into four distinct Ethnic/Geographic groups, namely Europe/USA, Middle East, North Asia, and Pacific Rim. Four American samples of likely African origin (AKS15, OKS7, OKS8 and AKS20) are also included in Figure 1A. The country of residence of each patient within the coded sample sets and the disease (PEL or KS) origin are indicated. Overall, the results show that all but four of the 136 samples analyzed cluster into one of eight distinctive ORF26 genotypes or subtypes designated as groups A/C, J, K/ M, D/ E, B, Q, R, or N.

When multiple samples gave exactly the same ORF26 sequence profile (eg. most of the Eurasian A/C samples in Table 1A, or the 29 different B2 subtype samples in Fig 1B), we also analyzed the mitochondrial HVS-1 locus by direct PCR sequencing (data not shown) in order to confirm that all of the KS and PBMC samples represented independent human samples that were distinct from each other, and from any of the known standard PEL cell line mitochondrial DNA patterns.

Several changes in nomenclature have been introduced in Table I compared to our previous analyses (i.e C2 and C2' are now K and K', C3 is now J, B3 is now R and B3' is now Q). In addition, two new subgroup patterns have been designated N and F. Importantly, all seven of the new N subtype samples came from South Africa, and the subset referred to as N2 match eight other samples from South Africa described as B5 in Treuernicht et al (2002). The designation N comes from the SALN6 and SALN7 samples, which were among the six samples originally described as having N subtype ORF75 genes by Alagiozoglou et al (Alagiozoglou, et al., 2000). Five of the six samples within the subgroup designated Q also came from South

Africa and are closely related to both the K and R groups, except for an additional change at 1122 G to T. The designation Q is based on that used previously for a novel clustered subtype exemplified by SAPB1, SAKS29, SAKS33 and SAKS35 within the T0.7/KAP gene locus (Zong, et al., 2002;Whitby, 2004). The latter shows considerably greater divergence from the A pattern than do the B, R, M or N patterns. The clustered patterns in the ORF26 locus that were originally designated as B1/B2, B3 and B4 (Poole, et al., 1999;Zong, et al., 2002) have been renamed as B, R and N respectively, whereas for the T0.7/KAP locus the clustered patterns originally designated as B1, B2 and B3/B4 (Poole, et al., 1999;Zong, et al., 2002) have been renamed as R, B and G, respectively.

As expected, both the Eurasian and African ORF26 patterns that were previously grouped together as either C2 or B3 patterns (Poole, et al., 1999) now resolve into two distinct groups (classified as K and R, respectively) when the ORF26 locus was extended 630-bp further to the right (Fig 1A and 1B). Both groups have the same additional polymorphisms at 1490 G to T, 1594 G to T and 1754 C to T, but only the Eurasian samples (K group) have the 1684 C to A change. In addition, the African subset (R group) are all deleted by 2-bp from the prototype 8 × poly-G indel between positions 1568 to 1573 (which lies within in a small intergenic block between the ORF26 and ORF27 gene coding regions). Similarly, the Eurasian (A/C) and Pacific rim (D/E) patterns that were identical in the previous shorter analysis (Poole, et al., 1999) now differ at three positions within the extended data, namely 1206 G to C, 1557 G to C and 1735 C to A, as well as in the poly-G indel.

One African sample HKS54 is the sole representative of a distinct ninth ORF26 pattern designated F that contains three novel polymorphisms at positions 1206 G to A, 2545 C to T and 1802 G to A (Fig 1B). Finally, three other African samples HKS22, HKS56 and SAKS25 represent intragenic chimeric recombinants (designated R/Q and Q/B) within ORF26.

Therefore, we recognize and define eight principle ORF26 patterns or clusters, of which four (B, N, Q and R) are found virtually exclusively among Sub-Saharan African samples, one (D/E) is found only within indigenous South Asian and Polynesian (Pacific Rim) populations, and three (A/C, J and K) are found to be almost exclusively of Eurasian (European, United States, North Asian and Middle Eastern) origin. The only exceptions to these groupings were two African examples of the Eurasian J pattern (HKS21 and HKS40), two A/C samples in Caucasian patients of Dutch descent from South Africa (SAKS21 and SAKS31), four B-subtypes from either Haiti or from African-American patients in the United States (VG1, AKS15, OKS7 and OKS8), plus one Q sample from a Bushman patient who emigrated to New Zealand from Mozambique (ZKS6). The ORF26 K subtype is almost exclusively associated with genomes that have M allele TMP (ORF15) genes, although there are also many other genotypes that have TMP-M alleles.

Linkage and Recombination between Multiple Genomic Loci

We summarize in Figures 1A and 1B the subtype patterns determined for nearly all of these KSHV samples at three of the other eight constant region loci that we routinely analyze. These designations for the VIP(K1), T0.7(KAP) and TMP(K15) loci, which lie at genomic map coordinates 1%, 85% and 98% (compared to at 24% for ORF26), are listed in the RHS columns alongside the assigned ORF26 designation. The six other constant region PCR loci analyzed generally show the same eight or nine sets of clusters, with either five or six distinct groups among Sub-Saharan African samples (B,N,Q,R,F and G), a different one in Pacific Rim samples (D/E) and either two or three more in Eurasian samples (A/C, J and K or M).

Comparison of the subtype assignments reveals that there is a great deal of linkage between the multiple loci analyzed for many KSHV genomes. Nevertheless, some genomes that are identical in ORF26 differ at other loci, and linkage and recombination patterns across the entire

KSHV genome can be complex. Furthermore, when all eight constant region loci plus the VIP (K1) and TMP (K15) genes are all taken into account, very few African genomes fail to show at least some chimerism (Hayward, 2006; Kajumbula, et al., 2006; Zong, et al., 2002). The situation is much simpler for Eurasian genomes, with only 30 to 50% showing one or more inter-subtype recombination events (Zong, et al., 2002).

Low Levels of Sporadic Variability

There are nine commonly observed nucleotide variations from the prototype BCBL-R sequence within the standard 330-bp ORF26 block (namely 935 A, 981 C, 1032 A, 1055 T, 1085 A, 1086 T, 1122 T, 1132 G and 1139 C). These are used as characteristic diagnostic polymorphisms for determining subtype or genotype assignments. Positions 1206 C and 1214 T could also be included here as diagnostic polymorphisms, but most available data for the short region does not include them. Surprisingly, the results shown in Figures 1A and 1B detected only seven additional sporadic nucleotide changes over the standard 330-bp ORF26 region, out of a total of 38,500 nucleotides encompassing positions 893 to 1222 from 136 different patients. Four of the seven sporadic variants (BCBL1, ASM70/80, HKS54 and TKS13) were confirmed by sequencing multiple PCR reaction products from either the same sample, or, in one case, from nine other skin or disseminated lesions from the same patient (ASM70/80), and therefore represent additional real subtype-specific variants. Position 1103 A is diagnostic for the BCBL1 PEL cell line, a commonly encountered contaminant. Therefore, only three nucleotides in our data out of the 38,500 have some potential to be PCR sequencing artifacts (0.007%). Finally, the C at position 1033 of the original Chang and Moore RDA sample (Chang, et al., 1994) was never found by us in any other sample and probably represents a PCR error.

Similarly, sporadic variants other than the 17 commonly encountered diagnostic polymorphisms, or within the position 1562 poly-G indel, were also extremely rare within the extended ORF26E region. With the exception of the three novel variant positions in the sole example of the postulated rare ninth or F genotype (HKS54), there was just one sporadic variant among all 30 samples in Table 1A and three among the 56 samples in Table 1B. This is a rate of four out of 55,900 nucleotides examined between positions 1206 and 1855 (0.007%).

DISCUSSION

Analysis of the expanded ORF26 locus indicates that eight commonly encountered KSHV subtype clusters can be unambiguously distinguished. A single example of a postulated ninth genotype and a small number of intratypic recombinants were also detected. Overall, our findings of very specific and different patterns of ORF26E genotypes being found in Sub-Saharan Africa and in the Pacific Rim, compared to Eurasia, closely parallel the results for the hypervariable ORF-K1 protein and other constant region loci, including T0.7 and ORF75. However, as described in a recent review of the structure and modern evolutionary history of KSHV genomes (Hayward, 2006), there is also a major difference in that there are many more highly diverged ORF26 (and other constant region) KSHV subtype clusters found in Africa than there are ORF-K1 variants in Africa.

Despite the complexities of the overall structure of KSHV genomes, and the incomplete picture obtained from data for such a small segment of the 170-kb KSHV genome, there is nevertheless considerable value for epidemiological studies and clinical diagnostic purposes of obtaining and interpreting ORF26 DNA sequence information. A particularly important finding here is the very low rates of sporadic variation found within both the standard ORF26 and extended ORF26E region sequences at nucleotide positions other than the 26 characteristic polymorphisms used to define the different genotypes or subtypes.

The information presented provides a solid context for assessing and confirming whether certain positive PCR results accurately detect the presence of KSHV genomes within the tissue samples. In the accompanying review by Zong et al (2007), we have utilized this information to evaluate likely sources of sequence artifacts and contamination in some KSHV PCR studies, as well as claims for biologically or clinically relevant correlations with particular ORF26 polymorphism patterns.

ACKNOWLEDGEMENTS

This work was supported at various times by Public Health Service Grants CA73585, CA81400, CA81422 and AI24576 to GSH from the National Cancer Institute.

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^ = Deleted; \$ = 1033 T to C is probably an RDA-PCR error.
^ = Variable number of Gs inserted between positions 1561 and 1564.
AKS and ASM = Maryland, New York; BKS = Texas and Tennessee; EKS = Egypt; IKS = Israel; KKS = South Korea;
MKS = Scandinavia; OKS = Florida; RGKS = Sicily; SKS = Saudi Arabia; TKS = Taiwan Chinese;
TKS(H) = Taiwan Hualien; VGS = Haiti; ZKS = New Zealand Caucasian; ZKS(P) = New Zealand Polynesian.
RDA-CM = Chang, 1994 #331; KSPN = [Neipel, 1997 #281].

ISOLATE NAME	POLYMORPHISMS (38 / 965 bp)																								ORF26E CLASS	VIP (K1)	T0.7	TMP (K15)	ORIGIN		
	9	9	9	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
	3	8	9	3	3	5	8	8	9	0	2	3	3	0	1	1	1	9	2	4	5	6	6	6	9	1	1	5	8	1	2
	5	1	4	2	3	5	5	6	4	3	2	2	9	6	4	3	1	0	0	5	7	2	8	9	4	2	8	5	4	7	3
BCBLR (PEL)	C	T	A	C	T	G	G	C	G	C	G	A	A	G	C	C	C	G	G	C	G	0	G	G	G	C	A	C	C	C	A
oSAKS21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-
oSAKS31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	C	A	C	A	C	A	C	G	T	G
HKS54	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	A
HKS21	-	C	-	A	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-
HKS40	-	C	-	A	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	-	-	-	-	-
391K	-	C	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	0	@	@	T	-	-	-	-	-	-	-
OKS3	-	C	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	0	@	@	T	-	-	-	-	-	-	-
ST1	-	C	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	0	@	@	T	-	-	-	-	-	-	-
#AKS20	-	C	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	0	@	@	T	-	-	-	-	-	-	-
RKS5	-	C	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	0	@	@	T	-	-	-	-	-	-	-
HKS7	-	C	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	0	@	@	T	-	-	-	-	-	-	-
HKS10	-	C	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	0	@	@	T	-	-	-	-	-	-	-
HKS15	-	C	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	0	@	@	T	-	-	-	-	-	-	-
HKS18	-	C	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	0	@	@	T	-	-	-	-	-	-	-
HKS29	-	C	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	0	@	@	T	-	-	-	-	-	-	-
HKS32	-	C	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	0	@	@	T	-	-	-	-	-	-	-
HKS33	-	C	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	0	@	@	T	-	-	-	-	-	-	-
HKS35	-	C	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	+1	@	@	T	-	-	-	-	C	-	-
HKS43	-	C	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	0	@	@	T	-	-	-	-	-	-	-
HKS66	-	C	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	0	@	@	T	-	-	-	-	-	-	-
HKS22	-	C	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	A	T	-	-	-
HKS56	-	C	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	A	T	-	-	-
SAKS24	-	C	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	A	T	-	-	-
SAKS29	-	C	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	A	T	-	-	-
SAKS33	-	C	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	A	T	-	-	-
SAKS35	-	C	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	A	T	-	-	-
HKS8	-	C	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	+1	-	-	-	-	-	A	T	-	-	-
#ZKS6	-	C	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	0	-	-	-	-	-	A	T	-	-	-
SAKS25/30	-	C	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
431K	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
OKS4	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
HKS11	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
HKS49	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
021K	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
ST2/ST3	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
RKS1/6	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
RKS2/7	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
RKS3/8	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
RKS4	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
OKS11	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
SAPB5	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
SAKS32	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
SAKS34	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
HKS9	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
HKS13	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
HKS19	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
HKS20	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
HKS23	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
HKS24	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
HKS27	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
HKS34	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
HKS36	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
HKS41	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
HKS47	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
HKS50	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
HKS52	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
HKS58	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
HKS59	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
HKS60	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
HKS61	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
HKS67	-	C	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0	T	A	-	-	-	-	-	-	-	C
SALN6	-	C	-	-	-	-	T	A	-	-	-	-	-	-	-	-	-	-	-	-	G	C	-	-	-	-	-	-	-	-	-
SALN7	-	C	-	-	-	-	-	T	A	-	-	-	-	-	-	-	-	-	-	-	G	C	-	-	-	-	-	-	-	-	-
FTKS8	-	C	-	-	-	-	-	T	A	-	-	-	-	-	-	-	-	-	-	-	G	C	-	-	-	-	-	-	-	-	-
SAKS22	-	C	-	-	-	-	-	T	A	-	-	-	-	-	-	-	-	-	-	-	G	C	-	-	-	-	-	-	-	-	-
SAKS28	-	C	-	-	-	-	-	T	A	-	-	-	-	-	-	-	-	-	-	-	G	C	-	-	-	-	-	-	-	-	-
SAKS23	-	C	-	-	-	-	-	T	A	-	-	-	-	-	-	-	-	-	-	-	G	C	-	-	-	-	-	-	-	-	-
SAKS26	-	C	-	-	-	-	-	T	A	-	-	-	-	-	-	-	-	-	-	-	G	C	-	-	-	-	-	-	-	-	-
SAKS27	-	C	-	-	-	-	-	T	A	-	-	-	-	-	-	-	-	-	-	-	G</										

Figure 1.
A: KSHV ORF26E VARIABILITY (NORTH AMERICAN, EUROPE, ASIA AND PACIFIC RIM)
B: KSHV ORF26E VARIABILITY (SUB-SAHARAN AFRICA ONLY)

Table 1

ORF26 PCR Primers Employed

Name	Sequence (5' to 3')	Orientation	Genome Start Position *	ORF26 Start Position **
LGH1701	GGATCCCTCTGACAACC	Rightwards	47,193	893
LGH1702	GGATCCGTGTTGTCTACG	Leftwards	47,522	1222
LGH2575	GTGCTTGACGATCTGTCC	Rightwards	47,539	1239
LGH2573	GGCTGATAGGATACAAAGG	Leftwards	47,560	1280
LGH2574	CAGAAACAGGGCTAGGTAC	Leftwards	48,157	1855

* Russo et al, 1996

** Chang et al, 1994