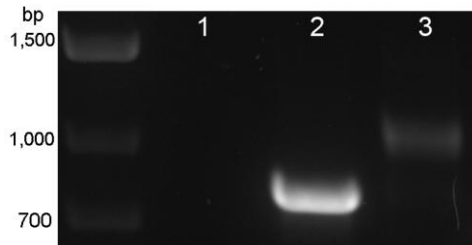


Novel Polyomavirus associated with Brain Tumors in Free-Ranging Raccoons, Western United States

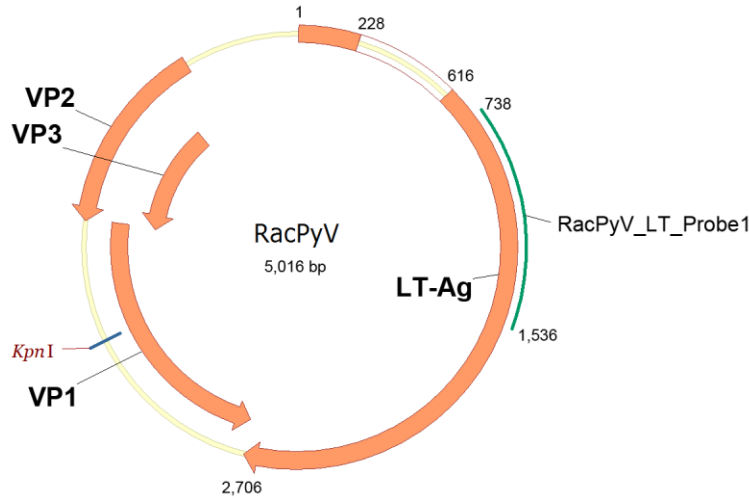
Technical Appendix

Technical Appendix Table. Primers used in the identification of raccoon polyomavirus

Name	Sequence (5' → 3')
PLMA_F3	GSNNGGTNGCNTGGTAY
PLMA_F5_1of2	GGNAARACNACNSTDGCN
PLMA_F5_2of2	GGNAARACHACHTTRGCN
PLMA_R1	YTGHCCTTACATCYTCAAA
PLMA_R4	NCCTTTHACATCYTCAAHAC
PLMA_R5_1of2	RTCRA TRGCRC AKCCYAGYTCRAA
PLMA_R5_2of2	RTCRA TRGCRC AKCCTAAYTCRAA
PLMA_LTag01_F	CATGTTGAAGGGACTCCTGCAATTATTTAC
PLMA_LTag02_R	AGTTTATCTGCTGGACAGTTGATGTTTAAGG
PLMA_F1	CAYCCNGAYAARGGNGGN



Technical Appendix Figure 1. Southern blot hybridization probe amplification verification. Digoxigenin (DIG)-labeled probe for Southern blot hybridization. Lane 1 is a water-negative control. Lane 2 is amplification of the 799-bp RacPyV_LT_Probe1 with regular dNTPs. Lane 3 is amplification of RacPyV_LT_Probe1 with DIG-dUTP. As expected, the DIG-labeled amplicon runs slower and appears fainter because of high-density DIG-labeling. RacPyV, raccoon polyomavirus.



Technical Appendix Figure 2. Southern blot hybridization probe and restriction site map. A highly conserved ($\geq 99.87\%$ identity) 799-bp region of RacPyV LT-Ag spanning nt 738–1,536 of the RacPyV4 genome was chosen as the probe for the Southern blot hybridizations. A unique, conserved *Kpn*I restriction site within VP1 linearizes the RacPyV circular genome. RacPyV, raccoon polyomavirus; LT-Ag, large T-antigen; VP, viral protein.

	PLMA_F5	PLMA_R5	PLMA_R4
BPV1	T A I L L F N W L F P P I I D V D V I I V D I L Q T W V K N P P K R R Y V I F K G P V N S G K T T L A A A I L L A L C T D A S L N V K G T P D R L G F E L D C A I T D G F M V L T E D V K G T P		
HPV16	Q A V A W L D C L Q P I I S F T T K L K E I L S I L T E N I P K R R N V L F K G P I N S G K T T L A A A I L D L V G G V S L N V N G T P D K I N F E L D C A I T D G F M G V I E D V K G T P		
HPV17	Q A V A W L D C L L P I I Q F T I K K E W L S Y V E N I P K R R N L L F K G P I N S G K T T L A A A I L D L L G G V A L N V N G S S D K I N F E L D C A I T D G F M V I I E D V K G T P		
HPV18	S G V A W Y T I L L D I I N S W D V F Q K I L Q L T T S D P K R N I I F K G P I N S G K T T L A S A F E W F F D D K A L N I N G P F A E K L L F E L D C A I T D G F C V L I D G V K G T		
CSLPV1	A G A A W L H C L L P I I R F E D T V Y D I I K A P T L N A P K N R Y F L F G G P L N S G K T T L A A A L L D L L G G K A L N I N I P K E K L S F E L D C A I T D G F M V F E D V K G K		
JCV	A G V A W I H C L L P I I Q W D T V I Y D F L K C I V L N I P K K R Y W L F K G P I D S G K T T L A A A L L D L C G G K S L N V N M P L E R L N F E L D V G I D G F M V F E D V K G T S		
HPV8	A G V A W L H C L L P I I K R D S V I F D F L W C I V E N I P K R R Y W L F K G P I D S G K T T L A A G L L D L C G G K A L N V N L P F M E R L T F E L D V A I D G F M V F E D V K G T S		
BPV1	A A V M W L N A L H P I I N N P D V I F N Y I K M V V E N K P Q R Y L L L K G P V N S G K T T V A A G L I G L C G G A V L N I N G P P E R L A F E L D M A I D G F T V F E D V K G K		
MeaPV1	A G V T W Y N N P F A Q I N W K D F V L E V L E C W V T N I P K K R Y W L F T O P V N S G K T T L A A A L L D L C G G K S L N I N T P F D K I N F E L D V A I D G F S V F E D V K G S		
BPV1	A G V C W F F C L L P E V N W K N F I L Q V L E C V V Q N V P K R F W C T I O P V N S G K T T L A A A L L D L C G G S L N V N M P P D K L N F E L D V A I D G F T V F E D V K G S		
HPV1	A S I A W Y T O L N K I I K I D E L V V R F L K L I V D N K P K R Y W L F K G P I N S G K T T L A A L L N L L C G G K A L N I N I P S E K L P F E L D V A L D G F M V F E D V K G S		
HPV2	A R V A W Y V G C L L E I I D P F O T L F K W L K L L T E N I P K R R N I L F R O P I N S G K T T L A A A L I B L L G G K S L N I N G P A D K L A F E L D V A D G F V V F E D V K G S		
HPV5	Q A V A W I D C F F P I I D F E M L L D I L L K L F F E N I P K R R N V L F R O P I N S G A T S A A I I W L L V G G V A L N V N D P A D K L N F E L D V A I D G F A V F E D V K G S		
HPV7	Q G V A W Y C C L F E I I Q F E K K L K L I L E L T E N I P K R R N I W F K G P I N S G K T S F A A A L L D L L E G K A L N I N G P S D K L P F E L D C A I D G F L V F E D V K G S		
HPV33	A G V A W Y S C L F E I I N I I E E V V T K I I I R L L V E N I P K R R N T L F R O P I N S G K T T L A A A L M N F L G G K T L N V N G P A D K L A F E L D C A I D G F V I F E D V K G S		
HPV35	A G V A W Y S C L F E I I N I I E E L V M K I L Q L L T E N I P K R R N V L F R O P I N S G K T S L A A A F M D L L E G K A L N V N G P P K L P F E L D C A I D G F A I V F E D V K G S		
HPV39	Q G V A W Y C C L F E I I E F E K K L S R I L E L T E N I P K R R N I W F K G P I N S G K T S F A A A L L D L L E G K A L N I N G P S D K L P F E L D C A I D G F L V F E D V K G S		
HPV45	A G V A W Y A N L F E I I N F D E L L F Q W L K L L T G N I P K Q R N I L F R O P I N S G K T T L A A A L V D L L G G R S L N V N G P A D K I N F E L D C A I D R F V I F E D V K G S		
WCV	A G V A W Y L D L N S I I K I D E L V Y R Y L K I V I V E N I P K R R Y W L F K G P I N S G K T T V A A A L L D L C G G K A L N I N I P A D R L N F E L D V A I D G F T V F E D V K G S		
HPV31	Q G V A W Y S C L F E I I N I D E V V T K I I L L V I V E N I P K R R N C L F R O P I N S G K T T F F A A A L M N F L G G K T L N V N G P A D K L P F E L D C A I D G F V I F E D V K G S		
MCV1	S G V A W Y C C L F E I I E F E K K L Q K I I Q L L T E N I P K V R N I W F K G P I N S B K T S F A A A L I D L L E G K A L N I N G P S D K L P F E L D C A I D G F M V F E D V K G S		

Technical Appendix Figure 3. Polyomaviruses used for consensus PCR primer design. The large T-antigens of 21 polyomaviruses as described in materials and methods were aligned and scanned for conserved regions to generate consensus PCR primers.