Development of a PCR-assay to detect papillomavirus in the snow leopard

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Introduction

Papillomaviruses (PVs) are DNA viruses that cause lesions in the skin and mucous membranes and after persistent infection, a subset of PVs can cause tumors such as cervical malignancies in humans. PVs from several species have been sequenced, thereby increasing our understanding of the viral oncogenesis and allowing for the development of molecular diagnostics for PV infection. In felids, PVs have been sequenced from oral lesions of wild species such as bobcats, Asian lions and snow leopards. Since some of these species are endangered, PV-associated disease and mortality are of interest and there is a need for molecular assays that identify infected animals.

The genome sequence of PV1 from the snow leopard was used to develop a PCR assay to detect viral DNA. DNA samples were collected from captive animals using Performagene™ saliva† collection kits (DNA Genotek, Inc). Primers flanking the two key viral oncogenes E6 and E7 were used and two DNA fragments encompassing these genes were amplified. Using this assay, viral DNA for E6 and E7 was detected in genomic DNA isolated from saliva, however, the same viral DNA was not detected in genomic DNA extracted from paired blood samples of snow leopards. The identity of these PCR products was further verified by restriction digest and DNA sequencing. The sequences were 100% identical to the published sequence of the UuPV1 genome. This PCR assay could be used for the development of a non-invasive molecular diagnostic to screen snow leopards for papilloma virus using saliva samples. This sample type is ideal as it corresponds to the anatomical site where lesions develop during later stages of viral infection and disease development.

Materials and methods

Sample collection

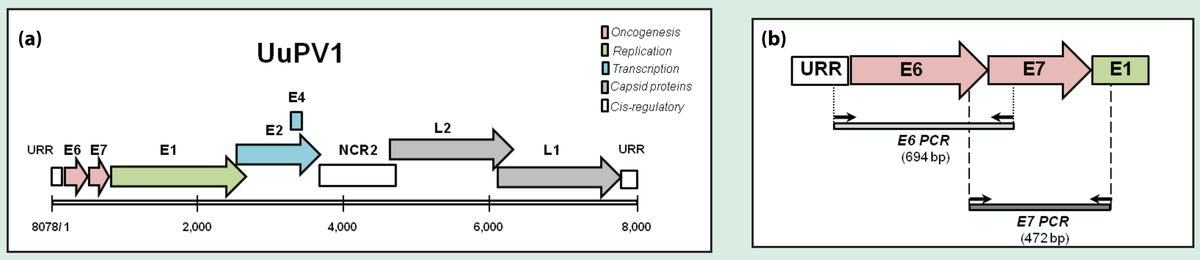
Figure 1: Collection of saliva samples from two captive snow leopards using the Performagene kit

(a) 7-week old female cub (b) 2-year old female

Because squamous cell carcinoma occurs within the snow leopard oral cavity, we were specifically interested in developing a strategy to test saliva samples from asymptomatic snow leopards for the presence of papillomavirus. Such an assay would allow for the detection of viral DNA in the animal as opposed to a serum test which detects past exposure, but may not correlate with current presence of virus. Captive snow leopards without lingual papilloma warts were selected for testing and saliva samples were collected using the Performagene kit.

PCR primer design

Figure 2: (a) Schematic of the PV1 genome and (b) of the E6 and E7 open reading frames indicating the positions of the two primer pairs used to detect viral DNA

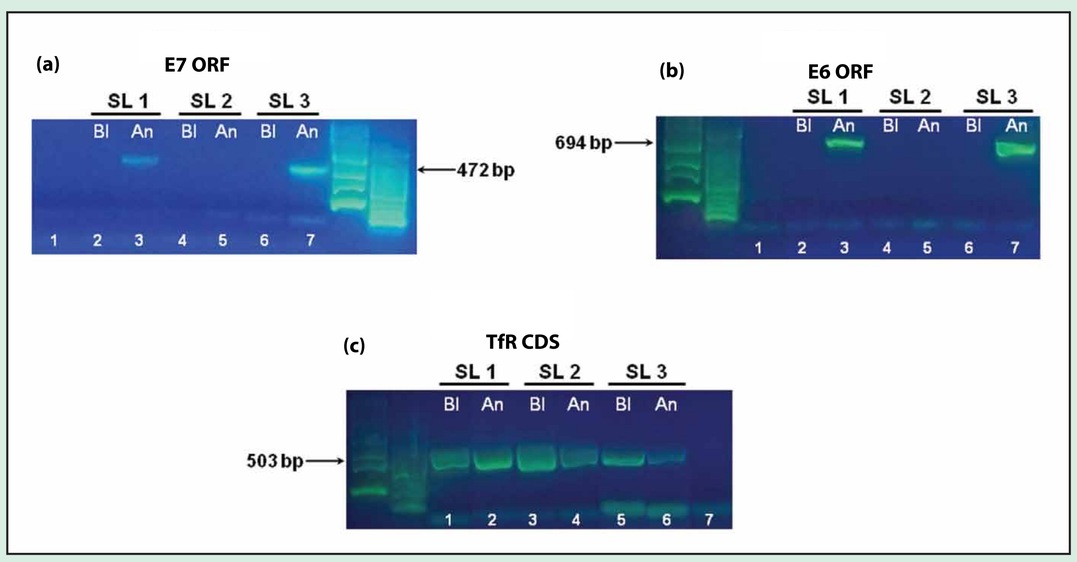


We used the published sequence of the uuPV1 genome to design primers to amplify the E6 and E7 oncogenes. Primer design was optimized such that the entire coding region of each oncogene would be amplified in a single PCR reaction. E6 and E7 were chosen since they are the key regulators of oncogenesis in Human Papillomaviruses, therefore sequencing and characterization of the E6 and E7 amplification products obtained from different animals could reveal sequence variants that directly affect the oncogenic potential of the virus. The primers did not produce any feline-specific amplification products as revealed by isPCR.

Results

PCR amplification of viral oncogenes from saliva

Figure 3: PCR amplification of the (a) E7, (b) E6 viral oncogenes and (c) of the feline transferrin receptor (TfR) from snow leopard paired saliva and blood samples.

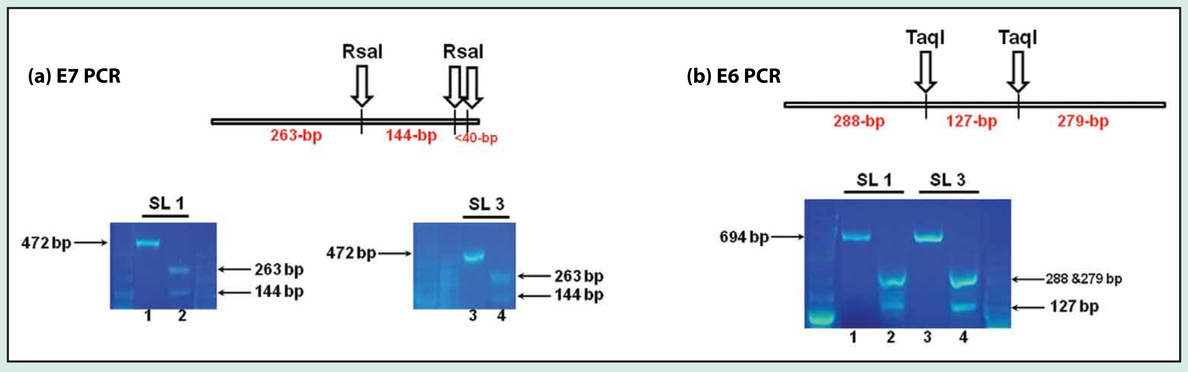


The E6 and E7 primer pairs were used to test paired blood and saliva samples obtained from three snow leopards for the presence of viral DNA. Amplification products were obtained with both E6 and E7 primers in the saliva samples from the two adult snow leopards, but not the cub. The amplification products were of the expected size (694-bp and 472-bp for E6 and E7 respectively). No amplification product was obtained using the paired blood samples from these animals. This wasn't due to the absence of DNA in the blood samples since positive control reactions amplified a region of the feline transferrin receptor (TfR) extracellular domain in both saliva and blood samples from all three animals.

† This data was generated using a previous version of the product (Oragene•ANIMAL). Please contact us for more information.

Restriction digest analysis of PCR products

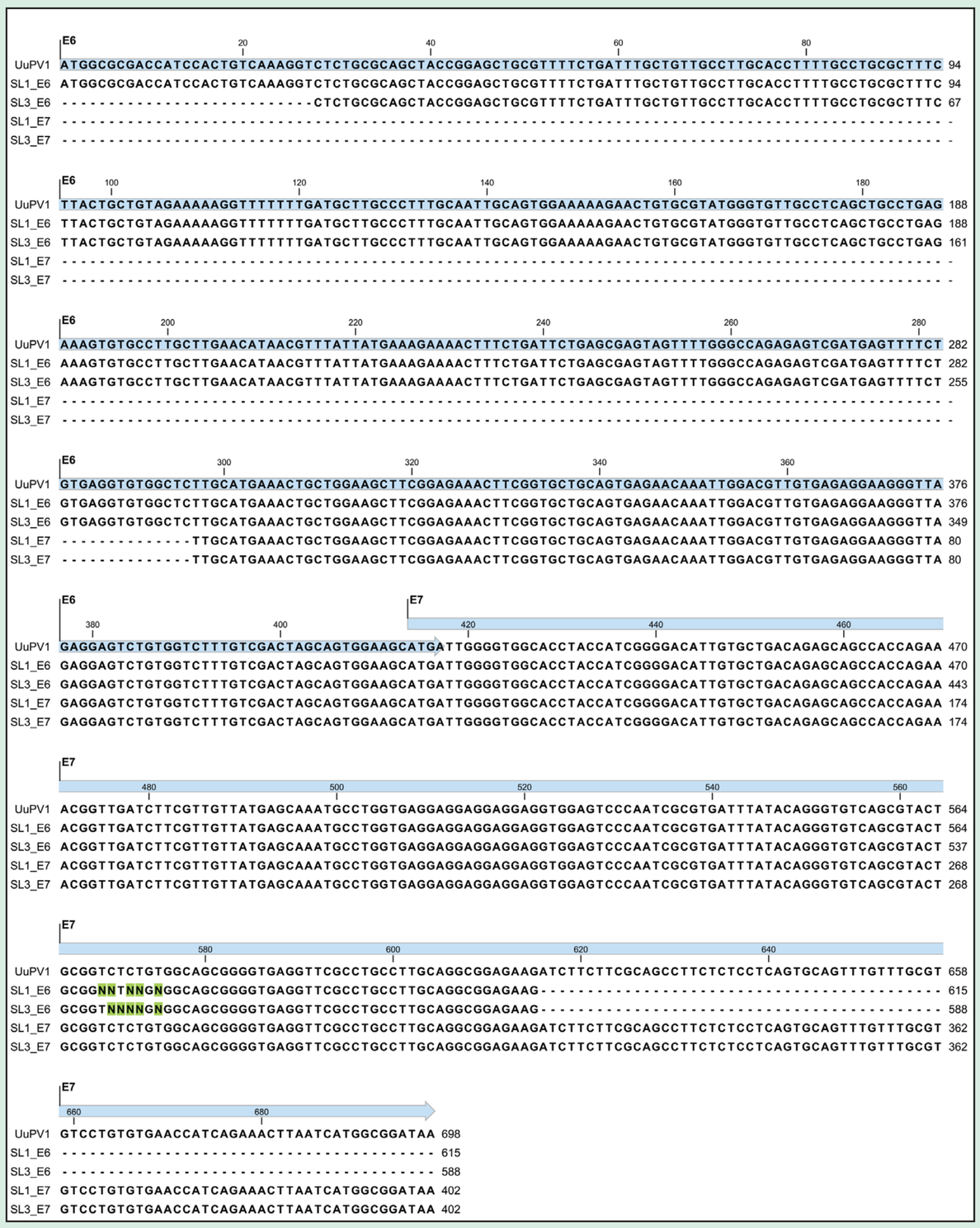
Figure 4: Validation of the (a) E7 and (b) E6 viral amplification products by restriction digest



Sequencing analysis of PCR products

Figure 5: Multiple sequence alignment of the snow leopard PV1 genome and E6 and E7 amplification products

The E6 and E7 PCR products obtained from the two adult snow leopards were gel purified and subjected to bi-directional DNA sequencing using the PCR primers. The sequencing reads were assembled into contigs and compared to the published uuPV1 genome sequence. The sequences were 100% identical to the public uuPV1 genome sequence, confirming the fact that we were able to amplify the E6 and E6 oncogenes from snow leopard saliva.



Discussion

We developed a PCR-based assay that reliably detects the E6 and E7 oncogenes of PV1 in snow leopard saliva. PCR-based testing is advantageous as compared to serotesting. It has increased sensitivity, and unlike serotesting which assesses past exposure of the animal to the virus, it detects viral DNA in the oral cavity of the animal, where papillomatous lesions and squamous carcinoma will later develop.

A molecular assay for PV1 can detect the virus prior to the development of disease and can be used for increased veterinary screening and to implement management changes in order to prevent transmission of the disease to other animals. Furthermore, the use of saliva as a biological specimen to detect papillomavirus presents a considerable advantage since it allows for non-invasive sample collection.