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Characterization of Human Papillomavirus Subtype 72b

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We report the characterization of human papillomavirus (HPV) subtype 72b of the genus Alphapapillomavirus isolated from an oral rinse sample of a healthy woman. The HPV72b L1 open reading frame (ORF) was 90.2% identical to that of HPV72, indicating a subtype close to the border of a novel HPV type.

In an oral rinse sample from a 44-year-old woman we identified a novel sub-genomic Forward Antonsen primer (FAP)-fragment, FA171, with closest sequence identity to HPV72 (1). After cloning of its complete genome we determined that the L1 open reading frame (ORF) was 90.2% identical to that of HPV72 (genus Alphapapillomavirus). Therefore, it was labeled as subtype HPV72b of HPV72, originally isolated from an oral papillomatous lesion of an HIV positive patient (2).

The complete genome of HPV72b (8,098 bp) was obtained using the PrimeSTAR GXL DNA polymerase kit (TaKaRa Bio, Shiga, Japan). Briefly, 2.5 μL purified DNA (Magna LC, Roche) was amplified in 25 μL containing a × PrimeSTAR GXL buffer, 200 μM of each dNTP, 0.2 μM of each primer (fwd 5’-TGA CTA CAA GCA AAC ACA GTT GCT T-3’ and rev 5’-ACA GAT ATA TTG TCC CGG CTG TC-3’ from DNA-Technology, Denmark), and 0.625 U PrimeSTAR GXL DNA polymerase. Amplification was performed for 45 cycles at 98°C for 10 s, 60°C for 15 s, and 68°C for 8 min in a thermocycler (Mastercycler Eppendorf, Germany). The amplicon was cloned using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA) and sequenced using primer-walking (Eurofins, Germany).

A novel HPV type shares less than 90% similarity to the closest papillomavirus (PV) type in the L1 ORF (3). Pairwise comparisons between L1 ORFs of HPV72b and HPV72 (second ATG) (2) demonstrated 90.2% nucleotide (nt) identity, whereas the other ORFs showed identities less than 90% (E6: 85.0%, E7: 85.4%, E1: 89.2%, E2: 85.5%, E4: 80.6%, E5: 69.5%, L2: 89.2%) and URR 78.6%. The overall similarity between HPV72b and HPV72 was 85.3%. There was also a segment of 101 nt (3,874 to 3,974) downstream of the putative start of E4 ORF was absent, as the ATG codon was changed to ACG.

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REFERENCES