Supplementary Materials for

Coordinated Expression of HPV-6 Genes with Predominant E4 and E5 Expression in Laryngeal Papilloma

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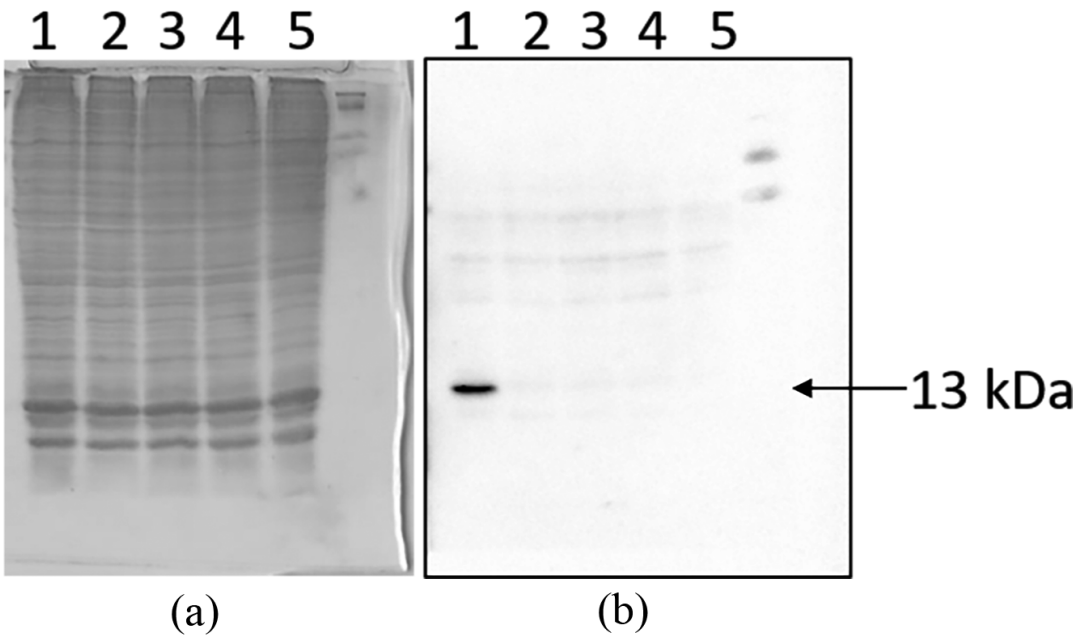

Figure S1. SDS-PAGE of cell lines and western blot analysis using the anti-E1^E4 antibody. SDS-PAGE analysis with Coomassie brilliant blue staining. (b) Western blot analysis with the anti-E1^E4 antibody. Lane 1 = HEK293T cells transfected with pcDNA3.1+ HPV-6 E4-3× FLAG; Lane 2 = HEK293T cells transfected with pcDNA3.1+HPV-11 E4-3× FLAG; Lane 3 = HEK293T cells transfected with pcDNA3.1+ CMTM7-3× FLAG; Lane 4 = HEK293T cells transfected with pcDNA3.1+; Lane 5 = HEK293T cells. A protein with a molecular weight of 13 kDa (E4) was observed only in lane 1.

Table S1. Primers used in the present study.

Screening primers	**Sequence (5′–3′)**
GP5+	TTTGTTACTGTGGTAGATACTAC
GP6+	GAAAAATAAACTGTAAATCATATTC
MY09	CGTCCMARRGGAWACTGATC
MY11	GCMCAGGGWCATAAYAATGG
PC04	CAACTTCATCCACGTTCACC
GH20	GAAGAGCCAAGGACAGGTAC
LCR-F	ACGTAAGCGCGCCAAAAC
LCR-R	ATGCATATGAATAAATCTCTGCTGTG
Cloning primers used for standard DNA	**Sequence (5′–3′)**
F1	AAAGTGCAAATGCCTCCACG
R1	TTTCTCTACCTGCGTTCCCG
F2	TTATGGCTGCACGGTACGC
R2	TAACCCCAACACCCCTACAA
F3	GTGTGTTACTGTCCCGCTTG
R3	CACACCTAATGGCTGTCCCC
F4	GCAGGGGACAGCCATTAGG
R4	TCCACATGACGCATGTACTCT
Real-time PCR primers and TaqMan probes	**Sequence (5′–3′)**
E6-F	GCGTGCTGCCTAGAATTTCAT
E6-R	CAACAGTTGTTGCATATCCAGCAT
E6-Probe	FAM-CAAAGTGTCTATATTGGTTAATTTTTC-MGB
E7-F	GACGAAGTGGACGGACAAGA
E7-R	ACACTGCACAACCAGTCGAA
E1-F	TAAGTCCACGATTGGACGCC
E1-R	TTTCTCTACCTGCGTTCCCG
E2-F	GGCAAACACCACCTAAACGC
E2-R	CAGGAGTCAGTGTCCTGCAC
E4-F	CGAGGAGTCCAACAGTCACC
E4-R	TCTTTGGTGCTGGTCGTGAT
E5a-F	ATGGAAGTGGTACCTGTACAAATAGC

E5a-R	AGCAGTGTTAGTACTAGCACAGATG
E5b-F	GGCTGGGTTTGTGGTTGTTAT
E5b-R	ATTTGGTGTGTTTATCGCCTTG
L2-F	GTGCGTCAGCTACACAGCTA
L2-R	TGCCTATACCCAACCCTCCA
L1-F	ACCACACGCAGTACCAACAT
L1-R	TCCACATGACGCATGTACTCT
β-Globin-F	TGGGTTTCTGATAGGCACTGACT
β-Globin-R	AACAGCATCAGGAGTGGACAGAT
β-Globin-Probe	FAM-TCTACCCTTGGACCCAGAGGTTCTTTGAGT-TAMRA
β-Actin-F	GCGAGAAGATGACCCAGATC
β-Actin-R	CCAGTGGTACGGCCAGAGG
β-Actin-Probe	FAM-CCAGCCATGTACGTTGCTATCCAGGC-TAMRA
Primers for ISH probes	**Sequence (5′–3′)**
E6-ISH-F	CCACGTCTGCAACGACCATA
E6-ISH-R	TTGTCCAGCAGTGTAGGCAG
E2-ISH-F	ATGGAAGCAATAGCCAAGCG
E2-ISH-R	GGAGTCAGTGTCCTGCACATA
E4-ISH-F	GGGAAGTATGTTATGGCAGCAC
E4-ISH-R	TCTTTGGTGCTGGTCGTGAT
E5a-ISH-F	AGCTGCAGGAACAACCAGCA
E5a-ISH-R	GCTGTGTGTTCACAATGTAGTGG
E5b-ISH-F	ATGATGCTAACATGTCAATTTAATG
E5b-ISH-R	CTAATTCATATATATATAATCACCATCAGTAG
Primers for pcDNA3.1(+)E1^E4 expression vector	**Sequence (5′–3′)**
HPV-6 E1^E4-F HPV-11 E1^E4-F	GAATTCACCACCATGGCGGACGATTCAGCAC
HPV-6 E1^E4-R	CTCGAGGCCACCGGATCCTAGGCGTAGCTGAA CTGTTACTGT
HPV-11 E1^E4-R	CTCGAGGCCACCGGATCCTAGGCGTAGCTGCA CTGTGA

R1 and R4 cloning primers used for standard DNA are the same as E1-R and L1-R real-time PCR primers, respectively. Since the HPV-6 sequence resembles that of HPV-11, HPV-6 E1^E4-F is the same as HPV-11 E1^E4-F.

Supplementary Table 2. Target gene, method, standard DNA, detection range, and amplification efficiency in real-time PCR.

Target gene	**Method**	**Standard DNA**	**Detection range**	**Amplification efficiency (%)**
E6	TaqMan MGB probe	p1478 HPV-6E6	10^1–10^7 copies	102.2
E7	SYBR Green	Clone A	$2.0 \times 10^1 – 2.0 \times 10^7$ copies	107.6
E1	SYBR Green	Clone A	$2.0 \times 10^1 – 2.0 \times 10^7$ copies	115.3
E2	SYBR Green	Clone B	$2.0 \times 10^1 – 2.0 \times 10^7$ copies	101.8
E4	SYBR Green	Clone B	$2.0 \times 10^1 – 2.0 \times 10^7$ copies	103.5
E5a	SYBR Green	Clone B	$2.0 \times 10^1 – 2.0 \times 10^7$ copies	102.0
E5b	SYBR Green	Clone C	$2.0 \times 10^1 – 2.0 \times 10^7$ copies	109.2
L2	SYBR Green	Clone C	$2.0 \times 10^1 – 2.0 \times 10^7$ copies	109.5
L1	SYBR Green	Clone D	$2.0 \times 10^1 – 2.0 \times 10^7$ copies	104.2

β-Actin	TaqMan probe	pCAG-mGFP-Actin	10^1–10^7 copies	108.2
β-Globin	TaqMan probe	Human placental DNA	0.3–300 ng	90.1