Characterization of novel bovine papillomavirus type 12 (BPV-12) causing epithelial papilloma

Wei Zhu[a#] • Jianbao Dong[ab#] • Erika Shimizu[c] • Shinichi Hatama[d] • Koichi Kadota[d] • Yoshitaka Goto[a] • Takeshi Haga[a,*]

a. Department of Veterinary Microbiology, University of Miyazaki, 1-1 Gakuen Kibanadai Nishi, Miyazaki, 889-2192, Japan.

b. JSPS Research Fellow DC (The United Graduate School of Veterinary Science, Yamaguchi University), Chiyoda, Tokyo, 102-8472, Japan.

c. Miyazaki Prefecture Tsuno Meat Inspection Center, Tsuno, 889-1201, Miyazaki, Japan.

d. Hokkaido Research Station, National Institute of Animal Health, 4 Hitsujigaoka, Toyohira, Sapporo 062-0045, Japan.

# These authors contributed equally to this work.

*Corresponding author. Tel: +81-985-58-7575, Fax: +81-985-58-7575

Email: a0d518u@cc.miyazaki-u.ac.jp

Running head: Characterization of BPV-12.

23 **Abstract** The bovine papillomavirus type 12 (BPV-12, putative type BAA1) was
24 detected in epithelial papilloma located on the tongue of infected cattle. Then the
25 whole genome was sequenced and phylogenetic analysis illustrated that it should be
26 classified in genus *Xipapillomavirus*. The viral genome is 7197 base pair in length and
27 contains five early ORFs (E1, E2, E4, E7 and E8), three late ORFs (L1, L2 and L3),
28 and a long control region which possesses replication regulatory elements. Meanwhile,
29 mRNA of each gene was detected in the papilloma sample. The papilloma was
30 identified as epithelial papilloma by histological and immunohistochemical
31 examination. Based on the genome information and pathological properties, BAA1
32 was designated as BPV-12 type in this study.
33

34 **Keywords** BPV-12 · Characterization · genome organization · epithelial papilloma
35

Introduction

Papillomaviruses (PVs) are widespread in nature and have been found in most mammals and birds, and more than 100 different types have been identified in humans [6, 9]. PVs have been classified into 30 genera (*Alphapapillomavirus* to *Dyoiotapapillomavirus*) according to the nucleotide sequence diversity in the open reading frame (ORF) of L1 gene and to their biological and pathological properties [5, 13]. For bovine papillomavirus (BPV), 11 types (BPV-1 to -11) have been characterized so far and classified into four genera: *Deltapapillomavirus* (BPV-1 and -2) cause fibropapillomas in cattle and sarcoids in horses, and exhibit a somewhat broader host range and tissue tropism than other types; *Xipapillomavirus* (BPV-3, -4, -6, -9, -10 and -11) are restricted to cattle and infect only epithelial cells to induce true epithelial papillomas; *Epsilonpapillomavirus* (BPV-5 and -8) appear to cause both fibropapillomas and true epithelial papillomas; and an unassigned genus (BPV-7) has been detected in healthy skin swab without pathopoiesis report [20, 21, 32, 33]. In addition, at least 16 novel putative BPV types (BAA1 to -4, BAPV3 to -5, BAPV7 to -10, BAPV11MY and BPV/BR-UEL2 to -5) have been detected in healthy skin swabs or cutaneous warts from cattle kept in Sweden, Japan and Brazil [1, 11, 20, 31].

BPV virion possesses a non-enveloped icosahedral structure of 55 - 60 nm diameter and the genome consists of a single molecule of circular double-stranded DNA approximately 7.2 - 8.0 kb in size, encoding some 8-10 proteins [7, 33]. The

58 whole genome can be divided into three parts depending on their function: long
59 control region (LCR), early region and late region. LCR is the region between the
60 early and late regions and contains several replication regulatory elements; the early
61 region encodes non-structural proteins designated E1-E8 (depending on the individual
62 virus) that exert important regulatory and replicative functions; the late region
63 encodes two structural proteins L1 and L2 that form capsid; BPV-4 also possesses L3
64 but the function is still unknown [34]. Although the genome is circular double-strand
65 DNA, the ORFs are located in the same strand, and the genome organizations are
66 similar to the known PVs.

67 During the last decade, the multiple-primed rolling circle amplification (RCA)
68 technique has been developed and optimized for rapid amplification of circular DNA
69 [14]. Then it was applied to amplify the whole genome of PVs [35-37] and became
70 one convenient tool for PVs molecular biological research. Recently, based on the
71 genome nucleotide sequence and biological and pathological properties, several new
72 BPV types were identified and designated from putative BPV types or clinical
73 samples. For example, BPV-7, -8, -9 and -10 were designated from putative BPV
74 types BAPV-6, -2, BPV-Type I and BPV-Type II, respectively [21, 32, 40]. Meanwhile,
75 BPV-11 was identified and designated from cutaneous warts in cattle [20]. Formal
76 designation is essential for BPV research to avoid misunderstanding caused by
77 complicated names.

78 BAA1 is one of the putative BPV types and was first detected in healthy skin
79 swab [1]. So far there has been no report regarding the pathogenicity of BAA1 and

80 just little information has been reported. In the present study, one BAA1 strain P38
81 was detected in the epithelial papilloma located on the tongue of infected cattle. The
82 whole genome was sequenced and mRNA was detected. Based on the ORF of L1
83 gene, phylogenetic analysis showed that BAA1 is a novel BPV type and should be
84 classified in genera *Xipapillomavirus*. According to the genome information and
85 pathological properties, BAA1 was designated as bovine papillomavirus type 12
86 (BPV-12).
87
88 **Materials and methods**
89
90 Histopathology and immunohistochemistry
91
92 Papilloma sample was collected by hand from the tongue of one infected cattle
93 (Fig.1A). Tissue samples were fixed in 10% buffered formalin, embedded in paraffin,
94 sectioned at 4μm, and stained with hematoxylin and eosin (HE) and Masson's
95 trichrome. Selected sections were labeled by the streptavidin-biotin-peroxidase
96 complex (SBC) method. As primary antibodies we used a rabbit polyclonal antibody
97 to bovine papillomavirus (Quartett GmbH, Berlin, Germany), mouse monoclonal
98 antibodies to cytokeratin (clone: MNF116) (Dako Corporation, Carpinteria, CA),
99 vimentin (Dako), and proliferating cell nuclear antigen (PCNA) (BioGenex
100 Laboratories, San Ramon, CA). Subsequent procedures were performed using
101 Histofine SAB-PO (R) and SAB-PO (M) kits (Nichirei, Tokyo, Japan).

102

103 DNA extraction and sequencing

104

105 DNA was extracted from the papilloma sample, which was located on the tongue
106 of infected cattle, with DNeasy blood and tissue kit (Qiagen, Maryland, MD). Then
107 PCR was performed with primer pair of FAP59/FAP64 to obtain 425 base pairs (bp)
108 of L1 gene [17]. Based on the known sequence, one pair of primers consisting of
109 BPV12F1 (5'-ATGCAGAAAACAGAACAAAGTACCC-3') and BPV12R1
110 (5'-GCAAAATTCTAAGCCTCTAATAGCC-3') was designed to amplify the
111 remaining part of the whole genome and PCR was performed with LA Taq kit (Takara,
112 Shiga, Japan) according to the protocol of the manufacturer. Subsequently, the PCR
113 products were gel purified by using a QIAquick gel extraction kit (Qiagen, Hilden,
114 Germany). Then primer walking sequencing was carried out with forward primers
115 BPV12F1, BPV12F2(5'-ATGAGGCAGACGAGAGAACA-3'),
116 BPV12F3(5'-TAGGTGCTTCACTATGTTTC-3'),
117 BPV12F4(5'-GCTCTGATGTGTCTGATTTG-3'), and
118 BPV12F5(5'-GCTGCTGTGTTTTGGTGGAA-3'); and reverse primers BPV12R1,
119 BPV12R2(5'-TCCAATGTCAGCAGCATCAT-3'),
120 BPV12R3(5'-ATCACTGCCATTTGTAGGAG-3'),
121 BPV12delR3(5'-CAAGACTAACCCCACTAATA-3'),
122 BPV12R4(5'-CGTCTGAAGCCTTTTAATGT-3'),
123 BPV12R5(5'-GCATTTTCAGGGTTATTATC-3'). In order to confirm the correct

124 sequence, the whole genome was sequenced at least three times for each reaction by
125 using a Bigdye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster
126 City, CA). Meanwhile, the whole genome was cloned into the pMD20-T vector
127 (Takara, Shiga, Japan) and sequenced. Sequencing was performed in an ABI Prism
128 3130 genetic analyzer (Applied Biosystems, Foster City, CA). To reconstitute the
129 full-length genome sequence, overlapping sequences were assembled into contiguous
130 sequences by using Seqman DNAStar software (Lasergene; DNAStar, Inc., Madison,
131 WI).

132

133 **Genome organization and phylogenic analysis**

134

135 For genome organization analysis, putative ORFs and their corresponding amino
136 acids were predicted by using the ORF finder tool
137 (http://www.ncbi.nlm.nih.gov/gorf/gorf.html), and similarity analysis was performed
138 with BLAST tool (http://blast.ncbi.nlm.nih.gov) of the National Center for
139 Biotechnology Information (NCBI). Peptide motif analysis was performed with the
140 TFSEARCH tool of Parallel Protein Information Analysis system
141 (http://www.cbrc.jp//research/db/TFSEARCHJ.html). Multiple nucleotide sequence
142 alignments and phylogenetic tree construction were performed by using the
143 neighbor-joining method with MEGA version 4 [39]. The GenBank accession
144 numbers of representative PV strains are the following: BPV-1 (X02346), BPV-2
145 (PPB2CG), BPV-3 (AJ620207), BPV-4 (X05817), BPV-5 (AJ620206), BPV-6

146 (AJ620208), BPV-7 (DQ217793), BPV-8 (DQ098913), BPV-9 (AB331650), BPV-10
147 (AB331651), BPV-11 (AB543507), CcPV-1 (EU493092), ChPV-1 (DQ091200),
148 COPV (L22695), CPV-2 (AY722648), CPV-5 (FJ492742), CRPV (K02708), EcPV
149 (AF498323), EcPV-2 (EU503122), EdPV-1 (AY684126), EhPV (FJ379293), FcPV
150 (AY057109), FdPV-2 (EU796884), FlPV-1 (EU188799), HaOPV (E15111), HPV-1
151 (V01116), HPV-4 (X70827), HPV-5 (M17463), HPV-16 (K02718), HPV-41 (X56147),
152 MnPV (U01834), PePV (AF420235), PsPV-1 (AJ238373), PePV (AF420235),
153 SsPV-1 (EF395818), TmPV-1 (AY609301),TtPV-1 (EU240894), UmPV-1
154 (EF536349).

155

156 Total RNA extraction and mRNA detection with RT-PCR

157

158 Total RNA was extracted from the papilloma sample with RNeasy Mini kit
159 (Qiagen, Maryland, MD). In order to perform gene expression analysis accurately and
160 eliminate the contaminating genomic DNA, total RNA was treated and
161 reverse-transcripted using PrimeScript RT reagent kit with gDNA eraser (Takara,
162 Shiga, Japan) according to the instructions of the manufacturer. Before reverse
163 transcription, PCR was performed to confirm that genomic DNA had been thoroughly
164 eliminated after treatment with gDNA eraser. Eight pairs of primer were designed to
165 detect the mRNA of each gene according to the ORFs in whole genome: E1F
166 (5’-TTGTGTGAATCTGGTTGCTC-3’) and E1R
167 (5’-AGTCACTTTGGCATTTCCTC-3’); E2F

168 (5'-CAGGAGGCAAGGAATAACTA-3') and E2R
169 (5'-GGTCAGTGTCAGACGAGGAA-3'); E4F
170 (5'-CCCAAGGACCACGACAACTC-3') and E4R
171 (5'-GTGCTGTCTCCTCGTCTGAA-3'); E7F
172 (5'-AACGATGACCCATACGCAGT-3') and E7R
173 (5'-AAGATTCAAAGAACAGGCAG-3'); E8F
174 (5'-TGTTTTGGTATTGCTTTGGT-3') and E8R
175 (5'-ATTCTTTGTACTAACAATGC-3'); L1F (5'-ATTAGACACGGATGACTTTG-3')
176 and L1R (5'-ACATCTGGACCTGCTTGGGA-3'); L2F
177 (5'-CAGTCGTGGCAGTAATGTTG-3') and L2R
178 (5'-TTCCCCTGGTAATTGTGTTG-3'); L3F (5'-TCTTTGGTGTCCAGCGATGT-3')
179 and L3R (5'-TGCCTCATCTCCTGTAGCCT-3'). The annealing temperature was
180 optimized with temperature-gradient PCR method by using Veriti 96-well thermal
181 cycler (Applied Biosystems, Foster City, CA). The optimized annealing temperatures
182 were the following: E1 (58°C), E2 (56°C), E4 (62°C), E7 (60°C), E8 (54°C), L1
183 (58°C), L2 (60°C) and L3 (60°C). The sensitivity of the PCR for each gene was
184 determined using serial dilutions of purified PCR products, and the result showed that
185 all PCRs can detect at least 10 attogram (ag). To confirm the mRNA detection result,
186 RT-PCR for each gene was carried out six times and the RT-PCR products were
187 sequenced. Then sequences of the products were aligned with the whole genome by
188 using MegAlign DNAStar software (Lasergene; DNAStar, Inc., Madison, WI).
189

190 Nucleotide sequence accession numbers

191

192 The nucleotide sequences of the BPV-12 whole genome has been deposited in the

193 GenBank sequence database under accession numbers: BPV-12 (JF834523).

194

195 **Results and discussion**

196

197 The papilloma sample was confirmed as epithelial papilloma

198

199 The neoplastic tissue was composed of sheet-like growths of squamous epithelial

200 cells (Fig. 1B). Basal cells were detected at the periphery of the growths, and their

201 surface was moderately hyperkeratotic. The squamous epithelial cells showed positive

202 immunelabeling for cytokeratin (Fig. 1C), whereas subepithelial connective tissue

203 cells expressed vimentin. The basal cells were weakly positive for cytokeratin and

204 intensely positive for proliferating cell nuclear antigen (PCNA) (Data not shown). On

205 the basis of the histological and immunohistochemical findings, a diagnosis of

206 epithelial papillomatosis was made.

207

208 Genome organization of BPV-12

209

210 The genome of BPV-12 is 7197 bp in length and possesses a common genomic

211 structure that consists of three regions of LCR, early and late regions which shared by

212 almost all known PVs. It contains five early (E1, E2, E4, E7 and E8) and three late
213 (L1, L2 and L3) genes which are located in the same strand (Fig. 2). The restriction
214 enzyme *HpaI* site or an analogous sequence has been used to number the genome of
215 PVs. But there is no appropriate *HpaI* site in the genome of BPV-12. In order to allow
216 direct comparison with other PVs, the single *Hind III* site (AAGCTT) has been
217 chosen to number the origin of the BPV-12 genome.

218 LCR is the non-coding-region that contains virus replication regulatory elements
219 and controls the transcription of transforming genes such as E5, E6 and E7 in PVs.
220 Some PVs, such as BPV-4, possess two non-coding-regions: the first one is located in
221 the upstream of the early region and the second is between the L2 and L1 ORFs [34].
222 In the genome of BPV-12, LCR is 488 bp in length, is located at nt 6926-216 (Fig. 2),
223 and has no second non-coding-region. In the previous studies, the results of transitory
224 replication assays illustrated that two viral proteins, E1 and E2, are essential for
225 genome replication [26, 41]. Both E1 and E2 proteins bind to the virus replication
226 origin located in LCR and activate the genome DNA replication. Most PVs possess
227 one E1-binding site (E1BS) and at least two E2-binding sites (E2BS). BPV-12 has
228 two putative E2BS (ACCGTTTTCGGT) located at nt 6932-6943 and 62-73. Besides
229 E2BS, other important transcription and replication regulatory elements have been
230 also identified in the LCR of BPV-12. There is one putative polyadenylation (polyA)
231 site (AATAAA) located at nt 7022-7027 for late mRNA. Two consensus sequences of
232 TATA boxes (TATAAAA) are located at nt 170-176 and 7193-2.

233 LCRs of mucosal epitheliotropic papillomaviruses possess similar organizations:

234 a promoter region, an enhancer region, and a highly conserved distribution of E2
235 DNA binding sites [15]. The enhancer of these viruses is specific to epithelial cells but
236 fails to activate transcription from heterologous promoters in non-epithelial cell types
237 [18]. BPV-12 was detected in epithelial papilloma located on the tongue. Therefore, it
238 is worthwhile to compare motifs of its LCR with other epitheliotropic
239 papillomaviruses. The epithelial cell-specific enhancers contain several binding sites
240 for cellular transcription factors including AP-1, Oct-1, NF-1, PEF-1, TEF-1, Sp1,
241 C/EBPbeta, glucocorticoid receptor, and so on [15]. In the LCR of BPV-12, the
242 consensus sequences of one NF-1, one C/EBPbeta and two Oct-1 sites were found.
243 NF-1 site acts positively [2, 3, 30], while C/EBPbeta and Oct-1 sites has been proved
244 to negatively regulate PVs enhancer activity [4, 22, 28].

245 The early region of PVs encodes non-structural viral proteins involved in viral
246 DNA replication, transcription and cell transformation. Depending on the individual
247 virus, the early region can encode 8 proteins (E1-E8) [23]. The early region of
248 BPV-12 contains ORFs of E8 (126 bp), E7 (273 bp), E1 (1791 bp), E2 (1254 bp), and
249 E4 (474 bp) in order, but E5 and E6 are absent. E5 was reported to be the major
250 transforming oncoprotein that destabilizes the function of many membrane proteins in
251 the infected cell for some certain BPVs [8], and E6 is also involved in cell
252 transformation and is clearly associated with carcinogenesis that is the stimulation of
253 the ubiquitin-mediated degradation of the p53 tumor suppressor protein [38]. In
254 BPV-12, E8 is located in the position of E5 or E6, and it has been reported to be
255 chemically and functionally similar to the E5 gene from some human

256 papillomaviruses and may also substitute for the E6 gene [24, 27]. Therefore, E8 may
257 play the role of E5 or E6 for BPV-12 transcription and other functions. The E7 ORF
258 of BPV-12 possesses a putative retinoblastoma tumor-suppressor protein-binding
259 domain (pRbBD: LxCxE), which is thought to be the biological significance of
260 epitheliotropic papillomavirus, including BPV-3, -4, -6, -9, -10 and -11 [10, 12, 16, 20,
261 21], while all PVs with pRbBD-absent E7 are fibropapillomaviruses such as BPV-1,
262 -2, -5, -7, EEPV and DPV [29, 32]. Therefore, it has been suggested that BPV-12 is an
263 epitheliotropic papillomavirus, which is consistent with the results of histological and
264 immunohistochemical examination. Moreover, this result contributed one more piece
265 of evidence that supports the above conclusion. One putative Zinc-binding domain
266 (ZnBD: CxxC-X_{29}-CxxC) was also found in E7 ORF, which works with pRbBD to be
267 responsible for immortalization and transformation of host cells [10, 25]. E4 ORF is
268 located inside E2 ORF in BPV-12 genome. This arrangement is similar to BPV-4, -7,
269 -8, -10 and -11.

270 The late region of BPV-12 contains three ORFs: L1 (1521 bp), L2 (1578 bp) and
271 L3 (234 bp). L1 and L2 code for the major and minor capsid proteins, respectively,
272 and cooperate with each other to package the viral DNA into the virion [23]. L3 ORF
273 overlaps with L1 and is only occasionally found in PVs. So far L3 has been detected
274 in BPV-4 (Xi-PV) and deer papillomavirus OvPV1 (Delta-2) [19, 34]. And L3 ORF of
275 BPV-12 shares 71.4% and 10.4% with that of BPV-4 and OvPV1. However the
276 significance is still unclear.

277

278 BPV-12 was designated and classified into genus *Xipapillomavirus*

279

280 L1 ORF of BPV-12 shares 55.1-69.0% sequence identity with other BPV types,
281 while the whole genome shares 12.3-61.9%. Moreover, other ORFs of BPV-12 share
282 9.5%-59.5% (E8), 4.0%-54.2% (E7), 34.6%-68.0% (E1), 13.8%-62.1% (E2),
283 8.5%-60.3% (E4), 2.8%-65.7% (L2) with other BPV types. It demonstrated that there
284 was no ancestral recombination events happened in BPV-12. Additionally, based on
285 the 425bp segment amplified with primer set of FAP59/64, BPV-12 possesses 96%
286 sequence identity with the putative BAA1 strain (AF485375) deposited in GenBank
287 and this strain was identical to the putative type BAA1. In 1995, the definition of new
288 PV types was released from the International Papillomavirus Workshop held in
289 Quebec. One PV strain can be recognized as a new type if the complete genome has
290 been cloned and the DNA sequence of the L1 ORF shares less than 90% homology
291 with the closest known PV type [13]. Based on this criterion, BPV BAA1 putative
292 type should be designated as a new BPV type. Moreover, L1 has been used for the
293 identification of new PV types by phylogenetic analysis during the past 20 years [13,
294 20]. In the present study, the type of BPV-12 was identified from the nucleotide
295 sequence of L1 ORF. As can be seen from the phylogenetic tree (Fig.3), BPV-12 was
296 clearly classified into the *Xi-papillomavirus* genera which includes BPV-3, -4, -6, -9,
297 -10 and -11, and these virus infect only epithelia cells to induce true epithelial
298 papillomas [20, 21, 32, 33]. Based on the criterion for a new PV type and the result of
299 phylogenetic analysis, BPV BAA1 putative type was designated as BPV-12 in the

present study.

Message RNA (mRNA) was detected for each gene in the papilloma sample

In order to confirm the etiological role of BPV-12 in epithelial papilloma, viral mRNA was detected from total RNA with RT-PCR. The result showed that mRNA of each gene was detected (Fig. 4) and identified with sequencing, suggesting the epithelial papilloma was caused by BPV-12 in infected cattle. The ORFs of E4 and L3 are located inside of E2 and L1, respectively. Therefore, the transcription of E2 and L1 might disturb the detection of E4 and L3 and further confirmation is still necessary for both. The transcription of PVs is complex because of the presence of multiple promoters, alternate and multiple splice patterns, and the differential production of mRNA species in different cells [23]. Although mRNA was detected for each gene in the present study, the transcription map of BPV-12 is still unclear. Therefore, further study is necessary to determine the promoters and mRNA species for BPV-12 in epithelial papilloma.

Acknowledgment This work was partly supported by the Project for Zoonoses Education and Research, University of Miyazaki, and by a grant from Japan Society for the Promotion of Science (no. KAKENHI22780272).

322

323 FIG.1 (A): Macroscopic view of the papilloma sample located on the tongue of an infected cattle.

324 Bar = 1cm (B): Histological section (H＆E staining). Bar = 40μm. (C): Immunohistochemical

325 staining of cytokeratin to confirm epithelial papilloma. Bar = 40μm.

326

327 FIG. 2 Genomic map of BPV-12. The circular genomes are represented in a linearized version. The

328 ORFs are depicted as a rectangle. Putative motifs of LCR are indicated with different symbols as

329 well as names.

330

331 FIG. 3 Phylogenetic tree of BPV-12 and PVs classified in genera *Alphapapillomavius* to

332 *Sigmapapillomavirus*. The tree was constructed with the Neighbour-joining method based on the

333 nucleotide sequence of L1 ORF. The number on the scale bar shows the percent occurrence in

334 1000 bootstrap replicates. Abbreviations: CcPV-1 (Caretta caretta PV-1), ChPV-1 (Capra hircus

335 PV-1), COPV (Canine oral PV), CPV (Canine PV), CRPV (Cottontail rabbit PV), EcPV (Equus

336 caballus PV), EcPV-2 (Equus caballus PV-2), EdPV-1 (Erithizon dorsatum PV-1), EhPV

337 (European hedgehog PV), FcPV (Fringilla coelebs PV), FdPV-2 (Felis domesticus PV-2), FlPV-1

338 (Francolinus leucoscepus PV-1), HaOPV (Hamster oral PV), HPV (Human PV), MnPV

339 (Mastomys natalensis PV), PePV (Psittacus erithacus timneh PV), PsPV (Phocoena spinipinnis

340 PV), RaPV-1 (Rousettus aegyptiacus PV-1), SsPV-1 (Sus scrofa PV-1), TmPV (Trichechus

341 manatus latirostris PV), TtPV-1 (Tursiops truncates PV-1), UmPV-1 (Ursus maritimus PV-1).

342

343 FIG.4 RT-PCR result of mRNA detection for each gene from the papilloma sample. Lane P:

344 positive control with purified PCR product; Lane N: negative control in which the template was

345 replaced with distilled water; Lane R: the template is total RNA treated with gDNA eraser; Lane S:

346 the template is cDNA reverse-transcripted from total RNA treated with gDNA eraser.

347

348

349

350 ## References

351

352 1. Antonsson A, Hansson BG (2002) Healthy skin of many animal species harbors
353 papillomaviruses which are closely related to their human counterparts. J Virol 76:
354 12537-12542

355 2. Apt D, Chong T, Liu Y, Bernard HU (1993) Nuclear factor I and epithelial cell-specific
356 transcription of human papillomavirus type 16. J Virol 67: 4455-4463

357 3. Apt D, Liu Y, Bernard HU (1994) Cloning and functional analysis of spliced isoforms of human
358 nuclear factor I-X: interference with transcriptional activation by NFI/CTF in a cell-type
359 specific manner. Nucleic Acids Res 22: 3825-3833

360 4. Bauknecht T, Shi Y (1998) Overexpression of C/EBPbeta represses human papillomavirus type
361 18 upstream regulatory region activity in HeLa cells by interfering with the binding of
362 TATA-binding protein. J Virol 72: 2113-2124

363 5. Bernard HU, Burk RD, Chen Z, van Doorslaer K, Hausen H, de Villiers EM Classification of
364 papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments.
365 Virology 401: 70-79

366 6. Bernard HU (2005) The clinical importance of the nomenclature, evolution and taxonomy of
367 human papillomaviruses. J Clin Virol 32 Suppl 1: S1-6

368 7. Borzacchiello G, Roperto F (2008) Bovine papillomaviruses, papillomas and cancer in cattle.
369 Vet Res 39: 45

370 8. Bravo IG, Alonso A (2004) Mucosal human papillomaviruses encode four different E5 proteins

371 whose chemistry and phylogeny correlate with malignant or benign growth. J Virol 78:
372 13613-13626
373 9. Campo MS (2002) Animal models of papillomavirus pathogenesis. Virus Res 89: 249-261
374 10. Chan HM, Smith L, La Thangue NB (2001) Role of LXCXE motif-dependent interactions in the
375 activity of the retinoblastoma protein. Oncogene 20: 6152-6163
376 11. Claus MP, Lunardi M, Alfieri AF, Ferracin LM, Fungaro MH, Alfieri AA (2008) Identification of
377 unreported putative new bovine papillomavirus types in Brazilian cattle herds. Vet Microbiol
378 132: 396-401
379 12. Dahiya A, Gavin MR, Luo RX, Dean DC (2000) Role of the LXCXE binding site in Rb function.
380 Mol Cell Biol 20: 6799-6805
381 13. de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H (2004) Classification of
382 papillomaviruses. Virology 324: 17-27
383 14. Dean FB, Nelson JR, Giesler TL, Lasken RS (2001) Rapid amplification of plasmid and phage
384 DNA using Phi 29 DNA polymerase and multiply-primed rolling circle amplification. Genome
385 Res 11: 1095-1099
386 15. Desaintes C, Demeret C (1996) Control of papillomavirus DNA replication and transcription.
387 Semin Cancer Biol 7: 339-347
388 16. Dick FA, Dyson NJ (2002) Three regions of the pRB pocket domain affect its inactivation by
389 human papillomavirus E7 proteins. J Virol 76: 6224-6234
390 17. Forslund O, Antonsson A, Nordin P, Stenquist B, Hansson BG (1999) A broad range of human
391 papillomavirus types detected with a general PCR method suitable for analysis of cutaneous
392 tumours and normal skin. J Gen Virol 80 (Pt 9): 2437-2443

393 18. Gloss B, Bernard HU, Seedorf K, Klock G (1987) The upstream regulatory region of the human
394 papilloma virus-16 contains an E2 protein-independent enhancer which is specific for cervical
395 carcinoma cells and regulated by glucocorticoid hormones. EMBO J 6: 3735-3743
396 19. Groff DE, Lancaster WD (1985) Molecular cloning and nucleotide sequence of deer
397 papillomavirus. J Virol 56: 85-91
398 20. Hatama S, Ishihara R, Ueda Y, Kanno T, Uchida I Detection of a novel bovine papillomavirus
399 type 11 (BPV-11) using xipapillomavirus consensus polymerase chain reaction primers. Arch
400 Virol
401 21. Hatama S, Nobumoto K, Kanno T (2008) Genomic and phylogenetic analysis of two novel
402 bovine papillomaviruses, BPV-9 and BPV-10. J Gen Virol 89: 158-163
403 22. Hoppe-Seyler F, Butz K, zur Hausen H (1991) Repression of the human papillomavirus type 18
404 enhancer by the cellular transcription factor Oct-1. J Virol 65: 5613-5618
405 23. Howley PM, Lowy DR (2007) Papillomaviruses. In: Knipe DM, Howley PM (eds) Fields Virology.
406 Lippincott Williams & Wilkins, Philadelphia, pp 2299-2354
407 24. Jackson ME, Pennie WD, McCaffery RE, Smith KT, Grindlay GJ, Campo MS (1991) The B
408 subgroup bovine papillomaviruses lack an identifiable E6 open reading frame. Mol Carcinog 4:
409 382-387
410 25. Liu X, Clements A, Zhao K, Marmorstein R (2006) Structure of the human Papillomavirus E7
411 oncoprotein and its mechanism for inactivation of the retinoblastoma tumor suppressor. J
412 Biol Chem 281: 578-586
413 26. Lu JZ, Sun YN, Rose RC, Bonnez W, McCance DJ (1993) Two E2 binding sites (E2BS) alone or
414 one E2BS plus an A/T-rich region are minimal requirements for the replication of the human

415 papillomavirus type 11 origin. J Virol 67: 7131-7139

416 27. Morgan IM, Campo MS (2000) Recent developments in bovine papillomaviruses.
417 Papillomavirus Rep 11: 127-132

418 28. Morris PJ, Dent CL, Ring CJ, Latchman DS (1993) The octamer binding site in the HPV16
419 regulatory region produces opposite effects on gene expression in cervical and non-cervical
420 cells. Nucleic Acids Res 21: 1019-1023

421 29. Narechania A, Terai M, Chen Z, DeSalle R, Burk RD (2004) Lack of the canonical pRB-binding
422 domain in the E7 ORF of artiodactyl papillomaviruses is associated with the development of
423 fibropapillomas. J Gen Virol 85: 1243-1250

424 30. O'Connor M, Bernard HU (1995) Oct-1 activates the epithelial-specific enhancer of human
425 papillomavirus type 16 via a synergistic interaction with NFI at a conserved composite
426 regulatory element. Virology 207: 77-88

427 31. Ogawa T, Tomita Y, Okada M, Shinozaki K, Kubonoya H, Kaiho I, Shirasawa H (2004)
428 Broad-spectrum detection of papillomaviruses in bovine teat papillomas and healthy teat skin.
429 J Gen Virol 85: 2191-2197

430 32. Ogawa T, Tomita Y, Okada M, Shirasawa H (2007) Complete genome and phylogenetic
431 position of bovine papillomavirus type 7. J Gen Virol 88: 1934-1938

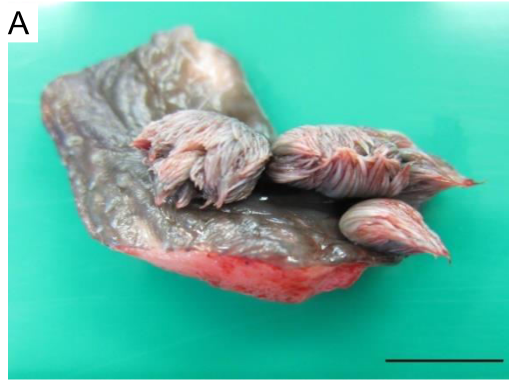
432 33. Parrish CR (2011) Papillomaviridae and Polyomaviridae. In: Maclachlan NJ, Dubovi EJ (eds)
433 Fenner's Veterinary Virology. Elsevier, Oxford, pp 213-223

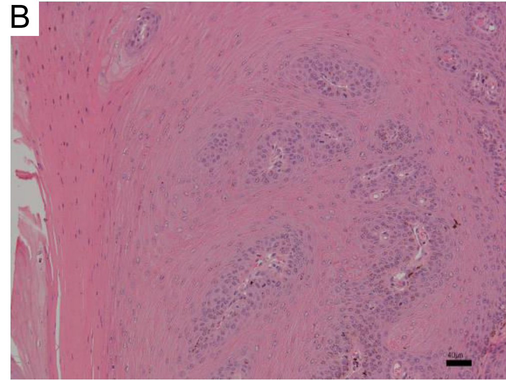
434 34. Patel KR, Smith KT, Campo MS (1987) The nucleotide sequence and genome organization of
435 bovine papillomavirus type 4. J Gen Virol 68 (Pt 8): 2117-2128

436 35. Rector A, Bossart GD, Ghim SJ, Sundberg JP, Jenson AB, Van Ranst M (2004) Characterization

437 of a novel close-to-root papillomavirus from a Florida manatee by using multiply primed
438 rolling-circle amplification: Trichechus manatus latirostris papillomavirus type 1. J Virol 78:
439 12698-12702
440 36. Rector A, Tachezy R, Van Ranst M (2004) A sequence-independent strategy for detection and
441 cloning of circular DNA virus genomes by using multiply primed rolling-circle amplification. J
442 Virol 78: 4993-4998
443 37. Rector A, Tachezy R, Van Doorslaer K, MacNamara T, Burk RD, Sundberg JP, Van Ranst M
444 (2005) Isolation and cloning of a papillomavirus from a North American porcupine by using
445 multiply primed rolling-circle amplification: the Erethizon dorsatum papillomavirus type 1.
446 Virology 331: 449-456
447 38. Scheffner M, Whitaker NJ (2003) Human papillomavirus-induced carcinogenesis and the
448 ubiquitin-proteasome system. Semin Cancer Biol 13: 59-67
449 39. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis
450 (MEGA) software version 4.0. Mol Biol Evol 24: 1596-1599
451 40. Tomita Y, Literak I, Ogawa T, Jin Z, Shirasawa H (2007) Complete genomes and phylogenetic
452 positions of bovine papillomavirus type 8 and a variant type from a European bison. Virus
453 Genes 35: 243-249
454 41. Ustav M, Ustav E, Szymanski P, Stenlund A (1991) Identification of the origin of replication of
455 bovine papillomavirus and characterization of the viral origin recognition factor E1. EMBO J
456 10: 4321-4329
457
458

Fig.1





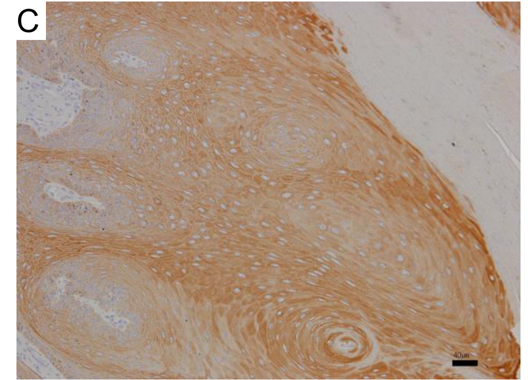
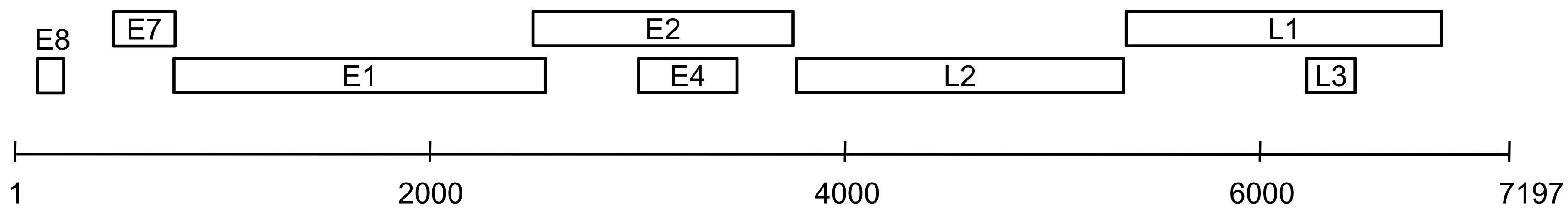


Fig.2

BPV-12



LCR

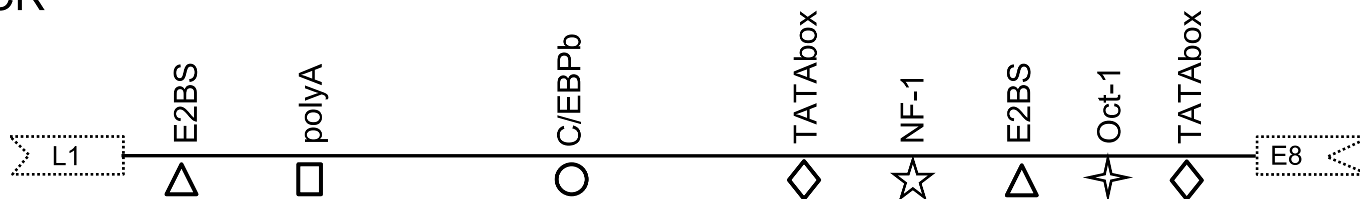


Fig. 3

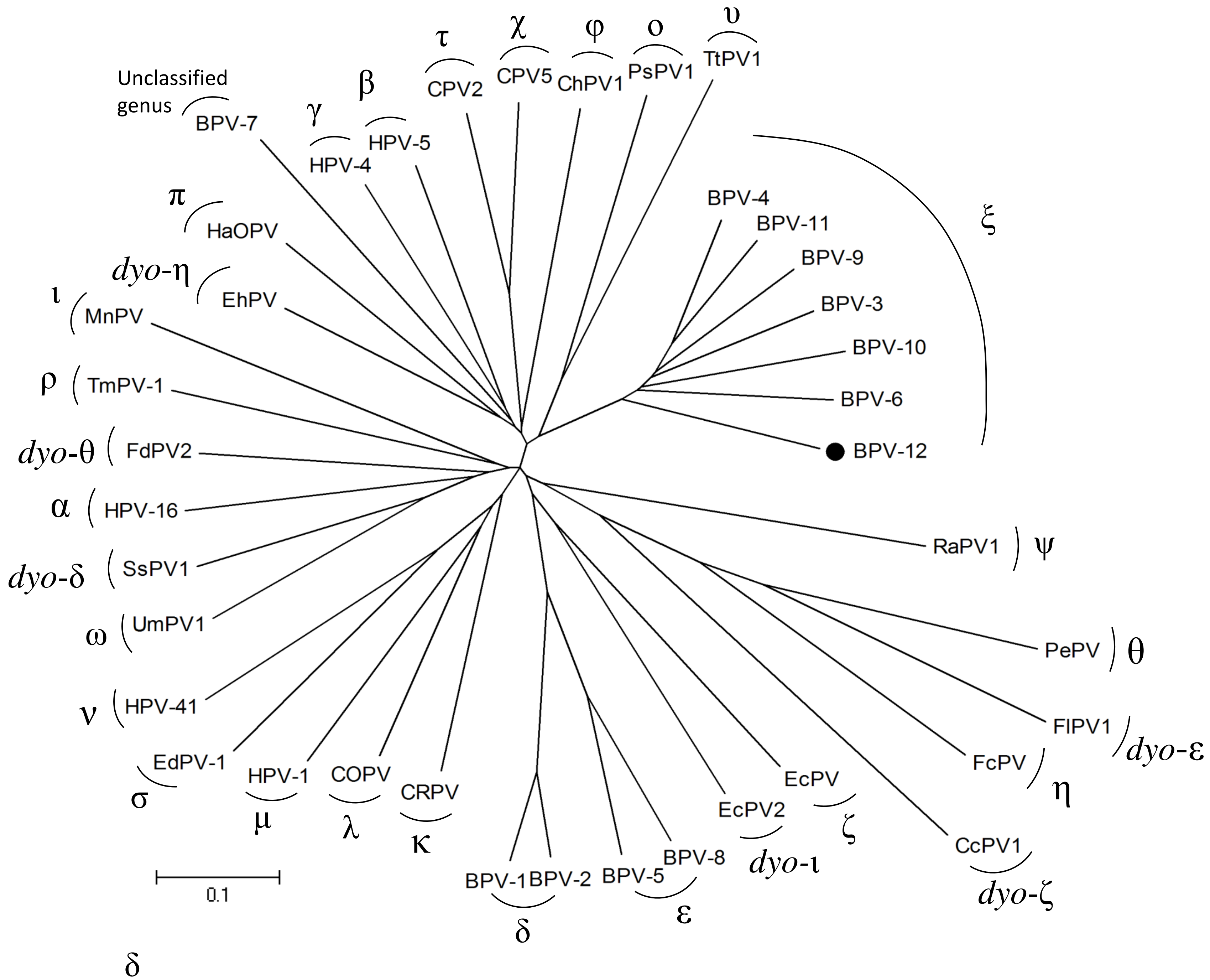


Fig. 4