

# Human Adenovirus B7d–Associated Urethritis after Suspected Sexual Transmission, Japan

## Appendix

### Additional References

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**Appendix Table 1.** Primer name and sequence used in study of human adenovirus B7d-associated urethritis, Japan.

Name	Sequence
Ad7_5'-1	TCTATTTAATATACCTTATAGATG
Ad7_5'-2	ACTCTTGAGTGCCAGCGAGAAGAG
Ad7_5'-3	AAGCTGCCAGCGATGTTTTAAGTTGG
Ad7_5'-4	TAAAATTATGTCAGCTGCTGAGTG
Ad7_5'-5	ACAATCTTAGACTGCGCCAGTGC
Ad7_5'-6	TAATAGATACACAAGATAAAGCAG
Ad7_5'-7	TCTTGCTACTGTGCATATCGTTTAC
Ad7_5'-8	AGTATTTAGCCCTTATCTGACGGGC
Ad7_5'-9	TTGAGAACTCGGTGGATCTTTCCAG
Ad7_5'-10	TCTGAAACATCATAGTTATGCTCC
Ad7_5'-11	AAAGAGTCTGTTGCAAGAGCTCGAGCC
Ad7_5'-12	AGGTCAGATCCGGCTCATCGGGGTC
Ad7_5'-13	TAGGAACGAGGAGGATTTGATATTGAC
Ad7_5'-14	TCTCGAACTGCCAGCGCGCTCATAGG
Ad7_5'-15	AGTACTCTTCGAGGGGAAACCCGTC
Ad7_5'-16	AGTGTTGAGGGGCCATTGTCGACG
Ad7_5'-17	TGACTTCCTTTGACGAGAAATTTAG
Ad7_5'-18	TGGTACTGCGCCCTGAGAAGACTCG
Ad7_5'-19	AGAAATACATGATCCATCGTCTCAGC
Ad7_5'-20	ATCGTCTCAAGATCCACGGGATCTG
Ad7_5'-21	ATTGTGTAGGAGCAGTTGGCCATGAC
Ad7_5'-22	AGCCGCAGCTAACGTGGTACTGGC
Ad7_5'-23	ACTGGCGCGCTGGGCGCACCATCG
Ad7_5'-24	TAACTACTGCGGCTGCAGCCGTCAGC
Ad7_5'-25	ATCAGCCCAGCTAGGGCACATGTGG
Ad7_5'-26	ACTCTGAGCGACGATCTGGGGGTG
Ad7_5'-27	AACCCACCCACGAGAAGGTCCTGG

Name	Sequence
Ad7_5'-28	TGCTGCTGCTGCTGGTATCC
Ad7_5'-29	AGTACGACATGCCCGACCCCAATG
Ad7_5'-30	TTGGGTGGAAGAGGAGGGGGCAACC
Ad7_5'-31	ATGGGGCGGTCATCTGAAGACCATC
Ad7_5'-32	ATGTATGAAGATCTTGAAGGGGG
Ad7_5'-33	AATGAGCAAGCCGTGTACTCTCAGC
Ad7_5'-34	TGCTCGTAATTATACTCCTACTGCGC
Ad7_5'-35	AAAAGCCCCGAAAATCAAGCGGGTC
Ad7_5'-36	AGCCTGAGGTCAAAGTAAGACCTATC
Ad7_5'-37	ATATGGCCCTCACTTGCCGCCTTCG
Ad7_5'-38	AAATAAGCTTAAGGAACAAAACCTCC
Ad7_5'-39	TCTGAACAGCATCGTGGGTCTGGGC
Ad7_5'-40	TAGCTTCAAGCCATATTCCGGCAC
Ad7_5'-41	AATTTGAAACTCCAGACAGCCATG
Ad7_5'-42	TTAATATCCAAGCTAATCTTTGGAG
Ad7_5'-43	ACTCTTGAAGCCATGCTGCGCAACG
Ad7_5'-44	AAACTTCCAGCCTATGAGCAGGCAGG
Ad7_5'-45	AGCTGCAGCCATGTCATGCGGGTC
Ad7_5'-46	AATCTCCCCAAGTGCAGCCCACCC
Ad7_5'-47	TGTCTCACGCTTGCCAGCACGGTCGG
Ad7_5'-48	TGCATCCATGAGCCACAGAGCGC
Ad7_5'-49	TGGTCTTTCTGGGCTTCTTCTTGGG
Ad7_5'-50	AAGAGATTGAGGCAGATGTGCGAGCAGG
Ad7_5'-51	TTACCTGATATAGCTTCCTTGAAGAG
Ad7_5'-52	ACCTTTAGACATGGCTTCGTGCGG
Ad7_5'-53	ATGGAGCCACTGCTACCTGTTCCGC
Ad7_5'-54	AGGATGTCCCATCGCCGAGGAAGC
Ad7_5'-55	ATTACTACCGTCACCTCCACAGCC
Ad7_5'-56	TTGGCAGCAGGCGCCTCCCAGGAC
Ad7_5'-57	TCTCCAGTTCGTGGAGGAGTTTACTCC
Ad7_5'-58	ACCAACCAGATCTTCCAGAAGACCC
Ad7_5'-59	ACTGCAGGTCCGTTGAAATTACAC
Ad7_5'-60	ATTTGGACCAAACCTTGAAGTGTTG
Ad7_5'-61	TTGGCTCAACATGTACACTACAAGG

Name	Sequence
Ad7_5-62	TATAGAATTATGATATTGTTTCAATC
Ad7_5-63	ACTTCTTAGGCTTATTTAAAACCATGC
Ad7_5-64	AACTACGCATCCGCCAGCAGCAGG
Ad7_5-65	TGTACCCACAATCTTCATGTCTTTC
Ad7_5-66	TAATTCTAACACTAGTTAAAAGTGG
Ad7_5-67	ACGACTGACAAATAAAGTTTAACTTG
Ad7_5-68	ACTTAGATTACTACAGTAGGTACAGC
Ad7_5-69	ACGAACACAACCTTACACTATGCATAG
Ad7_5-70	TAGATCGCGCAGATGGCATCTATCG
Ad7_5-71	TACAAGCGCAGACCTCCCCAATTGG
Ad7_5-72	ATGCTTAATCTTAAGTATAGCAAAGCC
Ad7_5_P7900	TTGTACAGACGGCCGCAGTACTCGC
Ad7_3_P9070	TTTTCAACTTTGCCGTGGACTTCTAC
Ad7_3-1	TCTATATAATATACCTTATAGATGG
Ad7_3-2	ATACTTAAGATTAAGCATAATTATACC
Ad7_3-3	TGTAGCGTCCCCTGCTATTGTTCCC
Ad7_3-4	ATTTGAAGTACTGCGAGATCGTTTGG
Ad7_3-5	TTCCTTGTTCTGCCAGCTTTACTGTTT
Ad7_3-6	TCAGAATGCGTTGCTGCGCGCACC
Ad7_3-7	TCTAATGTAGTAAAAGGTAAATGGAG
Ad7_3-8	ATTCAGTGGAGGCCATTATTTGACAG
Ad7_3-9	TTTGACATCCCCTTTAAAGTATGGAG
Ad7_3-10	TTTGCAATTGGTGAATTTGGATGAC
Ad7_3-11	ATTTTGATTGCGGTATTTCGGGATGG
Ad7_3-12	ATGTCAAATTTAGTAATGCATCAC
Ad7_3-13	ATAAGTTACAGCTGCAAGGCTAGTAATG
Ad7_3-14	AACCTCTGAAGTAATTGGGGGCC
Ad7_3-15	ACTTAATAAGAAGTCCACAGAGACG
Ad7_3-16	TTGTAAAGTCCTGATTGAAGAAGCGG
Ad7_3-17	TTCCGATGCCGCCCGAGCGGGGC
Ad7_3-18	ATTTGGGGCTGATAGCTCCACATG
Ad7_3-19	AAGTTCACACCGTGGTGAAGAGCAG
Ad7_3-20	AACCTTGTTGTTGAGCTCCTCACCGG
Ad7_3-21	AGAGGCACAAAGTCGGAGGGCAGCG

Name	Sequence
Ad7_3'-22	TAGTGCAGGTTCTCCTCTAGCTTGC
Ad7_3'-23	AGCTCCCGGACCCAAGTTGAGAAGGG
Ad7_3'-24	TTTCACATTATCCTGCGCCTGCATC
Ad7_3'-25	AGCATGTCCCTCTGCAAGACATCGGC
Ad7_3'-26	ATTATGAAGGCAAGGTGAAATGCC
Ad7_3'-27	TTCCAGTGTTGCAACCCAGGTACCG
Ad7_3'-28	TTTATTGAACACGGTTTTACATGAC
Ad7_3'-29	AAGCAAGAGGCTTCTTATGTGGTGGC
Ad7_3'-30	AAAAAGGAGTACATGCGATCCTTG
Ad7_3'-31	TGAAGCGGTGTTGTGAGCCATGGG
Ad7_3'-32	TTCCATAGCCAGATTGTTGCCTATGG
Ad7_3'-33	TTCCGTGTAAAGCACAAATTCAGGCG
Ad7_3'-34	TAATGTCAAAGAATGTGCTGGCCATG
Ad7_3'-35	AGACCCACGATGCTGTTCAAGTAC
Ad7_3'-36	AAGCTTTGTTCCCATAGGTTTTTACGG
Ad7_3'-37	AGGCAGCGTCAACAGTCATTAAGTGG
Ad7_3'-38	ATTTTCTCCAGTACGTCTTCTAGCC
Ad7_3'-39	TTTCATCCTTCACCGGTGGACCGTAG
Ad7_3'-40	TCGGCGACCACCTGGTTCGATCACATC
Ad7_3'-41	TAGAAACTCTTTGAGAAGACGGGC
Ad7_3'-42	TTTGAAACCCTCCTGGAATGGATGTC
Ad7_3'-43	TTCGTCAAAGTTGATGGTCTGGGTG
Ad7_3'-44	TCCAACACGCTGCTATCATCGGCAG
Ad7_3'-45	AAACCAGGTGGGGGCAGCCAGTGTG
Ad7_3'-46	TTGGGAGTCAGCAAGCTAGACACGGTC
Ad7_3'-47	AAAGGCGGTTGGCCTGGGGTTGCTG
Ad7_3'-48	AGAGTCATGCGCATGTAAAACCCATC
Ad7_3'-49	TCCTCGTCTTGCAGCACCCGTCTTCG
Ad7_3'-50	AGTAGTTACAGGAGCAGGAAGAGCC
Ad7_3'-51	TCTTCCAATTCCAGATCATAGGCGG
Ad7_3'-52	TGATCCGAGATTGAACCGGGGTAC
Ad7_3'-53	TTTGTGGACTTTGACGACTTCCAAG
Ad7_3'-54	TTGCGCGGAGTACCTACGGGGCAATTG
Ad7_3'-55	TCGTCACCGAGCACATCGCCACCAC

Name	Sequence
Ad7_3'-56	AACCTACCCGCGCGCGGCCGAC
Ad7_3'-57	ATTCATGAGGTGCATCCCGTGAATCG
Ad7_3'-58	TGCTCTCACTGACCCTACAGATCTCAC
Ad7_3'-59	AAGGATCGCGAAGAATACCTTCTC
Ad7_3'-60	AGATGACCTTGGATGATCCCACCACC
Ad7_3'-61	AACTGACAACCTGAGTGCAGAGGTC
Ad7_3'-62	AAACCGTCTGCGCCTCCTGCGGTGCG
Ad7_3'-63	AAATGCCCATGGCTGACGGGCTGAAG
Ad7_3'-64	TATCATTATGGATGAGTGCATGGAG
Ad7_3'-65	TGATCGAGACCGATGGTCCAGGGC
Ad7_3'-66	AAGCGCTTCCACTCATGGCAGCTGC
Ad7_3'-67	ATATTGCAATGTCCACCAGCGCAGG
Ad7_3'-68	ATTCCCTGATATGTAGCATGC
Ad7_3'-69	TCATCCTGCGAGCCTTCCATGTTC
Ad7_3'-70	TACATGACGTCACATTAATAAACAC
Ad7_3'-71	ATTCACTGCGGTATGGATGGACTGCTC
Ad7_3'-72	AACTCGTCAGGTTTAAATACCCTAGCG
Ad7_5_39_2	ATGCTTCGGAGTACCTGAGTCCGG
Ad7_5_40_2	ATATCAGCCAGAGCCTCAAGTTGG
Ad7_3_34_2	TTTCATTTGTTCCATCAATATCAG
Ad7_3_33_2	TTCAGTGTTCCTGTCTGCAAGTC
Ad7_up_3_1*	TTTTAGCCGTTACCCACAGCC
Ad7_up_3_2*	AATTTTTACTTGCATCCGCC
Ad7_down_5_1*	TTTTTTAAATTACCTCATTTC
Ad7_down_5_2*	AGGGAAAAGTACAGTTTCACTTCC

\*5' and 3' end of 293 genome was determined by direct sequencing using Ad7\_up3\_1, 3\_2, Ad7\_down\_5\_1 and 5\_2 with its genomic DNA.

**Appendix Table 2.** Primer set for PCR amplification and sequencing in study of human adenovirus B7d-associated urethritis, Japan\*

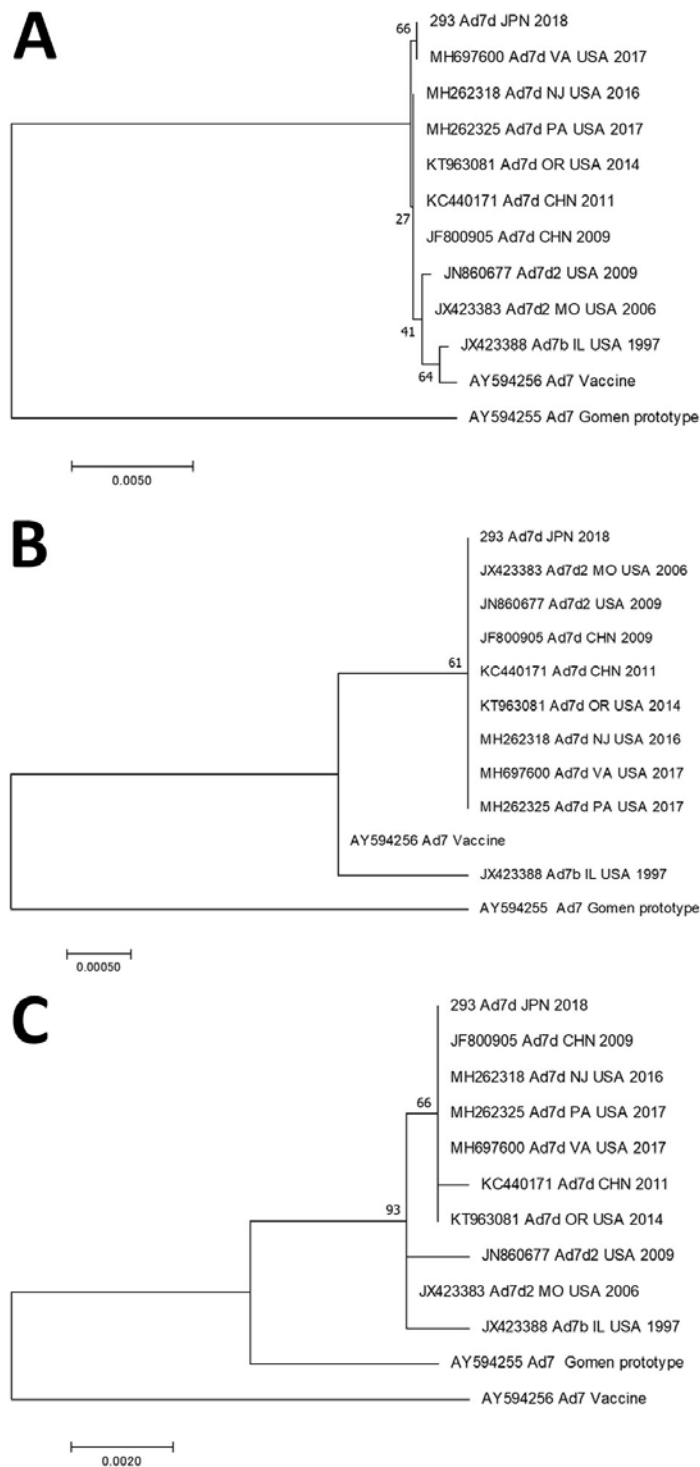
Fragment	1-3500	3000-6500	5500-8000	6000-9500	7500-10000	9000-12500	12000-15500	15000-18500	18000-21500	21000-24500	24000-27500	27000-30500	30000-33500	33000-35000
PCR forward primer	Ad7_5'-1	Ad7_5'-7	5-12	Ad7_5'-13	5-16	Ad7_5'-19	Ad7_5'-26	Ad7_5'-32	Ad7_5'-38	Ad7_5'-44	Ad7_5'-50	Ad7_5'-56	Ad7_5'-62	Ad7_5'-68
PCR reverse primer	Ad7_3'-66	Ad7_3'-60	3-57	Ad7_3'-54	3-53	Ad7_3'-47	Ad7_3'-41	Ad7_3'-35	Ad7_3'-29	Ad7_3'-23	Ad7_3'-17	Ad7_3'-11	Ad7_3'-5	Ad7_3'-1
Sequencing primer	Ad7_5'-1	Ad7_5'-7	Ad7_5'-12	Ad7_5'-13	Ad7_5'-16	Ad7_5'-19	Ad7_5'-26	Ad7_5'-32	Ad7_5'-38	Ad7_5'-44	Ad7_5'-50	Ad7_5'-56	Ad7_5'-62	Ad7_5'-68
	Ad7_5'-2	Ad7_5'-8	Ad7_5'-13	Ad7_5'-14	Ad7_5'-17	Ad7_5'-20	Ad7_5'-27	Ad7_5'-33	Ad7_5'-39	Ad7_5'-45	Ad7_5'-51	Ad7_5'-57	Ad7_5'-63	Ad7_5'-69
	Ad7_5'-3	Ad7_5'-9	Ad7_5'-14	Ad7_5'-15	Ad7_5'-18	Ad7_5'-21	Ad7_5'-28	Ad7_5'-34	Ad7_5'-39	Ad7_5'-46	Ad7_5'-52	Ad7_5'-58	Ad7_5'-64	Ad7_5'-70
	Ad7_5'-4	Ad7_5'-10	Ad7_5'-15	Ad7_5'-16	Ad7_5'-19	Ad7_5'-22	Ad7_5'-29	Ad7_5'-35	Ad7_5'-40	Ad7_5'-47	Ad7_5'-53	Ad7_5'-59	Ad7_5'-65	Ad7_5'-71
	Ad7_5'-5	Ad7_5'-11	Ad7_5'-16	Ad7_5'-17	Ad7_5'-20	Ad7_5'-23	Ad7_5'-30	Ad7_5'-36	Ad7_5'-40	Ad7_5'-48	Ad7_5'-54	Ad7_5'-60	Ad7_5'-66	Ad7_5'-72
	Ad7_5'-6	Ad7_5'-12	Ad7_3'-57	Ad7_5'-18	Ad7_5'-P7	Ad7_5'-24	Ad7_5'-31	Ad7_5'-37	Ad7_5'-41	Ad7_5'-49	Ad7_5'-55	Ad7_5'-61	Ad7_5'-67	Ad7_3'-1
	Ad7_5'-7	Ad7_5'-13	Ad7_3'-58	Ad7_5'-19	Ad7_3'-53	Ad7_5'-25	Ad7_5'-32	Ad7_5'-38	Ad7_5'-42	Ad7_5'-50	Ad7_5'-56	Ad7_5'-62	Ad7_5'-68	Ad7_3'-2
	Ad7_3'-66	Ad7_3'-60	Ad7_3'-59	Ad7_3'-54	Ad7_3'-54	Ad7_5'-26	Ad7_3'-41	Ad7_3'-35	Ad7_5'-43	Ad7_3'-23	Ad7_3'-17	Ad7_3'-11	Ad7_3'-5	Ad7_3'-3
	Ad7_3'-67	Ad7_3'-61	Ad7_3'-60	Ad7_3'-55	Ad7_3'-55	Ad7_3'-47	Ad7_3'-42	Ad7_3'-36	Ad7_5'-44	Ad7_3'-24	Ad7_3'-18	Ad7_3'-12	Ad7_3'-6	Ad7_3'-4
	Ad7_3'-68	Ad7_3'-62	Ad7_3'-61	Ad7_3'-56	Ad7_3'-56	Ad7_3'-48	Ad7_3'-43	Ad7_3'-37	Ad7_3'-29	Ad7_3'-25	Ad7_3'-19	Ad7_3'-13	Ad7_3'-7	Ad7_3'-5

Fragment	1-3500	3000-6500	5500-8000	6000-9500	7500-10000	9000-12500	12000-15500	15000-18500	18000-21500	21000-24500	24000-27500	27000-30500	30000-33500	33000-35000
	Ad7_3'-69	Ad7_3'-63		Ad7_3'-57	Ad7_3'-57	Ad7_3'-49	Ad7_3'-44	Ad7_3'-38	Ad7_3'-30	Ad7_3'-26	Ad7_3'-20	Ad7_3'-14	Ad7_3'-8	
	Ad7_3'-70	Ad7_3'-64		Ad7_3'-58	Ad7_3_P9	Ad7_3'-50	Ad7_3'-45	Ad7_3'-39	Ad7_3'-31	Ad7_3'-27	Ad7_3'-21	Ad7_3'-15	Ad7_3'-9	
					070									
	Ad7_3'-71	Ad7_3'-65		Ad7_3'-59		Ad7_3'-51	Ad7_3'-46	Ad7_3'-40	Ad7_3'-32	Ad7_3'-28	Ad7_3'-22	Ad7_3'-16	Ad7_3'-10	
	Ad7_3'-72	Ad7_3'-66		Ad7_3'-60		Ad7_3'-52	Ad7_3'-47	Ad7_3'-41	Ad7_3'-33	Ad7_3'-29	Ad7_3'-23	Ad7_3'-17	Ad7_3'-11	
						Ad7_3'-53			Ad7_3_33					
									_2					
						Ad7_3'-54			Ad7_3'-34					
									Ad7_3_34					
									_2					
									Ad7_3'-35					

\*PCR was performed with GXL enzyme [TAKARA BIO, Kyoto, Japan] as previously described (Biggs et al. [14]). DNA fragments was purified and performed sequencing with indicated primers by FASMAQ Company,

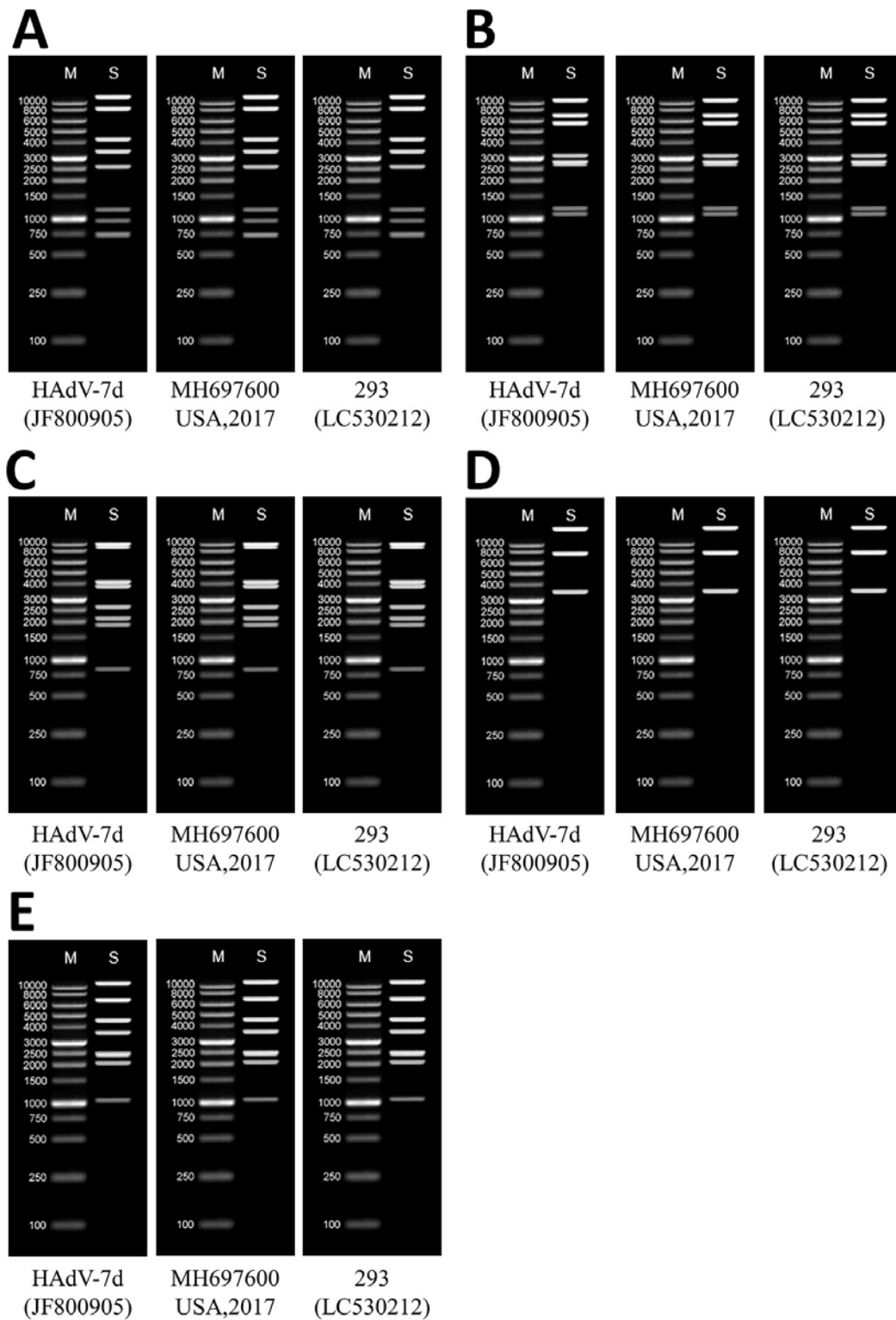
<http://fasmac.co.jp>.





**Appendix Figure 1.** Molecular phylogenetic analysis of human adenovirus 293 strain isolated in this study, compared with other human adenovirus type 7 reference strains. We aligned hexon (A), fiber (B) and penton (C) open reading frames using ClustalW (<http://www.clustal.org>) in

MEGA version 7 (<https://www.megasoftware.net>). We inferred the evolutionary history by maximum-likelihood method based on the Kimura 2-parameter model (A,C) or Tamura 3-parameter model (B). The tree with the highest log likelihood (A: -4462.55, B:-1392.74 and C: -2533.45) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. We obtained initial trees for the heuristic search automatically by applying neighbor-join and BioNJ (<http://bionj.org>) algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in number of substitutions per site. The analysis involved 12 nt sequences. All positions containing gaps and missing data were eliminated. The final dataset included a total of 2,805 positions (A), 978 positions (B), and 1,635 positions (C). We conducted evolutionary analyses in MEGA7. Sequence names are derived from the GenBank accession number, geographic location, year of sample collection, and virus type.



**Appendix Figure 2.** In silico restriction enzyme cutting pattern. A–E indicate restriction enzyme names used in this analysis: BamHI (A), BclI (B), BstEII (C), HpaI (D), and SmaI (5). HAAdV-7d

(JF800905) and MH697600 are reference strains of HAdV genome type 7d, and 293 (LC530212) is isolated strain in this study. In silico analysis with several enzyme were performed with whole-genome sequences of JF800905, MH697600 and LC530212 using online software (<http://www.molbiotools.com/restrictionanalyzer.html>).