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

Appendix

Additional References

16. Hanaoka N, Ito S, Konagaya M, Nojiri N, Yasuda M, Fujimoto T, et al. Infectious human adenoviruses are shed in urine even after disappearance of urethral symptoms. PLoS One. 2019;14:e0212434. PubMed https://doi.org/10.1371/journal.pone.0212434

17. Ito S, Hanaoka N, Shimuta K, Seike K, Tsuchiya T, Yasuda M, et al. Male non-gonococcal urethritis: from microbiological etiologies to demographic and clinical features. Int J Urol. 2016;23:325–31. PubMed https://doi.org/10.1111/iju.13044

18. Hiroi S, Kawahata T, Furubayashi K. First isolation of human adenovirus type 85 by molecular analysis of adenoviruses in cases of urethritis. J Med Microbiol. 2020;69:265–9. PubMed https://doi.org/10.1099/jmm.0.001149

19. Hawker GA, Mian S, Kendzerska T, French M. Measures of adult pain: Visual Analog Scale for Pain (VAS Pain), Numeric Rating Scale for Pain (NRS Pain), McGill Pain Questionnaire (MPQ), Short-Form McGill Pain Questionnaire (SF-MPQ), Chronic Pain Grade Scale (CPGS), Short Form-36 Bodily Pain Scale (SF-36 BPS), and Measure of Intermittent and Constant Osteoarthritis
**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 | AAGCTGCCAGCGATGTTTTTTAAGTTGG                 |
| Ad7_5'-4 | TAAAATTATGTCACTGTGAGTGTTCAC                  |
| Ad7_5'-5 | ACAATCTTAGATTACTGGCCAGTGCG                  |
| Ad7_5'-6 | TAATAGATACACAAGATAAAGCAG                    |
| Ad7_5'-7 | TCTTGCTACTGTGCAATATGCCTTCCAC                |
| Ad7_5'-8 | AGTATTTAGCCCTTATCTGACGGGC                   |
| Ad7_5'-9 | TTGAGAACTCGTGGATCTTTTCAG                    |
| Ad7_5'-10 | TCTGAAACATCATAGTTATGCTCC                   |
| Ad7_5'-11 | AAAGAGTCTGTGGAAGAGCTCGAGGCC                |
| Ad7_5'-12 | AGGTCAAGATCCGCTCAGGGGTGCC                 |
| Ad7_5'-13 | TAGGAACGAGGAGATTGTGATATGGAC                 |
| Ad7_5'-14 | TCTGCAACTCCAGCCGCCGTATAGGG                 |
| Ad7_5'-15 | AGTACTCTTTGGGGAACCGC                      |
| Ad7_5'-16 | AGTGTTGGGAGGCCCCCCATCTGCGACG                |
| Ad7_5'-17 | TGACTTCTTTGGCAGAAAAATTTCAG             |
| Ad7_5'-18 | TGGTACTGCGGCCCTGAGAAGACTCG               |
| Ad7_5'-19 | AGAAATACATGATCCCGTGTCACG                    |
| Ad7_5'-20 | ATCGTCTCAGGATCCACGGGACATCTG             |
| Ad7_5'-21 | ATTTGTGAGGAGCGATTGGGCAATGC                |
| Ad7_5'-22 | AGCCGCAGCAGCTGCGTGGTACCTGCG                |
| Ad7_5'-23 | ACTGGCCGGCTGGCGACCGCATCG                   |
| Ad7_5'-24 | TAATGACTGCGCCGTGAGAGGCACGTCAG            |
| Ad7_5'-25 | ATCAGCCCGAGCTAGGGCACATGTTGG               |
| Ad7_5'-26 | ACTCTGAGCGAGCATCTGGGGGTTG                |
| Ad7_5'-27 | AACCACCCACAGAGGACTCCTGG                     |
| Name     | Sequence                        |
|----------|---------------------------------|
| Ad7\_5'-28 | TGCTGCTGCTGCTGCTGTATCC         |
| Ad7\_5'-29 | AGTACGACATGCCGCCACCCCAATG     |
| Ad7\_5'-30 | TTGGTGGAAGAGGAGGAGGGGCAACC     |
| Ad7\_5'-31 | ATGGGGCGGTATCCTGAAGACCATC      |
| Ad7\_5'-32 | ATGTATGAGATCTTGAAGGGGGG       |
| Ad7\_5'-33 | AATGAGCAAGCCGTGTACTCTCAAGC    |
| Ad7\_5'-34 | TGCTCGTAATTATCTCTACTGCG       |
| Ad7\_5'-35 | AAAAGCCCCGCAAATCAACCGGGGTC    |
| Ad7\_5'-36 | AGCCTGAGATCAAGTACCTATC        |
| Ad7\_5'-37 | ATATGGCCCCCTCATTGGCCGCTTCCG   |
| Ad7\_5'-38 | AAATAAGCTTAAGGAACAAAAACTTCC    |
| Ad7\_5'-39 | TCTGAACACGATCGTGGGTCTGGGC     |
| Ad7\_5'-40 | TAGCTCAAGCCATATTCGCGAC        |
| Ad7\_5'-41 | AATTGGAAACTCCAGACAGGCATG      |
| Ad7\_5'-42 | TTAATATCCAAGCTAATCTTTGAG      |
| Ad7\_5'-43 | ACTCTTGAAGCCATGTCGGAAGC       |
| Ad7\_5'-44 | AAACCTCCAGCCTATGAGCAGGCAG     |
| Ad7\_5'-45 | AGCTGCAGCCCATGTCGCGGGTTC      |
| Ad7\_5'-46 | AATCTCCCAGTGCAGGCACCCACCC     |
| Ad7\_5'-47 | TGTCTCAGCTTGCCCAGCAGGTTCG     |
| Ad7\_5'-48 | TGCATCCATGAGCCCACAGGC         |
| Ad7\_5'-49 | TGCTCTTTCTGGCTTCTCTTTG        |
| Ad7\_5'-50 | AAGAGATTGAGCCAGATGTCGAGCAGG   |
| Ad7\_5'-51 | TTACCTGATATAAGCTTCTCTGGAGAG  |
| Ad7\_5'-52 | ACCTTATGACATGGCTCTTGCGG       |
| Ad7\_5'-53 | ATGGAGCCACTGCTACCTGTTCGGC     |
| Ad7\_5'-54 | AAGATGTCCCATCGCCGAGGAAGC      |
| Ad7\_5'-55 | ATCTACACGTCATCCACAGCCC       |
| Ad7\_5'-56 | TTGGCAGCCAGGCGCCCTCAGGAC      |
| Ad7\_5'-57 | TCTCCAGTCTGTTGAGAGTTTACTCC   |
| Ad7\_5'-58 | ACCAACCAGATCTTCCAGAAGACCC     |
| Ad7\_5'-59 | ACTGCAGTCCGTGGAAATTACAC      |
| Ad7\_5'-60 | ATTTGGACAAACTCGGAAGTGTG       |
| Ad7\_5'-61 | TTGGCTCAACATGTACACTACAAGG    |
| Name   | Sequence                                      |
|--------|-----------------------------------------------|
| Ad7_5'-62 | TATAGAATTATGATATTGTGTTCAATC                   |
| Ad7_5'-63 | ACTTCTTAGGCTATTTAAAACCATG                   |
| Ad7_5'-64 | AACCTAGCATCCGCCAGCACAGCAGG                   |
| Ad7_5'-65 | TGTACCACAATCTCTCATGTCTCTTC                   |
| Ad7_5'-66 | TAATTCTACACTAGTTAAAACCTGG                   |
| Ad7_5'-67 | ACGACTGACAAATAAAGTTTAAACTTG                 |
| Ad7_5'-68 | ACTTAGATTACGACTAGGTACAGC                   |
| Ad7_5'-69 | ACGAACACAACCTGACTATGATGAG                 |
| Ad7_5'-70 | TAGATCCGCAGATGTCATCTATCG                   |
| Ad7_5'-71 | TACAAGGCAGACTCTCCCAATTGG                   |
| Ad7_5'-72 | ATGCTTAATCTTATAGTATAGCAAAGCC                 |
| Ad7_5'_P7900 | TTGTACAGCGGCGCCGACTGAGC                    |
| Ad7_3'_P9070 | TTTTCAACTTGTGGCGACTTCTAC                   |
| Ad7_3'-1  | TCTATATAATATAACCTTATAGATGG                  |
| Ad7_3'-2  | ATACTTAAGATTAACGATAATATAACC                 |
| Ad7_3'-3  | TGATCGCCCTGCTTATGCTGTCC                   |
| Ad7_3'-4  | ATTTGAAGTACTGCAGATCGTCCGG                   |
| Ad7_3'-5  | TTCTCTGTTTCGCGACTTATTACGTTC                 |
| Ad7_3'-6  | TCAGAATGCGTGTGCGCAGGCGCC                    |
| Ad7_3'-7  | TCTATGTAGTAAAAGGGAATATGGAG                 |
| Ad7_3'-8  | ATTCACTGAGGCGCCATTTATGTACAG                |
| Ad7_3'-9  | TTGTACATCCCTTTAAAGTAGTGGAG                 |
| Ad7_3'-10 | TTTGCTTTGTGATCTTGGATAGC                    |
| Ad7_3'-11 | ATTTTGATTGCGTATTCGGGGATGG                   |
| Ad7_3'-12 | ATGTCAAAATTTATGATGCATCC                    |
| Ad7_3'-13 | ATAAGTTACAGCTGCAAGGCTAGTAAATG               |
| Ad7_3'-14 | AACCTCTGAAAGTAAATGGGCCC                   |
| Ad7_3'-15 | ACTTAATAAGAACTCCACAGCAGC                   |
| Ad7_3'-16 | TTGTAAAGCTCGATTTGGAAGAGCGC                 |
| Ad7_3'-17 | TCCGATGCGCCCGAGCGGGGG                    |
| Ad7_3'-18 | ATTTGGGGCTGTATAGCTCCACATG                  |
| Ad7_3'-19 | AAGTTCACACCGTGTTGGAAGACAG                 |
| Ad7_3'-20 | AACCTGTGGGATGCTCCACAGG                    |
| Ad7_3'-21 | AGAGGACAAAGTCCGAGGCGCAGCG                 |
| Name     | Sequence                                               |
|----------|--------------------------------------------------------|
| Ad7_3'-22| TAGTGCAGGTTTCTCCTCTAGCTTGC                           |
| Ad7_3'-23| AGCTCCCGGACCCAAGTGTAGAAGGG                           |
| Ad7_3'-24| TTTTACATTATCCTGCGCCTTCATC                           |
| Ad7_3'-25| AGCATGTCCCTCTGCAAGACATCGGC                           |
| Ad7_3'-26| ATTATGAGGCAAGGTTGAAATGCC                           |
| Ad7_3'-27| TTCCAGTTTGCAACCCAGTGTACC                           |
| Ad7_3'-28| TTTATGAGACAGGTTTACATGAC                           |
| Ad7_3'-29| AAGCAAGAGGCTTTCTTATGTGGTGCC                           |
| Ad7_3'-30| AAAAAAGGAGTACATGCAGATCCTTG                           |
| Ad7_3'-31| TGAAGGCGTTGTGTGAGCCATGGG                           |
| Ad7_3'-32| TTTCATAGCCAGATTGTGCTATGGG                           |
| Ad7_3'-33| TTTCCGTGTAAGCAACATTTCCAGGG                           |
| Ad7_3'-34| TAATGTCAAGAATGTGCTGGCAGATG                           |
| Ad7_3'-35| AGACCCACGATGCTTCCAGAGTAC                           |
| Ad7_3'-36| AAGCTTTGTTCCTCATAGGTTCATAGG                           |
| Ad7_3'-37| AGGCAGCGTCACAAGGTCAATTAGG                           |
| Ad7_3'-38| ATTTTCCTCACGTTCGTCATCC                             |
| Ad7_3'-39| TTTCACTCTCAGTGCCGTGATACATC                           |
| Ad7_3'-40| TCGGCAGGCACCTGGTGTCAGACATC                           |
| Ad7_3'-41| TAGAAACTCTTGTGAGACAGGCC                             |
| Ad7_3'-42| TTTGAACCTCCTGGAATGGATGTC                             |
| Ad7_3'-43| TCGTCAAGTGTTGTGATGTCGTTG                           |
| Ad7_3'-44| TCCAACACGCTGTATCATCGGCA                             |
| Ad7_3'-45| AAACCAGGTGGGGGCAGCAGTGTG                             |
| Ad7_3'-46| TTGGGAGTCAAGCATAGCAGACAGGTC                           |
| Ad7_3'-47| AAAGGCGGTTGGCTGGGTGGTGTTG                            |
| Ad7_3'-48| AGAGTCATGCGCATGTAACACCATC                           |
| Ad7_3'-49| TCCTCGTCTTGCAGACCCGTTCATC                           |
| Ad7_3'-50| AGTAGTACAGGAGCAGAAGAACCC                           |
| Ad7_3'-51| TCCTCAAATCCAGATCATAGCGG                             |
| Ad7_3'-52| TGATCGGAGATTGAACCCGGGTAC                             |
| Ad7_3'-53| TTTTGAGCTTCTGACAGTCCTCAAAG                           |
| Ad7_3'-54| TTGGCAGGAGTGACCTACGGCCAAATTCG                           |
| Ad7_3'-55| TCGTCACGGACACATCGCCACCA                               |
| Name          | Sequence                        |
|---------------|---------------------------------|
| Ad7_3'-56     | AACCTACCGCAGCGCGGCGAC           |
| Ad7_3'-57     | ATTCATGAGGTGCATCCCGTGAATCG      |
| Ad7_3'-58     | TGCTCTCACTGACCCTACAGATCTCAC     |
| Ad7_3'-59     | AAGGATCGCAAGAATAACCTTCTC        |
| Ad7_3'-60     | AGATGACCTTGGATGATCCCACCACC     |
| Ad7_3'-61     | AACTGACAACCTGAGTGCAAGGTC       |
| Ad7_3'-62     | AAACCGTCTGCGCTCTCCTGCGGTGCG    |
| Ad7_3'-63     | AAATGCCCATGGCTGAGGGCTGAAG      |
| Ad7_3'-64     | TATCATTATGGATGATGCAAGAG       |
| Ad7_3'-65     | TGATCGAGACCGAGTCACGGGC         |
| Ad7_3'-66     | AAGCGCTTCCACTCATGGCAGCTGC      |
| Ad7_3'-67     | ATATTGCAATGTCCACACAGCGAGG      |
| Ad7_3'-68     | ATTCCTGTATGATGCAAGC           |
| Ad7_3'-69     | TCCACCTGCGAGCTCCATGTTCC       |
| Ad7_3'-70     | TACATGACGTCACATTAATAAACAC      |
| Ad7_3'-71     | ATTCACGTGCGGTATGGATAGACTGCTC  |
| Ad7_3'-72     | AACTCGTCAGGTCAAATACCCCTAGCG   |
| Ad7_5_39_2    | ATGCTTCGGAGTACCTGAGTCCGG      |
| Ad7_5_40_2    | ATATCAGGCAGCGCTCAAGTTG        |
| Ad7_3_34_2    | TTTTCATTTGTTCCATCAATATCAG     |
| Ad7_3_33_2    | TTTACGTGTCTGTCTGCAAATC       |
| Ad7_up_3_1*   | TTTTAGCCGTTCACCACACGC         |
| Ad7_up_3_2*   | AATTTTTACCTGATCCGCGC          |
| Ad7_down_5_1* | TTTTTAAATACCTCATTTGC         |
| Ad7_down_5_2* | AGGGAAAAAGTACAGGTTCACCTCC  |

*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 | 7500- | 9000- | 12000- | 15000- | 18000- | 21000- | 24000- | 27000- | 30000- | 33000- |
|----------|-------|-------|--------|--------|--------|--------|--------|--------|--------|--------|
| 1-3500   | 3000-6500 | 5500-8000 | 6000-9500 | 10000 | 12500 | 15000 | 18000 | 21500 | 24500 | 27500 |
| 9000-12500 | 15000-18500 | 20000-24500 | 27000-30500 | 33000-35000 |

**PCR forward 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

**PCR reverse primer**
- Ad7_3’-66
- Ad7_3’-60
- Ad7_3’-54
- Ad7_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_5’-18
- 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_5’-71
- Ad7_5’-7
- Ad7_5’-13
- Ad7_5’-19
- 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_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_3’-66
- Ad7_3’-60
- Ad7_3’-59
- Ad7_3’-54
- Ad7_3’-43
- Ad7_3’-26
- Ad7_3’-54
- 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’-47
- Ad7_3’-42
- Ad7_3’-36
- Ad7_3’-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’-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 | 10000 | 12500 | 15000 | 18000 | 21000 | 24000 | 27000 | 30000 | 33000 |
|----------|--------|-----------|-----------|-----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Ad7_3'-69 | Ad7_3'-63 | Ad7_3'-57 | Ad7_3'-49 | 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'-50 | Ad7_3'-39 | Ad7_3'-31 | Ad7_3'-27 | Ad7_3'-21 | Ad7_3'-15 | Ad7_3'-9 |
| 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'-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), Hpal (D), and Smal (E). HAdV-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).