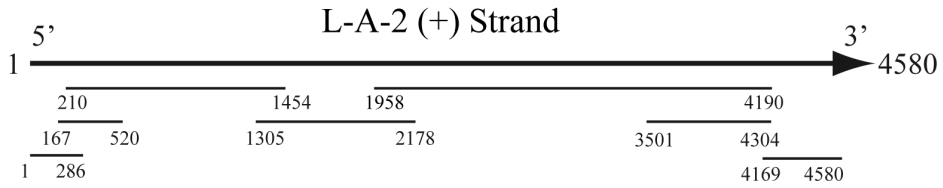
Figure S1.

A Sequencing strategy of L-A-2 cDNA clones. Independent clones with their nucleotide numbers are indicated.



B ClustalW alignment of nucleotide sequences of four L-A variants: L-A-28, L-A, L-A-lus, and L-A-2. Only the 5' region, the frameshifting region (red) and the last 400 nt at the 3' ends, which contain the encapsidation signal (green) are depicted. Since the comparisons were done on cDNAs T is shown instead of U.

Initiation

```
L-A-28          CGAATAATTTGAATATTCATATAACTCCCCATGCTTAGATTCGTTACTAAAAACTCTCAA   60
L-A             -GAAAAATTTTTAAATTCATATAACTCCCCATGCTAAGATTTGTTACTAAAAACTCTCAA   59
L-A-lus         -GAAAAATTTGAATAATCATATAACTCCCCATGCTTAGATTCGTTACCAAAAACTCTCAA   59
L-A-2           -GAATAATTTGAATATTCATACAACTCCCCATGCTTAGATTCGTTACTAAAAATTCTCAA   59
                 ***:***** :*:*:***** ************:***** ***** ***** ******

L-A-28          GACAAGTCCTCAGACTTGTTTTCTATCTGTTCAGACAGAGGAACTTTTGTCGCTCATAAT   120
L-A             GATAAATCGTCTGATCTATTCTCTATTTGTTCTGATCGCGGAACTTTTGTTGCCCATAAT   119
L-A-lus         GACAAGTCCTCCGATTTATTTTCTATTTGCTCTGACAAAGGTACTTTTGTTGCGCATAAT   119
L-A-2           GATAAATCTTCGGACTTATTCTCCATTTGCTCTGACAGGGGAACTTTTGTTGCTCATAAC   119
                ** **.** ** **  *.** ** ** ** **:** .. **:******** ** *****

L-A-28          AGAGTCAGAACTGATTTCAAGTTTGACAACTTAGTCTTTAACCGAGTCTACGGCGTGTCA   180
L-A             AGAGTTCGGACTGATTTCAAGTTTGACAACTTAGTATTCAACCGAGTTTATGGTGTTTCT   179
L-A-lus         AGGGTGAGGACTGACTTTAAATTTGACAACTTAGTTTTTAATCGAGTCTACGGCGTGTCA   179
L-A-2           CGAGTGAGAACTGATTTCAAGTTCGACAACTTGGTGTTTAACCGTGTTTACGGTGTGTCG   179
                .*.** .*.***** ** **.** ********.** ** ** **:** ** ** ** **

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L-A-28          CGGATAAATCTAGATTACGTCAAGCCTGTTTCCGCTGGCATCCAAGTCATCAATGCAGGT   1917
L-A             CGAATCAATCTTGATTATGTTAAGCCTGTTTCGACCGGCATTCAGGTGATCAATGCGGGC   1916
L-A-lus         AGGATTAATTTGGATTACGTCAAGCCTGTATCTGCAGGCATACAAGTCATTAACGCTGGT   1916
L-A-2           CGTATAAATCTTGATTATGTCAAGCCAGTTTCCGCGGGTATTCAGGTTGTTAACGCGGGA   1916
                .* ** *** * ***** ** *****:**:** .* ** ** **.** .* ** ** **

L-A-28          GAACTAAGGAACTATTGGGGTAGTGTCCGCCGCACTCAGCAGGGTTTAGGAGTGGTAGGT   1977
L-A             GAACTTAAGAACTACTGGGGTAGTGTGCGTCGTACTCAGCAGGGTTTAGGAGTGGTAGGT   1976
L-A-lus         GAACTAAGGAATTACTGGGGTAGTGTACGCCGTACGCAGCAGGGTTTAGGAGTGGTAGGT   1976
L-A-2           GAGTTGCGGAACTATTGGGGTAGTGTACGTCGTACTCAACAGGGTTTAGGAGTGGTAGGT   1976
                **. * ..*** ** *********** ** ** ** **.**********************

L-A-28          CTTACGATGCCAGCCGTAATGCCTACCGGAGAACGTACAGCTGGCACTGCCCACGAAGAG   2037
L-A             CTTACGATGCCAGCTGTAATGCCTACCGGAGAACCTACAGCTGGCGCTGCCCACGAAGAG   2036
L-A-lus         CTTACGATGCCAGCCGTAATGCCTACCGGAGAACGTACAGCTGGCACTGCCCACGAAGAC   2036
L-A-2           CTTACGATGCCAGCCGTAATGCCTACCGGAGAACGTACAGCTGGCACTGCCCACGAAGAT   2036
                ************** ****************** **********.**************

L-A-28          CTGATCGAACAGGTGGACGAGGTTTCAGTAGAGTAAACATAATAGAACCCAGTCATGGGA   2097
L-A             TTGATAGAACAGGCGGACAATGTTTTAGTAGAGTAAACGTAATCGAACCCTCACACGGAC   2096
L-A-LUS         TTGATCGAACAGACGGAAAATGTTTCAGTCGAGTAAACATAATAGAACCATCCCATGGGC   2096
L-A-2           CTGATCGAGCAGACCGAAGACGTTTCAGTAGAGTAAACATCTTCGAGCCAGCACATGGCC   2096
                ****.**.***.  **..* **** ***.********.*.:*.**.**.   ** ** .

--------------------------------------------------------------------------------

L-A-28          AAATAACGC-GAGCCCATCGGGTATGTGGAGGTATATCCACTGACCCATGGGCTCCAGTC   4136
L-A             GACATACACACAGCTCACAGGGTGTGTGGCGGTATCTCGACTGATACCTGGGCACCGGTT   4135
```

Frameshifting region

```
L-A-lus         CGAT-TTCTACAGCTCACAGAGTCTGCGGCGGTATATCAACCGACCCGTGGGCACCAGTT   4135
L-A-2           CTAT-AGCATCAGCCCATAGAGTTTGCGGAGGTATATCCACCGACATGTGGGCACCGGTA   4135
                  .: :     *** ** .*.** ** **.*****.** ** ** .  *****:**.**

L-A-28          GACACAAAAATACAAACAGACAACGAAGCATATGAAATACCATACGAAATAGATGATCCA   4196
L-A             GAAACTAAGATAATAACAGACAATGAAGCATATGAAATACCATACGAAATAGATGATCCA   4195
L-A-lus         ACTACAAAAATAAAGACAGACAACGAAGCATACGAAATACCATACGAAATAGATGATCCA   4195
L-A-2           AAGACAAAAATCAAAACAGACAACGAGGCATATGAAATACCATACGAAATAGATGATCCA   4195
                .. **:**.**..:.******** **.***** ****************************

L-A-28          TCATTTTGGCCAGGGGTAAATGATTATGCTTATAAAGTCTGGCAGAATTTCGGAGAGCGT   4256
L-A             TCATTTTGGCCAGGGGTAAATGATTATGCTTATAAAGTCTGGAAAAATTTCGGAGAACGA   4255
L-A-lus         TCATTTTGGCCAGGGGTAAACGATTATGCTTATAAAGTCTGGCAGAATTTCGGCGAAAGG   4255
L-A-2           TCATTTTGGCCTGGAGTAAATGATTATGCTTACAAAGTCTGGCAGAATTTCGGAGAACGG   4255
                ***********:**.***** ********** *********.*.********.**..*

L-A-28          CTAGAATTCAACAAGATTAAGGACGCAGTATCAAAAGGTAGTAGAAACACTATAGCACTG   4316
L-A             CTCGAATTTAATAAGATTAAAGATGCCGTAGCTAGAGGGAGTAGGAGCACTATAGCTCTG   4315
L-A-lus         CTGGAGTTTAACAAGATTAAAGACGCTGTTTCAAAGGGCAGCAGAAACACGATAGCCTTG   4315
L-A-2           CTGGAATTCAATAAGATTAAGGATGCAGTCGCAAAGGGCAGTCGAAACACCATTGCCTTA   4315
                ** **.** ** ********.** ** **  *:*..** ** .*.*.*** **:**  *.

L-A-28          AAACGTAAAGCTAAAATATCGGCGAAGAGCAATCCTTTTGTACACAAATCCGAATGGGAG   4376
L-A             AAACGTAAGGCTAGGATAACATCTAAGAAGAATGAATTCGCTAACAAGTCGGAATGGGAA   4375
L-A-lus         AAACGTAAGGCGAAGATTTCTGCAGTCAAGAACGACTTCGTCAATAAATCTGAATGGGAG   4375
L-A-2           AAACGTAAAGCAAAGATTACAGCTGTCACGAACGACTATATCACTAAATCAGAATGGGAA   4375
                ********.** *..**::*  * .: *  **  . *: .  .. **.** ********.

L-A-28          CGAACGATGTACAAAGCATACAAGGGTTTGGCAGTCTCATACTATGCCAACCTGAGTAAG   4436
L-A             AGGACAATGTACAAAGCCTATAAGGGTTTGGCAGTCTCATACTATGCTAACCTGAGCAAA   4435
L-A-lus         AGAACTATGTACAAAGCTTACAAAGGTTTAGCAGTATCTTATTATGCTAACTTGAGCAAA   4435
L-A-2           AGGACTATGTATAAAGCGTACAAAGGATTGGCAGTCTCATACTATGCTAATTTAAGCAAA   4435
                .*.** ***** ***** ** **.**:**.*****.**:** ***** **  *.** **.

L-A-28          TTTATGAGCATACCACCGATGGCTAATATCGAATTCGGCCAAGCACGGTACGCGATGCAG   4496
L-A             TTCATGAGTATACCACCAATGGCGAACATTGAATTTGGGCAGGCTAGATATGCTATGCAA   4495
L-A-lus         TTCATGAGCATACCACCCATGGCAAACATAGAATTCGGACAAGCTAGATTTGCGATGCAG   4495
L-A-2           TTTATGAGTATACCACCGATGGCGAATATAGAGTTTGGCCAAGCACGGTTCGCCATGCAG   4495
                ** ***** ******** ***** ** ** **.** ** **.**:.*.*: ** *****.

L-A-28          GCAGCTTTGGACAGTTCCGATCCTTTAAGGGCATTACAGATTTTCCTGTAGATAGCCCAA   4556
L-A             GCAGCCCTTGATAGTTCTGATCCACTCCGGGCATTACAGGTCATACTGTAATT--GCCAA   4553
L-A-lus         GCGGCCTTAGATAGTTCCGATCCTTTAAGAGCACTACAAATATTCTTATGAAGTGCTC-G   4554
L-A-2           GCTGCTTTAGACAGTTCAGATCCATTAAGAGCACTACAAGTCTTCTTGTGAGGTGCTC-A   4554
                ** **  * ** ***** *****: *..*.*** ****..* :*. *.*..      * .

L-A-28          AAAGATAATGGGAATTACCCATATGCCC   4584
L-A             AAAGATAATGGGAATTACCCATATGC--   4579
L-A-lus         AACGATGAGGGTTTTTACCCATATGC--   4580
L-A-2           AAAGAAGAGGGTTTATACCCATATGC--   4580
                **.**:.* ** :::***********
```

Encapsidation signal

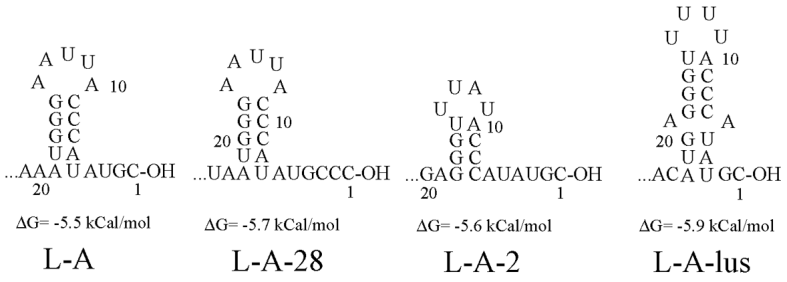
Termination

```
Clustal (% identity)

                  L-A-28    L-A       L-A-lus   L-A-2

1: L-A-28         100.00    74.67     76.44     75.76
2: L-A            74.67     100.00    73.87     74.25
3: L-A-lus        76.44     73.87     100.00    78.06
4: L-A-2          75.76     74.25     78.06     100.00
```

C Secondary structures at the 3’ ends predicted by MFOLD. Numbering is from the 3’ ends.



D Detection of Gag and Gag-Pol with anti-Gag antibodies from L-A.

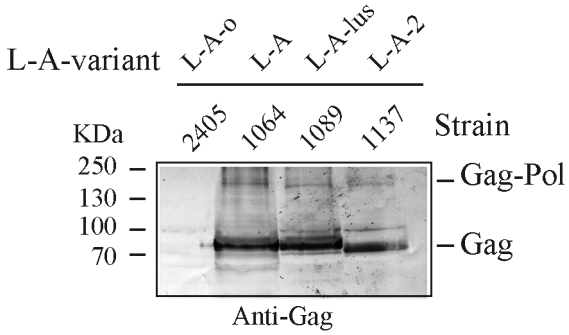
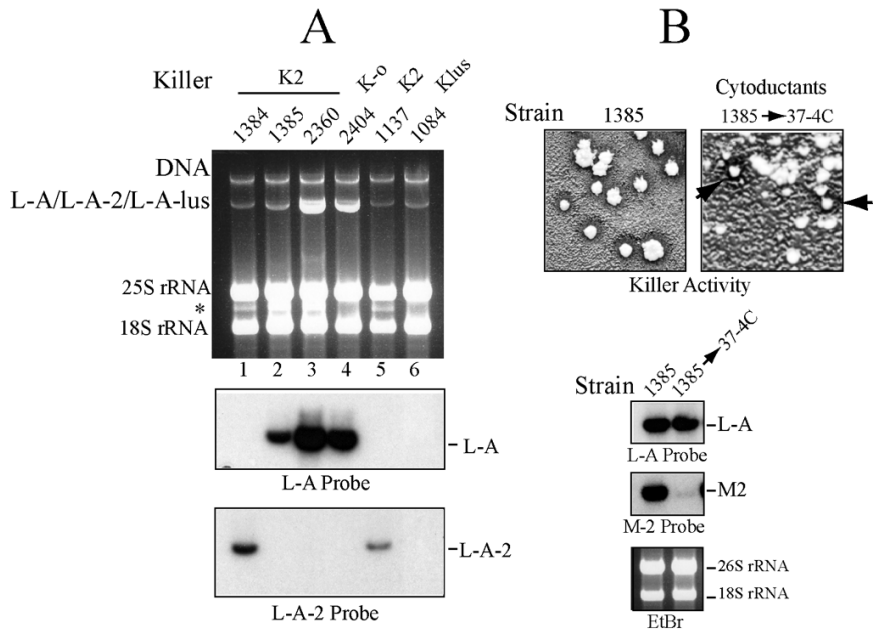


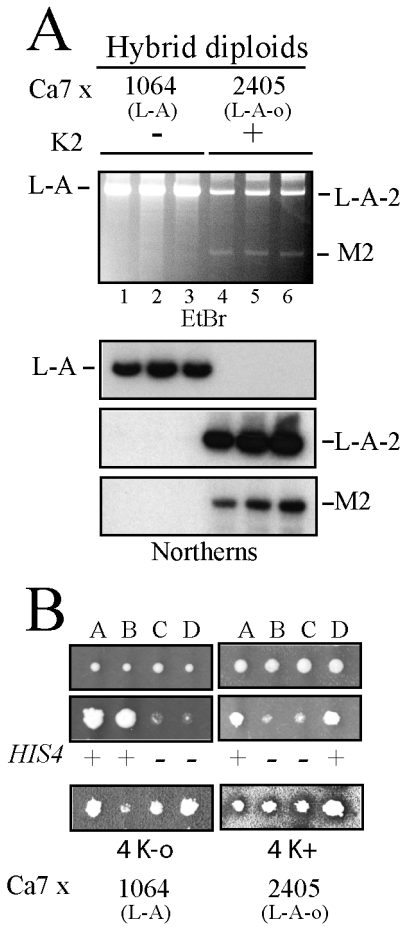
Figure S2



(A) Identification of L-A variants in laboratory K2 strains. A. Total nucleic acids from three K2 strains from Wickner's lab (1384, 1385 and 2360) were separated on an agarose gel and analyzed by Northern hybridization using specific probes for L-A or L-A-2. The upper panel shows the ethidium bromide stained gel and the lower panels the Northern autoradiograms. Strains 2404, 1137 and 1084 that harbor L-A, L-A-2 and L-A-lus, respectively, are included as controls. The asterisk indicates 23S RNA.

(B) M2 maintenance by L-A in strain 1385 is strain-specific. Cytoductants from strain 1385 (L-A and M2) into 37-4C (L-o) were streaked for single colony isolation and replica plated on an MB plate seeded with strain 5x47. The black arrowheads indicate two weak killer colonies. The rest were K-o. Below panels: RNAs from strain 1385 or from a weak killer cytoductant colony were prepared, separated in an agarose gel and probed with L-A- or M2-specific probes. While the M2 probe detected easily M2 in strain 1385 (middle panel, left lane), we needed to overexpose the filter to detect minimal amounts of M2 in the weak killer cytoductant (middle panel, right lane). The amounts of L-A in both strains were equivalent.

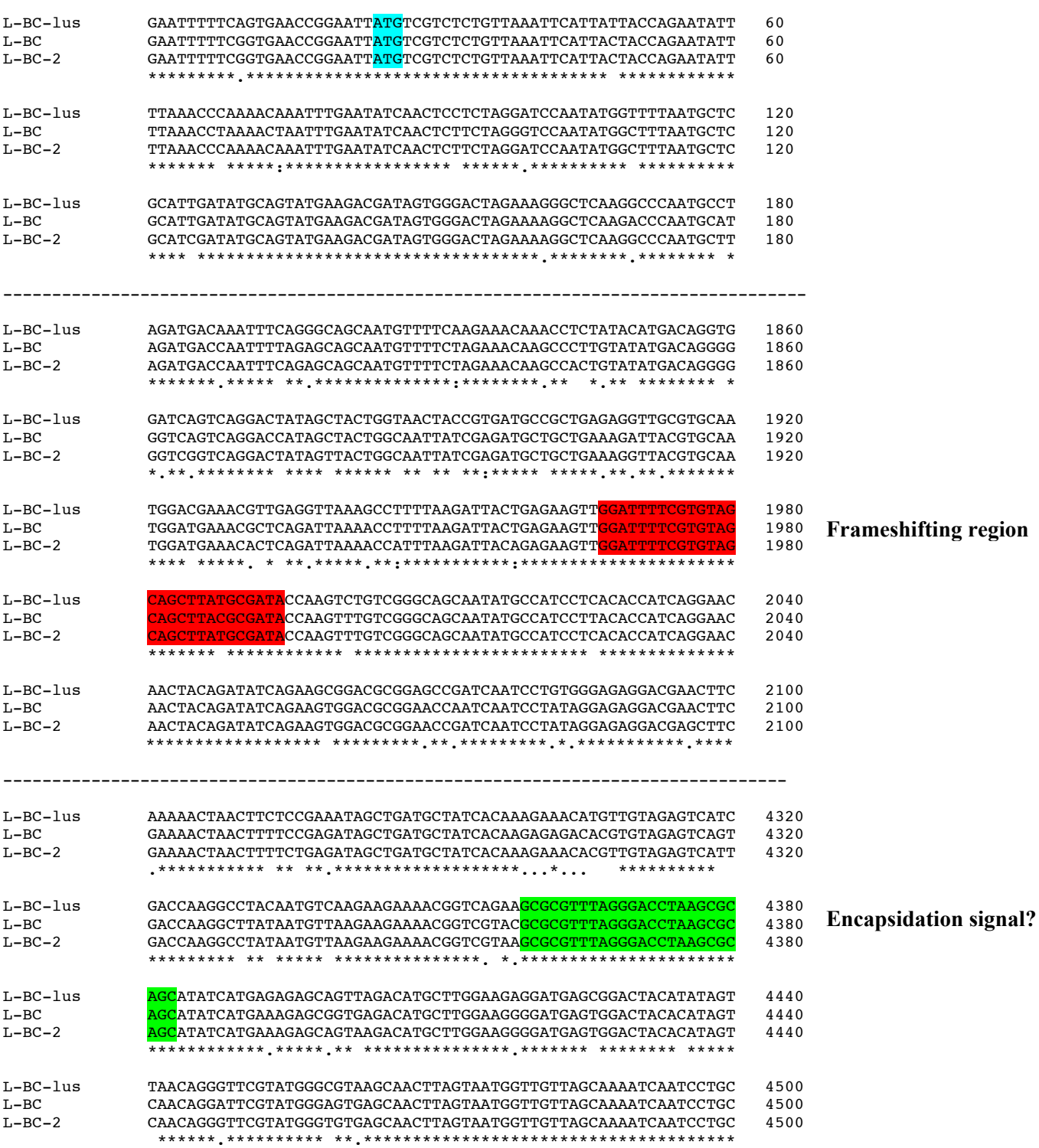
Figure S3



Exclusion of L-A-2 (and M2) by L-A in hybrid diploids. (A) Hybrid diploids were produced by mating the L-A-containing haploid strain 1064 to spore clones derived from K2 wine strain Ca7 (L-A and M2). RNAs from three independent diploid clones (lanes 1 to 3) were analyzed in an agarose gel after RNase A treatment in the presence of 0.5 M NaCl. As control, a parallel experiment was performed mating spore clones of Ca7 to the L-A-o strain 2405, isogenic to 1064 except for the absence of L-A (lanes 4 to 6). The ethidium bromide-stained gel is shown (EtBr) with the mobility of L-A/L-A-2 and M2 dsRNAs indicated. Below are Northern blots of the same samples hybridized to three different probes: The upper panel to L-A-, the middle panel to L-A-2- and the lower panel to M2-specific probe. (B) One diploid clone from each cross was further sporulated and tetrads were dissected and analyzed for *his4* meiotic segregation and K2 killer activity (at 20 ºC). Results from one representative tetrad of each cross are shown.

Figure S4

ClustalW comparison of the three L-BC variants (L-BC-lus, L-BC and L-BC-2) nucleotide sequences. Only the 5' region, the central part with the frameshifting region (red), and the 3' end region with a conserved putative encapsidation signal (green) are depicted. Initiation codons for Gag and termination codons for Pol are highlighted in blue. Below are the identity percentages and the MFOLD secondary structure prediction of the conserved putative encapsidation signal.



Termination

```
L-BC-lus        GAAAGCTAATGTGTTAGCCAAATCAGGGGATCCCACCAAATGGCTTGCTGTCCTTACATG   4560
L-BC            AAAAGCTAATGTGCTAGCCAAATCAGGAGATCCTACAAAATGGCTTGCAGTCCTTACATG   4560
L-BC-2          AAAAGCTAATGTGCTAGCCAAATCAGGTGATCCTACAAAATGGCTTGCAGTCCTTACATG   4560
                .************ ************* ***** **.***********:***********

L-BC-lus        ATATACAGGCAACAACATAAGACCTGAGAACAAAGAGTACATACGATACTACGC         4614
L-BC            ATATACAGGCAACCACATAAGACCTGAGAACAAAGAGTACATACGATACTACGC         4614
L-BC-2          ATATACAGGCAACCACATAAGACCTGAGAACAAAGAGTACATACGATACTACGC         4614
                *************.*************************************
```

CLUSTAL COMPARISON (% identity)

Nucleotide sequences

	L-BC-lus	L-BC	L-BC-2
1: L-BC-lus	100.00	88.30	88.56
2: L-BC	88.30	100.00	93.41
3: L-BC-2	88.56	93.41	100.00

Encoded Proteins

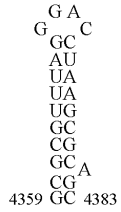
Gag

	L-BC-lus	L-BC-2	L-BC
1: L-BC-lus	100.00	96.99	97.56
2: L-BC-2	96.99	100.00	97.70
3: L-BC	97.56	97.70	100.00

Pol

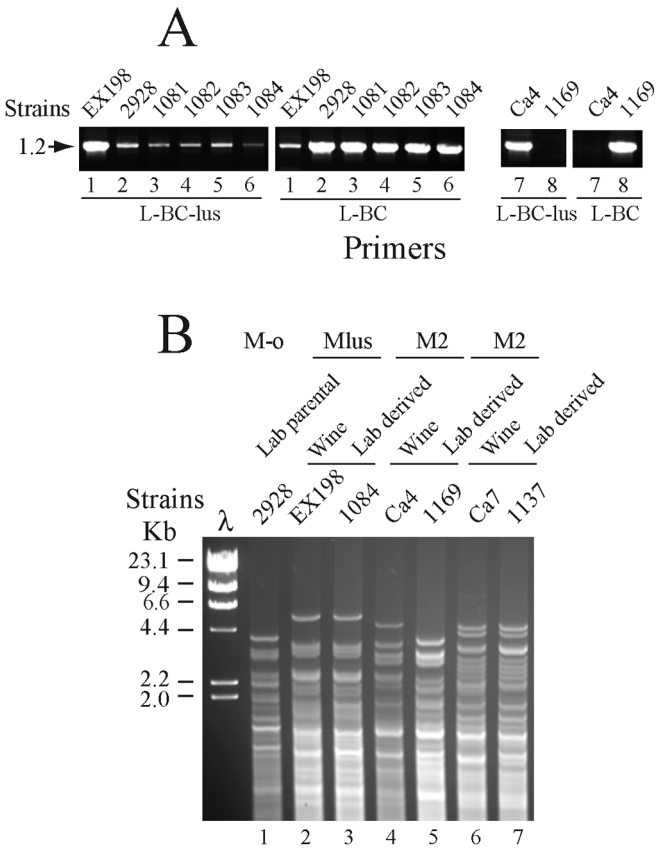
	L-BC	L-BC-2	L-BC-lus
1: L-BC	100.00	95.49	93.63
2: L-BC-2	95.49	100.00	95.49
3: L-BC-lus	93.63	95.49	100.00

Encapsidation signal?



ΔG= -11.35 Kcal/mol

Figure S5

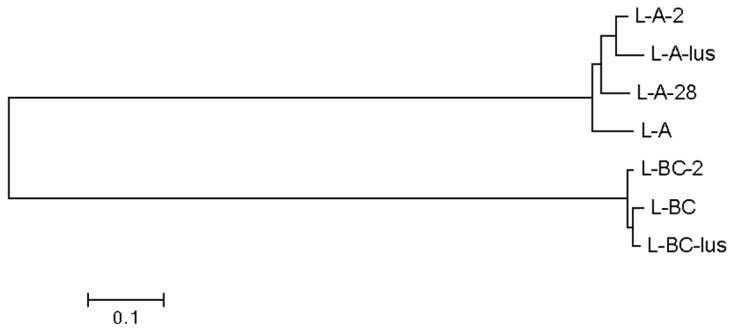


Inheritance of L-BC variants and mitochondrial DNA in laboratory strains derived from Klus or K2 wine strains. (A) RT-PCR analysis of the L-BC variants present in wine strain EX198 (lane 1), laboratory strain 2928 (lane 2) or in four Klus laboratory haploid strains (lanes 3 to 6) constructed in our previous work from strains EX198 and 2928 (Table 1, 34). Primers specific for L-BC-lus or for L-BC were used. Lanes 7 and 8 show RT-PCR fragments amplified from the strains indicated in an independent experiment. B. Total DNA from the strains indicated was digested with the restriction enzyme HinfI and the fragments generated were separated on an agarose gel. According to the pattern, strain 1084 (lane 3) or strain 1137 (lane 7) derived their mitochondria from the wine strain EX198 (lane 2) or Ca7 (lane 6), respectively, while mitochondria in strain 1169 (lane 5) came from laboratory strain 2928 (lane 1).

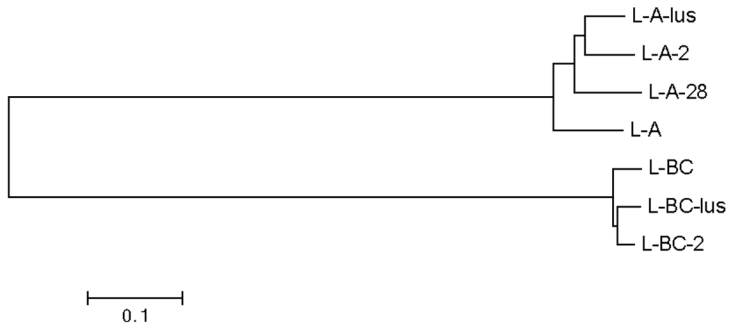
Figure S6

Phylogenetic trees of the Gag and Pol domains of different variants of L-A or L-BC totiviruses.

Gag



Pol



The evolutionary history was inferred using the Neighbor-Joining method [1]. The optimal tree with the sum of branch length = 1.808120792 is shown for Gag proteins. For Pol the sum of branch length is = 1.55825550. Trees are drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method [2] and are in the units of the number of amino acid substitutions per site. The analysis involved 7 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 654 positions in the final dataset for Gag and 843 positions for Pol. Evolutionary analyses were conducted in MEGA6 [3]

References:

1. **Saitou N, Nei M.** 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution **4**:406-425.

2. **Zuckerkandl E, Pauling L.** 1965. Evolutionary divergence and convergence in proteins, p 97-166. *In* Bryson V, Vogel HJ (ed), Evolving Genes and Proteins. Academic Press, New York

3. **Tamura K, Stecher G, Peterson D, Filipski A, Kumar S.** 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution **30**:2725-2729.

Table S7. Oligonucleotides used

Name	Sequence (5' to 3' end)	Description
NR67	GACTCGAGTCGAGCGGCCGCTTTTTTTTTTTTTTTT	OligodT 3'RACE
NR68	GACTCGAGTCGAGCGGCCGC	Oligo 3'RACE
NR80	AATTAGCGGCCGC**AGGGAAAATCATGTAAGG**	L-A-lus (1454-1437)
NR88	AATTAGGATCC**GTCTGTTTCAATGAGGG**	L-A-lus (210-226)
NR111	AATTAGAATTC**CATGAGTATCAAAAATGTC**	L-BC-lus (282-264)
NR118	**GTGTACGTTACATACCCT**	L-A-2 (1305-1322)
NR119	**GATACCGCCTGTACTCT**	L-A-2 (2178-2162)
NR121	**GGGTTTAGGAGTGGTAGGTC**	L-A-2 (1958-1977)
NR122	**CATCTATTTCGTATGGTATTTC**	L-A-2 (4190-4169)
NR125	AATTAGGATCC**ATGGCCAAACCACCATCAAC**	L-A-2 (286-267)
NR126	AATTAGGATCC**GAAATACCATACGAAATAGATG**	L-A-2 (4169-4190)
RE548	AATTAGTCGAC**GAAAAATTTTTAAATTCATATA**	L-A (1-22)
RE549	AATTAGGATCC**CGCAGCTACTGGAAATATCA**	L-A (1463-1444)
RE633	**CTGACGAAGTATTAGATGC**	L-A-2 (2556-2574)
RE634	**TAGCGTGCCCTTTAATTC**	L-A-2 (3589-3572)
RE635	**GATAATGGCCACGCCTGTCT**	L-BC (480-499)
RE636	**GCAGTTTGGCCTTCATACTTG**	L-BC-lus (1711-1690)
RE637	**GCAGTTTGTCCTTCATACTTC**	L-BC (1711-1690)
RE638	**GTGGATACGTGACAATTTTAC**	L-BC-lus (2524-2544)
RE639	**CATTGAATAACTTGCGAGCG**	L-BC-lus (4076-4057)
RE643	**CCTAAGCGCAGCATATCATG**	L-BC-lus (4372-4391)
RE647	**TTACGGTGTGTCGCAGAAGT**	L-A-2 (167-186)
RE648	**TCGATCGGCACATCAAGCTT**	L-A-2 (520-501)
RE649	**AGGCGATGGACTGGGTGTGC**	L-A-2 (3501-3520)
RE650	**TGTTTCGACTGCCCTTTGCG**	L-A-2 (4304-4285)
RE796	**TGGATTTTCGTGTAGCAGCTTA**	L-BC (1966-1987)
RE797	**GCTGCGCTTAGGTCCCTAAACG**	L-BC (4380-4359)
RE820	**GAATTTTTCAGTGAACCGGAA**	L-BC-lus (1-21)
RE821	**GAATTTTTCGGTGAACCGGAA**	L-BC (1-21)
RE822	**TCTGGAAATTCCAGGCCAGG**	L-BC-2 (607-588)
RE823	**GCATGATGGGGAACGATGTGA**	L-BC-2 (1591-1611)
RE824	**ATCGGTTCCGCGTCCACTTC**	L-BC-2 (2074-2055)
RE825	**CGGTGATTATACTGCATTCC**	L-BC-2 (4240-4259)
RE826	**GCGTAGTATCGTATGTACTCT**	L-BC (4614-4594)
RE827	**GGAGTACGATATGAGTA**	L-BC-2 (2851-2867)

Numbering corresponds to the sequences specific to each dsRNA (in bold), and the flanking non-bold nucleotides correspond to restriction sites and extensions needed for cloning. Oligonucleotides NR80, NR88 and NR119, originally designed from L-A-lus sequence contain some mismatches regarding L-A-2 sequence (shadowed).