Supplementary Information

# Diversity and modularity of tyrosine-accepting tRNA-like structures

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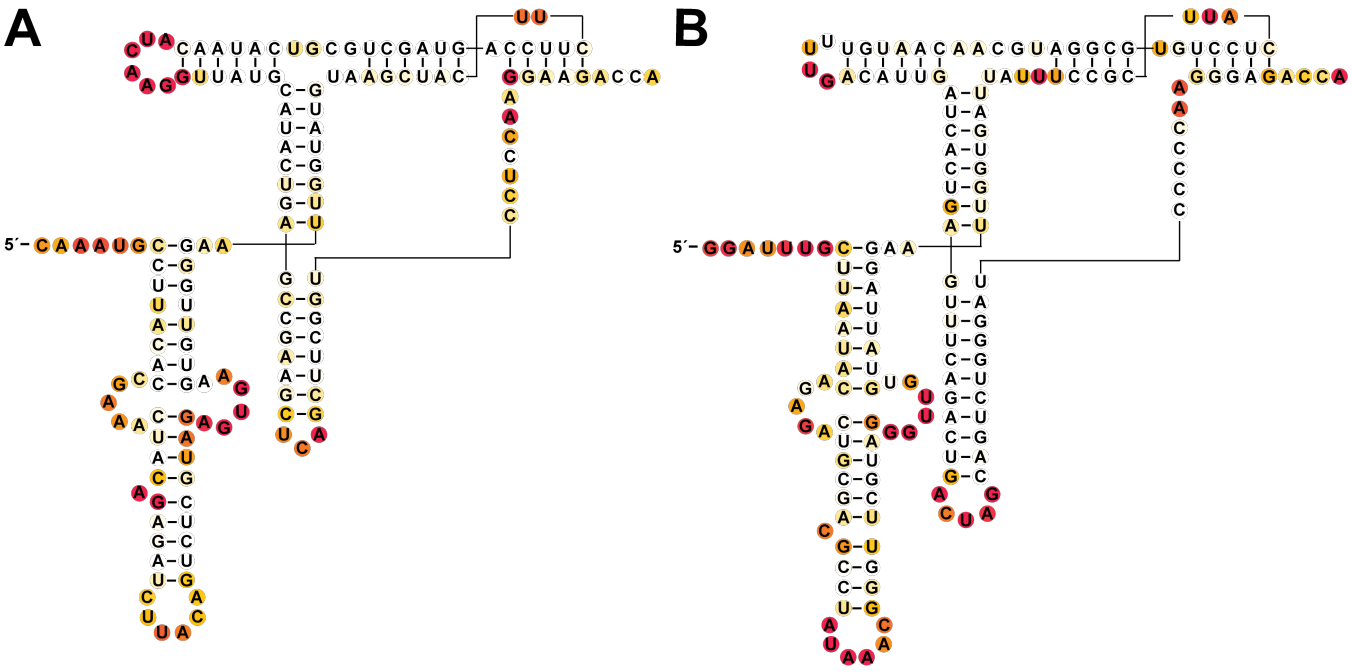
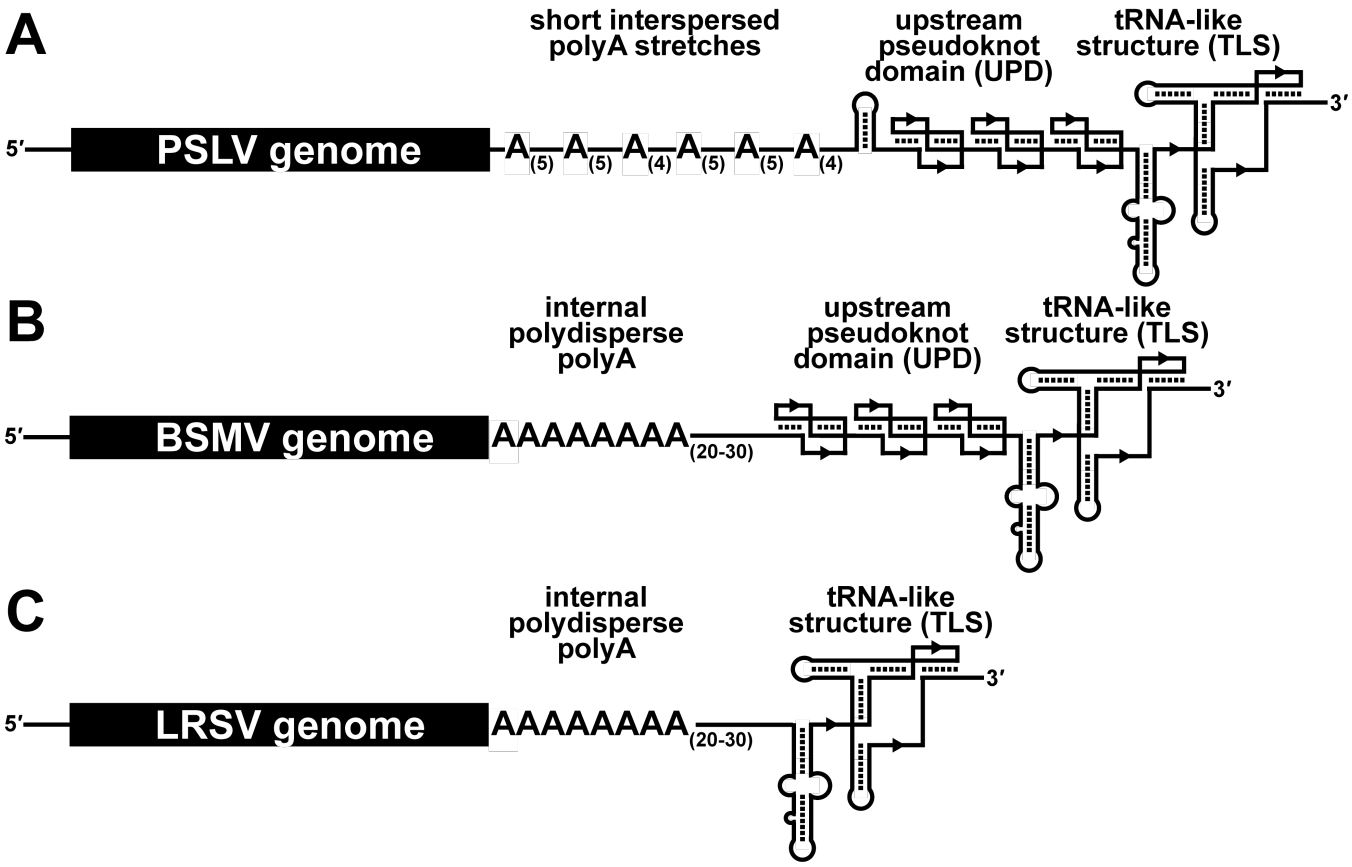
**Supplementary information contents:**

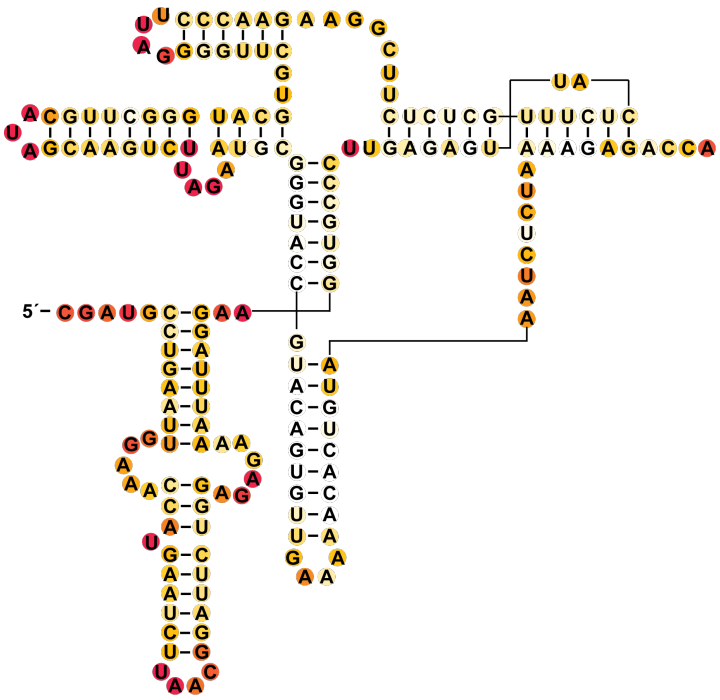
## FIGURES



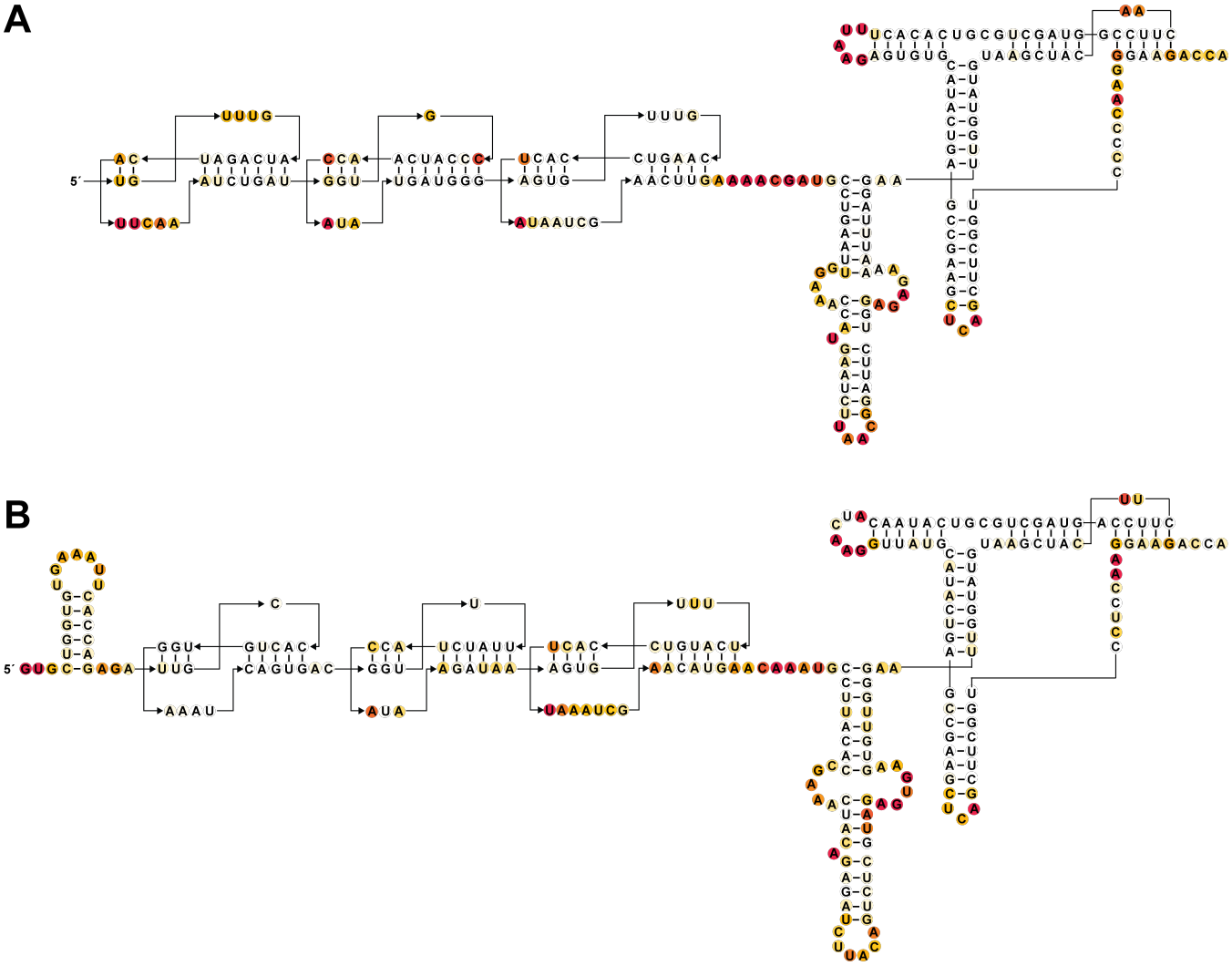

**SUPPLEMENTARY FIGURE S1.** Additional representative *hordeivirus* TLS$^{Tyr}$ RNAs conform to predicted secondary structure models. (*A,B*) Chemical probing of the TLS representative from (*A*) PSLV RNA1 and (*B*) LRSV RNA1 using the SHAPE reagent NMIA. See Figure 1 for chemical probing of the BSMV *hordeivirus* TLS$^{Tyr}$ RNAs and the legend for more details. See Supplementary Table S1 for complete sequence details.




**SUPPLEMENTARY FIGURE S2.** Architecture of RNA elements in all *hordeivirus* 3' untranslated regions. Cartoon diagrams of the primary sequence and secondary structure elements within the 3' UTRs of the genomic RNAs of (*A*) PSLV, (*B*) BSMV, and (*C*) LRSV. PSLV contains short (4-5 nucleotide) interspersed stretches of adenosines while BSMV and LRSV contain an uninterrupted, longer, heterogeneous poly(A) stretch. PSLV and BSMV contain a UPD, which is absent in LRSV.



**SUPPLEMENTARY FIGURE S3.** A non-aminoacylatable chimeric TLS folds consistent with the predicted secondary structure model. Chemical probing of the chimeric TLS representative from BMV with the R domain of BSMV in place of the native E and B3 domains. See also Figure 1 for chemical probing of the WT BSMV *hordeivirus* TLS$^{Tyr}$ RNAs and the legend for more details, and Figure 3 for functional data of chimeric RNAs. Chemical probing of the WT BMV TLS was previously reported (Bonilla et al. 2021). See Supplementary Table S1 for complete sequence details.




**SUPPLEMENTARY FIGURE S4**. Upstream pseudoknot domains conform to predicted secondary structure models and do not impact folding of the TLS domain. (*A,B*) Chemical probing of the entire 3′ untranslated region from (*A*) BSMV RNA1 and (*B*) PSLV RNA1 using the SHAPE reagent NMIA. The probing construct includes all sequence following the poly(A) stretches following the stop codon. The three pseudoknot structures that comprise the upstream pseudoknot domain (UPD) are drawn as previously proposed (Van Belkum et al. 1985; Zeenko et al. 2002). See also Figure 1 for chemical probing of the BSMV *hordeivirus* TLS$^{Tyr}$ RNAs and the legend for more details. See Supplementary Table S1 for complete sequence details.

**SUPPLEMENTARY TABLE S1.** DNA oligonucleotide sequences used in this study.

| DNA sequence | Description |
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| GCGAGTGAATTCTAATACGACTCACTATAGGAAA CGACTCGAGTAGAGTCGAAAACGATGCCTGAAT TGGAAACCATGAATCTTAACGGATTCTGGAGAGA AAATTTAGGAATTGGTATGTAAGCTACAACTTCC GGTAGCTGCGTCACACTTTAAGAGTGTGCATACT GAGCCGAAGCTCAGCTTCGGTCCCCCAAGGGAA GACCAGTTGGAGTCGAGTAGACTCCAACAAAAG AAACAACAACAACAACGGATCCGCGAT | Gene block (dsDNA) fragment including the WT TLS representative from barley stripe mosaic virus (BSMV) and flanking normalization hairpins (underlined) for chemical probing. Used as a template for chemical probing and aminoacylation experiments. |
| GCGAGTGAATTCTAATACGACTCACTATAGGAAA CGACTCGAGTAGAGTCGAAAAGATAGTCGTGGT TGACACGCAGACCTCTTACAAGAGTGTCTAGGT GCCCTTGAGAGTTACTCTTTGCTCTCTTCGGAAG AACCCTTAGGGGTTCGTGCATGGGCTTGCATAG CAAGTCTTAGAATGCGGGTACCgTACAGTGTTGA AAaACACTGTAAATCTCTaAAAGAGACCAGTTGG AGTCGAGTAGACTCCAACAAAAGAAACAACAACA ACAACGGATCCGCGAT | Gene block (dsDNA) fragment including the WT TLS representative from brome mosaic virus (BMV) and flanking normalization hairpins for chemical probing. Used as a template for aminoacylation experiments in this study. Previously used for chemical probing experiments (Bonilla et al. 2021). |
| GCGAGTGAATTCTAATACGACTCACTATAGGAAA CGACTCGAGTAGAGTCGAAAACAAATGCCTTAC ACCGAAACTACAGAGATcTTACaGTCTCGTAGAG TGAAGTGTTGGGAATTGGTATGTAAGCTACTTCT TCCAGTAGCTGCGTCATAACaTCAAGGTTATGCA TACTGAGCCGAAGCTCAGCTTCGGTCCTCCAAG GAAGACCAGTTGGAGTCGAGTAGACTCCAACAA AAGAAACAACAACAACAACGGATCCGCGAT | Gene block (dsDNA) fragment including the WT TLS representative from poa semi-latent virus (PSLV) and flanking normalization hairpins (underlined) for chemical probing. Used as a template for all chemical probing and aminoacylation experiments. |
| GCGAGTGAATTCTAATACGACTCACTATAGGAAA CGACTCGAGTAGAGTCGAAAAGGATTTGCTTAAT ACAGAGACTGCGACGCCTaTAAAcGGGTTCgTAG GGTTGTGTATTAGGAATTGGTGATTATTTCCGCT TACTCCTGTGCGGATGCAACAATGTTTTGACATT GATCACTGAGTTTCAGAcTgacTagcagTCTGGGAT CCCCCAAGGGAGACCAGTTGGAGTCGAGTAGAC TCCAACAAAAGAAACAACAACAACAACGGATCC GCGAT | Gene block (dsDNA) fragment including the WT TLS representative from lychnis ringspot virus (LRSV) and flanking normalization hairpins (underlined) for chemical probing. Used as a template for all chemical probing and aminoacylation experiments. |
| GCGAGTGAATTCTAATACGACTCACTATAGGAAA CGACTCGAGTAGAGTCGAAAATGTTTGATCAGAT CATTCAAATCTGATGGTGCCCATCAACCATATGA TGGGAGTGTTTGCAAGTCCACTATAATCGAACTT GAAAACGATGCCTGAATTGGAAACCATGAATCTT AACGGATTCTGGAGAGAAAATTTAGGAATTGGTA TGTAAGCTACAACTTCCGGTAGCTGCGTCACACT TTAAGAGTGTGCATACTGAGCCGAAGCTCAGCT TCGGTCCCCCAAGGGAAGACCAGTTGGAGTCGA GTAGACTCCAACAAAAGAAACAACAACAACAACG GATCCGCGAT | Gene block (dsDNA) fragment including the entire 3′ UTR of BSMV and flanking normalization hairpins (underlined) for chemical probing. Used as a template for chemical probing experiments. |

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| GCGAGTGAATTCTAATACGACTCACTATAGGAAA CGACTCGAGTAGAGTCGAAAAGTGCTGGTGTGA AATTCACCAGAGATTGCCACTGTGGAAATCAGTG ACGGTTTTATCTACCATAAGATAAAGTGTTTTCAT GTCCACTTAAATCGAACATGAACAAATGCCTTAC ACCGAAACTACAGAGATcTTACaGTCTCGTAGAG TGAAGTGTTGGGAATTGGTATGTAAGCTACTTCT TCCAGTAGCTGCGTCATAACaTCAAGGTTATGCA TACTGAGCCGAAGCTCAGCTTCGGTCCTCCAAG GAAGACCAGTTGGAGTCGAGTAGACTCCAACAA AAGAAACAACAACAACAACGGATCCGCGAT | Gene block (dsDNA) fragment including the entire 3′ UTR of PSLV and flanking normalization hairpins (underlined) for chemical probing. Used as a template for chemical probing experiments. |
| GCGAGTGAATTCTAATACGACTCACTATAGGAAA CGACTCGAGTAGAGTCGAAAACGATGCCTGAAT TGGAAACCATGAATCTTAACGGATTCTGGAGAGA AAATTTAGGAAGGTGCCTTTGAGAGTTACTCTTT GCTCTCTTCGGAAGAACCCTTAGGGGTTCGTGC ATGGGCTTGCATAGCAAGTCTTAGAATGCGGGT ACCGTACAGTGTTGAAAAACACTGTAAATCTCTA AAAGAGACCAGTTGGAGTCGAGTAGACTCCAAC AAAAGAAACAACAACAACAACGGATCCGCGAT | Gene block (dsDNA) fragment including the chimeric TLS representative with the R domain from BSMV and the 3′ domain from BMV and flanking normalization hairpins (underlined) for chemical probing. Used as a template for chemical probing experiments. |
| CGATGCCTGAATTGGAAACCATGAATCTTTCGG GATTCTGGAGAGAAAATTTAGGAATTGGTATGTA AGCTACAACTTCCGGTAGCTGCGTCACACTTTAA GAGTGTGCATACTGAGCCGAAGCTCAGCTTCGG TCCCCCAAGGGAAGACCA | Gene block (dsDNA) fragment including the TLS representative from BSMV with mutations to the R apical loop (underlined). Used as a template for aminoacylation experiments. |
| CGATGCCTGAATTGGAAACCATGAATCTTAACGG ATTCTGGAGAGAAAATTTAGGAATTGGTATGTAA GCTACAACTTCCGGTAGCTGCGTCACACTTTAAG AGTGTGCATACTGAGCCGAAGCTTCGGCTTCGG TCCCCCAAGGGAAGACCA | Gene block (dsDNA) fragment including the TLS representative from BSMV with mutations to the B2 apical loop (underlined). Used as a template for aminoacylation experiments. |
| ATAGTcgtggttgacACGCAGACCTCTTACAAGAGTG TCTATTGGTATGTAAGCTACAACTTCCGGTAGCT GCGTCACACTTTAAGAGTGTGCATACTGAGCCG AAGCTCAGCTTCGGTCCCCCAAGGGAAGACCA | Gene block (dsDNA) fragment including the chimeric TLS representative with the E/B3 domain from BMV and the 3′ domain from BSMV. Used as a template for chemical probing experiments. |
| CGATGCCTGAATTGGAAACCATGAATCTTAACGG ATTCTGGAGAGAAAATTTAGGAAGGTGCCTTTGA GAGTTACTCTTTGCTCTCTTCGGAAGAACCCTTA GGGGTTCGTGCATGGGCTTGCATAGCAAGTCTT AGAATGCGGGTACCGTACAGTGTTGAAAAACAC TGTAAATCTCTAAAAGAGACCA | Gene block (dsDNA) fragment including the chimeric TLS representative with the R domain from BSMV and the 3′ domain from BMV. Used as a template for chemical probing experiments. |
| GCGAGTGAATTCTAATACGACTCACTATAGG | Forward ssDNA primer to amplify all constructs with flanking hairpins for chemical probing experiments. |
| GTTGTTGTTGTTGTTTCTTTTGTTGGAGTCTACTC | Reverse ssDNA primer to amplify all constructs with flanking hairpins for chemical probing experiments. |

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| TAATACGACTCACTATAGGCGATGCCTGAATTGGAAACCATGAATC | Forward ssDNA primer to amplify the full-length BSMV TLS template for in vitro aminoacylation experiments |
| TAATACGACTCACTATAGGAATTGGTATGTAAGCTACAACTTCCG | Forward ssDNA primer to amplify the truncated (-R) BSMV TLS template for in vitro aminoacylation experiments |
| mUmGGTCTTCCCTTGGGGGACC | Reverse ssDNA (with two 2′ O-methyl bases at the 5′ end) primer to amplify all BSMV TLS template for in vitro aminoacylation experiments. |
| TAATACGACTCACTATAGGCAAATGCCTTACACCGAAACTACAGAG | Forward ssDNA primer to amplify the full-length PSLV TLS template for in vitro aminoacylation experiments |
| TAATACGACTCACTATAGGAATTGGTATGTAAGCTACTTCTTCCAGTAG | Forward ssDNA primer to amplify the truncated (-R) PSLV TLS template for in vitro aminoacylation experiments |
| mUmGGTCTTCCTTGGAGGACCGAAG | Reverse ssDNA (with two 2′ O-methyl bases at the 5′ end) primer to amplify all PSLV TLS template for in vitro aminoacylation experiments. |
| TAATACGACTCACTATAGGATTTGCTTAATACAGAGACTGCGACG | Forward ssDNA primer to amplify the full-length LRSV TLS template for in vitro aminoacylation experiments |
| TAATACGACTCACTATAGGAATTGGTGATTATTTCCGCTTACTCC | Forward ssDNA primer to amplify the truncated (-R) LRSV TLS template for in vitro aminoacylation experiments |
| mUmGGTCTCCCTTGGGGGATCC | Reverse ssDNA (with two 2′ O-methyl bases at the 5′ end) primer to amplify all LRSV TLS template for in vitro aminoacylation experiments. |
| TAATACGACTCACTATAGGATAGTCGTGGTTGACACGCAGAC | Forward ssDNA primer to amplify the full-length BMV TLS template for in vitro aminoacylation experiments |
| TAATACGACTCACTATAGGTGCCTTTGAGAGTTACTCTTTGC | Forward ssDNA primer to amplify the truncated (-E+B3) BMV TLS template for in vitro aminoacylation experiments |
| mUmGGTCTCTTTTAGAGATTTACAGTGTTTTTCAACA | Reverse ssDNA (with two 2′ O-methyl bases at the 5′ end) primer to amplify all BMV TLS template for in vitro aminoacylation experiments |
| 5-6 FAM-AAAAAAAAAAAAAAAAAAAAgcttatgccgtgcctcagatc | FAM labeled RT primer for chemical probing experiments |

## SUPPLEMENTAL REFERENCES

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Van Belkum A, Abrahams JP, Pleij C, Bosch L. 1985. Five pseudoknots are present at the 204 nucleotides long 3'noncoding region of tobacco mosaic virus RNA. *Nucleic Acids Research* **13**: 7673.

Zeenko VV, Ryabova LA, Spirin AS, Rothnie HM, Hess D, Browning KS, Hohn T. 2002. Eukaryotic elongation factor 1A interacts with the upstream pseudoknot domain in the 3′ untranslated region of tobacco mosaic virus RNA. *Journal of virology* **76**: 5678-5691.