Predominance of six different hexanucleotide recoding signals 3' of read-through stop codons

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ABSTRACT

Redefinition of UAG, UAA and UGA to specify a standard amino acid occurs in response to recoding signals present in a minority of mRNAs. This 'readthrough' is in competition with termination and is utilized for gene expression. One of the recoding signals known to stimulate read-through is a hexanucleotide sequence of the form CARYYA 3' adjacent to the stop codon. The present work finds that of the 91 unique viral sequences annotated as readthrough, 90% had one of six of the 64 possible codons immediately 3' of the read-through stop codon. The relative efficiency of these read-through contexts in mammalian tissue culture cells has been determined using a dual luciferase fusion reporter. The relative importance of the identity of several individual nucleotides in the different hexanucleotides is complex.

INTRODUCTION

Standard decoding is enriched, in probably all organisms, by special signals, often called recoding signals, embedded in a subset of mRNAs. One facet of recoding is the redefinition of stop codons in certain sequence contexts to specify an amino acid. The same stop codon in the great majority of other contexts in the same cell retains the standard function of specifying termination. Thus, this redefinition is distinct from global reassignment that occurs in certain organelles and in some organisms. Where UGA is redefined to specify the 21st amino acid selenocysteine (1), the identity of the amino acid specified is the important feature. In other cases, in response to different recoding signals, the important consequence is that a proportion of the ribosomes continue translation beyond the stop codon in the same reading frame, i.e. they read through the stop codon. Though unimportant in itself, the most common amino acid encoded in the read-through of UGA is tryptophan and of UAG is glutamine. Read-through utilized for gene expression is typically 100-1000-fold above the error rate for sensing stop codons even though commonly only 1-10% of ribosomes read through such redefined stop codons. Readthrough is utilized to synthesize a proportion of extended proteins that may have additional functions to that of the

standard product. Synthesis of the extended product may be regulatory (2), or perhaps ribosome movement 3' of the leaky stop codon may itself have regulatory significance for mRNA structure (3). UAA is often less efficiently read through than UAG, and UGA is more 'leaky' than UAG.

A recoding signal for read-through can be located many hundreds of nucleotides 3' of the redefined stop codon as discovered by Miller and colleagues in their studies on barley yellow dwarf virus (4). It can also be an elaborate pseudoknot 3' of the leaky stop codon as in the synthesis of the murine leukemia virus gag-pol precursor (5-7). However, in the original case of the RNA phage Q beta coat protein read-through (8,9) or in the case of Sindbis virus recoding (10) only the identity of the nucleotide 3' adjacent to the UGA utilized is thought to be important. Release factors recognize the triplet stop codon and adjacent nucleotides; in particular, they recognize the 3' adjacent nucleotide. In read-through cases the 3' adjacent nucleotide is not one favored for recognition by the release factor (11). While the identity of the following two nucleotides has diminishing importance for release factor recognition, subsequent bases are not known to have any direct effect on that recognition. Nevertheless, for read-through to synthesize a replicase component of tobacco mosaic virus (TMV), the identity of six nucleotides 3' adjacent to the stop codon, in the form CAR-YYA, is important (12-14).

The recent enormous increase in sequence information prompted us to assess nucleotide preferences in the vicinity of read-through stop codons. We have concentrated on readthrough in viral expression since the great majority of cases currently known are in viral decoding. The importance of the different nucleotides within the contexts found were tested in a dual luciferase fusion reporter designed for this purpose (15). The leaky terminator and surrounding context are placed between a *Renilla* luciferase reporter, which provides a measure of termination, and a *Photinus* luciferase reporter, which provides a measure of read-through product.

MATERIALS AND METHODS

Collection and examination of viral sequences

Using the Taxonomy browser at the National Center for Biotechnology Information (NCBI) web site (http://www.ncbi.nlm.nih.gov/ Taxonomy/taxonomyhome.html/), several representative nucleotide sequences from each currently accepted International Committee on Taxonomy of Viruses genus were examined to

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determine whether any members of that genus contained a read-through stop codon. In addition, an Entrez (http://www.ncbi.nlm.nih.gov/Entrez/) keyword search was performed for the terms 'read-through' or 'transl_except' and the resulting hits were further examined.

The uniqueness of each sequence was determined using criteria that allowed different strains of the same virus to be included, but excluded any sequences with identical names or known aliases as determined by their NCBI taxonomic entries. The 82 nt long segment of sequence from the 19th nucleotide 5' to the 60th nucleotide 3' of the leaky stop codon was extracted from each identified distinct sequence. The segments were then compared to one another, and one sequence was excluded from any pair of sequences that shared >90% identity. Sequence controls were obtained by extracting the 82 nt region surrounding the in-frame, non-leaky stop codon downstream of each leaky stop codon.

The nucleotide triplet immediately 3' of the stop codon (+1 triplet), was used to divide the sequences into groups. The non-randomness associated with each nucleotide position in each group was examined by chi-square (χ^2) analysis. The secondary structure 3' of the stop codon was examined using mFold (16,17). The folding was simulated at 37°C, and limitations were set so that no binding was allowed to occur with the stop codon or the sequence 5' of it.

Assessing the ability of the sequence groups to signal readthrough using p2luc

The dual luciferase reporter vector p2luc (DDBJ/EMBL/ GenBank accession number AF043450) (15) was constructed with one of seventeen 18 bp sequences, representing groups 1, 2, 3 and 4 of the identified read-through groups, inserted between the BamHI and SalI restriction sites. The insert of each construct was synthesized as a pair of complementary oligonucleotides, such that the coding strand sequence read 5'-GATCC-CCC-AAA-WWW-XXX-XXX-CAG-3', and the non-coding strand sequence read 5'-TCGAC-CTG-YYY-YYY-ZZZ-TTT-GGG-3'. GATCC and TCGAC were the complementary sticky ends of the BamHI and SalI sites respectively. The WWW was either TAG or TGA for the test sequences or CAG or TGG for the control sequences and the Zs represent the complement of the corresponding Ws. The Xs represent the +1 and +2 triplet nucleotide positions and the Ys represent the complement of the corresponding X. Each pair of oligonucleotides was then annealed and separately ligated into the digested p2luc vector. The raw results in relative light units (RLU), available at http://opbs.okstate.edu/Virevol/lucdat.html, were converted to %read-through (RT) using the formula RT = (RLU of test Photinus luc/RLU of test Renilla luc)/(RLU of control Photinus luc/RLU of control Renilla luc).

RESULTS

Collection and examination of viral sequences

Of the viral genera examined, 23 had read-through stop codons reported in their genome annotations. These 23 genera yielded 157 individual sequences for screening. The uniqueness of each sequence was determined as described in Materials and Methods, eliminating 66 sequences and leaving 91 unique sequences to be analyzed.

Since the nucleotides immediately 3' of the leaky stop codon were previously implicated in read-through (4,5,10,12,18–22), the sequences were first categorized according to these nucleotides. It was found that 6 of the 64 possible nucleotide triplets accounted for 90% of the triplets in the +1 position of the readthrough sequences, while being present in only two of the nonread-through control sequences. In contrast, the six most frequent triplets found in the +1 position of the non-readthrough control groups only accounted for 35.1%. Each one of the six triplets, CAA, CGG, GGG, GGA, GUA and CUA, formed the basis of a sequence group into which all sequences containing that +1 triplet were placed (Table 1). For the purposes of discussion we have considered the nucleotides in sets of three, even though the influence of the nucleotides probably occurs at the nucleotide level rather than the codon level.

Sequences in the +2 triplet position were also non-randomly distributed with the five most frequent accounting for 70.3%. These triplets were distributed among all the +1 groups. However, for five of the six +1 groups, there was a +2 triplet that was mostly non-random for each +1 triplet sequence: CAA-UUA (88.0%), CGG-UUU (55.0%), GGG-UGC (52.9%), GGA-GGC (66.3%) and GUA-GAC (80.0%). The sixth group, CUA, did not show any common +2 triplet sequence.

Examination of sequence variability within the groups using χ^2 analysis (Fig. 1) showed that both the +1 and, to a lesser extent, +2 triplets in the read-through groups were much more non-random than their counterparts in the corresponding control groups. Read-through groups 4, 5 and 6 contain other nucleotides that have χ^2 values as high as, or nearly as high as, their respective nucleotides in the +1 triplet. For group 4, the high level of background in both the read-through and control groups can be accounted for by the fact that this group only contains three viral sequences. For group 5, which consists mostly of luteoviruses, the other highly non-random nucleotides are expected because of the CCN-NNN repeat that has been shown to be important in signaling read-through for luteoviruses (4). This repeat is present to a limited extent in the other group 5 sequences, and in six of the nine ungrouped sequences, suggesting that this repeat may also play a role in signaling read-through in other genera. In group 6, the equality of other χ^2 values with that of the +1 triplet is due to the higher level of sequence identity among the coding regions of the read-through proteins of the alpha viruses, as compared to members of other groups. This is evident by comparing the χ^2 values 3' of the leaky terminator in the read-through group and the χ^2 values 5' of the read-through protein stop codon in the control group with those 3' of the control group.

The terminal dipeptide has been implicated in translation termination efficiency (19,23,24). The chemical characteristics of the penultimate amino acid have been shown to influence the efficiency of termination, with basic residues yielding more efficient read-through in *Saccharomyces cerevisiae* (19), and acidic and hydrophobic residues giving higher read-through in *Escherichia coli* (23). Greater read-through efficiency is also associated with a higher likelihood of the ultimate amino acid participating in the formation of α -helices or β -sheets in *E.coli* (24). We examined the properties of the terminal dipeptides in both our sample and control groups and found no bias in the chemical characteristics of the penultimate amino acid, or in the α -helical or β -sheet propensities of the ultimate amino acid,

 Table 1. Sequence groups based on nucleotides 3' adjacent to read-through stop codons

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|---|--|--|---|--|--|--|---|--|--|
| Genus | Virus | -3 | -2 | -1 | Stop | +1 | +2 | +3 | Accession # |
| TOBAMO | Tump vein cleaning | GGG | GUC | CAA | UAG | CAA | UUA | AUU | U03387 |
| | Chinese rape mosaic | GGU | ACC | CAA | UMG | CAA | UUA | CAG | U30944 |
| | Tobacco moslac | GGU | ACU | CAA | UAG | CAA | UUA | CAG | AF155507 |
| | Tobacco mosaic (8935A) | GGA | ACA | ÇAA | UAG | CAA | UUA | CAG | AJ011933 |
| | Tobacco mosiac (crucifer, Cg) | GGG | ACC | CAA | LIAG | CAA | UUA | CAG | D38444 |
| | Tobacco mosaic (Korean) | GGA | ACA | CAA | LIAG | CAA | UUA | CAG | X68110 |
| | Tobacco mosaic (K2) | GGU | ACU | CAA | UAG | CAA | UUA | CAG | Z92909 |
| | Tobacco mosaic (OM) | GGA | ACA | CAA | UAG | CAA | UUA | CAG | D78608 |
| | Tobacco mosaic (Tomato/L) | GGU | ACU | CAA | UAG | CAA | UUA | CAG | X02144 |
| | Tobacco mosaic (crucifer, Russian) | GGG | AUC | CAA | UMG | CAA | UUA | CAG | 229370 |
| | Tobacco mild green mosaic (U2) | GGU | AGU | AGA | UAG | CAA | UUA | CAG | M34077 |
| | Tobamovinus Ob | GUG | AGU | GCA | UAG | CAA | UUA | CAG | D13438 |
| | Pepper mid mottle (S) | UCG | ACU | CAA | LIAG | CAA | UUA | CAG | M81413 |
| | Cucumber groep mottle mosaic (SH) | CCU | ACC | AAA | LIAC | CAA | UUA | AUG | D12505 |
| | Outumber organ mottle mosaic (VODO) | UCC | CCC | 0.00 | LIAG | CAA | UUA | AUG | AB015145 |
| | Suno-bomo mosaic | ACC | CAA | A64 | LIAG | CAA | UUA | CAG | 147034 |
| | Odortoolossum ringsoot (Singsoore) | occ. | ALM | LILIA | LINC | CAA | UILLA | CAG | 1174505 |
| | Curumber fuit motile metain | 000 | ACC | 0.64 | LINC | CAA | URIA | CAG | AE321057 |
| BENY | Boot mercetic vallew usin (S) | cee | CCA | CAA | 1140 | CAA | THIA | CCU | DR4411 |
| DENT | Boot soil home mosain | 000 | ACC | 4611 | 11AC | CAA | UIIA | 144 | AEAGABER |
| DOMO. | Dest soll-borne (Alsom) | 1000 | CILL | CAA | LUNC. | CAA | UCA | ACU | 1854512 |
| CORO | Broad base socrask | 000 | 400 | OCA | 110.0 | CAA | IIIIA | 100 | D04512 |
| | Didade post ten // B | COL | 000 | oca | 1100 | CAA | UUN | 400 | 0400037 |
| | Potato mop top (0) | GCU | GGU | GGA | LUNK: | CAN | UCA | ALC: | 019013 |
| | Deet virus Q-R/042 | ALL | GOL | OCA | CONC. | CAA | UCA | AUG | AU223391 |
| UNGLASS | Borryos wrus F | GCU | GAN | CLA | USIA | CAA | UCA | C90 | AF 238854 |
| Citoup 24CCco | Children and the second parts | 1010 | 010 | | No. | 000 | | 0.00 | 4 10 1 2 2 2 2 2 |
| FURO | Chinese wheat mosaic (Yantai)-RNA1 | UUC | GAC | AAA | UGA | CGG | 000 | GGG | AJ012005 |
| | Chinese wheat mosaic (Yantai)-RNA2 | AGG | ouc | GAG | UUA | CGG | GAU | GGÇ | AJ012006 |
| | European wheat mosaic | UUG | GCG | AAA | LIGA | CGG | UUU | GGG | AJ132576 |
| | Cat golden stripe-RNA1 | ANU | GAG | 111 | UCA | CGG | uuu | GGG | AJ132578 |
| | Cat golden stripe-RNA2 | GGT | AGU | GCC | UGA | CGG | GGC | GGC | AJ132579 |
| | Soil-borne rye mosaic (0) | UUG | GUG | AAA | UGA. | CGG | UUU | GGG | AF145280 |
| | Soli-bome wheat mosaic (Japanese) | AAC | GGG | AAA | UGA | CGG | UUU | GGG | AB033689 |
| | Soli-borne wheat mosaic (US-N)-RNA1 | CUU | ACU. | AAA | UGA . | CGG | UUU | GGG | L07937 |
| | Soil-bome wheat mosaic (US-N)-RNA2 | GGU | UCG | AGU | UGA | CGG | GAC | GGC | L07938 |
| | Soil-borne wheat mosaic (UK-Kent) | GGU | UCG | AGU | UGA | CGG | GAC | GGC | AJ258070 |
| | Soli-bome wheat mosaic (UK-Wiltshire) | GGU | ACG | AGU | UGA | CGG | GAC | GGC | AJ258069 |
| | Sorghum chlorotic spot | CAU | ACC. | AAA | UGA | CGG | UUU | GGG | AB033691 |
| POMO | Beet virus Q-RNA1 | UCU | GUU | CAA | UAA | CGG | UUU | GGG | AJ223596 |
| PECLU | Peanut clump | CAG | ACC | AAA | LIGA | CGG | UUU | GGG | X78602 |
| TOBRA | Pepper ringspot (CAM) | GCU | GCC | UUA | LIGA | CGG | UGU | CGG | L23972 |
| | Tobacco rattle (North American) | ACC | GUC | ULIA | UGA | CGG | UUU | OGG | AF034622 |
| | Peoper ringsport (CAM) | GCU | GCC | ULLA | LIGA | CGG | UGU | CGG | 123972 |
| | Pea early browning | GCU | AUG | 86A | LICA | CGG | UGU | CGG | X14005 |
| COLT | Colorado tick fever | GGC | UGC | UGU | LINA | CGG | UGU | UGG | AE000720 |
| AL PHA | Venezuelan emine encenhaltis (83) 1434 | CAA | CAA | CAA | IICA | 000 | ULUI | CAC | 185362 |
| Call Serve. | Venezuelan equine enceptalitis (681/201 | CAA | CAG | CAA | ALC: A | CGG | UUC | GAC | 1134999 |
| Group 3 (666) | Tenecolum equino encephanto (obcice) | 0.01 | GHG | Court | | 000 | 500 | GHO | 331333 |
| Conus | Views | -1 | .2 | .1 | Ston | 1.1 | 12 | 43 | Accession # |
| AUDCUR | Virus Dather Interf (classes) | -0 CA11 | -2 | inte | Stop | 000 | 1000 | 10 | Autocession # |
| AUREUS | Pothos latent (pigeonpea) | GAU | GUC | UAC | UND | 000 | UGC | CUA | AJ243370 |
| MA714171 | Maize chlorotic mottle | GA/G | UUG | AAA | UAG | GGG | UGU | UCU | X14736 |
| MARCHED. | | | | | | | | | |
| ENAMO | Pea enation mosaic (At+) | GCC | UCC | CUC | LICIA | GGG | GAC | GAC | Y09099 |
| ENAMO | Pea enation mosaic (At+) Cardamine chlorotic flock | GCC | GUC | CUC | UNG | GGG GGG | GAC | GAC | Y09099 L16015 |
| ENAMO | Pea enation mosaic (At+) Cardamine chlorotic flock Carnation mottle (Shanghai) | GCC UUU UUU | GUC GUC CCC | CUC CGC AAA | UAG | GGG GGG GGG | GAC UGC GGC | GAC UUA CUG | Y09099 L16015 AF192772 |
| ENAMO | Pea enation mosaic (At+) Cardamine chlorotic flock Carnation mottle (Shanghai) Galinsoga mosaic carmovirts | GCC UUU UUU CUG | GUC GUC GCC GGC | CUC CGC AAA AAA | UAG UAG UAG | 666 666 666 666 | GAC UGC GGC UGC | GAC UUA CUG CUU | V09099 L16015 AF192772 Y13463 |
| ENAMO | Pea enation mosaic (At-) Cardamine chicrotic flock Carnation motile (Shanghai) Galinsoga mosaic carmovirts Hibiscus chicrotic ringspot | GCC UUU UUU CUG CCC | GUC GUC GGC GUG | CUC CGC AAA AAA AAA | UAG UAG UAG | 666 666 666 666 666 | GAC UGC GGC UGC UGC | GAC UUA CUG CUU CUU | Y09099 L16015 AF192772 Y13463 X86448 |
| ENAMO | Pea enation mosaic (At+) Cardamine chlorotic flock Cardation mottle (Shanghai) Galinsoga mosaic carmovirts Hibisous chlorotic ringspot Japanese ins necrotic ring | GCC UUU UUU CUG CCC UUC | UCC GUC GGC GGC GUS UCC | CUC CGC AAA AAA AAA AAA | UAG UAG UAG UAG UAG | 666 666 666 666 666 | GAC UGC UGC UGC UGC | GAC UUA CUG CUU CUU CUU | Y09099 L16015 AF192772 Y13463 X86448 D86123 |
| ENAMO | Pea enation mosaic (At-) Cardamino chiorotic flock Carnation mottle (Shanghai) Galinsoga mosaic carmovits Hibisous chiorotic ring Japanese iris neocrotic ring Motor neocrotic spot | GCC UUU UUU CUG CCC UUC UUG | UCC GUC GGC GUC GUC GUC | CUC CGC AAA AAA AAA AAC AAC | UAG UAG UAG UAG UAG UAG | 666 666 666 666 666 666 666 | GAC UGC UGC UGC UGC UGC | GAC UUA CUG CUU CUU CUG CUG | Y09099 L16015 AF192772 Y13463 X85448 D86123 M29671 |
| ENAMO CARMO | Pea enation mosaic (At-) Candamine chioretic ficek Canadam mutici (Shangha) Galinsoga mosaic carmovits Hibiscus chioretic migspot Japanese iris neorotic mig Melon neorotic spot Saquaro catus | GCC UUU UUU CUG CCC UUC UUC UUG UAC | UCC GUC GGC GUC GUC GUC CAC | CUC CGC AAA AAA AAA AAA AAC AAA | UAG UAG UAG UAG UAG UAG UAG | 666 666 666 666 666 666 666 666 | GAC UGC UGC UGC UGC UGC UGC | GAC UUA CUG CUU CUU CUG CUG CUG | Y09099 L16015 AF192772 Y13463 X86448 D86123 M29671 U72332 |
| ENAMO CARMO | Pre-ension mosaic (Ar-) Candamine chlorotic flock Candamine chlorotic flock Canadon mobile (Shanghai) Galinsoga mosaic cannovits Hibisous chlorotic ningspot Japanese ins necretic ning Melon necretic spot Saquaro cactus Turnio crintele | GCC UUU CUG CCC UUC UUC UUG UAC | UCC GUC GGC GUC GUC GUC GUC GUC | CUC CGC AAA AAA AAA AAA AAC AAC AAA CGC | UAG UAG UAG UAG UAG UAG UAG | 666 666 666 666 666 666 666 666 | GAC UGC UGC UGC UGC UGC UGC UGC | GAC UUA CUG CUU CUU CUC CUG CUA UUG | Y09099 L16015 AF192772 Y13463 X86448 D86123 M29671 U72332 M22445 |
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| ENAMO CARMO NECRO | Pre-ension mosaic (4-) Cardenine chickette Rock Carnation mottle (Shanghai) Galinsoga mosaic carnevits Histous chickette nngspot Japanese Ins necrotic nng Melon necrotis spot Saquaro cactus Tump critike Lask white shipe Tobacce necross (Twe A) | GCC UUU UUU CUG UUC UUC UUG UUU CAU CGG | UCC GUC GUC GUC GUC GUC GUC GUC GUC GUC | CUC CGC AAA AAA AAA AAA AAA CGC AAA AAA | UAG UAG UAG UAG UAG UAG UAG UAG UAG | 666 666 666 666 666 666 666 666 666 | GAC UGC UGC UGC UGC UGC UGC UGC UGC UGC | GAC UUA CUG CUU CUC CUC CUG CUG CUA UUG CUA | Y09099 L16015 AF192772 Y13463 X86448 D86123 M29671 U72332 M22445 X94560 X58455 |
| NECRO PANICO | Pre-ensuon mosaic (Ar-) Canadamino chioretic flock Canadami otto (Shangtua) Galinsoga mosaic carmovits Histous chicotte mospot Japanese ins neoratic ring Melon neoratic spot Saguaro cactus Turmo crinkle Losk white stripe Tobacco neorosis (Typo A) Panicum mosaic | GCC UUU CUG CCC UUC UUC UUG UUU CAU CGG UUU | UCC GUC GGC GUC GUC GUC GUC GUC GCC GCC | CUC CGC AAA AAA AAA AAC AAA AAA AAA AAA AA | UAG UAG UAG UAG UAG UAG UAG UAG UAG | 666 666 666 666 666 666 666 666 666 66 | GAC UGC UGC UGC UGC UGC UGC UGC UGC UGC UG | GAC UUA CUG CUU CUU CUC CUG CUA UUG CUA CUA | Y09099 L16015 AF192772 Y13463 X85448 D85123 M29671 U72332 M22445 X94560 X58455 U55002 |
| NECRO PANICO RETRO C | Pea enation mosaic (At-) Canatom motive (Shangha) Canatom motive (Shangha) Galinsoga mosaic carmovits Hibiscus chlorotic mgspot Japanese ins nocrotic mg Melon nocrotis spot Saguaro cathus Turnig crinkle Loak white stripe Tobacco nocross (Type A) Panicum mosaic Baboon enforcess (M7) | GCC UUU CUG CCC UUC UUC UUC UUU CAU CAU CGG UUU GAC | UCC GUC GGC GUC GUC GUC GUC GUC GCC GCC | CUC CGC AAA AAA AAA AAC AAA CGC AAA AAG GAA | UAG UAG UAG UAG UAG UAG UAG UAG UAG UAG | | GAC UGC UGC UGC UGC UGC UGC UGC UGC UGC UG | GAC UUA CUG CUU CUU CUC CUG CUA CUA CUA CUA CCU AUC CAG | Y09099 L16015 AF192772 Y13463 X86448 D86123 M29671 U72332 M22445 X94560 X58455 U55002 D10032 |
| NECRO PANICO RETRO C | Pre-existion mosaic (Ar-) Candamine chlorotic flock Cantadom mobile (Shanghui) Galinsoga mosaic carmovits Hisisous chlorotic mogisot Japanese ins neorotic mg Miorin neorotic spot Saquaro cactus Turmp cirrikle Lask white stripe Tobacco neorosis (Type A) Panicum mosaic Baboon endogeneus (M7) Miorine NetWein (S13-3) | GCC UUU CUG CCC UUC UUG UUU CAU CAU CGG UUU GAC UUU | UCC GUC GUC GUC GUC GUC GUC GUC GUC GUC | CUC CGC AAA AAA AAA AAC AAA AAC AAA CGC AAA AAG GAA GAA | UAG UAG UAG UAG UAG UAG UAG UAG UAG UAG | 666 666 666 666 666 666 666 666 666 66 | GAC UGC UGC UGC UGC UGC UGC UGC UGC UGC UG | GAC UUA CUG CUU CUU CUG CUG CUG CUA CUA CUA CCU AUC CAG | Y09099 L16015 AF192772 Y13463 X85448 D86123 M29671 U72332 M29455 X94560 X58455 U55002 D10032 AF189255 |
| NECRO PANICO RETRO C UNCLASS | Pea enstion mosaic (At-) Candamine childrel (At-) Cantadiam multic (Shangha) Galinsoga mosaic carmovirts Helsicus chicetter mospet Japanese ins necrotic ring Melon necrotic spot Saguaro cactus Turnijo crinkle Lask white stripe Tobacco necrosis (Typo A) Panicum mosaic Babaon endogencis (M7) Multine laskemia (SL3-3) Carent col data Munohins assee ENA | GCC UUU CUG CCC UUC UUC UUC UUU CAU CAU CAU CAU CAU | UCC GUC GGC GUC GUC GUC GUC GUC GCC GCC | CUC CGC AAA AAA AAA AAC AAA AAA AAA AAA GAA G | UAG UAG UAG UAG UAG UAG UAG UAG UAG UAG | | GAC UGC UGC UGC UGC UGC UGC UGC UGC UGC UG | GAC UUA CUG CUU CUC CUG CUG CUA UUG CUA CUA CUA CUA CUA CCU AUC CAG CUU | V00099 L16015 Ar192722 V13463 X86448 D66123 M29671 U72332 M29671 U72332 M22445 X94560 X58655 U55002 D10032 AF180256 AF180256 |
| NECRO PANICO RETRO C UNCLASS GENERALIZZA | Pre-ension mosaic (Ar-) Candamine chlorotic flock Canadami mobile (Shanghui) Galinsoga mosaic carmovits Hibisous chlorotic mogsot Japanese ins neorotic mg Melon neorotic spot Saguaro cactus Turing cirkle Lask white stripe Tobacco neorosis (Type A) Panicum mosaic Baboon endogencus (M7) Murne kaukemis (SL3-3) Carnot red kalf Jutoovirus assoc RNA | GCC UUU CUG CCC UUC UUG UUG CAU CAU CAU CAU CAU CAU UUU GAC UUA UAC | UCC GUC GGC GUC GUC GUC GUC GUC GCC GCC | CUC CGC AAA AAA AAA AAC AAA CGC AAA AAG GAA GAA | UAG UAG UAG UAG UAG UAG UAG UAG UAG UAG | 666 666 666 666 666 666 666 666 666 66 | GAC UGC UGC UGC UGC UGC UGC UGC UGC UGC UG | GAC UUA CUG CUU CUC CUG CUA UUG CUA CUA CUA CUA CCU AUC CAG CUU | V00099 L16015 Af192772 Y13463 X86448 D86123 M29671 U72332 M22445 X94560 X58455 U55002 D10032 AF158256 AF168256 AF020616 |
| NECRO PANICO RETRO C UNCLASS Group 4 (GAS | Pea enstion mosaic (At-) Candamine chiercie ficek Cantation motive (Shangtua) Galinsoga mosaic carmovits Hibisous chiercite mospet Japanese ins neoretic ring Melon neoratic spot Saguaro catus Turnip crinkle Lask white stripe Tobacco neorosis (Type A) Panicum mosaic Babaon endogenous (MT) Murine taskemia (SL3-3) Carnot rock loaf Jutovitus assoc RNA | GCC UUU UUU CUG CCC UUC UUG UUG CAU CAU CAU CAU CAU CAU UUU GAC UUU GAC | UCC GUC GGC GUC GUC GUC GUC GUC GCC GCC | CUC CGC AAA AAA AAA AAA AAA CGC AAA AAA GAA G | UAG UAG UAG UAG UAG UAG UAG UAG UAG UAG | | GAC UGC UGC UGC UGC UGC UGC UGC UGC UGC UG | GAC UUA CUG CUU CUG CUG CUG CUG CUG CUG CUG CUA CUG CUG CUA | V00099 L16015 AF192772 Y13463 X86448 D86123 M22641 U72332 M22445 X94560 X58455 U55002 D10032 AF1862256 AF020615 A1249740 |
| NECRO PANICO RETRO C UNCLASS Group 4 (GRA TOMBUS NECRO | Pre-ension mosaic (Ar-) Candamine chloretic Rock Carnation mobile (Shanghai) Galinsoga mosaic carnevits Hibisous chloretic ningspot Japanese ins neoratic ring Melon neorotis spot Saquaro cactus Turing cirikle Leak white stripe Tobacco neorosis (Typo A) Panicum mosaic Baboon endogencus (MT) Munne taskema (SL3-3) Carnot rock last Jutovitus assoc RNA Tomato bushy sture (statee) Tobacco neorosis (the | GCC UUU CUG CCC UUC UUG UUG UUU CAU CAU CGG UUU GAC UUU GAC UUA UUA UUA UUA | UCC GUC GGC GGC GUC GUC GUC GUC GCC GCC | CUC CGC AAA AAA AAA AAA CGC AAA AAA GAA GA | UAG UAG UAG UAG UAG UAG UAG UAG UAG UAG | | GAC UGC UGC UGC UGC UGC UGC UGC UGC UGC UG | GAC UUA CUG CUU CUG CUG CUG CUG CUG CUG CUG CUG | V00099 L16015 AF192772 Y13654 D85123 M29671 U72332 M22445 X38456 X38455 U55002 AF169255 AF020616 AJ249740 U82546 |
| NECRO PANICO RETRO C UNCLASS Group 4 (GEA NECRO LENTI | Pre-ension mosaic (Ar-) Canadamino chioretic flock Canadamino chioretic flock Canadami otto (Shangtua) Galinsoga mosaic carmovits Histous chioretic mogsof Japanese ins neoretic ring Melon neoretic spot Saguaro cactus Turmo crinkle Lask white stripe Tobacco neorosis (Typo A) Panicum mosaic Baboon endogencus (M7) Murne Isukemia (SL3-3) Carrot reci kar Mutovitus assoc RNA Tomato bushy sturr (statice) Tobacco neorosis (D) | GCC UUU UUU CUG UUC UUC UUC UUU CAU CAU CAU CAU GAC UUU UAC UUA UUA UAC | GUC GUC GUC GUC GUC GUC GUC GUC GUC GUC | CUC CGC AAA AAA AAA AAA CGC AAA AAA GAC AAA AAA | UAG UAG UAG UAG UAG UAG UAG UAG UAG UAG | GGG GGG GGG GGG GGG GGG GGG GGG GGG GG | GAC UGC UGC UGC UGC UGC UGC UGC UGC UGC UG | GAC UUA CUG CUU CUU CUU CUU CUU CUU CUU AUC CAG CUU CUA CUU CUA CUA | V00099 L16015 AF192772 Y13463 X86448 D65123 M22445 X94560 X58455 U72332 W22445 X58455 U55002 D10032 AF160255 AF020616 AJ249740 U82556 |
| NECRO PANICO RETRO C UNCLASS Group 4 (GEA TOMBUS NECRO LENTI | Pre-ension mosaic (Ar-) Cardamine chloretic Rock Carradion mobile (Shanghai) Galinsoga mosaic carmovits Hibisous chloretic ningspot Japanese ins necretic ring Melon necrolis spot Saquaro cactus Turing cirkle Leak white stripe Tobacco necrosis (Type A) Panicum mosaic Baboon endogencus (MT) Munne taskiema (SL3-3) Carrot rock futurovitus assoc RNA Tomato bushy sturt (statce) Tobacco necrosis (D) Simian immunodeficiency | GCC UUU CUG CCC UUC UUC UUC UUC UUU CAU CAU CAU GAC UUU GAC UUA UAC GGU UGG ACA | GUC GUC GUC GUC GUC GUC GUC GUC GUC GUC | CUC CGC AAA AAA AAA AAA CGC AAA AAA GAC AAA AAA | UAG UAG UAG UAG UAG UAG UAG UAG UAG UAG | GGG GGG GGG GGG GGG GGG GGG GGG GGG GG | GAC UGC UGC UGC UGC UGC UGC UGC UGC UGC UG | GAC UUA CUG CUU CUU CUC CUG CUA CUA CUA CUA CUA CUA CUA | V00099 L16015 AF102772 Y13463 X136448 D85123 M26471 U72332 M26471 U72332 X32455 X34455 X34455 U55002 D10032 AF150256 AF020616 AJ249740 U82546 M92675 |
| NECRO CARMO PANICO PANICO RETRO Croup 4 (GGA) VICLASS Croup 4 (GGA) LENTI Group 5 (GJA) | Pre-ension mosaic (Ar-) Candamine chioretic flock Carnation mobile (Shangtua) Galinsoga mosaic carmovits Hisisous chicetic mogspot Japanese ins neorotic ring Maion neorotic spot Saquaro cactus Turnip cirrikle Lask white sittipe Tobacco neorosis (Type A) Panicum mosaic Baboon endogeneus (M7) Murrie tautemis (SL3-3) Carrot red leaf luteovitus assoc RNA Tobacco neorosis (D) Stimian immunodeficiency | GCC UUU CUG CCC UUG UUG UUG CAU CGG UUU GAC UUU GAC UUA UAC | UCC GUC CCC GUC GUC GUC GUC GUC GUC GUC | CUC CGC AAA AAA AAA AAA CGC AAA CGC AAA CGC AAA AAA | UAG UAG UAG UAG UAG UAG UAG UAG UAG UAG | GGG GGG GGG GGG GGG GGG GGG GGG GGG GG | GAC UGC GGC UGC UGC UGC UGC UGC UGC UGC UG | GAC UUA CUG CUU CUU CUU CUU CUU CUU CUU CUU CUU | V00099 L16015 AF192722 Y13463 X06448 D85123 M29671 U72332 M22445 M22445 X58456 X58455 U55002 D10032 AF160255 AF020615 AJ249740 U82545 AF202675 |
| NECRO CARMO NECRO PANICO RETRO C UNCLASS Croup 4 (GEA LUTEO LUTEO | Pre-ension mosaic (Ar-) Cardamino chicretic Rock Carradion mobile (Shanghui) Galinsoga mosaic carmovits Hibicous chicretic ningspot Japanese ins necretic ring Melon necretic spot Saquaro cactus Turing cirités Lasik white stripe Tobacco necrosis (Type A) Panicum mosaic Baboon endogenous (MT) Munne taukame (SL3-3) Carrot rock kaf Autovitus assoc RNA Tomato bushy sturi (statce) Tobacco necrosis (D) Strian immunodeficiency Bahay yellow dend (TPAX-13) | GCC UUU CUG CCC UUC UUC UUC UUC CAU CAU CAU CAU UAC UUU CAU UAC UCA UUA CAU UAC CAU CAU | UCC GUC GGC GGC GUC GUC GUC GUC GUC GUC | CUC CGC AAA AAA AAA AAA CGC AAA AAA GAC AAA AAA | UAG UAG UAG UAG UAG UAG UAG UAG UAG UAG | GGG GGG GGG GGG GGG GGG GGG GGG GGG GG | GAC UGC GGC UGC UGC UGC UGC UGC UGC UGC UG | GAC UUA CUG CUU CUG CUG CUG CUG CUG CUG CUG CUG | V00099 V00099 L16015 AF102772 V13403 X85448 D65123 W26471 U72332 W22445 X84455 U55002 D10032 AF100256 AF0020616 AJ249740 U62546 M92675 AF203167 AF210205 |
| NECRO CARMO ARMO PANICO RETRO C UNCLASS Group 4 (GCA TOMBUS NECRO LENTI Croup 5 (GLA UUTEO | Pre-ension mosaic (Ar-) Candamine chioretic flock Carnation mobile (Shangtua) Galinsoga mosaic carmovits Hisiscus chicete mogspot Japanese ins neorotic ring Micin neorotic spot Saquaro cactus Turnip cirrikte Lask white stripe Tobacco neorosis (Type A) Panicum mosaic Baboon endogeneus (M7) Murne teutemia (SL3-3) Carrot red kaf luteovirus assoce RNA Tobacco neorosis (D) Saring retine (SL3-3) Carrot red kaf luteovirus assoce RNA Tobacco neorosis (D) Simian immunodificiency Bahoy etilow dendf (PAV-112) Bahoy yellow dendf (PAV-122) | GCC UUU CUG CCC UUC UUC UUC CAU CAU CAU CAU UAC UUU CAU UAC UUU CAU UAC UUU CAU UAC UUU CAU UAC CAU CAU | UCC GUC GGC GGC GUC GUC GUC GUC GUC GUC | CUIC CGG AAA AAA AAA AAA CGC AAA AAA GAA GA | UAG UAG UAG UAG UAG UAG UAG UAG UAG UAG | GGG GGG GGG GGG GGG GGG GGG GGG GGG GG | GAC UGC GGC UGC UGC UGC UGC UGC UGC UGC UG | GAC UUA CUG CUU CUU CUU CUU CUU CUU CUU CUU CUU | V00099 V00099 L16015 AF192722 V13463 X06448 D85123 M29671 U72332 M29445 X94560 X59455 U05002 D10002 AF169255 AF0020615 AJ249740 U82546 M2546 M2546 M2546 AF205167 AF218728 |
| NECRO CARMO NECRO PANICO RETRO C UNCLASS Group 5 (GGA LENTI COMBUS NECRO LENTI COMBUS SIGUA | Pre-ension mosaic (Ar-) Cardamine chicketic Rock Cardamine chicketic Rock Cardamine chicketic Rock Cardamine chicketic Rock Histocas chickets rings Histocas chickets rings Malon neorotis spot Saquaro cactus Tumip critike Lask white stripe Tobacco necrosis (Type A) Paricum mosaic Baboon endogencos (M7) Munne kautemia (SL3-3) Carrot rock karf Autovitus assoc RNA Tomato bushy sturit (statce) Tobacco necrosis (D) Simian immunodoficiency Barloy yellow dend' (DAV-11) Barloy yellow dend' (DAV-12) Barloy yellow dend' (DAV-12) Barloy yellow dend' (DAV-12) | GCC UUU CUG CCC UUC UUC UUU CAU UUU GAC UUU GAC UUU UUU GAC UUU UGG ACA ACG ACG | UCC GUC GGC GGC GUC GUC GUC GUC GUC GUC | CUIC CGC AAA AAA AAA AAA AAA AAA AAA AAA AA | UAG UAG UAG UAG UAG UAG UAG UAG UAG UAG | GEG GEG GEG GEG GEG GEG GEG GEG GEG GEG | CAC UGC GGC UGC UGC UGC UGC UGC UGC UGC UG | GAC UUA CUG CUU CUU CUU CUU CUU CUU CUU CUU CUU | V00099 V00099 L16015 AF102772 V13403 X86448 D66123 W26471 U72332 W26471 U72332 X86455 U55002 D10002 AF160255 AF020616 AJ249740 U62546 M32675 AF210708 U00865 D11022 D11028 D01028 |
| NECRO CARMO NECRO PANICO RETRO C UNCLASS Croup 4 (GGA NECRO LENTI Croup 5 (GIA | Pre-ension mosaic (Ar-) Cardamine chioretic flock Carnation mobile (Shanghui) Galinsoga mosaic carmovits Hisisous chicete magsot Japanese ins neoratic mg Meion neorode spot Saguaro cactus Tump crinkle Lask while shipe Tobacco neoross (Type A) Panicum mosaic Baboon endogencus (M7) Murne Isukarenis (SL3-3) Carrot rod Isaf Lutoovirus assoc RNA Tomato bushy sturit (statice) Tobacco neorosis (D) Stiman immunod6/ciency Barley yellow dwarf (PAV-13) Barley yellow dwarf (SAV-13) Barley yellow dwarf (SAV-13) Barley yellow dwarf (SAV-13) | GCC UUU CUG CCC UUC UUC UUC CAU CAU CAU CAU CAU CAU | UCC GUC GGC GGC GUC GUC GUC GGC GUC GGC GG | CUC CGC AAA AAA AAA AAA AAA AAA AAA AAA AA | UCAG UAG UAG UAG UAG UAG UAG UAG UAG UAG U | GEG GEG GEG GEG GEG GEG GEG GEG GEG GEG | CAC GGC GGC UGC UGC UGC UGC UGC UGC UGC UG | GAC UUA CUG CUU CUU CUU CUU CUU CUU CUU AUC CAG CAG CAG CUU CUA CUA CUA CUA CUA CUA CUA CUA CUA | V00099 V00099 L16015 AF1902722 Y13463 X06448 D85123 M29671 U72332 M29671 U72332 M22445 X04555 U00032 AF169255 AF020616 AJ249740 U82546 M92675 AF215167 AF215 AF215167 AF2157 AF21517 AF215167 AF2157 AF2157 AF2157 AF2157 AF2157 AF2177 AF2177 AF2177 AF2177 AF2177 AF2177 AF2177 AF217 |
| NECRO CARMO NECRO PANICO RETRO C UNCLASS Group 4 (GAR NECRO LENTI Croup 5 (GUA LENTI | Pre-ension mosaic (Ar-) Cardamino chicketic Rock Carnation mobile (Shanghai) Galinsoga mosaic carmovits Histous chickets nngspot Japanese ins necrotic rng Melon necrotis spot Saquaro cactus Tumis critike Lasik white stripe Tobacco necrosis (Type A) Paricum mosaic Baboon endogencos (M7) Munne kautemia (SL3-3) Carnot rock karf Autovitus assoc RNA Tomsto bushy sturit (statce) Tobacco necrosis (D) Simian immunodoficiency Barloy yellow denarf (DAV-11) Barloy yellow denarf (MAV) Barloy yellow denarf (MAV) Barloy yellow denarf (MAV) Barloy yellow denarf (MAV) | GCC UUU CUG CCC UUC UUC UUC UUU CAU UAC UUU GAC UUU GAC UUU GAC UUU GAC UUU GAC ACG ACA ACG ACA ACG ACU | UCC GUC GGC GGC GUC GUC GUC GUC GUC GCC GC | CUC CGC AAA AAA AAA AAA AAA AAA AAA AAA AA | UCAG UAG UAG UAG UAG UAG UAG UAG UAG UAG U | GEGE GEGE GEGE GEGE GEGE GEGE GEGE GEG | CAC UGC UGC UGC UGC UGC UGC UGC UGC UGC UG | GAC UUA CUG CUU CUU CUU CUU CUU CUU CUU CUA CUA CUA | V00099 V00099 L16015 AF102772 V13403 X86448 D66123 W26471 U72332 W26471 U72332 W22445 X94560 X58455 U55002 D10002 AF160255 AF020616 AJ249740 U62546 M92675 AF218788 U008865 D11028 L24049 |
| NECRO CARMO NECRO PANICO RETRO C UNCLASS Croup 4 (GEA NECRO LENTI Croup 5 (GUA LUTEO | Pre-ension mosaic (Ar-) Cardamine chlorotic flock Carnation mobile (Shanghui) Galinsoga mosaic carmovits Hisisous chlorotic mogsot Japanese ins neorotic mg Melon neorotic spot Saguaro cactus Turing cirkle Lak white stripe Tobacco neorosis (Typo A) Panicum mosaic Baboon endogencus (M7) Murne taukemis (SL3-3) Carnot cel kalf Jutoovirus assoc RNA Tomato bushly sturt (statice) Tobacco neorosis (D) Simian immundoß(ciency) Barloy gellow dwarf (PAV-13) Barloy gellow dwarf (SAV-10) Barloy gellow dwarf (SAV-10) | GCC UUU UUU CUG CUG CCC UUC UUU CAU CAU CAU CAU CAU UUU CAU UAC CGG UUU UAC CGG UUU ACA ACG ACG ACA ACU AAU AAC | UCC GUC GCC GCC GUC GUC GUC GCC GCC GCC | CUCC CGC AAA AAA AAA AAA AAA CGC AAA AAA CGC AAA AAA | UCAG UAG UAG UAG UAG UAG UAG UAG UAG UAG U | GGG GGG GGG GGG GGG GGG GGG GGG GGG GG | GAC UGC UGC UGC UGC UGC UGC UGC UGC UGC UG | GAC UUA CUG CUJ CUJ CUJ CUJ CUG CUG CUA CUA CUA CUA CUA CUA CUA CUA CUA CUA | V00099 V00099 L16015 AF102772 Y13463 X06448 D85123 W2041 U72332 W20445 X04560 X58455 U72032 D10032 AF160255 AF5020615 AF2020615 AF20206 AF20206 AF20206 AF20 AF20206 AF20206 AF20 AF20206 AF20 AF20 AF20 AF20 AF20 AF20 AF20 AF20 |
| NECRO CARMO NECRO PANICO RETRO C UNCLASS Group 4 (GAA UNCLASS Group 4 (GAA LUTEO | Pre ensor mosaic (4-) Cardenine Chieferic Rock Carnation motile (Shanghai) Galinsoga mosaic cannevits Histous chiedets nngspot Japanese ins necrotic rng Melon necrotis spot Saquaro cactus Tump crefke Lask white stripe Tobacco necross (Type A) Panicum mosaic Baboon endogenesa (M7) Murine leakemia (5L3-3) Carrot rock last Jutoovkus assoc RNA Tomsto bushy sturit (statce) Tobacco necross (0) Simian immunodoficiency Barley yellow dend' (FAV-13) Barley yellow dend' (FAV-13) Barley yellow dend' (SUV) Barley yellow dend (MAV) Barley yellow dend (MAV) | GCC UUU UUU CUG CCC UUU CAU UAC UUU CAU UAC UUU CAU UAC UUU GAC UUU UAC CGU UUU UAC CGGU UUU CAU CAU CAU CAU CAU CAU CAU CAU CA | UCC GUC GUC GGC GUC GUC GUC GAC GAC GAC GAC GAC GAC GAC GAC GAC GA | CUCC CGC AAAA AAAA AAAA AAAA AAAA CGC AAAA AAAA | UCAG UAG UAG UAG UAG UAG UAG UAG UAG UAG U | GEGE GEGE GEGE GEGE GEGE GEGE GEGE GEG | CAC GGC UGC UGC UGC UGC UGC UGC UGC UGC UG | GAC UUA CUG CUU CUU CUU CUU CUU CUU CUU CUU CUU | V00099 V00099 L16015 AF102772 V13403 X05448 D86123 X05448 D86123 X05448 X0455 U55002 U55002 U55002 U55002 AF160255 AF020615 AF202615 AF202615 AF202615 AF2218728 U05885 U05885 U11028 L24049 L25299 X13063 X13063 |
| NECRO CARMO NECRO PANICO RETRO C UNCLASS Group 4 (GRA Group 5 (GUA LUTEO | Pre-ension mosaic (Ar-) Cardamine chlorotic flock Carnation mobile (Shanghai) Galinsoga mosaic carmovits Hibisous chlorotic mojsot Japanese ins nocrotic mg Melon nocrotis spot Saquaro cactus Turung crinkle Leak white stripe Tobacco nocrosis (Typo A) Panicum mosaik (SL3-3) Carnot too kard futuovirus assoc RNA Tomiso bushy sturit (statce) Tobacco nocrosis (D) Simian immunodoficioncy Barley gellow dwarf (PAV-129) Barley gello | GCC UUU UUU UUU CUG CCC UUC UUC CALI CGU UUU CALI UUU CALI UUU CALI UUU CALI UUU CALI UUU CALI CGG UUU UAC UUU CALI CGG UUU CALI CGG CALI CGG CALI CGALI CALI CGALI CALI CGALI CALI CGALI CALI CGALI CALI CGALI CALI CGALI CALI CALI CALI CGALI CALI CALI CALI CALI CALI CALI CALI C | UCC GUC CCC GUC GUC GUC GUC GUC GAC GAC GAC GAC GAC GAC GAC GAC GCC GC | CUCC CGC AAA AAA AAA AAA AAA AAA CGC AAA AAA AAA | UDAG UAG UAG UAG UAG UAG UAG UAG UAG UAG U | GEGE GEGE GEGE GEGE GEGE GEGE GEGE GEG | CAC GGC UGC UGC UGC UGC CGC UGC UGC UGC UG | GAC ULIA CUG CUU CUU CUU CUU CUU CUU CUU CUU CUU | V00099 V00099 L16015 AF102772 Y13463 X06448 D85123 M20671 U72332 M22445 X04560 X042445 V04560 X042445 U55002 U10032 AF160256 AF020616 AF020616 AF020516 AF235107 AF235107 AF235107 AF235107 AF235107 AF23529 X13063 AF55029 |
| NECRO CARMO ARMO PANICO RETRO C UNCLASS Group 4 (GA NECRO LUTEO POLERO POLERO | Pre-ension mosaic (A-) Candamine chioretic flock Canadamine chioretic flock Canadami mobile (Shanghua) Galinsoga mosaic carmovits Hissous chicete mogspot Japanese ins neorotic ring Miorin neorotic spot Saquaro cactus Turnip cirrikle Lask white sitipe Tobacco neorosis (Type A) Panicum mosaic Baboon endogeneus (M7) Murrie taulaemis (SL3-3) Carrot red leaf lutcovirus assoc RNA Tomato bushy sturit (statce) Tobacco neorosis (D) Stimian immunodeficiency Barley yellow dent (PAV-112) Barley yellow dent (PAV-112) | GCC UUU UUU UUU UUU UUU UUU UUU UUU GAU UUU GAU UUU GAU UUU GAU UUU GAC UUU GAC UUU AC UUU AC ACA ACA ACA ACA ACA ACA | UCC GUC GUC GCC GUC GUC GUC GUC GUC GCC GC | CUCC CGC AAA AAA AAA AAA AAA AAA CGC AAA AAA AAA | UCAG UAG UAG UAG UAG UAG UAG UAG UAG UAG U | GEGE GEGE GEGE GEGE GEGE GEGE GEGE GEG | CAC UGC UGC UGC UGC UGC CGC UGC UGC UGC UG | GAC ULIA CUG CUU CUU CUU CUU CUU CUU CUU CUU CUU | V00099 V00099 L16015 AF102722 V13463 X05448 D86123 X05448 D86123 X05448 X0455 V15202 V12332 M22445 X04550 U55002 U55002 U55002 U55002 U55002 U55002 AF160255 AF102055 AF202055 AF2218788 U05865 U11028 L24049 L24049 L24049 L24049 L24049 X13063 AF157029 X13063 AF157029 X14060 |
| NECRO CARMO PANICO RETRO C UNCLASS MECRO LENTI Group & IGUA LUTEO POLERO CLOSTERO | Pre-ension mosaic (Ar-) Cardamine chlorotic flock Carnation mobile (Shanghai) Galinsoga mosaic carnovits Hibscus chlorotic ningspot Japanese ins nocratic ning Melon nocrotis spot Saquaro cactus Turing cirités Leak white stripe Tobacco nocrosis (Typo A) Panicum mosaic Baboon endogencus (MT) Munne taskemel (SL3-3) Carnot rock last Jutovitus assoc RNA Tomato bushy start (statce) Tobacco necrosis (D) Simian immunodoficioncy Barley yellow denart (PAV-129) Barley yellow denart (PAV-121) Barley yellow denart (PAV-123) Barley yellow denart (PAV-124) Barley yellow denart (PAV-124) Barley yellow denart (PAV-125) Barley yel | GCC UUU UUU UUU UUU CAU UAC UUU GAU UAC UUU GAU UAC UUU GAU UAC UUU GAC UUU GAC UUU GAC CAU AAC AAC AAC AAC AAC AAC | UCC GUC GUC GUC GUC GUC GUC GUC GUC GUC | CUCC CGC AAA AAA AAA AAA AAA AAA AAA AAA AAA AA | UCAG UAG UAG UAG UAG UAG UAG UAG UAG UAG U | GGGG GGGG GGGG GGGG GGGG GGGG GGGG GGGG GGGG | CAC C | GAC UIUA CUU CUU CUU CUU CUU CUU CUU CUU CUU C | V00099 V00099 L16015 AF102772 Y13463 X856448 D85123 W26471 U72332 W22445 X84455 U55002 D10032 AF150256 AF020616 AF020616 AF2020616 AF2020616 AF215702 AF2151067 AF2151067 AF2151067 AF2157067 AF2157067 L25299 X13068 AF157029 X14060 X75633 |
| NECRO CARMO CARMO NECRO PANICO RETRO C UNCLASS Group 4 (GCA UNCLASS Group 5 (GLA LUTEO POCERO CLOSTERO Group 5 (CUA | Pre-ension mosaic (A-) Cardamine chlorotic flock Carnation mobile (Shanghui) Galinsoga mosaic carmovits Hisisous chlorotic mogsof Japanese ins neorotic mg Micin neorotic spot Saquaro cactus Tump crinkle Lask white shipe Tobacco neorosis (Type A) Panicum mosaic Baboon endogencus (M7) Murne testalemis (SL3-3) Carrot red kalf lutoovirus assoc RNA Tomato bushy sturit (statice) Tobacco neorosis (D) Simian immunodificiancy Barley yellow deart (PAV-11) Barley yellow deart (PAV-11) Barley yellow deart (SGV-U) Barley yellow deart (SGV-U) Barley yellow deart (SAV-1) Barley yellow deart (SAV-1) | GCC UIUU UUUU CUG CCC UIUC UUU CAU UUU CAU CAU GAU UAC UUA GGU UAC UUA GGU UAC UGG ACA ACG AAC AAU AAC AAU AAC AAC | UCC GUC GUC GCC GCC GUC GUC GUC GUC GUC | CUIC CGC AAA AAA AAA AAA AAA AAA AAA AAA AA | UCAG UAG UAG UAG UAG UAG UAG UAG UAG UAG U | GGGG GGGG GGGG GGGG GGGG GGGG GGGG GGGG GGGG | GAC GGC UGC UGC UGC UGC UGC UGC UGC UGC UG | GAC UILA CUG CUG CUG CUG CUG CUG CUG CUA AUC CAG CUJ CUA CUA CUA CUA CUA CUA CUA CUA CUA CUA | V00099 V00099 L16015 AF102722 V13463 X05448 D86123 X05448 D86123 X0545 X04550 X02445 X04550 U55002 D10032 AF160255 M02675 M02675 AF020516 AF2315107 AF2315107 AF231507 AF231 X14600 X75331 |
| ERAMO CARMO CARMO PANICO RETRO C UNCLASS Group 4 (GLA Croup 5 (GLA LUTEO POLERO CLOSTERO CLOSTERO CLOSTERO | Pre-ension mosaic (Ar-) Cardamino chicrete Rock Carradom mobile (Shanghui) Galinsoga mosaic carmovits Hibscus chicrete ningspot Japanese ins necretic ring Melon neorotis spot Saquaro cactus Turing cirkle Lask white stripe Tobacco neorosis (Typo A) Panicum mosaic Baboon endogencius (MT) Munne kaukemis (SL3-3) Carrot rock fatuovitus assoc RNA Tomato bushy sture (statce) Tobacco neorosis (D) Santari terite (PAV-129) Barley yellow denet (PAV-121) Barley yellow denet (PAV-121) Barley yellow denet (PAV-121) Barley yellow denet (SAV-123) Barley yellow denet (SAV-124) Barley yellow denet (SAV-124) Barley yellow denet (SAV-125) Barley yellow denet (SAV-126) Barley yellow denet (SAV-127) Barley yellow denet (SAV-128) Barley ye | GCCU UUUU CUGU CUCU UUUG UUUG UUUG GACU UUUU GACU UUUA UUGA UUUA UUGA ACA ACG AACU AACU | UCC GUC GUC GUC GUC GUC GUC GUC GUC GUC | CUC CGC AAA AAA AAA AAA AAA AAA AAA AAA AA | UCAA UAAG UAAG UAAG UAAG UAAG UAAG UAAG | GEGE GEGE GEGE GEGE GEGE GEGE GEGE GEG | AAC JOC J | GAC UIUA CUG CUG CUG CUG CUG CUA AUC CCU AUC CAG CUU AUC CUA CUA CUA CUA CUA CUA CUA CUA | V00099 V00099 L16015 AF102772 Y13463 X80448 D85123 M26471 U72332 M26471 U72332 X80455 U55002 D10032 AF108256 AF020616 AF20506 AF20506 AF20507 AF215107 AF215107 AF215107 AF215708 U06686 D11028 L24049 L25299 X13063 AF105728 AF10578 AF10 AF10 AF10 AF10 AF10 AF10 AF10 AF10 |
| NECRO CARMO CARMO PANICO RETRO C UNCLASS Group 4 (GGA TOMBUS NECRO LENTI CROUP 5 (GIA LUTEO POLERO CLOSTERO CLOSTERO CROUP 6 (CUA | Pre-ension mosaic (Ar-) Cardamine chlorotic flock Carnation mobile (Shanghui) Galinsoga mosaic carmovits Hisisous chlorotic mogsof Hisisous chlorotic mogsof Nalion neorodic spot Saguaro cactus Tump crinkle Lask while shipe Tobacco neorosis (Type A) Panicum mosaic Baboon endogencus (M7) Murne Isukarenis (SL3-3) Carrot rod kaf Jutoovirus assoc RNA Tomato bushy sturit (statice) Tobacco neorosis (D) Simian immunodificiency Barley yellow denart (PAV-13) Barley yellow denart (PAV-13) Barley yellow denart (SUV-) Barley yellow denart (S | GCC UUU UUU CUU CUU UUU CUU UUU CUU CUU | UCC GUC CCC GCC GUC GUC GUC GUC GUC GUC | CUIC CAAA AAAA AAAA AAAA AAAA AAAA AAAA | UCAA UAAG UAAG UAAG UAAG UAAG UAAG UAAG | GEGE GEGE GEGE GEGE GEGE GEGE GEGE GEG | GAC UGC UGC UGC UGC UGC UGC UGC UGC UGC UG | GAC. UIUA CUG CUG CUG CUG CUG CUG CUG CUG CUG CUG | V00099 V00099 L16015 AF102772 Y13463 X06446 D85123 M29671 U72332 M29445 X04550 U72332 M22445 X04555 U00032 AF150225 AF020615 AF225167 AF225167 AF22556 D11028 L24049 L25299 X13063 AF157029 X13063 AF157029 X14600 X76931 |
| REARIO CARMO CARMO PANICO RETRO C UNCLAS Group & (GUA UNCLAS) NECRO LENTI Croup \$ (GUA LENTI Croup \$ (GUA LENTI Croup \$ (GUA ALPHA | Pre-ension mosaic (Ar-) Candamino chicrete Rock Carradom mobile (Shanghui) Galinsoga mosaic carmovits Hibiscus chicrete ningspot Japanese ins necretic ring Melon neorotis spot Saquaro cactus Turing cirités Lask white stripe Tobacco necrosis (Type A) Panicum mosaic Baboon endogencius (MT) Munne kaukamel (SL3-3) Carrot rock kaf Jutovitus assoc RNA Tomato bushy sturi (statce) Tobacco necrosis (D) Strian immunode/ficiency Barley sellow dend (PAV-113) Barley sellow dend (PAV-113) | GCCU UUUU CUGU CUCU UUUC UUUC UUUC UUUC | UCC GUC GUC GUC GUC GUC GUC GUC GUC GUC | CUIC CAAA AAAA AAAA AAAA AAAA AAAA AAAA | UCAA UAAG UAAG UAAG UAAG UAAG UAAG UAAG | GGGG GGGG GGGG GGGG GGGG GGGG GGGG GGGG GGGG | GAC GGC UGC UGC UGC UGC UGC UGC UGC UGC UG | GAC UILA CUG CUG CUG CUG CUG CUG CUG CUA CUA CUA CUA CUA CUA CUA CUA CUA CUA | V00099 V00099 L16015 AF102772 V13403 X86448 D65123 M26471 U72332 M26471 U72332 M26471 U72332 M26445 X84455 U55002 D10032 AF103254 AF203728 U5605 D11028 AF203728 U06665 D11028 L25299 X13053 AF103728 AF103728 AF103728 AF103724 |
| NECRO CARMO CARMO PANICO RETRO C UNCLASS Croup 4 (GGA UNCLASS NECRO LENTI Croup 5 (GIA LUTEO POLERO CLOSTERO CROUP 5 (CLIA ALPHA | Pre-ension mosaic (Ar-) Cardamine chioretic flock Carnation mobile (Shanghui) Galinsoga mosaic carmovits Hisisous chicete magsot Japanese ins neoratic mg Meion neorotes spot Saguaro cactus Turnip crinkle Lask while shipe Tobacco neorosis (Type A) Panicum mosaic Baboon endogencus (M7) Murine kakemis (SL3-3) Carnot red kaf Jutoovirus assoc RNA Tomsto bushly sturit (statice) Tobacco neorosis (D) Simian immunodificiency Barlay sellow denarf (PAV-13) Barlay sellow denarf (PAV-13) Barlay sellow denarf (MAV-130) Barlay sellow denarf (MAV-130) Barlay sellow denarf (SIX) Barlay sellow denar | GCCU UUUU CUGG CCCC UUUC UUUC CAU CAU CAU CAU CAU CAU | UCC GUC GUC GUC GUC GUC GUC GUC GUC GUC | CUCC CAAA AAAA AAAA AAAA AAAA AAAA AAAA | UCAG UAG UAG UAG UAG UAG UAG UAG UAG UAG U | GGGG GGGG GGGG GGGG GGGG GGGG GGGG GGGG GGGG | ALC GGC UGC UGC UGC UGC UGC UGC UGC UGC UG | GAC UILA CUG CUG CUG CUG CUG CUG CUA CUA CUA CUA CUA CUA CUA CUA CUA CUA | V00099 V00099 L16015 AF102772 Y13463 X06448 D85123 M20614 U72332 M20617 U72332 M22445 X04560 X58455 U00032 AF102255 AF102255 AF1020515 AF225167 AF225167 AF225167 AF215708 U06885 D11028 L24049 L25599 X13068 AF157029 X13068 AF157029 X14600 X75931 AF103728 A6002553 AF103728 AF10378 AF10378 AF1040 AF104 AF |
| REARIO CARMO CARMO PANICO RETRO C UNCLASS Group & (GLA Croup S (GLA LUTEO POLERO CLOSTERO CROUP & (CLA | Pre ensor mosaic (Ar-) Cardamino chicrete Rock Carradom mobile (Shanghui) Galinsoga mosaic carmovits Hibisous chicrete ningspot Japanese ins necretic ring Melon neorotis spot Saquaro cactus Turing cirkle Lask white stripe Tobacco neorosis (Type A) Panicum mosaic Baboon endogencius (MT) Munne kaukamet (SL3-3) Carrot red kaf futovitus assoc RNA Tomato bushy sturi (statce) Tobacco neorosis (D) Santari terite (SL3-3) Carrot red kaf futovitus assoc RNA Tomato bushy sturi (statce) Tobacco neorosis (D) Barley yellow dend (PAV-112) Barley yellow dend (PAV-112) Barley yellow dend (SAV-122) Barley yellow dend (SAV-123) Barley yellow dend (SAV-124) Barley yellow dend (SAV-124) Barley yellow dend (SAV-125) Barley yellow dend (SAV-125) Barley yellow dend (SAV-126) Barley yellow dend (SAV-127) Barley yellow dend (SAV-128) Barley y | GCCU UUUU CUGG UUUC UUUC UUUC UUUC CAU UUUU CAU UUUU CAU UUUU CAU UUUU CAU UUUU CAU UUUU CAU UUUU CAU UUUU CAU UUUU CAU UUUU CAU CA | UCC GUC GUC GUC GUC GUC GUC GUC GUC GUC | CUIC CARA AAAA AAAA AAAA AAAA AAAA AAAA AA | UCAA UAAG UAAG UAAG UAAG UAAG UAAG UAAG | GGGG GGGG GGGG GGGG GGGG GGGG GGGG GGGG GGGG | GAC G | GAC ULA CUG CUG CUG CUG CUG CUA CUA CUA CCUA C | V00099 V00099 L16015 AF102772 V13403 X86448 D85123 M2641 U72332 M2641 U72332 M2641 U72332 M2644 U72332 U75002 D10032 AF103255 AF020616 A720106 A720106 A720107 U00866 D11022 AF201708 U006865 D11028 AF2017029 L25299 X13063 AF103728 AF10378 AF10878 AF108 |
| NECRO CARMO CARMO PANICO RETRO C UNCLASS Croup 4: (GA TOMBJ5 NECRO LUNTEO POLERO CLOSTERO CROUP 5: (GUA ALPHA CRICKET P4L | Pre-ension mosaic (Ar-) Cardamine chicretic flock Carnation mobile (Shanghui) Galinsoga mosaic carmovits Hibisous chicretic mogsot Japanese ins neorotic mg Melon neorotic spot Saguaro cactus Turing cirkle Lak white stripe Tobacco necrosis (Typo A) Panicum mosaic Baboon endogencus (M7) Murne taukemis (SL3-3) Carnot red kaf Jutoovirus assoc RNA Tomato bushy sturt (statce) Tobacco necrosis (D) Simira immunod6/ckmy Barloy gellow dwarf (PAV-13) Barloy gellow dwarf (PAV-13) Barloy gellow dwarf (VAV-129) Barloy gellow dwarf (KAV-129) Barloy gellow | GCCU UUUU CUGG CCCC UUCU UUU CALL CGGU GALU UUUU GALU UUUU GAC UUUU GAC UUUU GAC UUUU GGU UUUU GAC AAC AAC AAC AAC AAC AAC AAC AAC AAC | UCC GUC GUC GUC GUC GUC GUC GUC GUC GUC | CUIC CAAA AAAA AAAC AAAA AAAA AAAA AAAA | UCAG UAG UAG UAG UAG UAG UAG UAG UAG UAG U | GGGG GGGG GGGG GGGG GGGG GGGG GGGG GGGG GGGG | GAC G | GAC. UIUA CUIG CUIG CUIG CUIG CUIA CUIA CUIA CUIA CUIA CUIA CUIA CUIA | V00099 V00099 L16015 AF102772 Y13463 X06448 D85123 M29671 U72332 M29445 X04560 X58455 U00032 AF102255 AF020615 AF20375 AF20375 AF20375 AF20375 AF20375 AF20375 AF20375 AF150229 X13068 D11028 L24049 L25299 X13068 AF157029 X13068 AF157029 X13063 AF157029 X14600 X15633 AF157228 A6002373 |
| REARIO CARMO CARMO PANICO RETRO C Group 4 (GCA Coroup 5 (GCA LENT) Croup 5 (GCA LUTEO POLERO CLOSTERO Croup 6 (CLA ALPHA CRECKET P4L LINDFOURSESC | Pre ensor mosaic (Ar-) Cardomino chicreti Rok Carradom mobile (Shanghai) Galinsoga mosaic carmovits Hibicos chicrotic moşot Hibicos chicrosis opot Saquaro cactus Turnio cretele Lasik white stripe Tobacco necrosis (Type A) Panicum mosaic Baboon endogenous (M7) Munne kaukamis (SL3-3) Carrot rock kaf Autovitus assoc RNA Tomato bushy sturi (statce) Tobacco necrosis (D) Simian immunodeficiency Barley yellow deart (SAV-12) Barley yellow deart (SAV-12) B | GCCU UUUU CUGC UUUU CAU UUUU CAU UUUU CAU UUUU CAU UUUU CAU UUUU CAU UUUU CAU UUUU CAU UUUU CAU UUUU CAU UUUU CAU UUUU CAU UUUU CAU UUUU CAU UUUU CAU UUUU CAU CA | UCC GUC GUC GUC GUC GUC GUC GUC GUC GUC | CUIC CAAA AAAA AAAA AAAA AAAA AAAA AAAA | UCAG UAG UAG UAG UAG UAG UAG UAG UAG UAG U | GGGG GGGG GGGG GGGG GGGG GGGG GGGG GGGG GGGG | ALL GGC GGC UGC UGC UGC UGC UGC UGC UGC UGC | GAC ULUA CUG CUG CUG CUG CUG CUG CUG CUG CUG CUG | V00099 V00099 L16015 AF102772 V13463 X86448 D65123 W26471 U72332 W26471 U72332 W22445 X94560 X36455 U55002 D10002 AF160255 AF020616 A249740 U62546 M92675 V1002 AF160255 AF210708 U06866 D11028 L25299 X13063 AF103728 AF103728 AF103728 AF103724 AF009456 J002253 AF103734 AF009456 J02246 AB0005331 |
| NECRO CARMO CARMO NECRO PANICO RETRO C UNCLASS COUPS (GUA LUTEO POLERO CLOSTERO COUPS (GUA LUTEO CRICKET PLL Ingroups S C | Pre-ension mosaic (Ar-) Cardamine chicretic flock Carnation mobile (Shanghai) Galinsoga mosaic carnovits Hisisous chicretic mojsot Japanesie ins neorotic mg Molen neorotic spot Saguaro cactus Turung cirkle Laak white stripe Tobacco necrosis (Typo A) Panicum mosaic Baboon endogencus (M7) Murne Istakemis (SL3-3) Carrot rock laaf Jutoovirus assoc RNA Tomsto bushy sturt (Sdatce) Tobacco necrosis (D) Strinan immunodificiency Barloy gellow devarf (PAV-129) Barloy gellow devarf (PAV-129) Barloy gellow devarf (RPV) Barloy gellow devarf | GCCU UUUU CUGG CCCU UUCU UUUU CAUU CAUUUUU CAUUUUUUU CAUUUUUUUU | UCC GUC GUC GUC GUC GUC GUC GUC GUC GUC | CUIC CAGC AAA AAA AAA AAA CGC AAA AAA AAA A | UCAG UAG UAG UAG UAG UAG UAG UAG UAG UAG U | GGGG GGGG GGGG GGGG GGGG GGGG GGGG GGGG GGGG | GAC GGC UGC UGC UGC UGC UGC UGC UGC UGC UG | GAC UIUA CUG CUG CUG CUG CUG CUG CUG CUG CUG CUG | V00099 V00099 L16015 AF102772 Y13463 X06448 D65123 M29671 U72332 M29471 U72332 X39455 U55002 U70332 AF150256 AF020616 AF020616 AF020616 AF02075 AF215167 AF215167 AF215167 AF215728 L25246 U006866 D11028 L24049 L25299 X15063 AF157029 X15063 AF157029 X15063 AF157029 X15063 AF103728 AF037324 AF079456 J32246 AF009456 J32246 AF009456 J32246 |
| REARIO CARMO CARMO PANICO RETRO COUNCLASS Group 4 (GCA Group 5 (GCA LENT) CRECRO LENT) POLERO CLOSTERO CRECKET P4L Ingrouped 56 RETRO C | Pre-ensor mosaic (Ar-) Cardemino chicretic Rock Carriation mobile (Shanghai) Galinsoga mosaic carmovits Hibicous chicrotic mogsoft Japanese ins necretic mg Saguaro cactus Turnip crinkle Lasik white stripe Tobacco necrosis (Styte A) Panicum mosaic Baboon endogencus (MT) Munne kaukemia (SL3-3) Carrot rock karf Autovitus assoc RNA Tomato bushy sturit (statce) Tobacco necrosis (D) Simian immunodeficiency Barlay yellow denarf (SAV-11) Barlay yellow denarf (SAV-12) Barlay yellow denarf (SAV-13) Barlay yellow denarf (SAV-14) Barlay yellow denarf (SAV-14) Barlay yellow denarf (| GCCU UUUU CUGC UUUG UUUG UUUU CAU UUUU CAU UUUU CAU UUUU CAU UUUU CAU UUUA CAU UUUA CAU UUUA CAU UUUA CAU UUA AAC AAC | UCC GUC GUC GUC GUC GUC GUC GUC GAC GAC GAC GAC GAC GAC GAC GAC GCC CCC C | CUIC CAGA AAA AAA AAA AAA AAA AAA AAA AAA A | UCAG UAG UAG UAG UAG UAG UAG UAG UAG UAG U | GEGE GEGE GEGE GEGE GEGE GEGE GEGE GEG | GAC GAC GAC GAC GAC GAC GAC GAC GAC GAC | GAC. ULUA CUIG CUIG CUIG CUIG CUIA AUC CUIA AUC CAG CUIU CUIA CUIA CUIA CUIA CUIA CUIA CUIA | V00099 V00099 L16015 AF102772 V13403 X85448 D66123 X85448 D66123 X82647 U72332 X82645 U72332 X82645 U55002 D10032 AF169255 AF020615 AF020615 AF020615 AF020615 AF020615 AF020615 AF020615 AF020615 AF0207 AF103728 AF03758 AF103728 AF03728 AF0378 |
| NECRO CARMO CARMO NECRO RETRO C UNCLASS CECUP 4 (GUA COMP 5 (GUA COMP 5 (GUA LUTEO POLERO CLOSTERO CROCKET PAL Ingroup 5 (GUA ALPHA CRICKET PAL | Pre-ensore mosaic (Ar-) Cardamine chicretic Reck Carradian mobile (Shanghui) Galinsoga mosaic carmovits Hibisous chicretic mojspot Japanesie ins neoratic mg Melon neorotis spot Saquaro cactus Tumio cirités Leak white stripe Tobacco necrosis (Typo A) Paricum mosaic Baboon endogencus (M7) Murne Istakemis (SL3-3) Carrot too land Jutioovitus assoc RNA Tomito bushy sturit (statce) Tobacco necrosis (D) Simian immunodoficioncy Barley gellow denaf (DAV-129) Barley gellow denaf (DAV-129) Barley gellow denaf (SAV-120) Barley gellow denaf (SAV-120) Ba | GCC UUUU CUG CCC UUC UUC UUC UUC CAU CAU UAC UUC CAU UAC UUA CAU UAC CGG GAU UUA CAU CAU CAU CAU CAU CAA ACA AAC AAC | UCC GUC GUC GUC GUC GUC GUC GUC GUC GUC | CUIC CGG AAA AAA AAA AAA AAA AAA AAA AAA AA | UCAG UAG UAG UAG UAG UAG UAG UAG UAG UAG U | GGGG GGGG GGGG GGGG GGGG GGGG GGGG GGGG GGGG | CARC GGC UGC UGC UGC UGC UGC UGC UGC UGC UG | GAC. UIUA CUG CUG CUG CUG CUG CUG CUG CUG CUG CUG | V00099 V00099 L16015 AF102772 Y13463 X56448 D85123 W2647 U72332 W2647 U72332 W2645 V32465 U55002 U10032 AF169256 AF020616 AF020616 AF020616 AF020616 AF02075 AF215107 AF215128 AF215107 AF215128 AF215 AF21 AF215 AF215 AF21 AF215 AF21 AF215 AF215 AF21 AF215 AF21 AF21 AF21 AF21 AF21 AF21 AF21 AF21 |
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| NECRO CARMO CARMO PANICO RETRO C UNCLASS Group & (GUA LUTEO POLERO CLOSTERO CLOSTERO CLOSTERO CAUSTERO CROCKET PL Ungroup of SGUA CROCKET PL Ungroup of SGUA | Pre ensor mosaic (Ar-) Cardamine chicrete Rock Carradom motile (Shanghai) Galinsoga mosaic carmovits Hibisous chicrete ningspot Japanese ins nocratic ring Melon nocrotis spot Saquaro cactus Turung cirrike Leak white stripe Tobacco nocrosis (Typo A) Panicum mosaic Baboon endogencus (M7) Murine taskenedi (St.3-3) Carrot too land futuovitus assoc RNA Tomito bushy start (statce) Tobacco nocrosis (D) Simian immunodoficioncy Barloy gellow denaf (PAV-129) Barloy gellow denaf (PAV-129) Barloy gellow denaf (PAV-129) Barloy gellow denaf (StA-0) Barloy gellow denaf (| GCC1 UUUU CUIG CCCC UUUG CCCC UUUG CAUU CAUU CAUU GGU UUUA CAUU GGU UUUA CAUU GGU UUUA CAU UGA CGG GAC UUUA AAC AACA AACA | UCC GUC GUC GUC GUC GUC GUC GUC GUC GUC | CUIC CGG AAA AAA AAAA AAAA AAAA AAAA AAAA | UCAA UAAG UAAG UAAG UAAG UAAG UAAG UAAG | GGGG GGGG GGGG GGGG GGGG GGGG GGGG GGGG GGGG | CARC GGC UGC UGC UGC UGC UGC UGC UGC UGC UG | GAC. ULUA CLUG CLUG CLUG CLUG CLUG CLUA CLUA AUCC CAG CLUA CLUA CLUA CLUA CLUA CLUA CLUA CLUA | V00099 V00099 L16015 AF102772 Y13443 X80448 D85123 M29671 U72332 M29671 U72332 K82645 U55002 D10032 AF150256 AF020616 AF0206368 D11028 L25299 X14600 X75031 AF103728 AF103728 AF103728 AF103728 AF103724 AF002431 M5493 AF052431 M90393 X97469 |
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suggesting that these 5' signals are not utilized to facilitate viral read-through. However, comparison of the 5' sequences revealed that there was a preference for adenine in the penultimate and ultimate nucleotide positions, accounting for 76 and 71%



Figure 1. Examination of non-randomness in read-through groups. Each data set represents the 82 nt region surrounding either the leaky (left column) or non-leaky (right column) stop codon for each of the six +1 triplet groups. For each data set, the *x*-axis represents the individual nucleotide position from the -19th to the +60th relative to the stop codon, and the *y*-axis is a measure of the non-randomness associated with each nucleotide position in the form of a χ^2 value.

respectively of the total nucleotides in those positions compared to only 28 and 25% respectively in the non-read-through control groups.

The secondary structure 3' of the stop codon was examined for stem–loop or pseudoknot structures reported to be important for efficient read-through in some viruses. Although some of the sequences examined displayed feasible structures, no consistent significant similarities were found with either the reported structures or with each other.

Construct analysis using luciferase reporter system

The test construct inserts were designed either to be exact replicas of the +1 and +2 triplets of the sequence groups, as was the case for constructs 1, 4, 11, 12 and 14 (groups 1, 3, 4, 2 and 2 respectively), or derivatives of those sequences with single nucleotide substitutions, as was the case for the remaining 10 constructs. Each of the 15 constructs inserted into the p2luc vector was tested for its ability to facilitate read-through



Figure 2. Read-through facilitated by p2luc constructs. Each of the 15 sequences listed on the *x*-axis were tested for their ability to facilitate read-through of *Photinus* luciferase using the fusion vector p2luc. Sequences 1, 4, 11, 12 and 14 represent exactly the +1 and +2 triplet sequences from groups 1, 3, 4, 2 and 2 respectively. The other 10 sequences were either substitutions of a single nucleotide position (sequences 2, 3 and 5–9) or a substitution of the stop codon (sequences 10, 13 and 15).

expression of *Photinus* luciferase. All the constructs produced values greater than the low background level for spontaneous read-through for this vector (15). The raw output of the assays was converted to average %RT (Fig. 2). The sequence for construct 1 was taken directly from group 1 (CAA-UUA) and constructs 2 and 3 each make a single substitution in that sequence (CAA-GUA and CAA-UUU respectively). While the U to G substitution at position +4 had little effect on read-through, the A to U substitution at position +6 significantly reduced the level of read-through observed. These results are consistent with the results obtained by Skuzeski *et al.* examining the TMV read-through context *in vivo* (12) and the *in vitro* work of Zerfass and Beier (14).

Constructs 4–10 were all based on group 3 (GGG). None of the single nucleotide substitutions made in the +1 and +2 triplets in constructs 5–8 had any significant effect on readthrough levels, however in construct 9, where the +6 nucleotide U was substituted with a G, read-through increased significantly. In construct 10, where the UAG stop codon was changed to a UGA, the read-through rose ~1.7-fold. A difference in readthrough between identical constructs with different stop codons also occurred in constructs 12–14, which are based on Group 2 (CGG); both the CGG-UUU and CGG-UGU constructs showed significantly higher levels of read-through when placed downstream of a UGA terminator compared to their UAG counterparts. Groups 5–6 were not tested.

When comparing the constructs that represent the nonsubstituted read-through group sequences, there also appeared to be variation in the percentage of read-through each sequence group facilitated. Group 1 showed the highest levels $(3.2\% \pm 0.4)$, followed by both group 2 constructs, themselves displaying variation $(2.3\% \pm 0.3 \text{ and } 1.9\% \pm 0.2)$. Group 4 had the third highest read-through levels $(1.06\% \pm 0.08)$, and group 3 had the lowest of the groups tested $(0.74\% \pm 0.05)$.

DISCUSSION

The degree to which the identity of the six nucleotides 3' of viral read-through stop codons is restricted is remarkable. The sequences examined are from RNA-containing viruses in

which mutation rates are notoriously high and different sequence combinations are undoubtedly frequently tested. Given the larger than triplet recognition in the release process (25), one would have expected the sequence 3' of 'tight' (non-read-through) stop codons to be more restricted than leaky stop codons but this is not so, as the level of non-randomness associated with this position in the control groups is low (Fig. 1). However, with the read-through stop codons, restriction extends to the sixth following nucleotide and is even pronounced at this position. While other recoding signals are known to be operative in some of the available sequences analyzed (4,6,10,12), and are likely involved, though unrecognized, in others, it is clear from the statistical and experimental analysis performed that the 3' hexanucleotide sequence is a major influence on read-through.

Stop codon recognition occurs in the ribosomal A-site. Stacking of the 3' adjacent base has an influence on codon interactions in the A-site and influences both termination (26–28) and frameshifting (29). How the identity of up to six nucleotides affects read-through is less clear. In one case of frameshifting, that for yeast Ty3, there is provocative evidence that a local 3' effect, which extends to 13 bases, is due to mRNA pairing with rRNA in the pre A-site (30).

An influence of specific rRNA segments on in-frame stop codon recognition has been proposed based on several experiments. These experiments suggest that in prokaryotes there is an interaction involving the stop codon and C1054 of helix 34 of the 16S rRNA (31). There is also evidence that a similar interaction is at work in eukaryotes because of the conservation of C1054 in yeast 18S rRNA (32). Also, the strength of the stop codon's interaction in this trio may be in part due to the nucleotide sequence surrounding the stop codon (33). This is supported by site-directed crosslinking experiments showing that nucleotide positions +1 (34) and +4 to +6 (35) can be crosslinked to release factor 2.

Read-through signals

The number of nucleotides that appear to be necessary for the signaling of read-through vary. Group 1 adhered to the CAR-YYA formula found to be important for the in vivo readthrough of the UAG of TMV (12) and the in vitro read-through of UAG, UAA and UGA in a TMV-specific context (14). Group 1 is also in agreement with the -CA(A/G)N(U/C/G)A- consensus sequence found to facilitate read-through in S.cerevisiae (36). The essential nucleotides in the spacer region between the stop codon and the beginning of the pseudoknot in MuLV (37) are mostly conserved in all of the members of group 3. The luteovirus proximal signaling sequence CCN-NNN is known to be necessary for read-through, whereas the +1 and +2 triplets appear to have no importance (4). This luteovirus signal appears to various extents in every group 5 sequence, as well as appearing in six of the nine ungrouped sequences. Therefore, perhaps group 5 should be redefined using the CCN-NNN repeat as the criterion instead of the +1 triplet, leaving only three ungrouped sequences.

The type of stop codon appears to be a determinant for the +1 triplet groups, as almost all the groups are stop codon specific, the only exceptions being broad bean necrosis virus and botrytis virus F in group 1, beet virus Q in group 2 and pea enation mosaic virus in group 3. Except for broad bean necrosis virus in group 1 and beet virus Q in group 2, the UAA

stop codon does not appear in any of the sequences examined, which is consistent with UAA(A/G) being one of the most preferred termination sequences in eukaryotes (25). However, the context for UAG read-through in MuLV has been shown to work with UAA and UGA *in vivo* and *in vitro* (22). The TMV UAG read-through context also appears to function for both UAA and UGA *in vitro* (14). And the UGA read-through context of Sindbis virus can facilitate read-through for UAA and UAG as well (38). These data suggest that the stop codon dependence of the sequence groups may be the result of some other factor and not a necessity of the +1 triplet sequence.

The relative abundance of adenine in the penultimate and ultimate nucleotide positions relative to the leaky terminator suggests that the 5' context may also play a role in signaling read-through. However, previous studies report conflicting evidence as to the influence of adenine in these positions raising questions, at least at the nucleotide level, about the importance of these positions (39,40).

It is interesting to note that the individual groups are not host specific, since members of Furovirus, Coltivirus and Alphavirus all appear in group 2, and infect plants, bacteria and mammals, respectively. Host non-specificity combined with the small number of groups that are sufficient to accommodate all but three of the examined sequences suggests one of two conclusions: that either there are only a limited number of sequences that can signal read-through, and the members of each group co-evolved the same sequence; or, less likely given the diversity of the members within a group, that the members of a group came from a common ancestor that possessed the signaling sequence or a precursor to it.

Although we were not able to categorize three of the examined sequences, this is likely to be a consequence of the limited number of complete viral genomes containing read-through stop codons that have been sequenced to date. Identification of more read-through sequences may lead to the addition of other groups of conserved sequences, allowing the ungrouped sequences here to be categorized, revealing the total number of signaling sequence groups and how they facilitate readthrough of a stop codon.

Read-through facilitated by the selected sequences in the p2luc

The varying amount of read-through observed with the different constructs suggests that some signals may play a larger role than others. The group 1 sequence CAA-UUA facilitates the highest amount of read-through of the tested sequences and reaches levels approaching the level of $\sim 5\%$ reported by Skuzeski et al. for TMV (12). The constructs representing group 3, in contrast, show much lower levels than the ~5% level reported by Jamjoom et al. for MuLV (41). However, both the pseudoknot and the spacer region are known to play a role in read-through signaling in MuLV (5,6), a member of group 3. So perhaps additional signaling sequences exist in the other members of group 3, and the CGG sequence is only part of a more complex signal. The presence of additional signals may also explain the presence of the CUA +1 sequence in group 5. As explained above, all the members of group 5 contain the CCN-NNN repeat known to be the proximal read-through signal in luteoviruses. Perhaps the CUA sequence increases the efficiency of the repeat.

Inspection of the luciferase assay results also reveals some differences in the importance of the individual nucleotide positions in the constructs. In all the constructs assayed, and in all but 4 of the 91 sequences examined, either a C or G is in the +1 nucleotide position. This supports evidence suggesting that the +1 position is critical for read-through in most systems, and that C or G in that position is important for efficient read-through to occur (10,18,42). In fact, C in the +1 nucleotide position is associated with all four constructs showing the highest levels of read-through.

The constructs that had substitutions in the +2 (construct 5), +3 (construct 6) and +4 (constructs 2 and 7) nucleotide position show no significant changes in the level of read-through facilitated, suggesting that these positions either have no role in signaling read-through or the nucleotides substituted are comparable to those they replaced. Previous work with the tobacco rattle virus (TRV) UGA context concluded that just a single nucleotide substitution was not sufficient to influence read-through (43). However, no substitutions to the +5 nucleotide were made in that study. Our results show that the +5 U to G substitution in TRV's UGA significantly increases readthrough in our system. In contrast, our data also indicate that the same substitution at the +5 position had no influence on either the group 2 UAG construct series or on the group 3 construct series, hinting that the importance of a position depends on the nature of the group and the stop codon. Alteration of the +6 position caused the most dramatic change in readthrough of any subset tested. An A to U substitution in position +6 of group 1 decreased read-through 3.8-fold. In contrast, substituting a G for the +6 U in the group 3 construct series increased read-through significantly. The terminator also appeared to have an influence on read-through levels in COS cells. For both the group 3 and group 2 construct series, higher levels of read-through were displayed when comparing constructs with identical +1 and +2 sequences, but having a UGA terminator instead of a UAG. These findings hint at the complexity of read-through signaling and demonstrate the need for additional constructs to further explore the importance of each nucleotide position.

Since the contexts tested here were taken from groups that include viruses whose hosts are in different kingdoms, readthrough signaling mechanisms may be universal. Indeed, in support of this view, the read-through analysis using luciferase reporter genes in mammalian COS cells is consistent with inferences derived primarily from viral sequences infecting plants. On the other hand, it may be possible that differences between the translation systems of the hosts would make the COS assay system used here a non-accurate representation of the performance of some of the contexts. Experiments are being designed to test the constructs used with COS cells in a plant based system.

With the mechanism of translation termination only partially understood, it is difficult to determine with certainty what role the sequence groups described here play in altering that mechanism. The sequences in these groups could influence the binding equilibrium of either release factor or amino-acyl-tRNA, through direct contact or indirectly through interactions with the ribosome. It is unlikely that these groups have no role because of their high level of non-randomness, their conspicuous under-representation in the control groups and the ability of the plant viral sequences to facilitate read-through even using an animal based assay system.

While this manuscript was in final preparation, another classification scheme for read-through stop codon contexts was published by Beier and Grimm (44). This classification differs from the one presented here in that the sequences are divided into three types. Type I represents plant viruses containing the CAA-UYA consensus sequence of TMV (12), similar to our group 1. Type II contains both plant and animal viruses that have either a CGG or CUA +1 triplet 3' of a UGA terminator, where we have each of these triplets separated into different groups. Type III is based on the linear, purine-rich octanucleotide found in the spacer region of MuLV.

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