Reference-guided annotation of viral genomes

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Most prevalent viral genomes in GenBank*

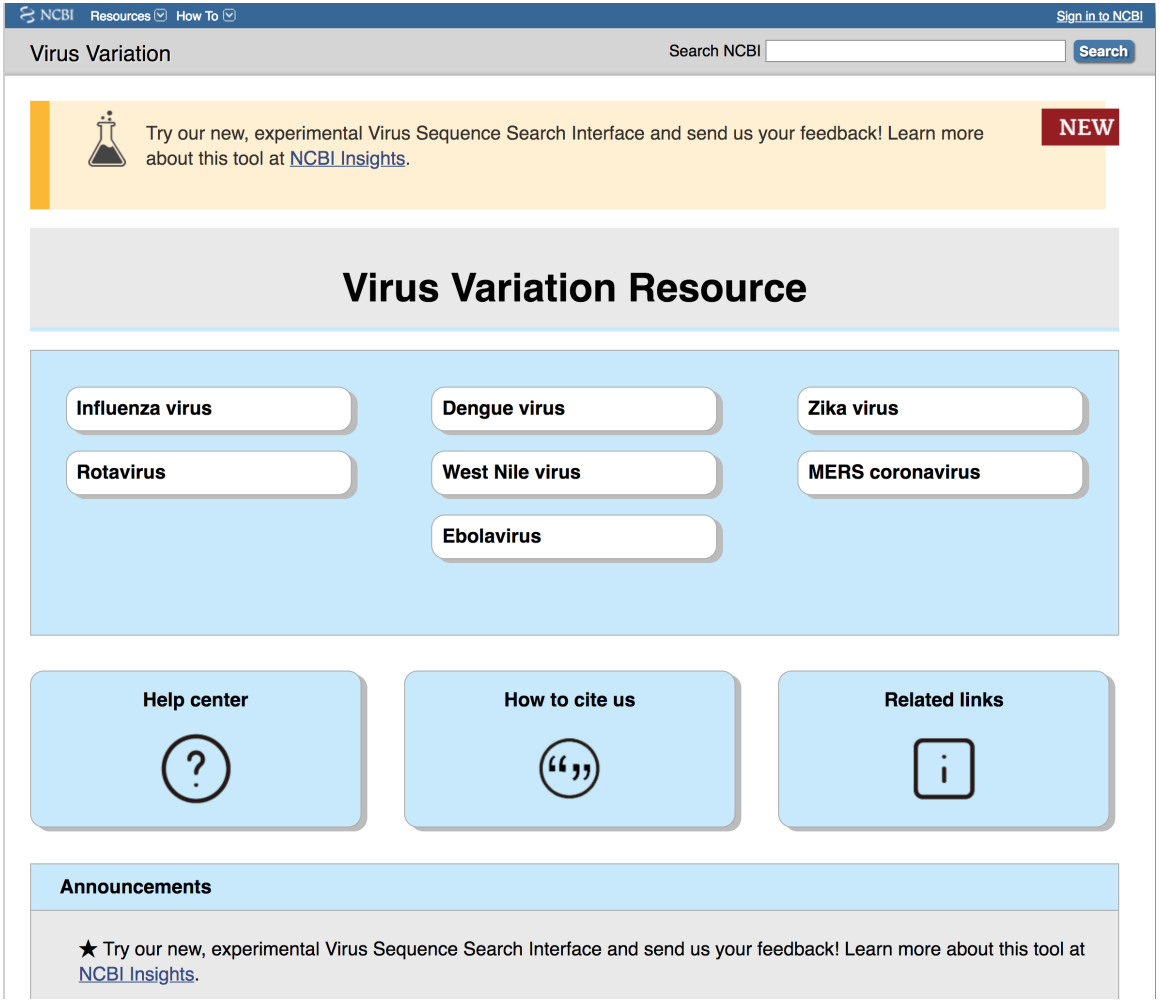
rank	species	#seqs	family	type	host	#CDS	#mature peptides
1	Influenza	503,115	Orthomyxoviridae	(-)ssRNA	humans+	11	-
2	Rotavirus A	58,405†	Reoviridae	dsRNA	humans	12	-
3	Hepatitis B	9211	Hepadnaviridae	dsDNA-RT	humans	7	-
4	Dengue	4853	Flaviviridae	(+)ssRNA	humans	1	14
5	HIV-1	2597	Retroviridae	ssRNA-RT	humans	10	14
6	Hepatitis C	2185	Flaviviridae	(+)ssRNA	humans	2	10
7	Porcine circovirus	1905	Circoviridae	ssDNA	pigs	3	-
8	West Nile	1667	Flaviviridae	(+)ssRNA	humans	3	16
9	Ebola	1384	Flaviviridae	(+)ssRNA	humans	9	-
10	Enterovirus A	1222	Picarnoviridae	(+)ssRNA	humans	1	11
11	RSV	1122	Orthopneumovirus	(-)ssRNA	humans	11	-
12	Norwalk virus	1009	Calciviridae	(+)ssRNA	humans	3	6
13	Maize streak virus	884	Geminiviridae	ssDNA	plants	4	-
14	Rabies lyssavirus	826	Rhabdoviridae	(-)ssRNA	humans+	5	-
15	Enterovirus C	765	Picarnoviridae	(+)ssRNA	humans	1	13

† sum of 11 segments

*Current as of March, 2018.

The Virus Variation Resource is a powerful tool for viral research

- value added database that includes annotations not in GenBank
- allows users to:
  - select subsets of data based on desired criteria (host, country, gene, etc.)
  - download alignments
  - compute trees
  - more...



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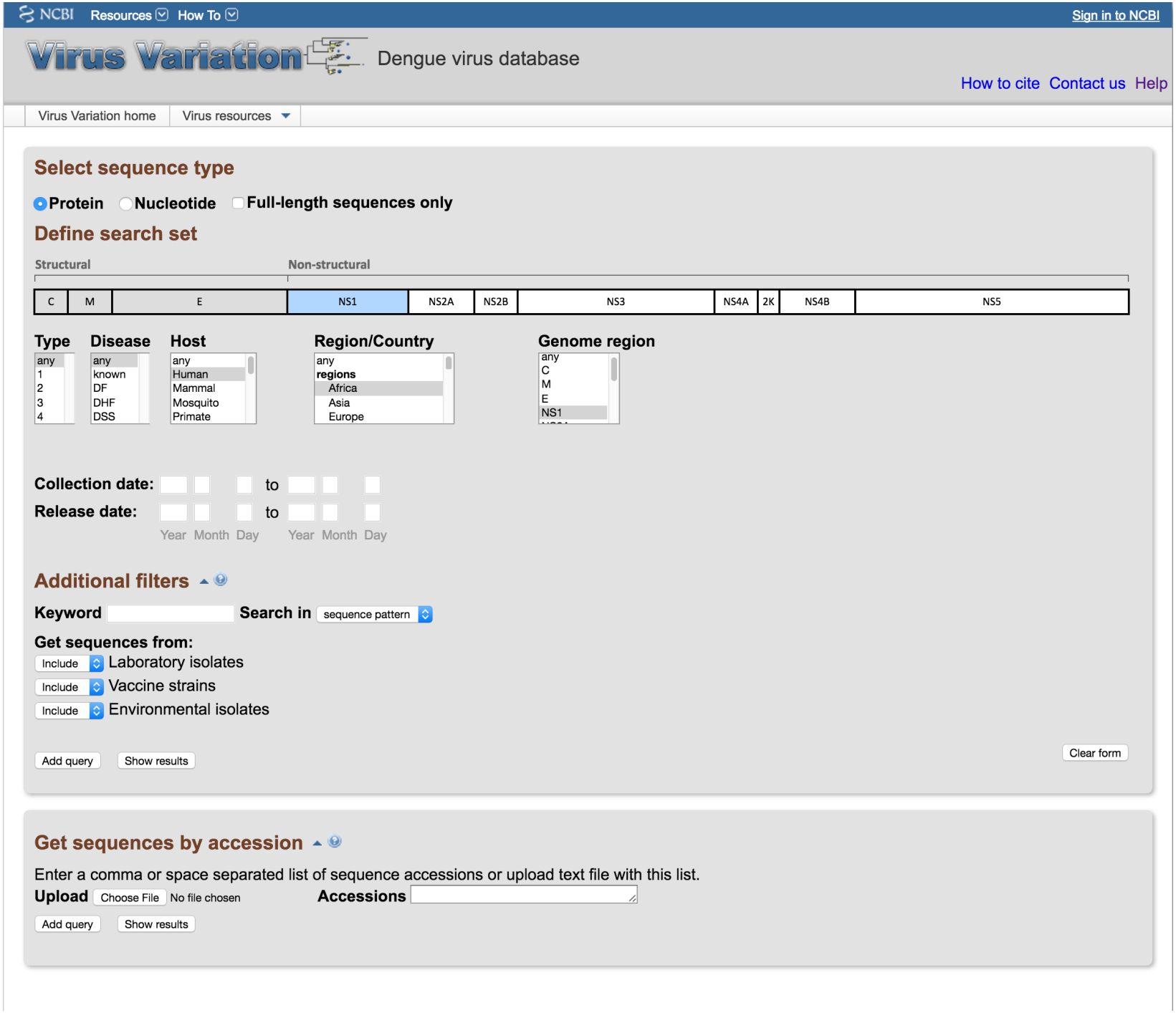

Our initial goal was to develop an annotation pipeline to benefit Virus Variation

- general method for annotating existing and new viral genome sequences for a species using trusted annotation for that species (e.g. RefSeq)

- identify interesting characteristics that Virus Variation can allow users to sort/select based on:
  - identification of high quality sequences that meet specific expectations
  - identification of sequences that deviate from expectations in various ways
    * early stop codon
    * above or below a specific fractional identity to reference
    * more...

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A new goal is to use this annotation pipeline to validate/annotate incoming submissions of sequences from commonly deposited viruses

- use unexpected features identified by pipeline to flag issues for indexers and submitters to deal with

- currently testing on Norovirus, and soon to be testing on Ebolavirus

Four pilot species were chosen from the 15 most prevalent

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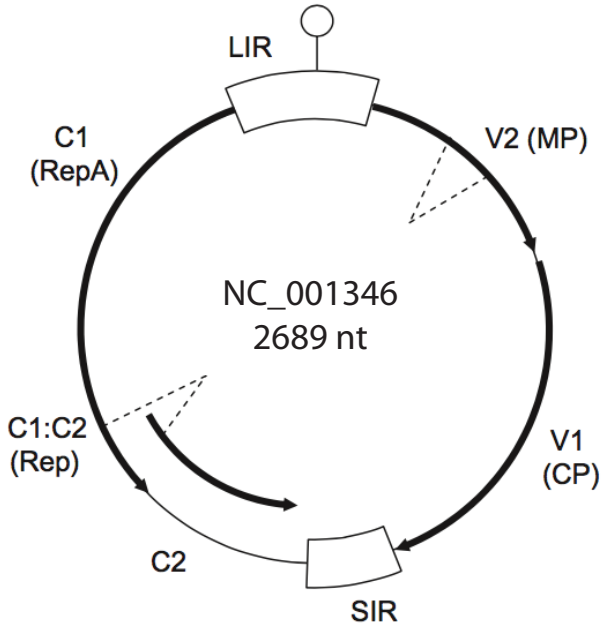
Overview of annotation pipeline for Maize Streak Virus

INPUT:

1. RefSeq annotation



2. Target sequence(s) to annotate:

Overview of annotation pipeline for Maize Streak Virus

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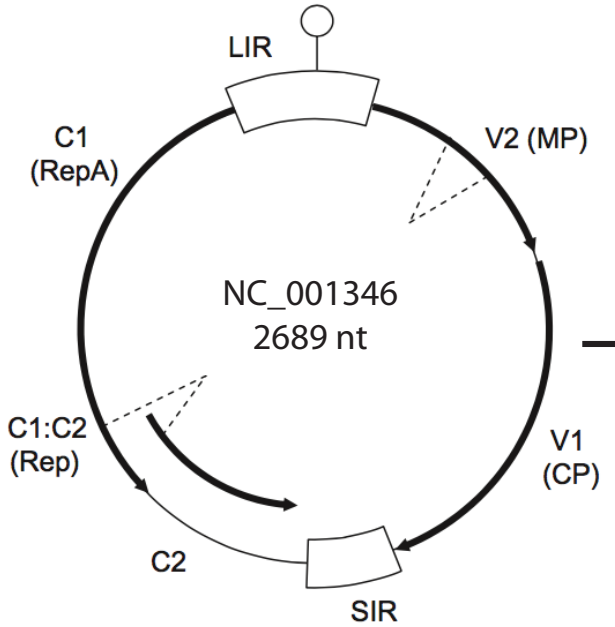
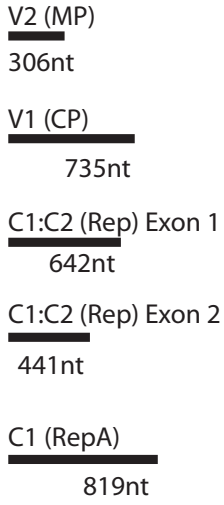
1. RefSeq annotation



Build homology models and search targets:



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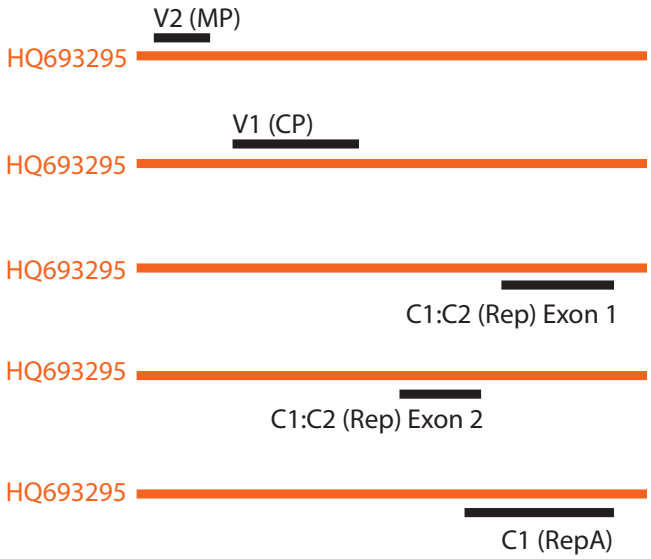

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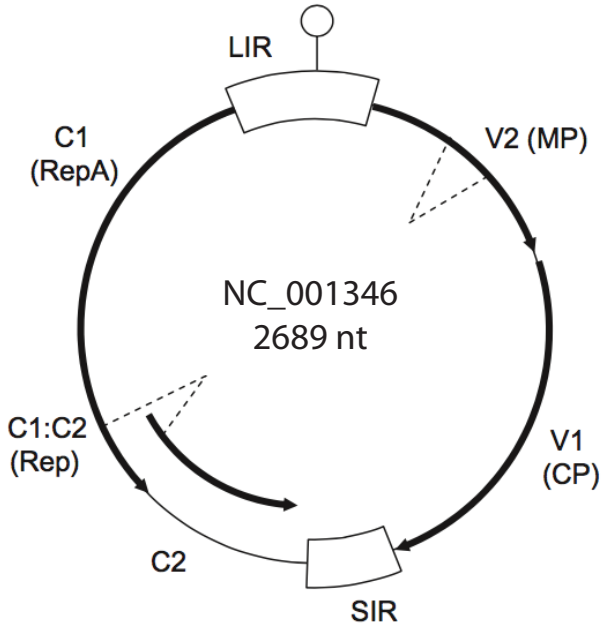
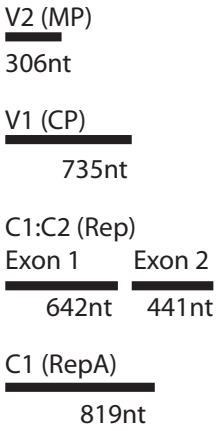
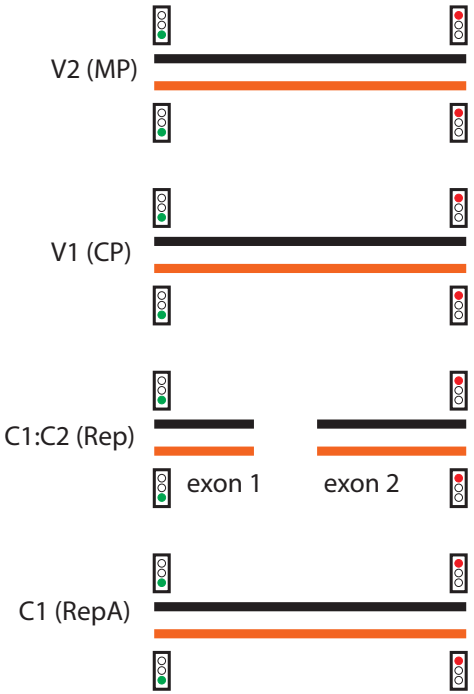


2. Target sequence(s) to annotate:

Combine models into 'features', translate, find stop codons, and identify errors:





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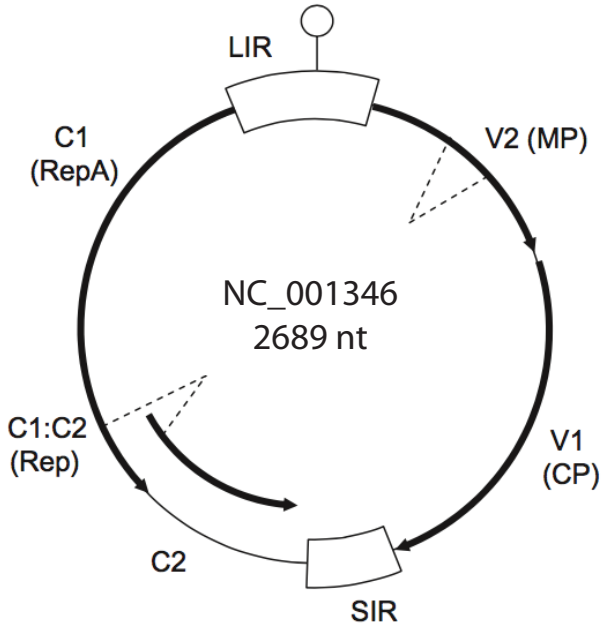

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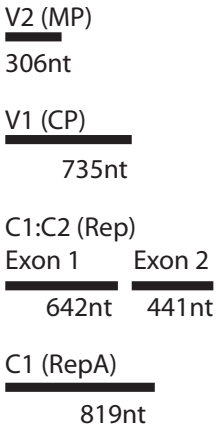
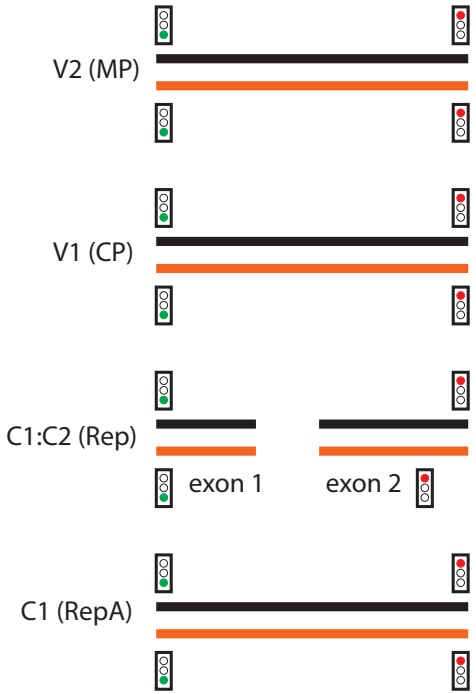




OUTPUT:

1. Tabular annotations of all features:

EU628610:MP:157-462
EU628610:CP:473-1207
EU628610:Rep:2530-1889,1796-1371
EU628610:RepA:2530-1712

2. List of all 'error codes':

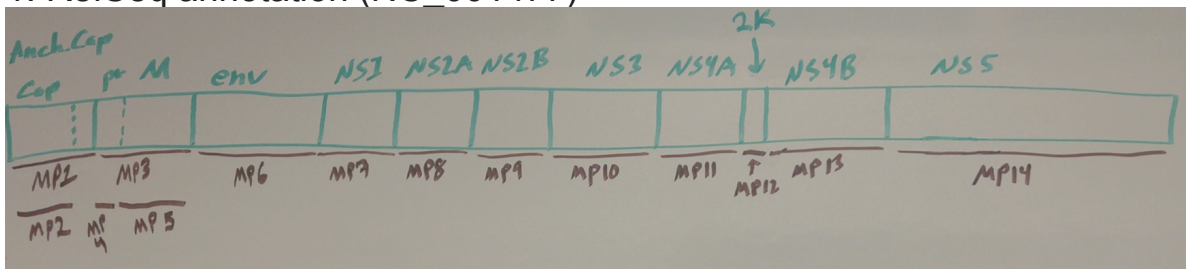
EU628610:Rep:trc

3. Nucleotide and protein multiple alignments (optional)

Overview of annotation pipeline for Dengue Virus

INPUT:

1. RefSeq annotation (NC_001477)

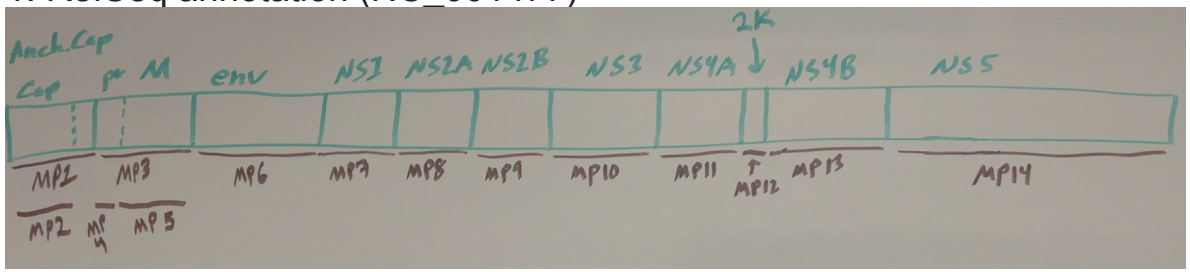


2. Target sequence(s) to annotate:

Overview of annotation pipeline for Dengue Virus

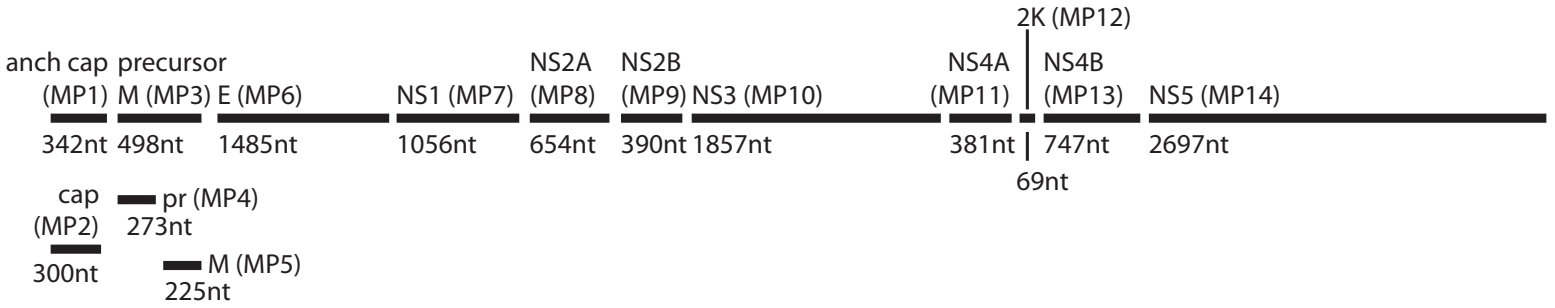
INPUT:

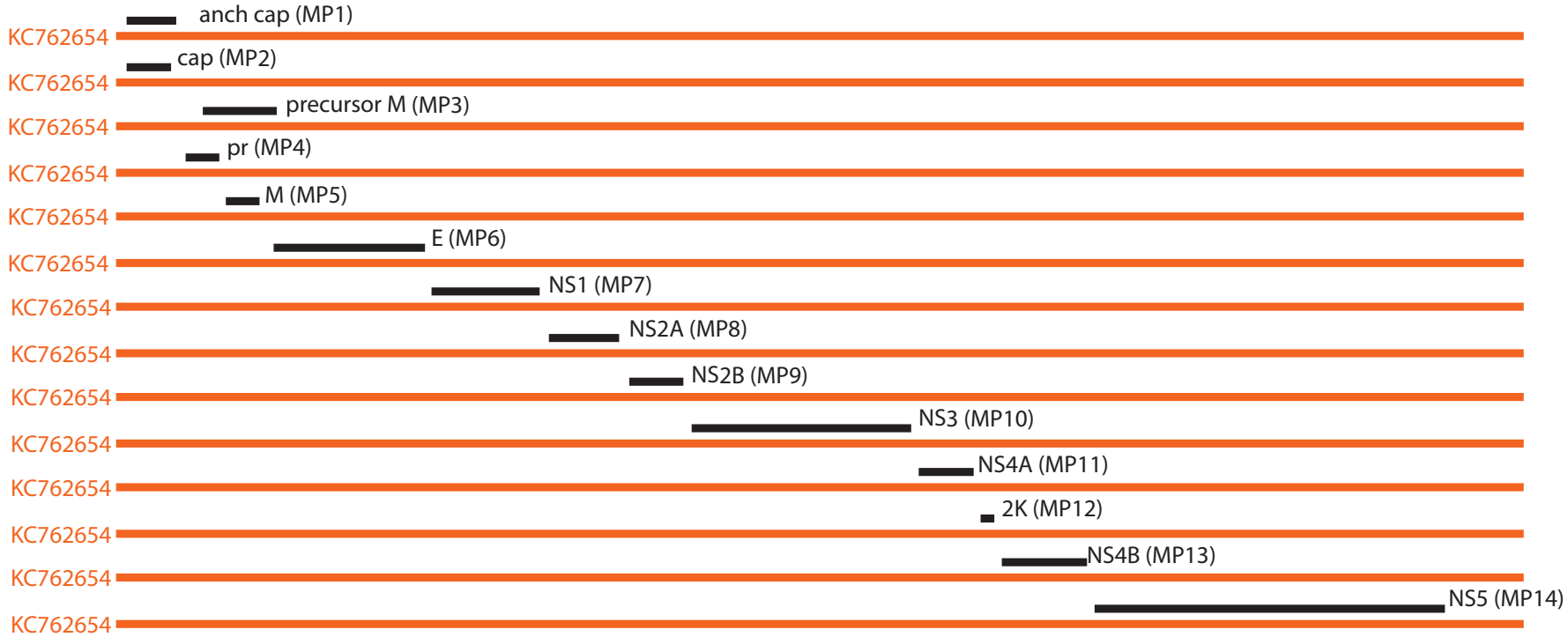
1. RefSeq annotation (NC_001477)



2. Target sequence(s) to annotate:

Build 14 homology models (one per mature peptide) and search targets:





Overview of annotation pipeline for Dengue Virus

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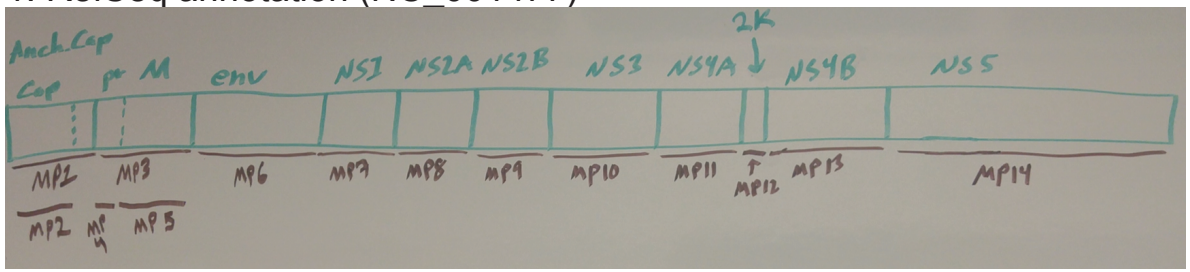
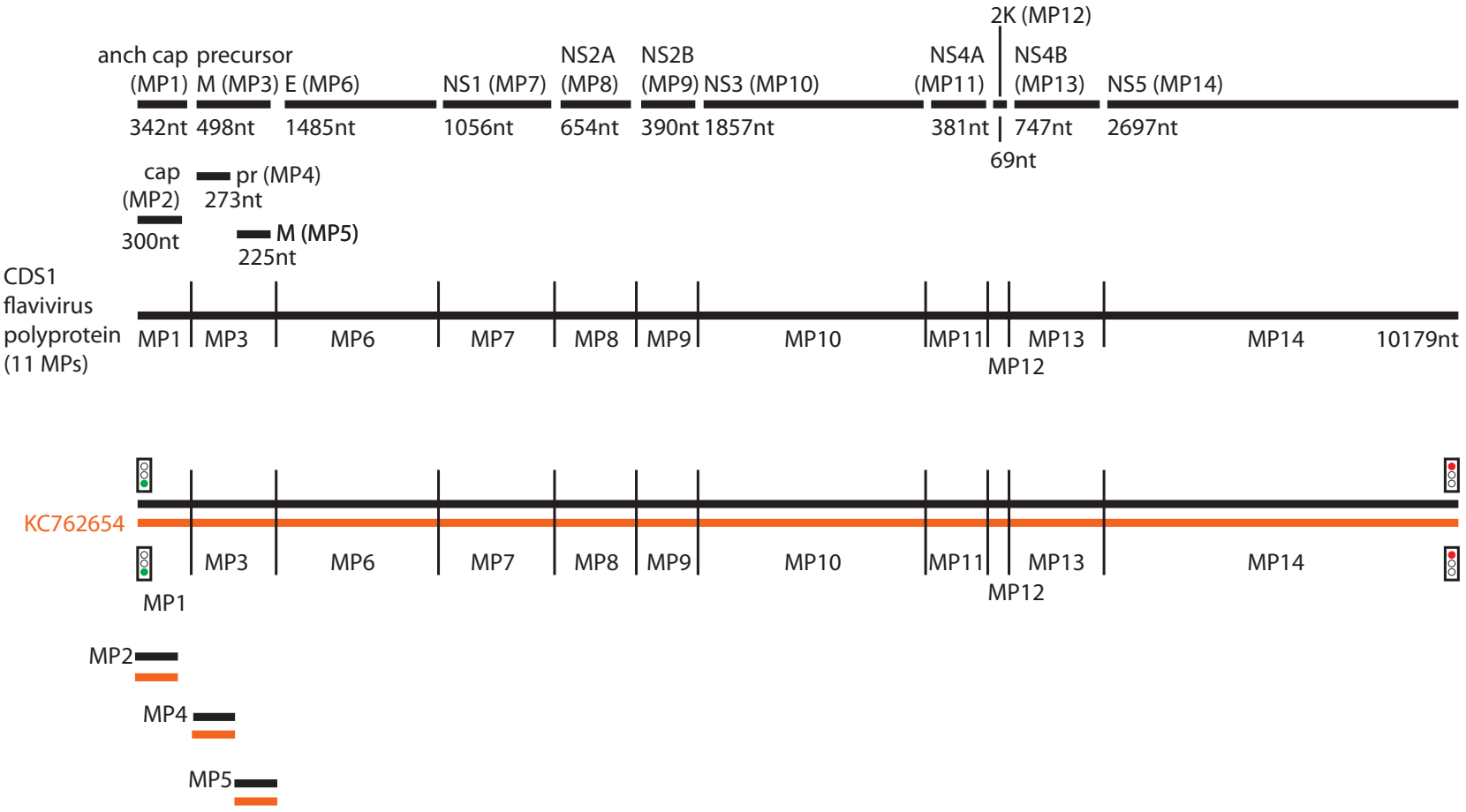
1. RefSeq annotation (NC_001477)



2. Target sequence(s) to annotate:

Combine models into 'features' (14 MPs and 1 CDS), translate, find stop codons, and identify errors:



Overview of annotation pipeline for Dengue Virus

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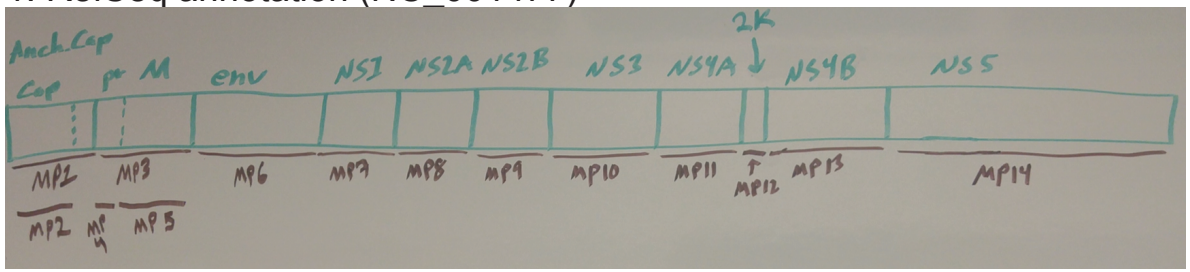
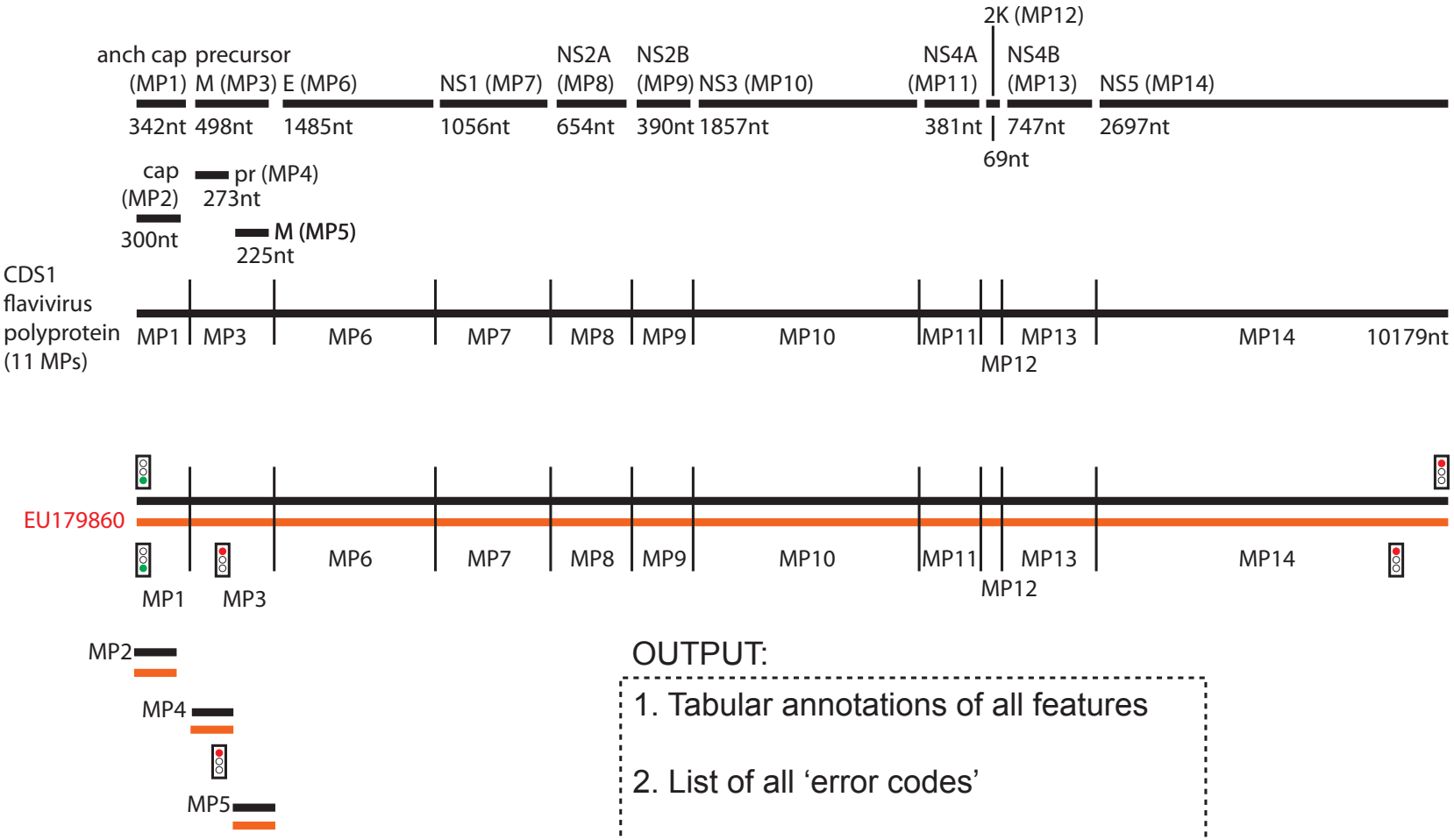
1. RefSeq annotation (NC_001477)



2. Target sequence(s) to annotate:

KC762654
EU179860
A75711
DQ193572

Combine models into 'features' (14 MPs and 1 CDS), translate, find stop codons, and identify errors:



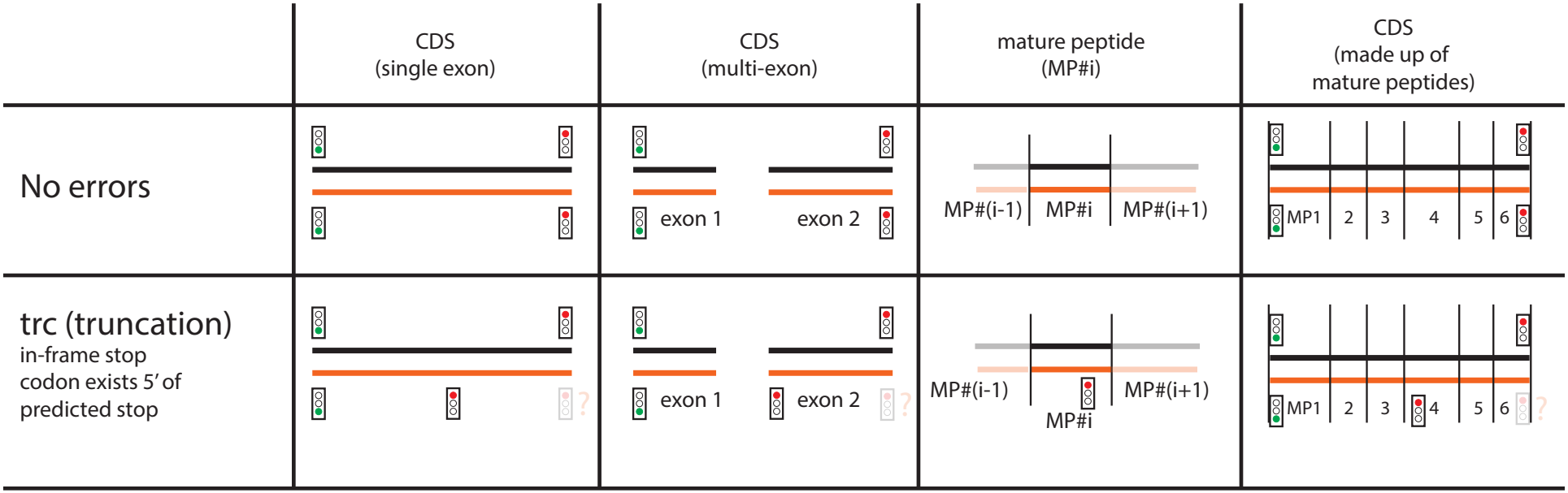
OUTPUT:

1. Tabular annotations of all features
2. List of all 'error codes'
3. Nucleotide and protein multiple alignments (optional)

Error codes: 17 abnormal situations

- Per-feature (e.g. CDS, mature peptide) errors:
  - Unexpected stop codon errors (trc, ext, nst, ntr)
  - Missing expected features (str, stp, nm3)
  - Problem with homology search prediction (bd5, bd3, nop)
  - Unexpected relationship to other features (olp, aja, ajb)
  - Problem annotating CDS due to mature peptide errors (aji, int, inp)
- Per-sequence errors:
  - Lack of exactly one origin sequence (ori)

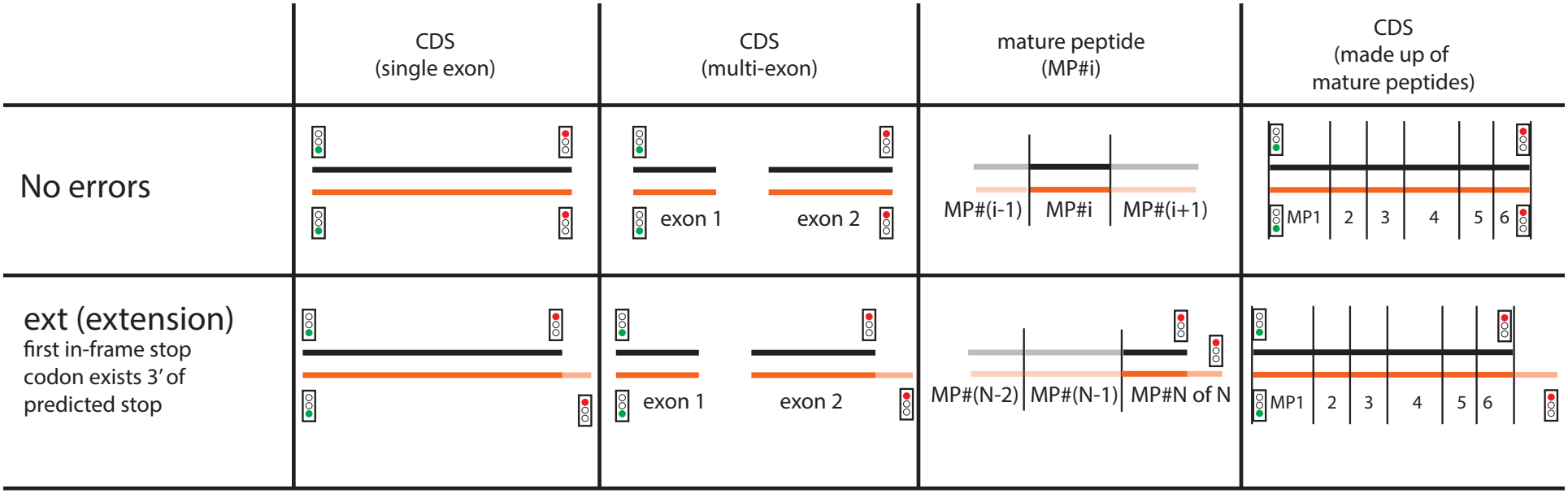
trc error code reports a truncation due to an early stop codon



Example output from annotation script:

```
EU628610      3  CDS#3       trc  in-frame stop codon exists 5' of stop position predicted by homology to reference
                                  [homology search predicted 1796..1356 exon 2 of 2 revised to 1796..1371 (stop shifted 15 nt)]
```

ext: extended feature due to a missing stop codon

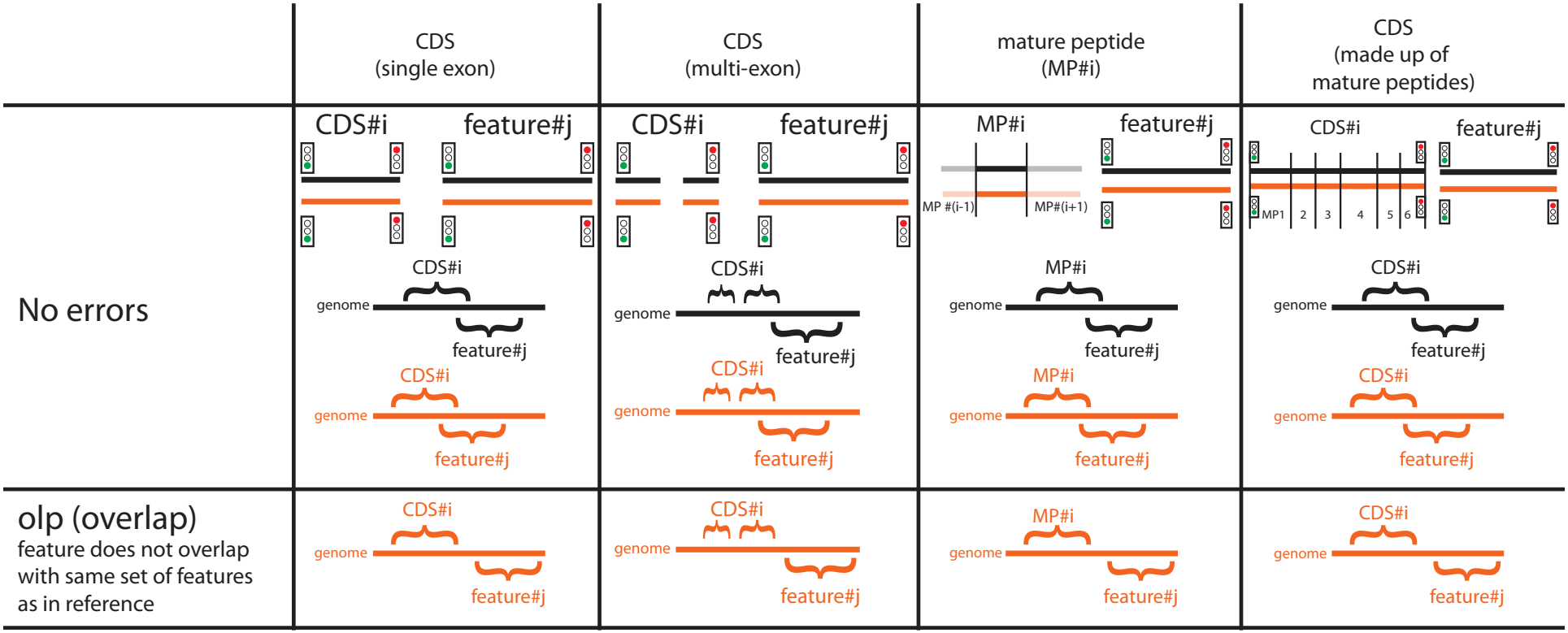


Example output from annotation script:

```
HM631854      14   MP#14        ext   first in-frame stop codon exists 3' of stop position predicted by homology to reference
                                      [homology search predicted 7544..10230 revised to 7544..10288 (stop shifted 58 nt)]

HM631854      15   CDS(MP)#1    ext   first in-frame stop codon exists 3' of stop position predicted by homology to reference
                                      [homology search predicted 74..10233 revised to 74..10291 (stop shifted 58 nt)]
```

olp: lack of an expected overlap with another feature



Example output from annotation script:

```
FJ562227      6  CDS#6       olp  feature does not overlap with same set of features as in reference [-(6.1,1.1),-(6.1,7.1)]
```

For screening submissions: mapping error codes to errors and warnings

- Error codes are too complex to return to submitters
- Sometimes error codes are legitimate (true early stop)
- Sometimes error codes reflect artefacts in the sequence (assembly error)
- Example: trc error code leads to misc_feature and note

```
>Feature NC_029646-1ntgap-outframe
5       5103    gene
                        gene    ORF1
5       5103    misc_feature
                        note    similar to nonstructural polyprotein; contains premature stop codon
                        gene    ORF1
5       994     misc_feature
                        note    similar to p48; polyprotein may not be translated
995     2091    misc_feature
                        note    similar to NTPase; polyprotein may not be translated
2092    2628    misc_feature
                        note    similar to p22; polyprotein may not be translated
2629    3027    misc_feature
                        note    similar to VPg; polyprotein may not be translated
3028    3570    misc_feature
                        note    similar to Pro; polyprotein may not be translated
3571    5100    misc_feature
                        note    similar to RdRp; polyprotein may not be translated
5084    6691    gene
                        gene    ORF2
5084    6691    CDS
                        product         VP1
                        gene    ORF2
6691    7470    gene
                        gene    ORF3
6691    7470    CDS
                        product         VP2
                        gene    ORF3

Additional note(s) to submitter:
ERROR: Deletion of Nucleotides: Contains internal deletion of 1 nucleotide in nonstructural polyprotein/NTPase
ERROR: CDS Has Frameshift: Contains internal deletion of 1 nucleotide(s) in nonstructural polyprotein/NTPase
```

Future directions (long term)

- Protein homology searches to supplement or replace nucleotide searches
- Multiple alignment (profile) based homology searches
- Annotate structural RNA features (Infernal is well-suited for this)

Acknowledgements

Alejandro Schäffer
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Olga Blinkova
Anatoly Mnev
Sergey Zhdanov
Yiming Bao