Validation and annotation of SARS-CoV-2 sequences for GenBank using VADR

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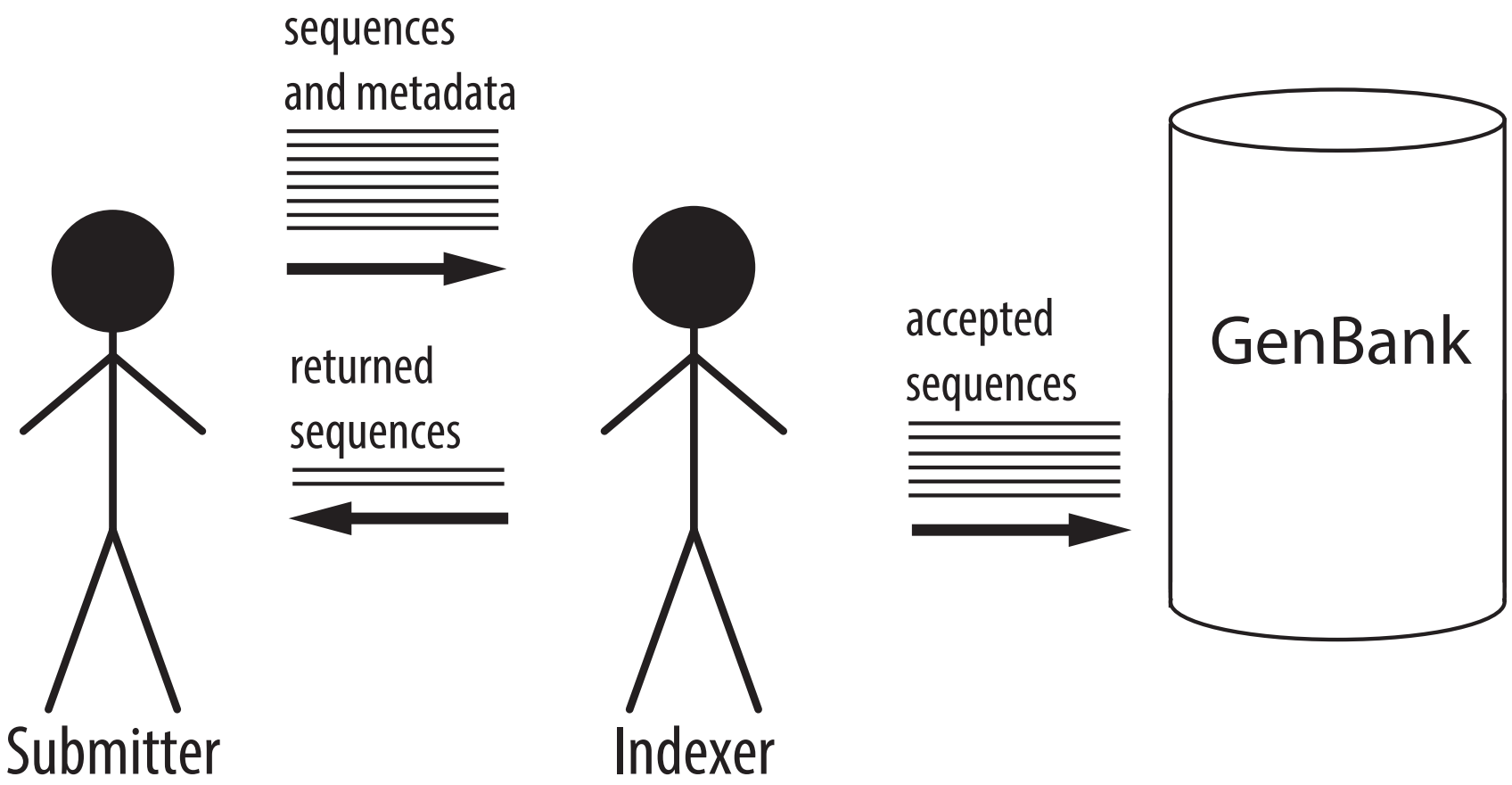
GenBank indexers handle incoming sequence submissions



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SOFTWARE Open Access

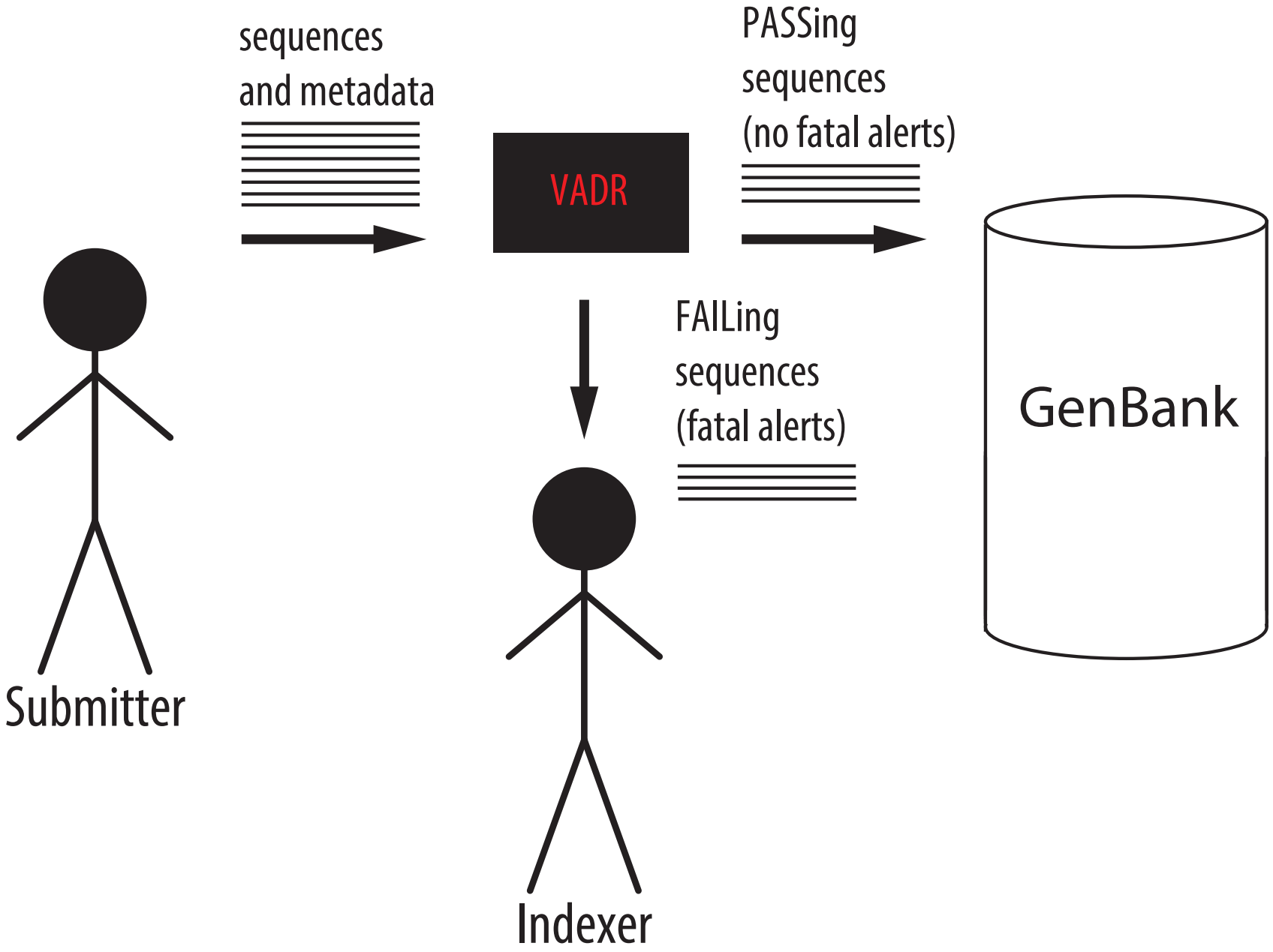
VADR: validation and annotation of virus sequence submissions to GenBank

Alejandro A. Schäffer[1,2], Eneida L. Hatcher[2], Linda Yankie[2], Lara Shonkwiler[2,3], J. Rodney Brister[2], Ilene Karsch-Mizrachi[2] and Eric P. Nawrocki[2*]

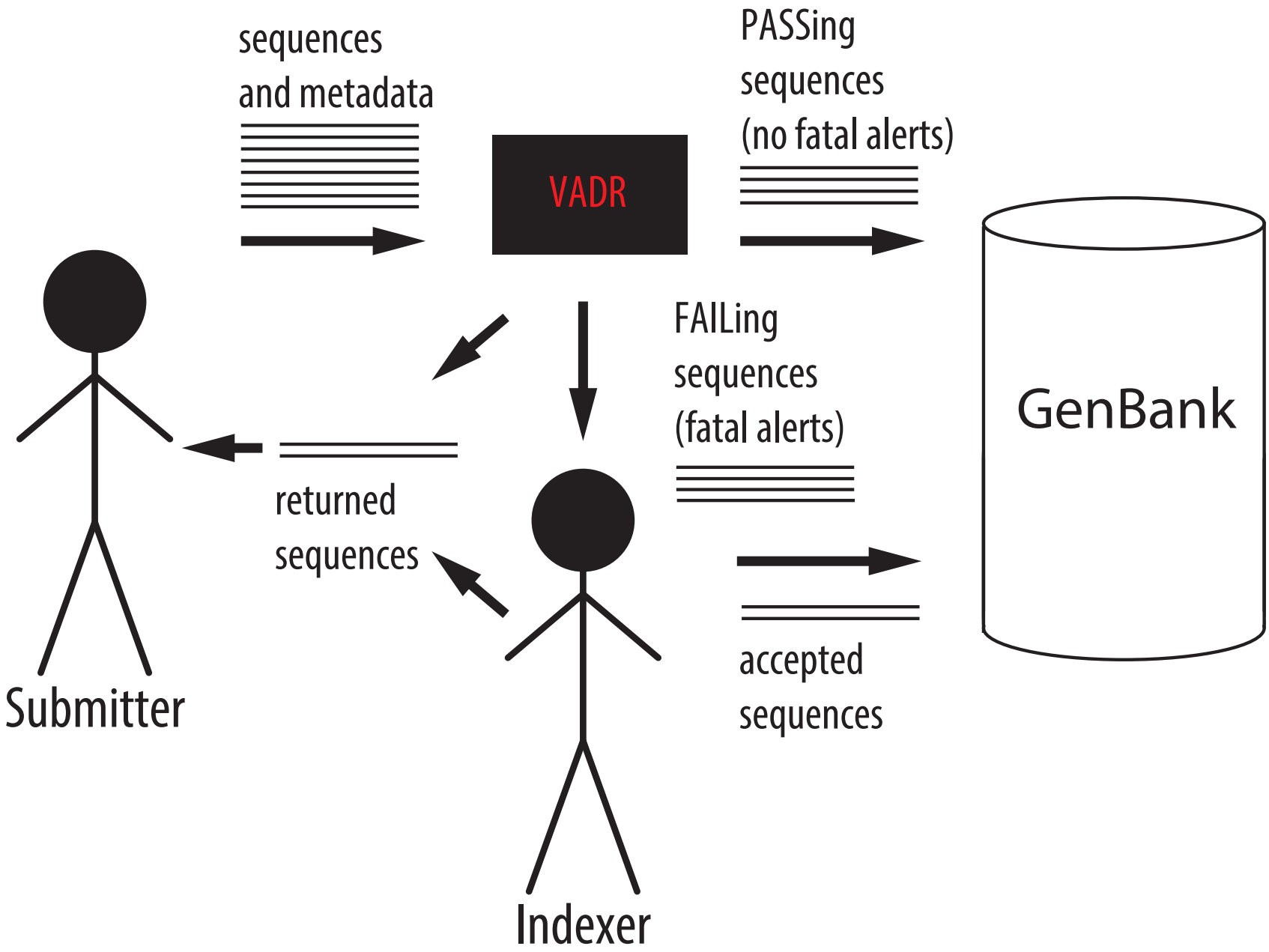
- general tool for reference-based annotation of viral sequences
- used for dengue virus and norovirus submissions since 2018
- used for SARS-CoV-2 submissions since March 2020

VADR assists GenBank indexers:

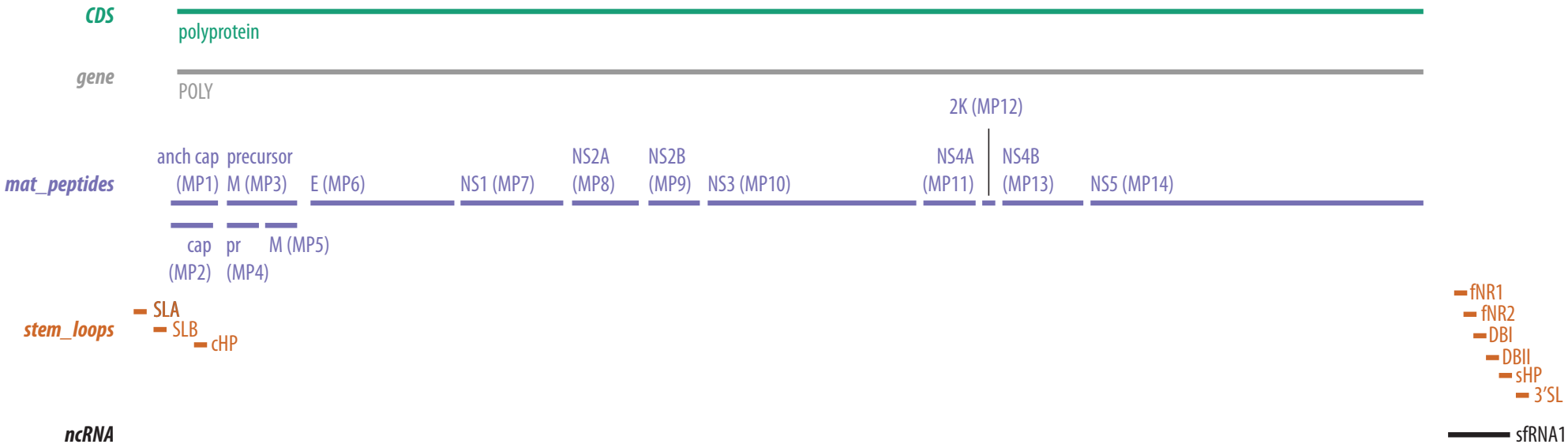
Each sequence PASSes or FAILs

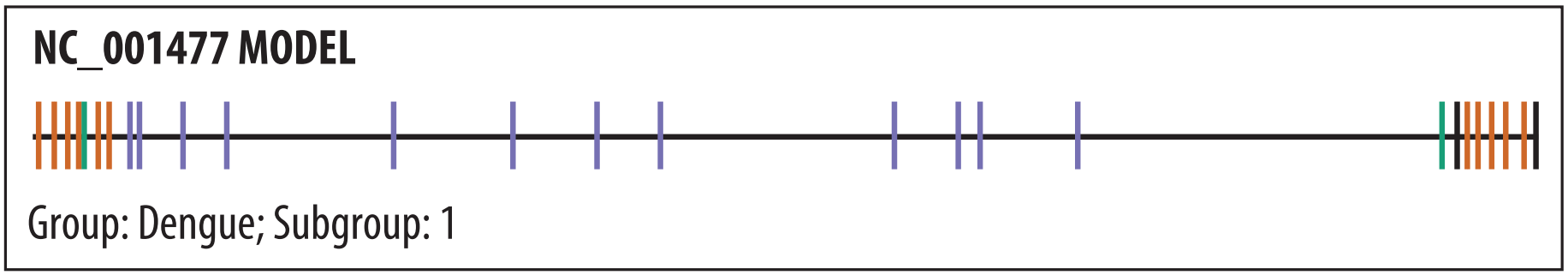


Indexers decide fate of most FAILing sequences
but some are sent directly back to submitter with error reports



VADR builds a reference model of a RefSeq and its features





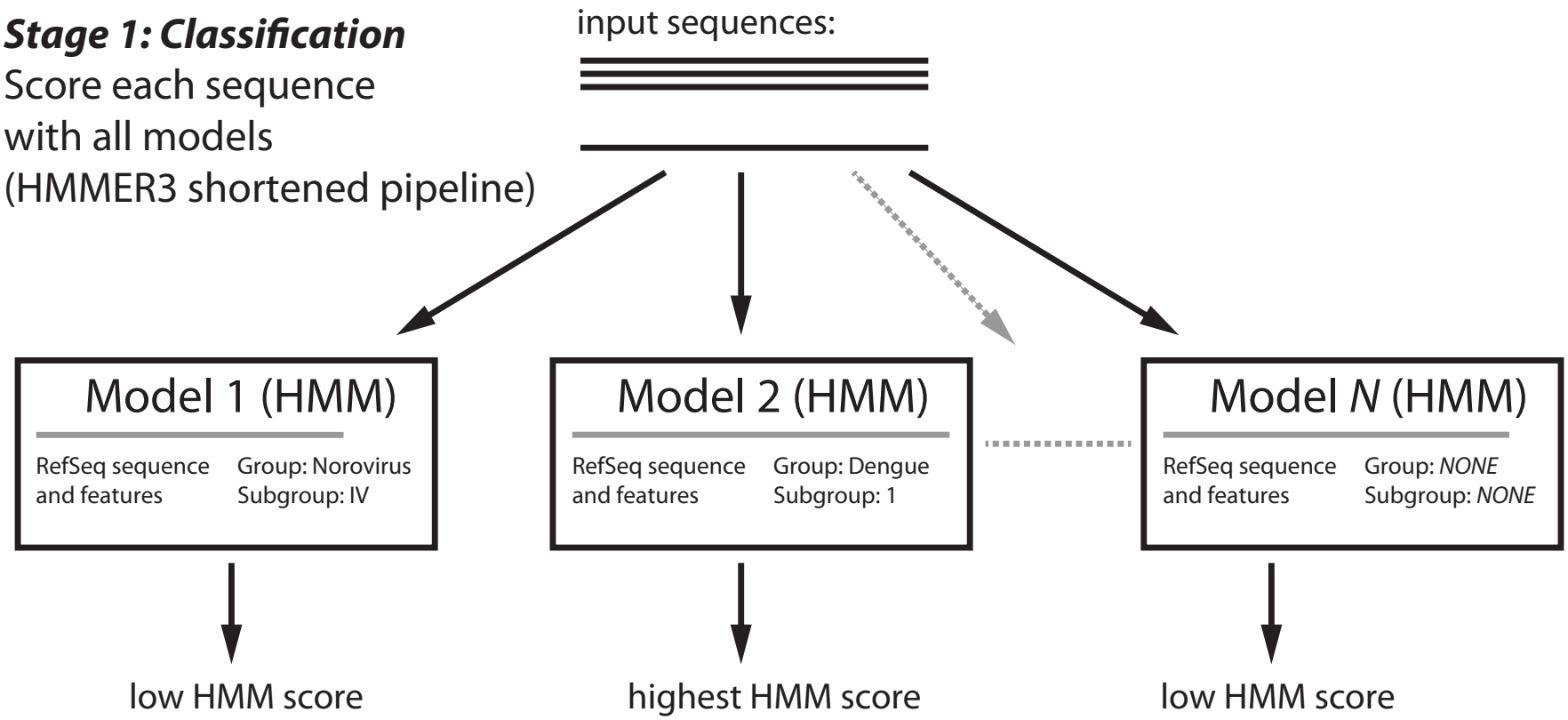
VADR validates and annotates each input sequence using its best-matching model

- Each sequence S proceeds through 4 stages:
  1. **Classification**
  2. **Coverage determination**
  3. **Alignment**
  4. **Protein validation**

Different types of alerts are identified and reported at each stage

Stage 1: Classification

Score each sequence
with all models
(HMMER3 shortened pipeline)



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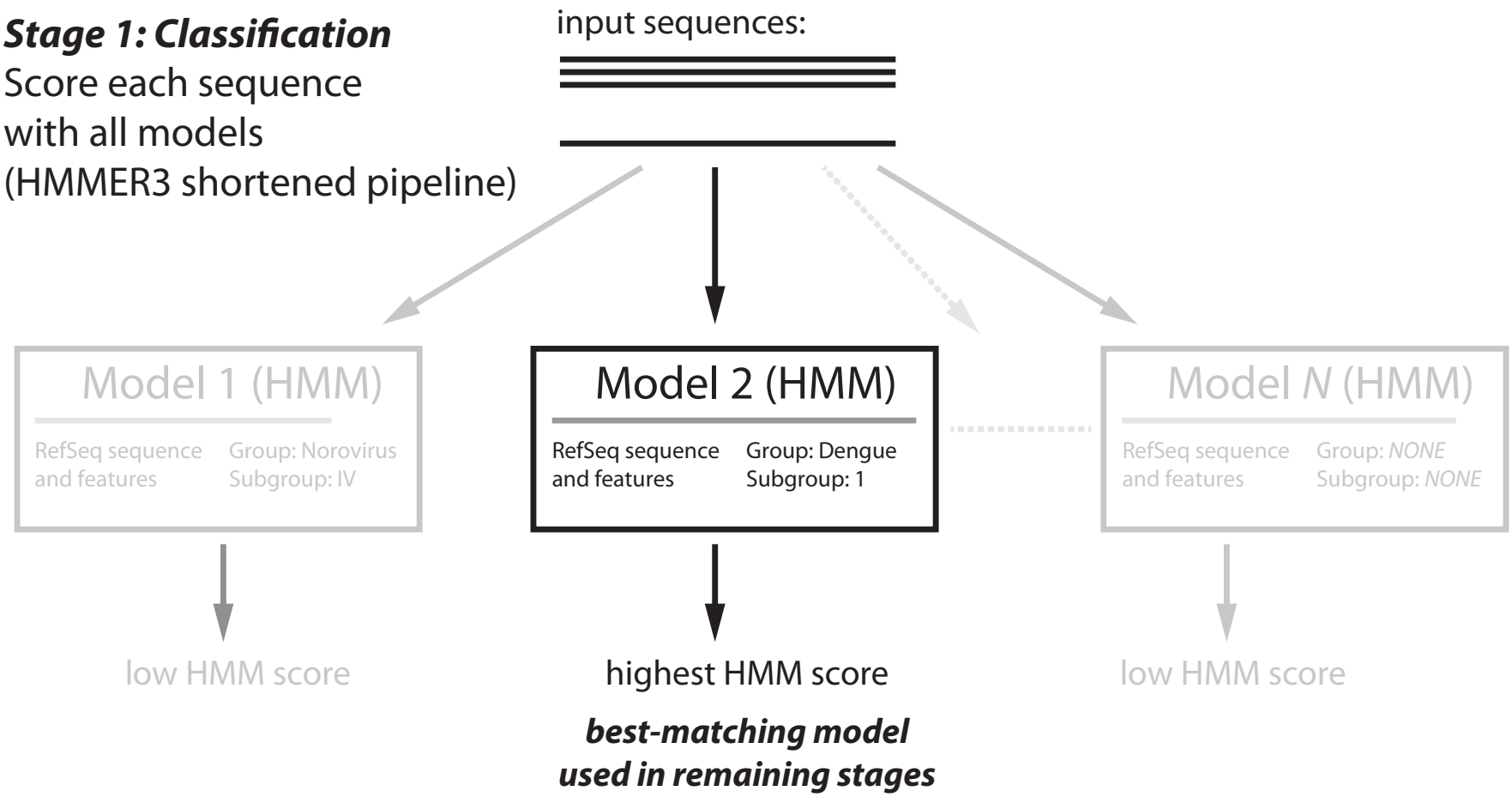
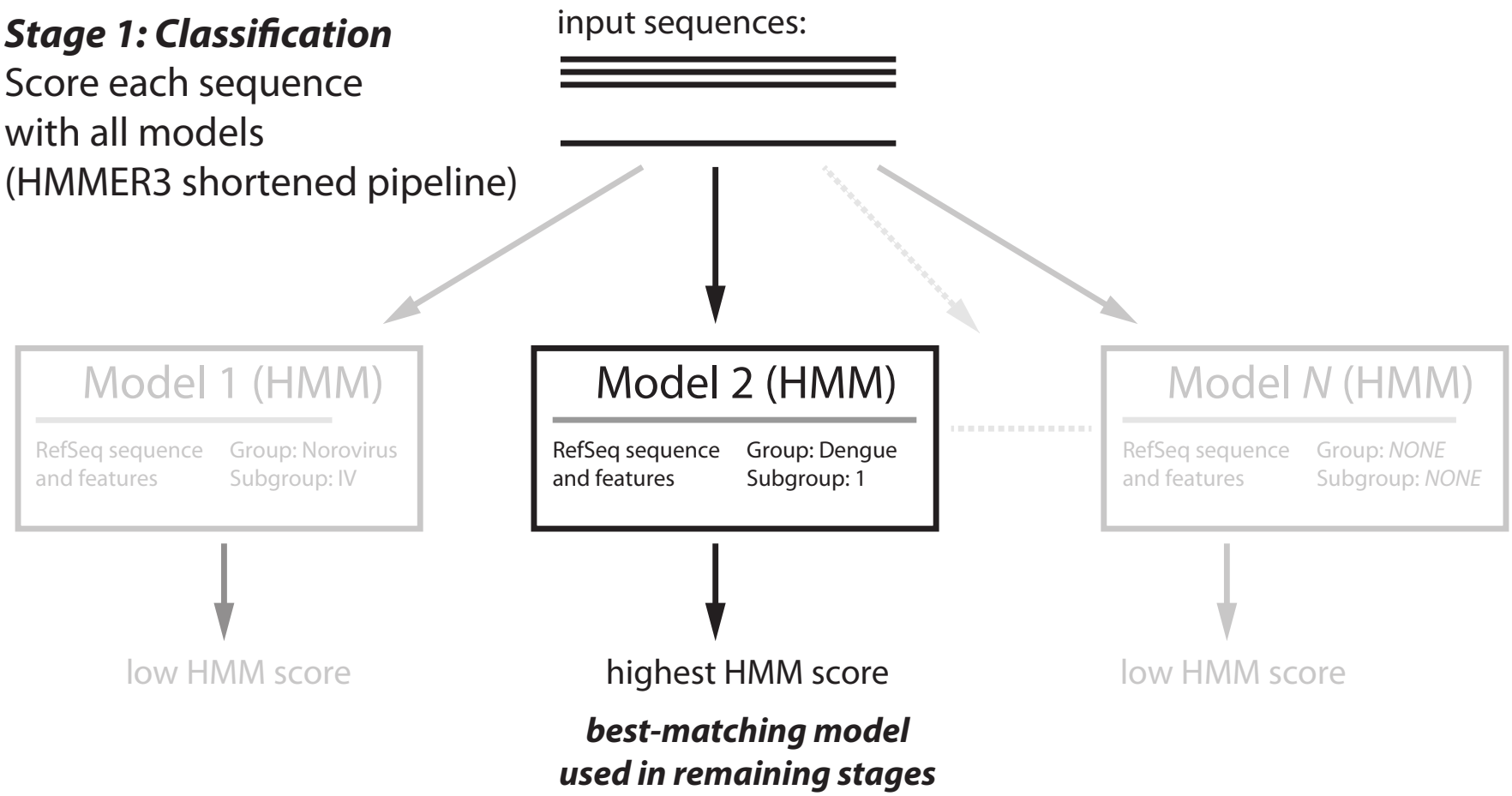
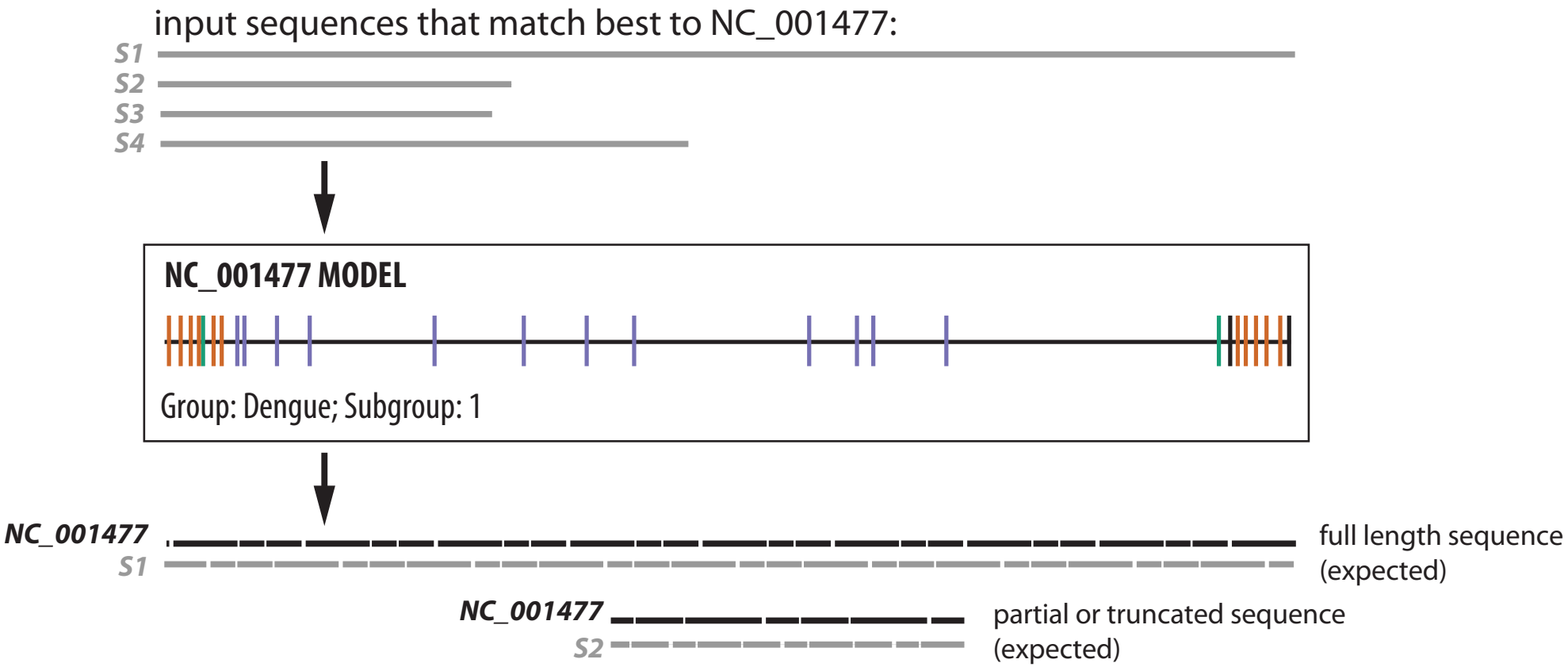




code	S/F	error message	description
Fatal alerts detected in the classification stage			
noannotn*	S	NO_ANNOTATION	no significant similarity detected
revcompl*	S	REVCOMPLEM	sequence appears to be reverse complemented
incsbgrp	S	INCORRECT_SPECIFIED_SUBGROUP	score difference too large between best overall model and best specified subgroup model
incgroup	S	INCORRECT_SPECIFIED_GROUP	score difference too large between best overall model and best specified group model
Non-fatal alerts detected in the classification stage			
qstsbgrp	S	QUESTIONABLE_SPECIFIED_SUBGROUP	best overall model is not from specified subgroup
qstgroup	S	QUESTIONABLE_SPECIFIED_GROUP	best overall model is not from specified group
indfclas	S	INDEFINITE_CLASSIFICATION	low score difference between best overall model and second best model (not in best model's subgroup)
lowscore	S	LOW_SCORE	score to homology model below low threshold

Stage 2: Coverage determination

Search each sequence with best-matching model (HMMER3 full pipeline)



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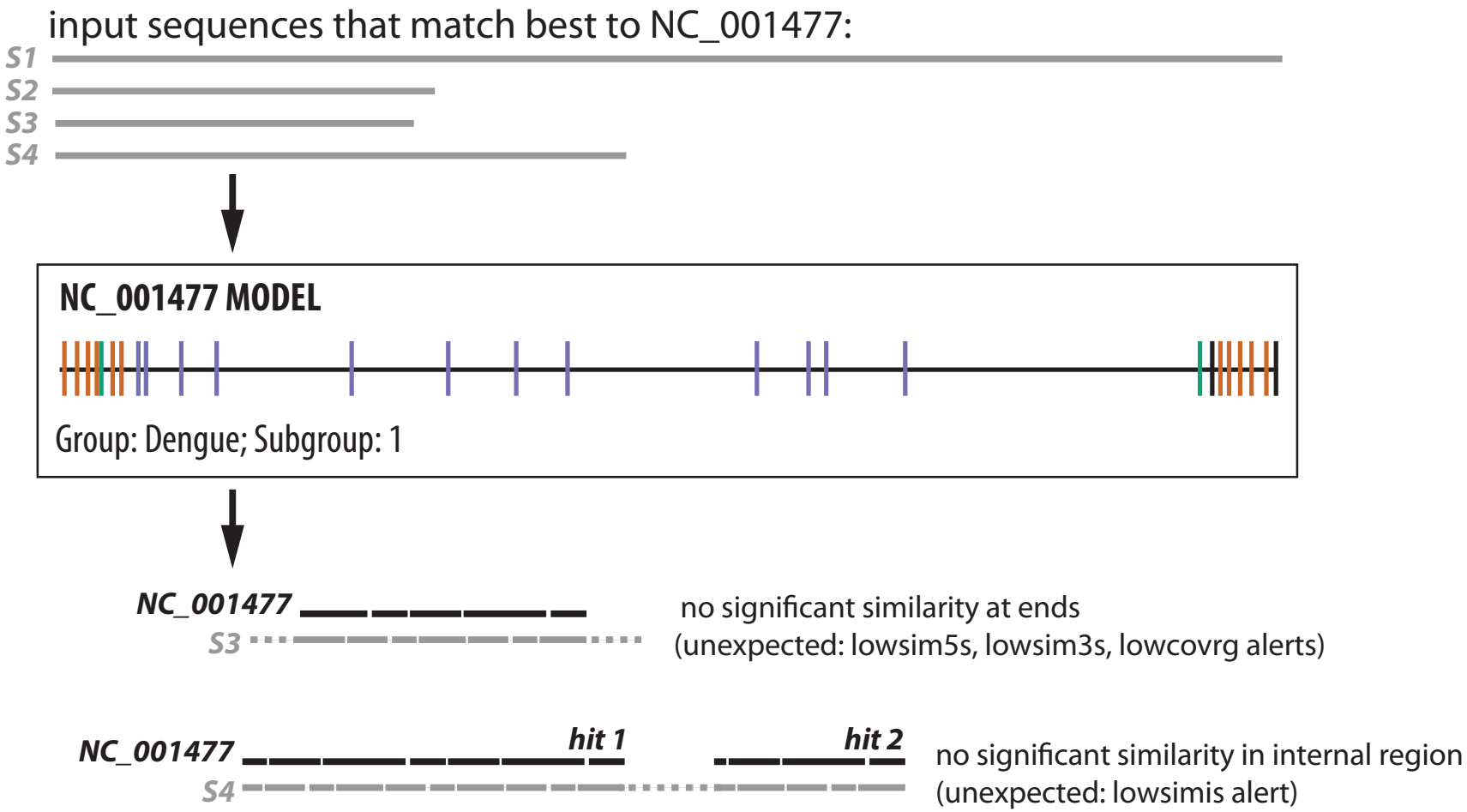
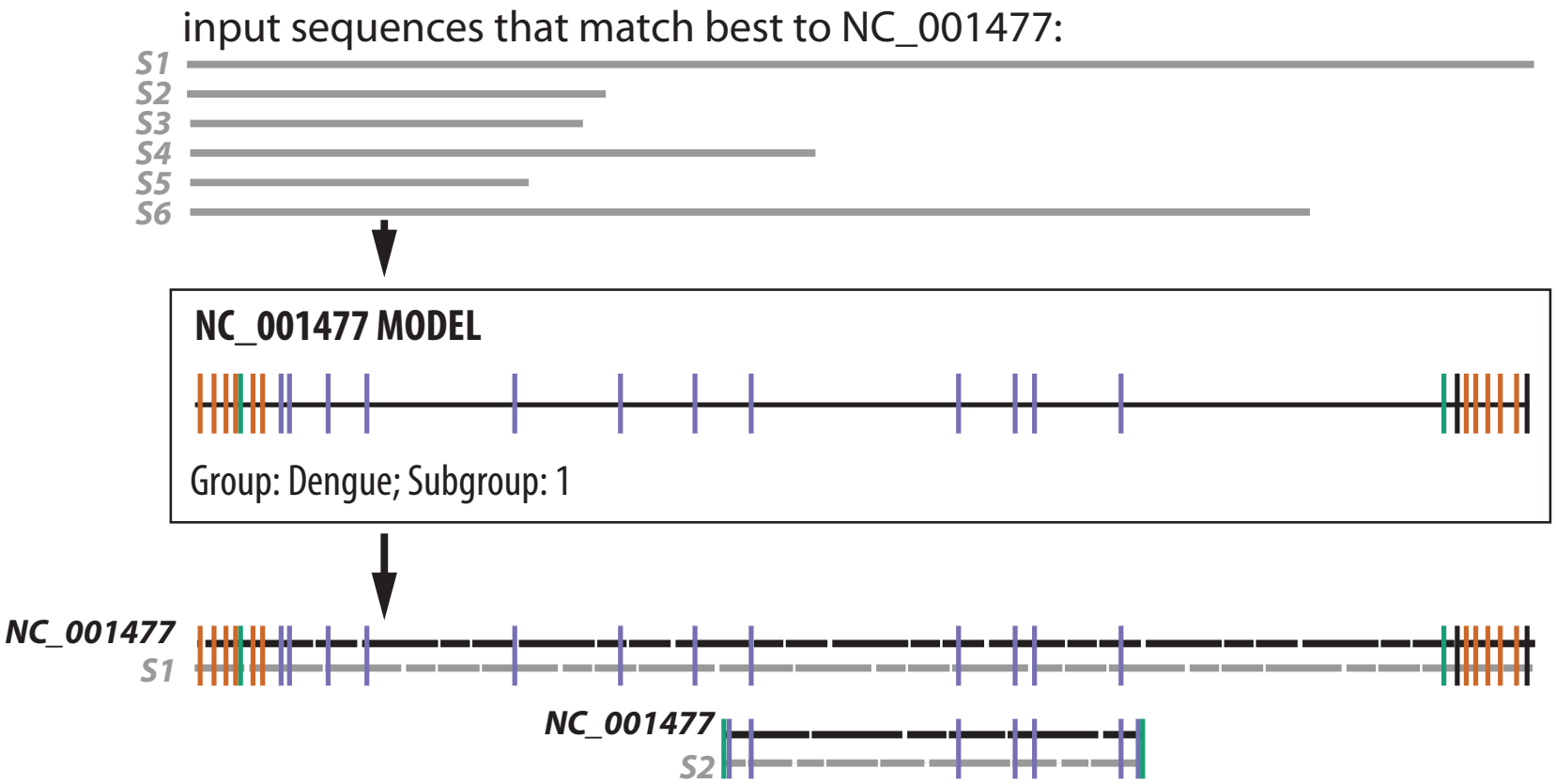


code	S/F	error message	description
Fatal alerts detected in the coverage stage			
lowcovrg	S	LOW_COVERAGE	low sequence fraction with significant similarity to homology model
dupregin	S	DUPLICATE_REGIONS	similarity to a model region occurs more than once
discontn	S	DISCONTINUOUS_SIMILARITY	not all hits are in the same order in the sequence and the homology model
indfstrn	S	INDEFINITE_STRAND	significant similarity detected on both strands
lowsim5s	S	LOW_SIMILARITY_START	significant similarity not detected at 5' end of the sequence
lowsim3s	S	LOW_SIMILARITY_END	significant similarity not detected at 3' end of the sequence
lowsimis	S	LOW_SIMILARITY	internal region without significant similarity
Non-fatal alerts detected in the coverage stage			
biasdseq	S	BIASED_SEQUENCE	high fraction of score attributed to biased sequence composition

Stage 3: Alignment and feature mapping

Align each sequence to its best-matching model (Infernal's cmalign)



Stage 3: Alignment and feature mapping

Align each sequence to its best-matching model (Infernal's cmalign)

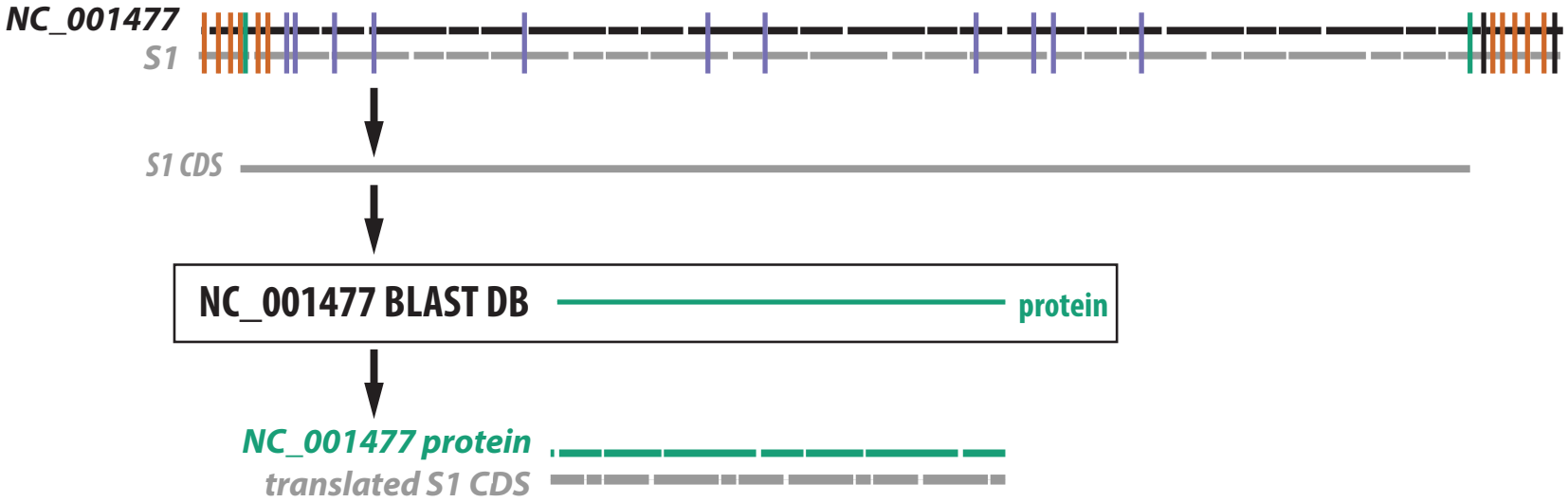
gap or low confidence at feature boundary
(indf5gap, indf3gap, indf5loc, indf3loc alert)

early stop codon in CDS
(cdsstopn alert)

code	S/F	error message	description
Fatal alerts detected in the annotation stage			
unexdivg*	S	UNEXPECTED_DIVERGENCE	sequence is too divergent to confidently assign nucleotide-based annotation
noftrann*	S	NO_FEATURES_ANNOTATED	sequence similarity to homology model does not overlap with any features
mutstart	F	MUTATION_AT_START	expected start codon could not be identified
mutendcd	F	MUTATION_AT_END	expected stop codon could not be identified, predicted CDS stop by homology is invalid
mutendns	F	MUTATION_AT_END	expected stop codon could not be identified, no in-frame stop codon exists 3' of predicted valid start codon
mutendex	F	MUTATION_AT_END	expected stop codon could not be identified, first in-frame stop codon exists 3' of predicted stop position
unexleng	F	UNEXPECTED_LENGTH	length of complete coding (CDS or mat_peptide) feature is not a multiple of 3
cdsstopn	F	CDS_HAS_STOP_CODON	in-frame stop codon exists 5' of stop position predicted by homology to reference
peptrans	F	PEPTIDE_TRANSLATION_PROBLEM	mat_peptide may not be translated because its parent CDS has a problem
pepadjcy	F	PEPTIDE_ADJACENCY_PROBLEM	predictions of two mat_peptides expected to be adjacent are not adjacent
indfantn	F	INDEFINITE_ANNOTATION	nucleotide-based search identifies CDS not identified in protein-based search
indf5gap	F	INDEFINITE_ANNOTATION_START	alignment to homology model is a gap at 5' boundary
indf5loc	F	INDEFINITE_ANNOTATION_START	alignment to homology model has low confidence at 5' boundary
indf3gap	F	INDEFINITE_ANNOTATION_END	alignment to homology model is a gap at 3' boundary
indf3loc	F	INDEFINITE_ANNOTATION_END	alignment to homology model has low confidence at 3' boundary
lowsim5f	F	LOW_FEATURE_SIMILARITY_START	region within annotated feature at 5' end of sequence lacks significant similarity
lowsim3f	F	LOW_FEATURE_SIMILARITY_END	region within annotated feature at 3' end of sequence lacks significant similarity
lowsimif	F	LOW_FEATURE_SIMILARITY	region within annotated feature lacks significant similarity

Stage 4: Protein validation (Alejandro Schäffer)

Compare each predicted CDS to model (RefSeq) proteins with BLASTX



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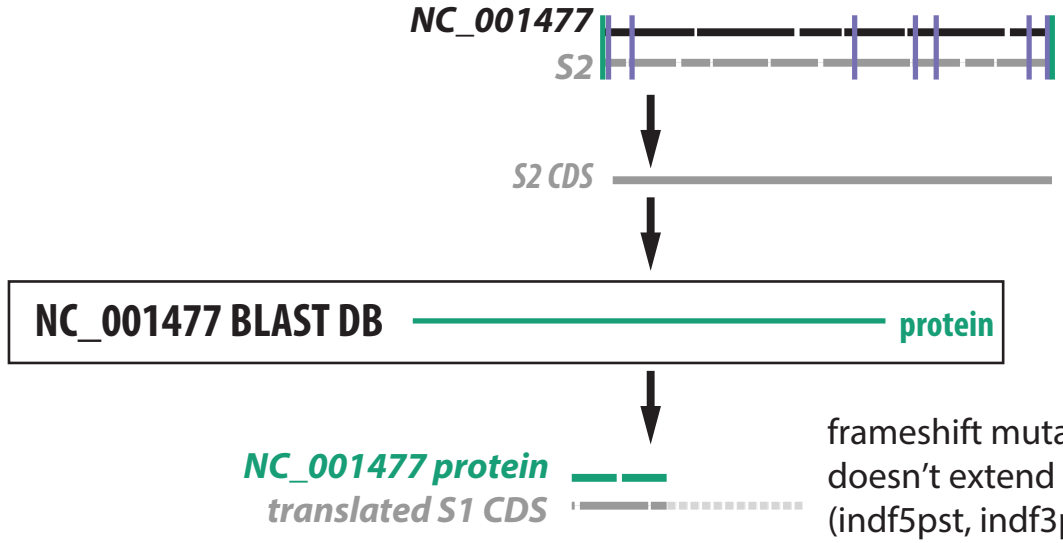
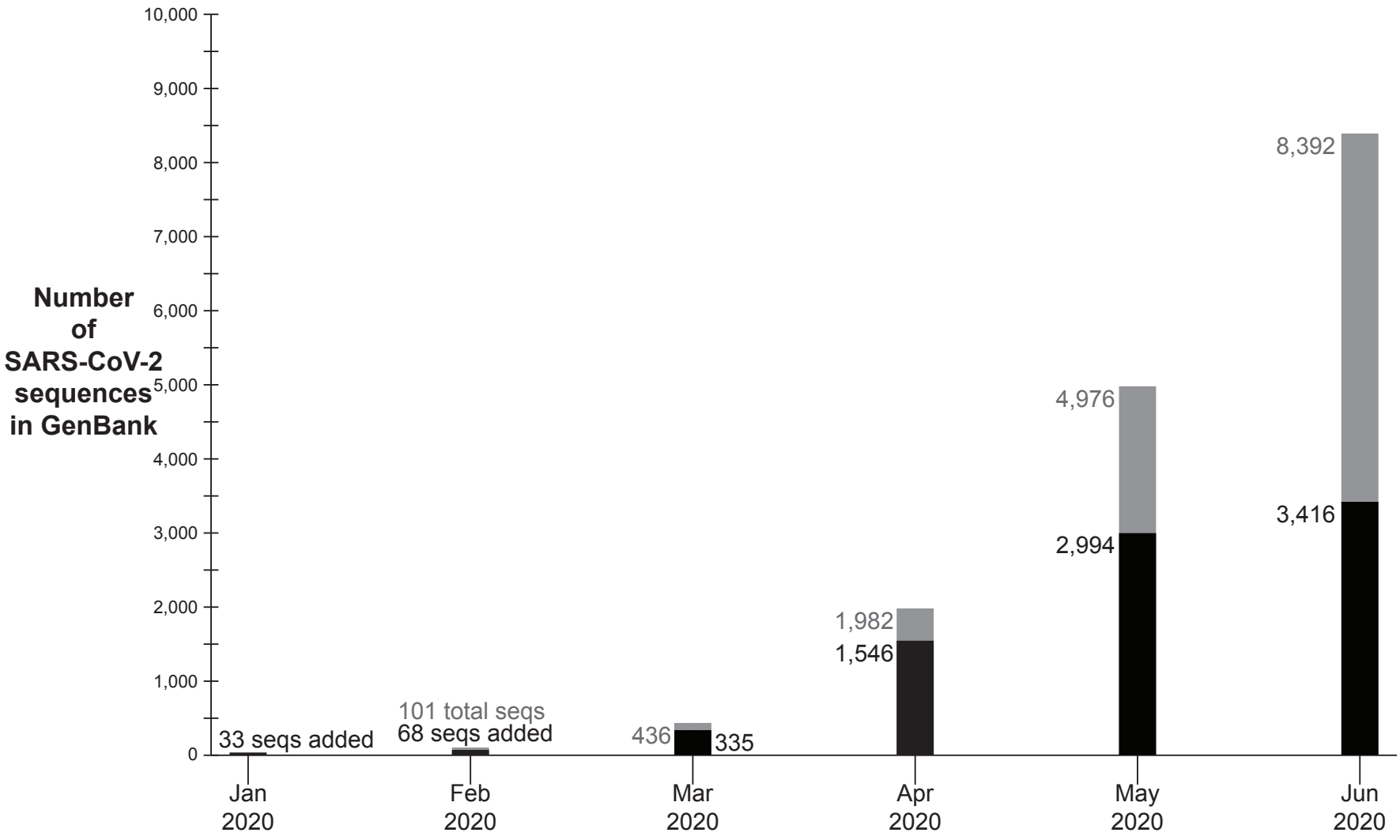


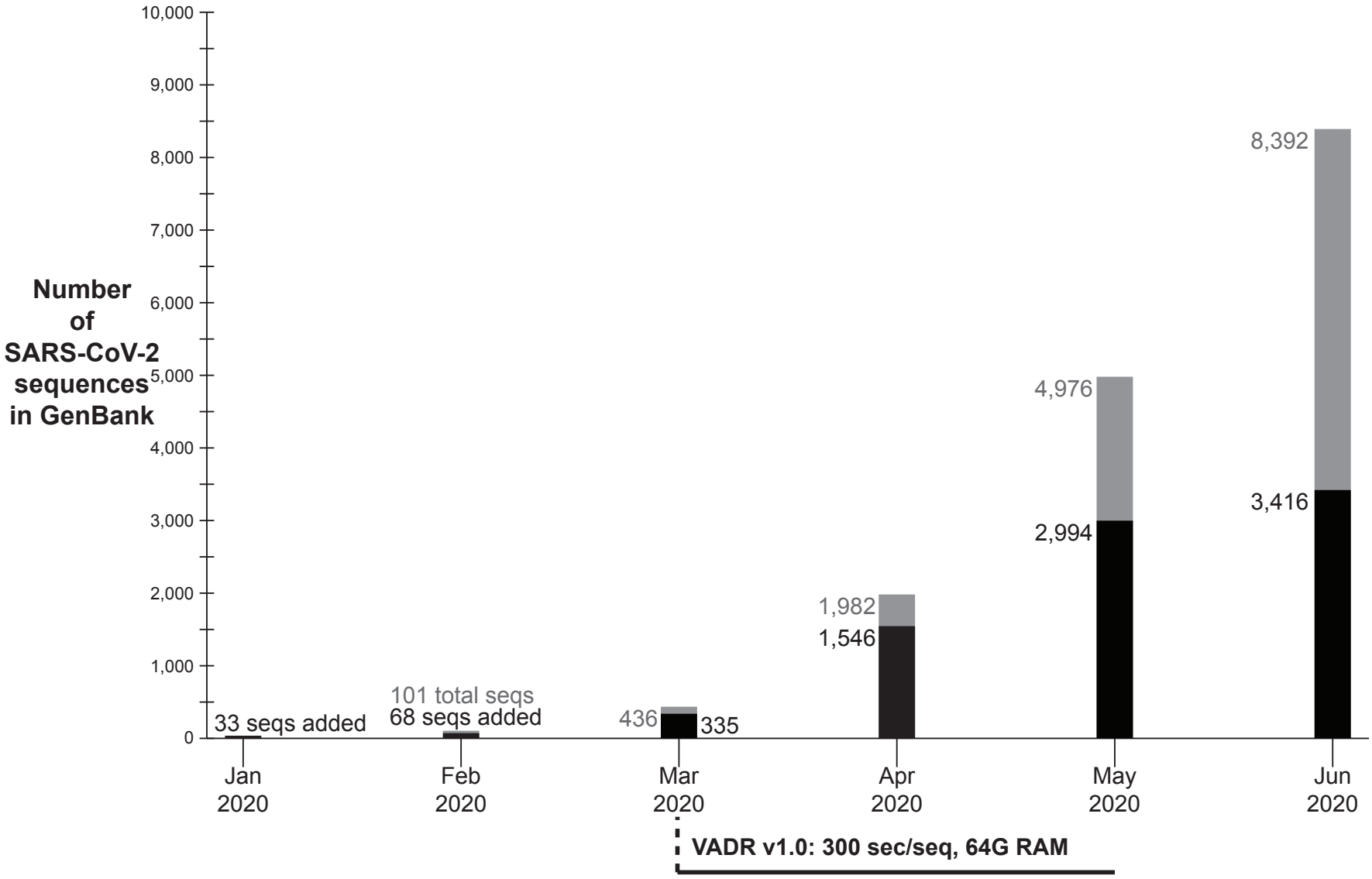
frameshift mutation; BLASTX alignment doesn't extend full length of CDS prediction (indf5pst, indf3pst alerts)

code	S/F	error message	description
Fatal alerts detected in the protein validation stage			
cdsstopp	F	CDS_HAS_STOP_CODON	stop codon in protein-based alignment
indfantp	F	INDEFINITE_ANNOTATION	protein-based search identifies CDS not identified in nucleotide-based search
indf5plg	F	INDEFINITE_ANNOTATION_START	protein-based alignment extends past nucleotide-based alignment at 5' end
indf5pst	F	INDEFINITE_ANNOTATION_START	protein-based alignment does not extend close enough to nucleotide -based alignment 5' endpoint
indf3plg	F	INDEFINITE_ANNOTATION_END	protein-based alignment extends past nucleotide-based alignment at 3' end
indf3pst	F	INDEFINITE_ANNOTATION_END	protein-based alignment does not extend close enough to nucleotide -based alignment 3' endpoint
indfstrp	F	INDEFINITE_STRAND	strand mismatch between protein-based and nucleotide-based predictions
insertnp	F	INSERTION_OF_NT	too large of an insertion in protein-based alignment
deletinp	F	DELETION_OF_NT	too large of a deletion in protein-based alignment

SARS-CoV-2 sequences in GenBank: Jan 2020 to June 2020



VADR 1.0: functional but slow



SARS-CoV-2 sequences have a lot of ambiguous nucleotides (Ns)

virus	% of nucleotides that are Ns	% of seqs w/stretch of Ns >= 50 nt
Dengue virus	0.0037%	0.0070%
Norovirus	0.296%	0.628%
SARS-CoV-2	1.12%	*26.4%*

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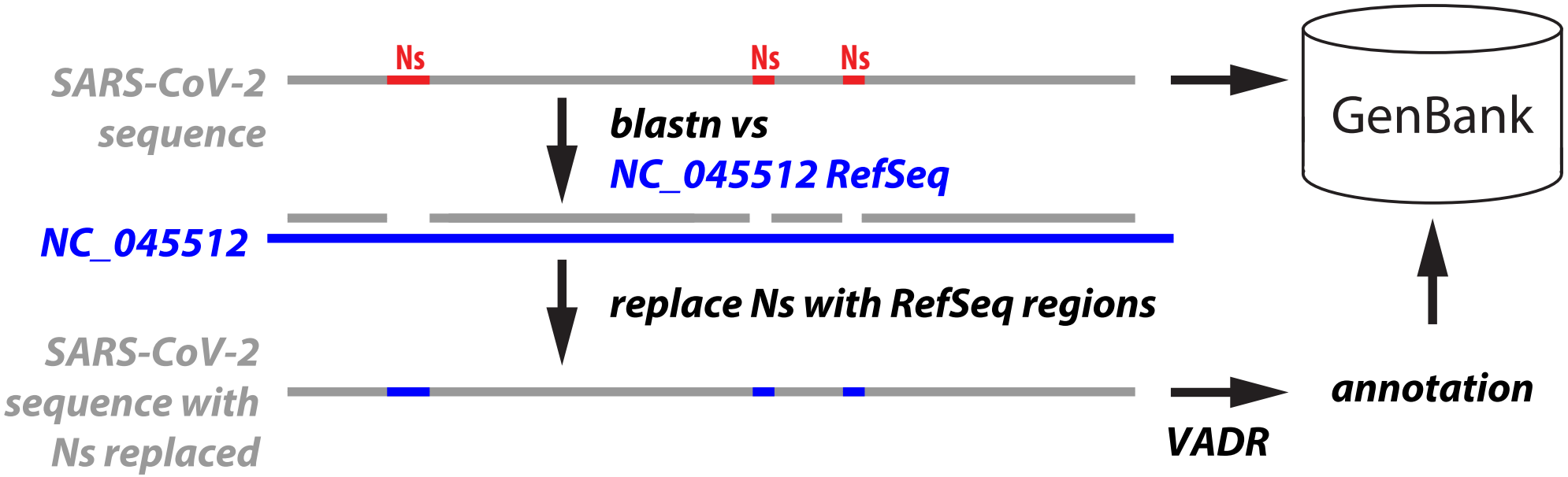
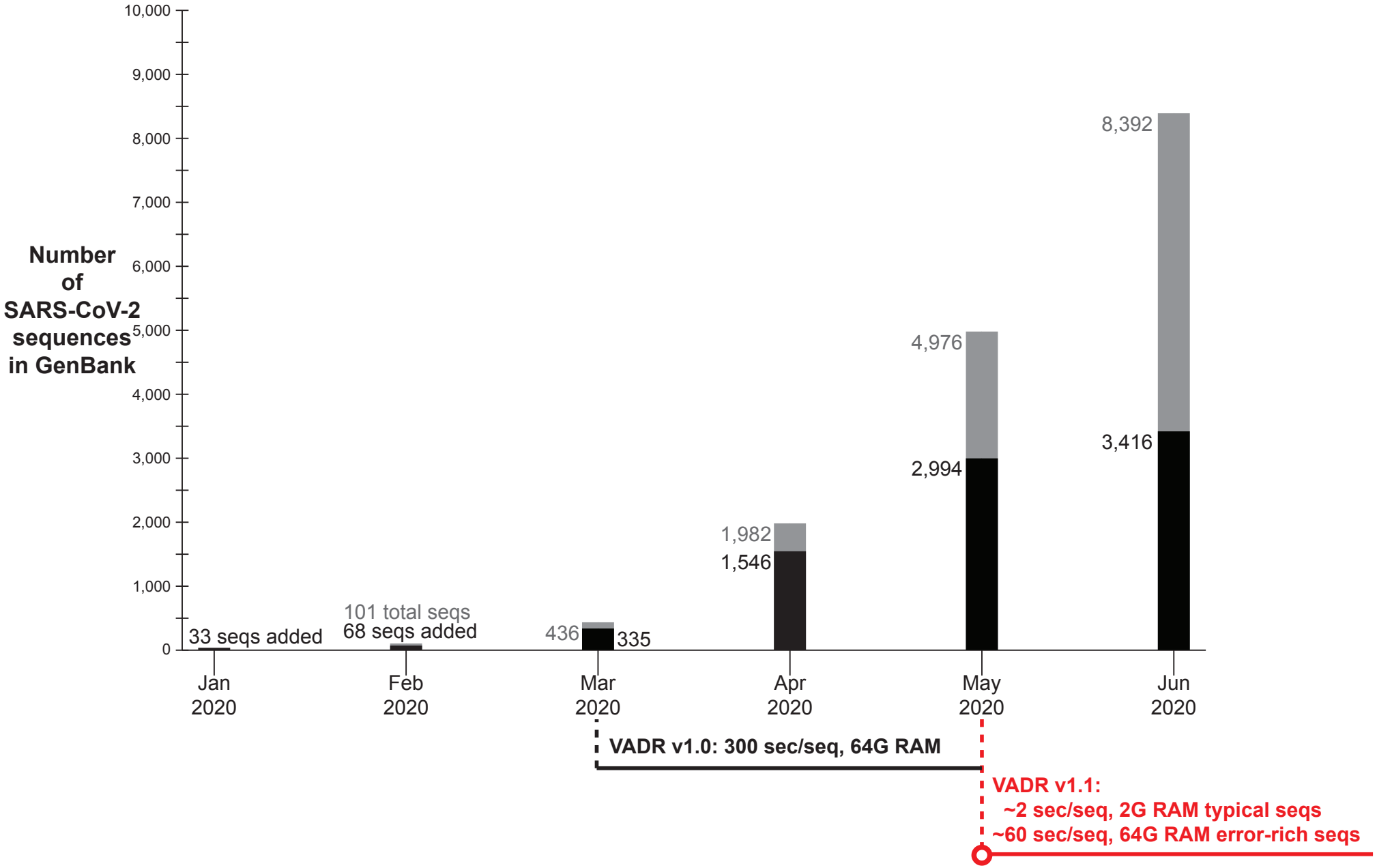


VADR 1.1 exploits high similarity (typically $> 99.5\%$) of SARS-CoV-2 sequences to the RefSeq

- blastn replaces hmmer3 in classification and coverage determination stages

- max ungapped blastn alignment region *seeds* the cmalign alignment

VADR 1.1: 150X speedup on typical sequences



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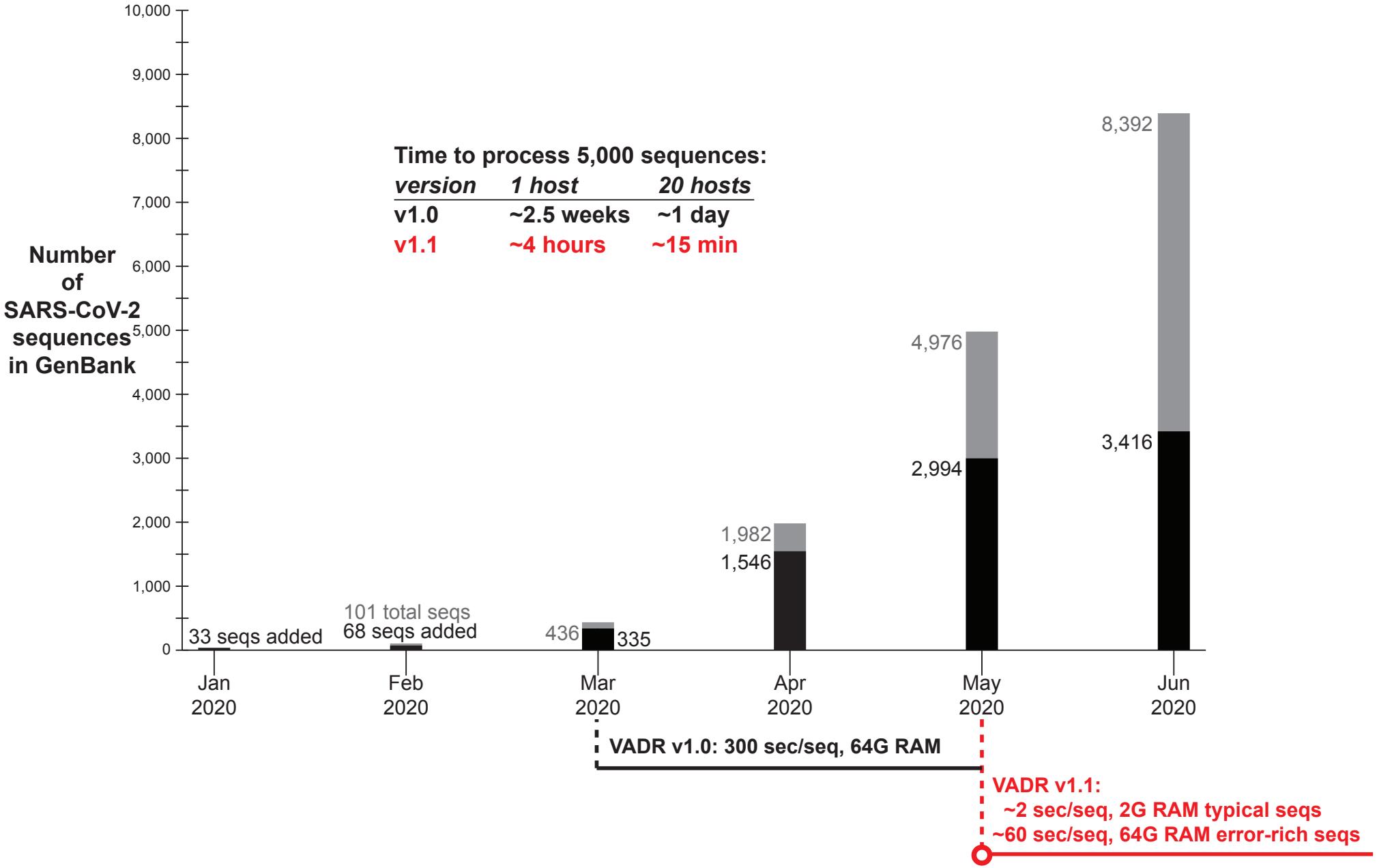
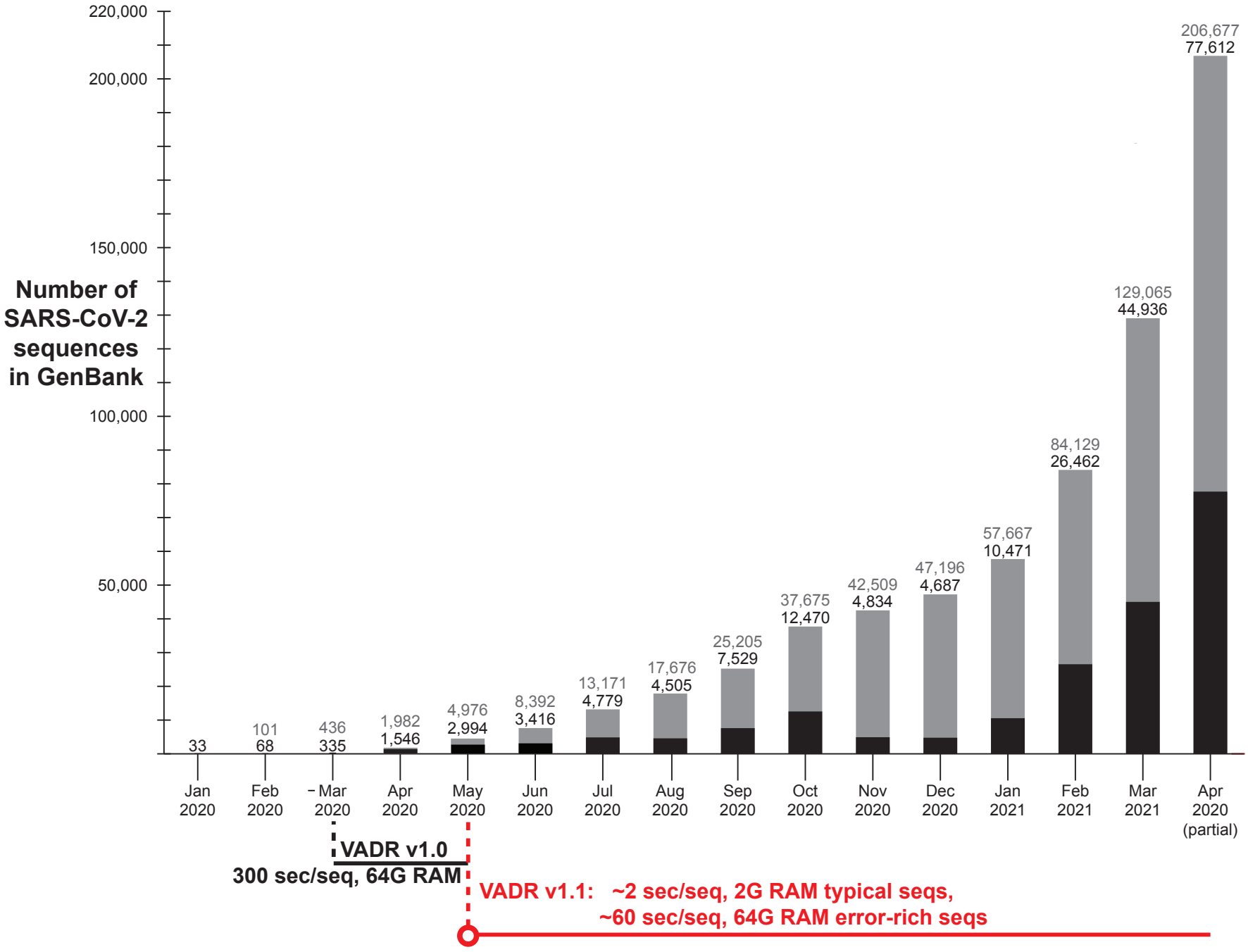
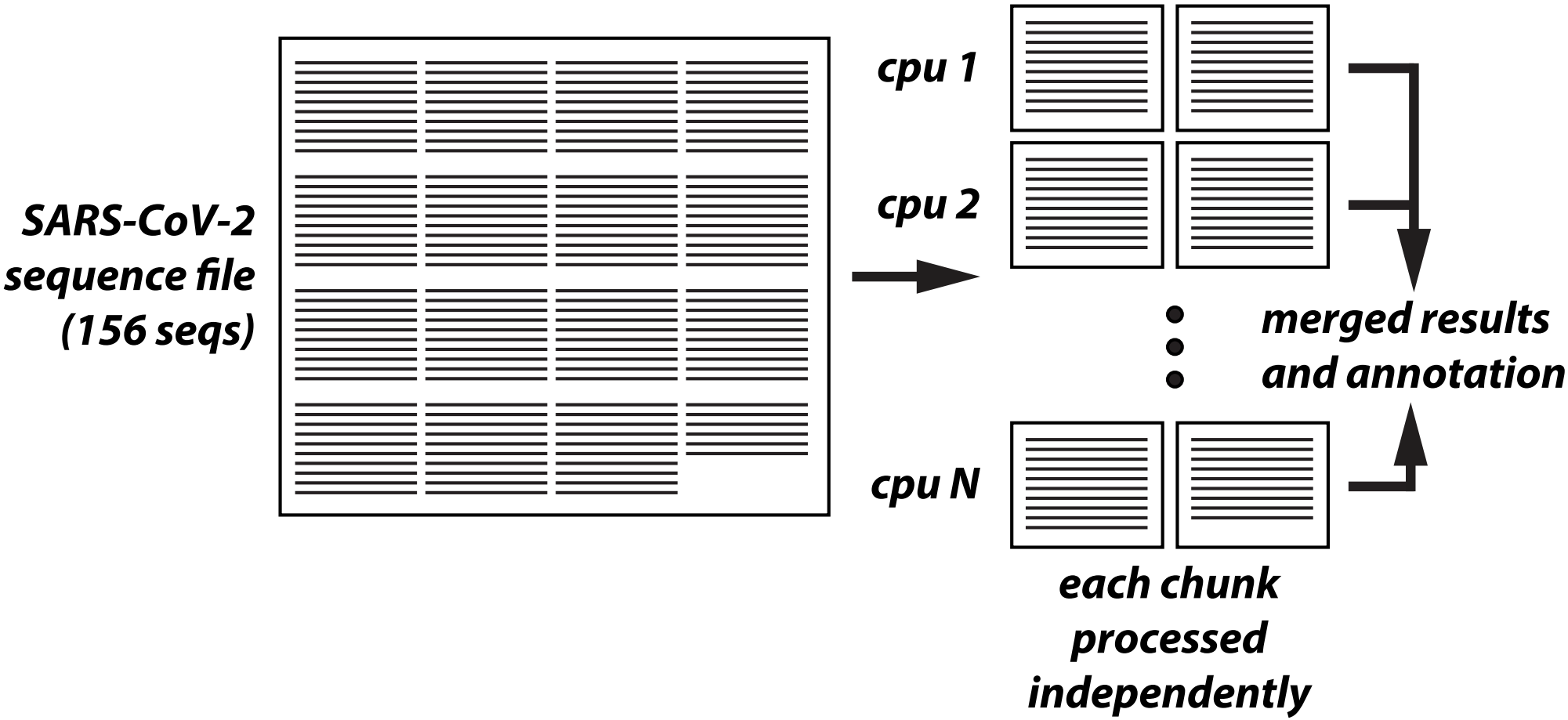


Sequence volume increased dramatically in 2021

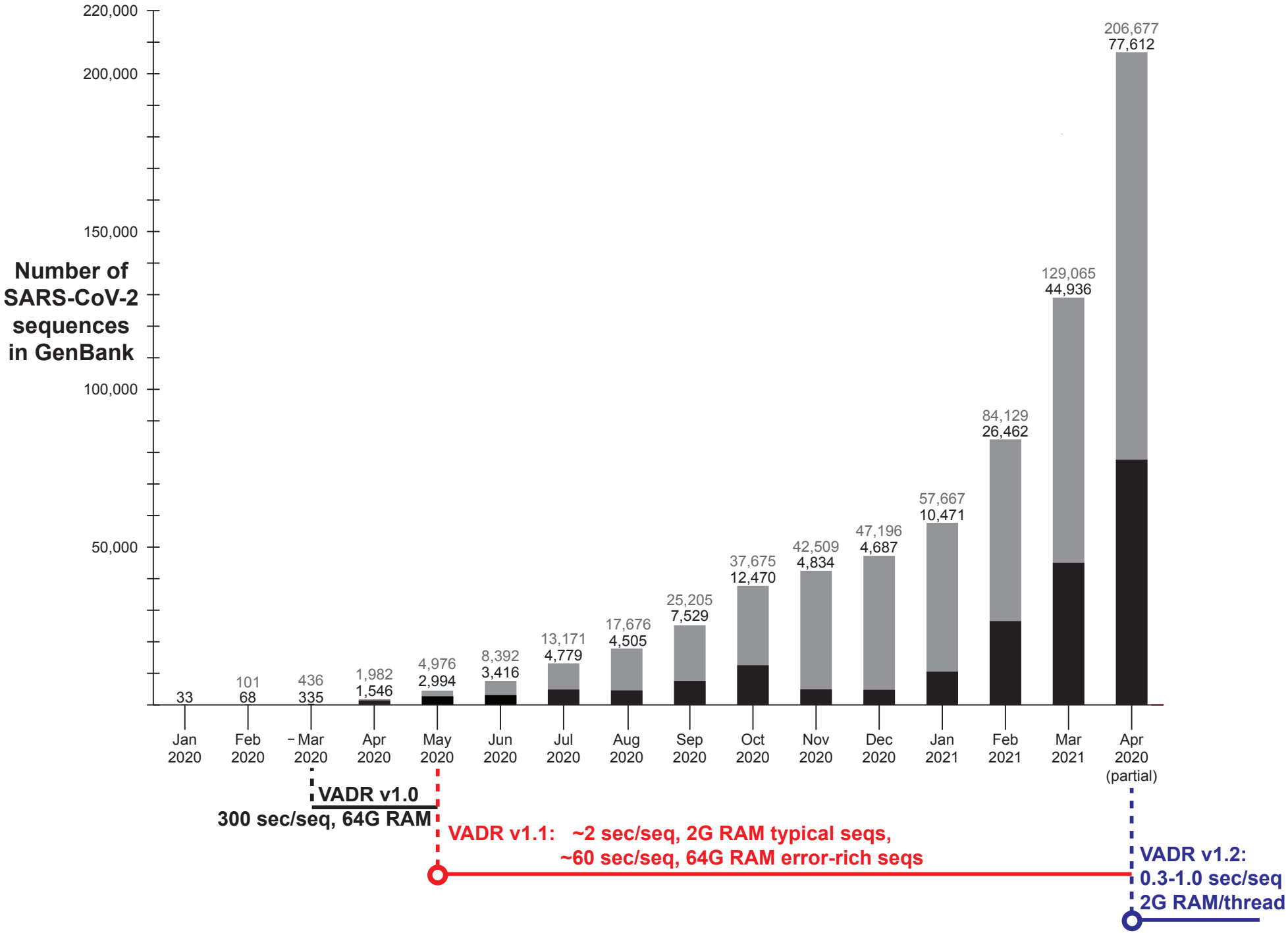


Speed and memory bottleneck in VADR 1.1 is cmalign

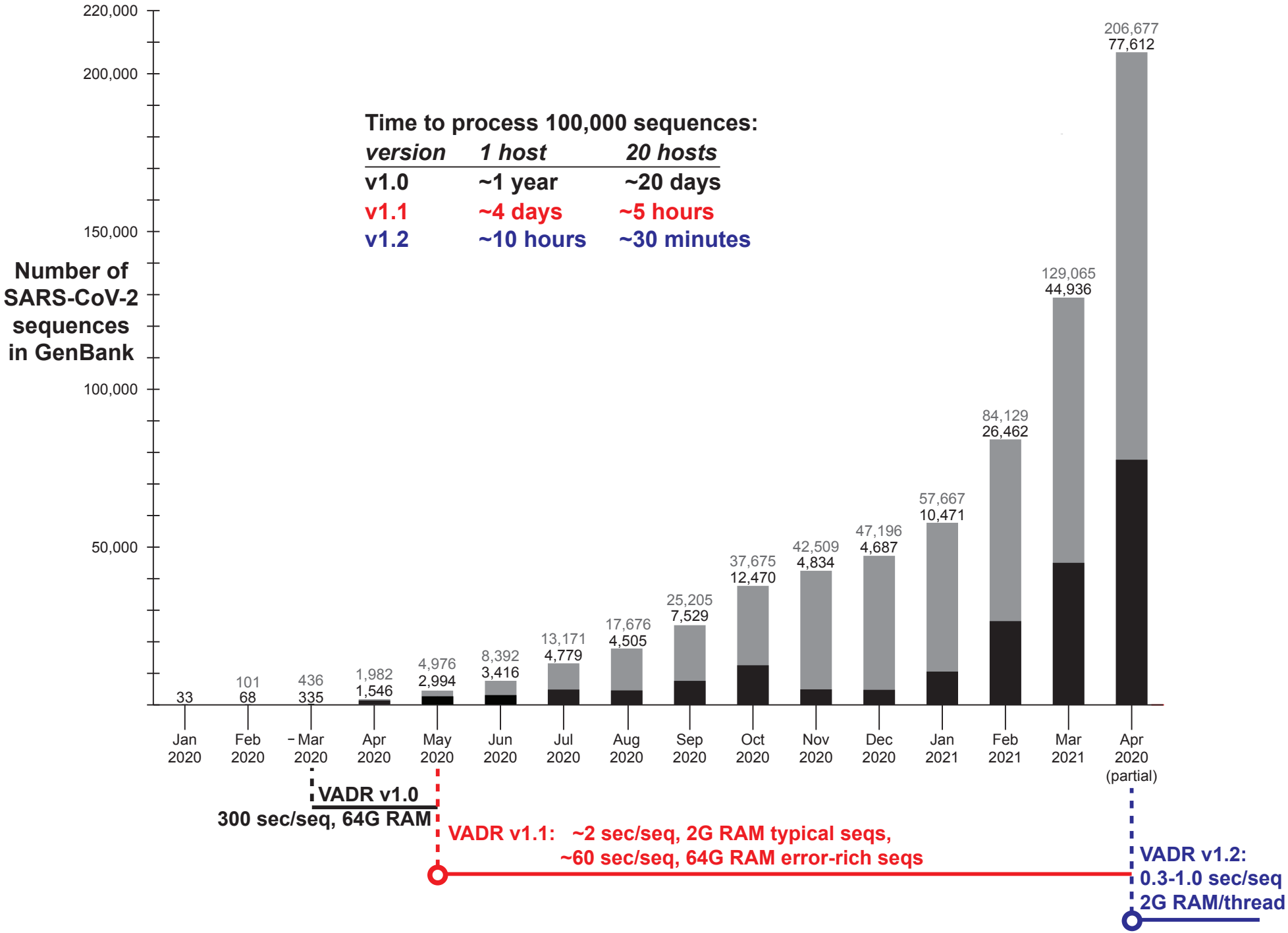
- VADR 1.2 replaces cmalign with glsearch ('glocal' alignment)
  - lower memory requirement (2G max) opens door for multi-threading



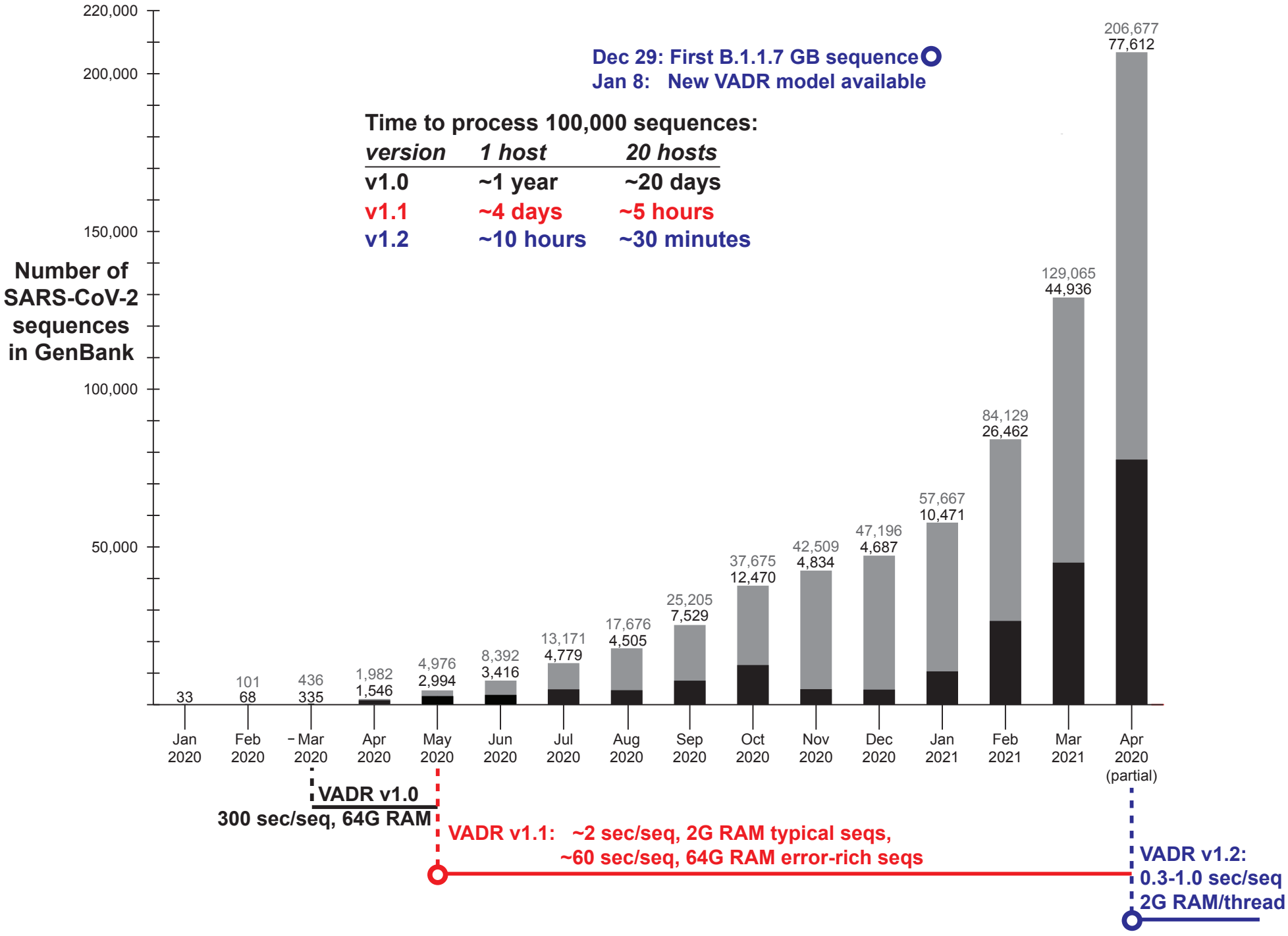
VADR v1.2 is about 10X faster than v1.1



GenBank is now better prepared for large sequence submissions



Besides getting faster, VADR has improved in other ways



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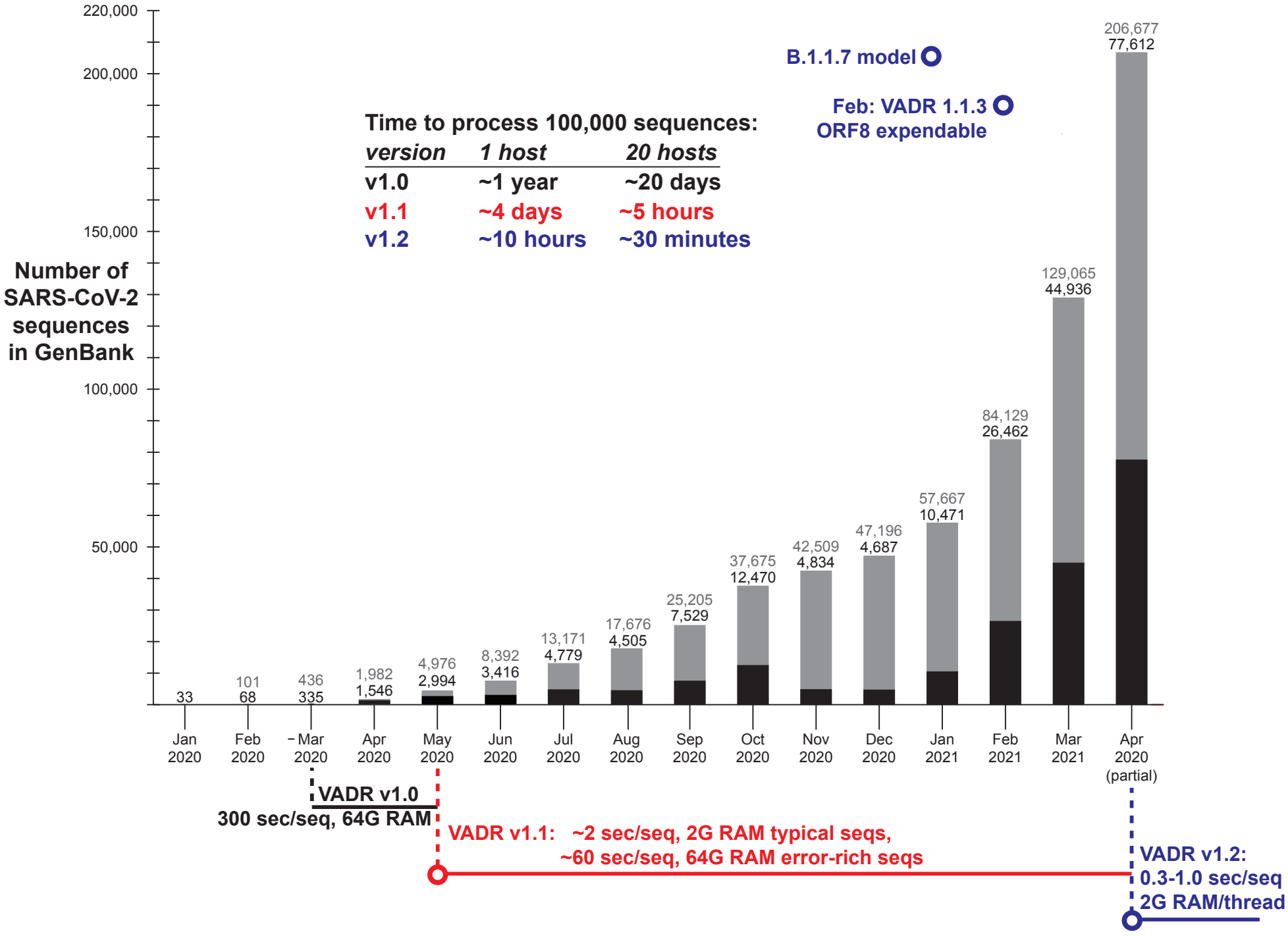

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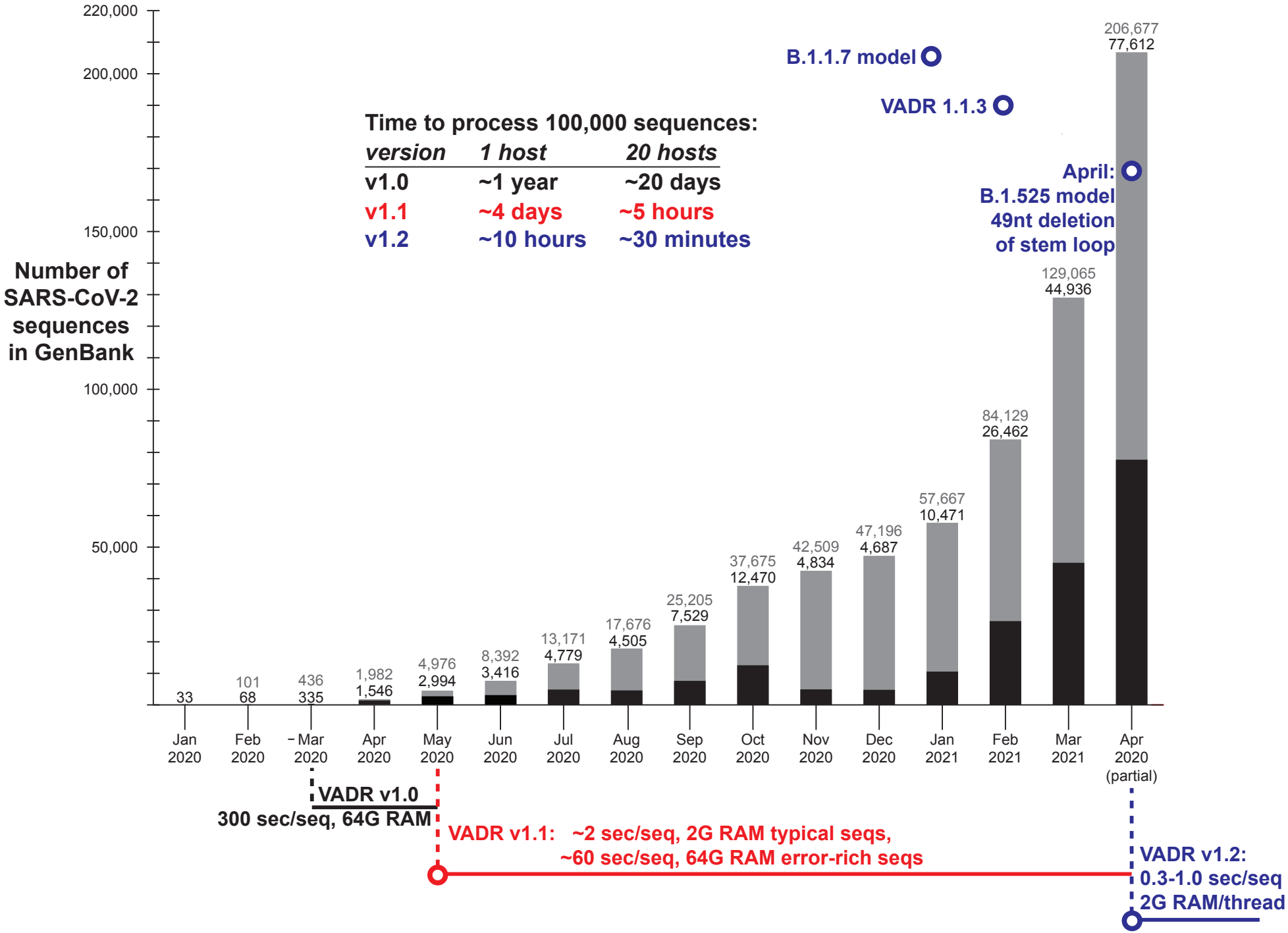
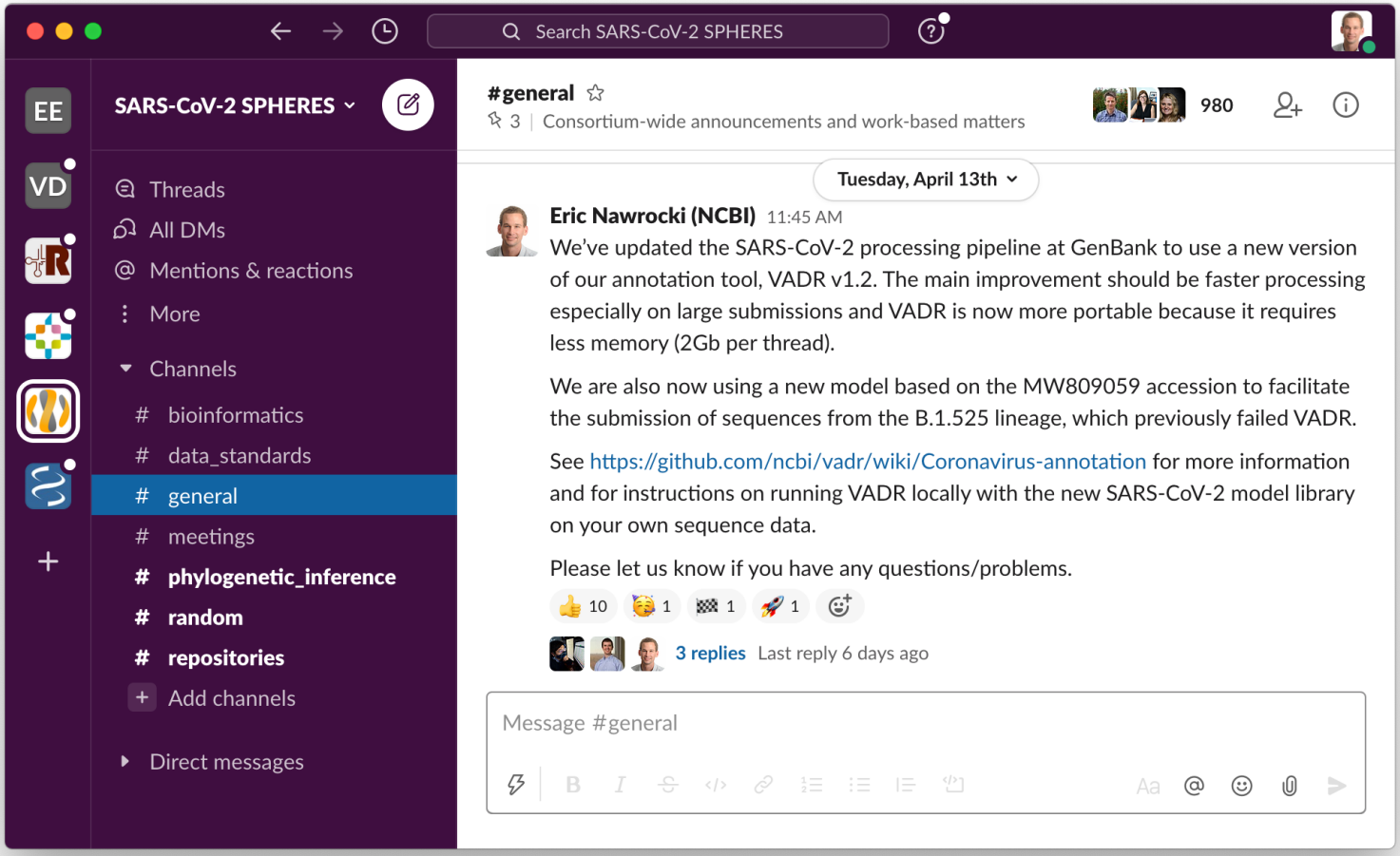


We actively support (and are helped by) the SPHERES community



- VADR is portable and is run locally by labs on their sequences prior to submission
- SPHERES/CDC alert us of problems with VADR and model coverage

Future improvements: VADR 1.2.1 TODO list

- Review list of seqs that fail VADR but should pass
  - make changes to code and/or models to accommodate them, if possible
- Review VADR error messages, and add parseable position data (SPHERES)
- Update handling of non NNN start/stop codons that translate to X (CDC)

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Kim Pruitt
Steve Sherry
Jim Ostell
David Lipman

NLM - leadership
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Jerry Sheehan
Valerie Florance

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Travis Wheeler (HMMER)
Tom Madden and BLAST team
William Pearson (FASTA/glsearch)
Michael Farrar (HMMER/glsearch)

NIH NLM

NCBI National Center for Biotechnology Information