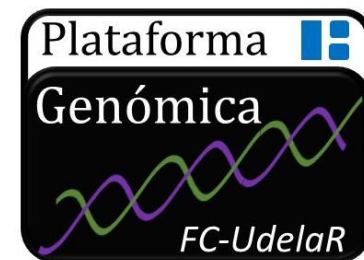
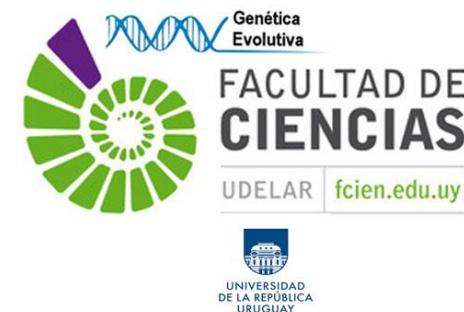




VIRUS CANINOS

DESARROLLOS METODOLÓGICOS PARA LA CARACTERIZACIÓN GENÓMICA

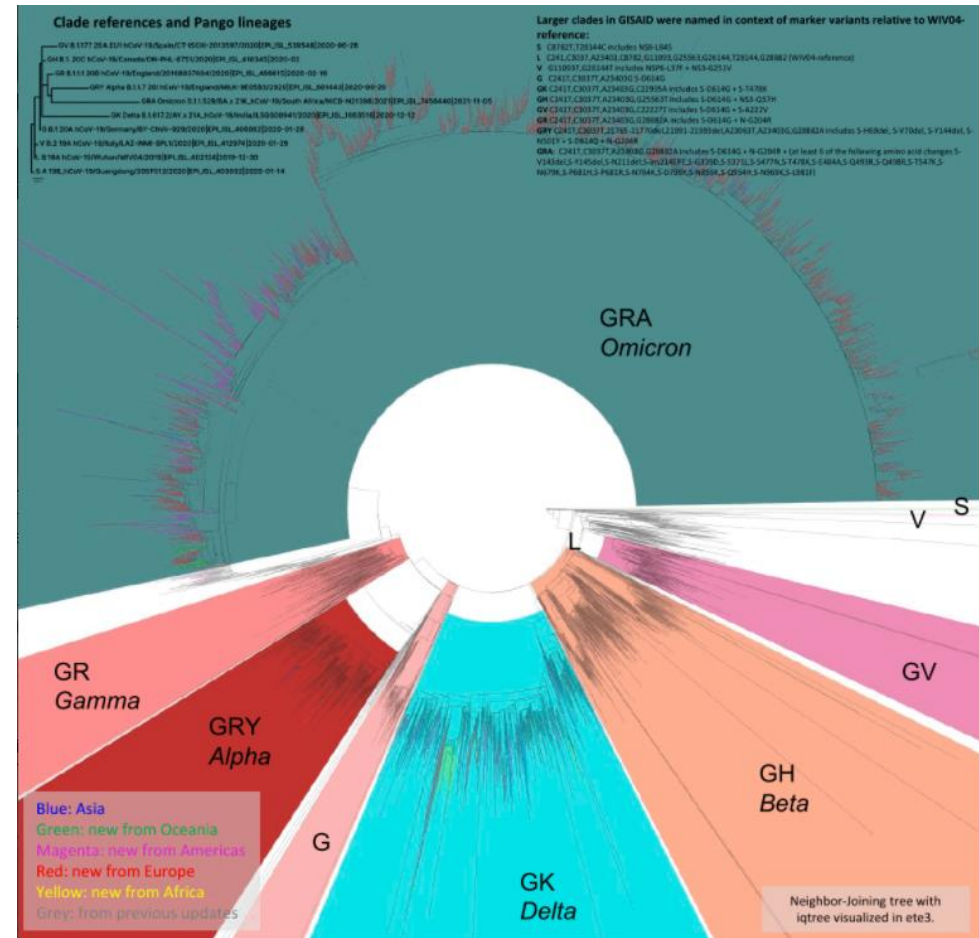


Dra. Yanina Panzera
ypanzera@fcien.edu.uy

Secuenciación masiva



Caracterización Genómica de SARS-CoV-2 en tiempo real



Sampled genome tree derived from all outbreak sequences
2022-03-29

Notable changes

8,866,293 full genomes (+72,016)
(excluding low coverage, out of 9,673,063 entries)

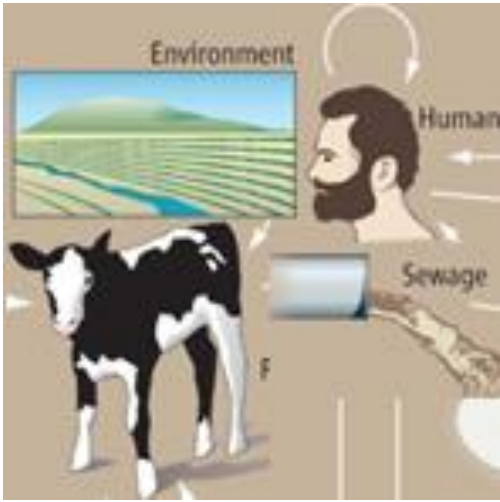
Updated clades
RBDx: relevant changes near receptor and antibody binding sites

- S clade [#RBDx] 15,947 [6,092] (+5 [+0])
- L clade [#RBDx] 5,957 [143] (+0 [+0])
- V clade [#RBDx] 6,675 [41] (+3 [+0])
- G clade [#RBDx] 281,585 [117,951] (+126 [+65])
- GR clade [#RBDx] 467,160 [242,421] (+148 [+117])
- GRY clade [#RBDx] 1,027,508 [112,764] (+110 [+22])
- GH clade [#RBDx] 489,403 [226,906] (+229 [+149])
- GV clade [#RBDx] 171,504 [9,632] (+1 [+1])
- GK clade [#RBDx] 3,994,737 [626,942] (+4,074 [+957])
- GRA clade 2,362,749 (+67,242)
- Other clade [#RBDx] 43,068 [29,646] (+78 [+75])

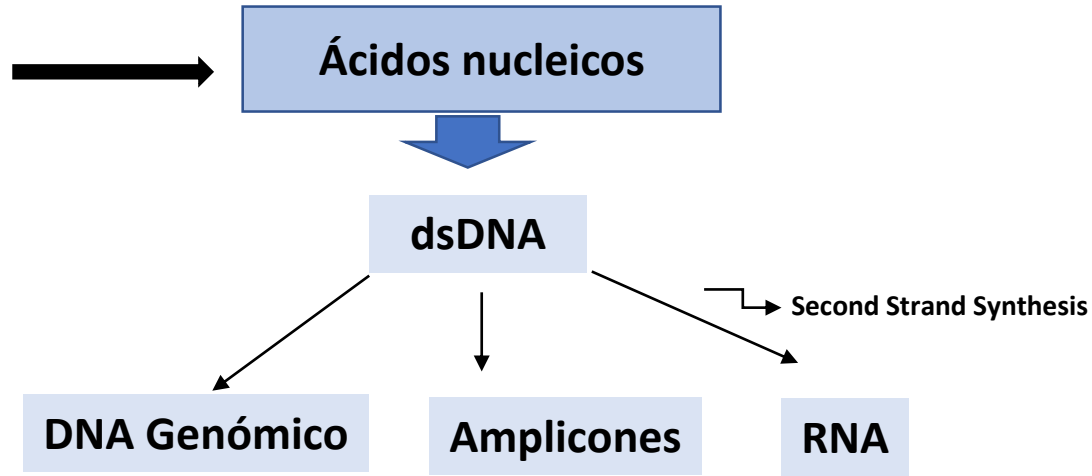
We gratefully acknowledge the Authors from Originating and Submitting laboratories of sequence data on which the analysis is based.



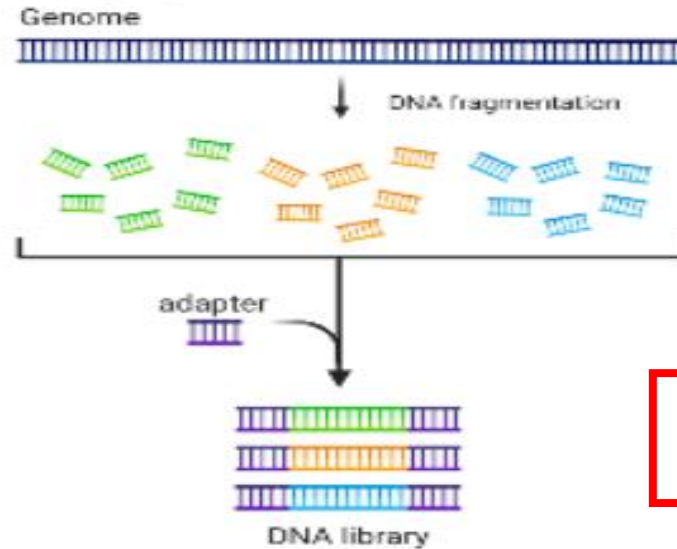
Secuenciación masiva



SCOTT A. MCEWEN and PETER J. COLLIGNON. 2018



1 Library preparation

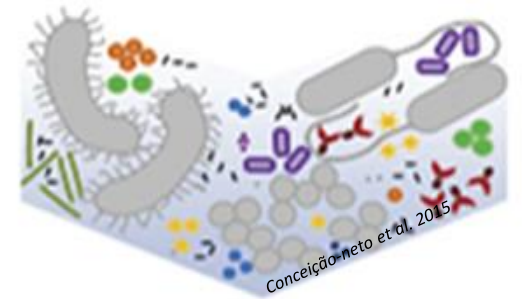


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SECUENCIACIÓN MASIVA APLICADA A VIRUS

PROBLEMA

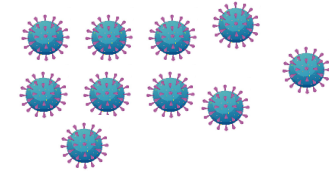
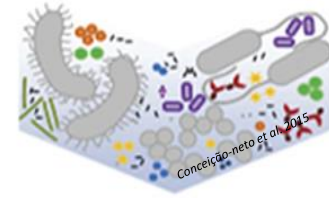
Virus con genomas pequeños infectando células con tamaños genómicos grandes



SOLUCIÓN

- **Secuenciar más**
- **Incrementar la proporción viral**

Incrementar la proporción viral ENRIQUECIMIENTO



- **Cultivos**
- **PCR**
- **Sondas de captura**
- **Aislamiento de partículas virales**

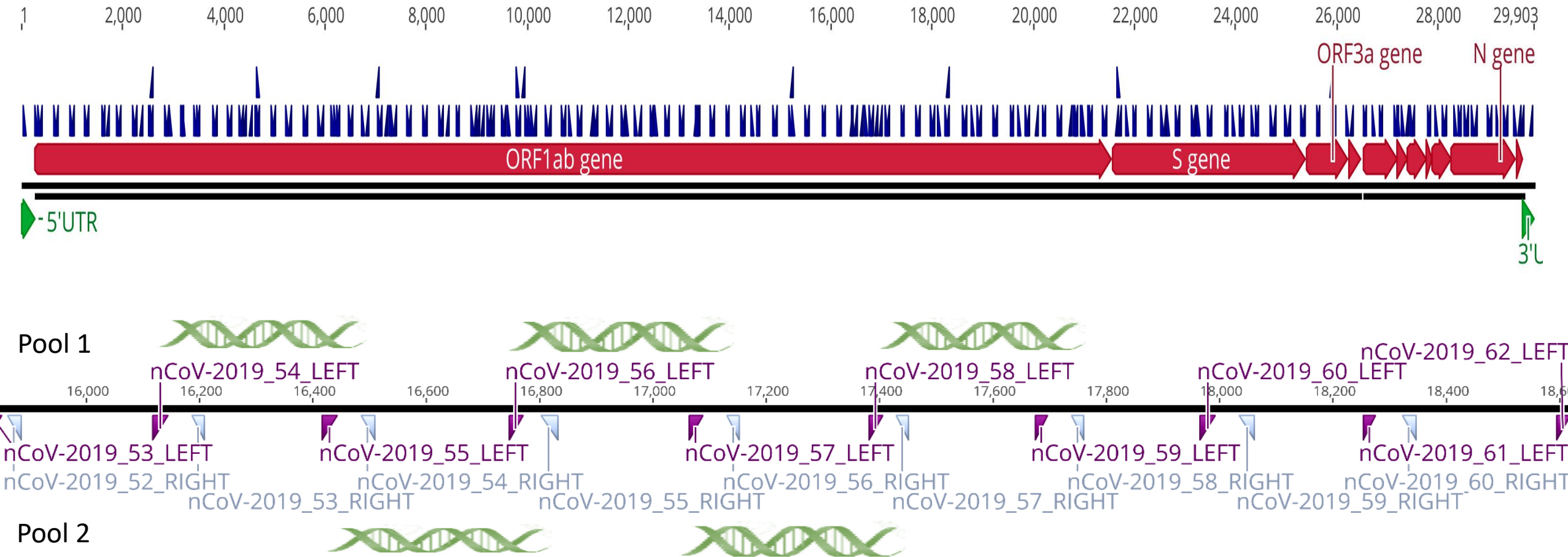
SARS-CoV-2

ARTIC (<https://artic.network/ncov-2019>)

Enrichment of viral sequences by PCR (ARTIC)

NGS of all the sequences

Ultrplex-PCR



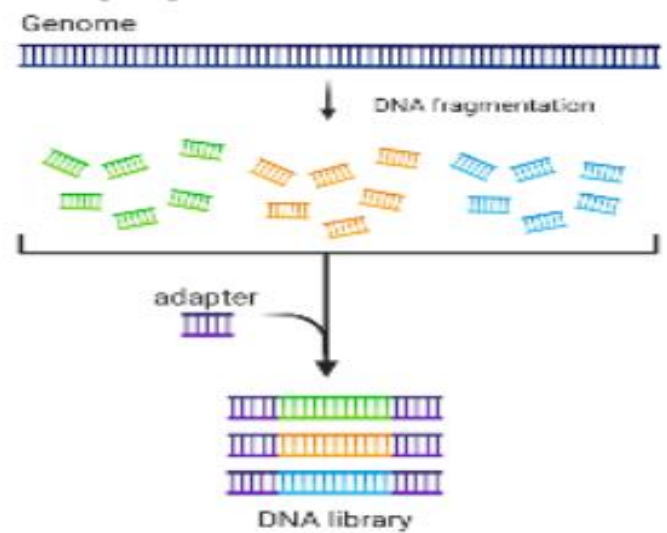
ORF1ab gene

S gene

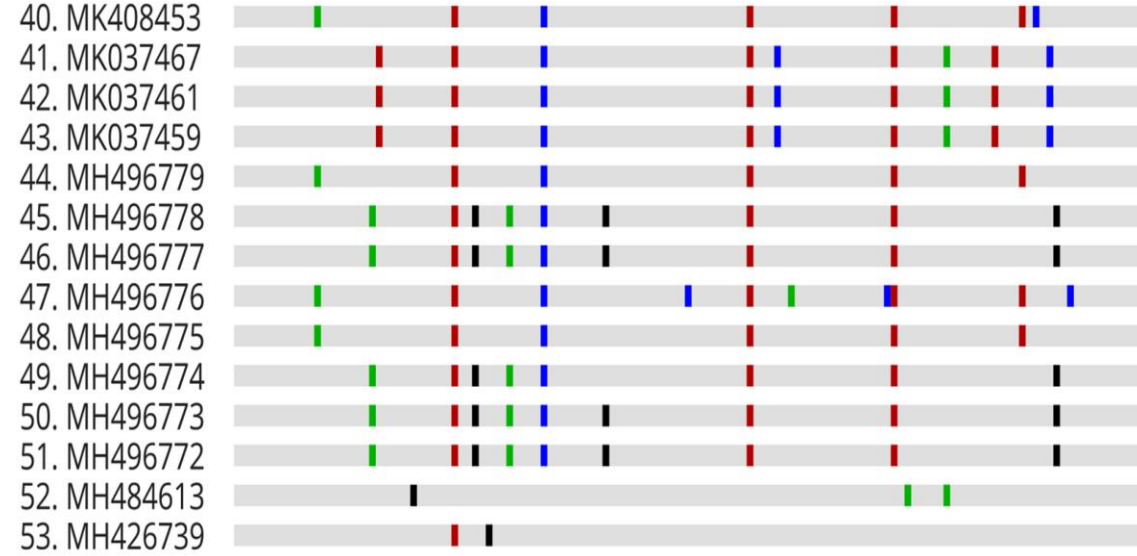
-5'UTR



① Library preparation



¿En qué virus podría aplicar una estrategia de enriquecimiento por PCR?



TTTTTAAATGTGACAACGTCCAACCTAAATGGAAAGGATGTTTCGCTGGA^CAACTATACCAAACCAATTCAAA
 TTTTTAAATGTGACAACGTCCAACCTAAATGGAAAGGATGTTTCGCTGGA^CAACTATACCAAACCAATTCAAA
 TTTTTAAATGTGACAACGTCCAACCTAAATGGAAAGGATGTTTCGCTGGA^CAACTATACCAAACCAATTCAAA
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VIRUS CANINOS

Parvovirosis

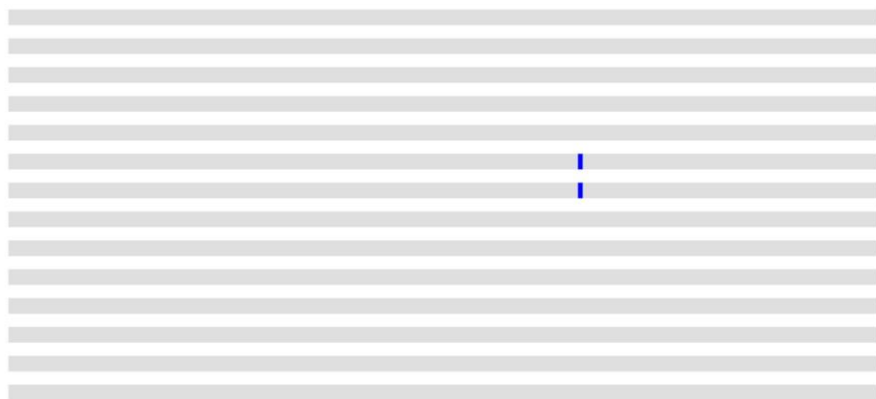
Carnivore

protoparvovirus 1

-ssDNA 5200 nt



- 14. CPV2-b_US_1979
- 15. CPV2-6_US_1980
- 16. CPV2b-411a_US_1998
- 17. CPV2b-410_US_2000
- 18. CPV2b-193_US_1991
- 19. CPV2b-395_US_1998
- 20. CPV2b-411b_US_1998
- 21. CPV2a-15_US_1984
- 22. CPV2a-31_US_1983
- 23. CPV2a-13_US_1981
- 24. CPV2a-339_NZ_1994
- 25. CPV2a-nj01_AS_2006
- 26. CPV2a-B_AS_2004
- 27. CPV2a-SC02_AS_2011



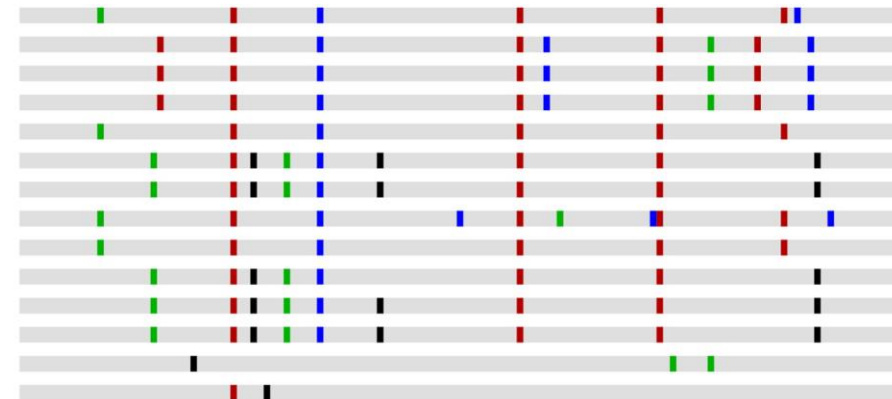
Distemper

Canine morbillivirus

-ssRNA – 15690 nt



- 40. MK408453
- 41. MK037467
- 42. MK037461
- 43. MK037459
- 44. MH496779
- 45. MH496778
- 46. MH496777
- 47. MH496776
- 48. MH496775
- 49. MH496774
- 50. MH496773
- 51. MH496772
- 52. MH484613
- 53. MH426739



DetECCIÓN Y CARACTERIZACIÓN GENÉTICA



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Veterinary Microbiology xxx (2007) xxx-xxx

**veterinary
microbiology**

www.elsevier.com/locate/vetmic

Carnivore parvovirus 1

Short communication

First detection of canine parvovirus type 2c in South America

Ruben Pérez*, Lourdes Francia, Valeria Romero, Leticia Maya, Ionacio López, Martín Hernández



Contents lists available at SciVerse ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic



Recent spreading of a divergent canine parvovirus type 2a (CPV-2a) strain in a CPV-2c homogenous population

Ruben Pérez*, Pablo Bianchi, Lucía Calleros, Lourdes Francia, Martín Hernández, Leticia Maya, Yanina Panzera, Katia Sosa, Stephanie Zoller

Sección Genética Evolutiva, Instituto de Biología, Facultad de Ciencias, Universidad de la República, Iguá 4225, 11400 Montevideo, Uruguay

Arch Virol
DOI 10.1007/s00705-012-1591-5

ORIGINAL ARTICLE

Phylodynamics analysis of canine parvovirus in Uruguay: evidence of two successive invasions by different variants

Leticia Maya · Lucía Calleros · Lourdes Francia · Martín Hernández · Gregorio Iraola · Yanina Panzera · Katia Sosa · Ruben Pérez



Virus Evolution, 2018, 4(1): vey011
doi: 10.1093/ve/vey011
Research article

Inter- and intracontinental migrations and local differentiation have shaped the contemporary epidemiological landscape of canine parvovirus in South America

Sofía Grecco,¹ Gregorio Iraola,^{1,2} Nicola Decaro,³ Alice Alfieri,⁴ Amauri Alfieri,⁴ Marina Gallo Calderón,⁵ Ana Paula da Silva,⁴ Daniela Name,^{1,4} Jaime Aldaz,⁶ Lucía Calleros,¹ Ana Marandino,¹ Gonzalo Tomás,¹ Leticia Maya,¹ Lourdes Francia,¹ Yanina Panzera,¹ and Ruben Pérez^{1,*†}

Canine morbillivirus Diagnóstico

Primer diagnóstico molecular y caracterización parcial del gen de la nucleoproteína del Virus Distemper Canino en Uruguay

Sarute, N.; Pérez, R.; Francia, L.; Hernández, M.; Bedó, G.; Bonilla, B.; Guasco, S.; Cardeillac, A.; Panzera, Y.



Contents lists available at SciVerse ScienceDirect

Virus Research

journal homepage: www.elsevier.com/locate/virusres



Short communication

Evidence of two co-circulating genetic lineages of canine distemper virus in South America

Yanina Panzera^{a,*}, Marina Gallo Calderón^b, Nicolás Sarute^a, Soledad Guasco^a, Arianne Cardeillac^a, Braulio Bonilla^a, Martín Hernández^a, Lourdes Francia^a, Gabriela Bedó^a, José La Torre^b, Ruben Pérez^a

OPEN ACCESS Freely available online



The Fusion Protein Signal-Peptide-Coding Region of Canine Distemper Virus: A Useful Tool for Phylogenetic Reconstruction and Lineage Identification

Nicolás Sarute¹, Marina Gallo Calderón², Ruben Pérez¹, José La Torre², Martín Hernández¹, Lourdes Francia¹, Yanina Panzera^{1*}



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Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev



Molecular phylogeography of canine distemper virus: Geographic origin and global spreading ☆

Yanina Panzera^{a,*}, Nicolás Sarute^a, Gregorio Iraola^{a,b}, Martín Hernández^a, Ruben Pérez^a

Caracterización genómica - NGS

PROTOCOLOS DE ENRIQUECIMIENTO

Carnivore protoparvovirus 1

Canine morbillivirus

OPEN ACCESS Freely available online



Phylogenetic and Genome-Wide Deep-Sequencing Analyses of Canine Parvovirus Reveal Co-Infection with Field Variants and Emergence of a Recent Recombinant Strain

Ruben Pérez^{1*}, Lucía Calleros¹, Ana Marandino¹, Nicolás Sarute¹, Gregorio Iraola¹, Sofía Grecco¹, Hervé Blanc², Marco Vignuzzi², Ofer Isakov³, Noam Shomron³, Lucía Carrau¹, Martin Hernández¹, Lourdes Francia¹, Katia Sosa¹, Gonzalo Tomás¹, Yanina Panzera¹

¹ Sección Genética Evolutiva, Instituto de Biología, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay, ² Institut Pasteur, Viral Populations and Pathogenesis Unit, Centre National de la Recherche Scientifique, Paris, France, ³ Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

Abstract

Canine parvovirus (CPV), a fast-evolving single-stranded DNA virus, comprises three antigenic variants (2a, 2b, and 2c) with different frequencies and genetic variability among countries. The contribution of co-infection and recombination to the genetic variability of CPV is far from being fully elucidated. Here we took advantage of a natural CPV population, recently formed by the convergence of divergent CPV-2c and CPV-2a strains, to study co-infection and recombination. Complete sequences of the viral coding region of CPV-2a and CPV-2c strains from 40 samples were generated and analyzed using phylogenetic tools. Two samples showed co-infection and were further analyzed by deep sequencing. The sequence profile of one of the samples revealed the presence of CPV-2c and CPV-2a strains that differed at 29 nucleotides. The other sample included a minor CPV-2a strain (13.3% of the viral population) and a major recombinant strain (86.7%). The recombinant strain arose from inter-genotypic recombination between CPV-2c and CPV-2a strains within the VP1/VP2 gene boundary. Our findings highlight the importance of deep-sequencing analysis to provide a better understanding of CPV molecular diversity.

Citation: Pérez R, Calleros L, Marandino A, Sarute N, Iraola G, et al. (2014) Phylogenetic and Genome-Wide Deep-Sequencing Analyses of Canine Parvovirus Reveal Co-Infection with Field Variants and Emergence of a Recent Recombinant Strain. *PLoS ONE* 9(11): e111779. doi:10.1371/journal.pone.0111779

Editor: Edward Ayuso, University of Nantes, France

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Data Availability: The authors confirm that all data underlying the findings are fully available without restriction. All sequence files are available from the GenBank database (accession numbers KM457102-KM457143).

Funding: Grant number: 2206, Comisión Sectorial de Investigación Científica (CSIC) (<http://www.csic.edu.uy/>) to KS. Grant number ANR-10-LABX-62-IBED, French Investissement d'Avenir program, Laboratoire d'Excellence Integrative Biology of Emerging Infectious Diseases (<http://www.agence-nationale-recherche.fr/investissementsdavenir/>) to MV. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* Email: rperez@fcien.edu.uy



First Genome Sequence of a Canine Distemper Virus Strain from South America

Nicolás Sarute,^a María V. Delgado,^a Lucía Carrau,^a Alejandro Benech,^b Lourdes Francia,^a Ruben Pérez,^a Yanina Panzera^a

^a Centro de Estudios de Virus y Vacunas, Instituto de Biología, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay, ^b Departamento de Microbiología y Parasitología, Facultad de Medicina, Universidad de Chile, Santiago, Chile



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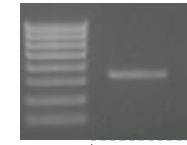
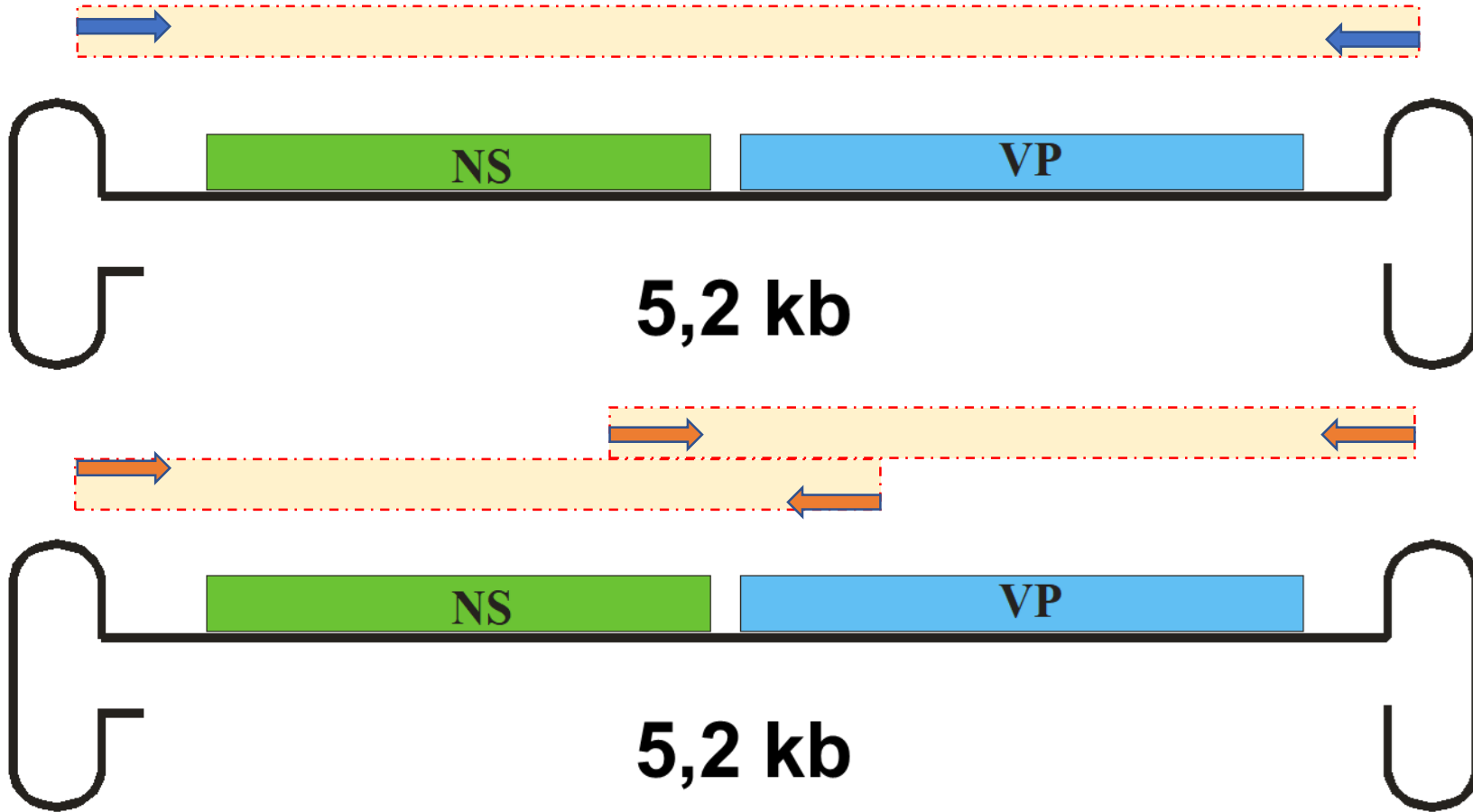
Virus isolation and full-length genome sequencing of a representative canine distemper virus wild type strain of the South America 2 clade

Romanutti Carina^a, Keller Leticia^b, La Torre José^a, Panzera Yanina^c, Fuques Eddie^c, Pérez Ruben^c, Gallo Calderon Marina^{a,*}

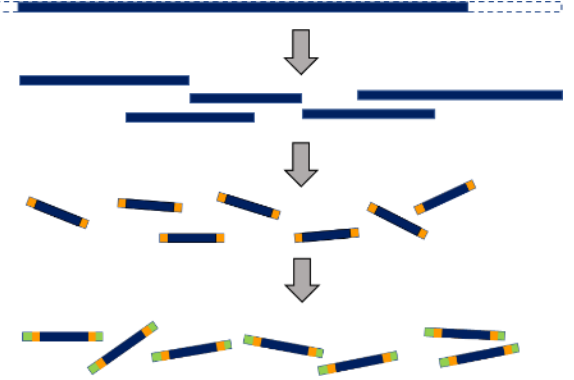


*Carnivore
protoparvovirus 1*

PCR



Amplicones CPV



CO-INFECCIÓN

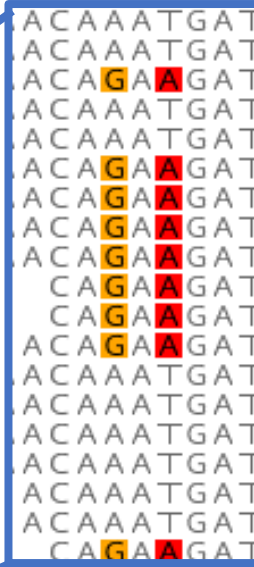
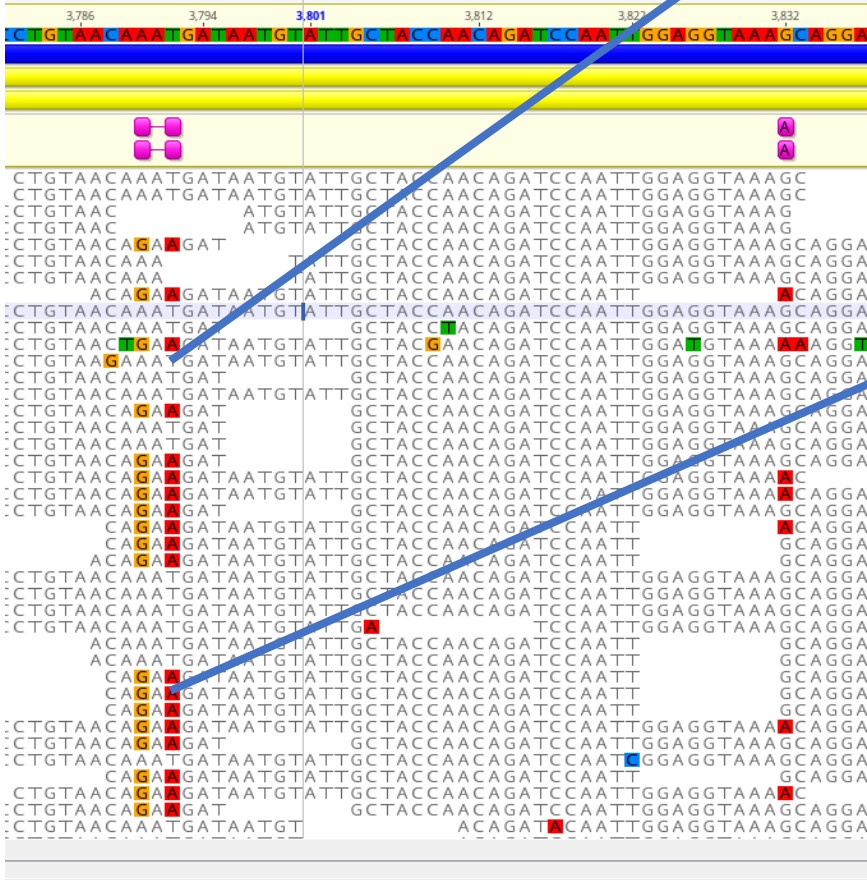
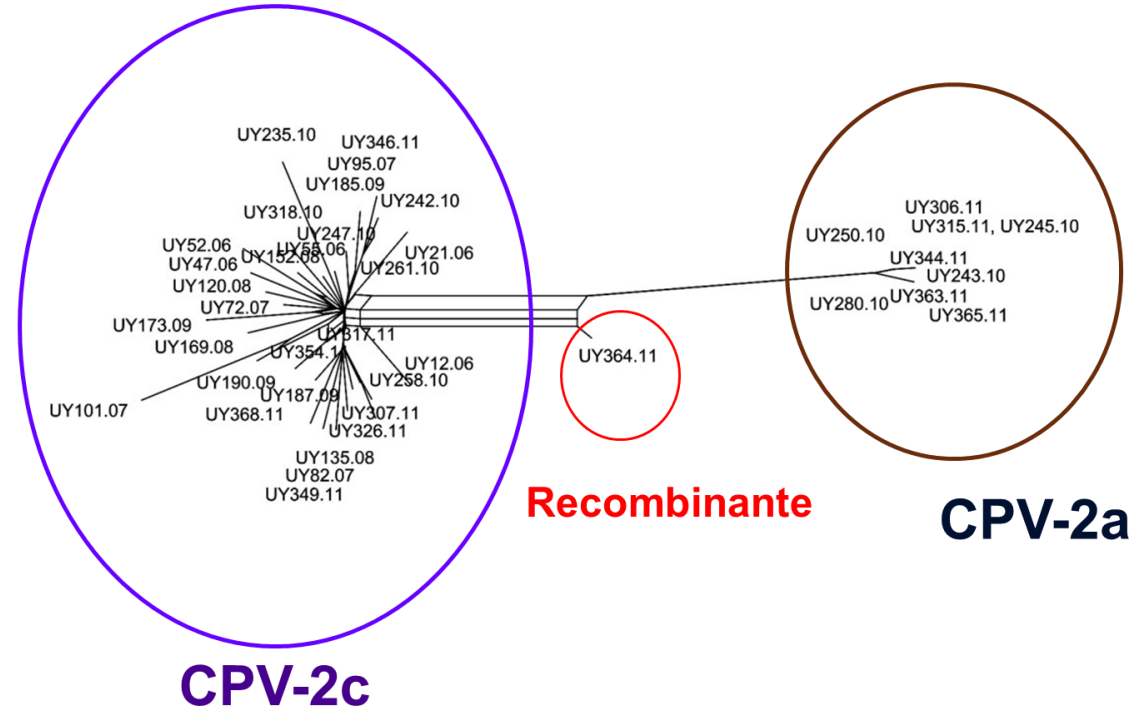


TABLE 3 Nucleotide and amino acid differences along the coding sequences of the CPV-2c, CPV-2a, and recombinant 364-rec strains

ORF	NS1							VP1				VP1/VP2												
Nucleotide position	81	342	516	1173	1542	1714	1875	1975	2063	2085	2086	2432	2550	2574	2817	3246	3314	3345	3484	3485	3790	3792	3832	4266
Amino acid identity and position	S27	C114	K172	G391	I514	K572E	Q625	L659	Int ^a	Int ^a	Int ^a	R140K	Q12	A20	T101	Y244	Y267F	H277	I324Y	I324Y	N426E	N426E	A440T	Y584
2a	A	C	A	C	C	A	A	T	G	G	A	G	G	T	C	C	A	C	A	T	A	T	G	C
364-rec	G	T	G	T	T	G	G	C	A	A	G	A	G	T	C	C	A	C	A	T	A	T	G	C
2c	G	T	G	T	T	G	G	C	A	A	G	A	A	A	T	T	T	T	A	G	A	A	T	

Pérez et al. 2014

^a VP1 Intron. Shaded nucleotides represent identical sequences.



Recombinante

CPV-2a

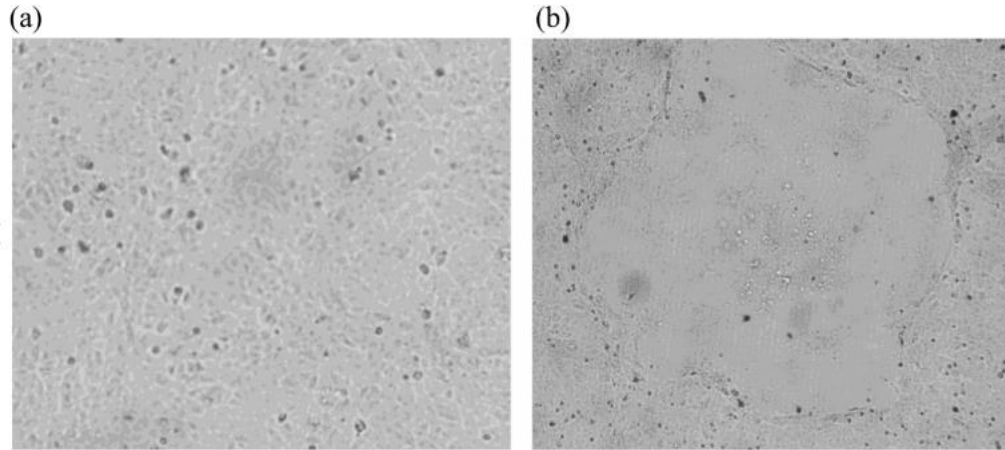
CPV-2c

Infecciones en cultivo celular

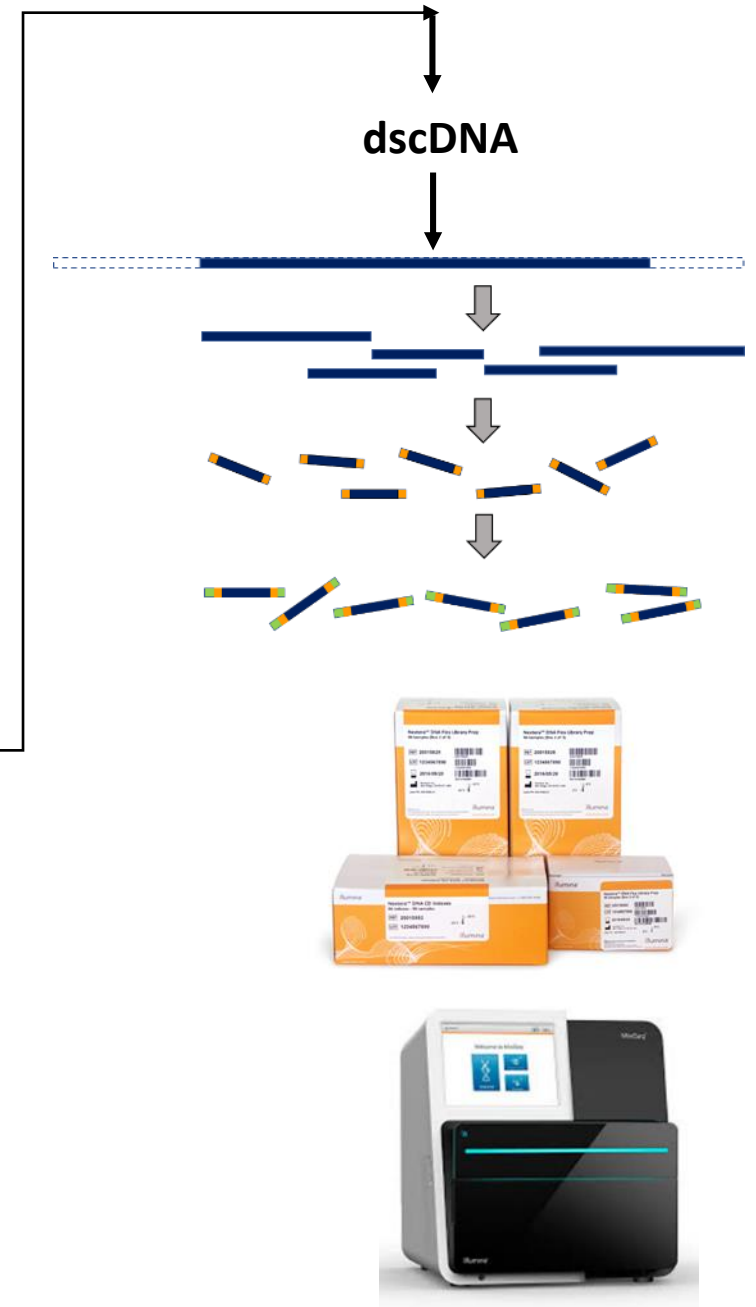
C. Romanutti, et al.

Journal of Virological Methods 279 (2020) 113857

Vero SLAM



ARN

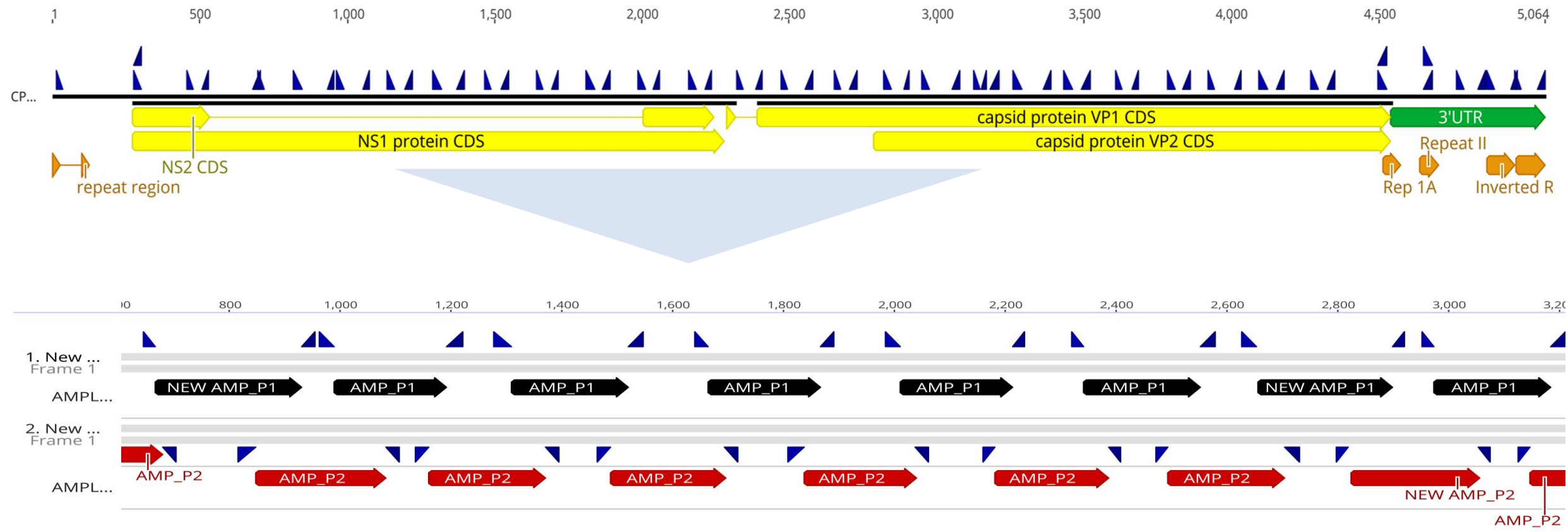


Caracterización genómica

PROTOCOS
DE
ENRIQUECIMIENTO

Ultrplex-PCR-NGS

*Carnivore
p*

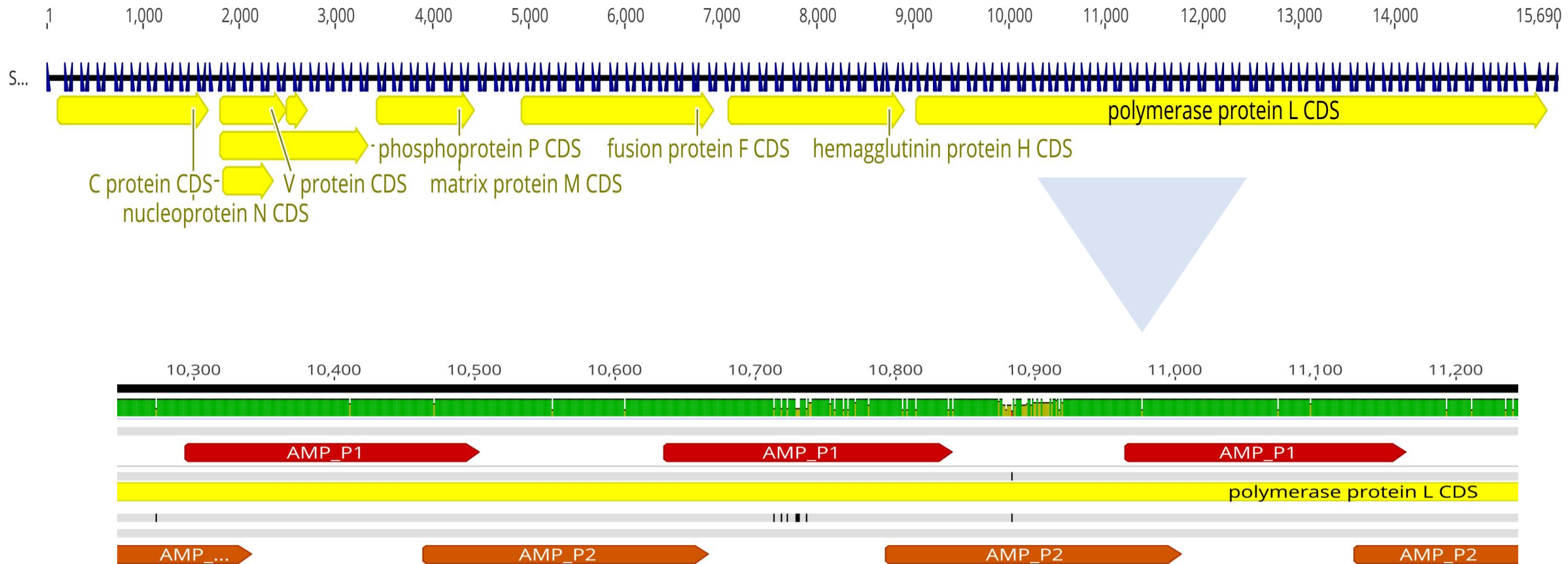


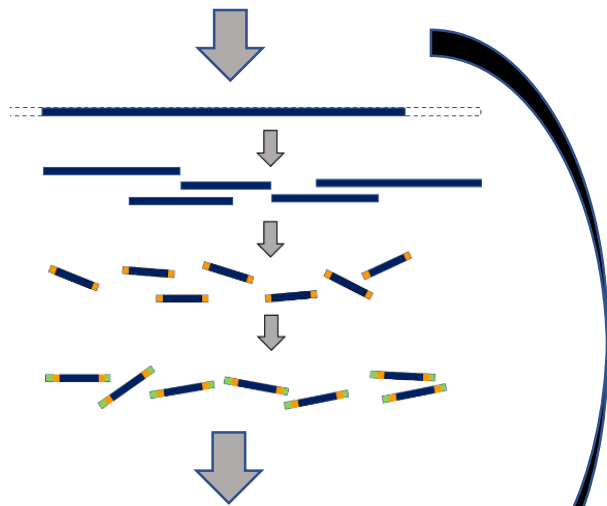
Caracterización genómica

PROTOS
DE
ENRIQUECIMIENTO

Ultrplex-PCR-NGS

Canine morbillivirus

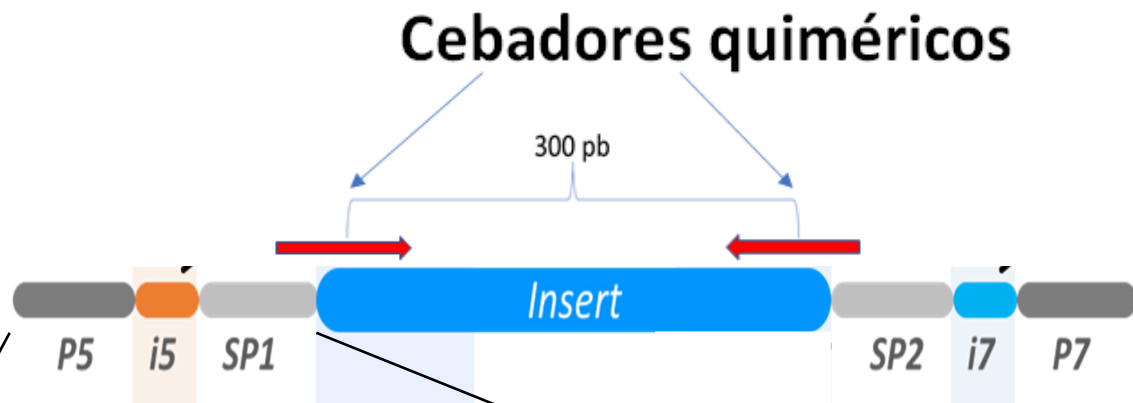




PCR

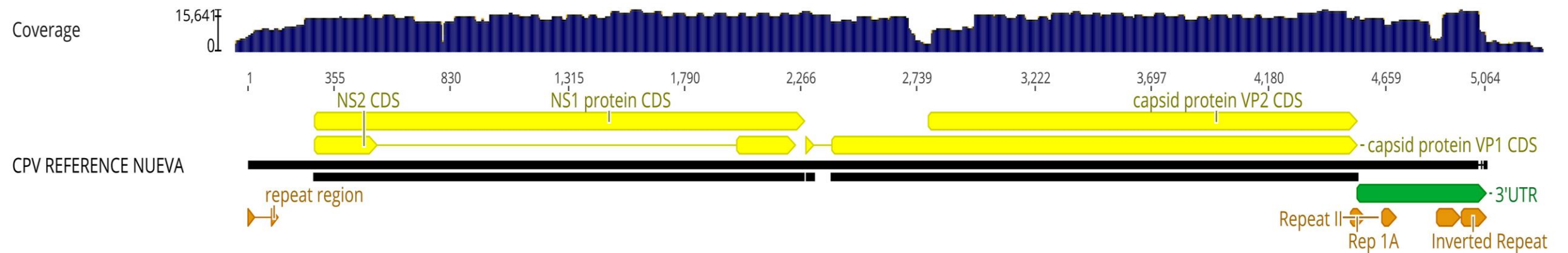
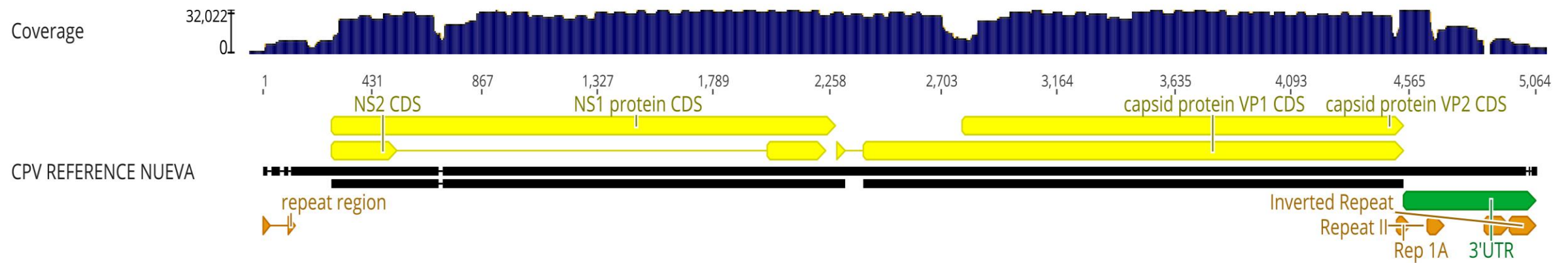
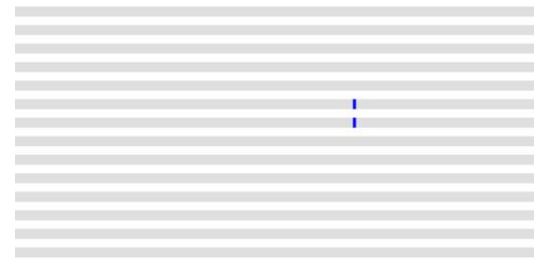


Cebadores quiméricos

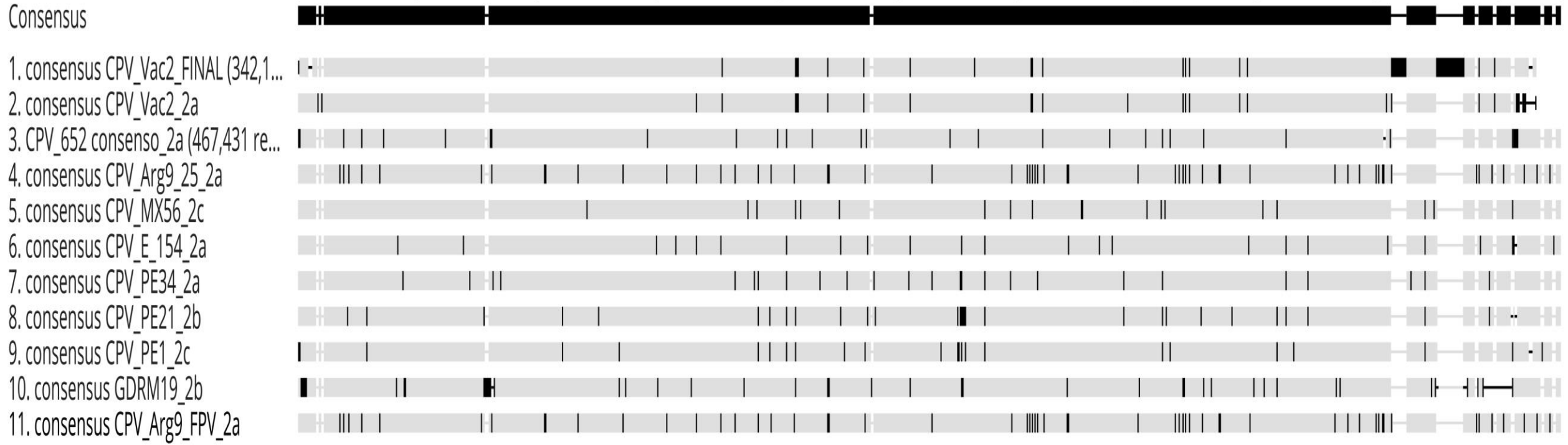


AATGATACGGCGACCACCGAGATCTACACNNNNNNNNNTCGTCCGGCAGCGTTCAGATGTTGTATAAGAGACAG

**Carnivore
protoparvovirus 1
Ultrplex-PCR-
NGS**

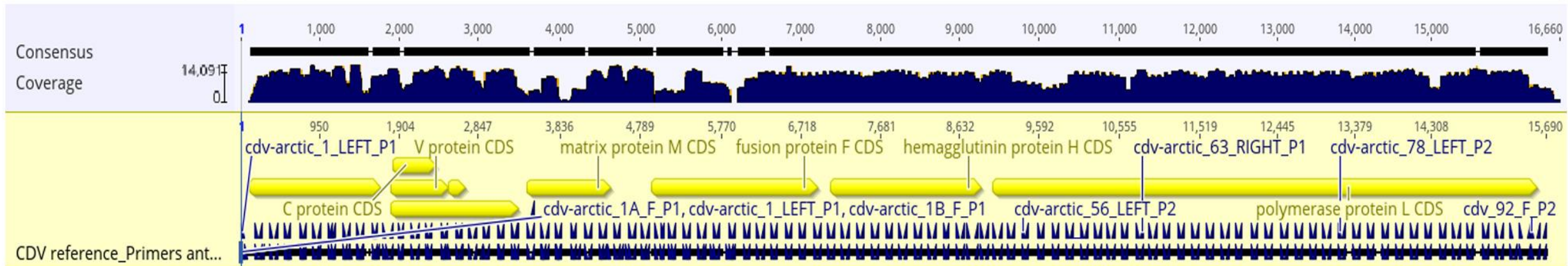
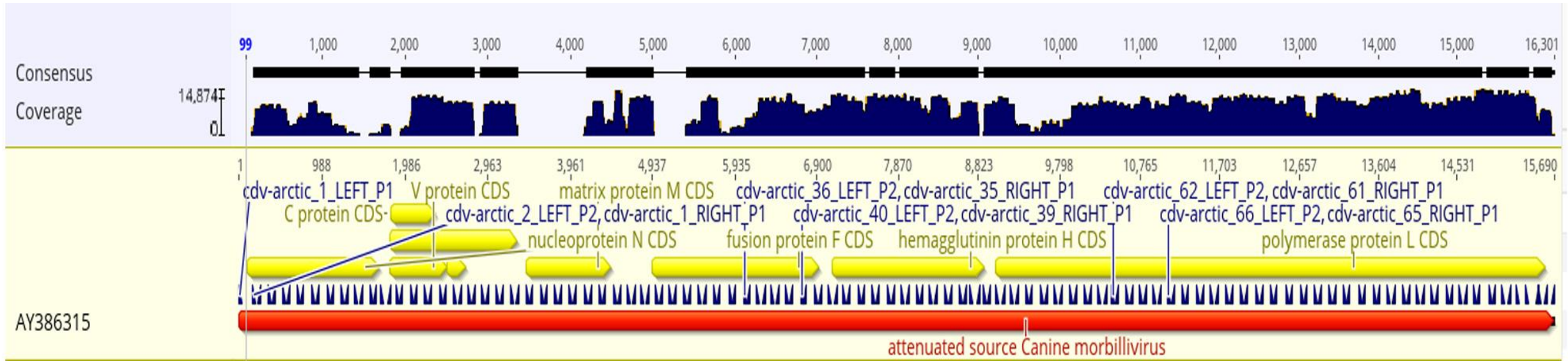
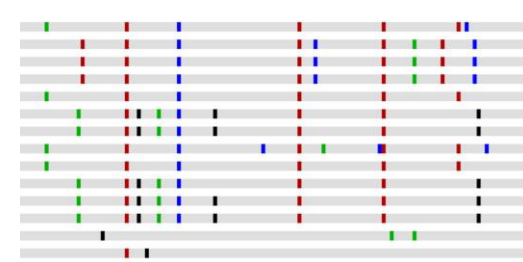


1 500 1,000 1,500 2,000 2,500 3,000 3,500 4,000 4,500 5,000 5,259



Canine morbillivirus

Ultraplex-PCR-NGS

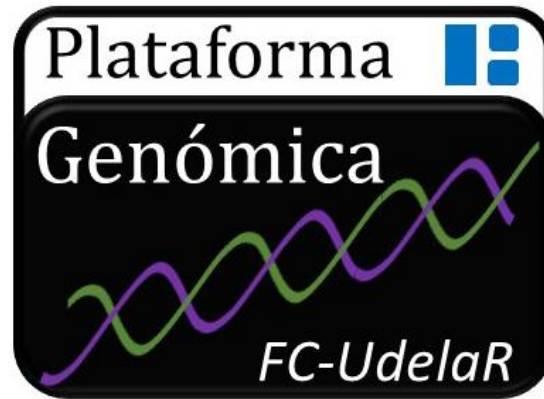


DESARROLLO METODOLÓGICO



Secuenciación masiva

Evolución genómica



Genómica funcional

Genómica comparativa

Diagnóstico

Ensayos clínicos

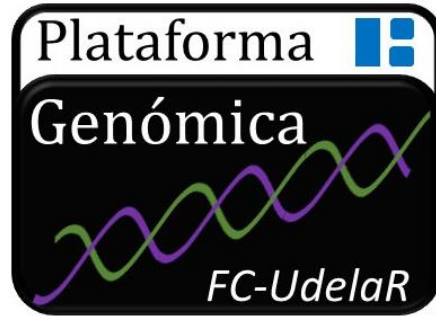
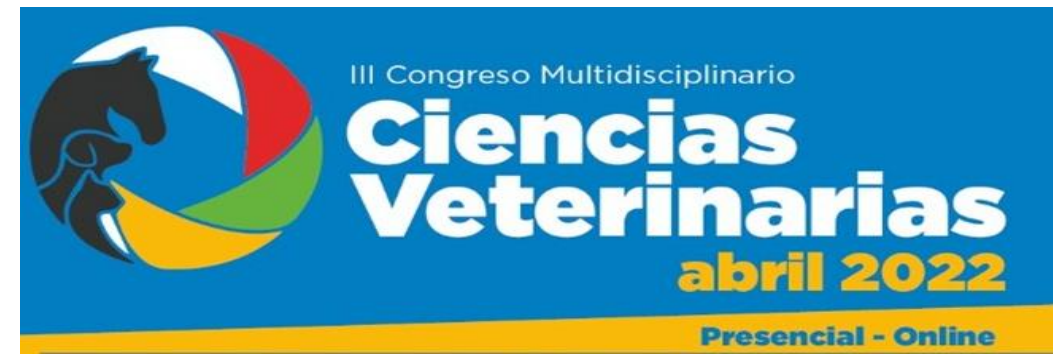
***Capacitar recursos humanos
Grado/Posgrado***

***Transferencia de tecnología
Proyectos***

Generación de convenios

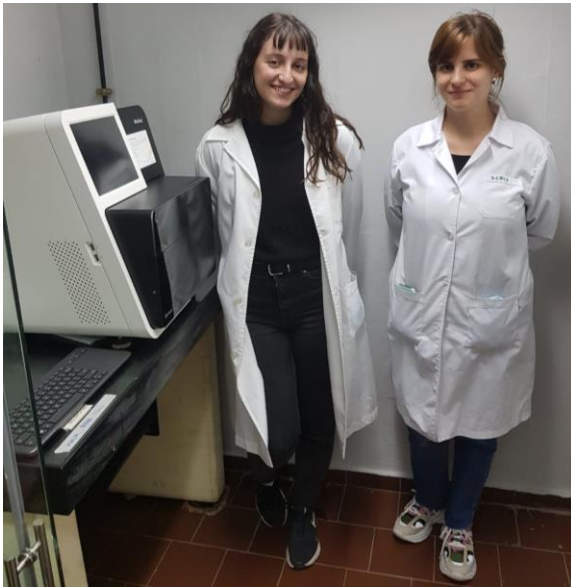


Genética de Microorganismos



ANII-FCE_1_2019_1_155660

“ANÁLISIS DE LA EVOLUCIÓN GENÓMICA EN VIRUS CANINOS”



Lic. Emma Condon

Lic. Sofia Grecco



GRACIAS

