March 2023, Issue 13

GFRA NEWS

The official newsletter of the Global FMD Research Alliance

Our Vision

A coordinated global alliance of scientists producing evidence and innovation that enables the progressive control and eradication of FMD.

Our Mission

To establish and sustain global research partnerships to generate scientific knowledge and discover the tools to successfully prevent, control and eradicate FMD.

Newsletter Highlights

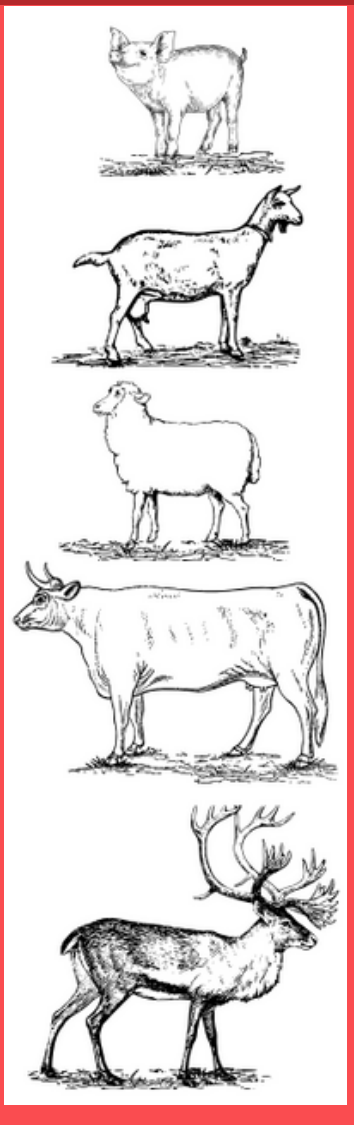
Welcome Remarks from GFRA President

Frank N. Mwiine

The Global Foot and Mouth Disease Research Alliance (GFRA) aims to establish and sustain global research partnerships that continues to generate scientific knowledge and tools to contribute to the successful prevention, control and where feasible eradication of Foot and Mouth Disease (FMD).

Distinguished GFRA Community, it has been two years since the successful virtual Scientific meeting that was initially scheduled to take place in Buenois Aires, Argentina in 2021 but changed due to COVID-19 pandemic. In addition, it is almost four years since the last physical GFRA Scientific meeting that was successfully held in Bangkok, Thailand. As you may be aware, our first physical post pandemic GFRA Scientific Workshop will be held at Munyonyo Commonwealth Resort in Kampala, Uganda from 8-10th November 2023. The meeting will provide an opportunity to connect with FMD collaborators and friends (old and new) and to get an update on advances in FMD research. Importantly, it will provide us with the opportunity to update the FMD GAP analysis report, a core component of the workshop. Whilst updates on FMD research activities have been possible through virtual webinars and training sessions (especially held during the COVID-19 pandemic period), these can never replace the valuable in-person discussions that a GFRA Scientific meeting affords. The GFRA 2023 scientific meeting in Uganda will bring together leading scientists with expertise in FMD all over the globe in order to analyze knowledge and research gaps.

We look forward to welcoming you to Kampala, Uganda.
Prof. Frank N Mwiine
President



A New Edition of the Traditional GFRA Gap Analysis Meeting in Buenos Aires

Alejandra Capozzo and Mariano Pérez Filgueira

Institute of Virology and Technological Innovations "IVIT", CONICET-INTA. Buenos Aires. Argentina.

The 6th GFRA Gap Analysis meeting was held in Buenos Aires on December 5 and 6, 2022, hosted by INTA. INTA´s GFRA members have organized all the GFRA GAP Analysis meetings since 2010, and Buenos Aires has traditionally been the venue city. The meeting enrolled experts from all around the world. This time, 31 experts from Argentina, Uruguay, Germany, Italy, UK, USA, Australia, South Africa, Pakistan, and Kenya got together to identify research gaps in Vaccine development, immunology, pathogenesis, virology, diagnostics and epidemiology. We also have representatives from the industry:

continued.

Biogenesis Bago, CDV, Senasa, INTA, CSIRO, FLI, FAO, OVI, Pirbright, Zoetis, USDA, the University of Minnesota, and national veterinary laboratories. The workshop included a short update on the progress of each discipline since the last GAP Analysis in 2018, and proposed research gaps presented by the chairs, afterwards discussed in deep by all the assistants. As Dr Gay said during the wrap-up session, the GFRA GAP analysis report is one of the most important contributions of our Alliance.

From Left to Right: Michael Nyberg CRDF, Michael Eschbaumer (FLKI), Toby Tuthill, (The Pirbright Institue), Melanie Chitray (ARC), Carolina Stenfeldt (PIADC) Alejandra Capozzo (INTA), Ana Taffarel (SENASA), Nagendra Singanallur (CSIRO), Romina Sierra (CDV)

This report is used as a source of information by colleagues and different international animal health organizations.

Apart from working, the GFRA team also enjoyed the warm December weather of Buenos Aires, great Malbec, and beef, in the trendy Buenos Aires neighborhood called "Palermo Hollywood".

From Left to Right: Andrés Perez, Manuel Borca, Mariano Pérez Filgueira, Guido König, James Zhu, Alejandra Capozzo and Cyril Gay

We were delighted to host this meeting in Buenos Aires, and look forward to meeting again soon to analyze the progress of FMD global research.

From Left to Right: Mariano Pérez-Filgueira, Alejandra Capozzo, Manuel Borca and Teresa de los Santos

Buenos Aires Gap Analysis Sessions:

Session 1: Vaccines and Biotherapeutics

- "New generation FMD vaccines: technologies at high readiness level"
  Elizabeth Rieder
- "Research achievements in vaccine development and biotherapeutics"
  Melanie Chitray

Chair	**Elizabeth Rieder,** United States, USDA PIADC
Co-Chair	**Melanie Chitray,** South Africa, OVI

Session 2: Diagnostics

- "The latest achievements in FMD diagnostics (2018 –2022)"
  Michael Eschbaumer
- Proposed research gaps: "What is missing?"
  Nagendra Singanallur

Chair	**Michael Eschbaumer**, Germany, Friedrich-Loeffler-Institut
Co-Chair	**Nagendra Singanallur**, Australia, CSIRO

Session 3: Epidemiology

- "The eradication process in Latin America"
  Prepared by Manuel Sánchez Vazquez, Brazil, PANAFTOSA
  Andrés Pérez
- "The latest achievements in epidemiology (2018 –2022)"
  Jonathan Arzt
- Proposed research gaps
  Andrés Pérez

Chair	**Andrés Perez**, United States, University of Minnesota
Co-Chair	**Jonathan Arzt**, United States, USDA PIADC

Session 4: Immunology

- "Immune responses to FMD infection and vaccination with special focus on cross-protection"
  Mariano Pérez Filgueira
- "Research Gaps on FMDV immunology: innate and adaptive immunity, new technologies and approaches applied for other viruses."
  Alejandra Capozzo

Chair	**Mariano Pérez Filgueira**, Argentina, CONICET-INTA
Co-Chair	**Alejandra Capozzo**, Argentina, CONICET-INTA

Session 5: Virology

- "Advances in Virology. Research achievements of the last 4 years"
  Toby Tuthill
- "Research Gaps, new technologies and perspectives"
  María Inés Gismondi

Chair	**Toby Tuthill**, United Kingdom, Pirbright Institute
Co-Chair	**María Inés Gismondi**, Argentina, CONICET-INTA

Session 6: Pathogenesis

- "Transmission dynamics in and between different species"
  Jonathan Artz
- "Identifying the research gaps in FMD transmission in the lab and in the field"
  Carolina Stenfeldt

Chair	**Jonathan Arzt**, United States, USDA PIADC
Co-Chair	**Carolina Stenfeldt**, United States, USDA PIADC

The First GFRA Grants:
Supporting Global FMD Research

GFRA aims to facilitate research collaborations between the global FMD research community and conduct strategic research to better understand FMD. Following these objectives, last year in 2022 the GFRA launched for the first time a funding call to support short projects executed by our member organizations.

This funding supported projects that addressed at least one of the strategic themes identified in the 2018 GAP Analysis Workshop.

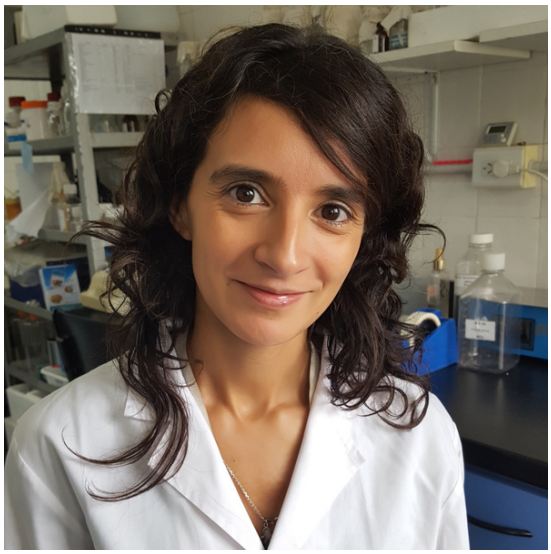
Special consideration was given to those projects proposing collaboration between different countries, as well as applicants who presented their research at the 2021 GFRA Scientific Meeting. Over twenty contributions were received, and of these four grants with a maximum of USD 5,000 per project were awarded.

Meet our winners, their trajectory, and their granted projects. We aim to keep on supporting young scientists in the future.

GFRA 's First Grant Winners

DR. NANCY CARDOSO

Ph.D. in Physiology Science



Dr. Cardoso graduated from the University of Buenos Aires in 2005. She also received her Ph.D. in Physiology Science at the same University in 2011. Dr. Cardoso was a postdoctoral fellow at the Institute of Virology and Technological Innovations from 2013-2015, studying the Immunophatogenic mechanism of Bovine Viral Diarrhea Virus. Since 2015, she has been a researcher at the National Council for Scientific and Technical Research (CONICET) at INTA. Her focus is on Animal Health, mainly in control of viral diseases affecting bovines.

DR. MOHAMED FAWZY

Ph.D. in Virology

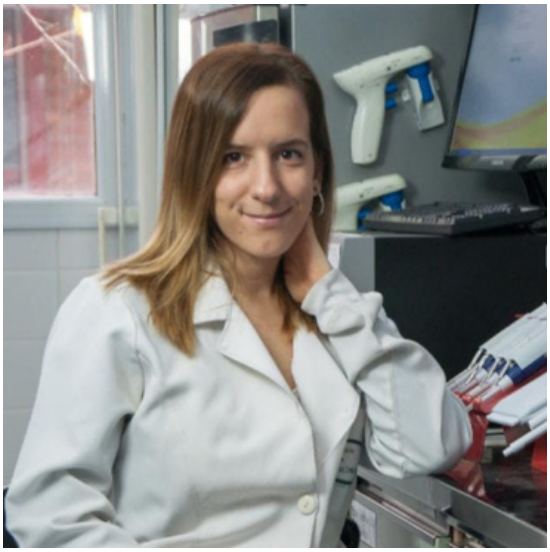
Dr. Mohamed Fawzy served as the associate professor of Virology and Faculty member of Veterinary Medicine at Suez Canal University. He published 41 international scientific articles in the fields of virology, Immunology, and Vaccinology with 12 H-Index. He supervised 13 Master and 7 PhD postgraduate students. Dr. Fawzy has completed many vaccinology courses in Africa and Europe, such as ADVAC, Afro-ADVAC, and VACFA. He is a reviewer for over 15 international journals and member of 6 scientific organizations. He has attended 11 national and 9 international conferences in Egypt, Thailand, Argentina, South Korea, Spain, Vietnam, and Italy.

GFRA 's First Grant Winners

DR. GUIDO KÖNIG

Ph.D. in Biological Sciences

Dr. König received his degree in Biological Sciences from the University of Buenos Aires in 1996, and his PhD from this university in 2004. Dr. König is currently working as a research at the National Institute of Agricultural Research (INTA), the Comisión Nacional de Investigaciones Ciéntificas y Técnicas (CONICET), and an Independent researcher. He is currently developing an FMDV epidemiological surveillance system, as well as working on molecular epidemiology of several animal viral diseases. He also oversees three doctoral students, and is the current leader of the viral evolution group.



DR. FLORENCIA MANSILLA

Ph.D. in Veterinary Sciences

Dr. Mansilla received a degree in Biological Sciences from Maimonides University in 2010, and her PhD in Veterinary Sciences from the University of Buenos Aires in 2015. Dr. Mansilla was a doctoral fellow at the Institute of Virology from 2010-2015, focused in the development of vaccines and immunomodulation in applied veterinary immunology. Her Post-doctorate was at the same institute, focused on developing and characterizing novel diagnostic tools for Foot-and-Mouth Disease Virus. She currently works as a researcher at the Institute of Virology (INTA), studying the innate immune response, aimed at establishing strategies to prevent viral infections in species of livestock interest.

Our next scientific meeting will be at the Speke Resort in

Kampala, Uganda

November 8th-10th, 2023

Plum Island Celebrates 68th Anniversary

Teresa de los Santos

On November 2022, Plum Island Animal Disease Center (PIADC) celebrated its 68th Anniversary during a two-day event. This was a coordinated project that brought together the three U.S. government agencies currently working in the facility, culminated with a stelar celebration held on site at Plum Island, and across the Long Island Sound in the Old Saybrook Inn and Resort in CT.

With the participation of over 120 scientists, including past and current PIADC employees and fellows, USDA ARS/APHIS and DHS administrators, colleagues from NBAF, collaborators from all over the World, including representatives of FAO, OIE (now WOAH), the Pirbright Institute (UK), CFIA (Canada), FLI (Germany), INTA/Conicet (Argentina), SENESICA (Mexico), COBAV Makerere (Uganda) and others, the event offered a historical prospective and summarized many accomplishments that have led Plum Island to be internationally recognized as a center of excellence for the prevention and control of transboundary animal diseases.

On the first day a historical perspective highlighted facts from the Island's discovery in the early 1600's, its purchase from resident native Americans, its use during several wars and for military training, and its final transfer to USDA in 1952. Emphasis was given to the impact of PIADC science on global animal health, highlighting the multiple strategic international research collaborations, and the establishment and growth of GFRA and GARA, which allowed PIADC to reach the strategic international position of today, and its expansion to the USDA National Bio and Agro Defense Facility in Manhattan, KS.

On the second day, focus was given to science. Four scientific sessions featured international guest speakers and PIADC scientists presenting the most updated research on transboundary diseases of interest at PIADC and the great accomplishments of PIADC researchers.

The Plum Island Animal Disease Center's motto is "to defend USA from the accidental, natural or intentional introduction of transboundary animal diseases including Foot-and-Mouth Disease (FMD), and African Swine Fever (ASF), among others". Three government agencies currently accomplish this mission: the USDA Agriculture Research Service (ARS), the USDA Animal and Plant Health Inspection Service (APHIS) and the U.S Department of Homeland Security (DHS) Science and Technology Directorate (S&T) office.

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These agencies conduct research, perform diagnostics, provide trainings to state, federal and foreign official institutions manage the North American FMD and national Animal Vaccine and Veterinary Countermeasures vaccine bank, as well as characterizing agricultural threats and supporting the development of veterinary medical countermeasures.

Photo:

Science at PI has resulted in several success stories such us: the licensing of the first recombinant FMD vaccine (Ad5 vectored FMD) approved to be produced in USA in the last 70 years, a novel safe modified whole antigen antigen FMDV vaccine that can be produced in the U.S mainland (leaderless FMDV with DIVA markers), the first ASFV live attenuated vaccine (ASFV-I177L) that is effective against the current pandemic virus and could also be produced in the U.S. mainland, and several diagnostics methods/kits for the detection of transboundary diseases.

Since the beginning, Plum Island leaders showed a visionary prospective that enabled scientific knowledge to flourish on site, and through a milieu of international collaborations, leading the development of what it is today, a worldwide recognized center of excellence in animal health. Major scientific contributions in foot-and-mouth disease, African swine fever, classical swine fever, and vesicular stomatitis, that started at Plum Island, have provided not only gap-filling knowledge at the basic level in viruses biology, pathogenesis, epidemiology, but also practical solutions in the form of improved diagnostics, novel vaccines and biotherapeutics that have reached the end user in the field.

A warm and well deserved congratulations goes out to all of those whose efforts have led to the Plumb Island Animal Disease Center's 68th anniversary.

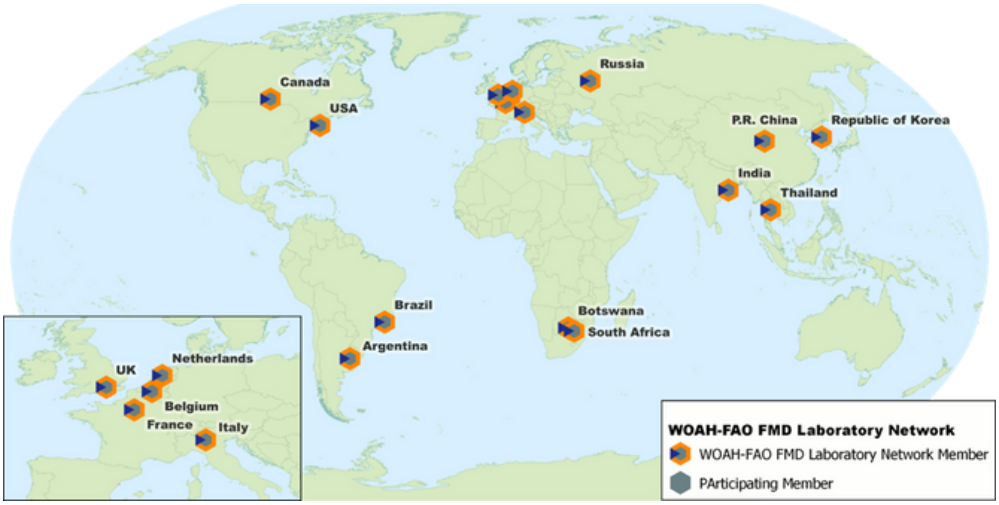
17th Annual WOAH/FAO FMD Reference Laboratory Network Meeting

Phaedra Eble

From 29th November – 1st December 2022 the 17th annual meeting of the WOAH/FAO FMD Reference Laboratory Network, was held at Wageningen Bioveterinary Research in Lelystad, the Netherlands. After two years of restrictions due to COVID, it was a pleasure to meet with colleagues in a face-to-face format and video conferencing was available for those that were not able to travel. The WOAH/FAO Reference Laboratory Network for FMD was established in 2004 as a forum to exchange laboratory and epidemiology data for FMD and FMD vaccines, as well as to harmonize and improve the quality of diagnostic testing carried out by national and international FMD laboratories.

The WRLFMD serves as the Network Secretariat and the meeting was supported by WOAH, EuFMD and sponsorship from commercial vaccine companies (Biogenesis Bago, Boehringer Ingelheim and MSD). The network has been adopted as a model in the creation of other WOAH Reference Laboratory Networks for African swine fever, peste des petits ruminants and rabies.

The hybrid meeting of 2022 was attended by approximately 26 persons present in Lelystad and up to 30 persons online. It was an excellent meeting with a lot of input from all partners, including those in FMD endemic regions. Topics that were discussed included new threats posed by FMDV lineages in Africa and Asia, FMD vaccine quality control, as well as new web-based tools that are being designed to display data to the FMD community.



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For those that are interested, minutes of this meeting can be retrieved from: https://www.foot-and-mouth.org/sites/foot/files/user-files/research-paper/pdf/02-23/WOAH-FAO%20Network%20Meeting%20Report%202022.pdf

WOAH/FAO Foot-and-Mouth Disease Reference Laboratories Network

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Evaluating recombination of codon deoptimized FMDV strains in cell culture

Gisselle N. Medina1,2, and Teresa de los Santos1

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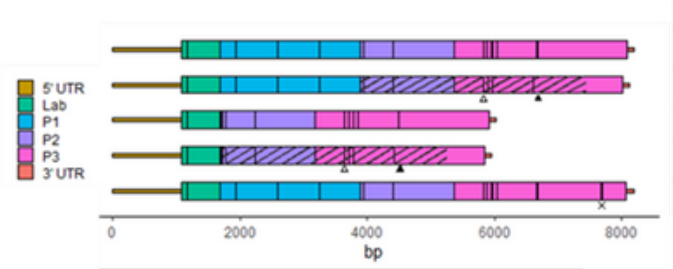
This article provides summarized data from a manuscript already published: Viruses 2023, 15(3), 670; https://doi.org/10.3390/v15030670

Codon deoptimization (CD) of a viral genome represents a promising technology for the development of live attenuated vaccines (LAVs) candidates against multiple viruses (Coleman et al., 2008, Wang et al 2021). We have applied the same technology to FMDV rendering strains containing DIVA markers that were attenuated in vitro and in vivo (Diaz-San Segundo et al 2016, 2021). However, safety is a predominant concern for this type of LAV due to the possibility of recombination between the CD strains and circulating WT viruses, that may lead to the loss and/or transfer of DIVA markers.

Nucleic acid recombination usually occurs in nature when two or more viral genomes infectthe same host cell and exchange genetic material, resulting in the creation of a new sequence that differs from the original parental genomes. The mechanism of RNA recombination in FMDV is thought to resemble that of poliovirus where the RNA-dependent RNA polymerase (RdRp) from one parental RNA genome (donor) switches to another RNA genome (acceptor) during negative strand synthesis. Co-circulation of distinct FMDV serotypes in endemic areas has been found to greatly facilitate the identification of RNA recombination events. In fact, recombination hotspots have been identified throughout the genome with a substantial number in the conserved regions encoding non-structural (NS) genes (Lewis-Rogers et al 2008, Brito et al 2018, Ferreti et al 2018, Lasecka-Dykes et al 2018, Bertram et al 2021, Palinski et al 2022). More recently, both intra- and inter-serotypic recombination was observed after experimental infection of natural hosts (Fish et al 2022, Ferretti et al 2020, Arzt et al 2021). However, thus far, no FMDV system has been reported to measure the rate of recombination in vitro in cell culture.

This article highlights the development of such an assay to quantitate the levels of recombination in cell culture using two in vitro synthesized constructs: the donor template A24-ΔP1 (ΔP1) and the acceptor template A24-ΔGDD (ΔGDD). By including in the design CD sequences, this system has allowed us to evaluate recombination events between our previously developed CD FMDV and WT genomes. Basically, the donor RNA template contained P2/P3 deoptimized sequences in the context of a ΔP1 backbone, and the acceptor RNA template featured WT A24 sequences throughout, except for a lethal mutation in the P3 (3DpolΔGDD) coding region (A24-ΔGDD). Neither construct could drive the formation of viable virus on their own. However, co-transfection of these RNA molecules could lead to formation of viable viruses only if recombination between them took place (Figure 1).



Template	Infectious	Replication Competent
A24WT	✓	✓
A24-P2P3Deopt	✓	✓
ΔP1	×	✓
ΔP1-P2P3Deopt	×	✓
ΔGDD	×	×

Figure 1: Schematic diagram of the FMDV RNA templates used during lipofectamine 2000 transfection. DIVA markers are indicated by open (3B1 deletion, RQKP$_{9-12}$→PVKV) and closed (3D $H_{27}N_{31}$→YR) triangles. Deoptimized regions are indicated by stripes. ΔGDD within the active site of 3D polymerase is indicated by an X

Co-transfection of ΔP1-P2P3Deopt × ΔGDD produced approximately the same number of viable particles as co-transfection of ΔP1 × ΔGDD RNAs at 24 hours post-transfection (hpt), indicating that recombination could still occur despite the presence of CD sequences (Figure 2).

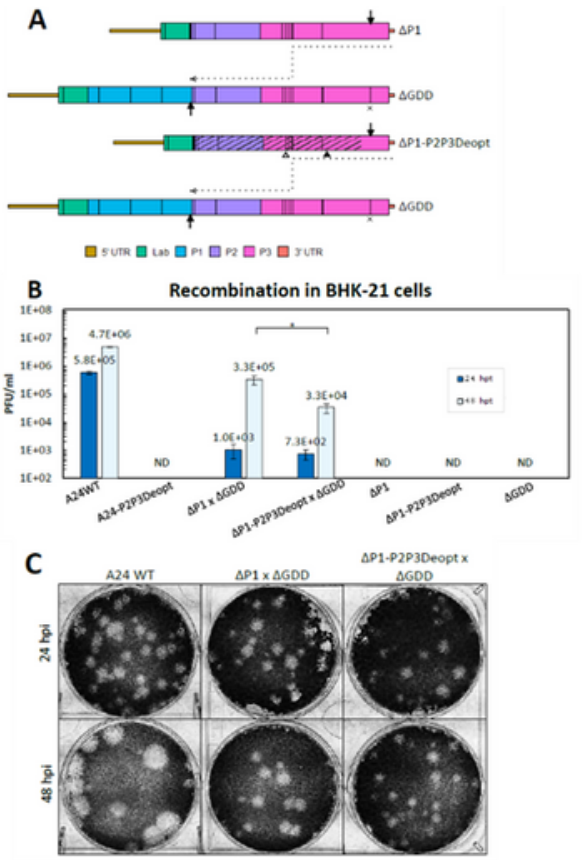


Figure 2. A. Schematic model of FMDV recombination with the given templates. DIVA markers are indicated by open and closed triangles. Deoptimized regions are indicated by diagonal stripes. The ΔGDD within the active site of RdRp encoded by 3D is indicated by an X. **B.** Number of infectious particles generated 24 h post transfection in BHK-21 cells. * Statistically significant difference between the number of infectious virions produced from the different donor RNA templates. ND-not detected. **C.** Plaque morphologies of WT and recombinants in BHK-21

Plaque purified viruses were analyzed by NGS to identify the general sites of recombination and the stability of the P2P3 deoptimized regions post-recombination. Illumina sequencing of the ΔP1-P2P3Deopt × ΔGDD recombinants revealed that at 24 hpt, most recombinants were fully composed of WT sequences at the consensus level and only very few recombinants contained deoptimized sequences at the similar level (Figure 3).
Data analyzed at the sub-consensus level, revealed that recombinants were much more likely to contain deoptimized sequences in the 3CD coding region, as only a few recombinants isolated at 24 hpt contained deoptimized sequences in the 2AB and 2C-3A coding regions.

Taken together, our data indicated that: (1) an in vitro assay to measure recombination for FMDV genome sequences was successfully established; (2) the majority of observed recombinants weregenerated by template switching that presumably occurred positionally very early during the reading of the template strand, either within a relatively small region (185 nt) of non-deoptimized sequence or within the first 750 nt of the 3507 nt deoptimized region in the negative strand; (3) in our system, codon deoptimization did not completely prevent recombination, but recombinants containing large regions of deoptimized regions at the consensus level were not observed and presumably displayed lower fitness than WT; (4) deoptimization of P2/P3 prevented the passing of DIVA markers between intra-serotype genomes.

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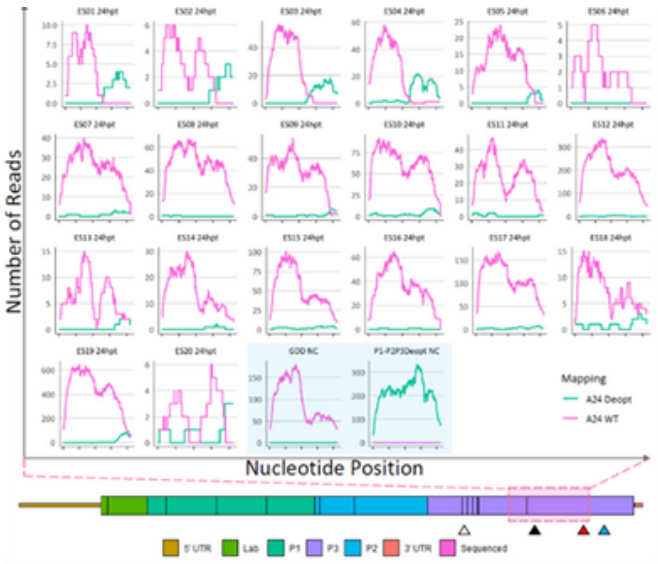

Figure 3. Sequence analysis of 24-hpt ΔP1-P2P3Deopt × ΔGDD recombinant plaque isolates and ΔP1-P2P3Deopt or ΔGDD transfection total cell supernatant (shaded blue box). Locations (x-axis) and counts (y-axis) of reads that preferentially map to either a A24-WT or A24-P2P3Deopt reference within a 1059 nt section of the 3CD coding region as indicated by the pink shaded box. Triangles indicate the location of the 3B DIVA marker (white), 3D DIVA marker (black), the boundary between deoptimized and non-deoptimized regions (red), and the GDD deletion (blue).

Our results suggest that the presented system served as a tool to evaluate potential recombination events between FMDV genomes in vitro, though validation using different cell lines including those that offer immune pressure remains to be tested. Hopefully, these systems will contribute to the development of improved FMD LAV candidates.

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