

# Technical Journal Club

30 June, 2020

Anna Henzi, MD PhD student

NZZ am Sonntag, May 17 2020

# Bern coronavirus clone goes "viral"

Researchers in virology and veterinary bacteriology at the University of Bern have cloned the novel coronavirus (SARS-CoV-2). The synthetic clones are being used by research groups worldwide to test corona samples, find antiviral drugs and develop vaccines as quickly as possible. The method developed in Bern can also be used in future to combat other highly infectious viruses.

In the high-security laboratory of the Institute of Virology and Immunology (IVI) of the Federal Food Safety and Veterinary Office (FSVO) in Mithelhäusern and at the Vetsuisse Faculty of the University of Bern, the researchers successfully reconstructed the coronavirus from synthetic DNA (deoxyribonucleic acid). DNA copies containing parts of the coronavirus genome were introduced into yeast cells and assembled into a complete copy. The researchers then used this to produce infectious coronaviruses.

[https://www.unibe.ch/news/media\\_releases/2020/bern\\_coronavirus\\_clone\\_goes\\_viral/index\\_eng.html](https://www.unibe.ch/news/media_releases/2020/bern_coronavirus_clone_goes_viral/index_eng.html)

Fortsetzung Seite 38

# Synthesizing viruses

- Viral isolates for development of diagnostics, therapeutics and vaccines, or in vivo models

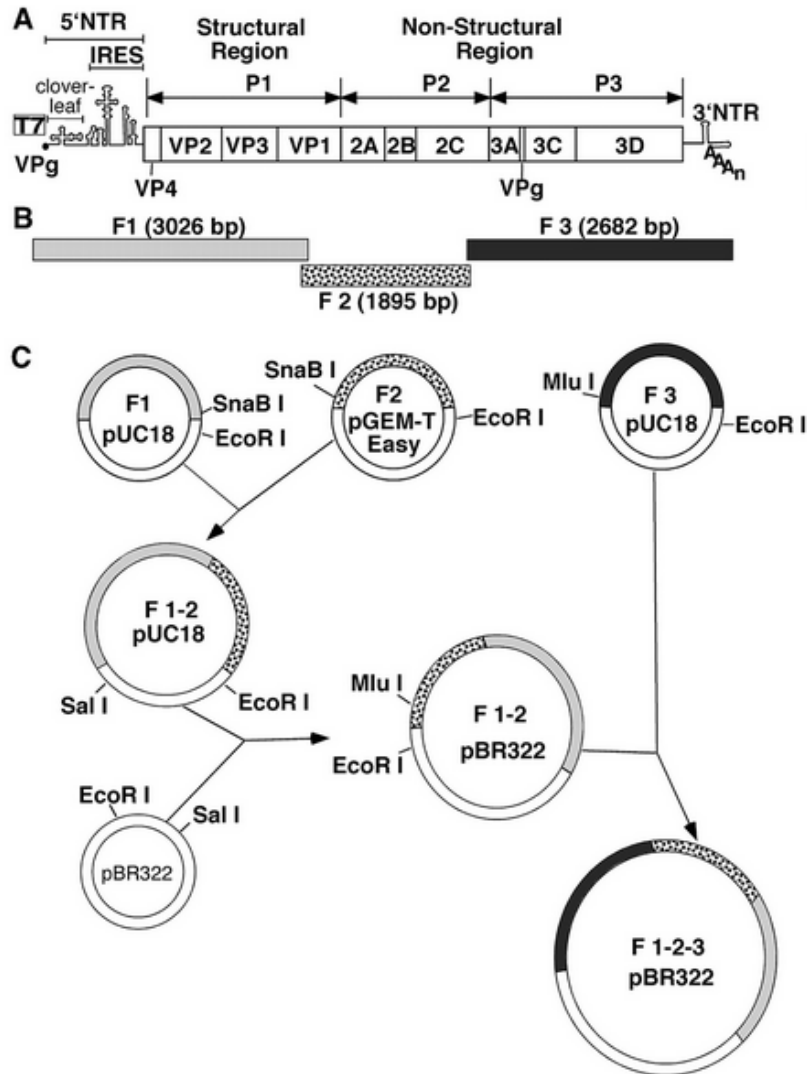
# Synthesizing viruses

Chemical Synthesis of Poliovirus cDNA: Generation of Infectious Virus in the Absence of Natural template

Poliovirus: small, nonenveloped, + ssRNA genome (7.5 kb)

One of the simplest genetic systems known for proliferation

# Synthesizing viruses



- Use of known sequence as the only instruction for engineering the genome
- Phage T7 promoter for RNA polymerase > transcription into infectious RNA
- Generation of virus in cell-free cytoplasmic extracts

# How to make recombinant Coronaviruses

- Thiel, J Gen Virol, 2001
- Coley, J Virol, 2005
- Thao, Nature, 2020

# Coronaviruses

- enveloped, + stranded RNA viruses
- large genomes: 27-31 kb
- Gene sequences toxic to E.coli
  - > not possible to clone into plasmid vectors or passage in procaryotic systems
- Bacterial artificial chromosomes or long range RT-PCR
- Alternative: optimization of in vitro DNA ligation, vaccinia virus eukaryotic cloning vector, cytoplasmic expression of transfected RNA

# Infectious RNA transcribed *in vitro* from a cDNA copy of the human coronavirus genome cloned in vaccinia virus

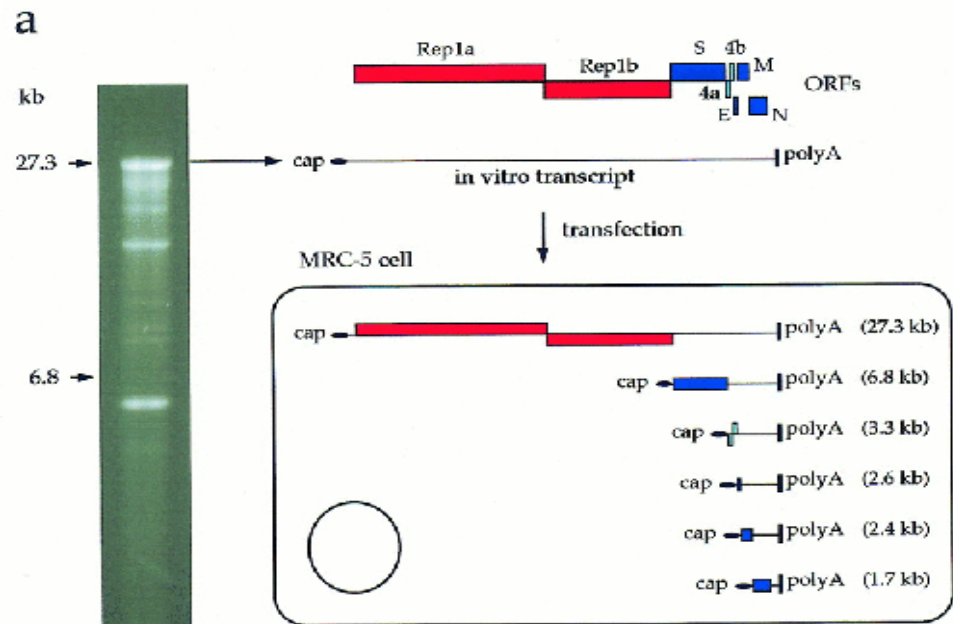
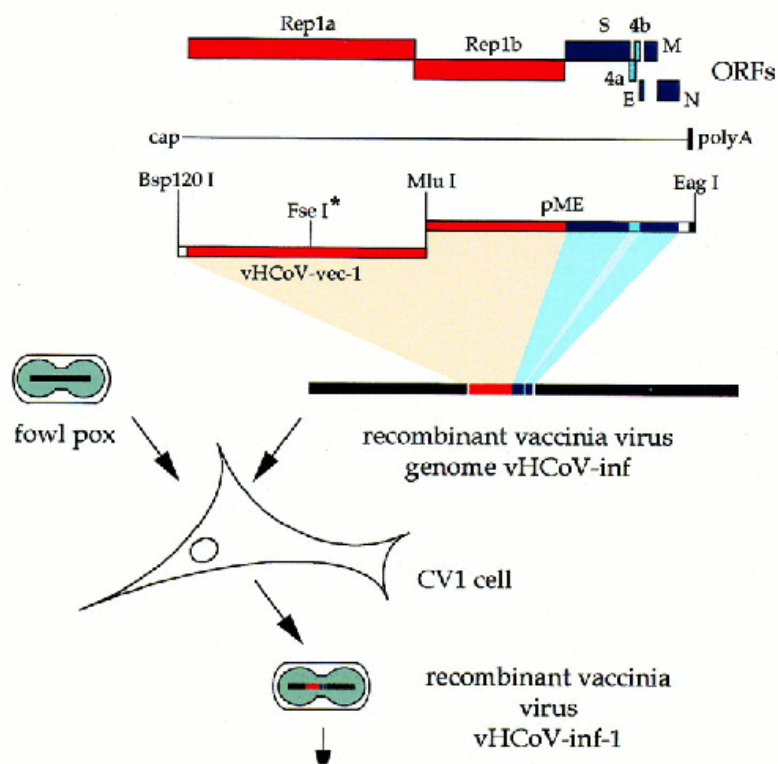
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- HCoV229E
  - Vaccinia virus (poxvirus)
    - eukaryotic cloning vector
- High stability of cloned insert cDNA
  - Retain infectivity, growth kinetics, stability regardless of insert size
  - Large cDNA fragments can be cloned efficiently into vaccinia virus genome





# Recombinant Mouse Hepatitis Virus Strain A59 from Cloned, Full-Length cDNA Replicates to High Titers In Vitro and Is Fully Pathogenic In Vivo

Scott E. Coley, Ehud Lavi, Stanley G. Sawicki, Li Fu, Barbara Schelle, Nadja Karl, Stuart G. Siddell, Volker Thiel

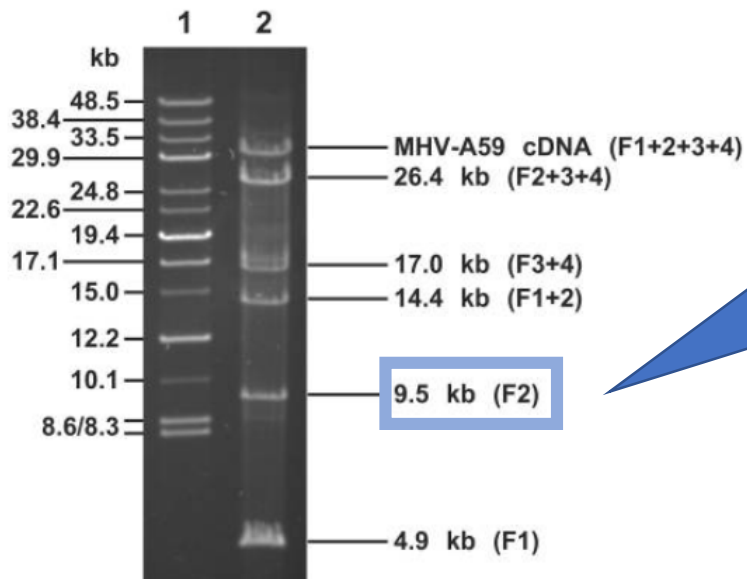
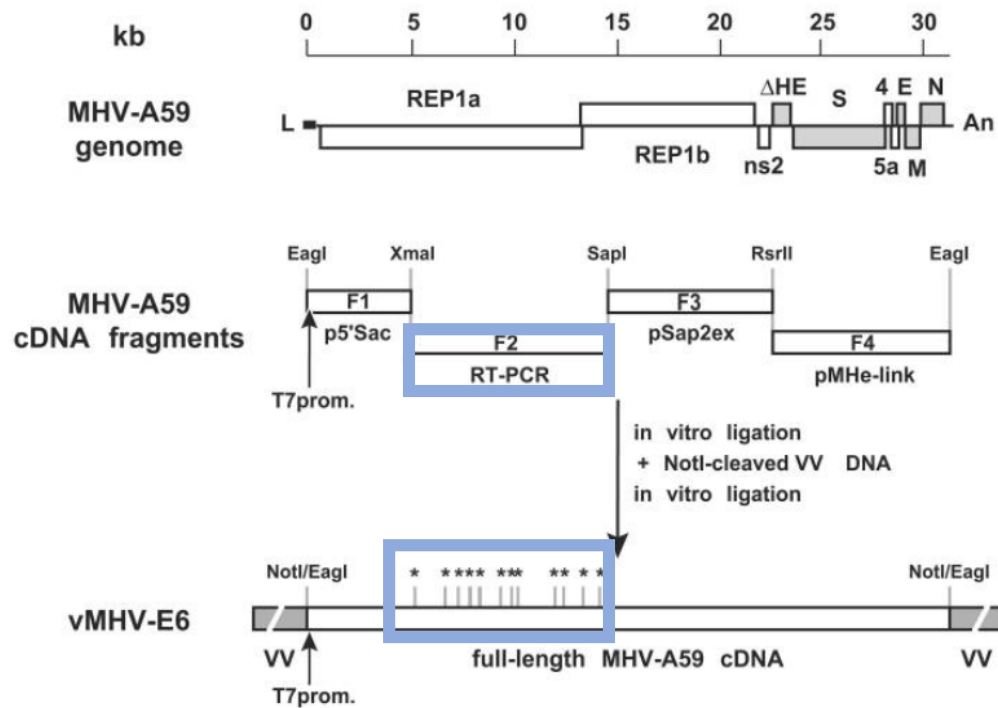
DOI: 10.1128/JVI.79.5.3097-3106.2005



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SOCIETY FOR  
MICROBIOLOGY

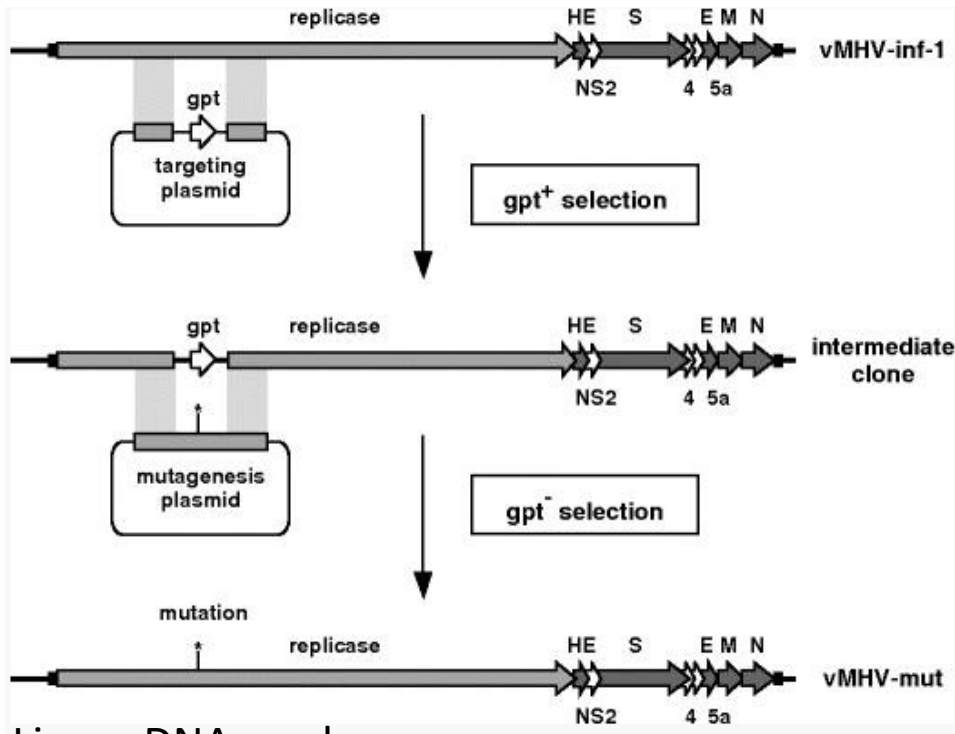
Journal of  
Virology

- Vaccinia virus
- MHV strain A59
  - Versatile, universal tool to study all aspects of MHV molecular biology and pathogenesis
  - Facilitation of mutagenesis
  - Rescue of recombinant coronaviruses by nucleocapsid protein-expressing cell line

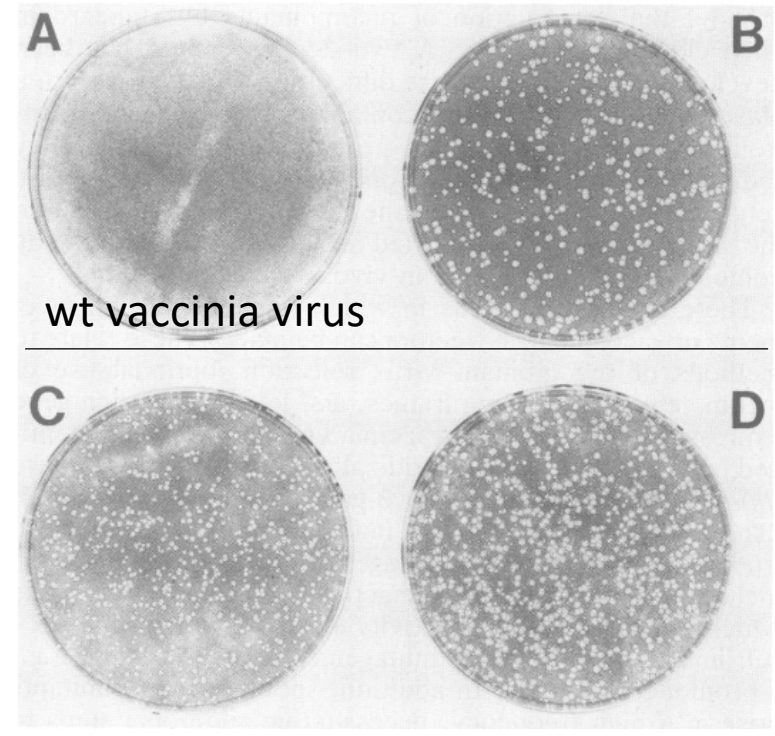


Replaced with correct  
sequence in 4 rounds of virus-  
mediated recombination (with  
+ and - selection)

# Modification of Coronavirus cDNA by Vaccinia Virus-Mediated Homologous Recombination



Linear DNA can be used instead of plasmids



Recombinant vaccinia virus (gpt+)

+MPA  
+(hypo)  
xanthine

No  
treatment

# Rescue of recombinant MHV-inf-1

- «rescue of recombinant coronaviruses is greatly facilitated under conditions that allow N protein expression”

# Rescue of recombinant MHV-inf-1

- Full length MHV RNA
  - in vitro transcription of DNA from
    - purified virions
    - Infected cells

+ synthetic mRNA  
encoding the MHV  
nucleocapsid protein

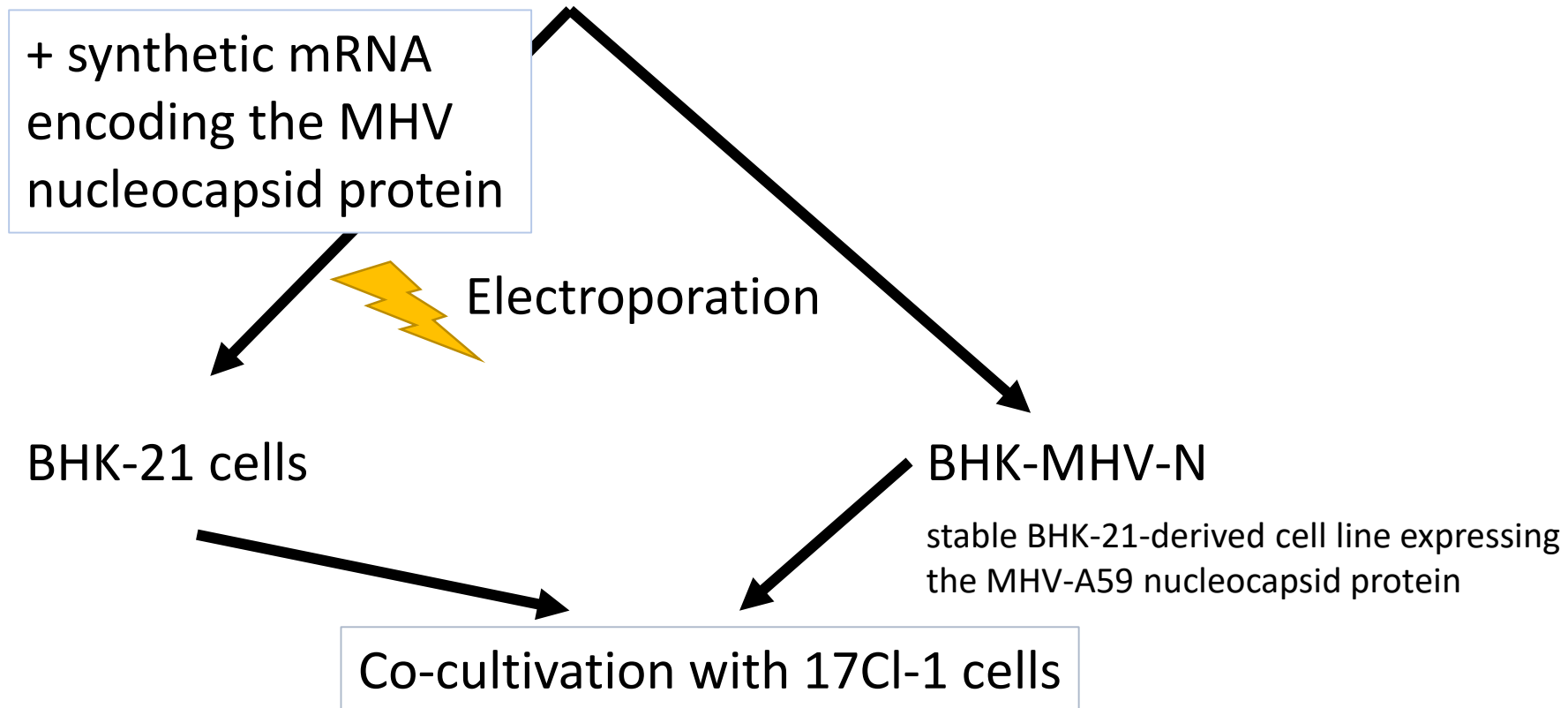
Electroporation

BHK-21 cells

BHK-MHV-N

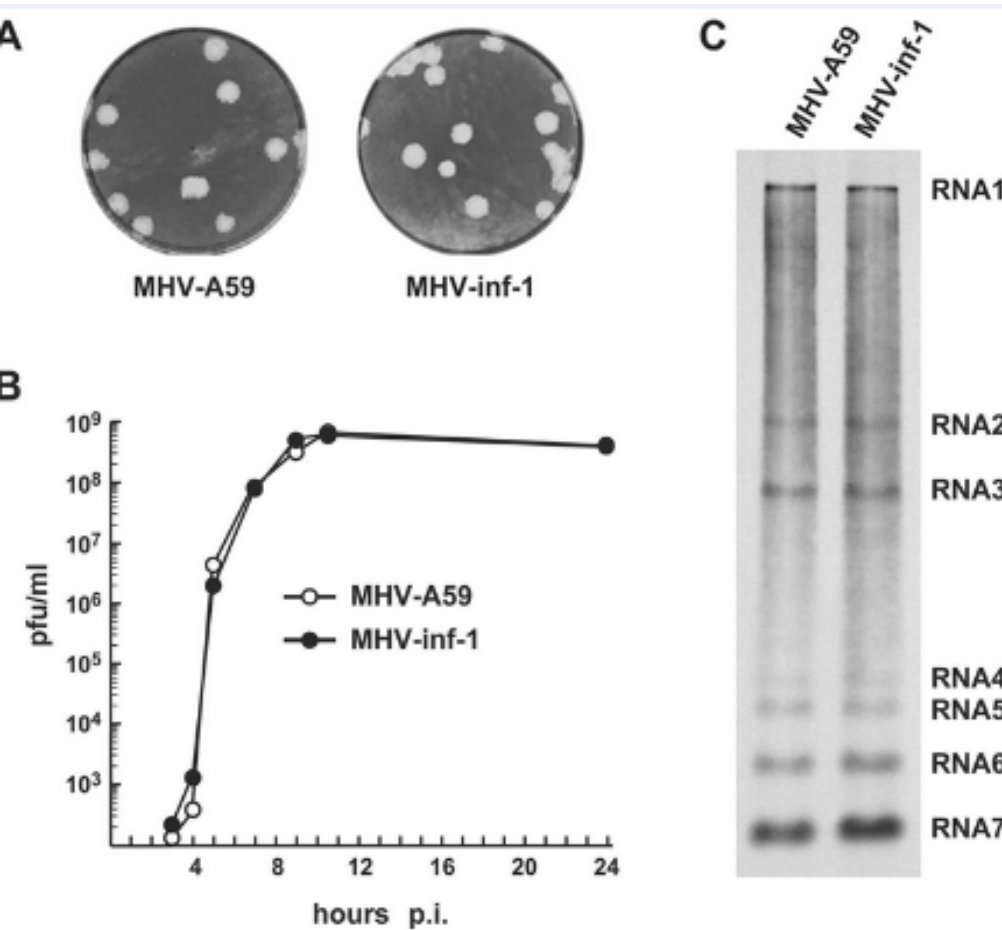
stable BHK-21-derived cell line expressing  
the MHV-A59 nucleocapsid protein

Co-cultivation with 17Cl-1 cells

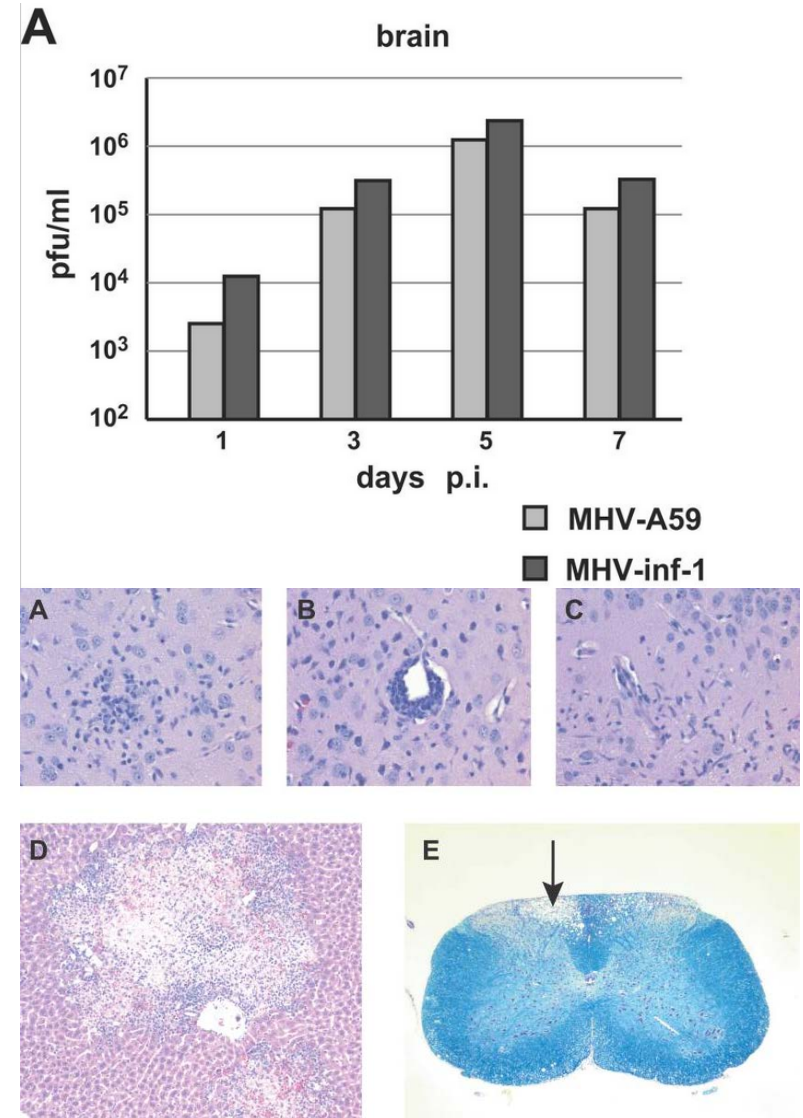


# Replication of recombinant MHV-inf-1

## In vitro



## in vivo



# Recombinant Mouse Hepatitis Virus Strain A59 from Cloned, Full-Length cDNA Replicates to High Titers In Vitro and Is Fully Pathogenic In Vivo

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Journal of  
Virology

- Plasmid DNA and RT-PCR derived products for vaccinia-virus mediated homologous recombination
- Infectious virus produced from 6-well dish
- Cell line expressing N protein (BHK-MHV-N) facilitates rescue
- MHV system to study similar coronaviruses like SARS-CoV



# Reverse genetics platform

- Feline coronaviruses (Tekes, J Virol, 2008, 2012)
- SARS-CoV (van den Worm, PloS One, 2012)

**Accelerated Article Preview**

# **Rapid reconstruction of SARS-CoV-2 using a synthetic genomics platform**

Received: 20 February 2020

Accepted: 24 April 2020

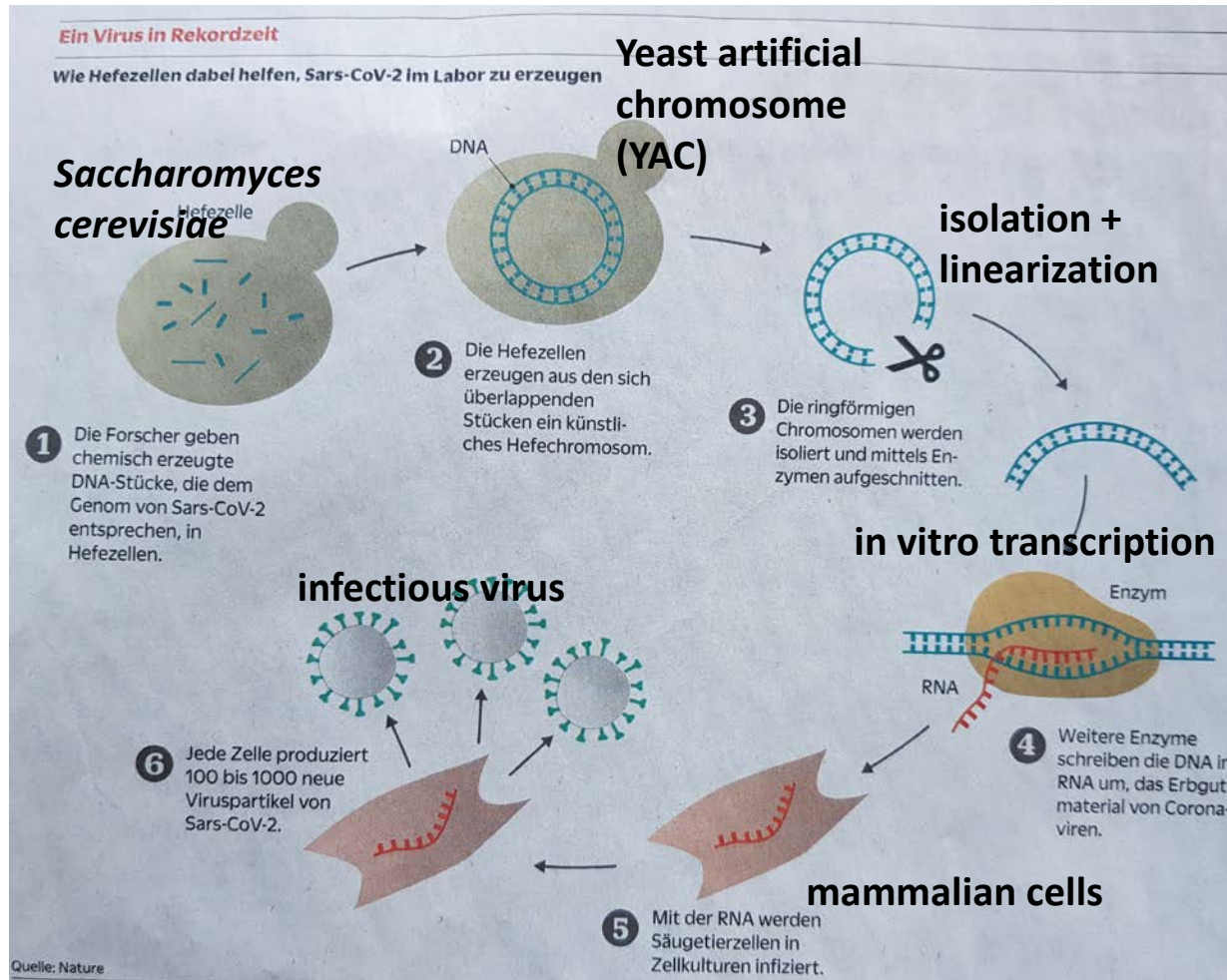
Accelerated Article Preview Published  
online 4 May 2020

Cite this article as: Thao, T. T. N. et al. Rapid reconstruction of SARS-CoV-2 using a synthetic genomics platform. *Nature* <https://doi.org/10.1038/s41586-020-2294-9> (2020).

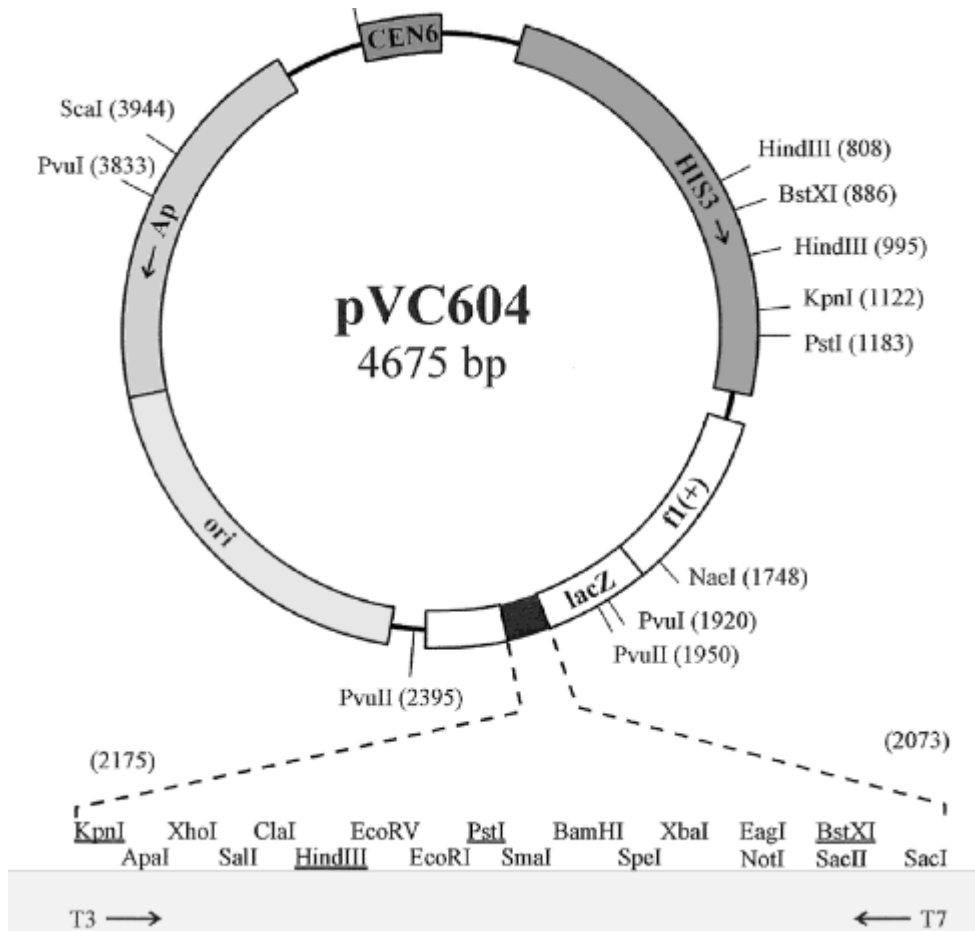
Tran Thi Nhu Thao, Fabien Labrousseau, Nadine Ebert, Philip V'kovski, Hanspeter Stalder, Jasmine Portmann, Jenna Kelly, Silvio Steiner, Melle Holwerda, Annika Kratzel, Mitra Gultom, Kimberly Schmied, Laura Laloli, Linda Hüsler, Manon Wider, Stephanie Pfaender, Dagny Hirt, Valentina Cippà, Silvia Crespo-Pomar, Simon Schröder, Doreen Muth, Daniela Niemeyer, Victor Corman, Marcel A. Müller, Christian Drosten, Ronald Dijkman, Joerg Jores & Volker Thiel

This is a PDF file of a peer-reviewed paper that has been accepted for publication. Although unedited, the content has been subjected to preliminary formatting. Nature is providing this early version of the manuscript as a service to our authors and

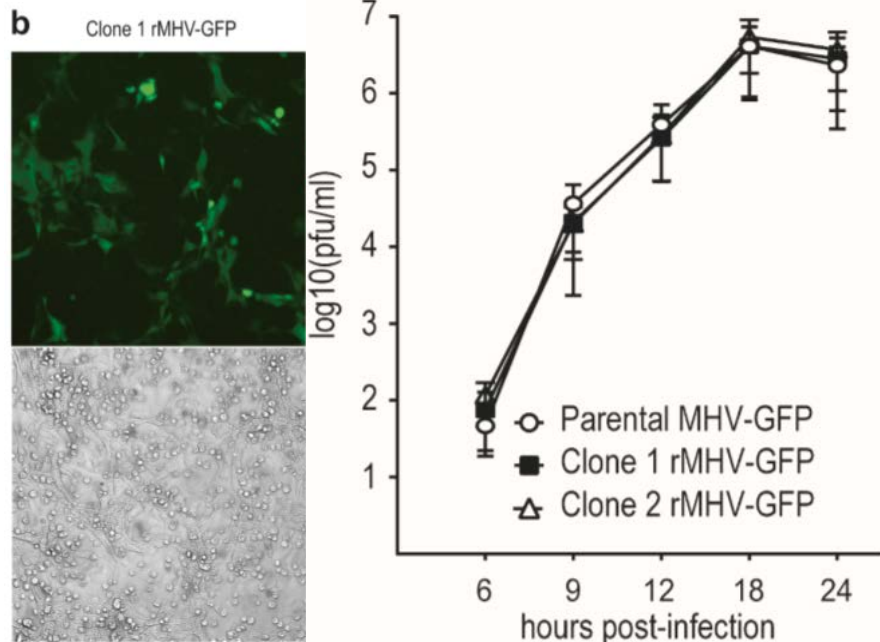
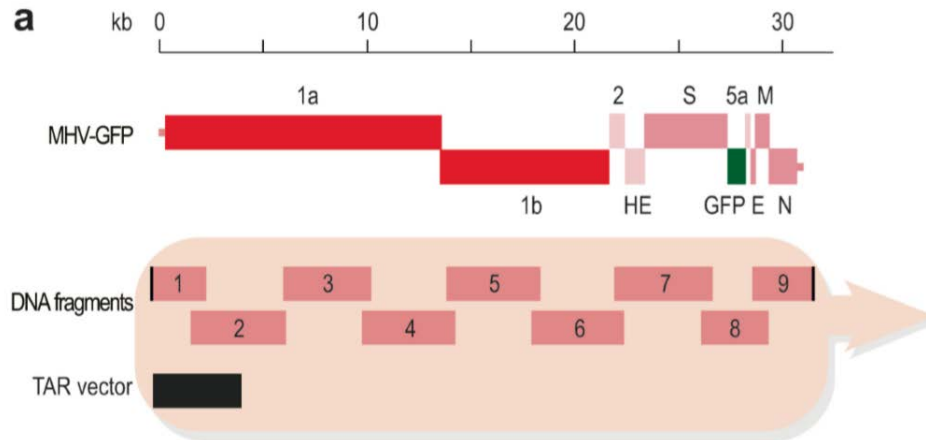
# Transformation-associated recombination (TAR) cloning



# TAR vector (Kouprina *et al.*)



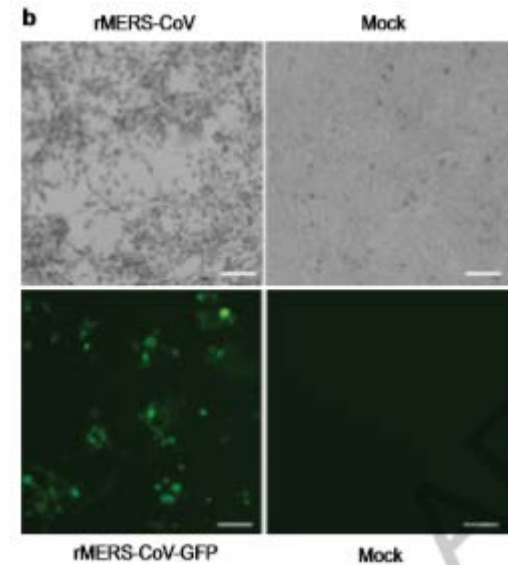
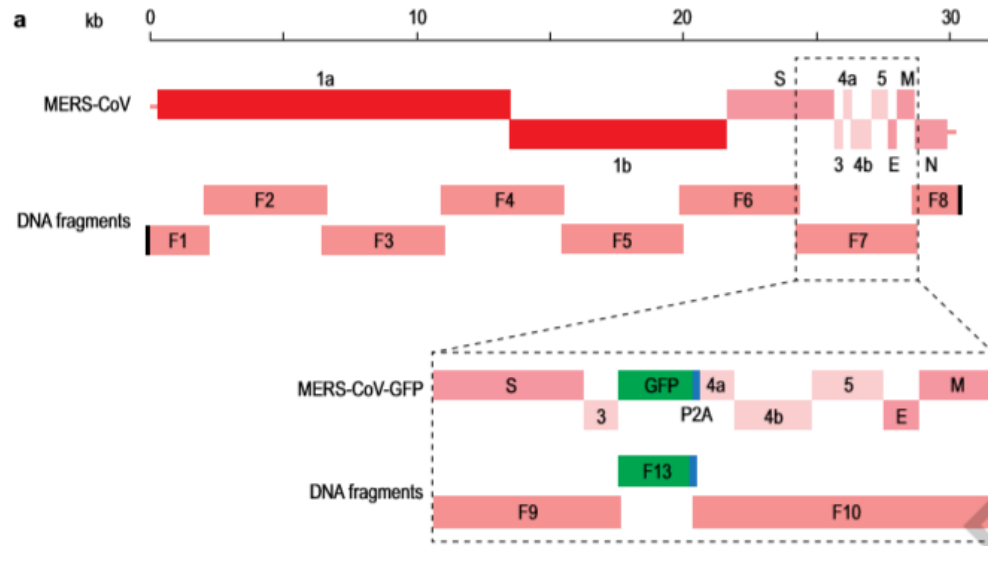
# Test platform with MHV-GFP



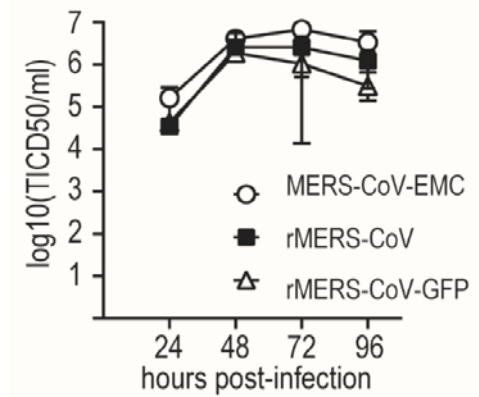
Virus rescue:

- 1) electroporation of **BHK-MHV-N** cells
- 2) co-cultivation with susceptible cells (17Cl-1)

# Application to other coronaviruses + mutagenesis



Synthetic genomics platform suitable to genetically modify coronavirus genomes.

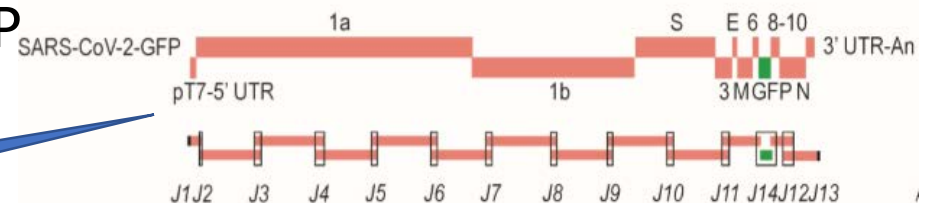
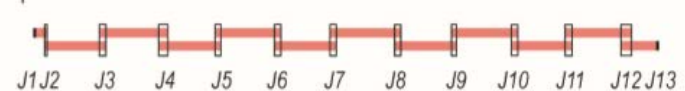
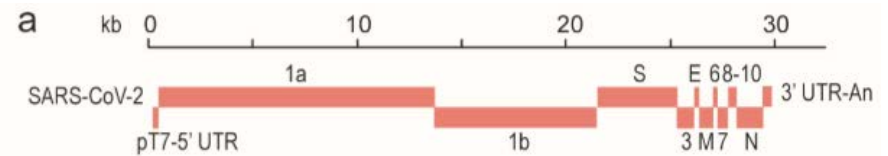


# Application to other viruses, families and sources

- Several other coronaviruses
- ZIKA virus (Flaviviridae)
- hRSV
- Cloning of nRSV-B from clinical sample (nasopharyngeal aspirate) without any prior information about the virus genotype using RSV consensus primers
- Synthetic genomics platform provided the technical advance to rapidly generate molecular clones of diverse RNA viruses by using virus isolates, cloned DNA, synthetic DNA or clinical samples as starting material.

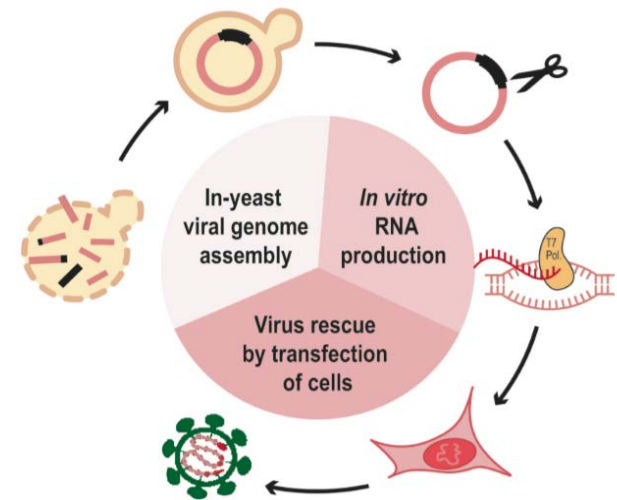
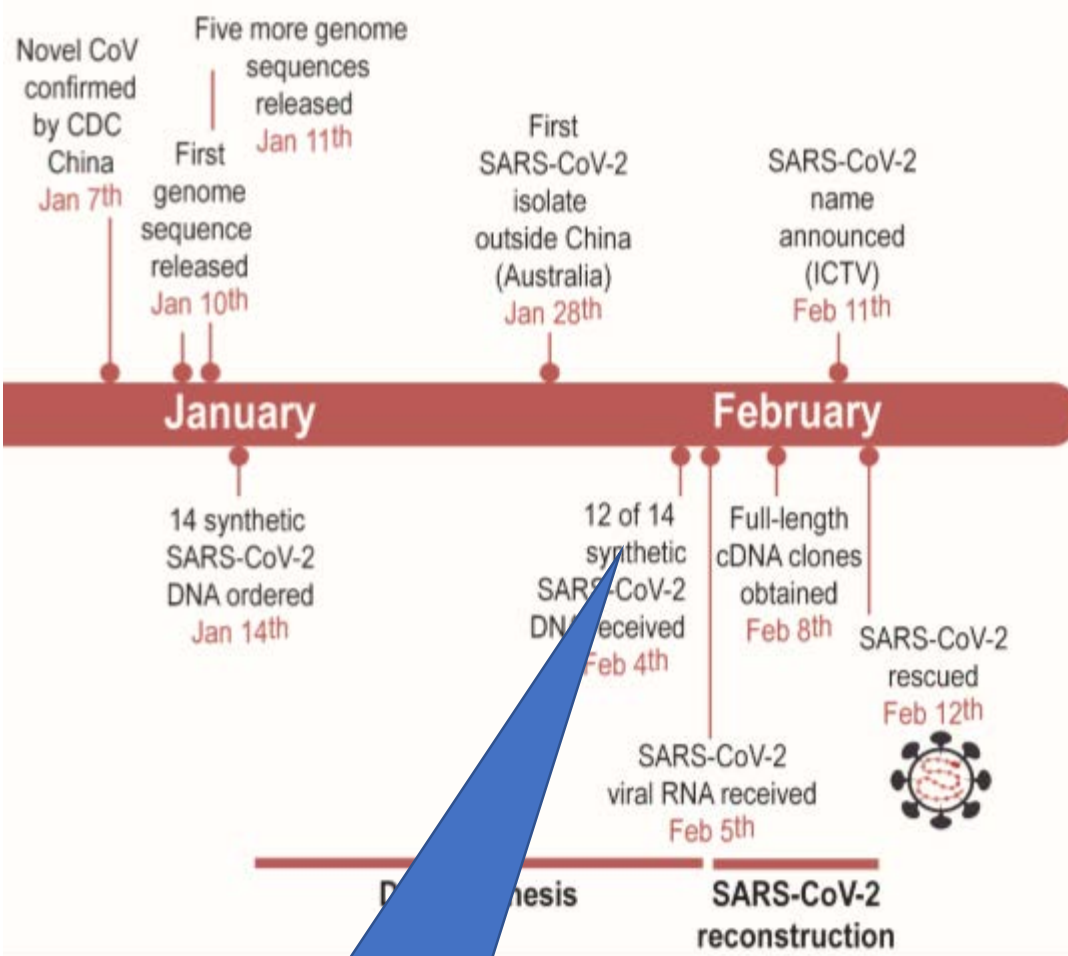
# SARS-CoV-2

- Genome sequences released 10.01.2020
- 12 overlapping DNA fragments
- In parallel: SARS-CoV-2-GFP

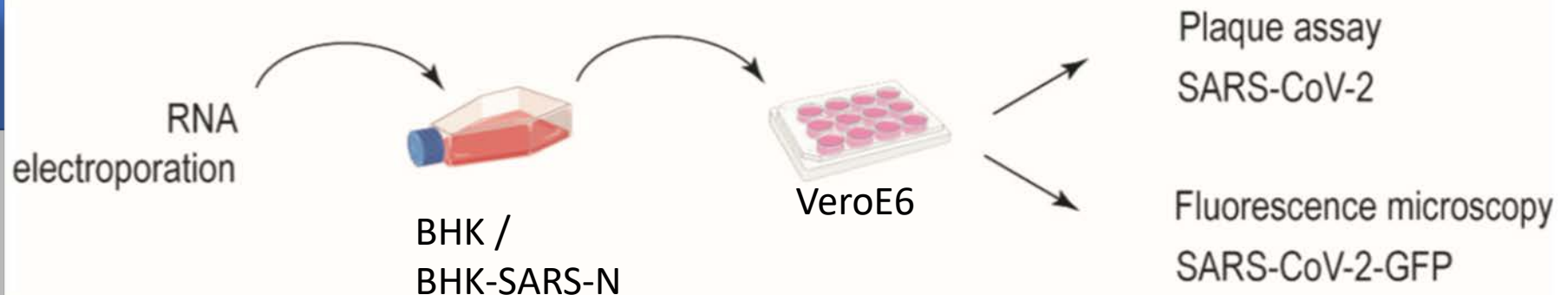


Variations in nt 3-5 >  
generate 3 versions +/- GFP





TAR cloning for all viruses > selection without sequence confirmation

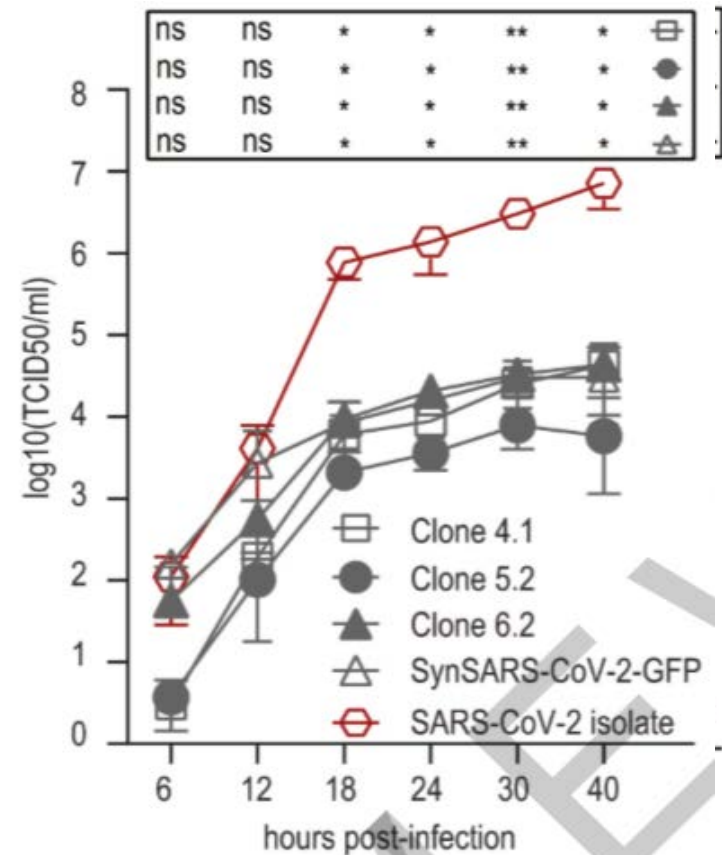


# Analysis of rSARS-CoV-2

- All cloned 5' ends retained
- similar replication kinetics

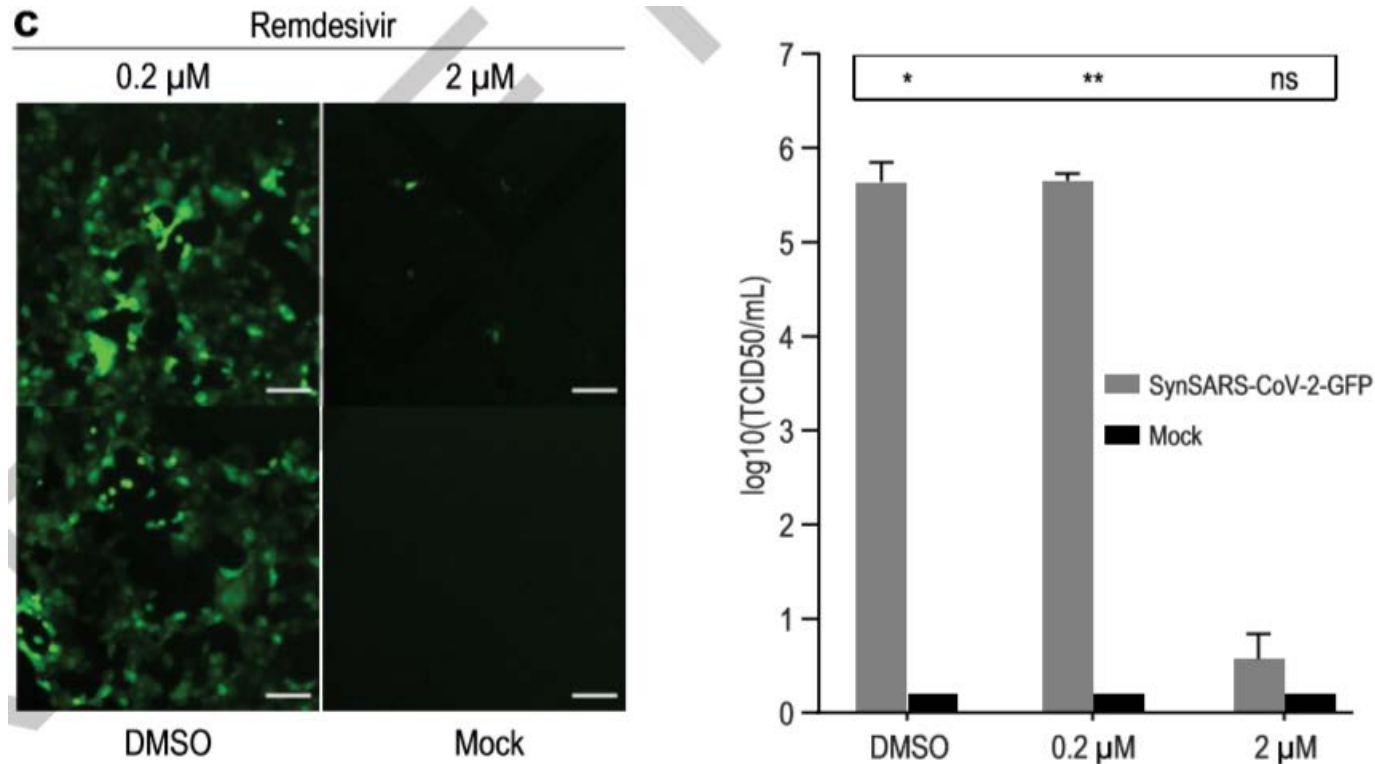
Replication affected by GFP  
and/or partial deletion of  
ORF

rSARS-CoV-2-GFP



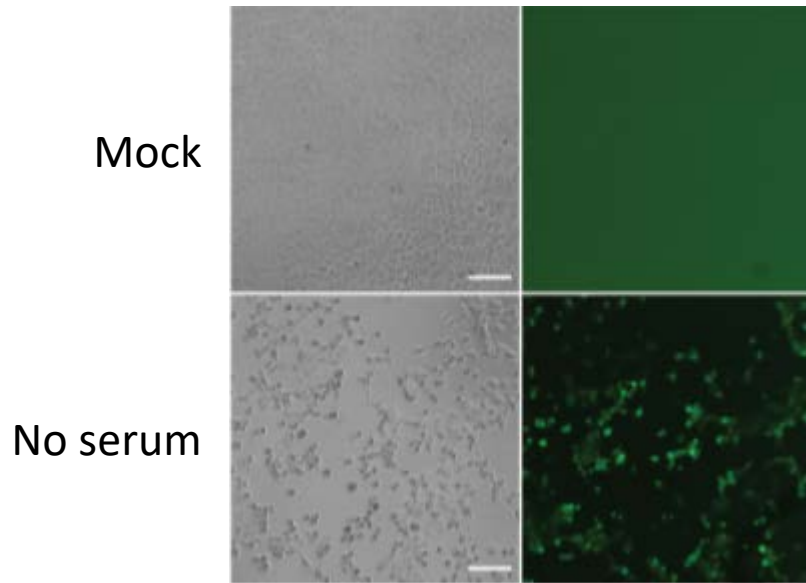
# Analysis of rSARS-CoV-2

- Simple GFP readout: Remdesivir



# Analysis of rSARS-CoV-2

- Simple GFP readout: serum

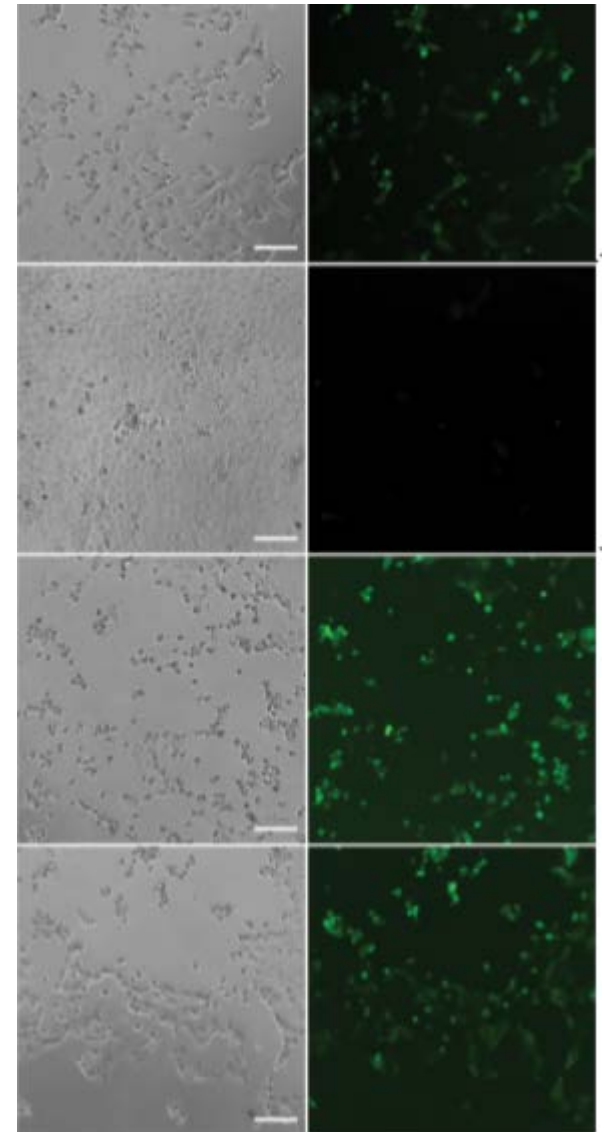


ctrl

Convalescent  
Human anti-  
SARS-CoV-2

Ctrl

Convalescent  
Human anti-  
SARS-CoV



# Synthetic genomics platform

- Provide infectious virus to health authorities and diagnostic laboratories without the need of having access to clinical samples
- Rapid introduction of emerging sequence variations into infectious clones > functional characterization

# Ethical concerns

- Synthesis of viruses
- Synthetic life

«If the ability to replicate is an attribute of life, then poliovirus is a chemical ( $\text{C}_{332,652}\text{H}_{492,388}\text{O}_{131,196}\text{-P}_{7501}\text{S}_{2340}$ ) with a life cycle.»

Thank you for your attention!

# Coronavirus Life Cycle

