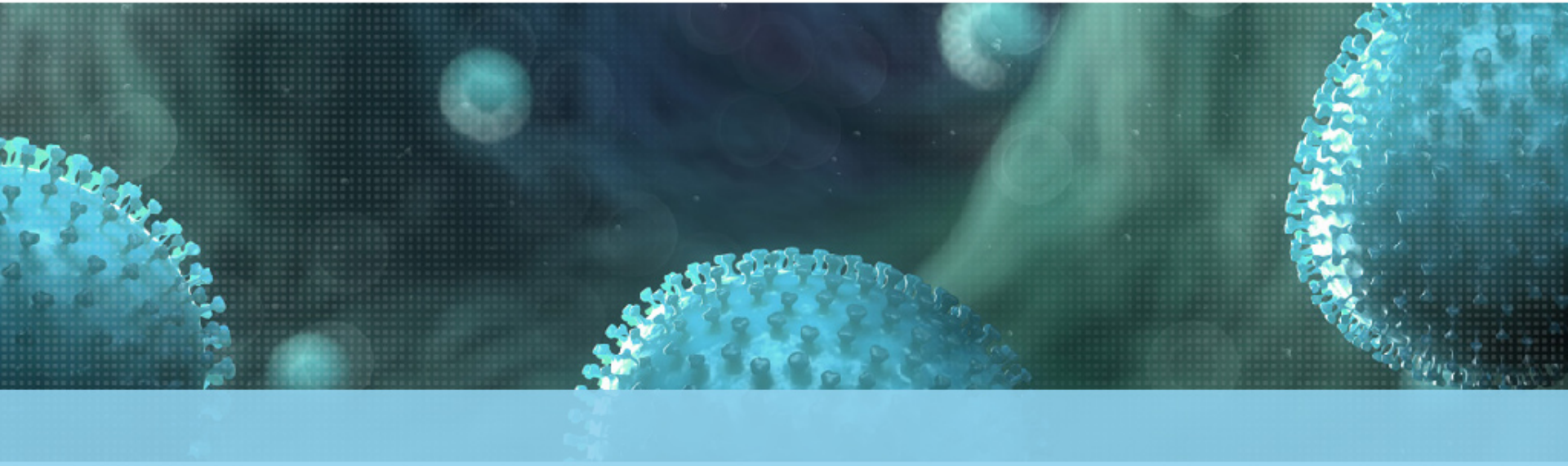
Erasmus MC

Viroscience lab

WHERE SKILLS MEET TO STUDY & PROTECT



Virus discovery

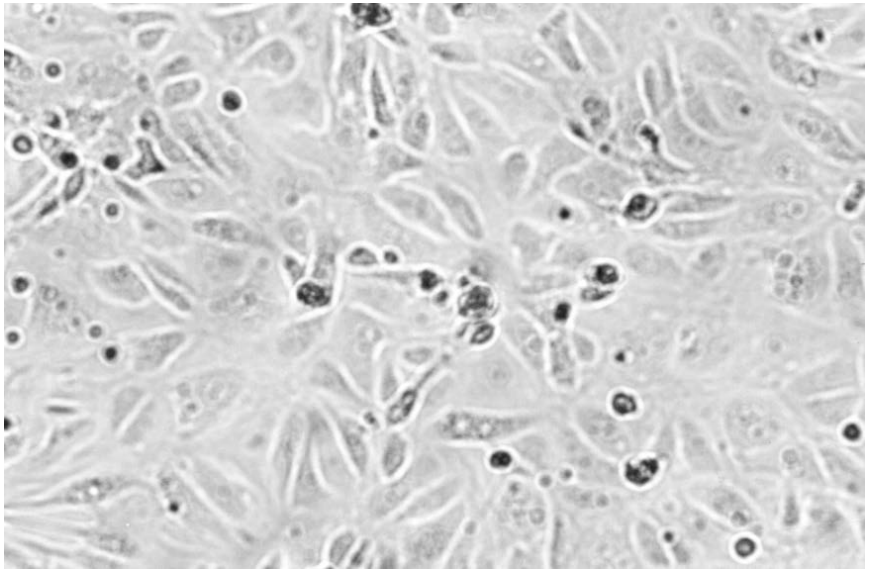
Ron A.M. Fouchier, PhD

Virus discovery, historical
- Required expertise -

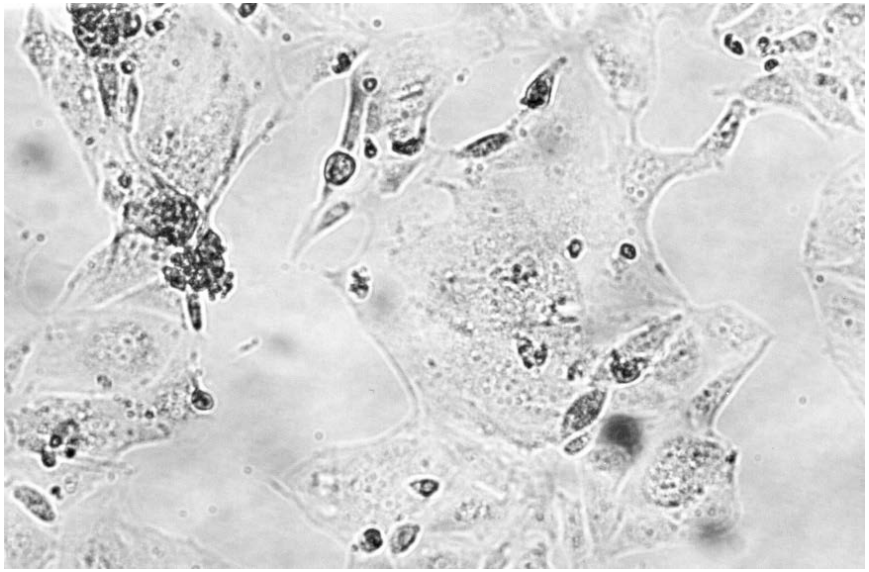
- Clinical diagnosis
- Pathology
- Epidemiology
- Laboratory
  - Classical virology
  - Electron microscopy
  - Serology
  - Animal models
  - Molecular biology

Cytopathic effect in cell cultures
- Classical virology (2001) -

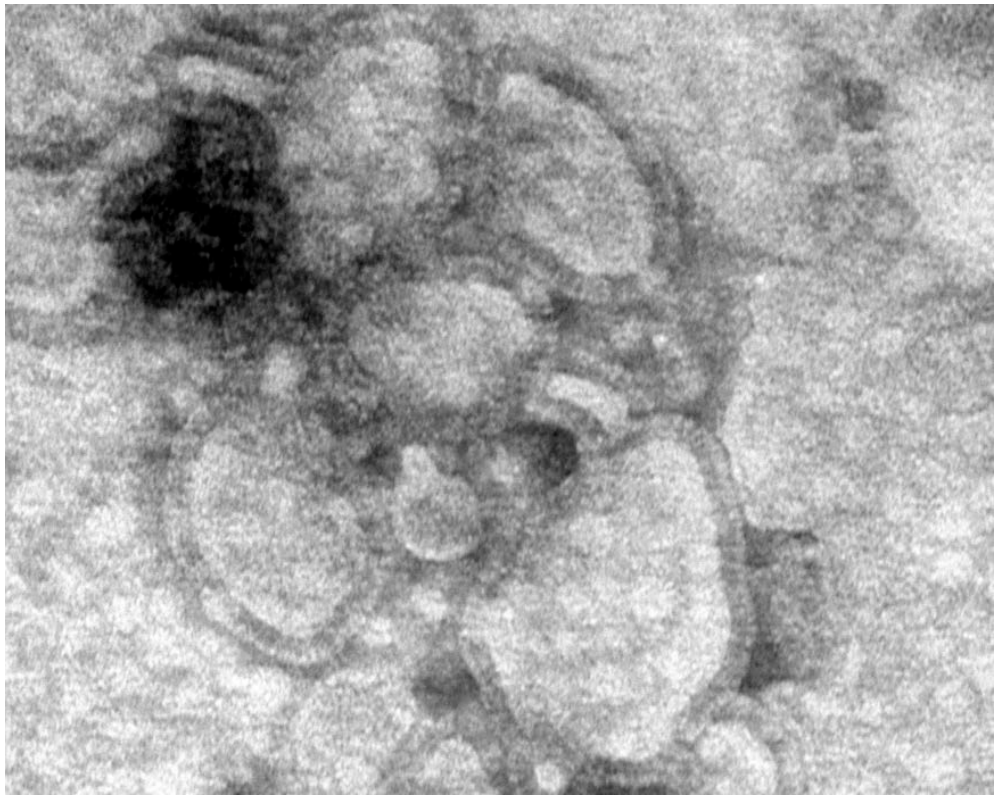
Control tMK



Infected tMK



Newly discovered paramyxovirus
- Electron microscopy -



- Pleiomorph

- Average size: 100-600 nm

- Envelope projections: 13-17 nm

- Paramyxovirus

Differential virus diagnostics

Negative results:

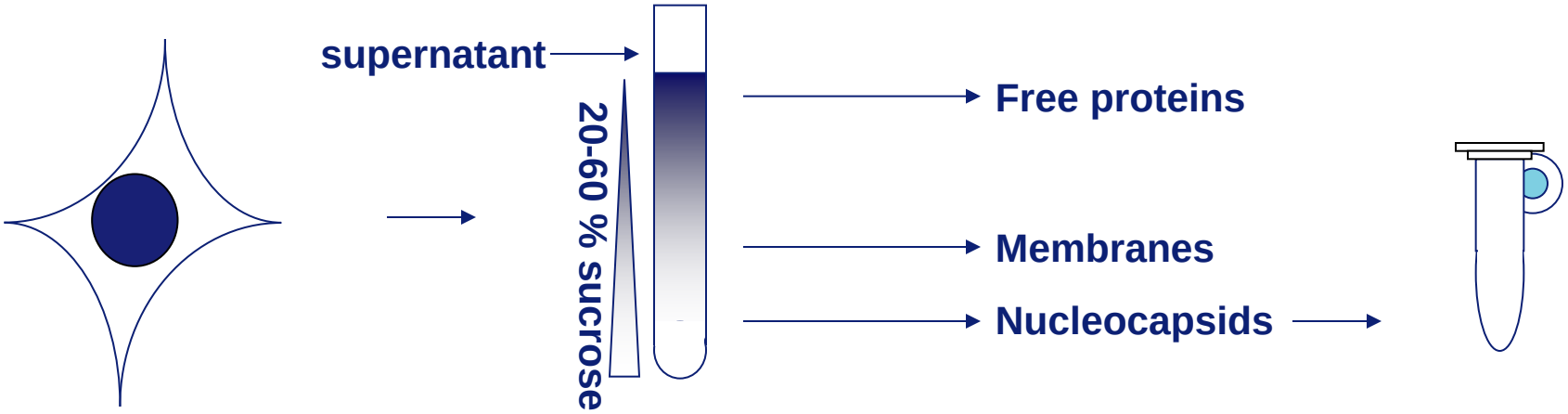
Serology

- Influenza A virus
- Influenza B virus
- Influenza C virus
- RSV
- hPIV-1
- hPIV-2
- hPIV-3
- hPIV-4
- PVM

Low stringency PCR

- Influenza A virus
- Influenza B virus
- RSV
- hPIV-1
- hPIV-2
- hPIV-3
- hPIV-4
- Sendai virus
- SV5
- Mumps virus
- Measles virus
- NDV
- Hendra
- Nipah

Purification of vRNA of an unidentified virus



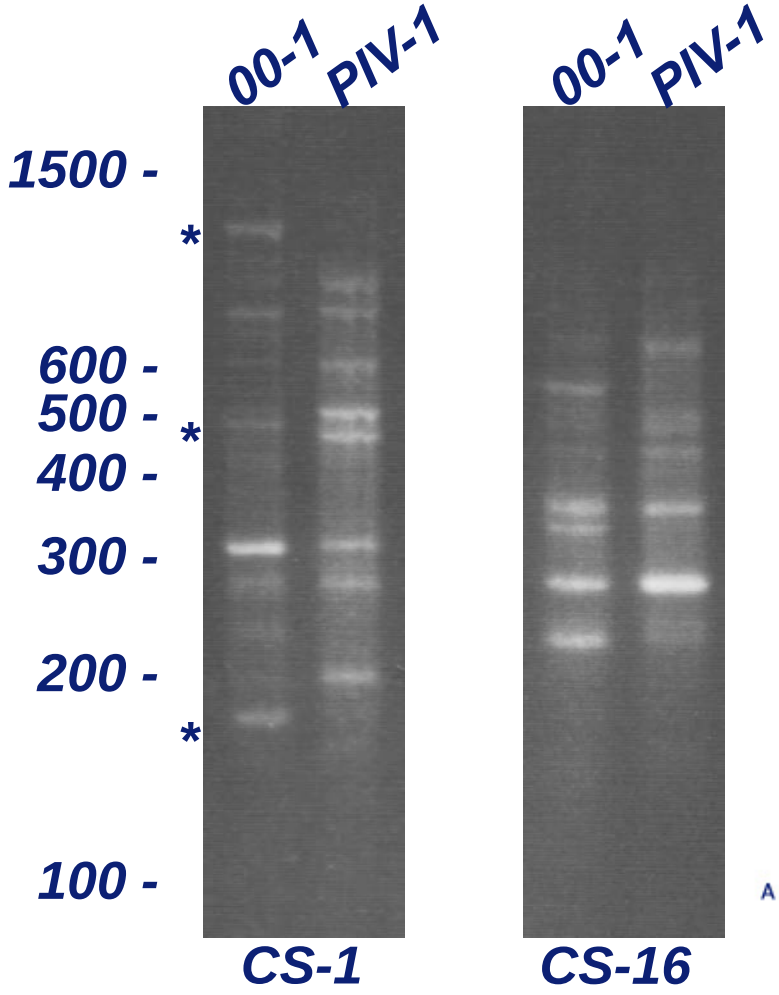
Infected tMK cells (hPIV-1 or 00-1)

Ultracentrifuge

Electron microscopy and SDS-PAGE (Ag-stain)

RNA isolation and RAP-PCR

Virus identification
- RAP PCR -



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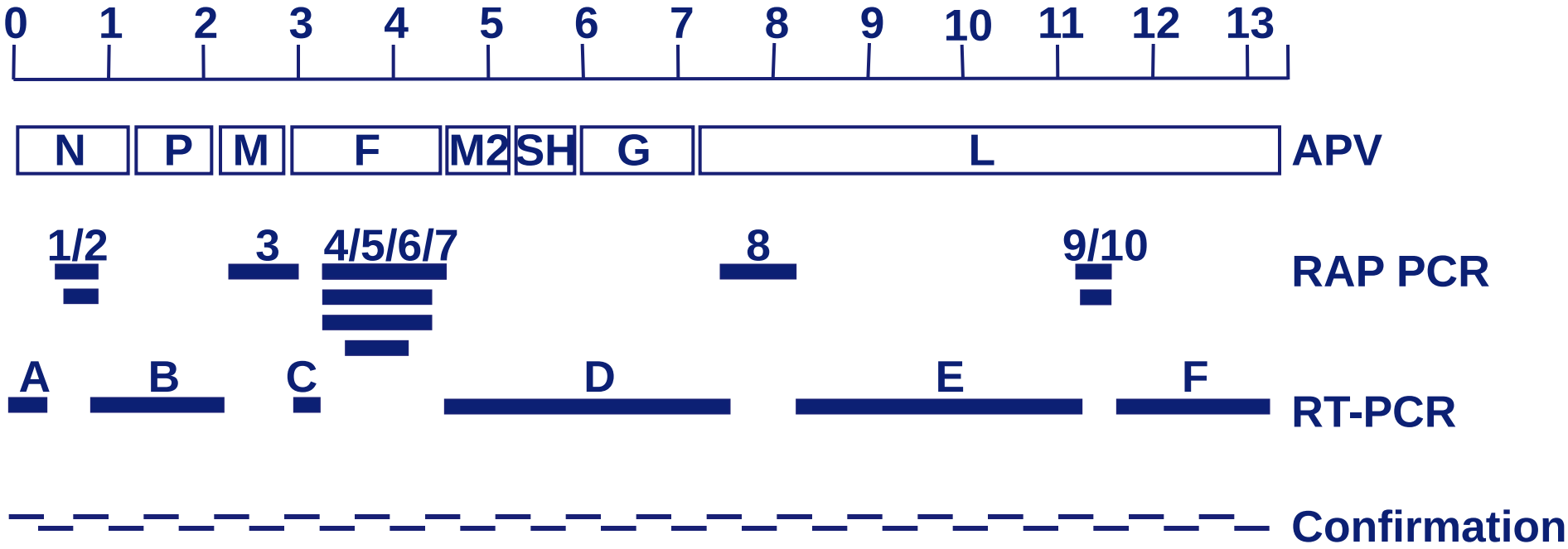
Identification of a new paramyxovirus
- Genome organisation -



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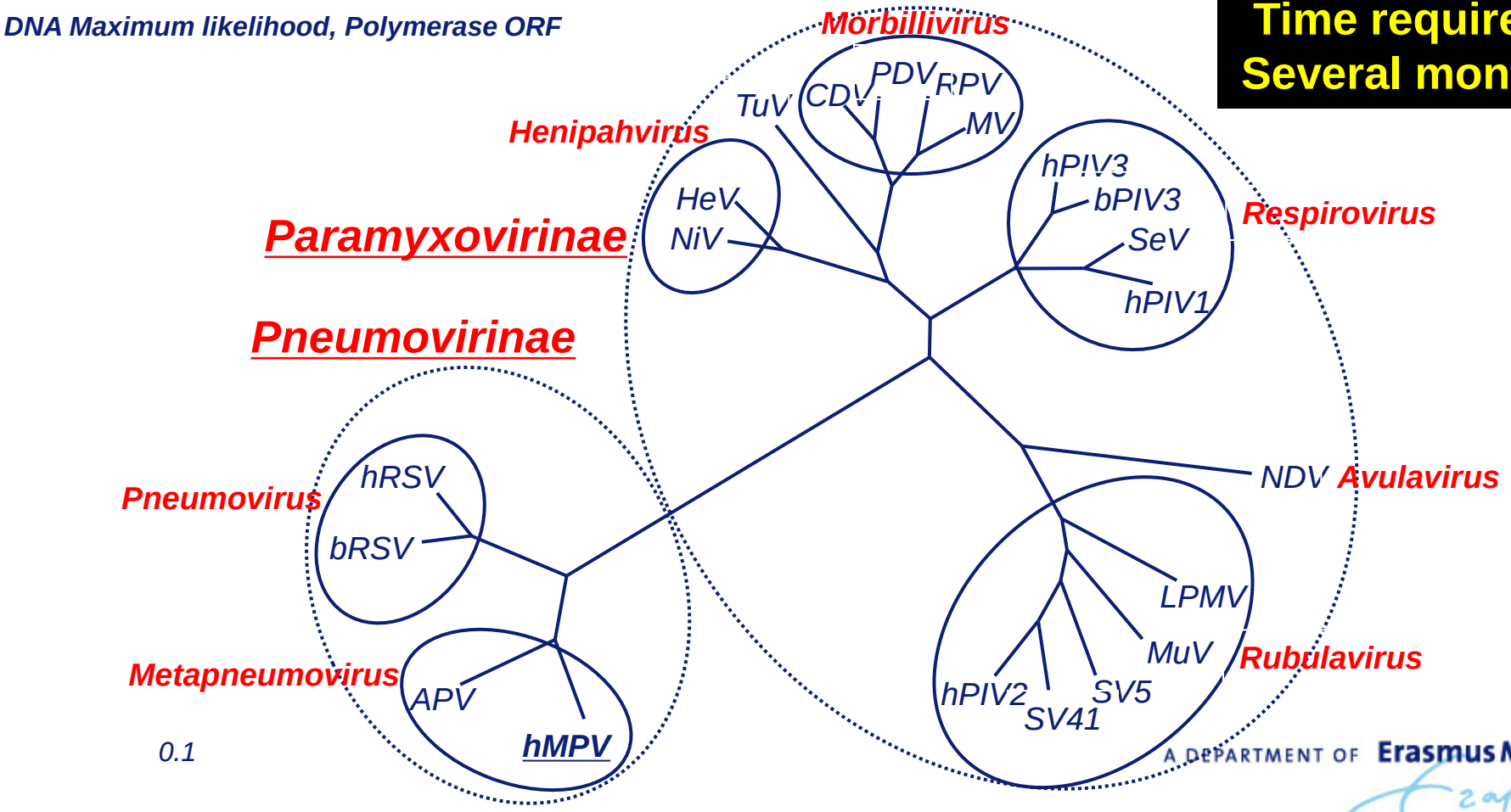
B.G. Van den Hoogen et al., Nature Medicine 2001

Order Mononegavirales, family Paramyxoviridae

Time required: Several months



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B.G. Van den Hoogen et al., Nature Medicine 2001

Erasmus MC
Universitair Medisch Centrum Rotterdam

SARS

Severe Acute Respiratory Syndrome

Genome of the SARS-associated coronavirus - 2003 -

Time required: Several weeks

Sciencexpress Research Article

The Genome Sequence of the SARS-Associated Coronavirus

Marco A. Marra,[1]* Steven J. M. Jones,[1] Caroline R. Astell,[1] Robert A. Holt,[1] Angela Brooks-Wilson,[1] Yaron S. N. Butterfield,[1] Jaswinder Khattra,[1] Jennifer K. Asano,[1] Sarah A. Barber,[1] Susanna Y. Chan,[1] Alison Cloutier,[1] Shaun M. Coughlin,[1] Doug Freeman,[1] Noreen Girn,[1] Obi L. Griffith,[1] Stephen R. Leach,[1] Michael Mayo,[1] Helen McDonald,[1] Stephen B. Montgomery,[1] Pawan K. Pandoh,[1] Anca S. Petrescu,[1] A. Gordon Robertson,[1] Jacqueline E. Schein,[1] Asim Siddiqui,[1] Duane E. Smailus,[1] Jeff M. Stott,[1] George S. Yang[1]

Francis Plummer,[2] Anton Andonov,[2] Harvey Artsob,[2] Nathalie Bastien,[2] Kathy Bernard,[2] Timothy F. Booth,[2] Donnie Bowness,[2] Michael Drebot,[2] Lisa Fernando,[2] Ramon Flick,[2] Michael Garbutt,[2] Michael Gray,[2] Allen Grolla,[2] Steven Jones,[2] Heinz Feldmann,[2] Adrienne Meyers,[2] Amin Kabani,[2] Yan Li,[2] Susan Normand,[2] Ute Stroher,[2] Graham A. Tipples,[2] Shaun Tyler,[2] Robert Vogrig,[2] Diane Ward,[2] Brynn Watson[2]

Robert C. Brunham,[3] Mel Krajden,[3] Martin Petric,[3] Danuta M. Skowronski[3]

Chris Upton,[4] Rachel L. Roper[4]

Sciencexpress Research Article

Characterization of a Novel Coronavirus Associated with Severe Acute Respiratory Syndrome

Paul A. Rota,[1]* M. Steven Oberste,[1] Stephan S. Monroe,[1] W. Allan Nix,[1] Ray Campagnoli,[1] Joseph P. Icenogle,[1] Silvia Peñaranda,[1] Bettina Bankamp,[1] Kaija Maher,[1] Min-hsin Chen,[1] Suxiong Tong,[1] Azaibi Tamin,[1] Luis Lowe,[1] Michael Frace,[1] Joseph L. DeRisi,[2] Qi Chen,[1] David Wang,[2] Dean D. Erdman,[1] Teresa C. T. Peret,[1] Cara Burns,[1] Thomas G. Ksiazek,[1] Pierre E. Rollin,[1] Anthony Sanchez,[1] Stephanie Liffick,[1] Brian Holloway,[1] Josef Limor,[1] Karen McCaustland,[1] Melissa Olsen-Rassmussen,[1] Ron Fouchier,[3] Stephan Günther,[4] Albert D. M. E. Osterhaus,[3] Christian Drosten,[4] Mark A. Pallansch,[1] Larry J. Anderson,[1] William J. Bellini[1]

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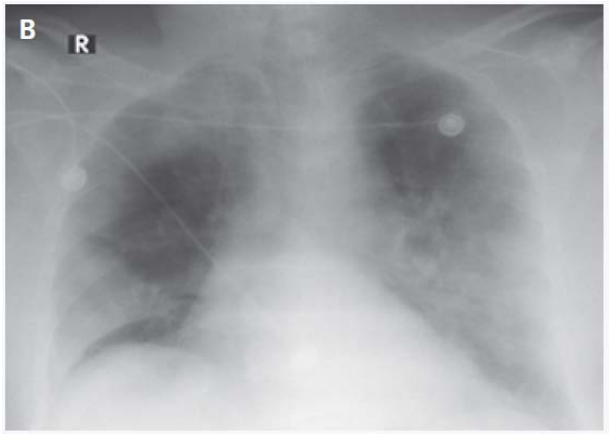
Novel coronavirus, 2012
- Middle East Respiratory Syndrome (MERS) -

The NEW ENGLAND JOURNAL of MEDICINE

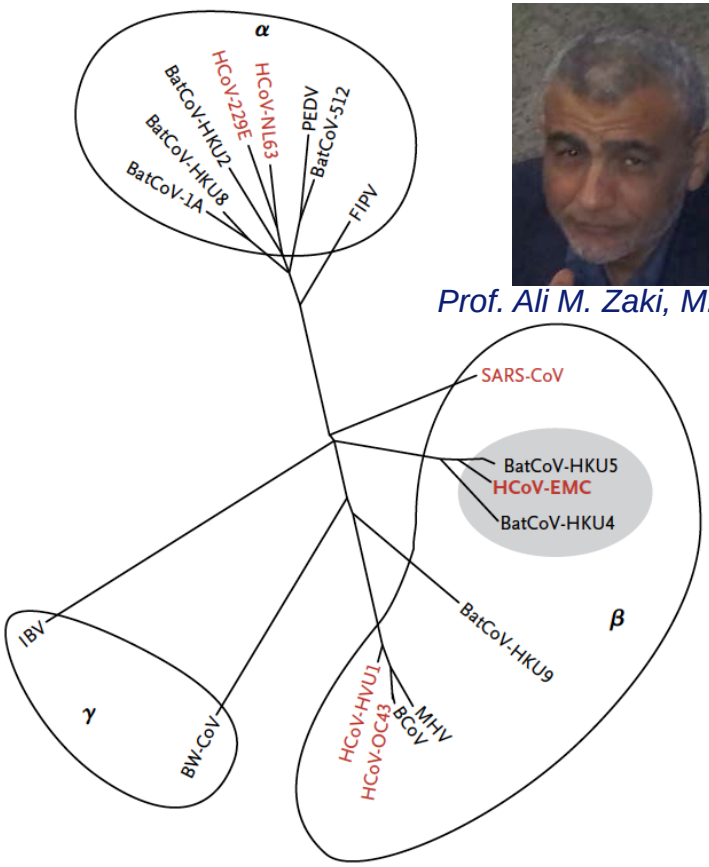
BRIEF REPORT

Isolation of a Novel Coronavirus from a Man with Pneumonia in Saudi Arabia

Ali Moh Zaki, M.D., Ph.D., Sander van Boheemen, M.Sc., Theo M. Bestebroer, B.Sc., Albert D.M.E. Osterhaus, D.V.M., Ph.D., and Ron A.M. Fouchier, Ph.D.



Prof. Ali M. Zaki, M.D.



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Zaki et al., N Engl J Med. 2012 367:1814-20

Novel coronavirus, Saudi Arabia
- 2012 -

- Virus @ Vero passage 5
- Cleaning
- Arbitrarily primed PCR
- 454 jr sequencer
- 87,256 sequence reads (>90% of genome)
- PCR 800 bp fragments, 100 bp overlap
- 3130XL capillary sequencer
- 5’ and 3’ RACE

A.M. Zaki et al., N.E.J.M. 2012
S. Van Boheemen et al., MBio 2012

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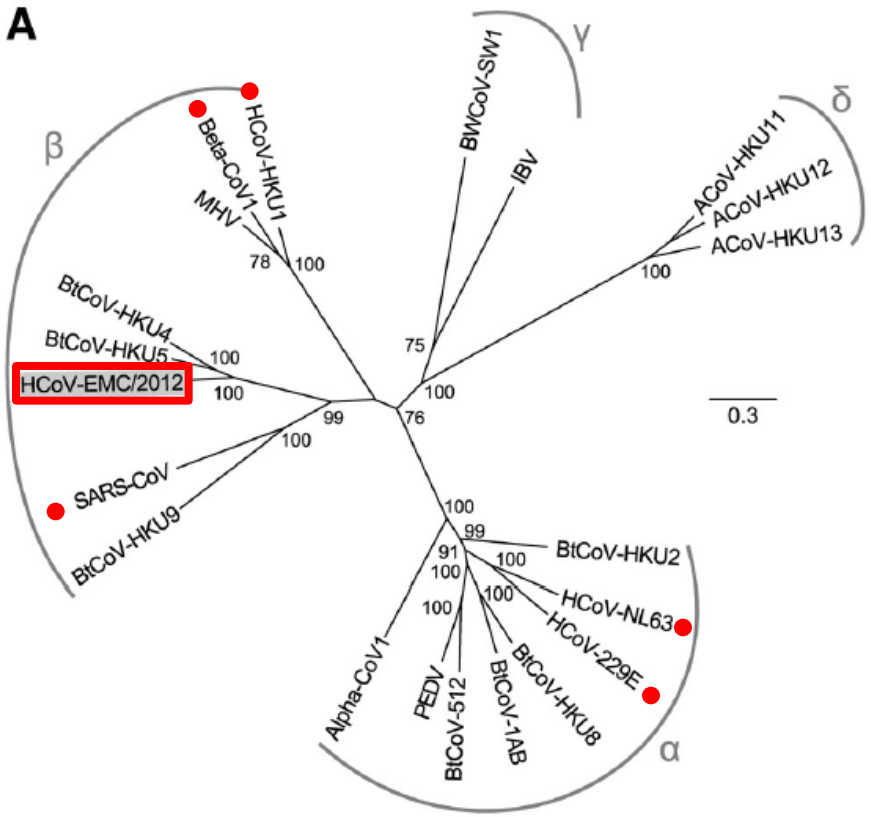
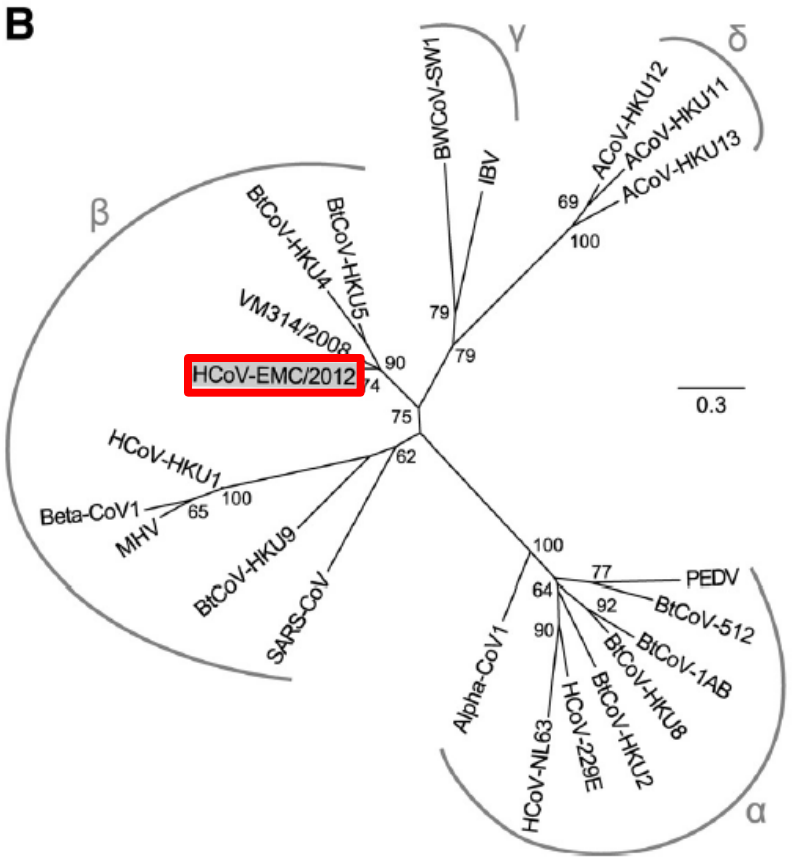
EUROPEAN MANAGEMENT PLATFORM FOR EMERGING AND RE-EMERGING INFECTIOUS DISEASE ENTITIES

Novel coronavirus
- Phylogeny -





Time required: Few days

S. Van Boheemen et al., MBio 2012

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Virus discovery
- NGS -

Opportunities

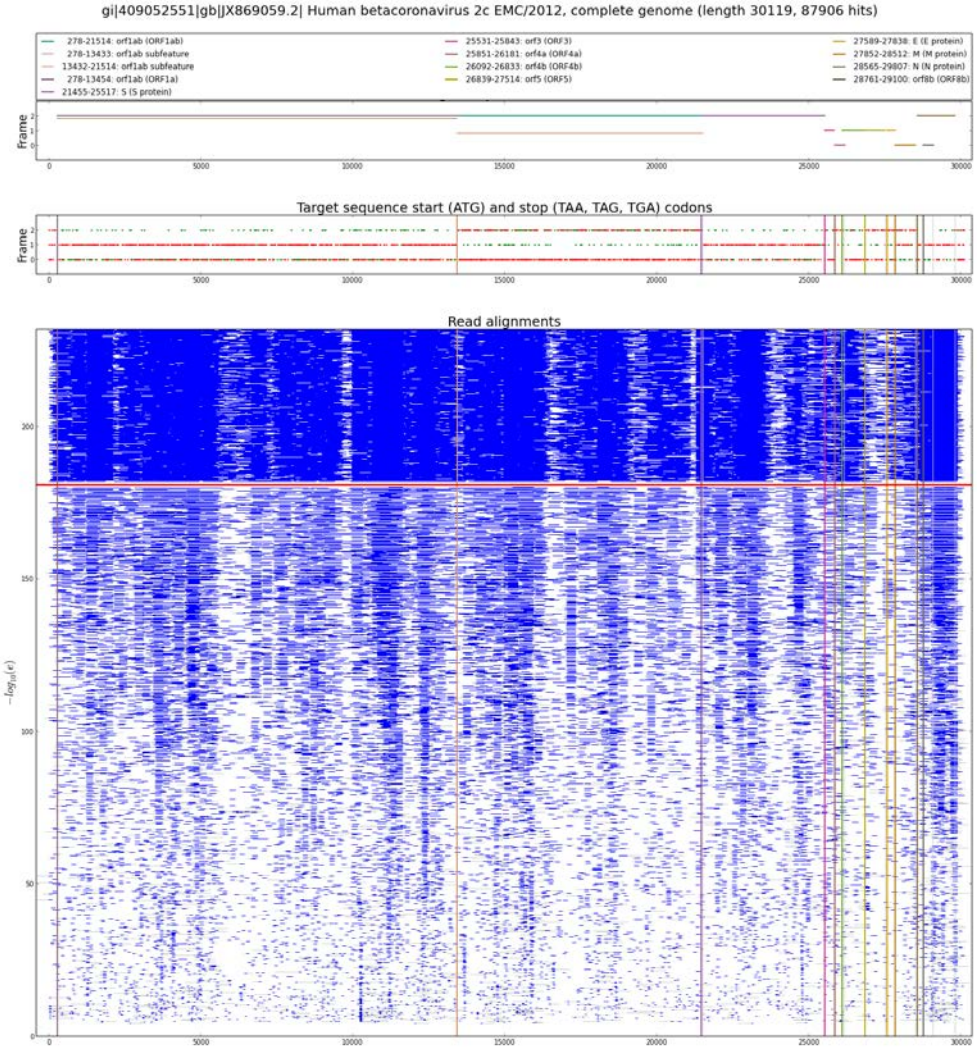
- *Fast, random, uninformed generation of pathogen sequence data*
- *Multiple samples at same time, systematic screens*
- *Multiple tissues, specimen collections, hosts*
- *No viable material needed & no need for pathogen isolation*
- *Multiple platforms, more coming*
- *Linking diseases of humans, animals, plants with unknown bugs*
- *….*

Virus discovery
- NGS -

Challenges

- *Shift in bottleneck from laboratory work to bioinformatics*

Bioinformatics challenges; MERS-CoV
- Blasting against self -



Terry Jones, Zoology,
University of Cambridge UK

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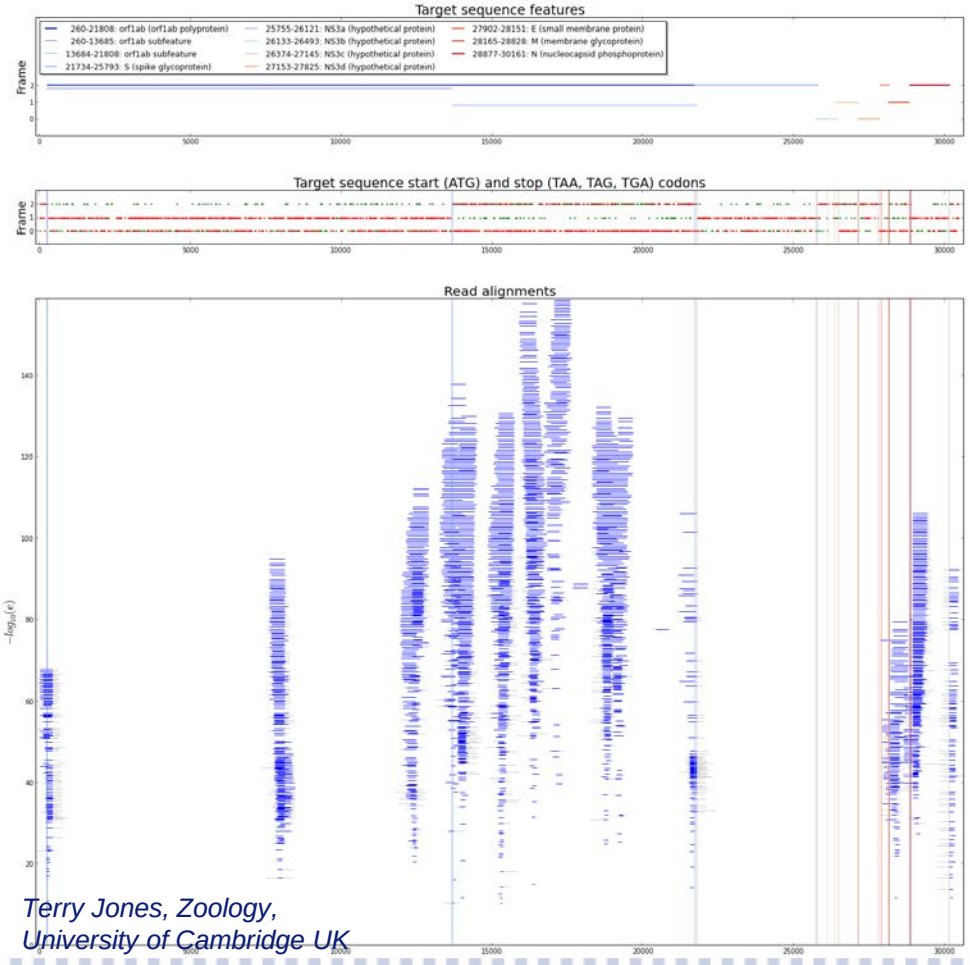
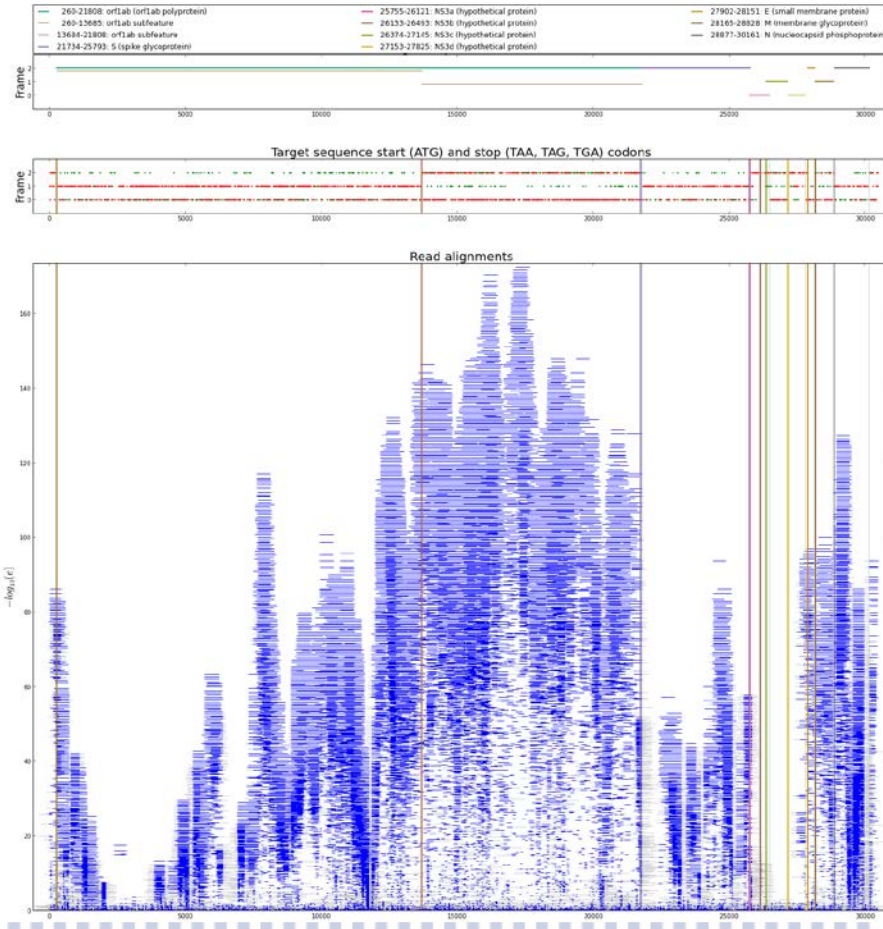
Bioinformatics challenges; MERS-CoV - Blasting with Megablast, blastn -

gi|124389437|gb|EF065509.1| Bat coronavirus HKU5-1, complete genome (length 30482, 9713 hits)



gi|124389437|gb|EF065509.1| Bat coronavirus HKU5-1, complete genome (length 30482, 88940 hits)



Terry Jones, Zoology,
University of Cambridge UK

Virus discovery, metagenomics
- NGS -

Challenges (hands-on work)

- *Shift in bottleneck from laboratory work to bioinformatics*
- *Real-time analysis (lab and computer)*
- *Association versus causation of disease (availability of cultured bugs)*
- *Omnipresence of bugs (e.g. plant viruses in mammal gut, ERVs, etc)*
- *Partial (virus) genome data*
- *Host sequence info (e.g. rRNA)*
- *….*

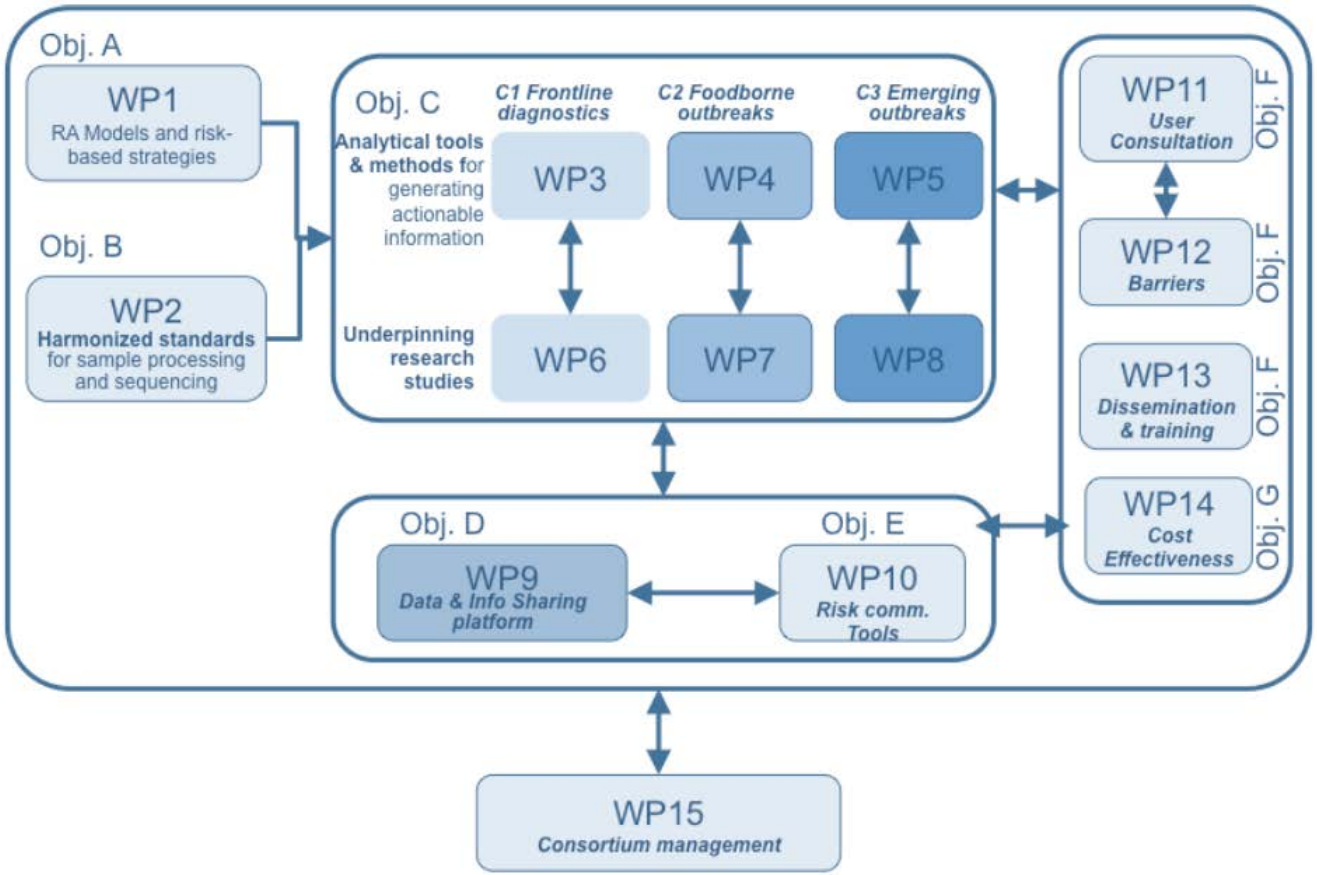
Virus discovery, metagenomics
- NGS -

Challenges (databases)

- *Sensitive info hidden in datasets (undiscovered bugs, personal genomes)*
- *Lack of uniformity of output, QA/QC*
- *Changing platforms*
- *SNP reporting*
- *Assembly/raw*
- *Amounts of data*
- *….*

Pathogen NGS
- EU Horizon-2020 COMPARE -

COllaborative **M**anagement **P**latform for detection and **A**nalyses of (**R**e-) **E**merging and foodborne outbreaks



EU No. 643476

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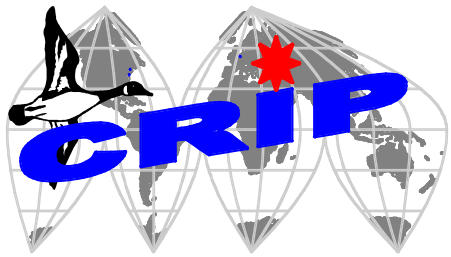
Acknowledgements



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