Twitter Thread by Billy Bostickson ■■&■ ■

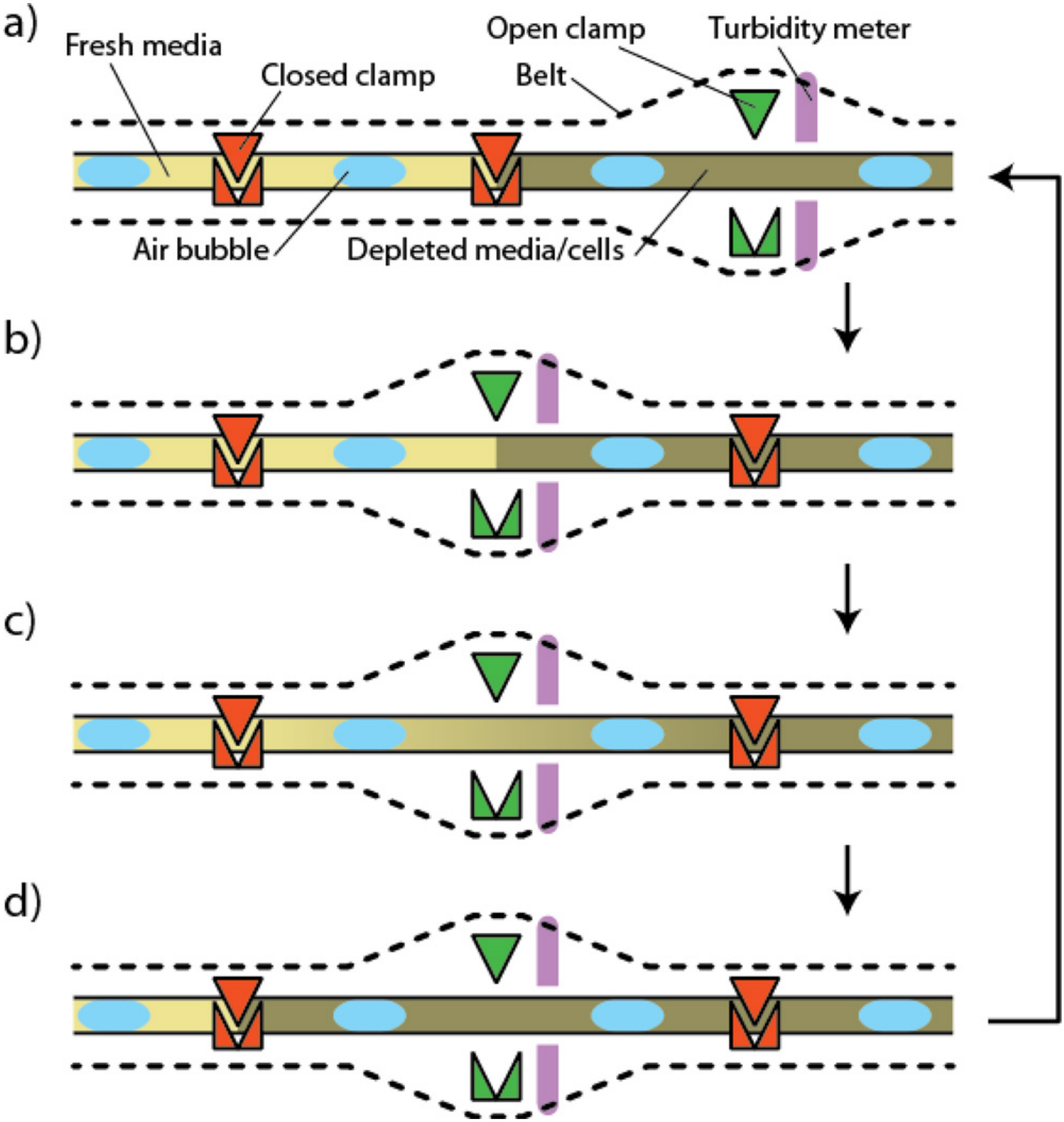


Billy Bostickson ■■&■ ■
@BillyBostickson

1/ Directed Evolution and Serial passage of RNA Viruses

A quick (I hope) thread on RNA viruses & some others, mainly for my own learning process, but also as evidence (as if it was needed) of what modern virology & microbiology is capable of doing to viruses in their laboratories

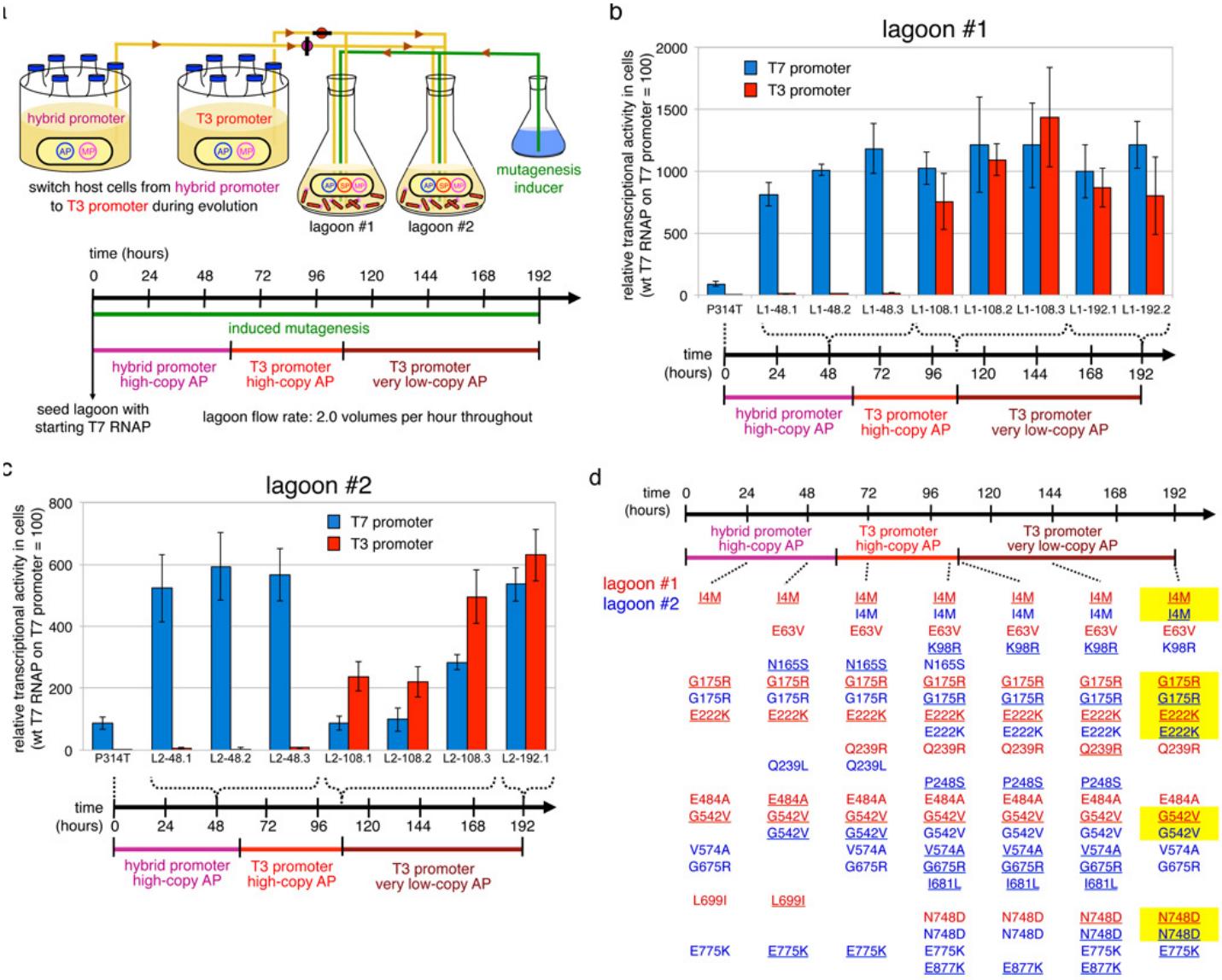
2/ In Vivo Continuous Directed Evolution
1 Recent advances in the field of in vivo continuous evolution
2 Advances in continuous culture of microbes enable continuous evolution
3 Viruses, prokaryotes, & eukaryotes mediate in vivo continuous evolution
https://t.co/BfR0B2Hwsf



3/ Continuous Directed Evolution of Biomolecules

By greatly accelerating laboratory evolution, PACE may provide solutions to otherwise intractable directed evolution problems and address novel questions about molecular evolution.

https://t.co/G3mIptI54H



4/ CDE for Strain & Protein Engineering

Inducible hypermutator systems accelerate strain evolution without compromising genome integrity.

Site-specific in vivo mutagenesis strategies have facilitated continuous protein engineering.

Automation possible!

https://t.co/pCxT250sKW

5/ Targeted mutagenesis:

A sniper-like diversity generator in microbial engineering

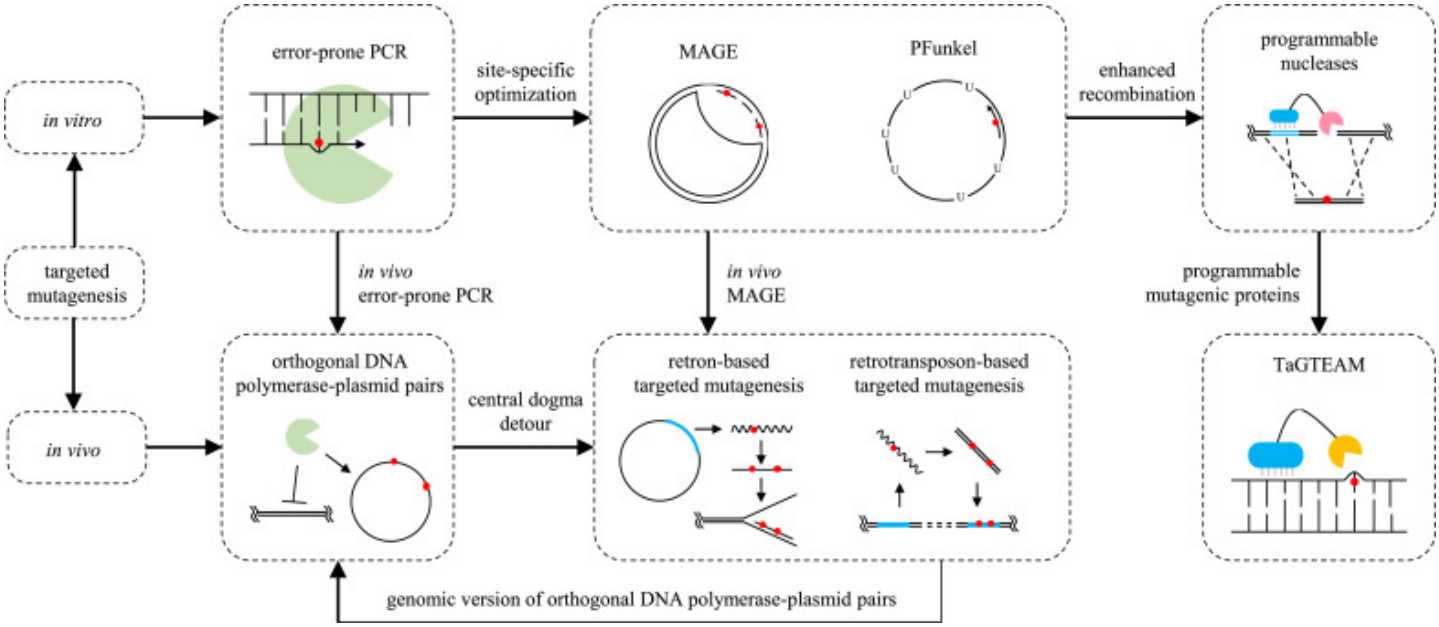
https://t.co/wdKyXghRYe

Advances in the directed evolution of proteins

https://t.co/1xXTtw1i3i

In vivo continuous directed evolution

https://t.co/xKRVSjVvo4



6/ Adaptable Platform for Directed Evolution in Human Cells
dynamic range for DE increased via application of small-molecule adenoviral protease inhibitor to modulate selection pressure during DE experiments. (paywall)
https://t.co/HHSm0u3I4m
support info:
https://t.co/F29aEZfkTU

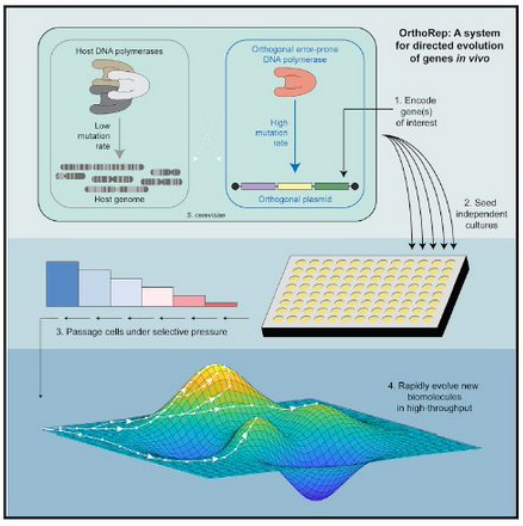
7/ Scalable, Continuous Evolution of Genes at Mutation
Rates above Genomic Error Thresholds:
(nice infographics, see attached images)
https://t.co/CNidbmviSR
Optimization of enzymes via directed evolution in vivo (thesis)
https://t.co/Cq7m8aDgVb

Resource

Cell

Scalable, Continuous Evolution of Genes at Mutation Rates above Genomic Error Thresholds

Graphical Abstract



Authors

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In Brief

An orthogonal DNA replication system with high mutagenicity allows for continuous directed evolution and investigation into experimental evolutionary trajectories, including the adaptive landscape of malarial drug resistance.

SUMMARY

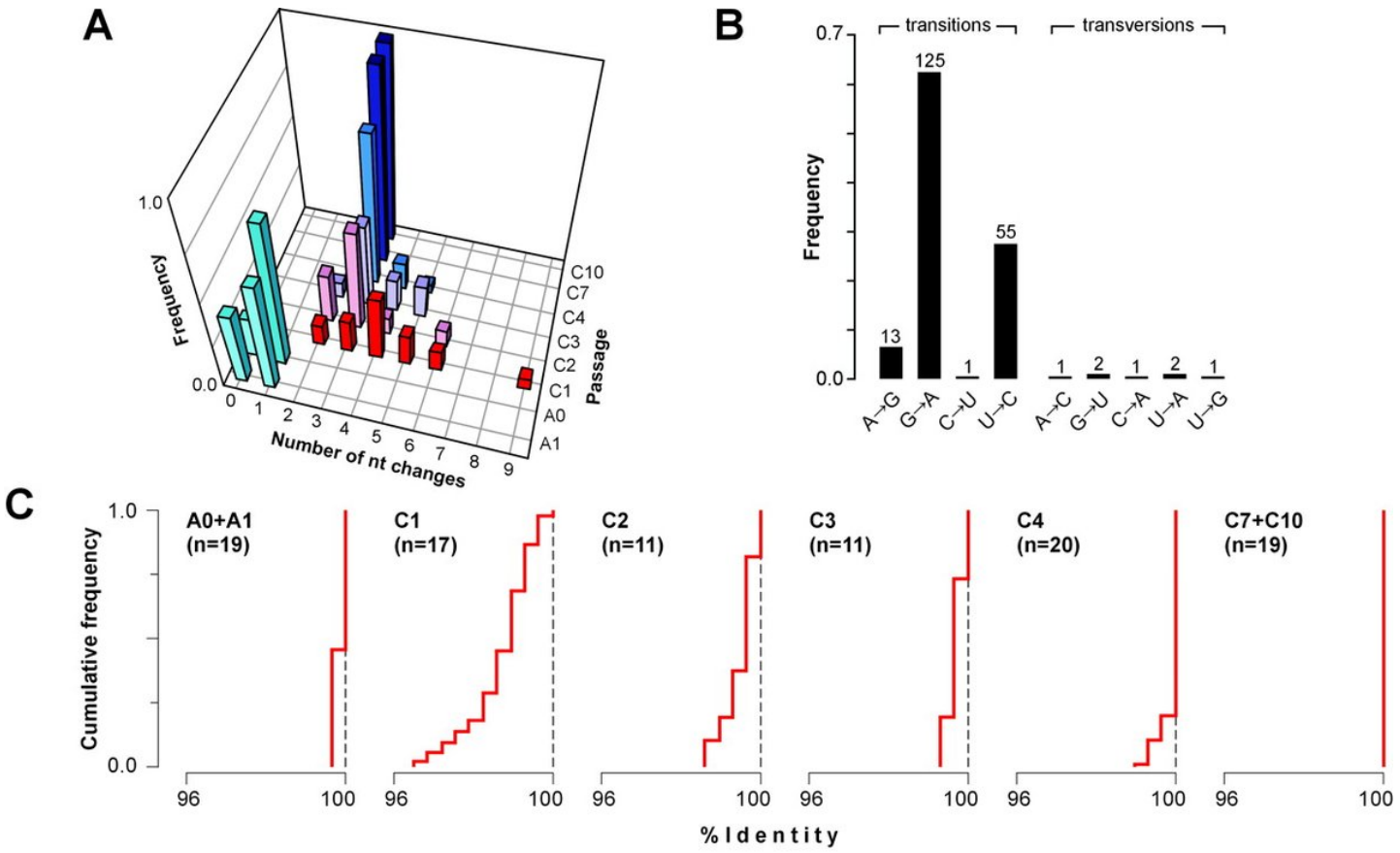
Directed evolution is a powerful approach for engineering biomolecules and understanding adaptation. However, experimental strategies for directed evolution are notoriously labor intensive and low throughput, limiting access to demanding functions, multiple functions in parallel, and the study of molecular evolution in replicate. We report OrthoRep, an orthogonal DNA polymerase-plasmid pair in yeast that stably mutates ~100,000-fold faster than the host genome *in vivo*, exceeding the error threshold of genomic replication that causes single-generation extinction. User-defined genes in OrthoRep continuously and rapidly evolve through serial passaging, a highly straightforward and scalable process. Using OrthoRep, we evolved drug-resistant malarial dihydrofolate reductases (DHFRs) in 90 independent replicates. We uncovered a more complex fitness landscape than previously realized, including common adaptive trajectories constrained by epistasis, rare outcomes that avoid a frequent early adaptive mutation, and a suboptimal fitness peak that occasionally traps evolving populations. OrthoRep enables a new paradigm of routine, high-throughput evolution of biomolecular and cellular function.

8/ Evolutionary Potential of an RNA Virus

in vitro evolution system using recombinant double-stranded RNA bacteriophage, ϕ6, containing a β-lactamase (bla) gene marker

Results show use of RNA virus as vehicle for directed evolution of heterologous proteins

https://t.co/VxXjHpc214



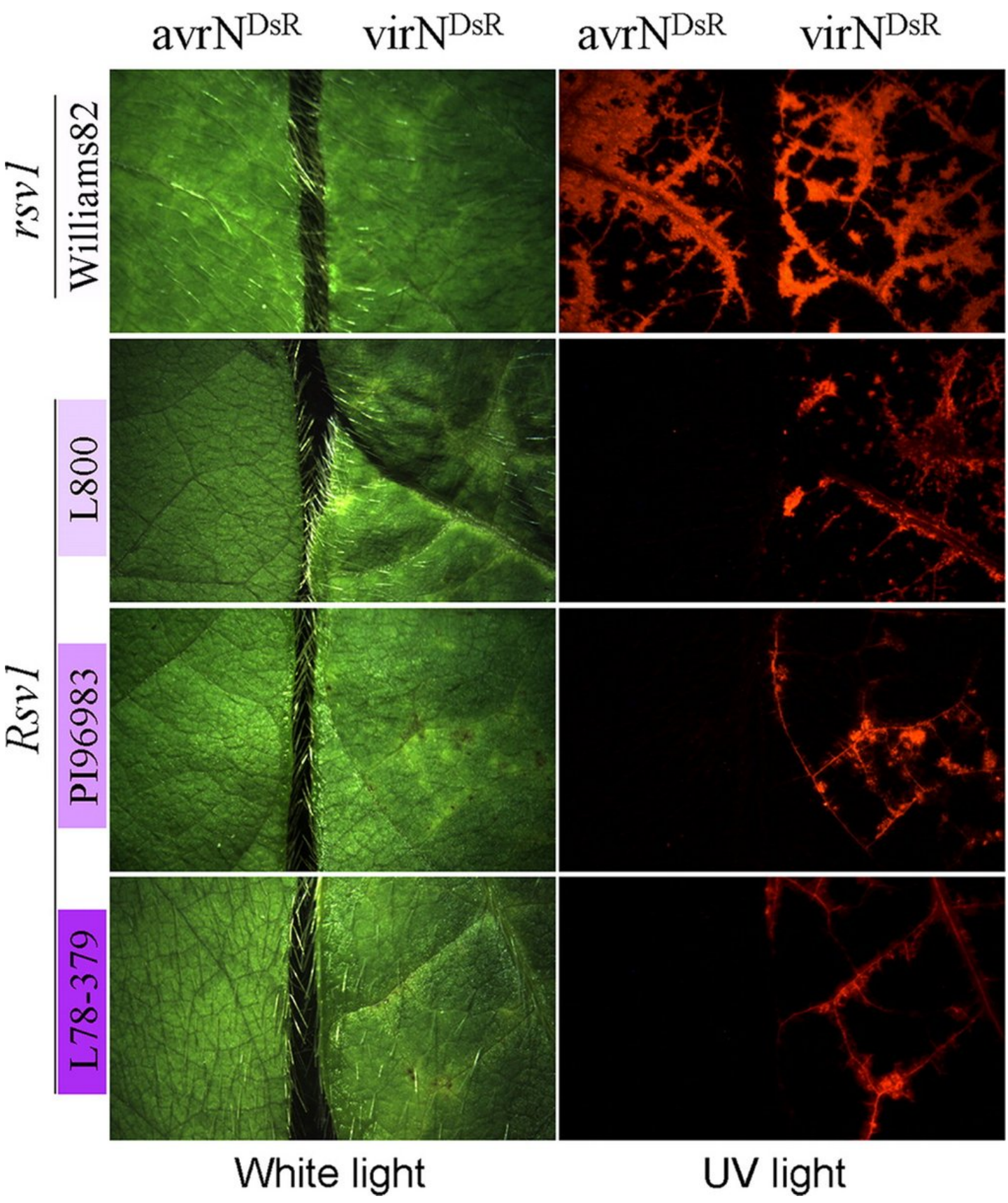
9/ Lab RNA Virus Mimics Natural Evolution!

Do lab evolved virulence determinants mimic natural ones?

Using a plant RNA virus, SMV, via serial in vivo passage experiment, they showed:

Experimentally evolved virulence determinants identical to natural ones

https://t.co/aNcoBOnSe4



10/ Nasty Title:

"Quantitative Modeling of Virus Evolutionary Dynamics and Adaptation in Serial Passages Using Empirically Inferred Fitness Landscapes"

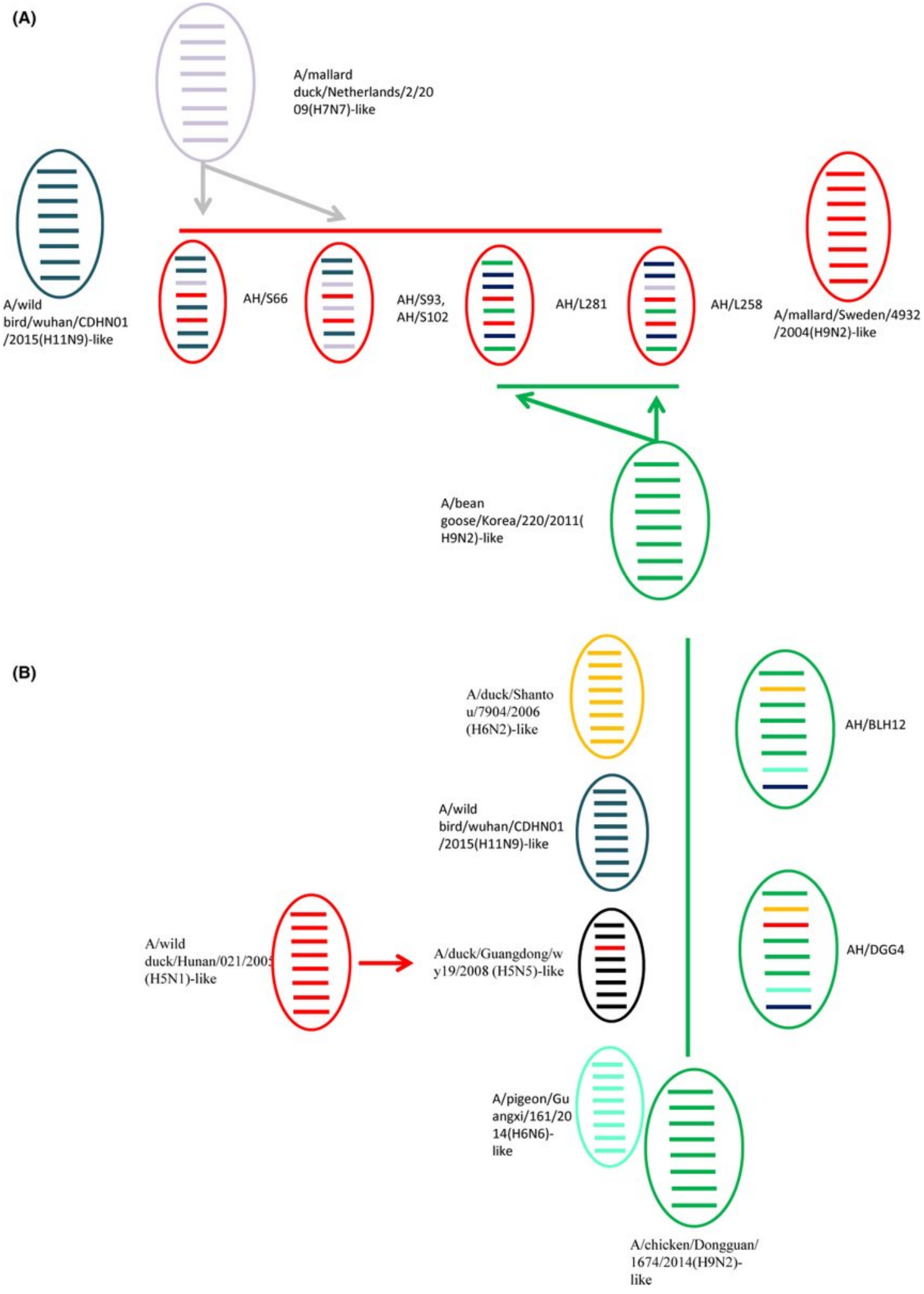
https://t.co/BctOBeyZBm

RNA Virus Mutations and Fitness for Survival

https://t.co/CNDcSCRiu2

11/ Serial passage in Wuhan with Deyin Guo!
To understand adaptation of H5N1 to mammals, a non-pathogenic H5N1 virus (HN021) in mice was passaged 15 times in mammals.
Experiment showed mouse-adapted variants highly pathogenic in mice after passages
https://t.co/QBLpvNVg0g

12/ Effect of PB2 Mutation 627K on Highly Pathogenic H5N1 Avian Influenza Virus Is Dependent on Virus Lineage (UK Research)
https://t.co/rUgqk2vHgT
A 627K variant in the PB 2 protein of H9 subtype influenza virus in wild birds (Harbin & Guandong)
https://t.co/Y6DM5eRcjU


(A)
A/mallard duck/Netherlands/2/2009(H7N7)-like
A/wild bird/wuhan/CDHN01/2015(H11N9)-like
AH/S66
AH/S93, AH/S102
AH/L281
AH/L258
A/mallard/Sweden/4932/2004(H9N2)-like
A/bean goose/Korea/220/2011(H9N2)-like
(B)
A/duck/Shantou/7904/2006 (H6N2)-like
AH/BLH12
A/wild bird/wuhan/CDHN01/2015(H11N9)-like
A/wild duck/Hunan/021/2005 (H5N1)-like
A/duck/Guangdong/wy19/2008 (H5N5)-like
AH/DGG4
A/pigeon/Guangxi/161/2014(H6N6)-like
A/chicken/Dongguan/1674/2014(H9N2)-like

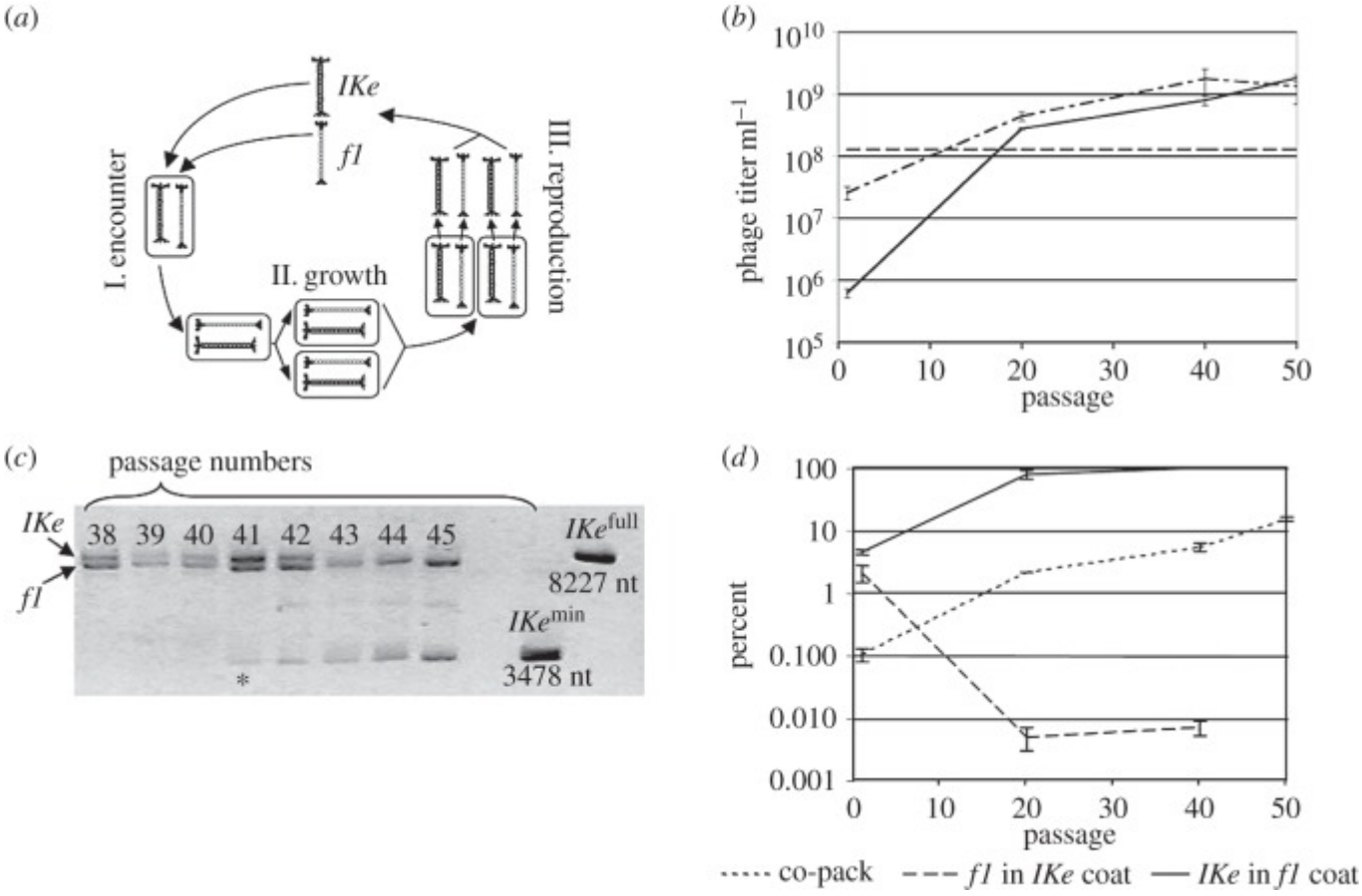
13/ RNA virus experimental evolution

1 New virus that infects otherwise inaccessible host & completely changes way it interacts with host regulation & metabolism

2 Cooperation & cheating during co-infection of same host.

3 Incorporation & loss of genes

https://t.co/A93ADvFlJK



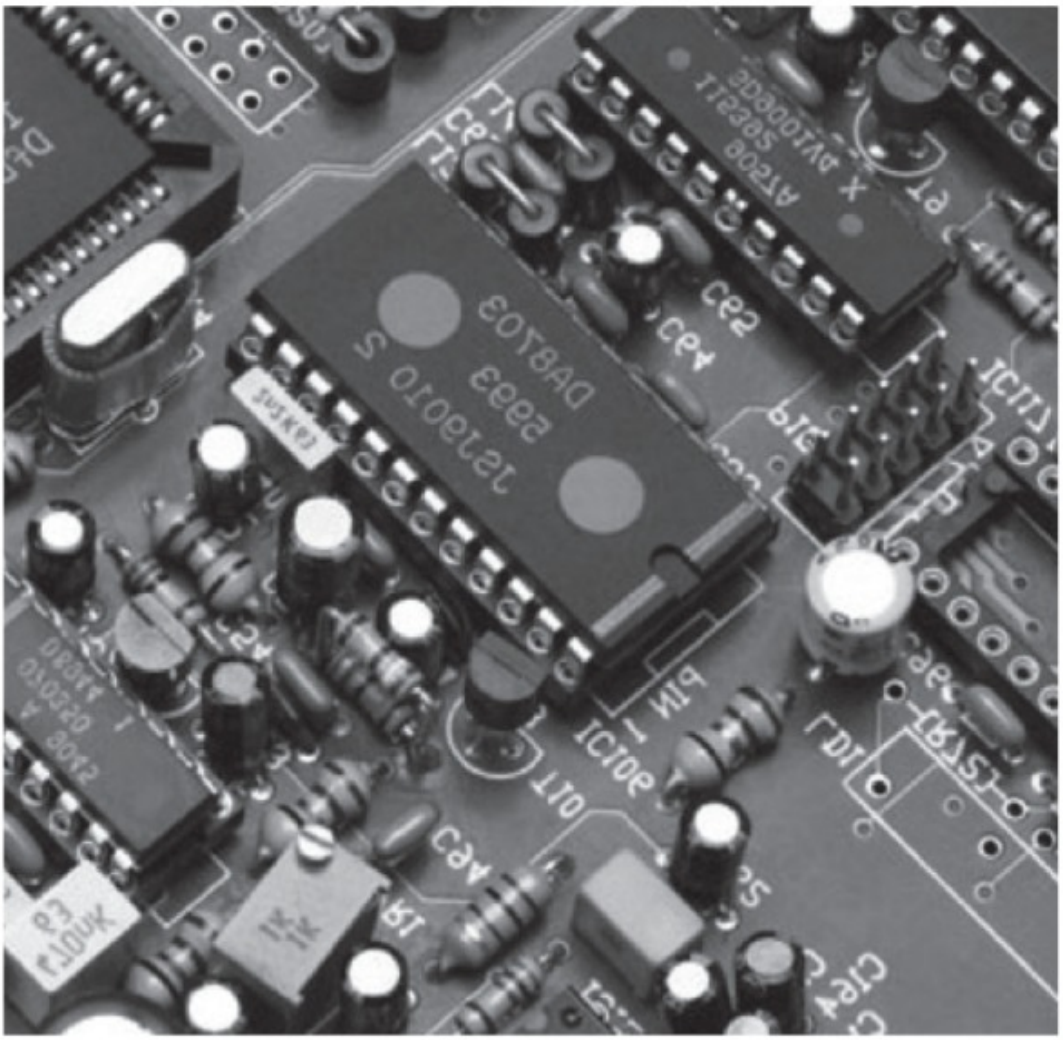
14/ Major Synthetic Evolutionary Transitions (Philosophy)

"parallel class of evolutionary transitions can be explored involving the use of artificial evolutionary experiments where alternative paths to innovation can be explored"

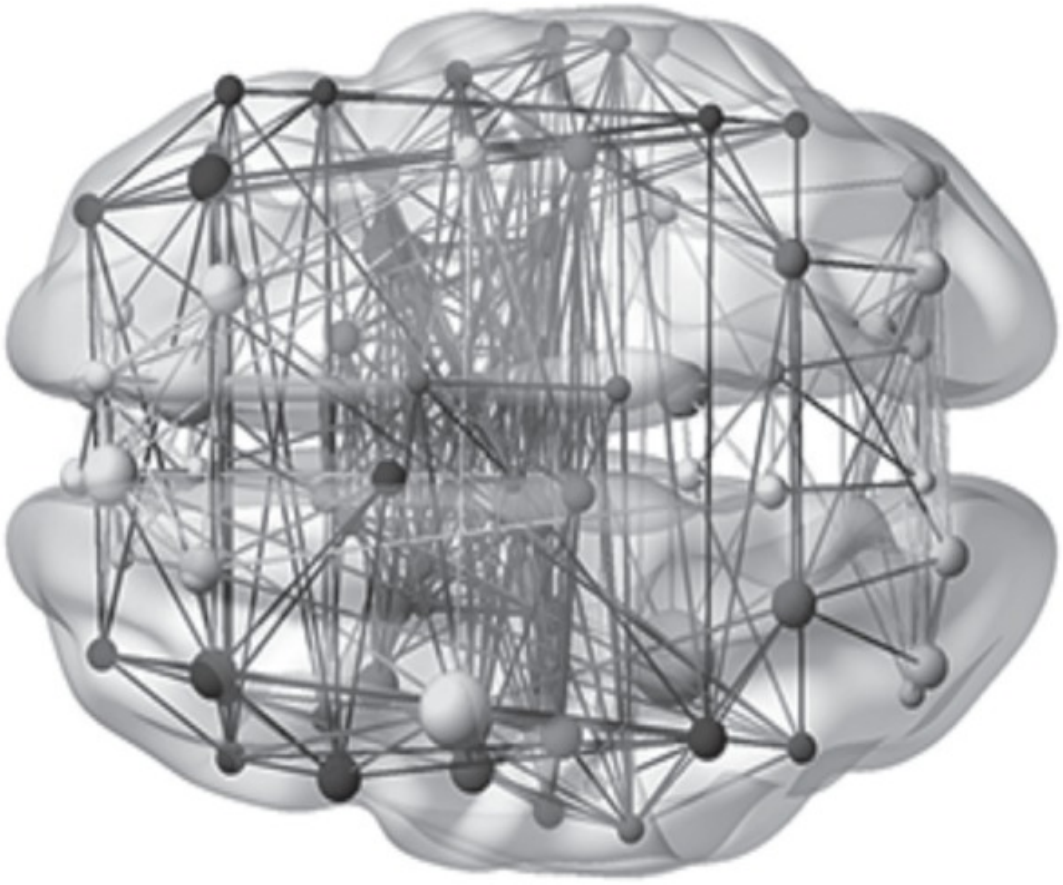
https://t.co/IzxFysxRME

https://t.co/nhTF4ZnTjP

(*a*)



(*b*)



15/ Evolutionary Pathway to Virulence of RNA Virus

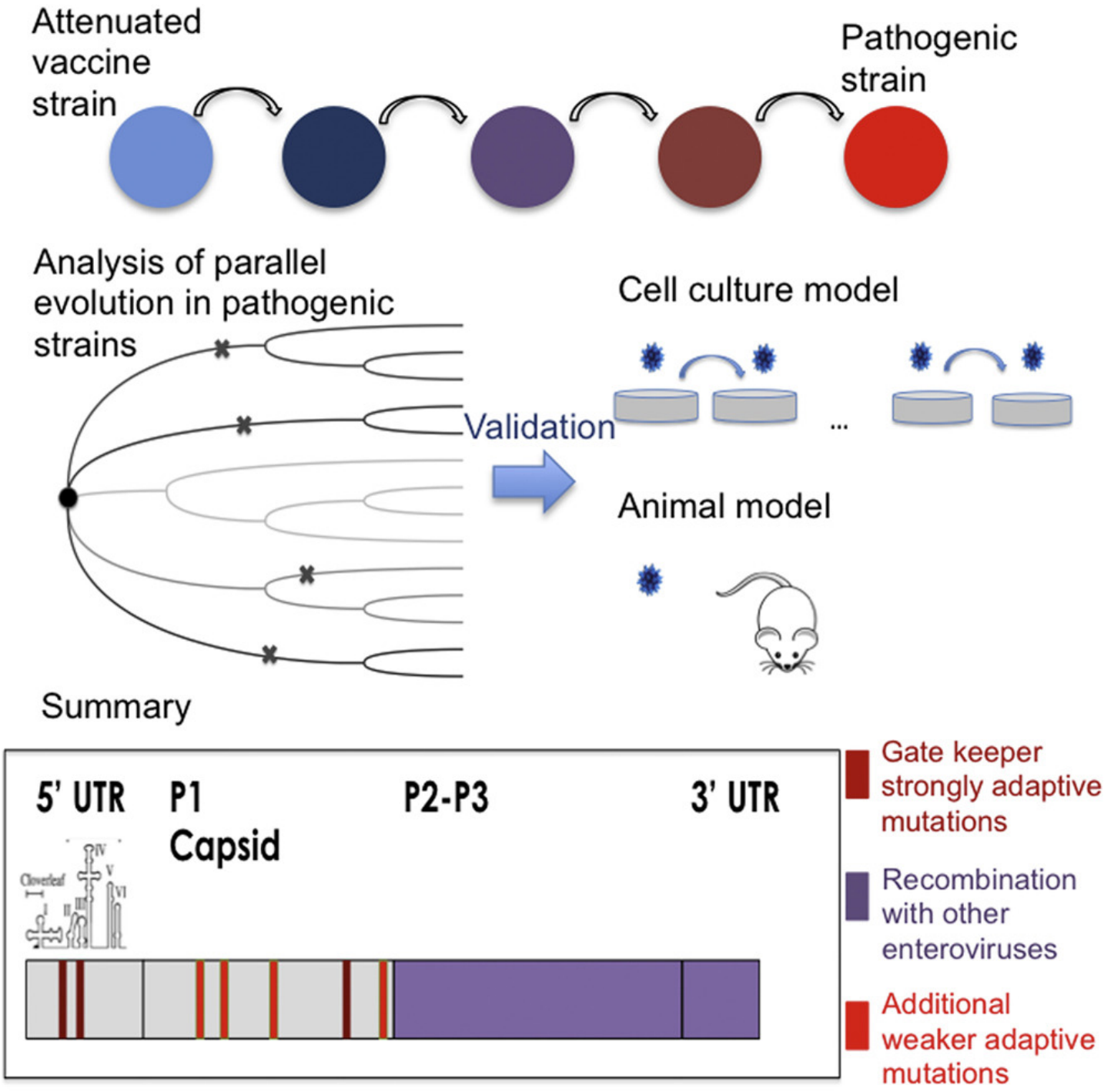
Video Abstract

https://t.co/RgYzp5nimc

1. Key substitutions adaptive in epidemics also observed in in vitro systems

2. Evolution of viral replication can be inferred in tissue culture over short time-frame

https://t.co/WFJXslscvq



16/ In vivo continuous directed evolution:

"advent of increasingly general methods for continuous directed evolution enables researchers to address increasingly complex questions & to access biomolecules with more novel or even unprecedented properties"

https://t.co/YAhLgfMMiK

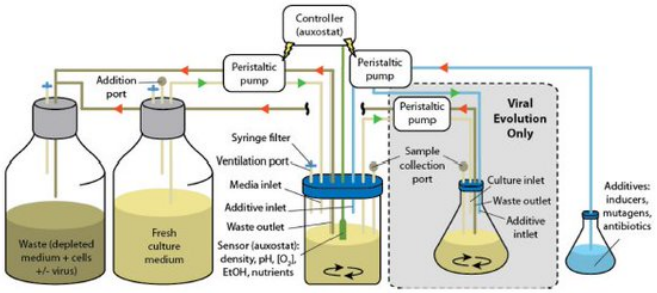


Figure 2. Auxostats and chemostats
Fresh culture medium is pumped into a pre-sterilized container containing the microorganism of interest. Medium with cells is pumped out of the culture container. The rates of medium input and output are held constant in a chemostat system, and the growth rate is regulated by the composition of the medium. In an auxostat system, the medium flow rates are dynamically regulated by a controller in response to measurements made in the growing culture, which correspond directly (turbidity meter) or indirectly (other sensors) to the culture density. In schemes for viral continuous evolution, the auxostat or chemostat culture is pumped into a new vessel where the virus of interest is supplied (cellstat). Both cultures can be supplemented with additives supplied through a dedicated inlet. Selection stringency can be regulated by varying flow rates, changing temperature, or adding compounds to the auxostat, chemostat or cellstat. The depleted medium, cells and virus are pumped to a waste container.

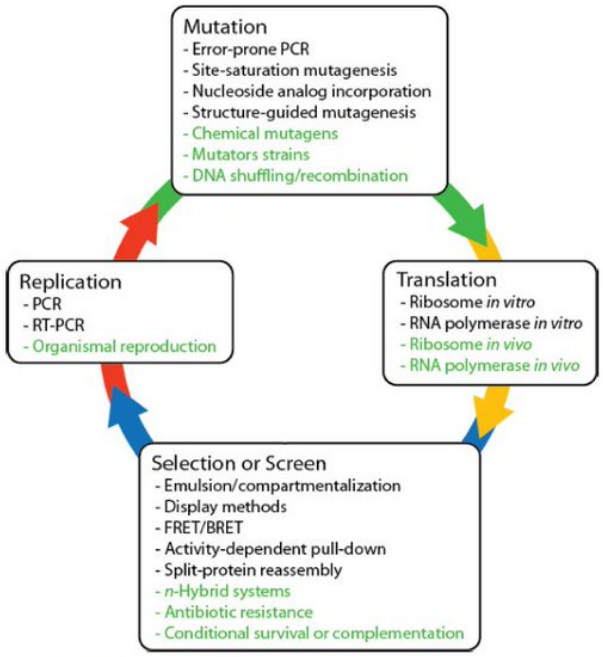


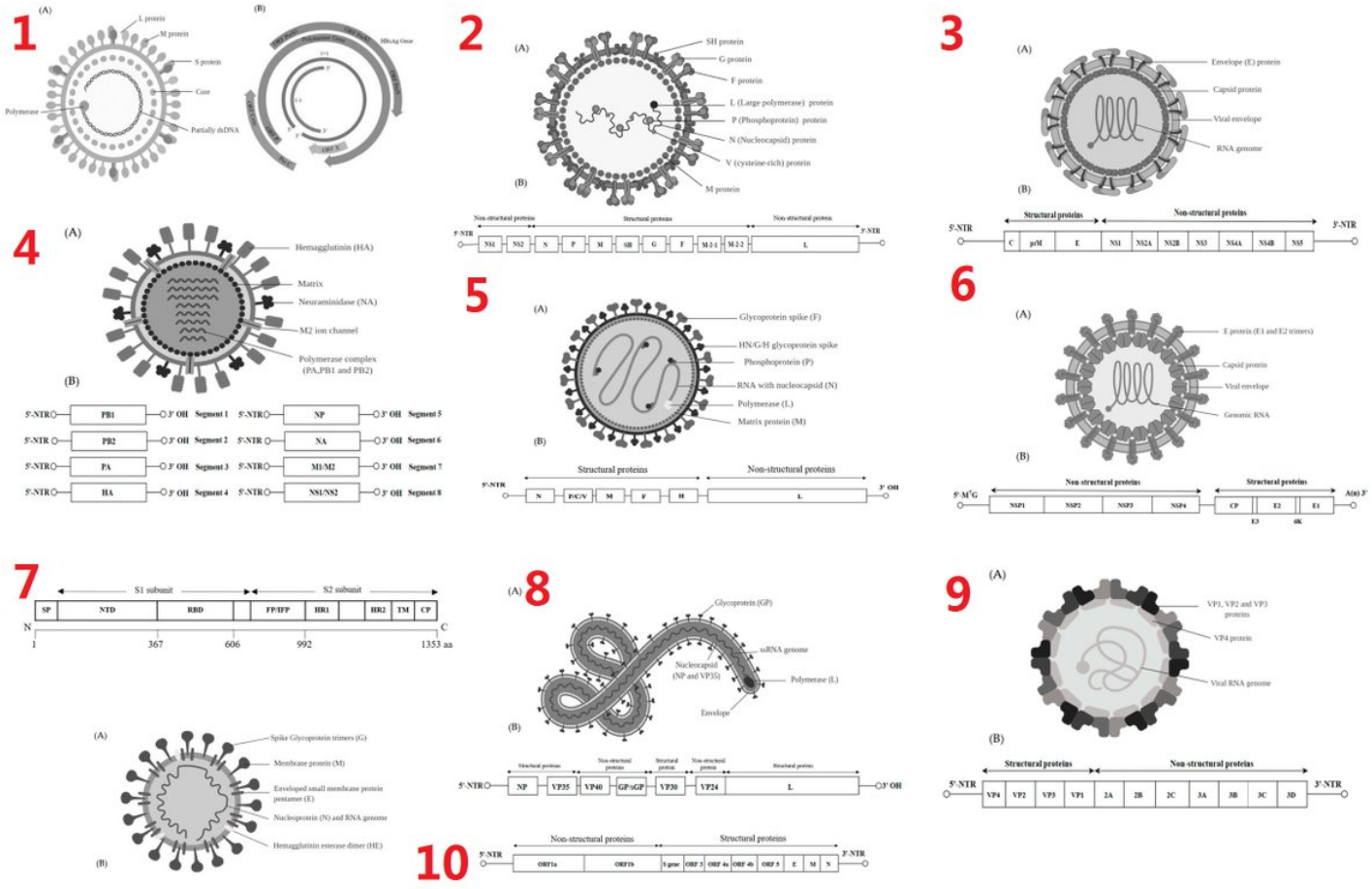
Figure 1. Schematic representation of directed evolution
All complete directed evolution methods must provide four major components: translation, selection or screening, replication, and mutation. Historical examples of each of these components are listed. Techniques that are particularly amenable to *in vivo* continuous evolution are shown in green.

17/ Time for a Game!

Impact of RNA Virus Evolution on Quasispecies

"We explore how genetic events such as spontaneous mutations could alter the genomic organization of RNA viruses in such a way that they impact virus replications & plaque morphology"

https://t.co/nfhsZI3f46



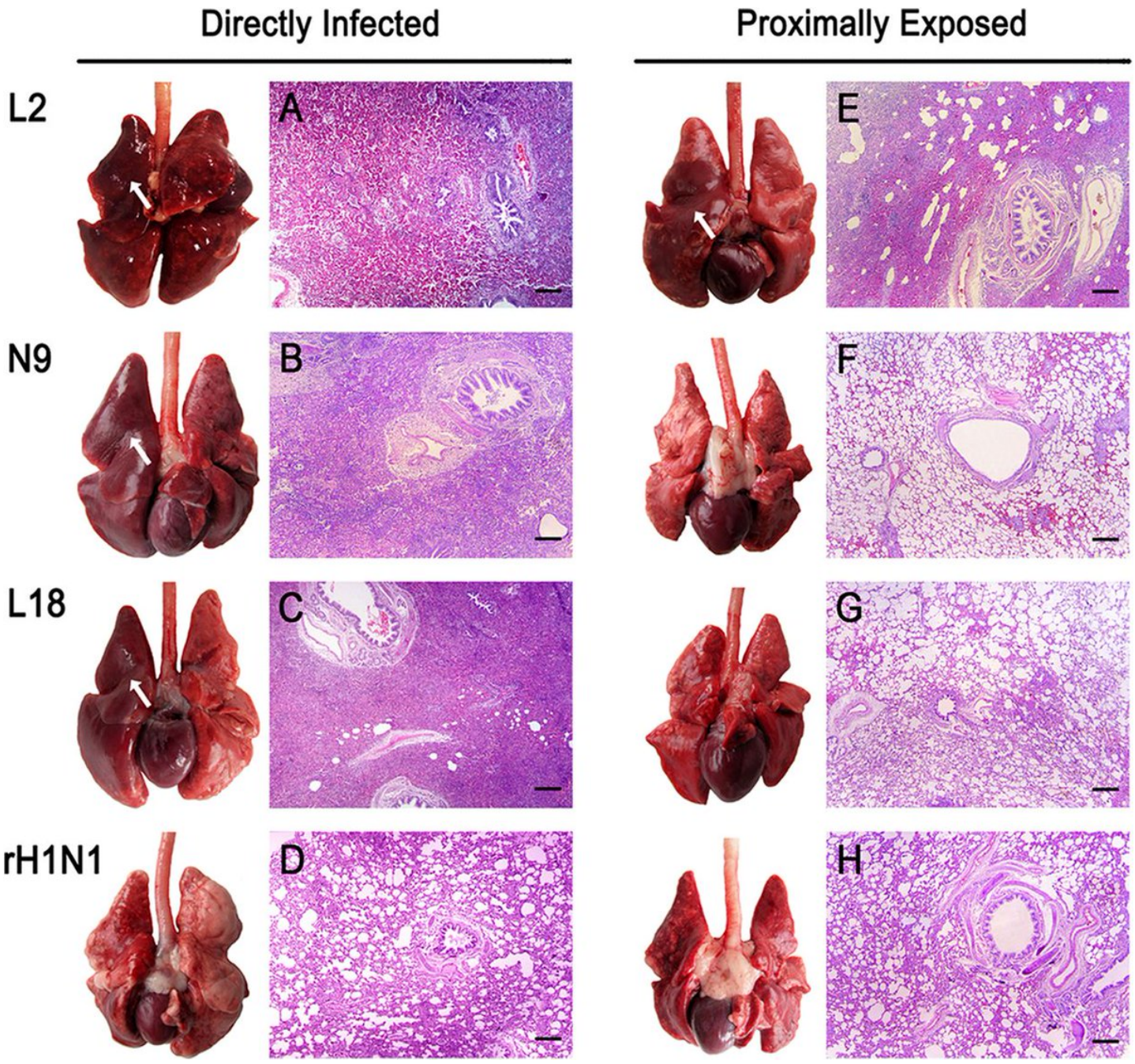
TRY TO IDENTIFY THE TEN VIRUSES

18/ Pigs, Guinea Pigs & Rabbits Butchered in Peking

Flu A Virus Acquires Enhanced Pathogenicity & Transmissibility after Serial Passages in Swine

After 9 serial passages in pigs, engineered swine flu virus had greatly enhanced virulence & transmissibility!

https://t.co/NflCS1d2ux

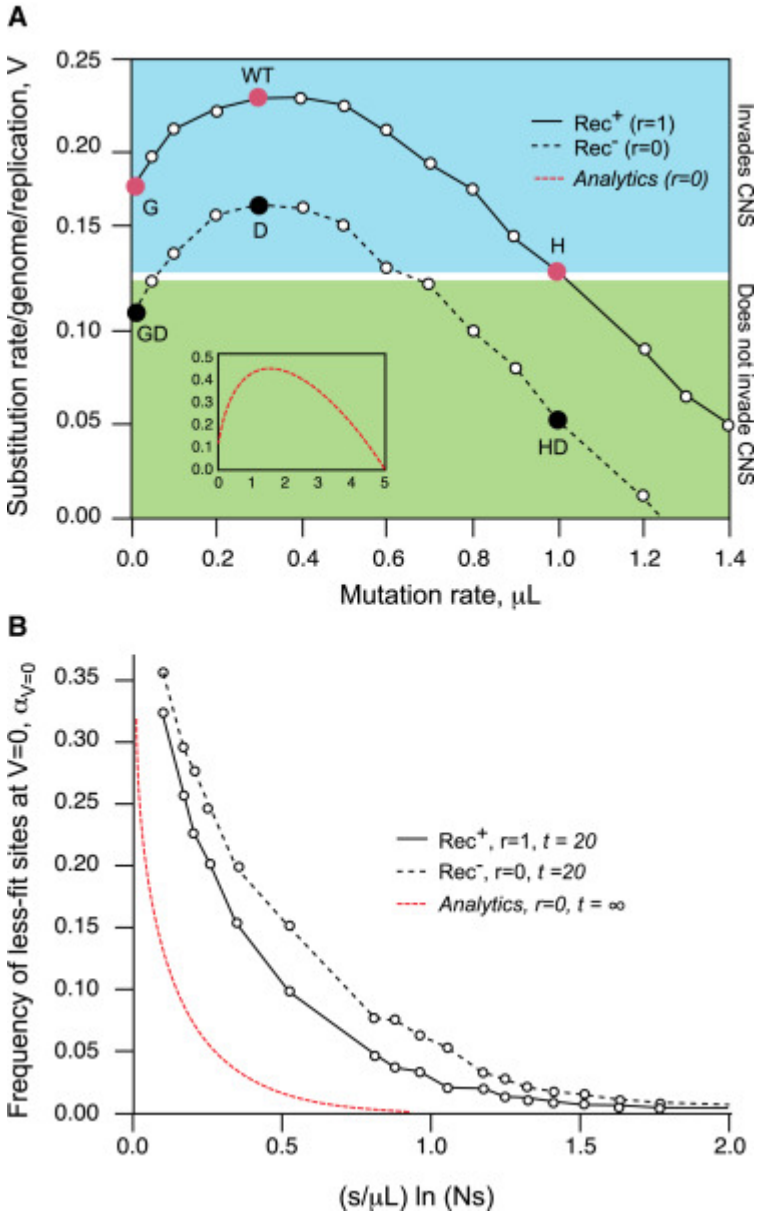


19/ RNA Recombination Enhances Adaptability & Required for Virus Spread & Virulence

1. Recombination significantly accelerates adaptation and evolution during acute virus infection.

2. Essential to enrich beneficial mutations & purge deleterious mutations

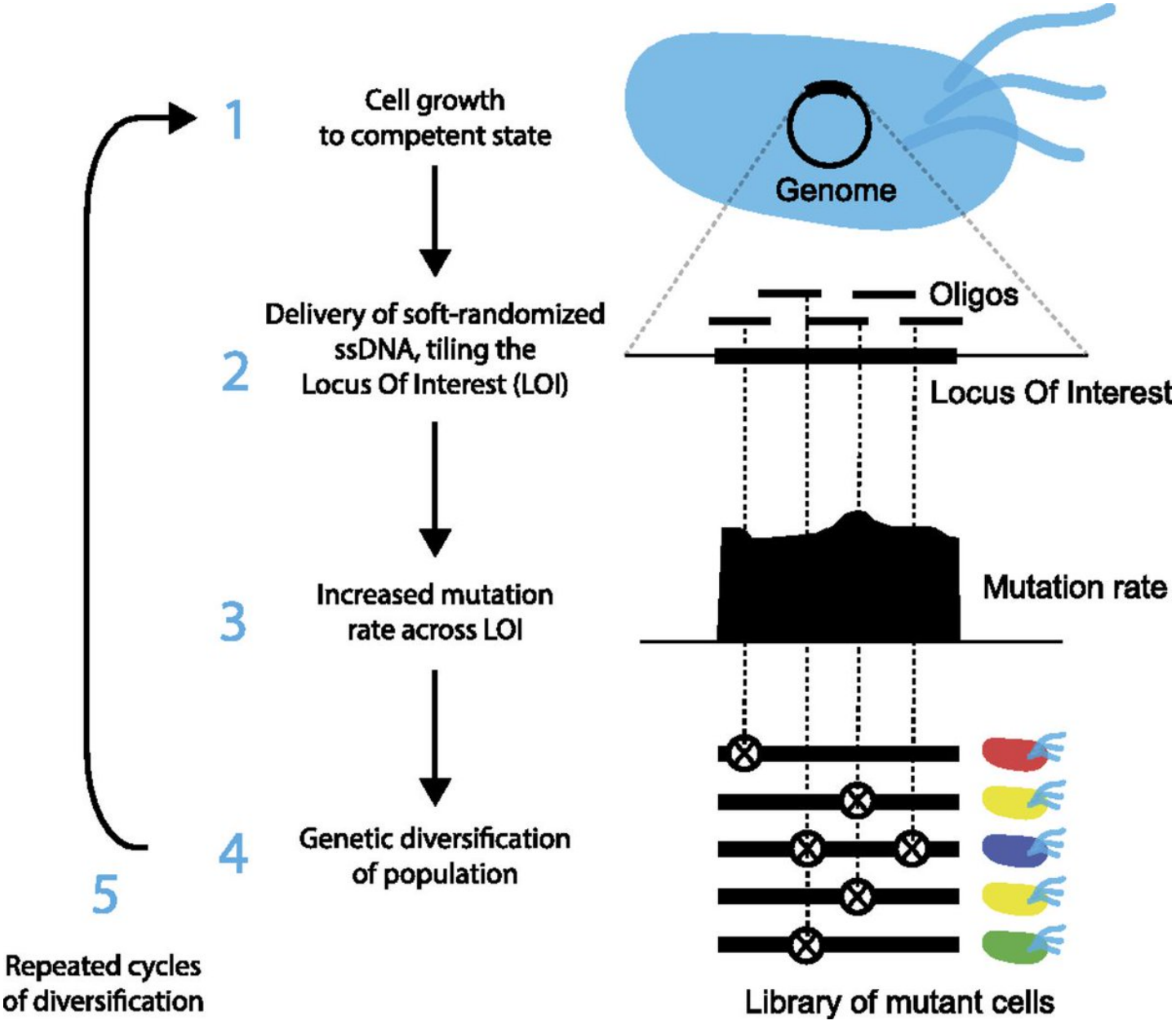
https://t.co/AgG8XVCRvR



20/ Directed evolution of multiple genomic loci

"By building on multiplex automated genome engineering, we developed a method that enables precise mutagenesis of multiple, long genomic segments in multiple species without off-target modifications.

https://t.co/sBdXdnTQH4



21/ Enough Directed Evolution of Tweets for one day!

Got some real work to do, will update tomorrow

