NGS analýza cirkulujících kmenů SARS-CoV-2

Implikace *Pro futuro*

MUDr. Soňa Peková, PhD.

TILIA LABORATORIES s.r.o.

1. Československá konference – COVID 19
27. ledna 2022

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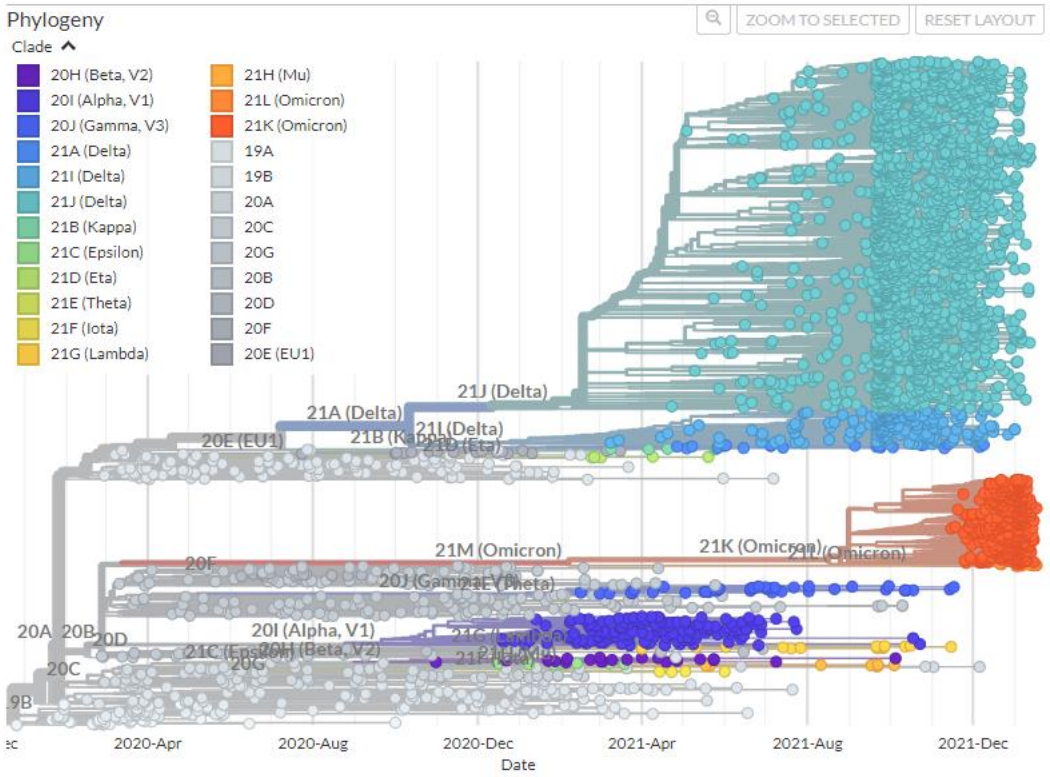
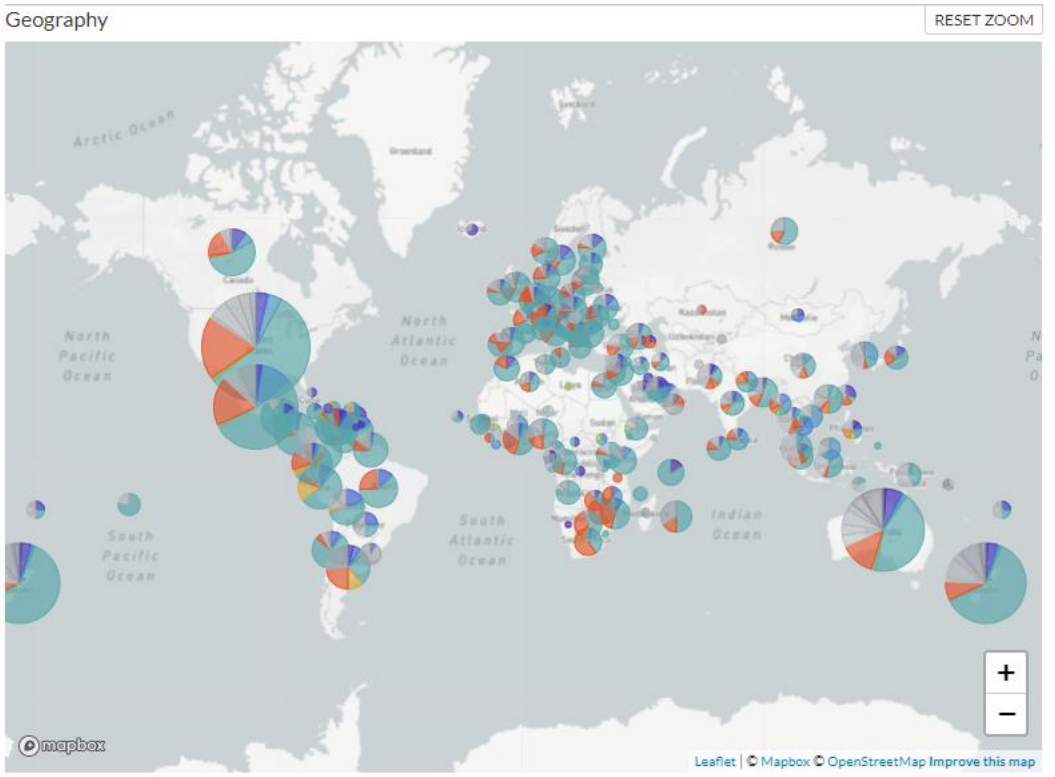
Leden 2022

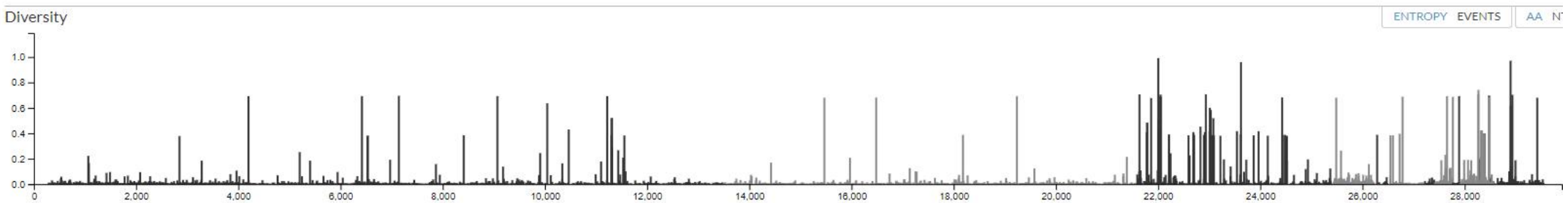
Genomic epidemiology of novel coronavirus - Global subsampling

Built with nextstrain/ncov. Maintained by the Nextstrain team. Enabled by data from GISAID.

Showing 3259 of 3259 genomes sampled between Dec 2019 and Jan 2022.

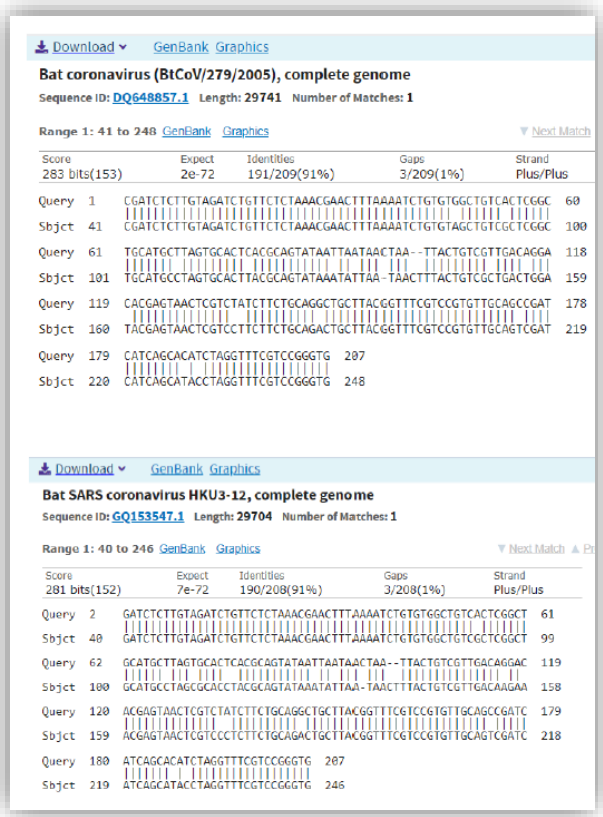


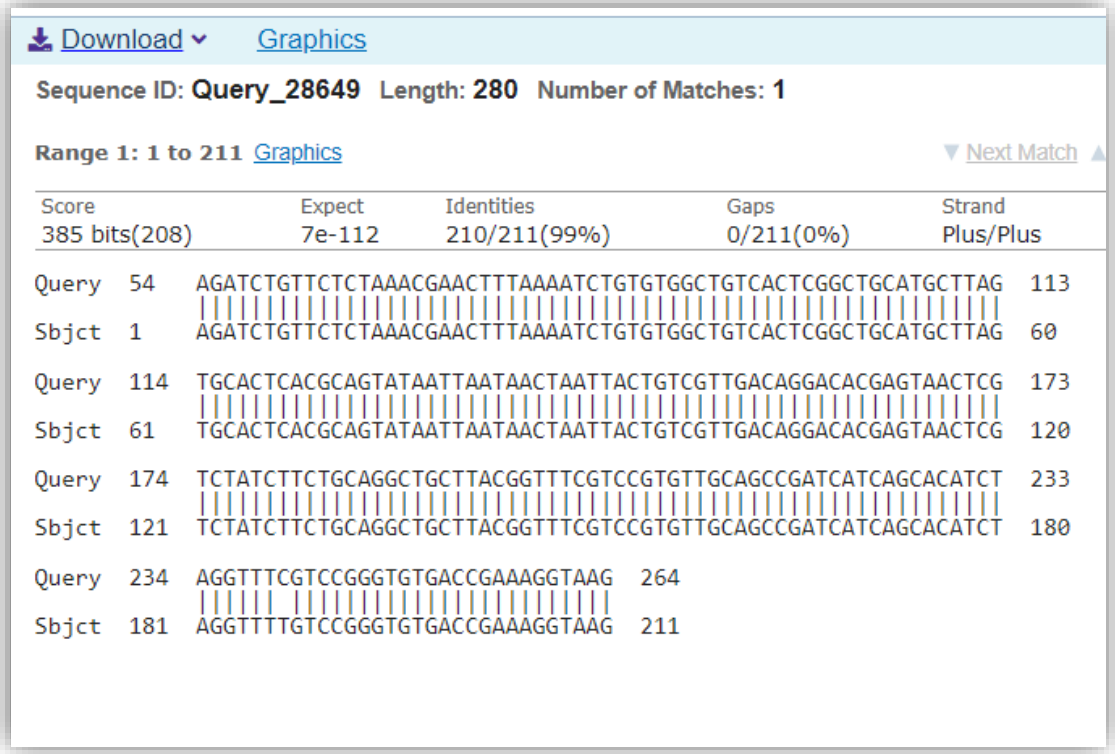




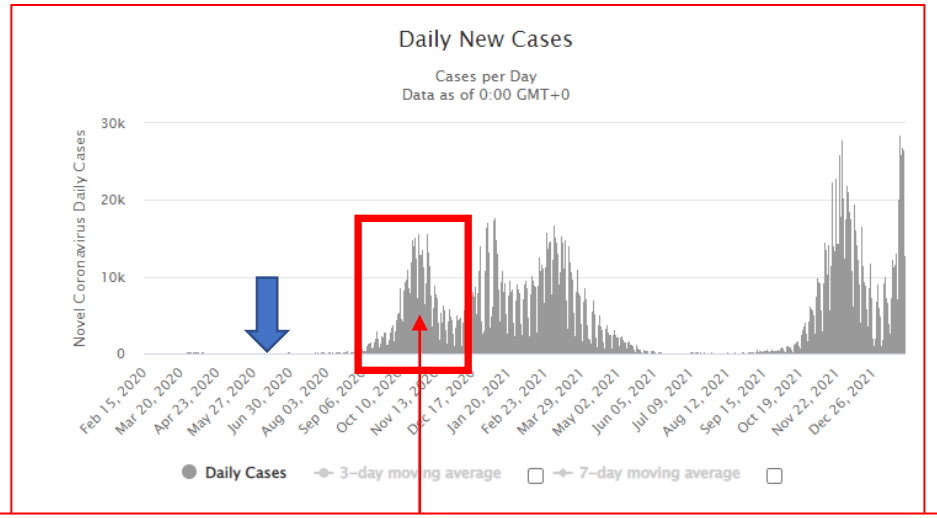
5‘ UTR SARS-CoV-2

- 27 záměn v sekvenci (265 bp) proti historickým izolátům netopýřích koronavirů
- nejbližší sekvenčně příbuzný SARS (2003)
- 5‘ UTR koronavirů - promotor – kontrola replikace a transkripce – **SARS-CoV-2 pravděpodobně silný promotor**
- predikce (březen 2020): vysoká mutagenita, mnoho kmenů, trvání epizody cca 3 měsíce, vývoj vakcíny nerelevantní
- 5‘ UTR SARS-CoV-2 doposud zcela stabilní, na rozdíl od downstream oblastí
- 5‘ UTR univerzální cíl pro detekci všech doposud cirkulujících kmenů SARS-CoV-2; EHK validovaná esej dostupná na požádání



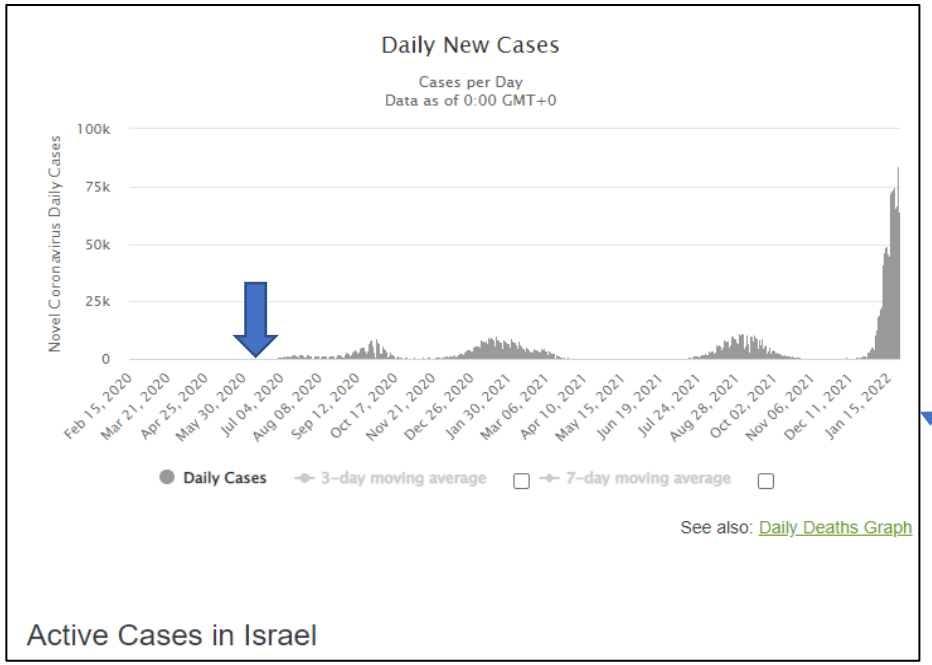


Původní kmen SARS-CoV-2 vs Omicron (alignment 5‘ UTR)



Vlnu genomicky charakterizovala RNDr. Jiřincová, SZÚ Praha;
9. listopad 2020, Zpráva NRL pro chřipku a nechřipková respirační onemocnění, 45. KT

Active Cases in Czechia



Active Cases in Israel

Druhá vlna je pravděpodobná, nástup může být velmi rychlý, očekává Prymula

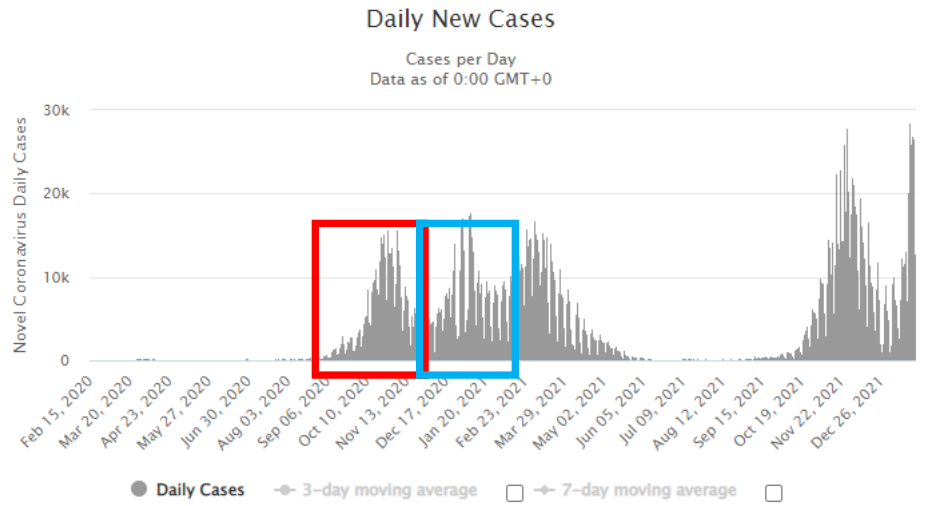
20. 6. 2020

Druhá vlna šíření nákazy covidem-19 nejspíše přijde, zásadní je, jestli se potom podaří udržet denní přírůstek počtu nakažených v řádu stovek, nebo bude ještě hůře. V **Interview ČT24** to řekl vládní zmocněnec pro výzkum ve zdravotnictví **Roman Prymula**. Zkušenost z Izraele přitom ukazuje, že nástup druhé vlny může být velmi rychlý – do deseti dnů. Je proto důležité, aby stále zůstalo v pohotovosti vše, co bylo vytvořeno ve snaze spoutat původní březnovou vlnu.

20. června 2020 – téměř nulový výskyt viru

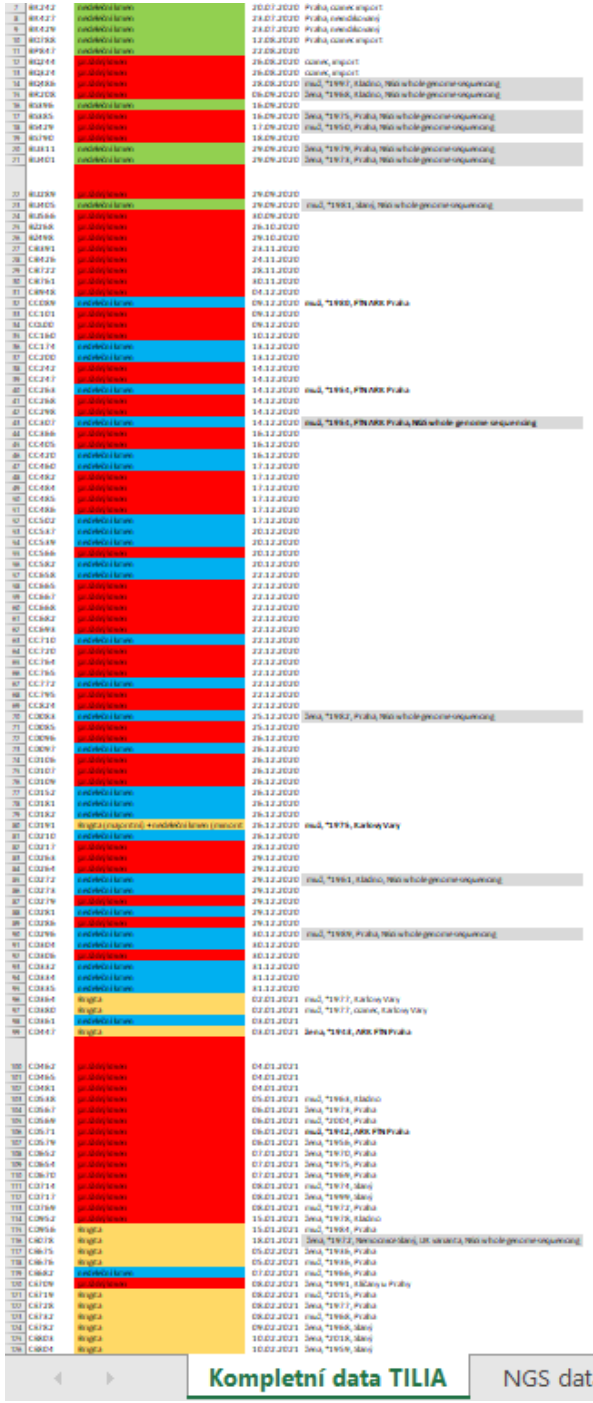
https://www.worldometers.info

Daily New Cases in Czechia



See also: Daily Deaths Graph

Active Cases in Czechia



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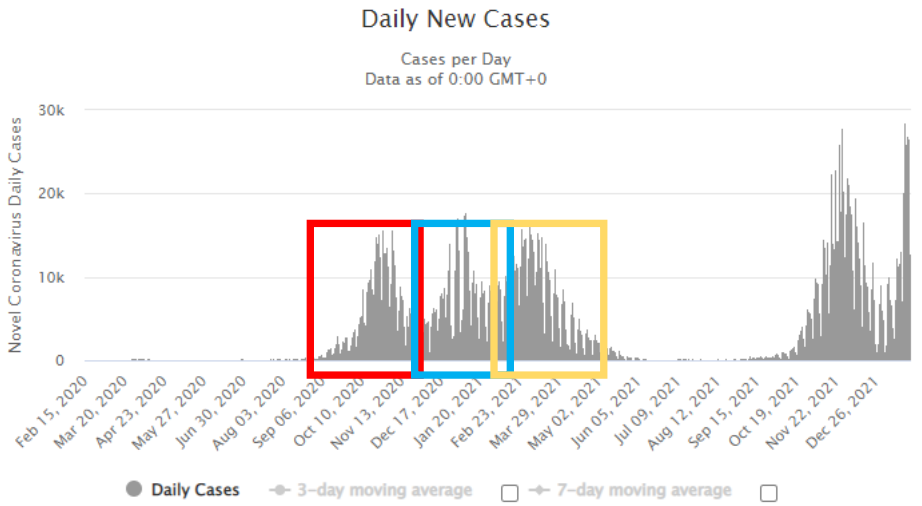
Daily New Cases in Czechia



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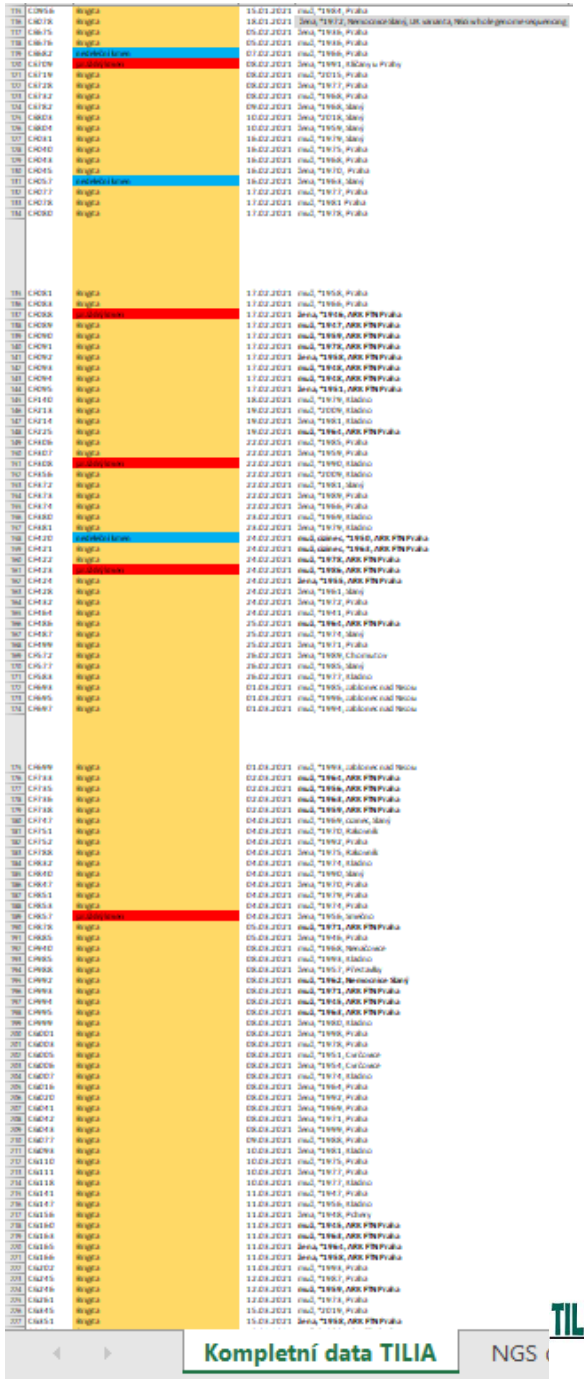


https://www.worldometers.info

- každá vlna od září 2020 tvořena distinktním kmenem s odlišnou genomickou sekvencí
- kmeny evolučně na sebe nenavazují (rozpor se zákonem evoluce mutací a genetiky uzavřených populací)
- přímý předchůdce Omicron nenalezen – genomický rozpor již příliš zjevný

Whole genome NGS sequencing SARS-CoV-2

Gen																											
Kmen	L16L	N160N	del141-143	V169V	Y195H	S216L	S302F	G392V	R402R	D448D	A498V	K517T	V868V	D909D	F924F	T1001I	V1025L	T1246I	A1298V	T1496I	T1543I	A1708D	F1907F	K2029N	S2030L	D2060D	A2080S
pražský / český																											
listopad 2020														X													X
britský (alpha)						X										X						X	X				
delta																											

Kmen	ORF1ab																											
	A2080S	A2192A	I2230T	S2224F	I2501T	Y2594Y	V2629V	L3027F	F3100F	M3221I	C3323R	L3535L	S3920S	V4137A	M4241I	Y4424Y	P4486L	D4683D	P4715L	P4804P	N4944N	H5005H	V5112I	D5130Y	T5198I	V5219V	T5304T	C5353C	N5426S
pražský / český				X	X	X					X				X				X				X						
listopad 2020	X	X						X			X		X						X										
britský (alpha)			X						X										X	X	X	X					X		
delta																													

Kmen																												
	N5426S	D5584Y	E5665D	P5815P	T5941I	H5614Y	T5856T	A5922S	D6249Y	D6256D	T6303I	N6525N	L6668L	N6729N	Y6776Y	T19R	A67V	del69-70	T95I	E96D	G142D	del143-145	del144-145	del156-157	R158G	R190R	del211	L212I	A222V
pražský / český						X		X					X	X	X			X											
listopad 2020																				X									
britský (alpha)																		X					X						
delta																X					X			X	X				

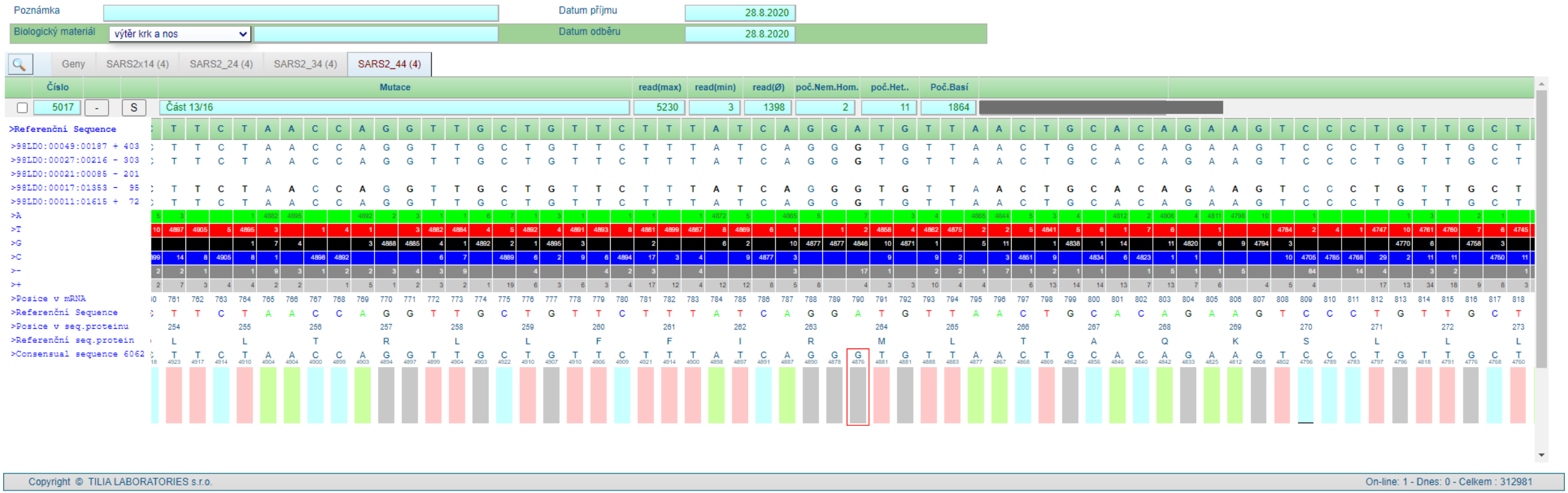
Kmen	S protein																			ORF3a					M		ORF8		
	A222V	N439K	L452R	T478K	N501Y	K558N	A570D	D614G	A626S	P681H	T716I	T723T	S982A	T1116T	D1118H	D1146D	F1148F	T1167T	L1196F	T12I	V13L	A59T	V112V	Y184H	R150R	E19*	Q27*	R52I	Y73C
pražský / český		X						X						X											X				
listopad 2020								X																					
britský (alpha)					X		X	X		X	X		X		X												X	X	X
delta			X	X				X		P681R																			

Kmen	ORF8	N protein													ORF10
	Y73C	D3L	T147A	N192K	S194L	N196Y	R203K	G204P	G204R	S235F	F274F	S318S	P302P	D341D	g.29542G/T
pražský / český															
listopad 2020			X												
britský (alpha)	X	X					X	X		X					
delta															

OMICRON:

Spike protein: A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F

TILIA LABORATORIES: analyzační software pro NGS analýzu dat



Protein S: D614G (GAT>GGT)

nature

https://doi.org/10.1038/s41586-020-2294-9

Accelerated Article Preview

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

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

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Tran Thi Nhu Thao[1,2,3,10], Fabien Labroussaa[2,4,10], Nadine Ebert[1,2,10], Philip V'kovski[1,2], Hanspeter Stalder[1,2], Jasmine Portmann[1,2], Jenna Kelly[1,2], Silvio Steiner[1,2,3], Melle Holwerda[1,2,3,5], Annika Kratzel[1,2,3], Mitra Gultom[1,2,3,5], Kimberly Schmied[1,2], Laura Laloli[1,2,3,5], Linda Hüsser[1,2], Manon Wider[5], Stephanie Pfaender[1,2,6], Dagny Hirt[1,2], Valentina Cippà[2,4], Silvia Crespo-Pomar[2,4], Simon Schröder[7], Doreen Muth[7,8], Daniela Niemeyer[7,8], Victor Corman[7,8], Marcel A. Müller[7,8,9], Christian Drosten[7,8], Ronald Dijkman[1,2,5], Joerg Jores[2,4,11]✉ & Volker Thiel[1,2,11]✉

Reverse genetics has been an indispensable tool revolutionising insights into viral pathogenesis and vaccine development. Large RNA virus genomes, such as from Coronaviruses, are cumbersome to clone and manipulate in *E. coli* due to size and occasional instability[1–3]. Therefore, an alternative rapid and robust reverse genetics platform for RNA viruses would benefit the research community. Here we show the full functionality of a yeast-based synthetic genomics platform to genetically reconstruct diverse RNA viruses, including members of the *Coronaviridae*, *Flaviviridae* and *Paramyxoviridae* families. Viral subgenomic fragments were generated using viral isolates, cloned viral DNA, clinical samples, or synthetic DNA, and reassembled in one step in *Saccharomyces cerevisiae* using transformation associated recombination (TAR) cloning to maintain the genome as a yeast artificial chromosome (YAC). T7-RNA polymerase has been used to generate infectious RNA to rescue viable virus. Based on this platform we have been able to engineer and resurrect chemically-synthetized clones of the recent epidemic SARS-CoV-2[4] in only a week after receipt of the synthetic DNA fragments. The technical advance we describe here allows a rapidly response to emerging viruses as it enables the generation and functional characterization of evolving RNA virus variants—in real-time—during an outbreak.

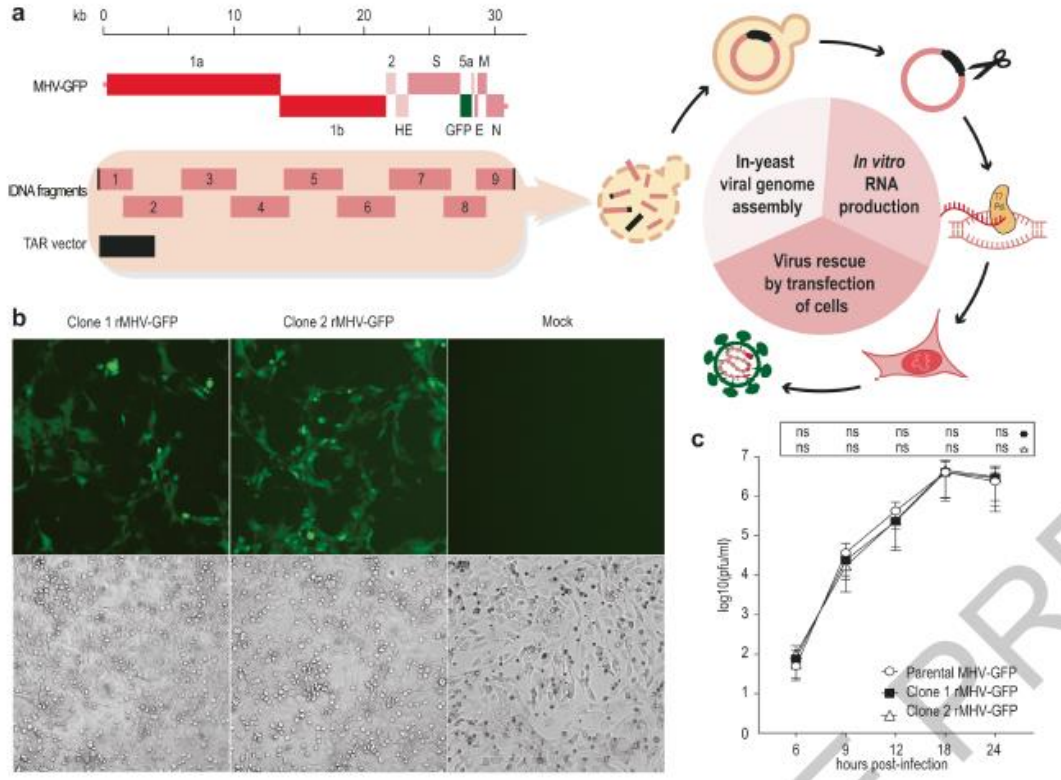

Fig. 1 | Application of the yeast-based TAR cloning to generate viral cDNA clones and the recovery of recombinant Mouse Hepatitis Virus-GFP. **a**, General workflow of TAR cloning and virus rescue. In-yeast genome reconstruction requires one-step delivery of overlapping DNA fragments covering the viral genome and TAR vector into yeast. Viral open reading frames (ORFs), the ORF for green fluorescent protein (GFP) are indicated. Transformed DNA fragments are assembled by homologous recombination in yeast generating a YAC carrying the full-length viral cDNA. *In vitro* production of infectious capped viral RNA starts with the isolation of the YAC, followed by plasmid linearization to provide DNA template for run-off T7 RNA polymerase-based transcription. Virus rescue is initiated with electroporation of BHK-MHV-N cells, followed by co-cultivation with susceptible cells for virus production and amplification. kb, kilobase. **b**, Recovery of infectious rMHV-GFP from yeast clones 1 and 2. Cell culture supernatants containing viruses produced after virus rescue from two MHV-GFP YAC clones were used to infect 17Cl-1 cells. At 48 hours post-infection, infected cells were visualised for GFP expression (top panels) and by bright field microscopy (bottom panels). Mock represents 17Cl-1 cells inoculated with supernatant from BHK-MHV-N cells electroporated without viral RNAs. Images are representative for two independent experiments. **c**, Replication kinetics of parental MHV-GFP and rMHV-GFP clones 1 and 2. L929 cells were infected (MOI = 0.1), and cell culture supernatants were harvested at indicated time points post-infection and titrated by plaque assay. Data represent the average of three independent biological experiments (n=3). Error bars: SD. Statistical significance was determined by two-sided unpaired student's t-test without adjustments for multiple comparisons. *P* values (left-right): top row - ns p=0.2905, ns p=0.3504, ns p=0.1817, ns p=0.9862, ns p=0.6738; last row - ns p=0.0835, ns p=0.1400, ns p=0.2206, ns p=0.8020, ns p=0.5894. pfu/ml, plaque forming units per millilitre.

SCIENTIFIC REPORTS
nature research

In silico comparison of SARS-CoV-2 spike protein-ACE2 binding affinities across species and implications for virus origin.

Piplani S, Singh PK, Winkler DA, Petrovsky N. Sci Rep. **2021 Jun 24**;11(1):13063. doi: 10.1038/s41598-021-92388-5.

Table 1 ACE2 RBD residues interacting with the S protein RBD from MD simulations of complexes.

From: In silico comparison of SARS-CoV-2 spike protein-ACE2 binding affinities across species and implications for virus origin

Species	Accession number	Position																% binding residues shared with hACE2
		19	24	27	28	30	31	34	37	38	41	42	79	83	330	353	393	
Homo sapiens (human)	Q9BYF1	S	Q	T	F	D	K	H	E	D	Y	Q	L	Y	N	K	R	100
Macaca fascicularis (monkey)	A0A2K5X283	S	Q	T	F	D	K	H	E	D	Y	Q	L	Y	N	K	R	100
Panthera tigris (tiger)	XP_007090142.1	S	L	T	F	D	K	H	E	**E**	Y	Q	L	Y	K	K	R	94
Bos Taurus (cow)	NP_001019673.2	S	Q	T	F	**E**	K	H	E	D	Y	Q	M	Y	N	K	R	88
Mesocricetus auratus (hamster)	A0A1U7QTA1	S	Q	T	F	D	L	Q	E	D	Y	Q	L	Y	N	K	R	88
Felis catus (cat)	Q56H28	S	L	T	F	**E**	K	H	E	**E**	Y	Q	L	Y	N	K	R	81
Rhinolophus sinicus (bat)	U5WHY8	S	*E*	**M**	F	D	K	T	E	D	H	Q	L	Y	N	K	R	75
Paguma larvata (civet)	Q56NL1	S	L	T	F	**E**	K	Y	E	**Q**	Y	Q	L	Y	N	K	R	75
Equus ferus caballus (horse)	F6V9L3	S	L	T	F	D	K	S	E	**E**	H	Q	L	Y	N	K	R	75
Mustela putorius furo (ferret)	Q2WG88	D	L	T	F	**E**	K	T	E	**E**	Y	Q	-	Y	N	K	R	69
Canis luparis (dog)	J9P7Y2	–	L	T	F	**E**	K	Y	E	**E**	Y	Q	L	Y	N	K	R	69
Mus musculus (mouse)	Q8R0I0	S	**N**	T	F	**N**	N	Q	E	D	Y	Q	Y	F	N	K	R	63
Manis javanica (pangolin)	XP_017505752.1	–	*E*	T	F	**E**	K	S	E	**E**	Y	Q	**I**	Y	N	K	R	63
Ophiophagus Hannah (snake)	ETE61880.1	Q	V	K	F	**E**	Q	A	–	D	Y	**N**	N	**F**	N	L	R	38

Residues interacting with the same S residue in different species ACE2, that differ from those in human ACE2, are in bold (conservative replacements), in italics (partially conservative replacements) or in underline (non-conservative replacements).

NEWS · 27 JANUARY 2020

US officials revisit rules for disclosing risky disease experiments

An expert panel is considering how much to reveal about a largely secret review process of 'gain-of-function' research.

Nidhi Subbaraman

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Engineered bat virus stirs debate over risky research

US plan to assess risky disease research takes shape

US suspends risky disease research

Viral-research moratorium called too broad

Making pathogens more dangerous can help researchers prepare for pandemics. Credit: Anna Schroll/Fotogloria/UIG via Getty

The discussion is the latest chapter in a long-standing debate about the value of potentially dangerous biological research. In 2014, after a series of accidents involving mishandled pathogens at the US Centers for Disease Control and Prevention, the NIH announced that it would stop funding gain-of-function research into certain viruses – including influenza, severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS) – that have the potential to unleash a pandemic or epidemic if they escaped from the lab. Some researchers said the broad ban threatened necessary flu-surveillance and vaccine research.

Surprising news

The government reversed course in January 2017 after the NSABB concluded that very few such experiments posed a risk to public safety. The NIH lifted its ban on funding gain-of-function research in December 2017, after the HHS and the White House developed a system for vetting proposed experiments – creating the expert panel in the process.

The related debate over how much to disclose about such research reignited in 2019 following media reports that the government had approved two gain-of-function experiments.

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CRISPR inventor calls for pause in editing heritable genes

By Robert Sanders, Media relations | DECEMBER 1, 2015

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A three-day international summit on the ethics of making permanent, hereditary changes in the human genome begins today in Washington, D.C., the fruit of an informal discussion organized in January by Jennifer Doudna, the inventor of the CRISPR-Cas9 technology that makes such changes cheap and easy.

The Nobel Prize in Chemistry 2020

Emmanuelle Charpentier and Jennifer Doudna were awardedthe Nobel Prize in Chemistry 2020 for discovering one of gene technology's sharpest tools: the CRISPR/Cas9 genetic scissors. Using components of the CRISPR system, researchers can add, remove, or even alter specific DNA sequences. This technology has introduced new opportunities in cancer therapies, curing inherited diseases and also in plant inbreeding.

NEWS FEATURE | 26 February 2019 | Clarification 11 March 2019

The CRISPR-baby scandal: what's next for human gene-editing

As concerns surge after a bombshell revelation, here are four questions about this fast-moving field.

David Cyranoski

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An Institution for the Do-It-Yourself Biologist

DIYbio.org was founded in 2008 with the mission of establishing a vibrant, productive and safe community of DIY biologists. Central to our mission is the belief that biotechnology and greater public understanding about it has the potential to benefit everyone.

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Latest news

Announcing DIYbiosphere: an open source project to connect DIYbio related activities worldwide

March 17th, 2018

With significant growth in activities globally, our

DIYbio events for the week of September 3

September 3rd, 2017

Here are your events for the week Sunday, September 3 Oakland, CA, USA – Open Insulin

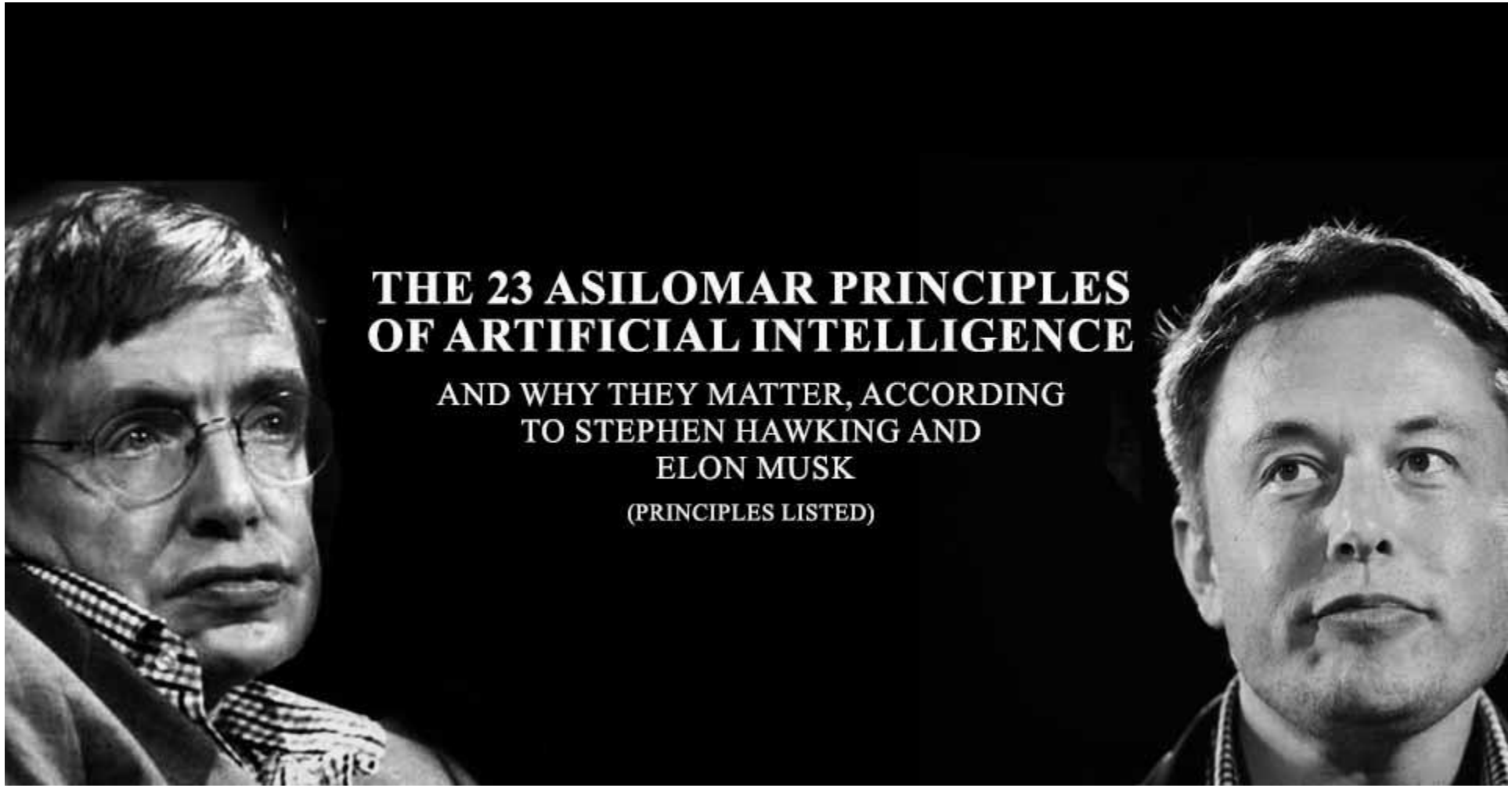
DIYbio events for the remainder of the week of August 27

August 27th, 2017

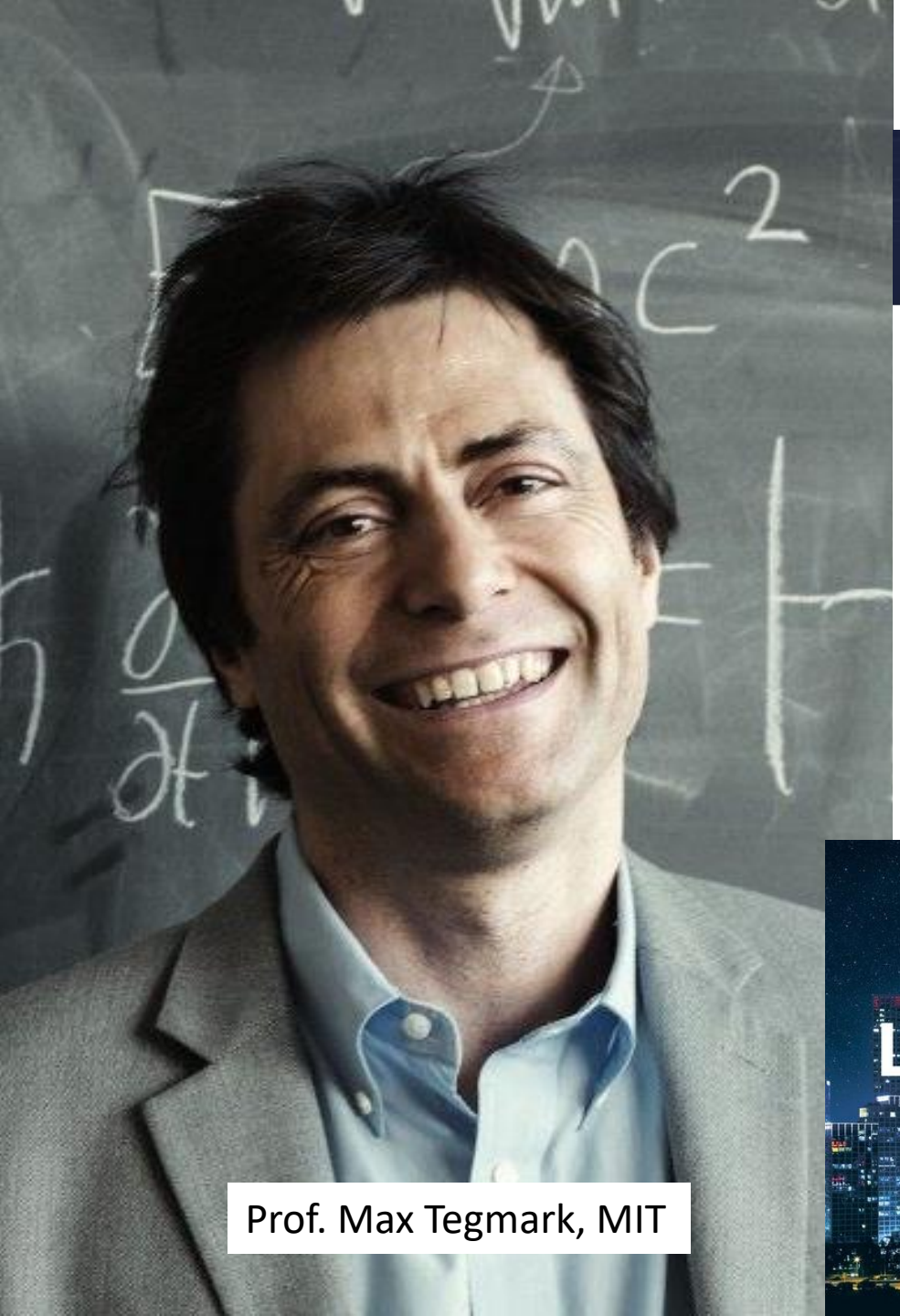
Here are your events for the remainder of the week Monday, August 28 Oakland, CA, USA –

Etika v molekulární biologii a genetice

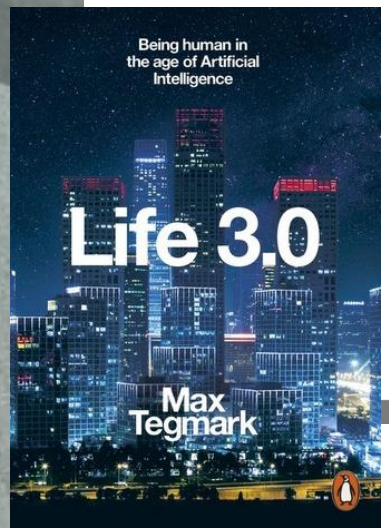
17. ledna 2017, Asilomar, CA, USA



Etika v oboru umělé inteligence (AI)



Prof. Max Tegmark, MIT



future of life INSTITUTE

Technology is giving life the potential to flourish like never before...

...or to self-destruct. Let's make a difference!

A Principled AI Discussion in Asilomar

January 17, 2017 / by The FLI Team

We, the organizers, found it extraordinarily inspiring to be a part of the BAI 2017 conference, the Future of Life Institute's second conference on the future of artificial intelligence. Along with being a gathering of endlessly accomplished and interesting people, it gave a palpable sense of shared mission: a major change is coming, over unknown timescales but across every segment of society, and the people playing a part in that transition have a huge responsibility and opportunity to shape it for the best.

Děkuji za pozornost