

# NGS analýza cirkulujících kmenů SARS-CoV-2

## Implikace *Pro futuro*

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TILIA LABORATORIES s.r.o.



1. Československá konference – COVID 19  
27. ledna 2022

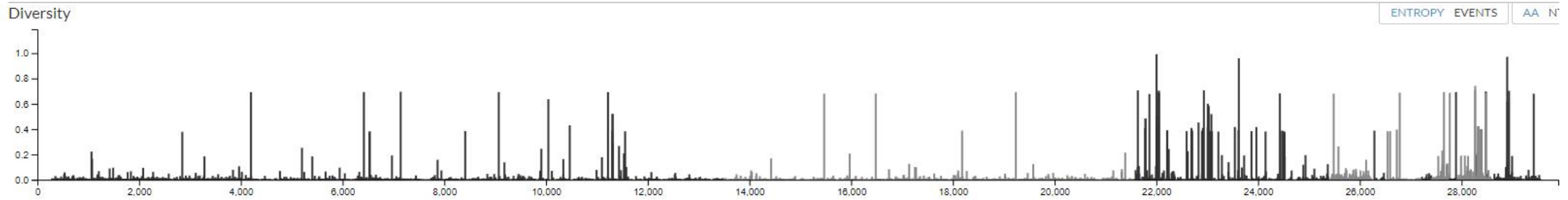
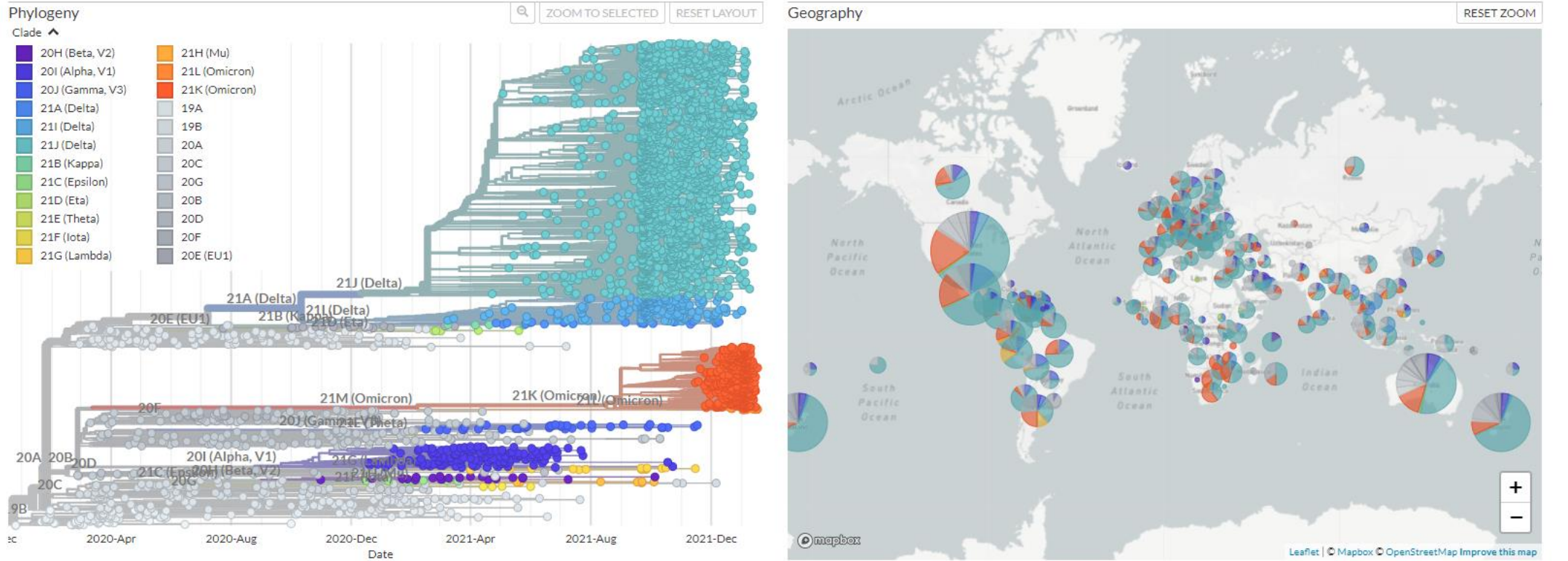


# Leden 2022

## 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

Download GenBank Graphics

**Bat coronavirus (BtCoV/279/2005), complete genome**  
Sequence ID: [DQ648857.1](#) Length: 29741 Number of Matches: 1

Range 1: 41 to 248 [GenBank](#) [Graphics](#) [Next Match](#)

Score	Expect	Identities	Gaps	Strand
283 bits(153)	2e-72	191/209(91%)	3/209(1%)	Plus/Plus

```

Query 1  CGATCTCTGTAGATCTGTTCTCTAAACGAACCTTAAAAATCTGTGGCTGTCACTCGGC 60
Sbjct 41  CGATCTCTGTAGATCTGTTCTCTAAACGAACCTTAAAAATCTGTGGCTGTCACTCGGC 100

Query 61  TGCATGCTTAGTGCACTCACGCAGTATAAATAAATAA--TTACTGCTGTTGACAGGA 118
Sbjct 101  TGCATGCTTAGTGCACTCACGCAGTATAAATAAATAA--TTACTGCTGCTGACTGGA 159

Query 119  CACGAGTAACTCGTCTATCTTCTGACGGCTGCTTACGGTTTCGTCGGTGTGCAECCGAT 178
Sbjct 160  TACGAGTAACTCGTCTTCTTCTGACGACTGCTTACGGTTTCGTCGGTGTGCAECCGAT 219

Query 179  CATCAGCACATCTAGGTTTCGTCGGGGTG 207
Sbjct 220  CATCAGCACATCTAGGTTTCGTCGGGGTG 248

```

Download GenBank Graphics

**Bat SARS coronavirus HKU3-12, complete genome**  
Sequence ID: [GQ153547.1](#) Length: 29704 Number of Matches: 1

Range 1: 40 to 246 [GenBank](#) [Graphics](#) [Next Match](#)

Score	Expect	Identities	Gaps	Strand
281 bits(152)	7e-72	190/208(91%)	3/208(1%)	Plus/Plus

```

Query 2  GATCTCTGTAGATCTGTTCTCTAAACGAACCTTAAAAATCTGTGGCTGTCACTCGGC 61
Sbjct 40  GATCTCTGTAGATCTGTTCTCTAAACGAACCTTAAAAATCTGTGGCTGTCACTCGGC 99

Query 62  GCATGCTTAGTGCACTCACGCAGTATAAATAAATAA--TTACTGCTGTTGACAGGAC 119
Sbjct 100  GCATGCTTAGTGCACTCACGCAGTATAAATAAATAA--TTACTGCTGCTGACTGGA 158

Query 120  ACEAGTAACTCGTCTATCTTCTGACGGCTGCTTACGGTTTCGTCGGTGTGCAECCGATC 179
Sbjct 159  ACEAGTAACTCGTCTTCTTCTGACGACTGCTTACGGTTTCGTCGGTGTGCAECCGATC 218

Query 180  ATCAGCACATCTAGGTTTCGTCGGGGTG 207
Sbjct 219  ATCAGCACATCTAGGTTTCGTCGGGGTG 246

```

Download Graphics

Sequence ID: **Query\_28649** Length: 280 Number of Matches: 1

Range 1: 1 to 211 [Graphics](#) [Next Match](#)

Score	Expect	Identities	Gaps	Strand
385 bits(208)	7e-112	210/211(99%)	0/211(0%)	Plus/Plus

```

Query 54  AGATCTGTTCTCTAAACGAACCTTAAAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAG 113
Sbjct 1  AGATCTGTTCTCTAAACGAACCTTAAAAATCTGTGTGGCTGTCACTCGGCTGCATGCTTAG 60

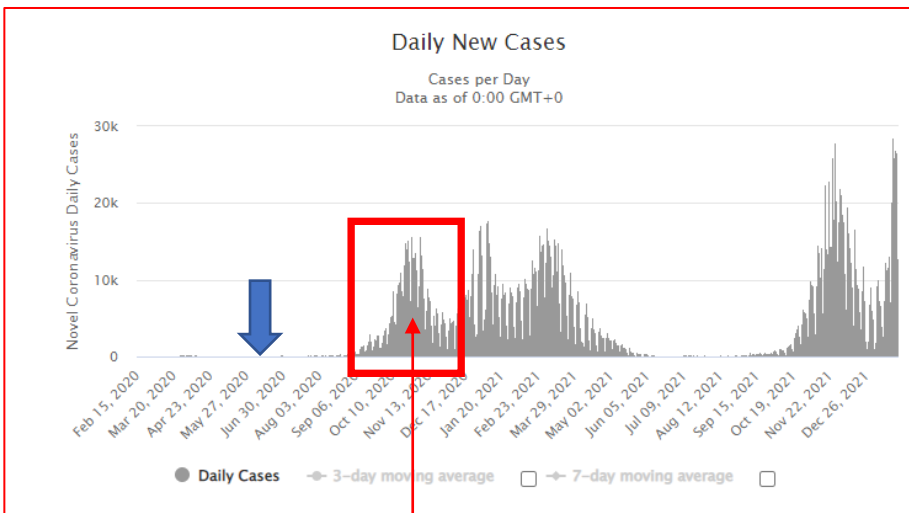
Query 114  TGCACCTCACGCAGTATAAATAAATAAATAA--TTACTGCTGTTGACAGGACACGAGTAACTCG 173
Sbjct 61  TGCACCTCACGCAGTATAAATAAATAAATAA--TTACTGCTGTTGACAGGACACGAGTAACTCG 120

Query 174  TCTATCTTCTGACGGCTGCTTACGGTTTCGTCGGTGTGCAECCGATCATCAGCACATCT 233
Sbjct 121  TCTATCTTCTGACGGCTGCTTACGGTTTCGTCGGTGTGCAECCGATCATCAGCACATCT 180

Query 234  AGGTTTTCGTCGGGGTGTGACCGAAAGGTAAG 264
Sbjct 181  AGGTTTTCGTCGGGGTGTGACCGAAAGGTAAG 211

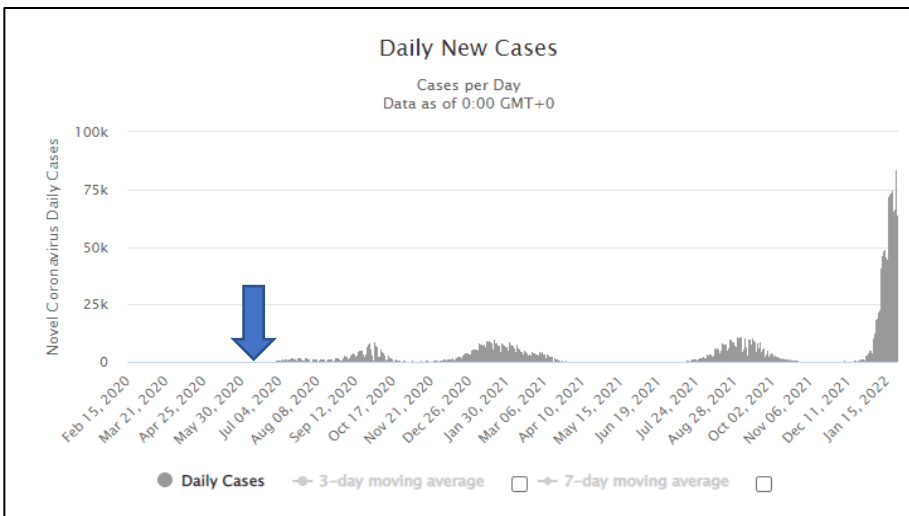
```

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

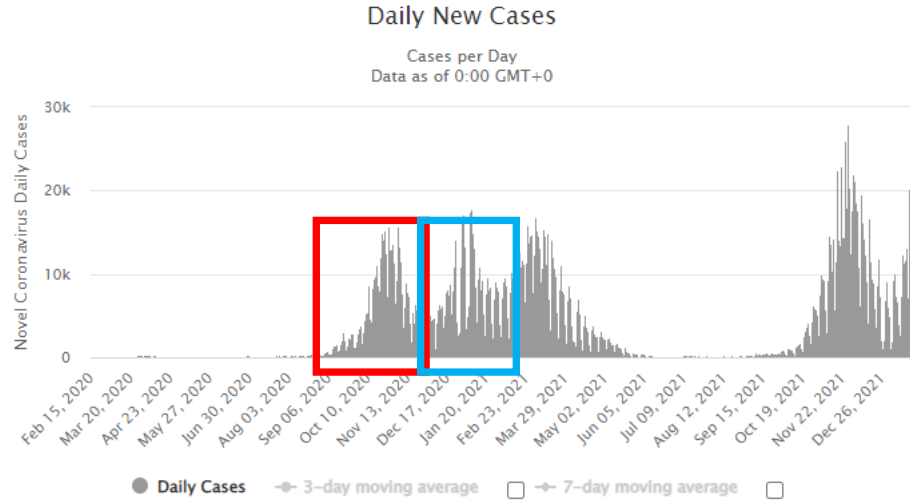
Druhá vlna šíření nákazy covidem-19 nejspíše přijde, zásadní je, jestli se potom podaří udržet denní přírůstek počtu nakažených v řádu stovek, nebo bude ještě hůře. V **Interview ČT24** to řekl vládní zmocněnec pro výzkum ve zdravotnictví **Roman Prymula**. Zkušenost z Izraele přitom ukazuje, že nástup 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



## Daily New Cases in Czechia



See also: [Daily Deaths Graph](#)

## Active Cases in Czechia

7	81212	COVID-19 (Nov)	20.07.2020	Praha, covid import
8	81217	COVID-19 (Nov)	21.07.2020	Praha, covid import
9	81226	COVID-19 (Nov)	21.07.2020	Praha, covid import
10	81238	COVID-19 (Nov)	22.07.2020	Praha, covid import
11	81247	COVID-19 (Nov)	22.07.2020	
12	81244	COVID-19 (Nov)	23.07.2020	Praha, covid import
13	81244	COVID-19 (Nov)	24.07.2020	Praha, covid import
14	81256	COVID-19 (Nov)	24.07.2020	Praha, covid import
15	81269	COVID-19 (Nov)	25.07.2020	Praha, covid import
16	81296	COVID-19 (Nov)	26.07.2020	Praha, covid import
17	81335	COVID-19 (Nov)	27.07.2020	Praha, covid import
18	81379	COVID-19 (Nov)	28.07.2020	Praha, covid import
19	81420	COVID-19 (Nov)	29.07.2020	Praha, covid import
20	81461	COVID-19 (Nov)	30.07.2020	Praha, covid import
21	81501	COVID-19 (Nov)	31.07.2020	Praha, covid import
22	81539	COVID-19 (Nov)	01.08.2020	
23	81585	COVID-19 (Nov)	02.08.2020	
24	81628	COVID-19 (Nov)	03.08.2020	
25	81669	COVID-19 (Nov)	04.08.2020	
26	81708	COVID-19 (Nov)	05.08.2020	
27	81745	COVID-19 (Nov)	06.08.2020	
28	81780	COVID-19 (Nov)	07.08.2020	
29	81813	COVID-19 (Nov)	08.08.2020	
30	81844	COVID-19 (Nov)	09.08.2020	
31	81873	COVID-19 (Nov)	10.08.2020	
32	81900	COVID-19 (Nov)	11.08.2020	
33	81925	COVID-19 (Nov)	12.08.2020	
34	81948	COVID-19 (Nov)	13.08.2020	
35	81969	COVID-19 (Nov)	14.08.2020	
36	81988	COVID-19 (Nov)	15.08.2020	
37	82005	COVID-19 (Nov)	16.08.2020	
38	82020	COVID-19 (Nov)	17.08.2020	
39	82033	COVID-19 (Nov)	18.08.2020	
40	82044	COVID-19 (Nov)	19.08.2020	
41	82053	COVID-19 (Nov)	20.08.2020	
42	82060	COVID-19 (Nov)	21.08.2020	
43	82065	COVID-19 (Nov)	22.08.2020	
44	82068	COVID-19 (Nov)	23.08.2020	
45	82069	COVID-19 (Nov)	24.08.2020	
46	82068	COVID-19 (Nov)	25.08.2020	
47	82065	COVID-19 (Nov)	26.08.2020	
48	82060	COVID-19 (Nov)	27.08.2020	
49	82053	COVID-19 (Nov)	28.08.2020	
50	82044	COVID-19 (Nov)	29.08.2020	
51	82033	COVID-19 (Nov)	30.08.2020	
52	82020	COVID-19 (Nov)	31.08.2020	
53	82005	COVID-19 (Nov)	01.09.2020	
54	81988	COVID-19 (Nov)	02.09.2020	
55	81969	COVID-19 (Nov)	03.09.2020	
56	81948	COVID-19 (Nov)	04.09.2020	
57	81925	COVID-19 (Nov)	05.09.2020	
58	81900	COVID-19 (Nov)	06.09.2020	
59	81873	COVID-19 (Nov)	07.09.2020	
60	81844	COVID-19 (Nov)	08.09.2020	
61	81813	COVID-19 (Nov)	09.09.2020	
62	81780	COVID-19 (Nov)	10.09.2020	
63	81745	COVID-19 (Nov)	11.09.2020	
64	81708	COVID-19 (Nov)	12.09.2020	
65	81669	COVID-19 (Nov)	13.09.2020	
66	81628	COVID-19 (Nov)	14.09.2020	
67	81585	COVID-19 (Nov)	15.09.2020	
68	81539	COVID-19 (Nov)	16.09.2020	
69	81490	COVID-19 (Nov)	17.09.2020	
70	81439	COVID-19 (Nov)	18.09.2020	
71	81386	COVID-19 (Nov)	19.09.2020	
72	81331	COVID-19 (Nov)	20.09.2020	
73	81274	COVID-19 (Nov)	21.09.2020	
74	81215	COVID-19 (Nov)	22.09.2020	
75	81154	COVID-19 (Nov)	23.09.2020	
76	81091	COVID-19 (Nov)	24.09.2020	
77	81026	COVID-19 (Nov)	25.09.2020	
78	80959	COVID-19 (Nov)	26.09.2020	
79	80890	COVID-19 (Nov)	27.09.2020	
80	80819	COVID-19 (Nov)	28.09.2020	
81	80746	COVID-19 (Nov)	29.09.2020	
82	80671	COVID-19 (Nov)	30.09.2020	
83	80594	COVID-19 (Nov)	01.10.2020	
84	80515	COVID-19 (Nov)	02.10.2020	
85	80434	COVID-19 (Nov)	03.10.2020	
86	80351	COVID-19 (Nov)	04.10.2020	
87	80266	COVID-19 (Nov)	05.10.2020	
88	80179	COVID-19 (Nov)	06.10.2020	
89	80090	COVID-19 (Nov)	07.10.2020	
90	80000	COVID-19 (Nov)	08.10.2020	
91	79908	COVID-19 (Nov)	09.10.2020	
92	79814	COVID-19 (Nov)	10.10.2020	
93	79718	COVID-19 (Nov)	11.10.2020	
94	79620	COVID-19 (Nov)	12.10.2020	
95	79520	COVID-19 (Nov)	13.10.2020	
96	79418	COVID-19 (Nov)	14.10.2020	
97	79314	COVID-19 (Nov)	15.10.2020	
98	79208	COVID-19 (Nov)	16.10.2020	
99	79100	COVID-19 (Nov)	17.10.2020	
100	78990	COVID-19 (Nov)	18.10.2020	
101	78878	COVID-19 (Nov)	19.10.2020	
102	78764	COVID-19 (Nov)	20.10.2020	
103	78648	COVID-19 (Nov)	21.10.2020	
104	78530	COVID-19 (Nov)	22.10.2020	
105	78410	COVID-19 (Nov)	23.10.2020	
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107	78164	COVID-19 (Nov)	25.10.2020	
108	78038	COVID-19 (Nov)	26.10.2020	
109	77910	COVID-19 (Nov)	27.10.2020	
110	77780	COVID-19 (Nov)	28.10.2020	
111	77648	COVID-19 (Nov)	29.10.2020	
112	77514	COVID-19 (Nov)	30.10.2020	
113	77378	COVID-19 (Nov)	31.10.2020	
114	77240	COVID-19 (Nov)	01.11.2020	
115	77100	COVID-19 (Nov)	02.11.2020	
116	76958	COVID-19 (Nov)	03.11.2020	
117	76814	COVID-19 (Nov)	04.11.2020	
118	76668	COVID-19 (Nov)	05.11.2020	
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120	76370	COVID-19 (Nov)	07.11.2020	
121	76218	COVID-19 (Nov)	08.11.2020	
122	76064	COVID-19 (Nov)	09.11.2020	
123	75908	COVID-19 (Nov)	10.11.2020	
124	75750	COVID-19 (Nov)	11.11.2020	
125	75590	COVID-19 (Nov)	12.11.2020	
126	75428	COVID-19 (Nov)	13.11.2020	
127	75264	COVID-19 (Nov)	14.11.2020	
128	75100	COVID-19 (Nov)	15.11.2020	
129	74934	COVID-19 (Nov)	16.11.2020	
130	74766	COVID-19 (Nov)	17.11.2020	
131	74596	COVID-19 (Nov)	18.11.2020	
132	74424	COVID-19 (Nov)	19.11.2020	
133	74250	COVID-19 (Nov)	20.11.2020	
134	74074	COVID-19 (Nov)	21.11.2020	
135	73896	COVID-19 (Nov)	22.11.2020	
136	73716	COVID-19 (Nov)	23.11.2020	
137	73534	COVID-19 (Nov)	24.11.2020	
138	73350	COVID-19 (Nov)	25.11.2020	
139	73164	COVID-19 (Nov)	26.11.2020	
140	72976	COVID-19 (Nov)	27.11.2020	
141	72786	COVID-19 (Nov)	28.11.2020	
142	72594	COVID-19 (Nov)	29.11.2020	
143	72400	COVID-19 (Nov)	30.11.2020	
144	72204	COVID-19 (Nov)	01.12.2020	
145	72006	COVID-19 (Nov)	02.12.2020	
146	71806	COVID-19 (Nov)	03.12.2020	
147	71604	COVID-19 (Nov)	04.12.2020	
148	71400	COVID-19 (Nov)	05.12.2020	
149	71194	COVID-19 (Nov)	06.12.2020	
150	70986	COVID-19 (Nov)	07.12.2020	
151	70776	COVID-19 (Nov)	08.12.2020	
152	70564	COVID-19 (Nov)	09.12.2020	
153	70350	COVID-19 (Nov)	10.12.2020	
154	70134	COVID-19 (Nov)	11.12.2020	
155	69916	COVID-19 (Nov)	12.12.2020	
156	69696	COVID-19 (Nov)	13.12.2020	
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158	69250	COVID-19 (Nov)	15.12.2020	
159	69024	COVID-19 (Nov)	16.12.2020	
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162	68334	COVID-19 (Nov)	19.12.2020	
163	68100	COVID-19 (Nov)	20.12.2020	
164	67864	COVID-19 (Nov)	21.12.2020	
165	67626	COVID-19 (Nov)	22.12.2020	
166	67386	COVID-19 (Nov)	23.12.2020	
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168	66900	COVID-19 (Nov)	25.12.2020	
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170	66406	COVID-19 (Nov)	27.12.2020	
171	66156	COVID-19 (Nov)	28.12.2020	
172	65904	COVID-19 (Nov)	29.12.2020	
173	65650	COVID-19 (Nov)	30.12.2020	
174	65394	COVID-19 (Nov)	31.12.2020	
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177	64614	COVID-19 (Nov)	03.01.2021	
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180	63816	COVID-19 (Nov)	06.01.2021	
181	63546	COVID-19 (Nov)	07.01.2021	
182	63274	COVID-19 (Nov)	08.01.2021	
183	63000	COVID-19 (Nov)	09.01.2021	
184	62724	COVID-19 (Nov)	10.01.2021	
185	62446	COVID-19 (Nov)	11.01.2021	
186	62166	COVID-19 (Nov)	12.01.2021	
187	61884	COVID-19 (Nov)	13.01.2021	
188	61600	COVID-19 (Nov)	14.01.2021	
189	61314	COVID-19 (Nov)	15.01.2021	
190	61026	COVID-19 (Nov)	16.01.2021	
191	60736	COVID-19 (Nov)	17.01.2021	
192	60444	COVID-19 (Nov)	18.01.2021	
193	60150	COVID-19 (Nov)	19.01.2021	
194	59854	COVID-19 (Nov)	20.01.2021	
195	59556	COVID-19 (Nov)	21.01.2021	
196	59256	COVID-19 (Nov)	22.01.2021	
197	58954	COVID-19 (Nov)	23.01.2021	
198	58650	COVID-19 (Nov)	24.01.2021	
199	58344	COVID-19 (Nov)	25.01.2021	
200	58036	COVID-19 (Nov)	26.01.2021	
201	57726	COVID-19 (Nov)	27.01.2021	
202	57414	COVID-19 (Nov)	28.01.2021	
203	57100	COVID-19 (Nov)	29.01.2021	
204	56784	COVID-19 (Nov)	30.01.2021	
205	56466	COVID-19 (Nov)	31.01.2021	
206	56146	COVID-19 (Nov)	01.02.2021	
207	55824	COVID-19 (Nov)	02.02.2021	
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210	54846	COVID-19 (Nov)	05.02.2021	
211	54516	COVID-19 (Nov)	06.02.2021	
212	54184	COVID-19 (Nov)	07.02.2021	
213	53850	COVID-19 (Nov)	08.02.2021	
214	53514	COVID-19 (Nov)	09.02.2021	
215	53176	COVID-19 (Nov)	10.02.2021	
216	52836	COVID-19 (Nov)	11.02.2021	
217	52494	COVID-19 (Nov)	12.02.2021	
218	52150	COVID-19 (Nov)	13.02.2021	
219	51804	COVID-19 (Nov)	14.02.2021	
220	51456	COVID-19 (Nov)	15.02.2021	
221	51106	COVID-19 (Nov)	16.02.2021	
222	50754	COVID-19 (Nov)	17.02.2021	
223	50400	COVID-19 (Nov)	18.02.2021	
224	50044	COVID-19 (Nov)	19.02.2021	
225	49686	COVID-19 (Nov)	20.02.2021	
226	49326	COVID-19 (Nov)	21.02.2021	
227	48964	COVID-19 (Nov)	22.02.2021	
228	48600	COVID-19 (Nov)	23.02.2021	
229	48234	COVID-19 (Nov)	24.02.2021	
230	47866	COVID-19 (Nov)	25.02.2021	
231	47496	COVID-19 (Nov)	26.02.2021	
232	47124	COVID-19 (Nov)	27.02.2021	
233	46750	COVID-19 (Nov)	28.02.20	



- 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		L16L	N160N	del141-143	V169V	Y195H	S216L	S302F	G392V	R402R	D448D	A498V	K517T	V868V	D909D	F924F	T100I	V1025L	T1246I	A1298V	T1496I	T1543I	A1708D	F1907F	K2029N	S2030L	D2060D	A2080S			
Kmen																															
pražský / český																															
listopad 2020															X														X		
britský (alpha)							X										X						X	X							
delta																															
ORF1ab		U2080S	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	
					X	X	X					X				X				X				X							
X	X								X			X		X							X										
			X							X											X	X	X	X					X		
S protein		U5426S	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	
							X		X					X	X	X				X											
																						X									
																		X							X						
S protein		U222V	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	
			X						X						X												X				
									X																						
					X			X	X		X	X		X		X													X	X	X
				X	X				X		P681R																				
N protein		Y73C	D3L	T147A	N192K	S194L	N196Y	R203K	G204P	G204R	S235F	F274F	S318S	P302P	D341D	ORF10															
																g.29542G/T															
				X																											
X	X							X	X		X																				

### 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

Poznámka  Datum příjmu

Biologický materiál  Datum odběru

Geny SARS2x14 (4) SARS2\_24 (4) SARS2\_34 (4) SARS2\_44 (4)

Číslo	Mutace	read(max)	read(min)	read(Ø)	poč.Nem.Hom.	poč.Het.	Poč.Basí
5017	Část 13/16	5230	3	1398	2	11	1864

>Referenční Sequence  
 >98LDO:00049:00187 + 403  
 >98LDO:00027:00216 - 303  
 >98LDO:00021:00085 - 201  
 >98LDO:00017:01353 - 95  
 >98LDO:00011:01615 + 72

>A  
 >T  
 >C  
 >G  
 >+  
 >Posice v mRNA  
 >Referenční Sequence  
 >Posice v seq.proteinu  
 >Referenční seq.protein  
 >Consensual sequence 6062

Protein S: D614G (GAT>GGT)





Accelerated Article Preview

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

Received: 20 February 2020

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online 4 May 2020

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

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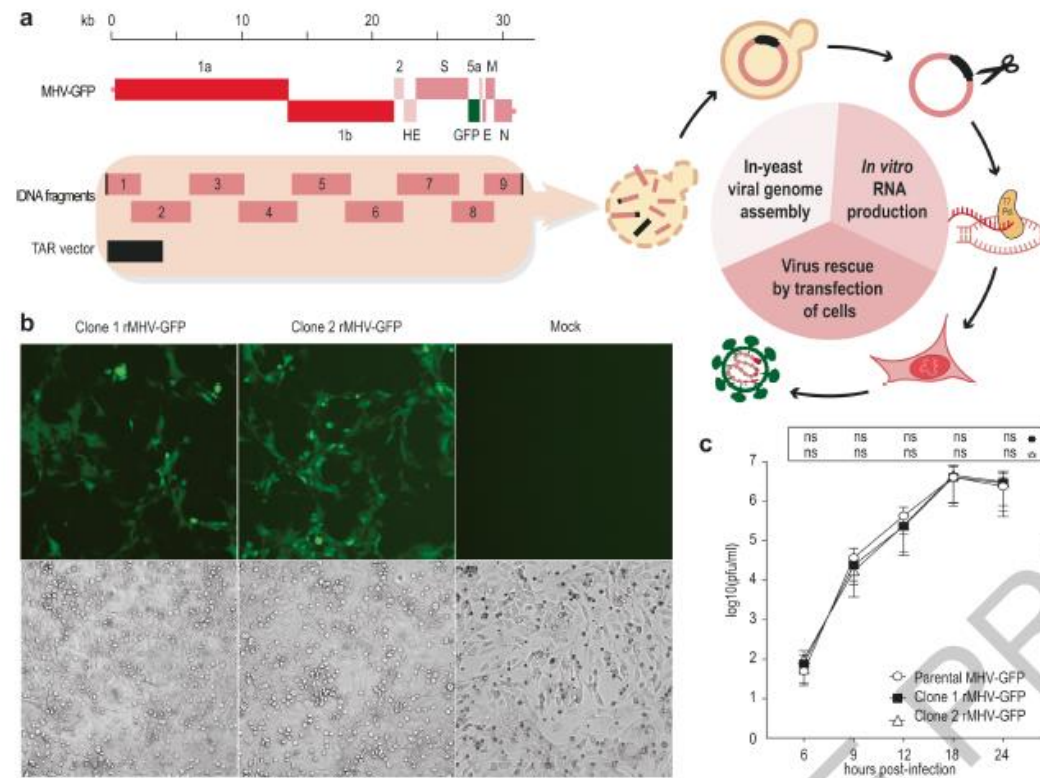
Accepted: 24 April 2020

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

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<sup>1–3</sup>. 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-synthesized clones of the recent epidemic SARS-CoV-2<sup>4</sup> 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.

**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
<i>Homo sapiens (human)</i>	Q9BYF1	S	Q	T	F	D	K	H	E	D	Y	Q	L	Y	N	K	R	100
<i>Macaca fascicularis (monkey)</i>	A0A2K5X283	S	Q	T	F	D	K	H	E	D	Y	Q	L	Y	N	K	R	100
<i>Panthera tigris (tiger)</i>	XP_007090142.1	S	L	T	F	D	K	H	E	<b>E</b>	Y	Q	L	Y	K	K	R	94
<i>Bos Taurus (cow)</i>	NP_001019673.2	S	Q	T	F	<b>E</b>	K	H	E	D	Y	Q	<u>M</u>	Y	N	K	R	88
<i>Mesocricetus auratus (hamster)</i>	A0A1U7QTA1	S	Q	T	F	D	<u>L</u>	<u>Q</u>	E	D	Y	Q	L	Y	N	K	R	88
<i>Felis catus (cat)</i>	Q56H28	S	<u>L</u>	T	F	<b>E</b>	K	H	E	<b>E</b>	Y	Q	L	Y	N	K	R	81
<i>Rhinolophus sinicus (bat)</i>	U5WHY8	S	<i>E</i>	<b>M</b>	F	D	K	<i>I</i>	E	D	<u>H</u>	Q	L	Y	N	K	R	75
<i>Paguma larvata (civet)</i>	Q56NL1	S	<u>L</u>	T	F	<b>E</b>	K	<u>Y</u>	E	<b>Q</b>	Y	Q	L	Y	N	K	R	75
<i>Equus ferus caballus (horse)</i>	F6V9L3	S	<u>L</u>	T	F	D	K	<u>S</u>	E	<b>E</b>	<u>H</u>	Q	L	Y	N	K	R	75
<i>Mustela putorius furo (ferret)</i>	Q2WG88	<u>D</u>	<u>L</u>	T	F	<b>E</b>	K	<i>I</i>	E	<b>E</b>	Y	Q	-	Y	N	K	R	69
<i>Canis luparis (dog)</i>	J9P7Y2	-	<u>L</u>	T	F	<b>E</b>	K	<u>Y</u>	E	<b>E</b>	Y	Q	L	Y	N	K	R	69
<i>Mus musculus (mouse)</i>	Q8R0I0	S	<b>N</b>	T	F	<b>N</b>	<u>N</u>	<u>Q</u>	E	D	Y	Q	<u>Y</u>	<u>E</u>	N	K	R	63
<i>Manis javanica (pangolin)</i>	XP_017505752.1	-	<i>E</i>	T	F	<b>E</b>	K	<u>S</u>	E	<b>E</b>	Y	Q	<b>I</b>	Y	N	K	R	63
<i>Ophiophagus Hannah (snake)</i>	ETE61880.1	<u>Q</u>	<u>V</u>	<u>K</u>	F	<b>E</b>	<u>Q</u>	<u>A</u>	-	D	Y	<b>N</b>	<u>N</u>	<b>F</b>	N	<u>L</u>	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).

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

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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.

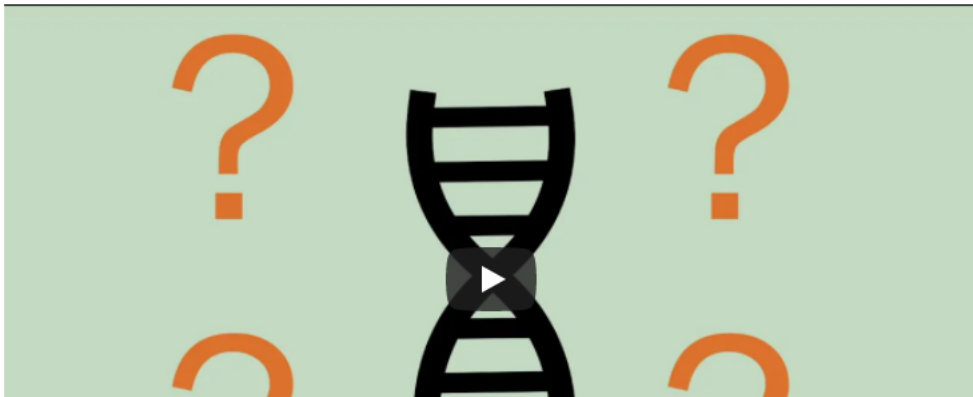
MIND & BODY, RESEARCH, SCIENCE & ENVIRONMENT

## 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 awarded the 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](#)



# Do It Yourself



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September 3rd, 2017

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DIYbio events for the remainder of the week of August 27

August 27th, 2017

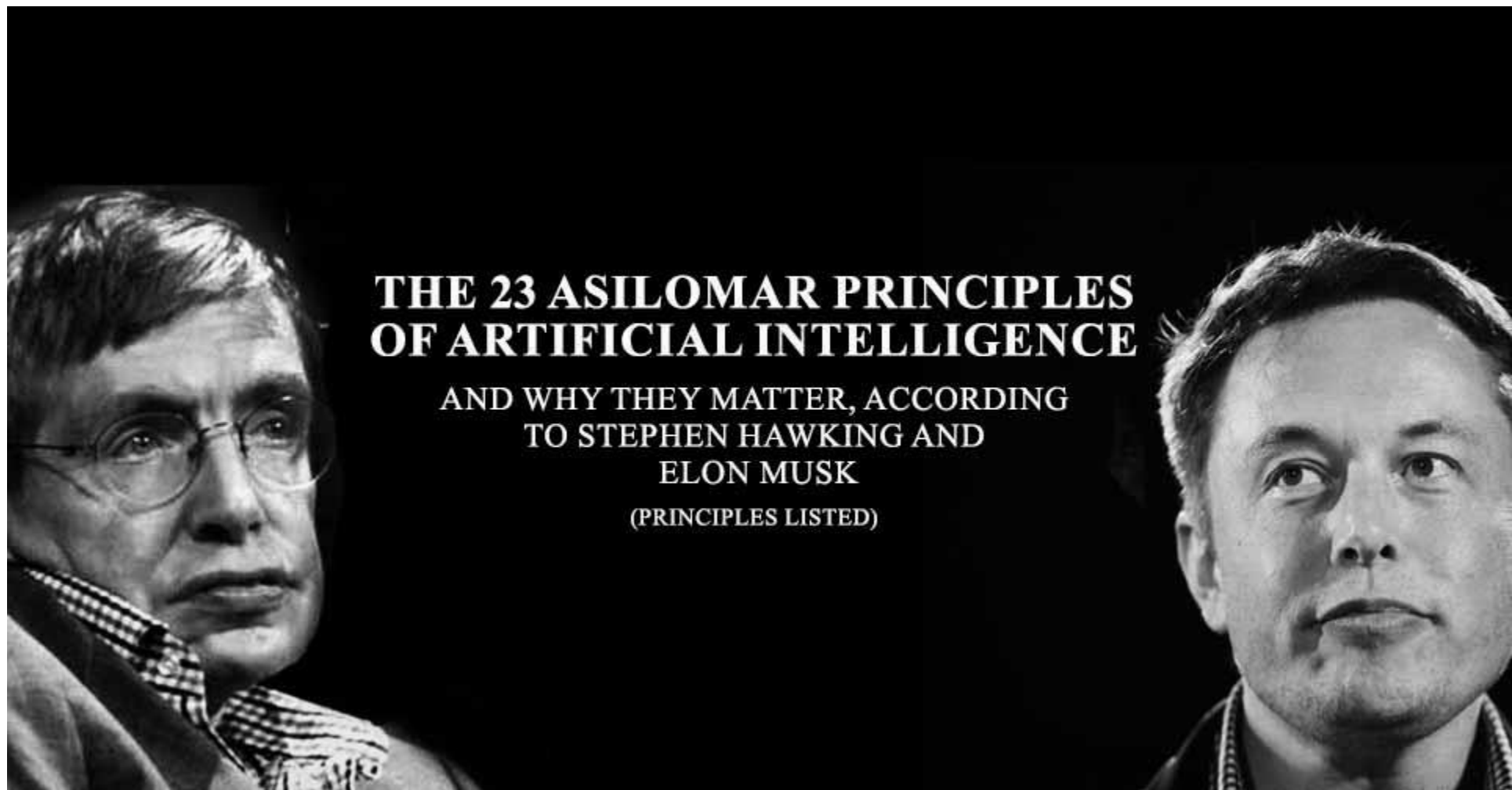
Here are your events for the remainder of the week Monday, August 28 Oakland, CA, USA –





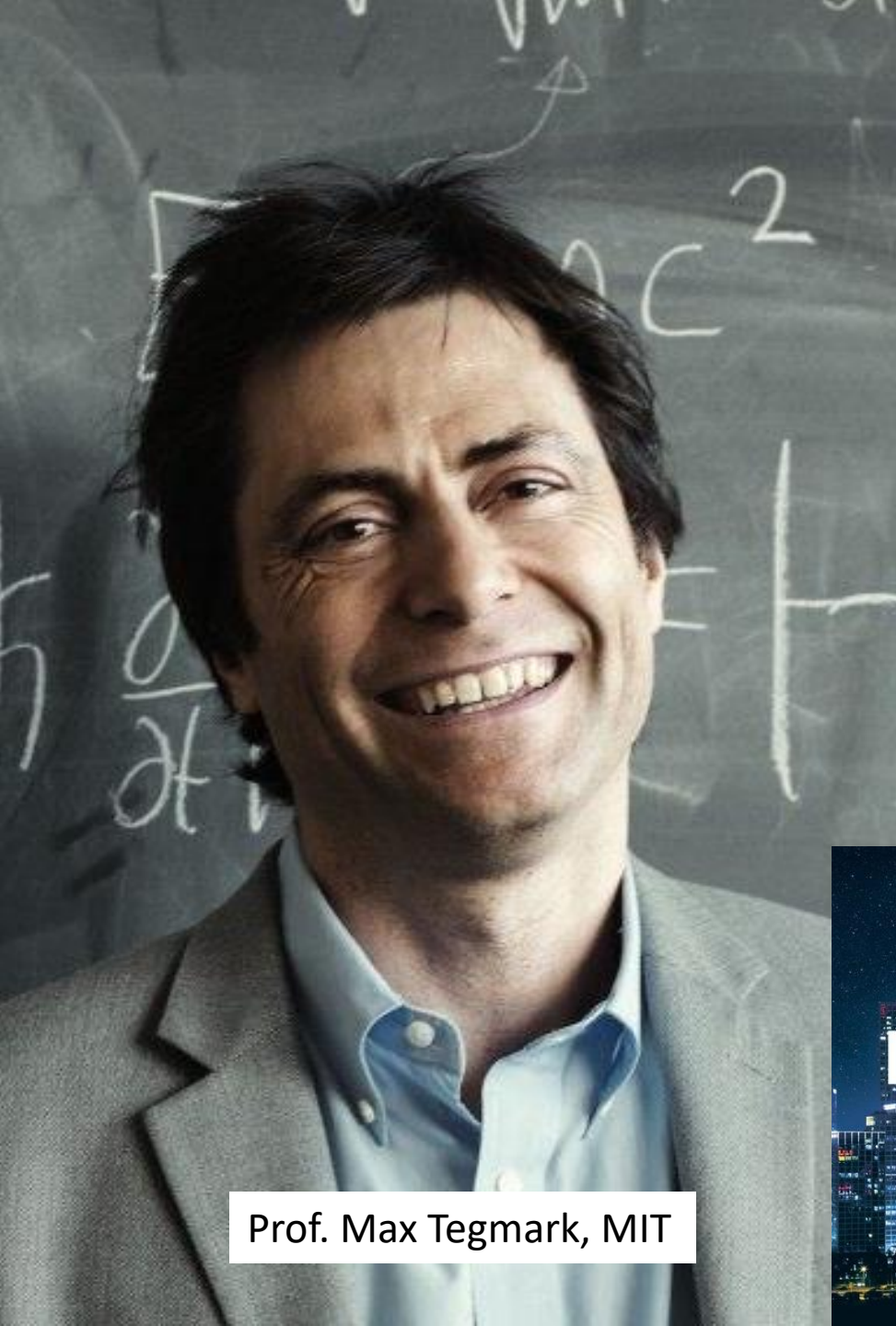
Etika v molekulární biologii a genetice

17. ledna 2017, Asilomar, CA, USA



Etika v oboru umělé inteligence (AI)





Prof. Max Tegmark, MIT



## 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