(U) Intelligence Community assessment

(U) 1. The attached document includes assessments of SARS-CoV-2 origin from a National Intelligence Council memorandum issued on 8 April. Do you agree with these assessments? If not, could you explain why?

(U) Research methods and capabilities

(U) 2. Can you distinguish between an engineered virus and one that is resultant from natural evolution? If so, how?

(U) 3. If the COVID-19 was engineered, what processes would be used? Would CRISPR/CAS9 or forced cell passage be the most appealing means? Or would other means be most appealing and why? Do investigators in China, particularly in Wuhan, have the capability to perform such processes? If so, does their N-terminal domain modifications conducted at Wuhan have any relevance?

(U) 4. Does previous gain-of-function research have an relevance to the question of SARS-CoV-2 origin—particularly, generation of a virus with SARS backbone and bat coronavirus spike protein (at UNC; Menachery et al. Nat Med 2015), and the H5N1 GOF studies?

(U) 5. If the COVID-19 virus has ribozymes or aptazymes in its sequence, would this be inserted by engineered means only? Does China have the capability to perform such viral modifications in your view, based on published work in mammalian cell lines?

(U) 6. What cell types are used to culture bat coronaviruses and SARS-CoV-2, and why? When repeated passage is performed in certain cell types, how often do certain sequences of the cell get inserted into the virus? Is this more common in certain types of cell lines?

(U) SARS-CoV-2

(U) 7. Have you found any interesting features in the COVID-19 sequence? If so, please explain.

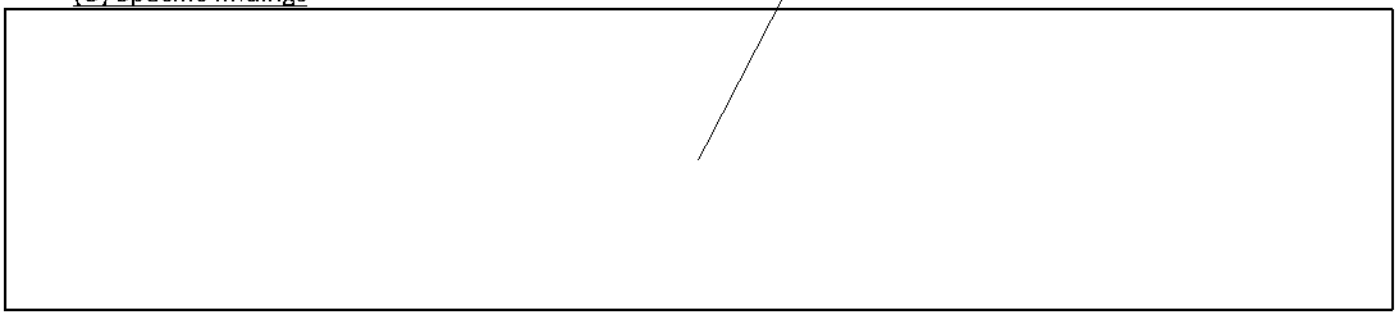
(U) 8. How does the COVID-19 sequence differ from other lineage 2B-coronaviruses if at all? What are your thoughts on why the COVID-19 virus differs from other bat coronaviruses? Is this due to natural evolution or recombination?

(U) 9. Based on your inquiry into COVID-19 RNA sequence, could the COVID-19 be an engineered virus? If so, discuss the specific viral features that would indicate engineering processes. Could this be the result of gain of function research? If so, why?

(U) 10. Have you inquired about the presence of ribozymes or aptazymes in the COVID-19 sequence? If so, do you see any evidence in the COVID-19 sequence? Would this be part of a normal viral sequence inquiry?

(b)(3):50 USC 3024(i); Sec. 1.4(c); Sec. 1.4(e)

(U) Specific findings



(U) 12. Letko et al. (Nature Microbiol 2020) wrote that "the RBD for SARS-CoV-2 . . . forms a distinct clade". Do you agree? Why or why note? Could you comment on the significance of this determination?

(U) 13. Coutard et al. (Antiviral Res 2020) wrote that "Strikingly, the 2019-nCoV S-protein sequence contains 12 additional nucleotides upstream of the single Arg↓ cleavage site 1 leading to a predictively solvent exposed PRRAR↓SV sequence, which corresponds to a canonical furin-like cleavage site (Braun and Sauter, 2019; Izaguirre, 2019; Seidah and Prat, 2012). This furin-like cleavage site, is supposed to be cleaved during virus egress (Mille and Whittaker, 2014) for S-protein "priming" and may provide a gain-of-function to the 2019-nCoV for efficient spreading in the human population compared to other lineage b betacoronaviruses. This possibly illustrates a convergent evolution pathway between unrelated CoVs." Do you have any comments on this statement?

(U) 14. Coutard et al. (Antiviral Res 2020) wrote that "the 2019-nCoV S-protein conserves site 2 sequence AYT↓M may still be cleaved, possibly after the preferred furin cleavage at the site 1. Since furin is highly expressed in lungs, an enveloped virus that infects the respiratory tract may successfully exploit this convertase to activate its surface glycoprotein (Bassi et al., 2017; Mbikay et al., 1997). Before the emergence of the 2019-nCoV, this important feature was not observed in the lineage b of betacoronaviruses." Do you have any comments on this statement?

(U) 15. Zhou et al. (bioRxiv 2020) wrote that "RmYN02 (novel bat-derived coronavirus) was characterize by the insertion of multiple amino acids at the junction site of the S1 and S2 subunits of the spike protein. This provides trong evidence that such insertion events can occur in nature." Do you think it is appropriate to say the insertion of 3 residues, PAA, at the junction of S1 and S2 in RmYN02, is strong evidence that a recombination event is responsible for the 4 amino acid substitutions at the junction of S1 and S2 in SARS-CoV-2? Are there any red-flags in this paper regarding scientific methodology that should cast doubt on its validity?

(U) 16. Lu et al. (Lancet 2020) wrote that "despite its occurrence, recombination is probably not the reason for emergence of this virus. Do you have any comments on this statement?

(U) 17. Poon et al. (J Med Virol 2005) reported only deletions and single nucleotide substitutions with repeated passage of SARS-CoV. Is this a good indicator of what would happen if repeated passage with SARS-CoV-2 was performed (i.e., no changes in sequential amino acids)? Is the answer different for cell line vs. animal model?

(U) 18. Zhang et al. (arXiv 2020) refuted previous studies that reported unique similarities between SARS-CoV-2 spike insertions and HIV-1. Do you find this analysis convincing?

(U) 19. Could you comment on SARS-Cov-2's unique affinity for the human ACE2 receptor, which reportedly is 10 to 20 times greater than SARS. Could this be due to vaccine-related research using gain of function, and is the taxonomy similar to the H5N1 research in the recent past?

<u>Anything else</u>

(U) 20. What other questions about SARS-CoV-2 should we be asking as part of the origin assessment? Could you share answers for any of them?