Synthetic virology: the experts speak

Even though only a few labs around the world have the means to engineer a purely synthetic virus, debate on the origins of SARS-CoV-2 has resurfaced concerns about the risks and benefits of synthetic virology.

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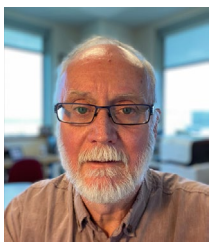
Synthetic virology—the re-creation and manipulation of viruses to study their properties—provides a powerful way of investigating how viruses cause infections and how to combat pathogenic subtypes. This is particularly true for hard-to-culture viruses. However, this approach also raises the prospect that bad actors could create more deadly viruses. Over a decade ago, the World Health Organization (WHO) issued a warning that "advances in genome sequencing and gene synthesis would render substantial portions of [variola] accessible to anyone with an internet connection and access to a DNA synthesizer," leading to concerns about future attempts to engineer viruses from the smallpox family. Here, *Nature Biotechnology* convenes a group of experts and a biohacker (see Box 1) to discuss the current state of synthetic virology. How far has the technology has advanced, what is currently possible, and what might the future hold in terms of best practices for advancing scientific knowledge and promoting biosecurity?

How have DNA synthesis and virus creation advanced in the past ten years?

David Evans: The primary advance in the past ten years has been the rapid reduction in the cost of assembling larger and larger DNA clones. This has 'democratized' access to the technology. Chemistry and instrumentation continue to improve alongside improved methods for assembling large DNA clones in vitro (for example, Gibson assembly) and in yeast (yeast artificial chromosomes; YACs) and bacteria (bacterial artificial chromosomes; BACs).



David Evans, University of Alberta.

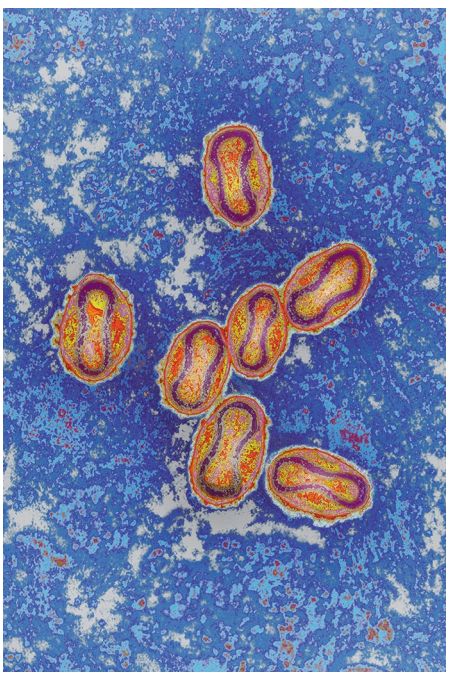
Nicholas Evans: We've gotten better at it. The number of base pairs we can synthesize has gone up, as has the size of the fragments we can produce prior to full genome synthesis. Techniques for stitching partial sequences together have become more reliable and may not create the effect seen by Wimmer in 2002 in which a lack of attention to supposed non-coding regions of the poliovirus synthesis rendered it less pathogenic.

The most common proxy for ease is cost per base pair, which has gone down considerably. Currently, it is estimated at 30 cents a base pair, which is still expensive for non-traditional science communities, but will continue to go down.

Finally, life sciences techniques have sprung up around RNA and DNA synthesis that will make viral synthesis easier. An additional technique arising from the 2018 horsepox synthesis by David Evans' group (Fig. 1) was the use of helper viruses to generate a live horsepox virus.

David A. Markowitz, IARPA.

David Markowitz: Although commercially available methods of DNA synthesis remain the same, their cost and quality have improved substantially. Likewise, there have been many advances in the genome assembly, amplification and verification processes available to researchers with appropriate technical expertise. For example, in the time since Eckard Wimmer's paper, several techniques for gene assembly and amplification have been introduced in the academic literature and/or commercialized, including Gibson assembly and polymerase cycling assembly, with overlap extension



Pox virus. Image produced using high-dynamic-range imaging (HDRI) from an image taken with transmission electron microscopy. Credit: BSIP SA / Alamy Stock Photo

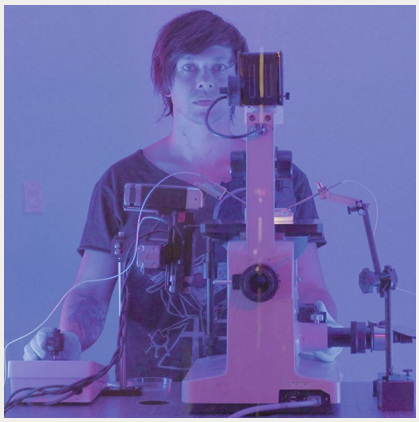
Box 1 | A biohacker's view

How realistic is the prospect of a bioterrorist synthesizing a pathogenic virus? More likely than you think.

Josiah Zayner: OK, let's do a thought experiment here. Not a fun Einstein *gedankenexperiment*, but an experiment that will scare you. How easy would it be for a bioterrorist to create a pandemic virus?

The main thing that has kept science siloed isn't expensive equipment but a lack of knowledge and training. This is changing rapidly as biomedical information is disseminated online. Nowadays, you can also find online any equipment and reagents you need and have them shipped to your home. If biotech companies won't ship what you are looking for to a residential address, you can usually find it on eBay or Alibaba or have it sent to a Post Office box. My company sells a pretty complete molecular biology lab for $1,600, and we make a profit. Cost is not a limiting factor. The future is here, people can easily do advanced molecular biology and genetic engineering in their kitchen, and there is no way to trace it or document it.

Once a bioterrorist has picked a virus to synthesize, it's not hard to find its sequence online. Synthetic DNA can be ordered from lots of companies all over the world, both large corporations like Genscript, IDT and Thermo and small mom-and-pop shops. Small companies are less likely to screen ordered DNA for pathogen sequences, but



Josiah Zayner, ODIN.

even many big companies don't claim to screen. Which companies screen sequences for pathogens? The International Gene Synthesis Consortium (IGSC), a group of companies that do screen, provides a list! The list includes 18 of the hundreds of companies that sell synthetic DNA to scientists and consumers. Although the list may not be complete, it seems like a major security hole to identify these companies to any potential bioterrorists. What's more, the IGSC graciously discloses what is screened: double-stranded DNA that is 200 base pairs or larger. Even if the companies tried to catch people who order, say, 190-base-pair sequences, it would not be hard to split the virus genome into sequences of 150 base pairs. Or to order large sequences as single-stranded DNA. Or to make many small orders from different companies. Or to disguise the sequences by changing the DNA codons while preserving the protein sequences.

Once one has the sequences in hand, simple molecular biology is all that is needed to create a virus. How to assemble viruses from plasmid DNA is described in papers. Growing and transfecting Vero or HEK293 cells can be done in simple media with no need for a CO_2 incubator or other fancy equipment. You can buy kits for pretty much the whole assembly and purification process, and using them doesn't require much more than pipettes and a centrifuge. Because a bioterrorist is more likely to work in a kitchen than in a BSL-4 lab, their biggest problem would be not infecting and killing themselves or the people they come in contact with.

This scenario scares me because the only thing between a would-be bioterrorist and a pandemic-creating virus is some thousands of dollars and being a psychopath. If the best idea we have on how to protect ourselves from bioterrorism is a flimsy consortium, something's got to change. I wish the US government cared about DNA synthesis companies. There are no laws or regulations requiring these firms to screen for any sort of bad DNA, and even if there were, a bioterrorist could just order from another country or even synthesize DNA themselves. Think about it: no one is arguing that SARS-CoV2 couldn't have come from a lab—just that it didn't.

PCR for assembly and rolling circle amplification for full-genome amplification. These techniques eliminate the need for plasmids and facilitate gene assembly from small fragments in a single reaction.

Similarly, several enzymatic error-correction technologies have been created that can be applied by appropriately skilled users to select error-free double-stranded DNA after the assembly process. These methods rely on either mismatch-binding proteins or mismatch-cleaving proteins to recognize distortions of the double helix that arise from indel (insertion/deletion) and substitution errors. Accompanying all laboratory technology innovations have been advances in bioinformatics tools that assist appropriately skilled users in genome analysis, design and verification. There are many open-source databases that contain extensively annotated genomic sequences across all virus families (such as the Virus Pathogen Database and Analysis Resource, ViPR). Both open-source and proprietary software tools are now available to guide the genome assembly process, from primer/overlap/restriction site design and melting temperature, GC content and codon optimization to the elimination of secondary structure and repeats.

Using commercial vendors, it now takes only 24 hours to receive primers needed to make modifications to a recombinant DNA construct, and in general, 5-kilobase gene fragments can be procured in less than one week. As a result, it is now possible for skilled researchers to produce a fully quality-controlled synthetic 30-kilobase RNA genome in two to three weeks. This capability was completely unavailable ten years ago.

Is there any practical or theoretical limit on the size of a virus genome that someone with limited knowledge could make?

David Evans: Synthetic virology relies on reverse genetics technologies, and these were first used to assemble viruses cloned using traditional methods. For example, influenza virus was produced from cloned DNA in 1999, six years before the reconstruction of the 1918 strain from synthetic fragments. However, the development of these methods takes a great deal of experimental skill. There's probably no theoretical limit to the size of virus that could be assembled as a DNA clone, but if there's no established way of reactivating the virus, then the clone could not be used to recover an infectious derivative.

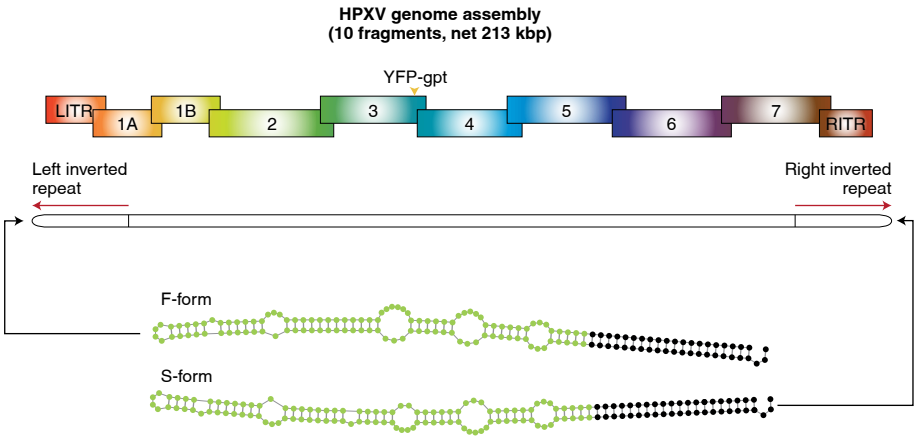


Fig. 1 | Assembling horsepox virus. The virus was assembled from ten different overlapping 1-kilobase-pair duplex DNA fragments, which were transfected into cells previously infected with a helper virus. Full-length genomes were created through virus-mediated homologous recombination. The inverted terminal repeats (red arrows) create two mismatched hairpin-ended structures (the F and S forms, shown enlarged at bottom). To replicate these features, two hairpins were ligated to the ends of the two fragments encoding the left and right inverted terminal repeats (LITR and RITR) before the transfection step. A gene encoding a drug-selectable yellow fluorescent protein (YFP-gpt) was used to recover reactivated virus. Courtesy of David Evans.

David Markowitz: With adequate technical knowledge and resources, there is a clear path to synthesizing whole multi-gigabase genomes of plants and animals within a decade. There is no practical limit on the ability to produce virus genomes—the largest of which is about a megabase. A non-expert is likely to highly rely on commercial vendors and software/hardware tools from the synthetic biology ecosystem to automate much of the design, synthesis, assembly and quality-control steps involved in virus creation. Currently, this is not an easy workflow to implement at any scale without significant domain expertise and access to molecular biology laboratory facilities.

Reshma Shetty, Gingko Bioworks.

Reshma Shetty: It is important to emphasize that generation of a DNA copy of a viral genome is not the same thing as generation of a replicating virus; indeed, substantial additional specialized expertise is required to achieve the latter. With respect to generation of a viral genome, there is no theoretical limit on the size of genome that can be made, other than perhaps what is found in nature (for example, human cytomegalovirus has the largest genome of any known human virus at 236 kilobases in size). Generally speaking, DNA synthesis and assembly gets harder above 10 kilobases, and much harder above 50 kilobases. However, there are companies and academic labs and consortia that have demonstrated even larger DNA syntheses, such as those of entire bacterial genomes or yeast chromosomes, though it is not trivial for others to achieve.

Eckard Wimmer: There is no theoretical limit.

How easy it is to acquire and cultivate cells in which to grow viruses?

David Evans: Polio was assembled in vitro, but influenza and horsepox viruses were assembled by transfecting DNA clones into cells (Fig. 1). Most cell lines are widely available in academic laboratories; however, viruses exhibit host range, and specific cell types may be difficult to acquire.

David Markowitz: Conventional tissue culture capabilities generally require laboratory infrastructure. However, the cells used to rescue viruses are dependent on the type of virus being rescued. A poorly characterized virus can pose a challenge for identifying a viable cell line for rescue. Cell-free systems can overcome this issue, but these systems are not established across all viruses and can be challenging to work with.

Reshma Shetty: Standard animal and human cell lines are readily available. Handling such cell lines and maintaining them in culture without contamination, however, requires specialized equipment and training. In addition, each specific virus requires a different receptor or other factors for successful propagation, so the cell line and propagation protocol must be compatible with a given virus, further increasing the barriers to this type of work.

Volker Thiel: The main hurdle for most viruses is the rescue procedure, meaning the steps required to get a virus replicating and isolated from a cloned nucleic acid. This step is dependent on the type of virus that is used, and for many viruses it requires ample expertise. In the future DNA synthesis, cloning and rescue procedures may become easier, but it will remain dependent on expertise.

How likely is it that people working outside traditional facilities would be able to create synthetic viruses?

David Evans: It's difficult to imagine any near-term advances that would substantially simplify the task of making synthetic viruses. The cloning and assembly steps will always require access to well-equipped molecular virology laboratories and supporting infrastructure. These facilities are expensive to assemble and operate, and difficult to acquire without being noticed. However, there is a thriving 'secondary market' for lab equipment, and if it continues to grow in an unregulated manner, it may create opportunities for people working outside the mainstream.

Nicholas G. Evans, University of Massachusetts Lowell.

Nicholas Evans: Right now, it is very unlikely. As of 2017 and the horsepox synthesis, there were still major technical and financial hurdles to viral synthesis. Viral synthesis is getting easier, but it is still technically challenging. The horsepox virus 'only' cost $100,000 to produce, according to one estimate: that's still too much for a non-traditional lab like a community lab.

The future is less certain. What we do know is there isn't an exceptional amount of interest [in virus synthesis] in non-traditional forums relative to the interest in traditional forums. There is also

recent social scientific evidence that these non-traditional spaces don't have the interest or incentive structure to pursue these kinds of advanced techniques that may be costly and might not provide utility above older techniques. Where it will happen, it is likely, as a recent horizon scan noted, to arise from the use of 'cloud labs' or other outsourcing methods that are going to emerge as a theme over the next decade of life sciences work.

David Markowitz: The ability to synthesize viruses with bespoke properties would have broad utility for applications in basic research, medicine and even agriculture. As such, there are strong market incentives for tool developers to make it easier for end users to synthesize viruses. Currently, it is very difficult to create a synthetic virus outside a traditional laboratory; however, this is likely to change with future improvements in cell-free systems, broader access to whole-genome sequencing and more accessible tools for nucleic acid synthesis through commercial vendors. To manage risk, it will also be important to ease the implementation of biosafety protocols by non-traditional virology facilities

Reshma Shetty: Given the present state of technology, regulations and DNA synthesis screening protocols, it's unlikely (but not impossible) that a person working outside traditional facilities would be able to create a biologically active synthetic virus of concern. Even within a well-equipped, traditional facility, there are limitations to performing such work.

Members of the International Gene Synthesis Consortium (IGSC) have agreed on a harmonized screening protocol to guard against the delivery of concerning DNA sequences and to ensure compliance with the US Department of Health and Human Services' Screening Framework Guidance for Providers of Synthetic Double-Stranded DNA Providers and other international standards. In the event that someone wanted to assemble many small fragments of otherwise innocuous sequences into a complete viral genome, moderate to advanced molecular biology skills would be required to produce a contiguous, error-free molecular clone.

Readers should also note that it is possible to obtain full-length viral genomes starting with infectious material directly from patient samples. In this approach—several examples of which have been published since the start of the COVID-19 pandemic—researchers recover infectious viral clones through RT-PCR of extracted viral RNA from patient samples that contain the virus. This approach is limited by the fact that it requires physical access to an infected patient or patient sample, meaning that it is only practical for viruses that are already widespread.

In each of the above cases, just making genetic material is not enough. Generating live virus from molecular clones requires in vitro/cell-free assembly (for a few classes of viruses) or tissue culture, along with the ability to deliver the viral genome into the cells. These advanced molecular and cell culture techniques are not trivial outside traditional facilities, and may be further restricted due to cost.

Even in the absence of de novo creation of a synthetic viral genome, it is also possible to deliver viral RNA extracted from patient samples into cells cultured in the laboratory, where the viral genomes can be 'booted up' and begin replicating again. If the recombinant virus generated in cell culture possesses human tropism, it could pose a threat to the actor in the absence of appropriate biocontainment. For example, in the case of research where 'live' SARS-CoV-2 is propagated, there are added technical requirements for laboratory containment at Biosafety Level 3 (BSL-3), which is not a widespread capability in traditional labs.

Improvements in technology are expected to make this work more feasible in the future. During particular events, such as a pandemic, there is an urgency to get samples and isolates of pathogens to the research, biotech and pharmaceutical communities so they can begin work immediately to respond to the pandemic. Modern technology has accelerated this process, enabling the re-creation and distribution of pathogens, in some cases even without actual access to the original pathogen itself. Although this can accelerate timelines for pathogen characterization and countermeasure development, new risk is introduced through the relative ease of distribution of the lab-generated components.

Eckard Wimmer, Stony Brook University.

Eckard Wimmer: Well-educated laboratory technicians can assemble a viral DNA genome (even horsepox virus genomes) or, in the case of the much shorter RNA virus genomes, the cDNAs corresponding to RNA virus genomes. Transfection of the DNA genome into suitable cells will produce virus. Similarly, transfection of genomic RNA obtained by transcription of viral cDNA with T7 RNA polymerase will produce (in most cases) virus. The procedure is more complicated with minus-strand or double-stranded RNA viruses. The availability of error-free commercial DNA fragments will make it easier to assemble full-length genomic DNA or cDNAs.

Gigi Kwik Gronvall, Johns Hopkins University. Credit: Larry Canner

Gigi Gronvall: At the time David Evans' horsepox work was published [in 2018], we thought that it was not going to be straightforward for people working outside traditional facilities to use that knowledge to make another orthopox virus, smallpox. Tacit knowledge, specifically designed tools, specialized expertise and some R&D would be required to do that, as well as a delivery mechanism if someone wanted to use it for a weapon. But this may not always be the case, so it helps to have a systematic way to work through the problem to see if conditions have changed and risks and vulnerabilities have increased. A US National Academies report, Biodefense in an Age of Synthetic Biology, also called the Imperiale report, aims to do just this. [I served on the committee that prepared this report.]

Are DNA viruses easier to make than RNA viruses? Which viruses might be the lowest-hanging fruit for a do-it-yourself virus builder?

David Evans: It's not so much the type of nucleic acid, but rather whether it's naturally infectious. A small plus-strand RNA virus like poliovirus can be made in vitro, and a duplex DNA virus like SV40 can be retrieved in an infectious form by simply transfecting the DNA into cells. Influenza or poxviruses require more complicated systems to reactivate transfected templates.

Nicholas Evans: The answer to the second part of this question is that we should ask what constitutes 'low-hanging fruit' to the DIYbio community. In academic and commercial labs, there are clear incentives that drive use: efficacy, cost-effectiveness, novelty, commercialization potential, among others. These might be substantially different for the DIYbio community, especially as the community evolves from early pioneers who came from academia and professional work but wanted a

non-traditional space, to individuals without those backgrounds whose aims may be very personal, or simply curiosity-driven. I find it hard to think that someone who just wants to know more about how their local biome works is going to start synthesizing poxviruses.

Reshma Shetty: We'd rather not speculate on what viruses might be easy for a DIY virus builder. As discussed elsewhere, the equipment and know-how to cultivate mammalian cell culture and produce active virus at any reasonable scale are likely to be a much higher barrier to entry than either building a viral genome or recovering infectious viral clones from patient samples.

Volker Thiel, University of Bern.

Volker Thiel: Irrespective of DNA or RNA, it depends on virus life cycle. Generally, the smaller, the easier, but the rescue procedure is different for different types of viruses. For example, for positive-sense RNA viruses it is usually sufficient to transfect cells with an RNA that corresponds to the virus RNA genome. In this type of virus, the genome is translated within the transfected cells to give rise to the proteins that build the RNA synthesis machinery, which then, in turn, replicates and expresses the virus genome. In the case of negative-sense RNA viruses, a RNA polymerase component, which may differ in complexity depending on the virus family, has to be co-delivered with the viral RNA to initiate the virus life cycle. Similarly, DNA viruses are quite different in regard to rescue procedures depending on the biology of the virus life cycle. The lower-hanging fruit is probably small positive-sense RNA viruses, such as poliovirus. However, there are exceptions: for example, norovirus, which is difficult to propagate in cell culture.

How much equipment is needed to build a virus and test its function and viability?

Nicholas Evans: That depends on the virus and its test. On one account, it can be as little as the appropriate growth media and cell cultures, a cell culture incubator and a BSL-2 safety cabinet. For testing, that depends on whether you are using a virus in vivo in animal tests or in therapeutics, as a diagnostic or in basic microbiological work. If we're talking human infectiousness,

Not your father's molecular biology lab. Working with potential human pathogens requires a BSL-3 laboratory. Credit: REUTERS / Alamy Stock Photo

you'd likely need at least human cells but possibly a human (or multiple humans) to infect, at which point we've likely crossed more than a couple of ethical lines.

You'd also need the tacit knowledge, the actual skill, to do the work. Although David Evans has said that you don't need exceptional biochemical knowledge or skills, significant funds, or significant time" to do what he did, that would then mean he thinks that being one of the leading orthopox researchers in the world isn't "exceptional," and possession of $100,000 isn't "significant." Both of which I suspect aren't quite right.

Reshma Shetty: As noted above, there are several strategies that can be used to 'build a virus'. Methods that seek to synthesize a virus from scratch without access to patient samples or isolates of the target virus require equipment corresponding to a basic molecular biology lab, such as fridges, freezers, PCR machines, incubators and gel electrophoresis apparatus as well as access to DNA sequencing and DNA synthesis. If one has access to patient samples or virus isolates, access to DNA synthesis will not be required. In either case, to render such a clone biologically active is substantially more difficult and would require transfection into a permissive cell line (typically of human or other animal origin), which requires equipment associated with cell culture work, such as fridges, freezers, biosafety cabinets, shakers and specialized incubators. The biologically active virus would then need to be tested for function and viability, which would typically be done in a BSL-2, -3 or -4 laboratory, depending on the viral properties.

Are there any issues around storing the genomes of synthetic viruses compared with those of pathogens isolated from the field?

Nicholas Evans: Depends on what you mean by 'storing' these viruses. Both can be stored digitally, assuming we have the tools to resynthesize them, and the time to want to spin these back up into real, infectious agents. But this might be a liability for synthetic viruses, if the data were corrupted or destroyed. Anthrax exists everywhere; your custom bug might only exist on your computer, which is a vulnerability if it breaks. Conversely, depending on the pathogen there might be nothing uniquely risky about *your* anthrax sequence, but it's possible your custom bug is yours and yours alone to release onto the internet and potentially into the wrong hands.

Reshma Shetty: All else being equal, from a purely technical point of view, there is no difference in either the digital or physical storage requirements of DNA copies of synthetic versus wild viral genomes or indeed synthetic versus wild pathogens.

Volker Thiel: No, storage is usually regulated according to rules and laws of

the county where the work is conducted. Depending on the biosafety level, permissions are required, and there should be regulations in place in each country.

Eckard Wimmer: I do not think that it is permitted to store complete DNA corresponding to smallpox.

How many of the tools needed to construct a synthetic virus are available openly?

David Evans: Most suppliers of these materials operate under 'know-your-client' policies, much as with common chemicals. Academic institutions and commercial industries would have few difficulties, private individuals much more so. Commercial suppliers of synthetic DNAs perform BLAST searches and will not fulfill orders for toxin and pathogen sequences unless the recipient can offer appropriate assurances that they are permitted to acquire such materials.

Nicholas Evans: Physically, most of it—although tacit knowledge may remain a strong limitation. Sequence information has been available for quite a number of viruses for more than 30 years, predating our ability to synthesize viruses by a decade or more. Cost might still be a factor, but I doubt the actual physical materials would be hard to get.

Reshma Shetty: The genomes of many viruses are available in public sequence databases, though some available viral sequences likely have nucleotide sequence errors. Methods for culturing and handling viruses are also available in the published literature. However, cell culture, in particular, would be difficult without some expert training and access to specialized equipment.

What might this mean for biosecurity?

Gigi Gronvall: The openness of scientific research information has been a longstanding debate. In 1982, the US National Academies published a report—called the Corson Report after its chair—that pushed back against calls to make more scientific research secret to protect US technologies from theft by the Soviets. The report maintained that "security by accomplishment" would better protect US national security than security by secrecy and classification, and that accomplishments in the sciences rely on an open environment to share ideas and results. This philosophy was encapsulated in National Security Decision Directive 189 (NSDD189), which was signed by US President Ronald Reagan, supported again by President George W. Bush after the 9/11 attacks and remains in effect today.

Years later, in 2011, the issue of how much research information should be in the public domain came up when debating so-called 'gain-of-function' influenza experiments. The results of those experiments—that H5N1 avian influenza could become transmissible, and that transmissibility was associated with certain mutations—had clear importance for public health surveillance of a deadly flu. However, the US National Advisory Board for Biosecurity (NSABB), a federal advisory committee to the US National Institutes of Health (NIH), was concerned that such information could be misused to deliberately create a transmissible avian flu bioweapon, and recommended that the research not be published. NSABB searched for a mechanism so that public health workers around the world could access that information and use it for public health purposes, but protect the information from others. They found no mechanism suitable. Eventually, the work was published.

Given a binary choice between openness and secrecy, most research is shared without restriction, including genetic sequences of pathogens. Decisions about what is acceptable to have available in the public domain, however, rest on a complex interplay of concerns about perception of risk and perceived benefits of openness, and not everyone sees these qualities the same way, even if they have access to the same information.

Recently, I asked my students—who had learned about the history of bioterrorism, biowarfare and misuse of biotechnologies—if they were given the power to go back to 1994 and stop the publication of the sequence of smallpox, would they do it? After all, the fact that the sequence is available means that it can be synthesized and made in the laboratory, and it will always be a biological weapons threat. Slightly more than half of the students said they would still publish the sequence because of all the benefits to our knowledge the availability of this sequence has given us. I expect that in years to come, there will be a great deal of back-and-forth about what scientific information and tools should be open and what should be secret, and there will be no clear resolution or general agreement. Whether scientific information should be restricted or not will ultimately come down to the person or institution who has the power to make the decision. However, I hope that people remember that you can't prepare for threats you don't know about, and keeping things secret can inhibit preparedness.

What is the go-to list of agents for deciding what sequences should not be synthesized?

David Evans: A commonly employed list is that of the Australia Group. However, individual countries have their own lists. Our group in Canada is regulated under the Human Pathogens and Toxins Act.

David Markowitz: IGSC membership uses its Regulated Pathogen Database plus a collection of dangerous sequence databases as a basis for screening orders. These databases source from, but are not limited to, agents and sequences listed by the US Federal Select Agent Program, US Commerce Control, the Australia Group, and the European Union. However, bio-risk assessments can be unique to the entities making the assessment, and come with many dependencies, including location, protocols utilized, researchers involved and infrastructure available.

Although lists of dangerous sequences have shared elements, it is unlikely a single standardized threat list across all stakeholders can be developed. At Intelligence Advanced Research Projects Activity (IARPA), we are developing the IARPA Functional Genomic and Computational Assessment of Threats (Fun GCAT) tools that do not exclusively rely on matching threat lists by using advancements in artificial intelligence to make assessments of novel sequences.

Reshma Shetty: In the United States, the Screening Framework Guidance for Providers of Synthetic Double-Stranded DNA recommends the use of the Select Agent and Toxins List. For international customers, the Export Administration Regulations (EAR) Commerce Control List is also recommended. In addition, the IGSC has developed a Harmonized Screening Protocol for screening of synthetic DNA sequence orders against the IGSC's Regulated Pathogen Database. Screening protocols and screening databases should be updated regularly to avoid obsolescence as new human, plant and animal pathogens emerge. The Nuclear Threat Initiative (NTI) and World Economic Forum (WEF) published a report recommending a system to globally expand synthetic DNA screening practices.

How much of a concern is it that horsepox virus wasn't picked up by the screening systems used by the IGSC?

David Evans: Horsepox virus did not fall under the lists of regulated pathogens. It is also so similar to vaccinia virus that it is widely suspected that it is the ancestor of vaccinia virus. Consequently, horsepox sequence would not be flagged as posing a biological risk according to these algorithms.

Gigi Gronvall: David Evans' work was legitimate research with institutional approval and typical funding mechanisms, and Evans himself is one of the top pox virologists in the world and even a member of the WHO Smallpox Advisory Group. He was a known customer. The work was also performed in the open using legitimately purchased laboratory equipment and space. If you need to hide what you are doing, it becomes harder to order supplies and carry out the work.

The Evans study certainly demonstrated that making horsepox—and thus, smallpox—is technically feasible. This wasn't really in doubt, especially after J. Craig Venter Institute scientists synthesized and booted up a much larger bacterium in 2010. But the Evans lab work doesn't mean that it would be as straightforward for a non-state actor with malevolent intent to do the same thing.

So are the national and international regulations and IGSC screening systems sufficient to prevent the synthesis of DNA from harmful pathogens?

Nicholas Evans: Sufficient for what? The algorithms alone aren't totally capable of stopping bad actors. But this isn't a productive way to assess security or safety risks. No series of regulations will stop a truly determined actor. All they can do is create certain kinds of barriers that involve both costs and benefits. The better question is, "Are the benefits of these systems proportionate to their costs, and would improving those benefits come with additional, undue costs?" The scientific community thus far has been unwilling to adopt any additional layer of oversight, presumably because it would be seen to potentially slow scientific progress, or some other claim of the kind.

David Markowitz: The IGSC is industry led and monitored, and membership in the consortium requires adherence to its guidelines under its Harmonized Screening Protocol. The systems used by the IGSC provide critical barriers to prevent the synthesis of DNA sequences, but these barriers will require continual improvement as technologies evolve. One current limitation is not screening double-stranded DNA sequences smaller than 200 base pairs; however, it's important to note that adoption of expanded screening standards must be commercially viable. New bioinformatics technologies for quickly and inexpensively evaluating DNA sequences may offer a path forward—this is a focus of the IARPA Fun GCAT program.

Gigi Gronvall: The systems in place for gene synthesis security aren't sufficient, but it isn't because of the IGSC. In the years since the United States released its 2010 guidance for gene synthesis providers, the market for gene synthesis products has changed. Costs for gene synthesis products have gone way down, but biosecurity screening relies on expert judgment, which costs more for personnel. So over the years, companies performing screening and hiring expert staff to do basic checks are increasingly at a competitive disadvantage to companies that don't bother to check what customers may be ordering. Legislation recently introduced in California and Maryland is aimed to fix this problem, requiring state research dollars to only be used to order gene synthesis products made by companies that do responsible screening. I hope that this will become federal regulation, so that federal research dollars can only be spent on companies which perform screening, and that it will make it a competitive advantage to do basic biosecurity screening.

Gene synthesis is an international business, with companies based all over the world, so oversight and monitoring is complicated. It would be great to get multiple countries on board so that public research funds may be used only for those companies that screen. It's not a panacea; there are plenty of ways to go around screening if a person or group is intent on synthesizing genetic material and booting it up to make a pathogen they shouldn't have. But the idea is to make misuse more difficult. It should be hard for a person with nefarious intent to order the genetic material to make smallpox from a gene synthesis company. It should be hard for a person to purchase the genetic material to make Ebola virus using a credit card and ship it to a non-laboratory address.

The US National Academies' Biodefense in an Age of Synthetic Biology report advances a framework so that one can systematically analyze the risks and vulnerabilities of a new advance like de novo virus creation, enabling a determination of whether some new development actually makes us more vulnerable. By considering factors such as the potential use of a weapon, the attributes of actors who could command such a capability, the capability for mitigation of an attack, etc., one can have a better picture of risks, and it is a better approach than just reacting to scary headlines.

Reshma Shetty: Mitigating risk will require that the protocols, standards and guidance keep pace with biotechnological advancement. Potentially, molecular safeguards should be incorporated wherever possible to build in intrinsic biosecurity, thereby reducing the risk to the user and the potential for accidental or intentional misuse.

Eckard Wimmer: As Joshua Lederberg put it in 1998, "There is no technical solution to the problem of biological weapons. It needs an ethical, human and moral solution—if it's going to happen at all." Obviously, investigators in the realm of 'dual-use research' need a high degree of responsibility in research and teaching. Frankenstein was not trained properly, so once he had produced a monster, he did not know how to deal with it.

Given the utility of synthetic virology for understanding the origins of viruses and guiding future vaccine development, how should the benefits be balanced with potential risks?

David Evans: There's no simple answer to this question. Synthetic gene technologies are now widely used as a tool in most branches of modern biotechnology. Virology is just one application and can't be considered in isolation from the bigger issues. The question really encompasses the much broader question of whether any form of technology associated with genetic engineering offers benefits that outweighs the risks. What are the pros and cons of CRISPR–Cas9 technologies or the capacity to make transgenic organisms?

Nicholas Evans: In any type of dual-use research like this, a common answer is threefold. The research must have a good reason. Its risks must be outweighed by its benefits. And there should be no less risky way to achieve those benefits than the research in question.

The central questions to balance these terms are as follows. Do these synthesis tools really connect to valuable projects? What are the scope of the risks and benefits of these experiments? And what are the alternatives, and what do their risk and benefit profiles

look like? These aren't easy questions, but they often do have answers, if you're willing to do the work to find them.

David Markowitz: The production of infectious, replication-competent viral particles will always have some associated risk of infection or release. Since the 1975 Asilomar Conference, researchers have recognized the dual-use concerns with this technology, and research practices, regulations and laws have evolved with the technology to mitigate the risks. For example, one approach to safely enabling research on viral evolution is to produce replication-incompetent viruses that are only able to replicate when specific genes are externally provided under specific conditions.

Reshma Shetty: This area of research remains highly controversial, given the inherent challenges of and uncertainty in weighing benefits versus risks. Understanding novel viruses and minimizing the potential for misuse must be pursued as simultaneous objectives. However, synthetic viruses may manifest in similar ways and pose similar risks to wild viruses, and far greater attention needs to be paid to developing and maintaining flexible public health infrastructure to respond to either type of threat in the future. Furthermore, as COVID-19 has illustrated, the generation of vaccine candidates is much cheaper and faster than (1) the clinical trials required to demonstrate vaccine safety and efficacy, (2) the manufacture of approved vaccines at scale and (3) the logistics of administering vaccine doses to an entire population. Substantial resources should be dedicated toward reducing those timelines.

Eckard Wimmer: The elegant work showing that HIV originated from chimpanzees provided compelling evidence for the virus' origin. That, in turn, lowered the fear that HIV is a threat coming from numerous unknown sources. Synthetic virology doesn't always point a straight path to a viral vaccine; it has not yet resulted in the development of an HIV vaccine, for example; however, lentiviral vectors are contributing important advances in gene therapy

What do you see as the next viruses that synthetic virology could tackle, and the pros and cons of some of such projects?

David Evans: The primary potential lies in the area of personalized cancer therapeutics. For example, the technology allows one to rapidly assemble personalized oncolytic viruses encoding and delivering (in special contexts) collectives of individualized tumor neoantigens. These can nowadays be predicted with continually improving accuracy using tumor sequencing and epitope prediction algorithms. Given the vast amount of human suffering caused by cancer, I see few reasons not to try and develop better treatments.

Nicholas Evans: It depends on the incentives. Rourke and colleagues, for example, have postulated that one impetus for the synthesis of horsepox was to avoid engaging with Mongolian authorities around fair access and benefit sharing associated with their research and development activities. The authors there only cited Evans' concerns about "commercial 'freedom to operate,'" but they identified a longstanding concern that synthetic virology is a potential tool to appropriate natural resources without providing benefits to local communities. If true, then the researchers involved would have been engaging in a form of biological piracy to avoid just involvement of communities in research deriving from their natural environments. I would suspect that viral synthesis will continue to be driven by these kinds of considerations, as synthesis will increasingly be easier than—to scientists—time-consuming acts of politics and equity.

David Markowitz: Synthetic virology has clear promise for advancing our understanding of betacoronaviruses and seasonal influenza. However, the ongoing SARS-CoV-2 pandemic underscores the need for scientific infrastructure that enables us to quickly detect, characterize and treat infections due to any and all zoonotic viral pathogens. The genetic diversity of viruses makes the preparation of a standardized viral detection and treatment methodology a major challenge.

Volker Thiel: Synthetic virology has its advantages in cases where virus isolates are not available and where procedures to rescue the virus from cloned DNA are feasible. For viruses that are of wide importance, as in the case of SARS-CoV-2, the benefits outweigh the risk because synthetic virology can be used to study the impact of individual mutations or genes on the virus phenotype, such as pathogenicity, transmissibility, immune escape and other aspects that are of medical or socioeconomic importance. Synthetic virology can reduce the time and effort that is needed to conduct such studies because the availability of a virus isolate is not a limiting factor anymore. Nevertheless, clear regulations have to be in place that also consider biosecurity.

Do any new issues arise from this technology being in the hands of the DIYbio community?

Nicholas Evans: I don't know that DIYbio presents too many new issues. 'Open biology', about which I've written elsewhere, may present new issues around increased access to tools and knowledge for bad actors, and there's a slight possibility that DIYbio, as a species of that kind of biology, might pose the same risks. But as a specific culture, DIY biologists are mostly hobbyists, and the movement has very specific aims to do with education, creativity and community engagement that don't set it up as breeding ground for near-future viral synthesizers.

More likely, the massive proliferation of biological laboratories staffed with highly educated scientists into the private sphere will pose a greater risk. Increasingly, science can be done solely with private funds, away from any governance measures, which in the United States at least are all tied to things like the provision of federal funding. The misuse of these technologies by skilled scientists funded and directed by the wrong people is more of a threat, to me, than DIYbio.

David Markowitz: There is evidence that the DIYbio community has established processes to guide and advise its members on assessing dual-use risks. Given the substantial technical expertise and laboratory facilities required to support synthetic virology, the same biosecurity issues would apply to an academic lab or a DIYbio team operating in this space. Both require common-sense oversight to ensure safe, but otherwise minimally constrained, progress.

Reshma Shetty: The DIYbio community has been very forward-looking in engaging with biosecurity leaders and law enforcement, as well as setting its own guidelines. Ultimately, these community-based labs don't have access to the same resources as academic, government and company labs, but they are pretty clear about their intent to engage the community and do their work out in the open. I encourage the DIYbio to continue to be mindful of the potential for unintentional accidents (just as all institutional labs should). In addition, the International Genetically Engineered Machine competition (iGEM) has done pioneering work around biosafety and

biosecurity education at the undergraduate and high-school levels.

Gigi Gronvall: One of the great things about the DIYbio community is that they are focused on things that are immediately important to people, which would never be the subject of an NIH RO1 grant. DIY biologists do big projects to learn what microbes are living in the water, or the open insulin project, which raises awareness about the unnecessarily high costs of insulin, or educational classes that draw people in who may be interested in biotechnology. The community laboratories have been super responsible and have had strict biosafety protocols. I'd like to see more interest and much more support for them, especially as an educational tool, to give students new experiential learning opportunities to get them into the biosciences and synthetic biology.

Are there any other issues surrounding synthetic virology that you wish to raise?

David Evans: I would note that the synthetic mRNA technology that has been used to make such successful COVID-19 vaccines is basically identical to the technology that Wimmer used to synthesize poliovirus. That's dual use, and it shows how it is impossible to separate 'good' technologies from 'bad'.

Nicholas Evans: I think, honestly, the possibility of the use of synthetic biology to take the indigenous natural resources of other nations, develop them and then exploit them for gain without just or fair recompense is the key issue for synthetic biology, and one to which we don't give enough attention.

David Markowitz: Synthetic biology is inherently dual use: it provides tools for engineering microbes and viruses that can power the engine of the rapidly growing bioeconomy, but those same tools can be used to produce pathogens. It is important to build awareness of this fact and to educate researchers, policymakers, journalists and the public about the importance of safe oversight of the synthetic biology community to enable industry growth while preventing misuse.

Reshma Shetty: The best approach to mitigating the risk from wild pathogens and synthetic viruses alike is a robust public health infrastructure that spans early detection and sequencing, test-and-trace methods to limit spread, and rapid response countermeasures, including both therapeutics and vaccines. It is likely in the interests of most countries to maintain a domestic biotechnology capacity for molecular testing, genome sequencing, and antibody and vaccine manufacturing that can be used in an ongoing way for commercial and academic purposes and re-purposed to quickly respond at scale when threats arise.

With respect to legitimate academic or commercial research involving synthetic virology, it is prudent to assess whether a fully pathogenic viral genome needs to be created to achieve research goals. For example, several synthetic attenuated or 'pseudovirus' constructs can be created to complete many laboratory characterizations and experiments that present a much reduced risk and can be handled at a lower biosafety level. Molecular tools like these to maximize experimental output while minimizing pathogenic risk should be employed whenever feasible.

Volker Thiel: I advocate for continued general discussion about the risks and benefits, similar to the dialog around the development and establishment of recombinant DNA technology.

Eckard Wimmer: Teaching of the ethics and dangers of modern synthetic biology remains inadequate. The numerous conspiracies that ordinary people have accepted are a sign of incredible ignorance of science and its dividends. Which US institutions of higher education plan to teach a course discussing the science and societal implications of our pandemic? Without such efforts, we will continue to battle specious conspiracy theories blaming Bill Gates or China for developing and then letting loose SARS-CoV-2. Ignorance is nothing new; a similar disaster was the conspiracy theory 30 years ago claiming that HIV was developed to punish gay people.

Most people in the USA do not know that currently it would be impossible to develop a virus as sophisticated as SARS-CoV-2 or HIV in a lab.

Interviewed by Laura DeFrancesco

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