DNA is the New Silicon

The Era of Experimental Evolution

By Joe Chiarella

DNA is the New Silicon. The Era of Experimental Evolution.

CRISPR/Cas9 is to DNA what photolithography was to Silicon. It is the accelerant in the new era of programmable biology, instead of silicon. It offers humanity the power to experiment with evolution itself. Are we ready?

The silicon era:

Who could have predicted it? About 60 years ago in 1960, a single lifetime, we were looking up at the rising silicon era. Excited scientists used room-sized computers for calculating orbital re-entry paths for astronauts. Who was thinking that today we'd be carrying far more powerful computers in our pockets? (And definitely not calculating orbital re-entry paths with them.)

Who, back then, would have predicted that we would use them today for live voice and video communications with someone on the other side of the planet? And for free! Or for watching movies? Or to identify star constellations by holding our phone up to a night sky? Or to ask and answer an obscure molecular biology question? Or the name of an actor in a play? And do it all in a handful of seconds? And for a few hundred dollars? No, not even Dick Tracy could have predicted that.

To make this possible, we carry computers in our pockets. Computers with dual 64-bit chips operating at 2 billion cycles per second. Computers with 128 billion bytes of high-speed memory. Computers with 5+/- radio antennas. And with screen resolution sharper than the human retina can distinguish. Nevermind cameras and video recorders that movie cameramen in 1960 would have thought impossible.

Let me try to put this into a little perspective. 30 or so years ago, I worked on an IBM mainframe computer with a single 32-bit chip (not dual 64s) operating at 38 million cycles per second (not 2 billion). It had 8 million bytes of memory and about 90 million bytes of offline storage. The computer was water-cooled, filled a raised-floor air-conditioned room nearing 1000 square feet. It nearly required its own connection to the power grid. It cost about $10,000,000 and a team of a few dozen college educated specialists managed it.

It didn't do videos. It wasn't connected to anything like the Internet (the Internet didn't really exist yet). It wasn't capable of making phone calls. You were lucky if you could get the printed sales summary report you needed by the morning after the sales happened. Still, it was an amazing tool for the $1 billion/year retail company with 1200 stores that paid to have it.

Today, IN YOUR POCKET, you carry more than 4 billion times the processing power and speed, and 4000 times the storage with more functionality, ease of use and convenience than that $10 million mainframe. But it's not just about the incredible inverted price to power ratio, it's about how it is being used. NOBODY in 1960 or 1970 or 1990 could have predicted this. And, as we all know, there is good and bad in what these devices do.

How did we get here?

In the mid 1950's a new kind of photolithography entered common use. This primary technique for imprinting circuits on silicon made silicon 'programmable'. While there were other technological contributors, it was this singular advancement that initiated the steady march that has brought us today to smartphones, the Internet, digital voice assistants and, yes, those Dick Tracy kinds of watches.

The key takeaway here is that in the 1950's and 1960's when the silicon era was emerging, NOBODY could have predicted where it would go.

This unrelenting march of silicon progress will continue, about 70 years and counting now. Still, the era of Silicon is waning and another, much more impactful one, is rising.

The programmable biology era.

Something called CRISPR/Cas9 is to DNA what photolithography was to Silicon. And it is going to usher in the new era in "programmable biology" instead of programmable silicon. Nearly within our grasp right now – is the ability to experiment with that which has always been the domain of nature: evolution.

Just as the developers of photolithography 70 years ago could not predict what computing would look like today, neither can the folks behind CRISPR predict what it will mean to our society a mere lifetime into the future. However, if the prior era is any indicator, we can expect power and price to invert. We can anticipate the democratization of the technology that puts it in the hands of non-technical users to use it for something as different from launching astronauts into space as playing Candy Crush. But can any of us predict what that use will be?

This incredible increase in Silicon-based power has been, so far, external to our very selves. But in the new era, we will be changing (programming) our very selves. This has significant implications. Is anyone thinking about it?

A perfect convergence (storm):

Without the benefits of the silicon era, CRISPR may never have been found. More pointedly, it is certain that without the kind of computing power we have today, CRISPR would be meaningless. We wouldn't be able to track, predict, and apply CRISPR to human, animal, plant and micro-organismal DNA. Computing power is important to the new era, but it is not the catalyst; CRISPR is.

But CRISPR, just like photolithography, while foundational – also benefits from some other supporting and converging technologies. There are other technologies emerging and converging that will fan the flame of this next era.

These forces are:

1. CRISPR and CRISPR-like variants
2. Gene sequencing and synthesis
3. High Performance Computing through "the cloud"
4. Assorted forms of artificial intelligence like machine learning, deep learning and more
5. Synthetic biology
6. {Still nascent} "Delivery mechanisms": Somatic cell therapy, germline editing
7. The BRAIN Initiative

In order to better understand why this convergence is so important, let's first look at each force individually, before we examine how they will cooperate to usher this new era.

CRISPR:

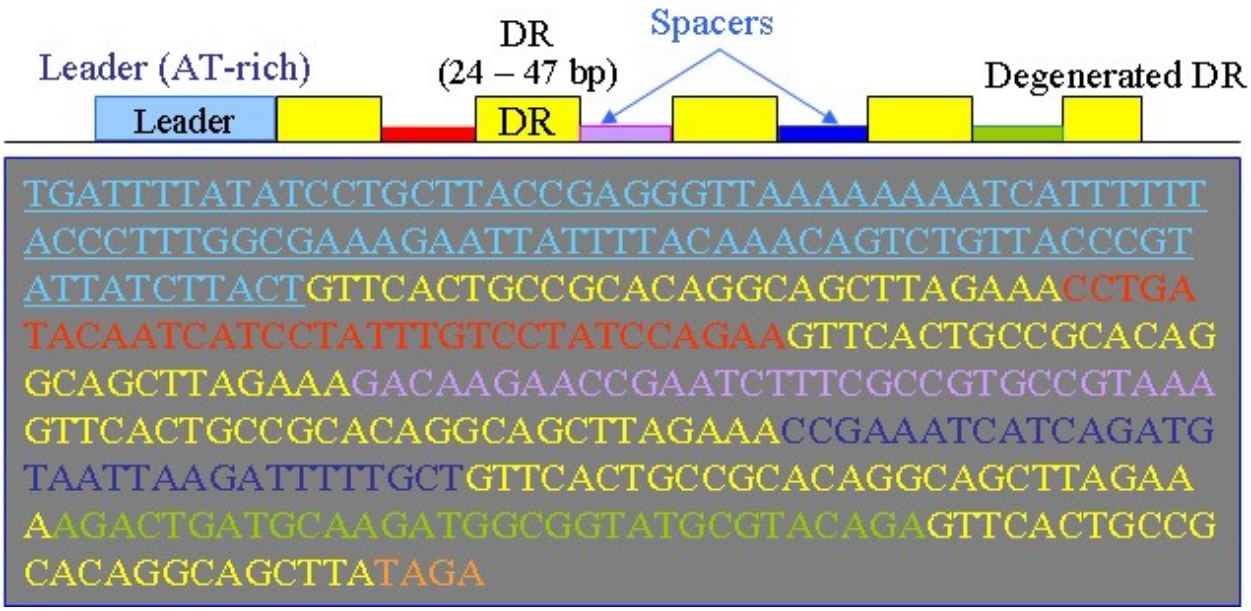
CRISPR is actually an acronym that stands for Clustered Regularly Interspaced Short Palindromic Repeats – discovered back in the early 1990's. What this refers to is an amazing adaptive immune system that certain bacteria use to protect themselves from virus (bacteriophages to be more precise) attacks.

https://en.wikipedia.org/wiki/CRISPR

The phages are relatively simple organisms with their own DNA. They replicate by attaching to a bacteria's outer cell wall, penetrate that wall and inject their DNA. Then the bacteria's own "DNA replication engine" does the work of replicating many copies of the phage's DNA. These phage DNA then do what DNA does – and create proteins etc. that eventually become little phages themselves. These new phages then destroy the bacteria's cell wall and burst forth to go and attack other bacteria in the same way. However, some percentage of the attacked bacteria do survive long enough to replicate, before they die.

What was discovered in the mid 2000's was that the CRISPR mechanism protects the bacteria from this kind of attack for specific phages. Imagine an attack whereby the phage DNA replication fails to destroy the bacteria and it manages to live another day or replicate. In this mechanism, a short piece of the phage's DNA is snipped and "stored" between a pair of the Short Palindromic Repeats in the bacteria's own DNA. Having survived, the bacteria replicates and its offspring have this "signature" as well. When a phage with that same DNA sequence attacks one of these bacterial offspring – the bacteria recognizes the DNA sequence and uses an enzyme called "Cas9" to cut the phages DNA into pieces thus destroying it. The bacteria thus survive the attack. Those bacteria now have another "signature" in its "inventory" of signatures stored within its CRISPR sequence. Over time and generations, bacteria can amass a considerable inventory of sequences – and thus survive many different future phage attacks. Resilient little buggers aren't they? In this scenario, we can see the "survival of the fittest" in biology playing out very clearly.

Here is an illustration of a CRISPR sequence:



CREDIT: http://crispr.i2bc.paris-saclay.fr/index.php?page=about

In this illustration – the yellow blocks and the yellow letters are the "Short Palindromic Repeats" or SPR part of CRISPR. Between these yellow blocks of DNA are the so-called "spacers" shown in red, purple, blue and green sequences of DNA that represent four different snipped DNA segments from past attacking viruses. The length of the SPR and the spacers are roughly 24 to 47 base pairs (DNA letters) in length. Pretty short all things considered.

However, with four letters to occupy a 47 letter word – there are between 4**24 = 281,474,976,710,656 (281 trillion) to 4**47 = 19,807,040,628,566,084,398,385,987,584 ways (almost twenty trillion-trillions) those four letters can be arranged in that tiny sequence.

About five years ago two scientists, Dr. Jennifer Doudna and Dr. Emanuelle Charpentier, were studying this mechanism and realized that it could be harnessed to do "surgery" on any DNA they wanted.

By injecting a CRISPR sequence with what is called a "Guide RNA (gRNA)" and some Cas9 enzyme into a cell from the liver of a pig (for example) – the CRISPR mechanism will find the RNA sequence in the pig's liver cell that pairs to the gRNA that was injected. The CRISPR/Cas9 mechanism then automatically snips the DNA at the targeted point using the Cas9 enzyme.

If you snip the DNA at two ends of a particular gene, you can perform a "gene knockout" – thus eliminating that gene. If you snip the DNA at two ends of a portion of a gene, and flood the cell with a different DNA sequence (RNA really) then the cells own DNA repair mechanism will replace the sequence you snipped out – with the replacement you injected. In other words, you can "edit" virtually any portion of DNA that you want.

There were technologies for doing this before CRISPR/Cas9, like TALENS and Zinc Finger Nucleases (ZFN). However, by comparison these are to CRISPR what "vacuum tubes" were before transistors on silicon. CRISPR is orders of magnitude faster, cheaper, easier and more precise. This increase in effectiveness and efficiency along with the $120 price tag is orders of magnitude better than ZFN and TALENS.

This is why CRISPR is such a big game changer; it's got the triple threat. It's better, faster and cheaper.

Today, CRISPR/Cas9-based gene editing has been proven to work in plants, animals, bacteria, and humans. Within three years of discovery, virtually every lab in the world that did any form of animal modeling or gene research and more – or wanted to but were unable – are using CRISPR/Cas9 to do all their gene editing. This includes poor developing nations with limited funding and technical expertise.

Again, CRISPR/Cas9 is to DNA what photolithography (the ability to "print" transistors onto silicon) was to the printed circuit. It changes everything.

Anyone with a credit card can buy a Do-It-Yourself bacterial gene editing CRISPR/Cas9 kit for about $120 with a quick Google search on the Internet.

CRISPR is not without challenges. There are limits to the length of DNA you can "knockout" or change. And the "delivery mechanism" (that Guide RNA) of CRISPR itself is quite large and too large for certain applications. And, sometimes, CRISPR can cause what are called "off target effects" which simply means that you ended up editing a piece of the DNA you didn't want to – often with either no observable effect – or a dramatic one. Finally, assuming you do manage to edit the DNA of a cell or a group of cells – what then?

Let's pretend there is a genetic defect in your liver that causes your liver to over-produce the bad kind of cholesterol (Low Density Lipoprotein or LDL). Can CRISPR/Cas9 cure you of this defect? Well, the theory goes that some of your liver cells would be removed and the bad DNA sequence is replaced with a correct DNA sequence. Then all we have to do is put those cells back in your liver – right? Not so fast. There are a few problems here.

Since ALL of the liver cells were not edited, even once the edited ones are put back in the liver – there is still going to be a much larger number of bad liver cells to corrected ones. How do we get the bad ones to die faster than the good ones so the good ones can "overrun" the bad? Or, recall that the DNA in a liver cell is the same DNA in the cells in your cornea, or bone marrow, etc. Our bodies are prone to rejecting stuff that isn't the same (which is why liver transplant patients take anti-rejection drugs for the rest of their lives to prevent the body from rejecting the new liver). So, what is to prevent the immune system from knowing that those cells have "alien" DNA and try to kill them?

These are very real and challenging problems to solve. This problem, called somatic cell therapy, is the most significant hurdle to get over at the moment. But researcher's the world over are working day and night to solve it. And, solve it they may, eventually. NOTE: this is the 6th bullet listed above in our list of six converging forces. And here's the good news – if scientists can indeed harness CRISPR for somatic cell treatments – AMAZING things are possible from correcting the aforesaid liver problem, to an incredibly wide range of maladies with little or no long-term repercussions. It's a future we would all welcome.

Of course, CRISPR can also be used at the very earliest of life stage – the single cell "embryo" of any animal, including human. By changing the DNA inside this single cell, or perhaps a stem cell, all the downstream cell divisions will carry that change. And here is where the possibilities and implications become profound.

What if the goal is to "knockout" the genetic defect that causes that overproduction of LDL? If you edit that out in the human embryo, then when that cell divides and divides and eventually divides into the

first liver cell – then all the liver cells that come after that will be the same corrected form and, poof, you no longer die of high cholesterol (at least the genetically caused form).

But what if when we are editing that human embryo for just that little genetic fix, CRISPR happens to also accidentally edit some other part of the DNA? What if it edits something in the DNA that causes early onset Alzheimer's? That human will be born and live free of high cholesterol – but will reach age 54 and suddenly, viciously, suffer from and die an ugly death from Alzheimer's – and who will know that it was the fault of CRISPR? Or perhaps the accidental edit changes something else in the DNA for which we don't yet understand what it does? That change will become part of that person's DNA to be passed on to their offspring. In this case, we are now "experimenting" on future generations and subjecting them to entirely new diseases that we ourselves wrought. And, pragmatically, just how will we even know that the 2nd or 3rd generation suffering the disease was the result of the "edit" done to the 1st generation?

Some might say that isn't a problem because, well, we'll just edit those changes out of THEIR offspring's embryo. We'll just fix what we broke. And perhaps that is a reasonable argument. Perhaps in the 20 or 30 or 40 years after that person is born with that "editing mistake" – we'll have improved our ability to make embryonic edits without causing unknown side edits. But what if we don't?

Others might say that Mother Nature is not exactly a benevolent scientist either. Nature mingles the DNA of two parents and who knows what results? Sometimes, the result is "positive" and a healthy baby emerges. Sometimes, unexpected mutations happen – like Downs or Trisomy 13. "How is nature a better 'engineer' than us?" some will argue. And perhaps that is a reasonable argument. Perhaps nature is "blind" and "dumb." Perhaps we are better equipped to "design" our future than an unpredictable process. But what if there is more we don't yet understand about how nature works – than we think? Doesn't that make us "blind" and "dumb" too? Where nature will take generations and hundreds or thousands of years to make a significant change to human (or other) DNA, we'll have the ability to do it year after year after year reducing the "intentional mutation" time to milliseconds in evolution-time.

Gene sequencing and gene synthesis:

Gene sequencing is the technology by which a sample of DNA is "scanned" and all of its base pairs are identified and recorded. Current gene sequencing technologies have enabled the sequencing of over 6000 species – including humans. There are an estimated 8 million species on the planet (6 million land-based and 2 million aquatic), so, we have a lot more sequencing to do in order to know the exact sequence of nucleotides in all DNA for all life on the planet.

The human genome, comprised of some 20,000 genes spread throughout 23 chromosomes, is about 3.1 billion letters (or nucleotides) long. A mere 16 years ago, not even a lifetime, though perhaps a generation, we didn't even know the entire human genome. It had not yet been fully sequenced. Millennials are the first generation to have only known a world where the human genome was "known."

All DNA is comprised of the same four nucleotides: Adenine, Guanine, Cytosine and Thymine known by their popular letters of A, G, C and T respectively. The DNA double-helix can be thought of as a twisted ladder with 3.1 billion rungs. Each rung is a pair of nucleotides. Nucleotides always pair up C with G and A with T. So, by knowing either half of any rung on the whole ladder, you automatically know the other.

SIDE NOTE: The DNA of all life is made up of the same four nucleotides. There is no DNA that is made up of five or six nucleotides. There is no DNA that has four different nucleotides. In fact, the oldest fossils

with any DNA intact that we can find are comprised of the same four nucleotides. In 2009 researchers found and sequenced DNA from bacteria that had been dead for over 400 million years and it still was made of the same four nucleotides. On a planet with 8 million diverse species still around, and uncounted extinct species, it is curious that no other forms of DNA have ever been found. Apparently, for hundreds of millions of years – the basic "programming language" of life has not changed. Evolution tends to exercise, over great or short periods of time depending on the environment, changes that favor life. And yet we cannot find any changes to this basic programming language. How do we know? We know by "sequencing."

Sequencing technology today is about 99.9% precise. It is tempting to consider that pretty good. But let's do some simple arithmetic. If the human genome is like a book that is 3,100,000,000 letters long, then a precision of 99.9% means that about 3,100,000 letters are wrong (1 in a thousand). Simple math. Is 3.1 Million *incorrect* letters a big deal?

Of the 3.1 Billion letters in the "human book", it is estimated that less than 2% of those actually account for the protein-encoding parts that make us human. That means under 62 million letters make us, us. The question is, if we are sequencing YOU, and the error rate is about one letter in a thousand – then it is likely that your sequenced DNA will have about 3.1 million errors where about 62,000 of those really matter because they'll be errors in the actual parts of the DNA that make you, you. A single letter mistake in reading the "book of you" could mean the difference between thinking you have sickle-cell anemia or cystic fibrosis, or not. What does 62 thousand mistakes mean?

The precision of gene sequencing is continuously improving. Better technology and better methods are coming out every year. But, even today, 99.9% is still not good enough. Let's examine this with an abstract example.

What if the gene sequencer made these mistakes and we construct a sequence of letters based on what the sequencer told us – but the sequencer was wrong? What if the sequence it told us, just for illustration purposes, was "ACCCctggTAAACG"? What if the "ctgg" (lowercase used for convenience) portion of that sequence caused a particular disease where the sequence "GTAA" did not? And we decide to use CRISPR to edit the CTGG to make it GTAA. To make sure we get the right "CTGG" we have to "create" the larger sequence shown above to use as our guide (so we don't change a CTGG sequence somewhere else in the DNA). What if it turns out that the real sequence in the DNA is "ACCCgtgaTAAACG" instead of the "ACCCctggTAAACG" we thought it was? Then the CRISPR edit will fail to alter the CTGG to GTAA.

OK, then no big deal. We tried to make a change and it failed. No harm done, right? But what happens if that sequence ACCCctggTAAACG we thought existed at the location we cared about, but didn't, actually DID exist somewhere else in our DNA in reality? Then the CRISPR edit would work to replace the CTGG with a GTAA, but it would be in the wrong place… with unknown consequences.

All because the sequencing technology was only 99.9% right.

And there is more we don't yet understand. For example, again, we used to think that the 62 million or so letters that comprise the protein-coding part of our DNA (our 20,000 or so genes that make us humans) were all that mattered. The rest was once literally called "junk DNA" because we didn't think it did anything. We now know that while other parts of our DNA don't code for proteins – they can do

things like turn genes on and off and more. We're learning, in short, that we still have a lot to learn about all the things DNA does. Or, by analogy, we're still reverse engineering the programming language that drives ALL life on the planet.

If I were attempting to make edits to a computer program, I'd want to first make sure the code in front of me was the right code. Gene sequencing isn't all the way there yet. However, speaking of computer programs, new techniques like using machine learning are coming rapidly now and aimed specifically at improving this percentage.

What about gene synthesis? (Sometimes called "DNA printing")

Technology exists today for "printing" or synthesizing each of the four nucleotides and assembling them into RNA or DNA. Several companies have already emerged to offer DNA synthesis services to virtually anyone who is willing to pay the cost. In just a few short years, the cost of doing this has dropped considerably and hovers today around $0.09 per pair of nucleotides (A with T or C with G). To synthesize the 3.1B pairs of DNA of a human would cost about $280M (talk about the cost of a human life!). But time is also a factor. DNA printing is presently slow. At current technology, it would take nearly three months non-stop to synthesize one complete human genome and by the time it was done, the first nucleotides synthesized would likely be breaking down. So, today, "designing and creating a human from scratch" is realistically out of our reach. But for how long?

Some scientists are deeply committed to changing the speed and economics of gene synthesis. One project, GP-Write, aims to get the cost of creating an entire human genome down to $0.01/basepair or only $31 million for a "custom human."

Why is DNA synthesis important? There are several reasons. In the case of CRISPR/Cas9, the demand for "guide RNA (gRNA)" is on the rise, dramatically. Scientists can find their own ways to generate the gRNA they need – or they can order it online and have it show up in a few days. Scientists focused on re-animating extinct species (yes, this is an active area of research and development) like the wooly mammoth – need DNA synthesis technologies, at favorable scale and cost. And scientists actively working on creating entirely new forms of life (the J. Craig Venter Institute created the first self-replicating synthetic life form in 2010 – a new kind of bacterial microbe) can perform far more experiments in the same time period, if they can rapidly generate DNA on demand.

These technologies (gene sequencing and gene synthesis) are evolving rapidly. Reaching economies of scale to democratize their use – is likely to be within reach in the next few (single digit) years.

With only 6 thousand of the 8 million (0.075%) known species on the planet having their genome decoded and catalogued – there is still a lot of work to be done to complete that exercise – and this is as much a compute constraint issue as anything else. That too is changing…

High Performance Computing through "the cloud":

The human genome is 3.1 billion base pairs long, with about 2% of those base pairs making up the 20,000 or so protein-coding genes that make us, us. There may be a temptation to think that humans, being the highest order living creature on earth – would have the longest DNA or the most complex. It isn't so.

For example, the worm *Caenorhabditis elegans*, has just over 100 million base pairs and 19,735 protein encoding genes. This worm, then, has about the same number of genes as we do – though only 3% of our DNA length. What about the classic Christmas tree (coniferous Norway spruce)? The genome for this tree has 20 billion base pairs – and about 27,000 protein-coding genes – way more than us humans but not nearly as "functionally rich". The marbled lungfish actually has the longest animal DNA at 133 billion base pairs (about 43 times as long as ours). And the longest known DNA to date belongs to a freshwater amoeboid called *Polychaos dubium* which has 670 billion base pairs (200x a human) in its DNA. I don't think anyone would disagree that humans are more capable than an amoeboid; why such a long DNA? Nobody knows. Why so much variation in length and protein-coding functionality? Nobody knows for sure.

Some believe that our DNA is like a history book whereby as we evolve and DNA is rendered "defunct" that DNA segment isn't "deleted" from our chromosomes, it remains as an inert artifact. Therefore, a species with considerable longevity and a high rate of evolution – like an amoeboid – could end up with a very long DNA that is mostly "inactive".

An analogy here might be a very long computer program. Such a computer program might have a million lines of code, but has been modified year after year and millennia after millennia, and every time a better chunk of code replaces the prior one – the prior one isn't deleted, it is just rendered inert. In programming parlance, it is 'commented out'.

Here's the thing – it is one thing to sequence a single amoeboid or worm or human – it is another thing entirely to "reverse engineer" the parts of that DNA that are active and what they do. For that, you need to sequence LOTS of amoeboid, worms and humans and then using computation – and a LOT of data about behavior and maladies – to tease out the differences and functions of the genes contained within that DNA.

More importantly, identifying genetic causes or contributors to diseases ranging from cystic-fibrosis to cancer – requires either decades in lab work – or as little as a few hours of compute time with a large enough cluster of computers and a solid machine learning algorithm.

Until recently, such compute power wasn't economically available. The advent of "cloud computing," with rentable massive server farms, has brought previously unheard of computing power to anyone with a credit card and a little know-how.

That means that we are on the verge of being able to catalog the DNA of all 8 million species on the planet – AND – to discern the function of each and every gene in all that DNA – as well as a substantive number of mutations. Once all that is done and in a rather large database, it becomes easily searchable.

Need a gene sequence that causes some species of aquatic life to withstand the massive deep sea pressures? No problem! It's a database search away!

Need the gene sequence that makes a bird's feathers bright purple? No problem! It's a database search away!

You can easily see where this goes when combined with DNA Synthesis…

Assorted forms of artificial intelligence like machine learning, deep learning and more:

As mentioned briefly above, machine learning algorithms (a kind of artificial intelligence) – particularly at large scale – can discover patterns and variations in patterns far faster and more reliably than any human can. This technology is advancing rapidly.

DNA analysis is very well suited to this kind of computational work for a variety of reasons outside the scope of this document. Suffice it to say that advances in the actual science of artificial intelligence will be an accelerant in the race to understand all manner of DNA.

Without it, we'll still eventually learn how DNA works, key genes, and more – it will just take longer.

With it, we'll speed up that cycle. But not without some risks. First, machine learning and AI are not foolproof. Errors still happen. Second, and perhaps more concerning, is that once a machine learning algorithm is 'trained' – it does what it does – opaquely. Which is to say – we don't necessarily understand how any given machine learning algorithm has reached its particular conclusion. Without that transparency – the scientific process is hampered. Imagine, for example, a scientist being interviewed for a major research publication on some new finding they are sharing and the interviewer asks "can you tell us the process that led you to your conclusion?" and the scientist is forced to answer "well, um, I mean, well… I'm sorry, actually, I don't know. The computer told me and I just believed it."

The main point here is not that AI is a problem from a transparency standpoint; rather, it will foster and dramatically speed up our discovery on gene function, and yes, in some cases, blindly.

Synthetic Biology:

The J. Craig Venter Institute in 2010 created, essentially from scratch, a new species of bacterial microbe with a genome of 1.08 million nucleotide-pairs. He even "signed" the DNA. This was a "proof of concept" for Dr. Venter – just a way to prove that it was possible. Since then, in 2016, he and his team successfully "refined" this DNA down to 531,560 base pairs contributing to 473 genes for the smallest known DNA of a living organism. They called this their "minimal cell" research project. He and others, like Dr. George Church at the MIT/Harvard's Broad Institute, are actively working on more sophisticated forms of synthetic biology.

Synthetic biology, like Venter's microbe, is still a fairly crude process. In that case, he "borrowed" DNA from other organisms or synthesized DNA in a lab, then had to effect a "nuclear transfer" whereby he removed the original DNA from an existing microbe – and replaced it with his own synthetically constructed form. Then "triggered" it back to life. There were a lot of failures of both the synthetic DNA not working once transferred and failures in the transfer or in the "triggering" mechanism.

But once he got his first success, and that microbe was able to self-replicate, the first truly man-made life form became reality. That was the smallest of tips of the largest of icebergs.

The BRAIN Initiative:

One of the initiatives of the Obama Administration was the creation of the BRAIN Initiative. In this title – the word BRAIN is actually an acronym for Brain Research through Advancing Innovative Neurotechnologies. Put plainly, it is for brain research what the Human Genome Project was for the human genome. It aims to dramatically improve our understanding of how the human brain works. Here is a quick link to the project: https://en.wikipedia.org/wiki/BRAIN_Initiative

You may be wondering “What does this have to do with DNA and CRISPR?” Quite a lot actually.

There is the short-term answer and the long-term answer.

The short-term answer is like the gene sequencing problem above. We cannot edit our DNA toward a specific intervention – if we don’t first have an accurate map of our DNA and an understanding of how a specific segment of it does whatever it does. This is also true of our brain.

Take the example of schizophrenia. Some recent research suggests that a certain process that most of our brains use to “prune” synapses – is broken for people suffering from schizophrenia. Fixing or eliminating this horrible disease requires two parts: understanding the broken machinery of the brain that causes it – then understanding the broken gene(s) that cause the broken brain machinery.

Right now, we understand so very little about how the brain works. What makes one person better at spatial reasoning than another? Why is it that some people can have a photographic memory, and the vast majority of us cannot? Before we can trace the root cause of these in our genes, we must first understand the biology of the brain much, much better.

Furthermore, we cannot _enhance_ the brain function of future generations through genetic engineering without it. What if everyone could be “engineered” to be a “genius”?

Going forward, longer term, were we to be able to identify how the brain works and which genes are responsible for various intellectual abilities – it certainly is reasonable to believe that future parents would “want the best” for their children and “order up” a smart kid. And succeed!

What happens when the species is that much smarter? Or perhaps just a few future scientists are “intellectually enhanced” and interested in things like CRISPR. Will those enhanced intellects accelerate our progress in this science? This seems likely.

Today’s BRAIN Initiative is both noble and appropriate and by all rights should continue with our support. However, in the near future it holds the potential – when married to CRISPR – to radically accelerate a scientific pursuit that is already moving faster than society can handle.

Putting it all together:

1. **CRISPR/Cas9** + gene sequencing + gene synthesis + high performance computing + AI & machine learning + synthetic biology + Delivery mechanisms + BRAIN initiative = what exactly?

What happens as these seemingly disparate technologies mature? And converge? Where does this lead?

Today, our ability to construct viable life forms from nothing is limited. Sure, DNA synthesis is expensive today, but we are conquering the cost and speed problems. As the combination of gene sequencing, high performance computing and AI mature, our inventory and understanding of existing genomes will rapidly accelerate.

Then, with tools like CRISPR and DNA synthesis, we’ll be able to “modify” existing life forms at ever increasing pace and variety. But why stop there?

Synthetic biology, aided by these other fundamental technologies, will “borrow” from the vast inventory of 8 million species worth of genomes to combine genes in various creative ways – to produce ever more diverse life.

Where does that lead? Science will move fast on this. There are a number of reasons for this.

First, the tools are democratizing very fast – so the economics are making this kind of research more accessible than ever before. That means more people doing research.

Global Race:

Second, this is a GLOBAL race.

China is investing immense amounts of money and people in CRISPR alone. They have 2000 unique modifications of mice in a lab. And they have edited the DNA of dozens of human embryos. And the now infamous case of Dr. He Jiankui – a Chinese researcher who CRISPR-edited twin human embryos that were implanted in a mother who carried them to term. Sisters, LaLa and NaNa, now have an uncertain future. And apparently there is another set of twins somewhere in China who also were CRISPR-edited as embryos. Dr. Jiankui eventually was sentenced by China to three years in prison and forever barred from practicing any form of medical research. See: https://en.wikipedia.org/wiki/He_Jiankui

Brazil is already releasing CRISPR edited mosquitos in the wild in a desperate attempt to contain the Zika virus. The UK has authorized human embryo testing as well. Virtually every nation on earth is doing something with CRISPR. And most nations already have access to the other resources like high performance computing (all they need is a credit card and an Internet connection for that) and DNA sequencing equipment (or the online databases of sequenced genomes) and synthesis (again, with just a credit card). When the race is global, the power of national competition alone will drive speed.

Who is thinking about the risks? Who is considering the deeply human questions of dignity and fairness? How do we, as a global species of wildly diverse viewpoints, come together on the much slower and indeed harder questions of the ethics of it all?

Unintended Consequences:

There are great possibilities ahead. Sure, we *may* learn how to eradicate malaria – who wouldn't want that? However, what if – in order to eliminate malaria – we have to eliminate all mosquitos on the planet? Do we know what the full ecological consequence is of doing that? (Never mind that it is unlikely that all nearly 200 nations would agree to voluntarily do it.) What if eliminating all mosquitos caused the collapse of two or three other species of creature that eat them? What if one of those species is the sole source of some compound that is used to relieve the symptoms of Parkinsons? (I'm making this stuff up just to illustrate the point – not to offer specific possibilities.)

Without careful consideration and regulation, it becomes perfectly ok for any consumer with a credit card to order a CRISPR kit and some synthetic DNA online – and use it on themselves to make their hair naturally purple or one arm stronger than the other (https://en.wikipedia.org/wiki/Josiah_Zayner). But it is then also equally possible for any terrorist to scan a database for a genetic marker for middle-eastern Jews, then order a CRISPR kit and some synthetic DNA, aided by some code on Amazon's AWS, to engineer a virus that is violently and always fatal to middle-eastern Jews only.

These examples, from presumably mundane, to extremely terroristic – are the outliers. These are certainly possible and maybe even probable. But it is all the unforeseen cases that we cannot imagine today that are the most concerning. Often, it is the seemingly simple and innocuous implementation or use case – that takes an unexpected turn or – dare I say it – mutation – that is potentially cataclysmic.

Who decides which future reality is acceptable and which isn't? How do we go about a global conversation to reach consensus? When do we do it? After the first species collapses? After the first genetically targeted plague executes global genocide on a specific class of humans? When?

And what about the ancillary costs? It is easy to say "we can eradicate cystic fibrosis from the human species" – but is that true? And if it is true – at what cost? Let's dig a bit deeper on this idea.

Editing Future Generations, Today:

How would CRISPR eliminate cystic fibrosis or beta-thalassemia? To do this would require "editing" the genetic mutation that causes each. Let's look at CF. CF is, being of genetic origin, inherited. So, depending on the genetic composition of the CFTR gene in each parent, the child will or won't inherit the disease. Obviously, if both parents have CF then both copies of their CFTR gene are defective. In which case 100% of their children will have CF. If neither parent has a faulty CFTR gene copy – then 0% of their children will have CF. But there are four other "in between" conditions for the parents resulting in 16 possible child outcomes. Of these 16, 4 result in CF, 4 result in no chance of CF, and 8 result in the child being a carrier but not actually have CF. So, in order to eradicate CF from the planet, we would have to "edit" the DNA of ALL embryos having at least one parent with CF or being a carrier.

But how do we do that? First of all, we cannot (yet) edit an embryo at the single cell stage in the womb and, arguably, will never be able to do so. So, this means that whenever at least one parent has CF or is a carrier for it, then ALL children MUST be "produced" via In Vitro Fertilization (IVF). In IVF, multiple eggs are inseminated artificially and when the embryo is still in the single-cell stage, regardless of the fact that it only has a 1 in 4 chance of producing a CF human, we would "CRISPR" it to correct the CFTR gene. If both copies of the CFTR gene are already ok, then the "CRISPRing" will do nothing (perhaps – but I'll come back to this). But if either copy of the CFTR gene is defective, the CRISPR process should correct it. If all goes as planned, the embryo then develops into a CF-free human.

Are there risks? Yes. There is no guarantee that any of the artificially inseminated and CRISPRed embryos will survive. Additionally, and perhaps more importantly, CRISPR is not 100% error proof. That is to say that the sequence of nucleotides that are "wrong" in the CFTR gene _could_ appear elsewhere in the genome of that embryo. Consequently, the "edit" that is targeted at the CFTR gene _could_ edit that gene – or not – and/or could edit the location that was NOT targeted for "correction" thus correcting something that doesn't need it. In which case – some other gene could be mutated by "accident". The mutation could be completely benign. Or it could be far worse than the disease we were trying to fix.

Let's consider the cost. First, we must put the female through IVF treatments to hyper stimulate her ovulation. This is costly in terms of both cash and impact on the female's reproductive system. Then we must "create" multiple embryos and CRISPR them all (whether they need it or not) and implant the two or three most viable embryos back in the womb for one or more to reach full term. One or more may reach full term and be born. If all the CRISPRing went as intended, with no "side effects" (other portions of the genome were also edited mistakenly) – this baby(ies) will be born CF-free as hoped. But if the CRISPRing accidentally edited the DNA – anything is possible. What then?

It is conceivable that when CRISPR is used in this fashion, it is likely that doctors (and parents) will want to do an amniocentesis at some point to make sure all is as planned. What if the test shows CRISPR didn't take and the baby(ies) is found to present CF? What if the test shows the baby(ies) have some

other unknown genetic mutation – the results of which we cannot predict? What if the unintended mutation is a blood-based issue? In this case, it is possible – since the baby and mother are co-mingling blood supplies – the mutation could be harmful to the mother? In which case do the parents decide to abort?

All of that is about the direct biological risks to just those parents and the child they understandably desire to be born CF-free. But are we thinking big enough? Are we considering the larger social issues? For example, in order to eradicate CF from the human species – does this mean that ALL humans of child-bearing or child-fathering age could be "forced" into an IVF procedure to ensure the elimination of CF? What if those parents object to IVF on religious grounds? Will they still be forced into the procedure? If so, do they choose to not have children at all? Or are legally forced (gasp!) to not reproduce? And what about the parents that do elect such a process, and a child is born with some new disease never before seen? If it is fatal – that is painful enough. But what if it isn't? What if it is a permanent and disabling, but not fatal, disease? What if it isn't a visible thing – like an issue with the formation of or chemistry of the brain – resulting in a whole new kind of schizophrenia? Does society now bear the burden of caring for this human being? What kind of life will they have? Is this diseased human _allowed_ to procreate? To pass on their unique genetic disease? Or are they forced, also, into an IVF/CRISPR process to edit out what was edited in? What if _that_ IVF/CRISPR process fixes _that_ genetic error, but introduces another?

Who is thinking about these implications?

About Human Dignity:

And what about the question of human dignity?

Today, when someone is born "naturally" with an illness or a genetic issue that causes blindness, for example, our society looks upon that person with dignity and we make responsible and caring accommodation for them. We do this because, in our current view, this situation is unavoidable. A 'sad accident' of genetics.

What about tomorrow? What happens when everyone being born is 'engineered' in one way or another to eliminate a disease or 'enhance a feature' like strength, speed, or intelligence? When the 'norm' is 'perfection' (or at least a lack of visible defects) – how will society look upon the 'poor natural' who is less perfect?

At the core, this is about recognizing that the human person is a created gift, not a manufactured product.

Summary:

In the 1950's when photolithography was first applied to silicon – the world changed in that moment. A singular technological advance, like photolithography or CRISPR, supported by other related technologies – has the power to dramatically change the world. And it did. The unending march of digital technology has gone in directions NOBODY could have foreseen in 1950. There were good and bad consequences of that era.

CRISPR is to DNA as photolithography was to Silicon. We are entering a new era where DNA is the new silicon – where we "program" our own DNA to "fix or enhance" all life forms.

Mind you, I am not saying that this is all bad. Nor am I saying that all research should stop. Not at all. I am very much in favor of man's quest to understand this universe and, where prudent, intervene natural processes in ways that improve lives. But not all interventions are created equal and some make short-term positive changes at the cost of long-term negative impacts. And sometimes, when the line between the two is either thin or blurry, and where there is a void of public policy clarity, unrestrained experimentation with the natural evolution of existing life forms WILL result.

Furthermore, in this era we will also create entirely new DNA and, indeed, introduce entirely new life forms into an ecosystem that has absolutely no anticipation of them, nor ability to integrate them.

In short, whereas nature has taken millions of years to evolve our complex biological ecosystem – we now intend to experiment and bend selected (and presumed tiny) parts of it to our will. And we intend to do that in minutes, hours or days of research and experimentation in our classically non-holistic way. Research today definitely leans toward studying the curve on the vein on the underside of the leaf of the tree – NOT toward studying the forest as an extremely interconnected system.

Does science consider the greater metaphoric ecosystem and evolutionary hurricane resulting from the so-called butterfly effect of some supposed tiny tweak to the DNA of this or that species?

This is not a future possibility. It is not a science fiction novel. It is a present reality. These technologies are here today. We are within a breath of the convergence of them. NOW is the time to act. Let's get the conversation started…

Unless and until the "everyday" non-scientist starts to ask the hard questions about how all this will be used – scientists (an absolutely well-meaning bunch!) will do what scientists do – research, experiment and push the boundaries of knowledge. But knowledge is not wisdom and, sometimes or even often, even brilliant scientific minds can be blind to the wise and unwise dividing line of their own research. That line MUST be drawn by you and I. The everyday non-scientist that will nonetheless have to live with the unintended consequences of what the scientists do.

You can do your part. Read. Listen. *Think*. Then reach out to your state and federal legislators, the National Institutes of Health, the Department of Health and Human Services, local universities and all manner of media to engage the conversation.

In this Era of Experimental Evolution – the future of ALL future generations of every living created living thing (not just human) on this planet depend upon an open, informed, civil and constructive dialog by all the highest order creatures living here: you.