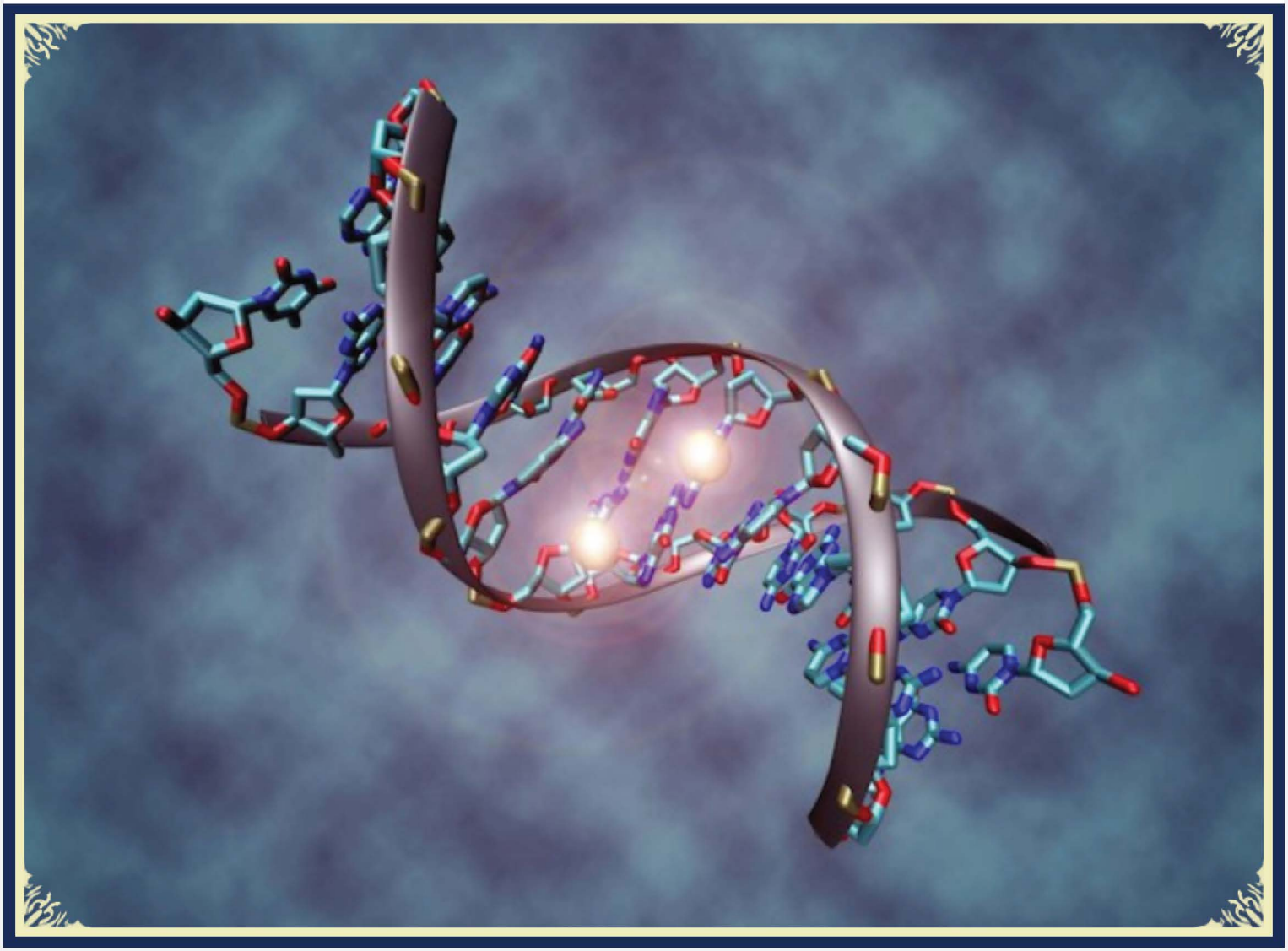
FREE-MAN'S PERSPECTIVE
How Life, Liberty & Sanity Can Win

A FRONTIER TECHNOLOGY REPORT

CRISPR: Science Fiction Now



Contents

Forward

Some things are so compelling that you have to alter your life and get involved with them. And that's what CRISPR has been to me. CRISPR stands for "Clustered Regularly Interspaced Short Palindromic Repeats." They are what give us a "handle" on DNA in order to edit genetic code. I learned that bio-hackers were interested in it by 2015, but I didn't have time to spend on it then. I was writing a trilogy of novels, the culmination of 40 years' work, and I had family matters to attend to as well. I wanted to evade new projects, not jump into them.

And then in early 2016 in Panama, I ran into Jeff Brown, editor of the *Exponential Tech Investor* newsletter. I sat in a small meeting room as he explained why CRISPR was "the biggest thing since penicillin." Knowing what a gigantic thing penicillin was to my parents' generation, this got my attention. Jeff wasn't too far from my own age, and he was clearly both informed and serious. This wasn't an empty marketing slogan.

I had the right scientific background to understand the power of this technology. And as he continued, the scope of what he was describing dawned upon me. And then something else also did, one of the more useful lessons of my life: That to really understand something, I need to drill down to essences, and at the earliest opportunity. So, I waited for a natural break in the presentation (and as I mentioned, this was a small group) and posed a few questions. They went like this:

> "Are you saying that this is the mechanism that nature uses to splice genes?"
>
> "Yes," he answered flatly and waited for me to digest that fact.
>
> "And you're saying that we've learned how to use this mechanism purposely and precisely?"
>
> Yes," he answered in the same way.
>
> "And this isn't restricted to certain types of organism?"
>
> "No," he said, "it works on any and all organisms."
>
> "And we can do this right now?"
>
> He answered "yes" again, firmly and seriously… and I believed him.

In response, and involuntarily, I started laughing.

This was not a laugh of ridicule; it was the laugh you see in a small child when he or she figures out how to do something. The child laughs and claps his hands for the joy of discovery, for the feeling of competence, and for the knowledge that he has gained an essential ability. *That's* how I laughed.

And that's how I've felt about it ever since, as I got together with bio-hackers, did my own research, and became clear on the fact that this was something I couldn't ignore.

And that's why I'm producing this report now, before it really makes sense from a marketing standpoint: CRISPR will change the future. I want as many people as possible to understand it at the earliest possible date.

CRISPR really is that important.

Insanely Great

Steve Jobs was famous for saying that he wanted new products that were not just good, but "insanely great." I've never seen many products I could define that way (even Jobs' didn't reach that level for me), but CRISPR does. This technology *is* science fiction come to life… and ahead of schedule.

And to illustrate just how big I think this is, consider this conclusion of mine: *We're about to change the way humanity sees itself*.

That's how big.

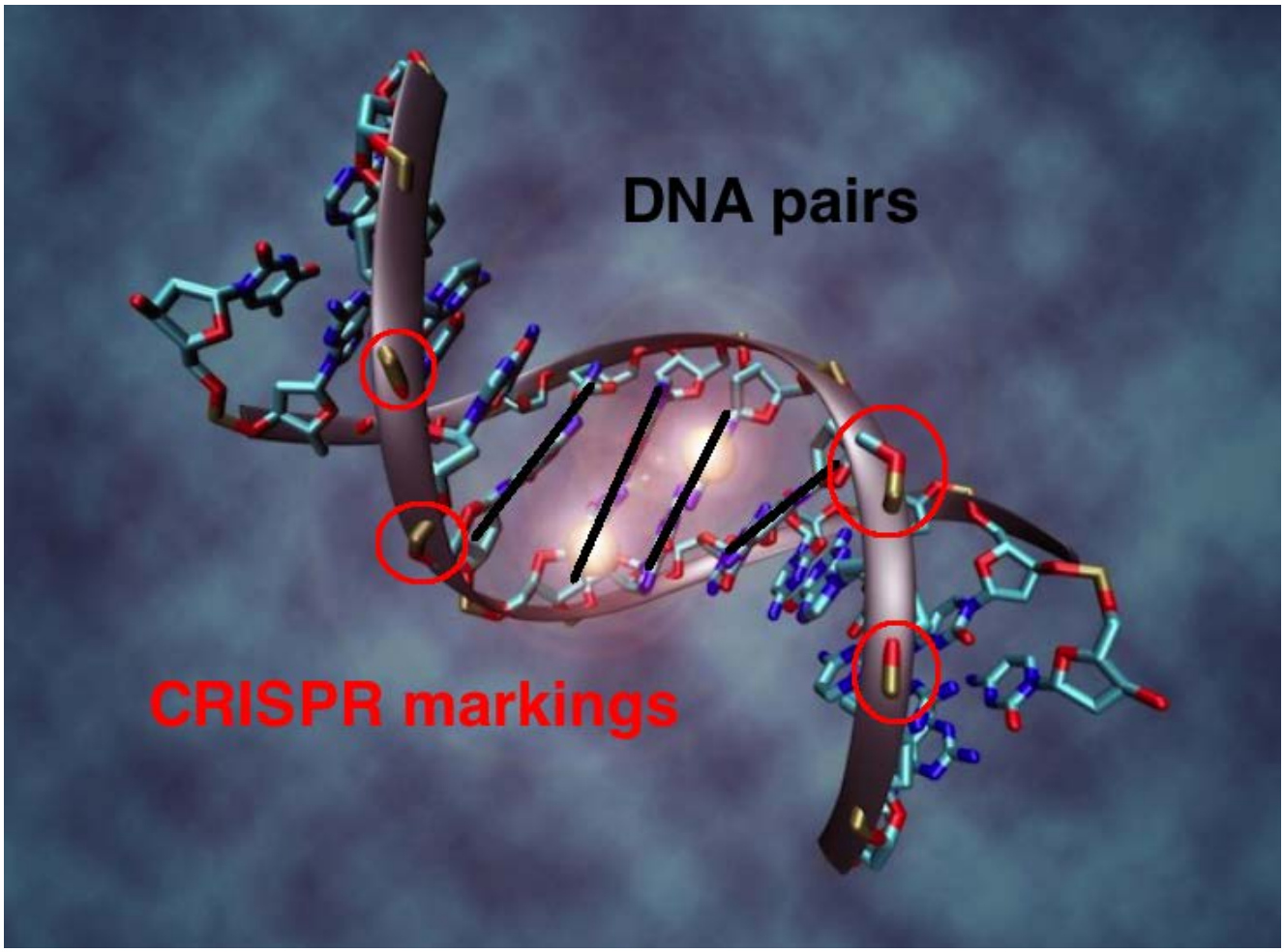
The opportunities associated with CRISPR are likewise big… and many. There are literally millions of applications for CRISPR. Anyone who wants to get involved, given motivation and a bit of ability, stands to thrive in the years ahead, while healing mankind of its diseases and improving the world. That's not a bad prospect.

And I'm not exaggerating. As you go through this report, you'll see what I mean. This field is huge, and it will be difficult to monopolize, even for mega-governments and their friendly mega-corporations. CRISPR is at its essence a "people's technology."

You'll see that for yourself as we continue.

Getting Clear

First, however, I want you to have a very clear understanding of how CRISPR operates. And so, please take a moment to examine this image:



What you're looking at here is a small section of DNA. I've highlighted in black the pairs of molecules that form the "rungs of the ladder" in a string of DNA. These rungs are called "base pairs." We'll talk more about these later, but first I'd like you to be clear on the fact that DNA is shaped like a long, twisted ladder and that each rung of the ladder is made of two molecules.

You'll also notice the gray "rails of the ladder" and the little colored pieces that are shown on them – and that I've circled some of them in red. These are the CRISPR molecules.

The CRISPR molecules are nature's DNA addressing system. They define locations along the ladder just like street addresses define building locations along a street.

The CRISPR system alters DNA by using special molecules (called *Cas* molecules) that find addresses on the DNA and clip the rails of the ladder at those points. They cut pieces out of the ladder, so they can be replaced with more useful pieces.

And that's really it. Actually doing this requires time, training, and so on, but this is the essence. Here it is in steps:

1. CRISPR molecules define the spot.
2. Cas molecules find the spot and cut out a piece of DNA.
3. We insert better DNA to take its place.
4. Mother Nature does the rest.

Take a look at that image again: rungs of paired DNA molecules and rails with markings. Hold that imagine in mind.

What This Report Provides

There are nine particular things this report is designed to give you:

1. A deep understanding of what CRISPR is and what it can do.
2. A clear understanding of the biology involved. And by "clear understanding," I mean that you'll understand what these things are and how they work together. I do not mean that I'll feed you a pile of terminology. (And don't let the terms we do use throw you. We'll continue to explain them and we have a glossary at the end of the report.)
3. Who the current players in this development are, some background on how they came into the process, and how it may turn out.
4. Who the long-term players in the field are likely to be, how the first of them became involved, how more will become involved, and how they're likely to be included or excluded.
5. Where this is heading; how this technology and all its parts are likely to unfold. We'll identify winners and losers for each major scenario.
6. How CRISPR will change the world we've known… by changing us.
7. Why scientific changes are far more potent and enduring than mere cultural changes… and how this relates to CRISPR.
8. The ability to read and understand CRISPR literature.
9. The ability to judge CRISPR technicians, independent of certifications, licenses, or whatever.

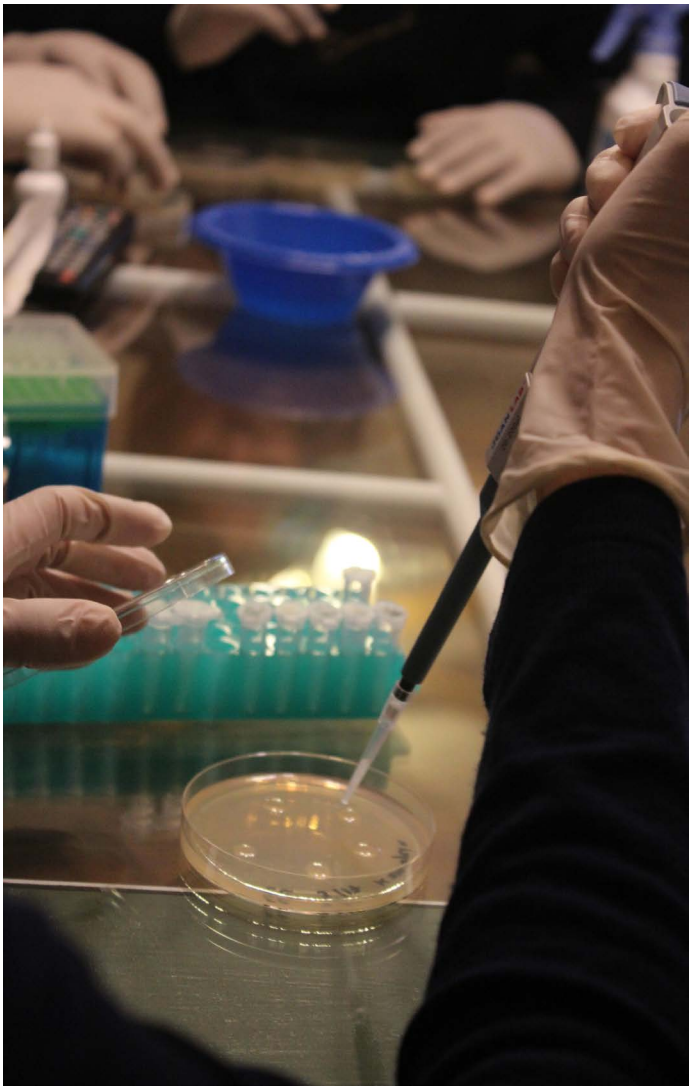
Claims

Before we go any further, I want to give you some specific claims – statements of precisely what CRISPR can do. So, when I say that CRISPR can do very important things, here's what I mean:

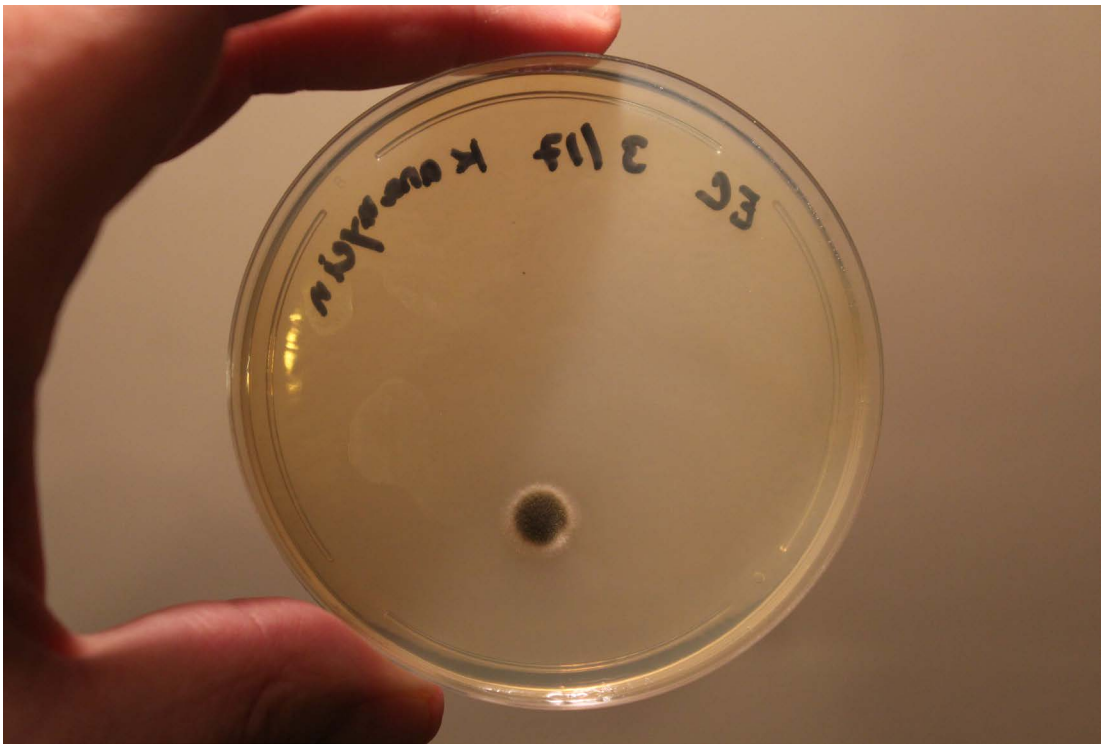
- CRISPR can probably reverse all of the 6,000 known genetically related diseases. And I really do mean *all of them*. Think about that for a moment.
- Huge numbers of cancers can be wiped out.
- Obesity can be eliminated.
- Alzheimer's disease can almost certainly be prevented.
- Muscular dystrophy can be eliminated.
- Down's syndrome can almost certainly be prevented.
- Allergens in peanuts can almost certainly be edited out.
- Any plant can be modified.
- Any animal can be modified.
- Any microorganism can be modified.

These are just the most obvious items on the list. From this point we can move into industrial processes, new types of food, improving ourselves, and even just-for-fun items like fluorescent beer.

Now to give you a gut-level feel for how accessible CRISPR is, here's a photo taken recently. It shows a group of us splicing DNA at a kitchen table. We called it a CRISPR Party, and it involved a dozen people or so, only one of whom had experience with lab equipment and techniques. All the materials for the party cost in the range of $400, a good deal of which will be carried to the next demonstration.



And here is the result, a colony of a simple (and harmless) bacteria, growing where it has no business being[1]. This type of bacterium can grow on a treated plate of this type only if it has been genetically altered.



So, CRISPR is not a technology that requires expensive laboratories and years of training. It's something that any motivated person can learn to do, and they can do it in a basement, spare bedroom, almost anywhere. This technology is extremely accessible. Almost anyone can learn to do it, with very little investment.

Something very powerful has come into the world. In my opinion, this development deserves both our attention and our informed efforts. CRISPR is part of nature, and as such, it's our natural inheritance. And being that our relationship to CRISPR is biological, it stands above our relationship to kings, conquests, and overlords.

CRISPR is nature's gift to intelligent beings. It's ours to use… and we should use it enthusiastically.

– Paul Rosenberg, February 2018

[1] To be precise, the bacteria is *E. coli* HME63 and is shown growing on a plate containing a Strep/Kanamycin agar.

Introduction:
What CRISPR Is Doing to the World

Imagine humans being able to control their own physical evolution… to improve their bodies promptly, durably, and precisely… each change individually chosen from a palette of possibilities… with all the miraculous and terrifying aspects that are implied in those thoughts.

We could eliminate thousands of maladies, including things like obesity and a great percentage of all cancers. But at the same time, secret government agencies could create super-soldiers with twice the muscle mass of a normal human. Overlords could withhold the technology from the public or even use it against them, breeding themselves into a super-class.

Yes, I know this sounds like the promotion for a science fiction film, and that's my point: *CRISPR is the stuff of science fiction, and it's here, right now*.

Something very powerful has come into the world, and we're just at the beginning of it. From a marketing standpoint this report is way too early – the larger world has just barely started to comprehend CRISPR's effects, and mass ignorance doesn't make for great sales numbers. But Free-Man's Perspective has never been just about sales, it's more about things that matter. And this matters a very great deal. And so I want our readers to get involved early, not just to profit, but to birth this thing into the world properly.

New technologies take the paths the early pioneers set, and I want those paths to be directed into life, liberty, and the pursuit of happiness… not toward restriction, monopoly, and elitist control.

CRISPR challenges many of the institutions and ideas that currently dominate mankind. With CRISPR, we can increase our lifespans in any of several ways. This perhaps won't bother people too greatly, but what happens when we can edit our children's DNA as they are conceived? What if we eliminate genetic defects, as well as making children healthier, smarter, and freer from stress? How many people will freak out at that? Some will call them *Frankenbabies* and scream that they should be banned.

But what right does anyone have to withhold improvement from mankind? And particularly, how dare anyone tell a parent that they can't improve their child? Is that anything but blind tyranny?

These are the challenges that lie before us, and they're already here. Human embryos have been edited successfully with CRISPR. So far as is known, this has not proceeded to a live birth. But the first live birth is only a matter of time, and the first few will probably be done in secret anyway.

One way or another, this is about to be thrust upon the world, and restricting it comes down to forbidding parents – with force if they don't comply – from improving their own children.

Already, mere genetic testing (generally called "Preimplantation Genetic Diagnosis," or PGD) has been banned in several countries and restricted in others. Why? Because it might lead to genetic modification. So, restating this plainly (and honestly), *genetic testing of embryos is forbidden, because parents might find out that their child has a defect and might want to fix it.*

Read that over if you think I'm being too dramatic. And these edicts have been put in place before any embryo modifications have gone badly[2].

[2] And eventually something will go badly, as it does in any area of human endeavor.

Once CRISPR is used to create healthier babies, the enforcers of the world will almost certainly move to ban or restrict it, merely because it is new and because it threatens the dogmas so many people live by.

CRISPR places us in a position of power… of being powerful enough to control our own physical evolution. And this new image of humanity as potent and powerful – which can hardly be avoided when we're purposely improving our own race – is a threat to many religions and very definitely a threat to rulership, which thrives on its subjects feeling small, weak, and powerless.

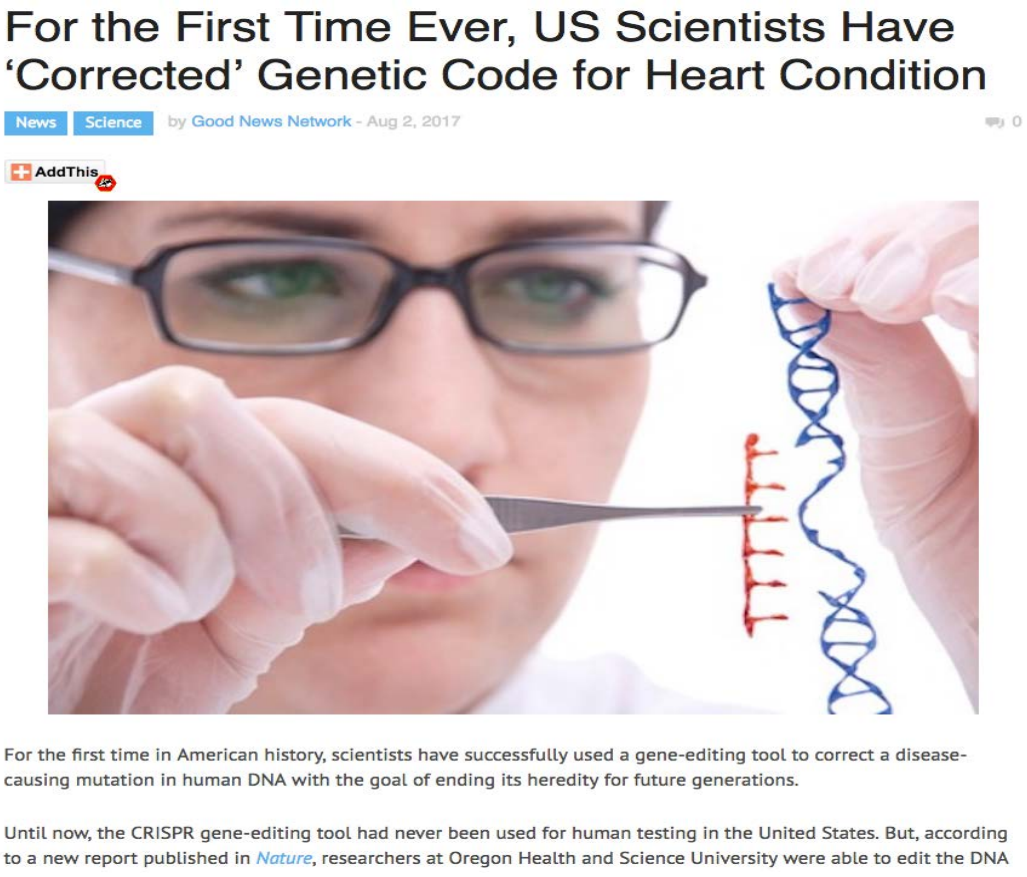
And this may bring us to a great crisis: By banning CRISPR from such uses, people will understand that the possibility of improving their children is real. How long then will it be until a couple with long and tragic histories in their families of heart disease or mental retardation decides that they're going to protect their babies anyway? And what if this procedure can be done in a small laboratory? And what if it's affordable?

If you were that parent, what would you do? Would you keep the rules or save your child from retardation?

That choice has just arrived. Most people simply aren't aware of it yet.

And yes, this technology is so fast, cheap, and easy that more or less every part of it can be done in small facilities.

I'd like you to see just a couple of images to support this. These are some of the first bits of CRISPR news to reach the general public. Here's CRISPR editing out heart disease:



For the First Time Ever, US Scientists Have 'Corrected' Genetic Code for Heart Condition

News Science by Good News Network - Aug 2, 2017 0

AddThis

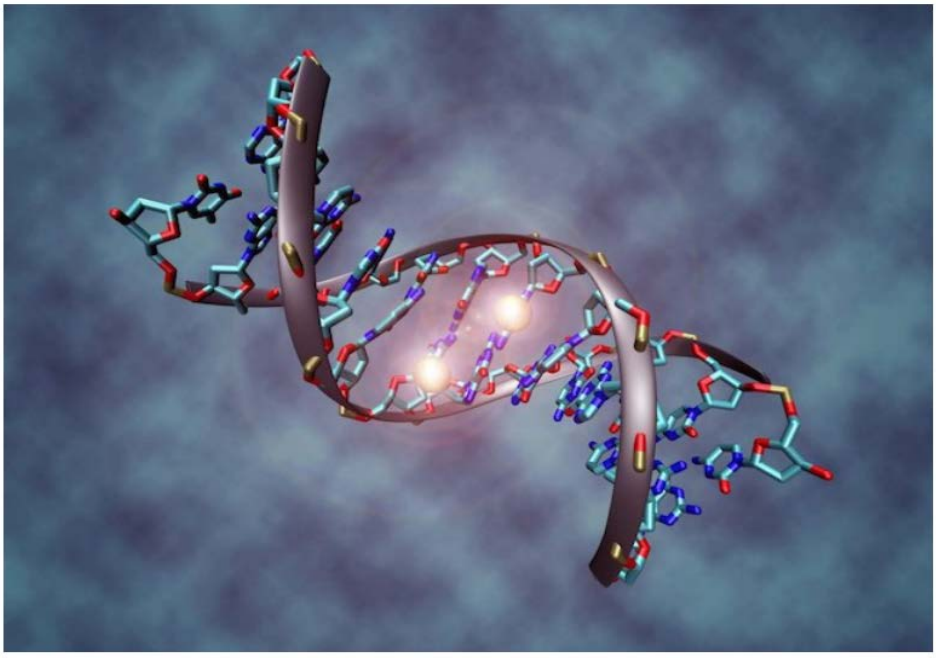
For the first time in American history, scientists have successfully used a gene-editing tool to correct a disease-causing mutation in human DNA with the goal of ending its heredity for future generations.

Until now, the CRISPR gene-editing tool had never been used for human testing in the United States. But, according to a new report published in *Nature*, researchers at Oregon Health and Science University were able to edit the DNA

And here's CRISPR removing HIV from mice… not from mouse cells, mind you, but removing HIV from a full, living organism. That's a very big deal.

Scientists Just Deleted HIV in Mice Using Gene-Editing Technique

News Health by Good News Network - Jun 9, 2016



Scientists at Temple University successfully removed HIV DNA from living animals, another step forward in their effort to develop a cure for the virus that causes AIDS.

And here's a scientific paper on the same topic:

Molecular Therapy
Original Article

AMERICAN SOCIETY of GENE & CELL THERAPY

In Vivo Excision of HIV-1 Provirus by saCas9 and Multiplex Single-Guide RNAs in Animal Models

Chaoran Yin,[1,6] Ting Zhang,[1,6] Xiying Qu,[2,6] Yonggang Zhang,[1] Raj Putatunda,[1] Xiao Xiao,[1] Fang Li,[1] Weidong Xiao,[3] Huaqing Zhao,[4] Shen Dai,[1] Xuebin Qin,[1] Xianming Mo,[5] Won-Bin Young,[2] Kamel Khalili,[1] and Wenhui Hu[1]

[1]Department of Neuroscience, Center for Neurovirology and the Comprehensive NeuroAIDS Center, Temple University Lewis Katz School of Medicine, 3500 N. Broad Street, Philadelphia, PA 19140, USA; [2]Department of Radiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15219, USA; [3]Department of Microbiology and Immunology, Temple University Lewis Katz School of Medicine, 3500 N. Broad Street, Philadelphia, PA 19140, USA; [4]Department of Clinical Science, Temple University Lewis Katz School of Medicine, 3500 N. Broad Street, Philadelphia, PA 19140, USA; [5]Laboratory of Stem Cell Biology, State Key Laboratory of Biotherapy, West China Hospital, West China Medical School, Sichuan University, Chengdu 610041, China

CRISPR-associated protein 9 (Cas9)-mediated genome editing provides a promising cure for HIV-1/AIDS; however, gene delivery efficiency in vivo remains an obstacle to overcome. Here, we demonstrate the feasibility and efficiency of excising the HIV-1 provirus in three different animal models using an all-in-one adeno-associated virus (AAV) vector to deliver multiplex single-guide RNAs (sgRNAs) plus *Staphylococcus aureus* Cas9 (saCas9). The quadruplex sgRNAs/saCas9 vector outper-

utilized in basic science and pre-clinical settings for the potential treatment of genetic diseases, cancer, and infectious diseases. The majority of these applications are attributed to the successful development of the *Streptococcus pyogenes* Cas9 (spCas9), a 1,368-amino acid (aa) protein that recognizes a protospacer adjacent motif (PAM) of NRG nucleotides to the 3′ target site.[1] However, several smaller Cas9 proteins from other bacterial species have been garnering more attention for their greater feasibility in viral gene therapy,

We'll continue into the nuts and bolts of CRISPR now, but I don't want you to lose track of the magnitude of this:

We're about to change the way humanity sees itself.

Where CRISPR Came From

CRISPR[3] has been around for millions of years… far longer than humanity has been around. We just didn't know about it till recently.

To put it very simply, CRISPR is a marking scheme. These "palindromic repeats" are little dabs of enzymes along the sides of DNA, appearing in recognizable patterns… patterns that CRISPR-associated molecules (*Cas* molecules) are designed to sense and react to.

CRISPR markings allow any specific spot on a string of DNA to be targeted. They are the markings that allow other molecules to find a specific "address" of DNA… *any* specific address of DNA.

Now, I'll restate that to make sure it's very clear:

> CRISPR is an addressing system, and it's present in all DNA.

In addition,

> There are complimentary molecules that find these addresses, connect to the DNA at precisely that spot, then perform certain actions, such as cutting the DNA.

Finally, as we've mentioned earlier, *we have now learned how to control and use these tools… however we wish*.

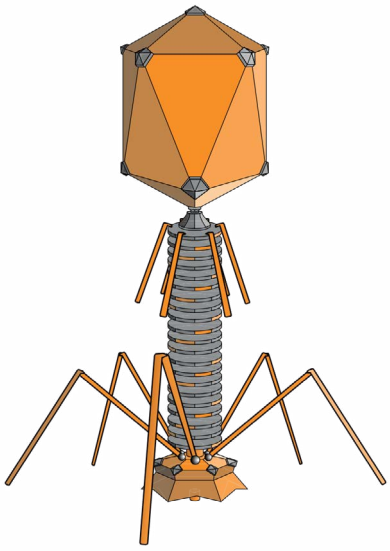
So, we have a tremendously powerful tool in our hands. We can change literally any organism on the planet.

But before we go forward, let's understand where this comes from and how it was developed.

CRISPR is nature's way of protecting cells from viruses. Viruses have caused a lot of pain in the world, and we fight them all the time. But they are also very impressive little bio-mechanical machines. And once we learn how to tame these things, there will be fascinating applications for them. Before dogs were house pets, as I'm sure you know, they were terrifying and predatory wolves that killed children among many other things. Viruses are taking the same path at the moment, although it will be some time before they complete it.

Viruses are very, very small, usually in the range of 100 nanometers or so in diameter. That is, 100 billionths of a meter, meaning that about 250,000 of them, lined up end to end, would be one inch long.

Viruses are typically very mechanical-looking things – like tiny machines. The image to the right is of a common virus.

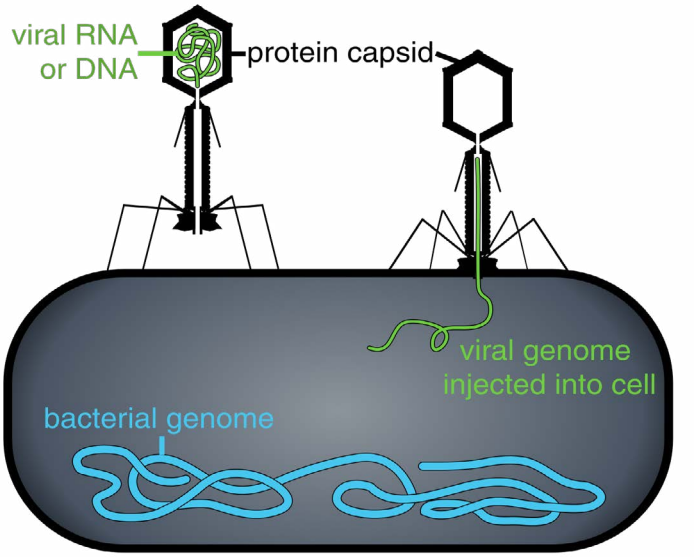


(Source: Wikipedia)

[3] CRISPR stands for *Clustered Regularly Interspaced Short Palindromic Repeats*.

The central action of a virus is to change the DNA of another organism. The only way viruses can replicate their DNA is to do it inside of living cells, and so they are very good at attaching to cells, breaking through the membrane (the wall) of the cell, and injecting their own DNA into the cell.

Once *viral* DNA (the DNA of the virus) is inside the cell, it's likely to mix itself with the DNA of the organism. This diagram shows how a virus changes the DNA of a bacterium (*bacterium* being the singular form of *bacteria*).



(Source: Wikipedia)

A *genome*, by the way, is simply the organism's DNA, and the *capsid* is just the "box" part of the virus that holds its DNA, which happens to be built out of proteins. (Don't let the terms throw you. We'll continue to explain them and we have a glossary at the end of the report.)

So, viruses mess with the DNA of other organisms. As you can imagine, this can be a big problem. And so, nature has developed a method of fighting back… and that method is CRISPR.

Here's how this defense process works:

1. A virus attacks a *bacterium* (a single bacteria cell) and alters its DNA.
2. The bacterium's Cas molecules carry templates of which DNA sequences should be at each place on its cell's DNA. Sensing that the altered DNA isn't matching the template, the Cas molecule locks itself into place.
3. The Cas molecule clips out the damaged DNA, just like a pair of scissors cutting a piece out of a string.
4. Once the enemy DNA has been clipped out, it floats away and soon enough dissolves.
5. The cell then, by its natural processes, replaces the DNA segment.

Thus the damage is repaired. And bacteria cells do this all the time.

This process, however, was only recently discovered. And as such things usually go, one of the primary breakthroughs came with a conversation along the lines of, "That's funny; I wonder what this thing is."

One of the groundbreaking moments came in the early 2000s, at a Danish yogurt lab, where bacteria (being critical to the yogurt-making process) was being carefully studied. Scientists Philippe Horvath, Rodolphe Barrangou, and their colleagues ran into CRISPR while analyzing the DNA of a bacterium used in yogurt and cheese production. Initially they had no idea what the CRISPR markings they saw were for, but as they examined more and more strains of the bacteria, they put the pieces together.

"That was an eye-opening moment when we first thought of the link between CRISPR sequencing content and phage resistance," said Barrangou. (A *phage*, short for *bacteriophage*, is a virus that infects bacteria… as in the illustration above.)

The general timeline of CRISPR development went like this:

1987: First report of clustered repeats.

2000: Recognition that CRISPR was present in all single-cell organisms.

2007: Proof that CRISPR carried out immune functions.

2008: Proof that CRISPR acted upon DNA targets.

2010: Proof that Cas9 (a molecule involved with many CRISPR processes) could be willfully guided to cut specific sections of DNA

2013: First demonstration of genetic editing in single-cell organisms.

2015: First demonstration of genetic editing in human embryos.

So, you can see how recent this development is. In fact, the patent wars over these technologies are still raging. (We'll cover them later.)

There are two essential facts here:

1. CRISPR is nature's tool for repairing DNA, and
2. CRISPR is easy for us to guide and control.

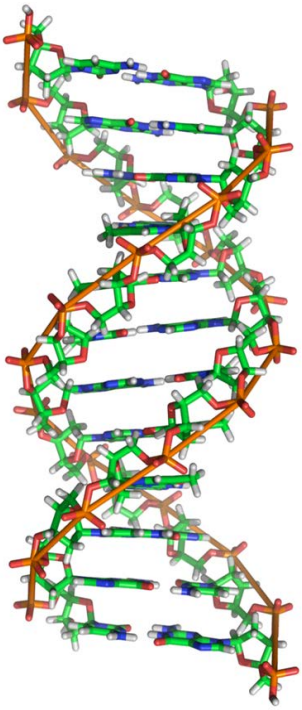
CRISPR is immensely efficient, fast, and accurate. What we're doing now is adapting it for intentional use. And what we've found is that it's easily adaptable to more or less any gene alterations we want to make. *We can control it at will.*

So, to more or less everyone's surprise, a cheap and universally available tool for editing any DNA anywhere has fallen into our laps. Science fiction has been made real, and far ahead of schedule.

What Exactly Are DNA and RNA?

All of us have heard of DNA and RNA, but to understand CRISPR, we need to know how they work. Fortunately, it isn't hard.

Both DNA and RNA exist in the familiar "twisted ladder" shape:



(Source: Wikipedia)

The sides (the rails) of these ladders are made of phosphate molecules, which are relatively unremarkable. It's the rungs of the ladder where the magic happens. Each rung of the ladder (the "rungs" are called *base pairs*) is composed of two molecules, which makes it possible for these long ladders to zip and unzip themselves, right down the middle. And when they do, each half-zipper can reassemble itself perfectly, creating two complete, identical zippers.

This works because each "rung" molecule has a natural compliment. For DNA, there are four molecules that can be used in the rungs: cytosine (represented as *C*), guanine (*G*), adenine (*A*), and thymine (*T*). And they match up with each other in specific ways:

G matches up with C

A matches up with T

So, if a split ladder has a G as one of its half-rungs, it will naturally connect to a C molecule and will refuse to connect with anything else. If it has a T, it will connect to an A and to nothing else.

It's really that simple: DNA and RNA unzip and then grab on to their complimentary molecules to form two identical ladders. (A cell nucleus has many such molecules floating around in it.) This process is what makes cell replication, and thus life, possible.

RNA differs from DNA in that is uses a molecule called uracil (U) in place of thymine (T) So, in RNA,

C matches up with G

A matches up with U

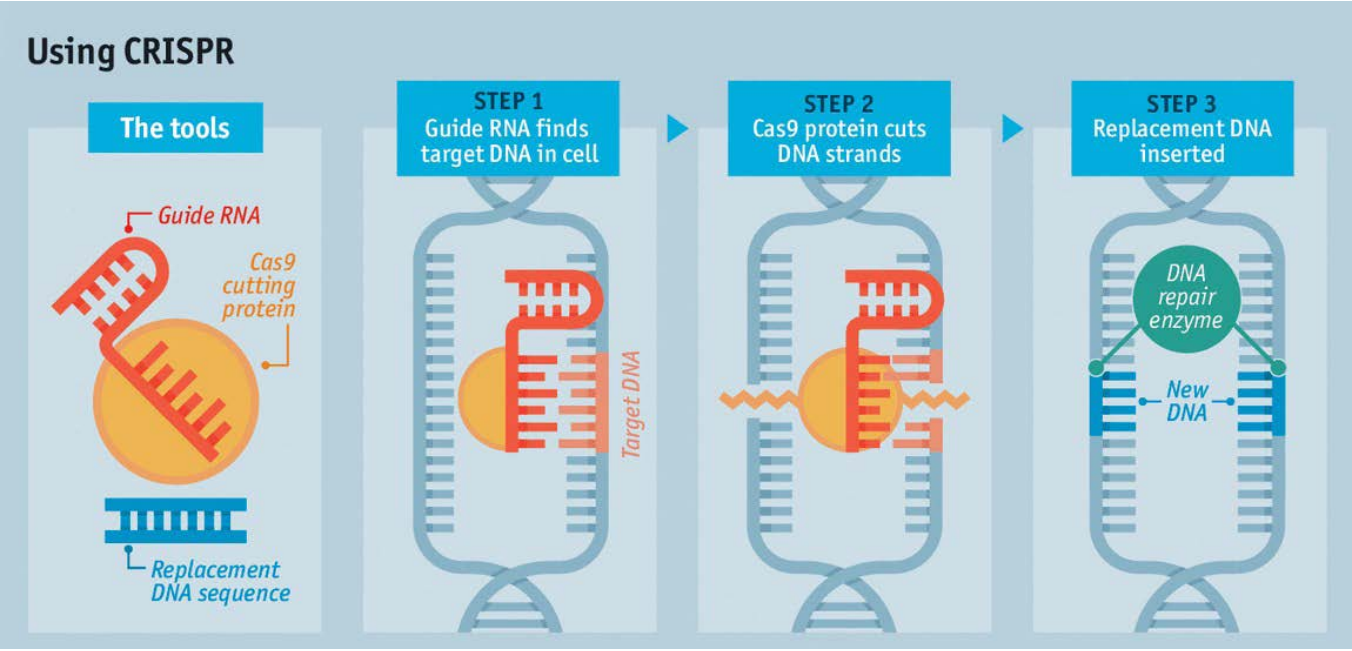
There are complexities to these huge molecules, but this is the essence of what they are and how they work.

The CRISPR Process

The CRISPR process breaks down into six basic steps, which are these:

1. Identify the section of DNA we want to change.
2. Manufacture *guide RNA* (to define the right location) and *template DNA* (to replace the bad DNA).
3. Get the appropriate Cas protein molecules. (*Cas* means *CRISPR associated*.)
4. Get the CRISPR materials inside the targeted cells. (These things will work by themselves, if you get them all into the right places.)
5. Wait for the process to work. It does take time, but usually only a certain number of hours.
6. Check that it worked properly.

We'll go through each of these in some detail, but first I'd like you to see the process graphically. So, take a good look at this diagram:



Source: *The Economist*

On the far left you can see the tools that are used. Don't worry about remembering all the terminology right now; we'll keep explaining as we go. And once you understand what these pieces are doing and how they work with each other, remembering becomes far easier.

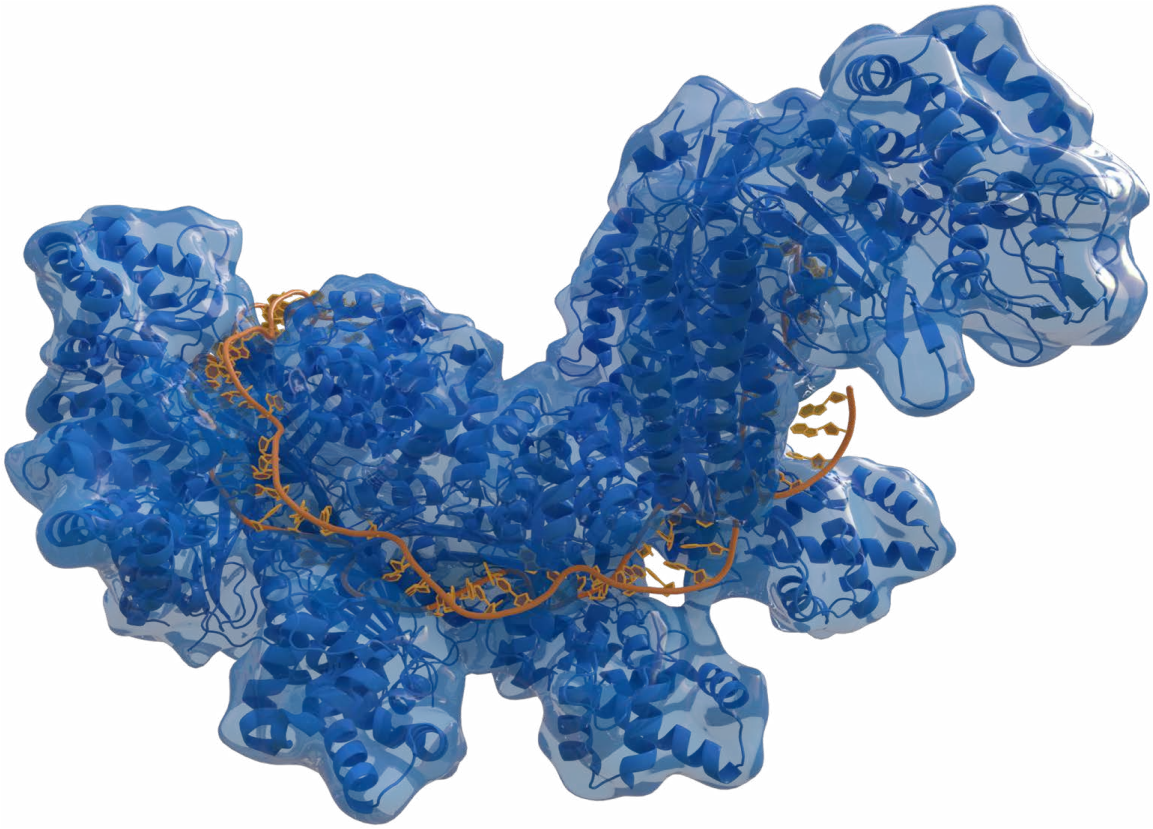
So, on the left, we see three things:

1. The guide RNA: This guides the Cas molecule to the right place on the long string of DNA. *This is shown as Step 1 in the diagram above.*
2. The Cas9 cutting protein. Cas9 is one of the most important Cas molecules, but there are others, and newer ones are being found. Cas9, after it's guided to its target, will simply clip both strands of the DNA at that place. *This is shown as Step 2 in the diagram above.*

3. Replacement DNA: These are short, pre-manufactured strings of DNA that will fill the gap made by Cas9's cuts. These are the improved bits of DNA that we want in place of the removed section. And once the "bad" section is removed, these will naturally move into place and stick. *This is shown as Step 3 in the diagram above.*

In the end, we have a repaired or upgraded string of DNA.

Now, take a good look at this image:



(Source: Wikipedia)

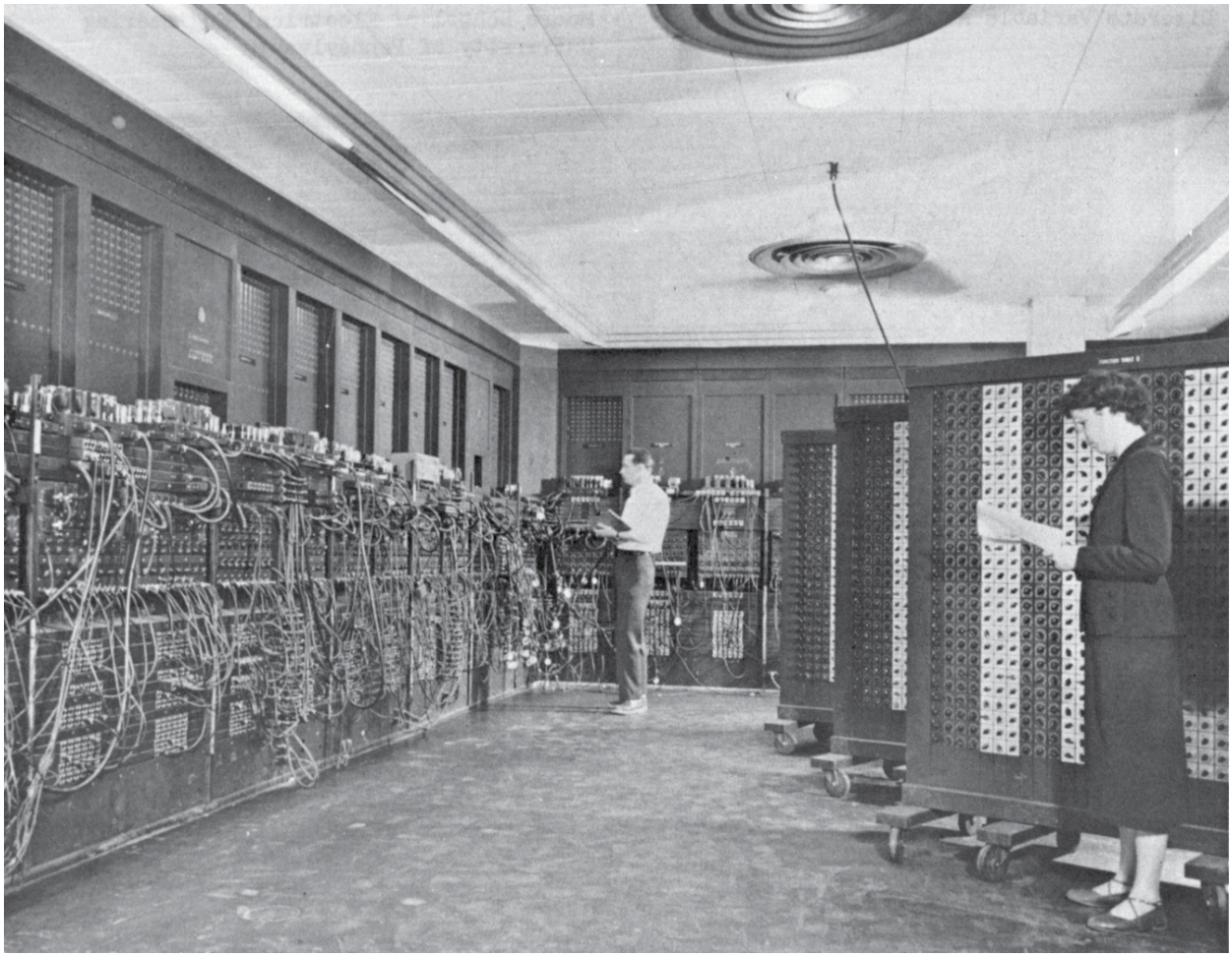
That's a Cas4 molecule. It's similar to the Cas9 molecule that's so useful for CRISPR, but this image works better as an illustration. Note how the blue Cas molecule wraps around the orange string of DNA and wraps the DNA around itself. Each little element of this very large molecule reacts to the individual molecules in the DNA. In fact it senses, electromagnetically, the sequences of DNA molecules. And it reacts to them in preset ways.

A Cas molecule then is a molecular machine. You can think of it as a complicated set of old-fashioned electrical relays: The molecules it reacts to inside the DNA all have positive and negative electrical charges of various levels. And as each exposes its charge to a part of the Cas molecule, a reaction occurs, changing the structure of the Cas molecule, passing the charge along, or both. Once the right charges line up between the DNA and the Cas molecule, Cas will cut the DNA.

In scientific terminology, it is said that a Cas molecule "interrogates and cleaves" DNA, which means precisely the same as "senses and cuts."

In a way, these molecules seem to be intelligent. They're not of course, but they are large and intricate. If a very large set of electrical controls were put together properly, it could do the same things…. and

that's precisely what the first computers were. Here, for example, is a picture of ENIAC, one of the very first computers, operating with relays, vacuum tubes, diodes, and lots of wire:



Our modern computers operate in basically the same way, save that we've replaced the relays with tiny transistors.

So, you can think of the Cas molecule as complicated sets of electro-mechanical controls or as a primitive type of computer. And these Cas molecules are the workhorses of the CRISPR process.

How to Understand Bio-speak

Biology, like many fields, has developed its own terminology. The hard part about biology is that most of the terms are in Latin or Greek. This is a carryover from the European Middle Ages, when an educated man proved his education by speaking, reading, and writing in both Latin and Greek.

Obviously Latin and Greek are no longer used in commerce or literature, but they've hung on in scientific terminology. So, we'll have to deal with that.

There are really just two tricks for dealing with scientific terminology:

1. Don't try to memorize. Rather, try to understand.

2. Don't let yourself get lost. Slow down, stop, and look things up.

Humans aren't built for memorization; that's work for computers.

Humans are built for understanding. If we do that, memorization comes along for the ride. As Boris Sidis, one of the more important early psychologists, wrote:

> Don't try to memorize. Just understand. Then you can't help but memorize.

So, don't let your mind cramp up trying to remember things. Rather, work to understand what these things are and why they act as they do. Go slowly and read the same passage over again when you need to. Get the understanding.

Likewise, don't let your mind become confused with terms you don't understand. I think we've all made the mistake of reading something, not understanding what the author is talking about, but continuing anyway. When we do that, confusion remains in our minds and increases as we go farther along.

So, when you hit a term that you don't know, stop and look it up. (Wikipedia is usually a good tool for this, and there's a glossary at the end of this report.)

And as you look things up, work to understand what the various pieces are and how they work together. The names aren't nearly as important as understanding how these things react with each other.

If you do these things, you'll get through the biological terminology with a minimum of pain and a maximum of understanding.

Now that you understand how these pieces work (and if you don't, read the "Bio-speak" sidebar), let's move on to the specific parts of the CRISPR process:

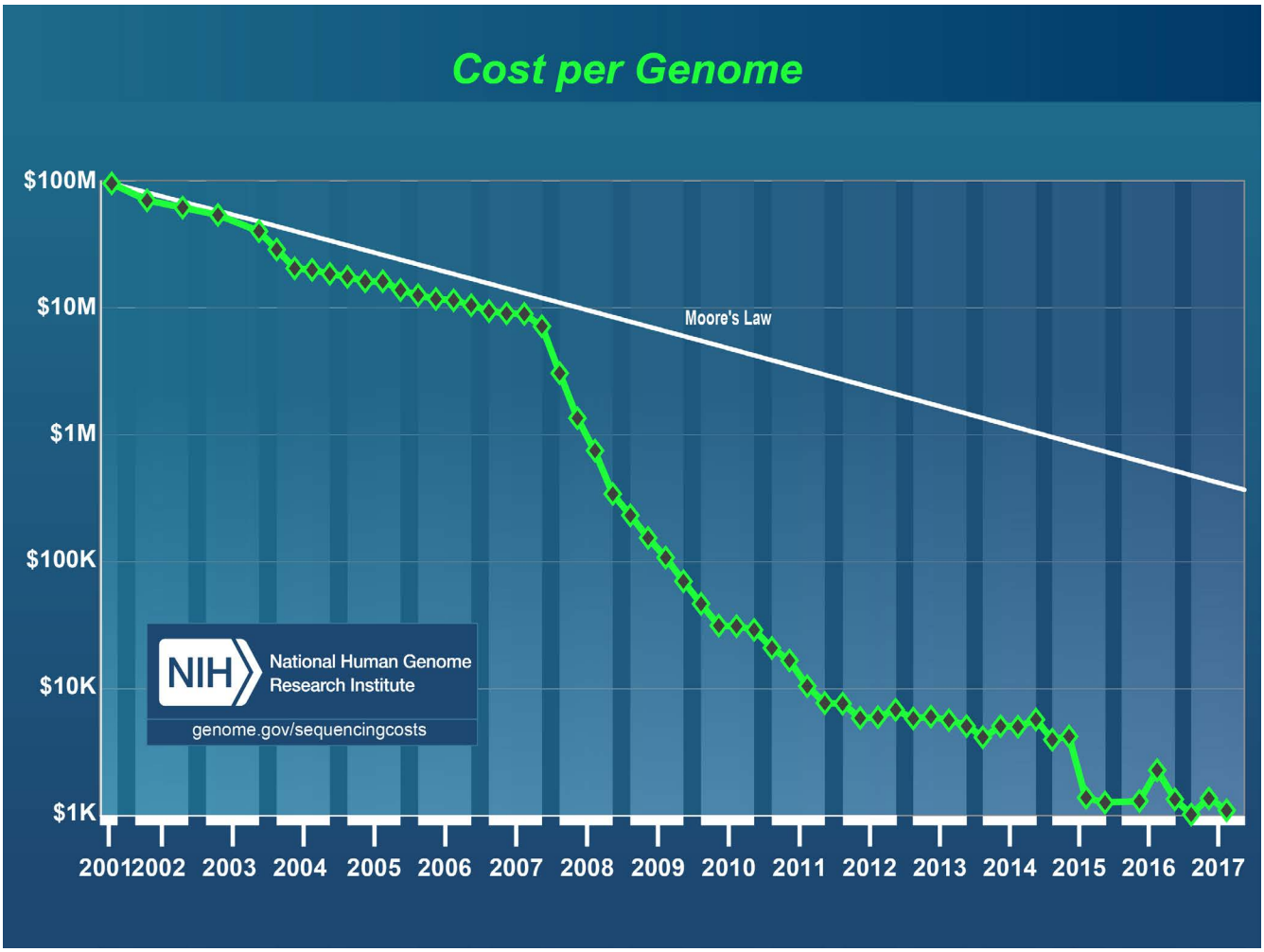
STEP ONE: Identify the DNA we want to change.

This is an obvious first step, and it has to be done precisely and with specialized scientific equipment.

DNA is not entirely identical from one individual to another. It's awfully close, but not fully identical. So, whether human, plant or bacteria, we have to verify that we have the right DNA to cut. This is done with a process called *DNA Sequencing*, which literally reads the DNA. Sequencing can be done in many ways, and it's a large subject in its own right. So, we won't go through it here. If you'd like to get into that area, you can start at Wikipedia.

The exciting thing about DNA sequencing (aside from the fact that it can be done at all) is that its cost is plummeting.

Take a look at the graph below. It shows the historical costs for total human genome sequencing and does so using a *logarithmic scale*. That is, each measurement on the left side of the graph is ten times the one below it. From the bottom, it runs $1,000, $10,000, $100,000, $1 million, $10 million, and $100 million.



(Source: genome.gov)

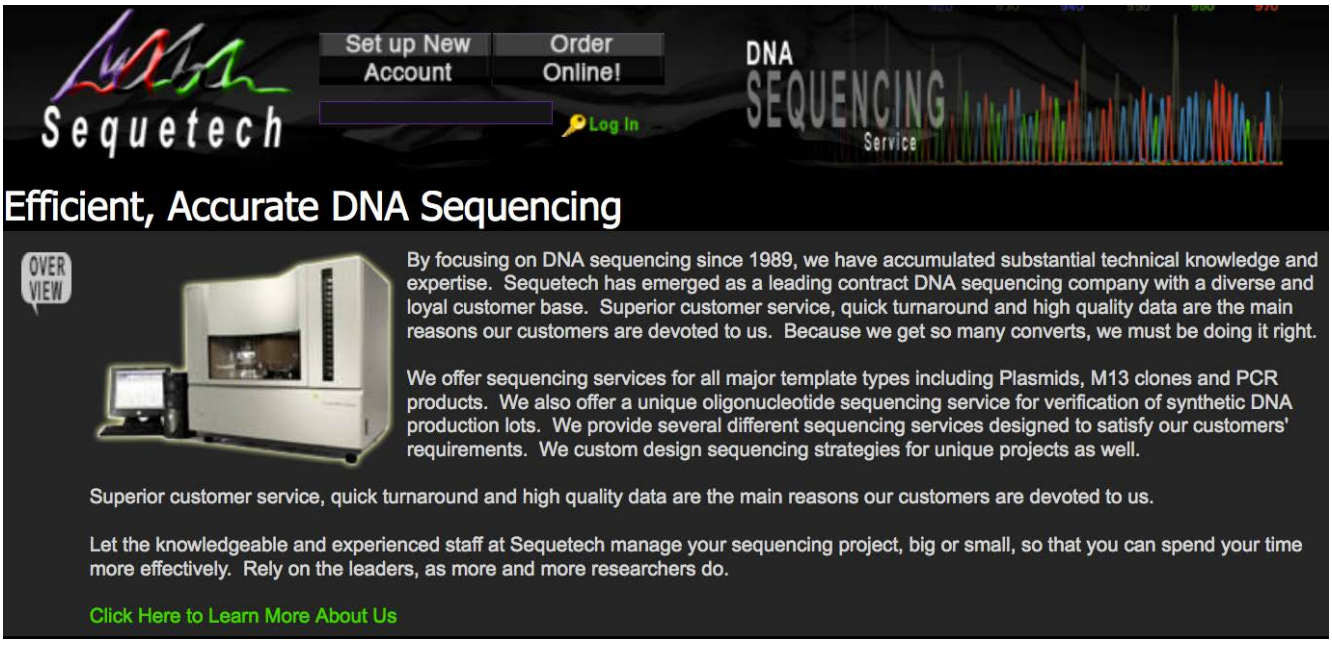
You can see from this graph that the cost of sequencing an entire human genome has fallen from $100 million in 2001 to about $1,000 today. That's a stunning reduction of cost.

The graph, however, shows the cost of sequencing an *entire* human genome, with some 3.2 billion pairs of Gs, Cs, As, and Ts. That much information is never necessary for CRISPR, where we change only a few dozen *base pairs*. (A base pair is one rung on the twisted ladders of DNA and RNA.) That's a very, very small fraction of an entire genome.

Testing a short sequence of DNA is downright cheap these days. You may have seen ads for the popular 23andMe genome test. This service sequences only part of the human genome, but it's enough to determine genetic ancestry and it sells for just $100. A more extensive version of the test, determining specific genetic health factors, sells for $200.

Also note that sequencing a whole genome is unnecessary in nearly all CRISPR applications, where the locations of problem DNA are usually known very precisely. It's only necessary to verify that everything is as we expect it to be.

Here is an advertisement for a DNA sequencing service (chosen almost at random from an internet search), advertising prompt, quality service:



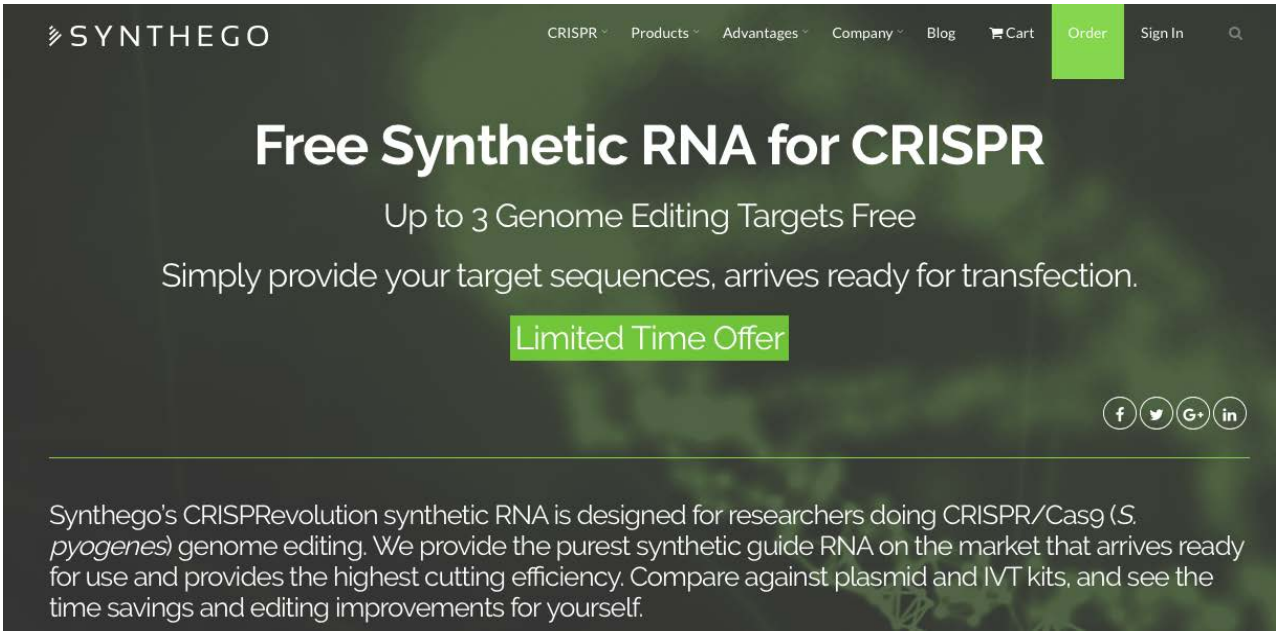
There are many others of course. And since they are competing in a more or less unregulated marketplace, companies like this will improve quality and delivery as a way to improve their profits[4].

STEP TWO: "Guide sequence RNA" and "template DNA" have to be manufactured.

Once the precise segment of DNA is known, special *guide sequences* get the CRISPR-associated molecules to the right place on the DNA, and *template sequences* replace the old DNA that's cut out. Both of these must be manufactured.

[4] If and when regulation comes, this beneficial arrangement will be diminished, and the companies will have to worry more about regulations than quality. And unless they're especially ethical operations, they'll end up competing to pay off legislators, who will then be engaged to write laws and regulations that favor their company and/or hurt their competitors.

But again, this process has become affordable. Consider, for example, this recent advertisement:

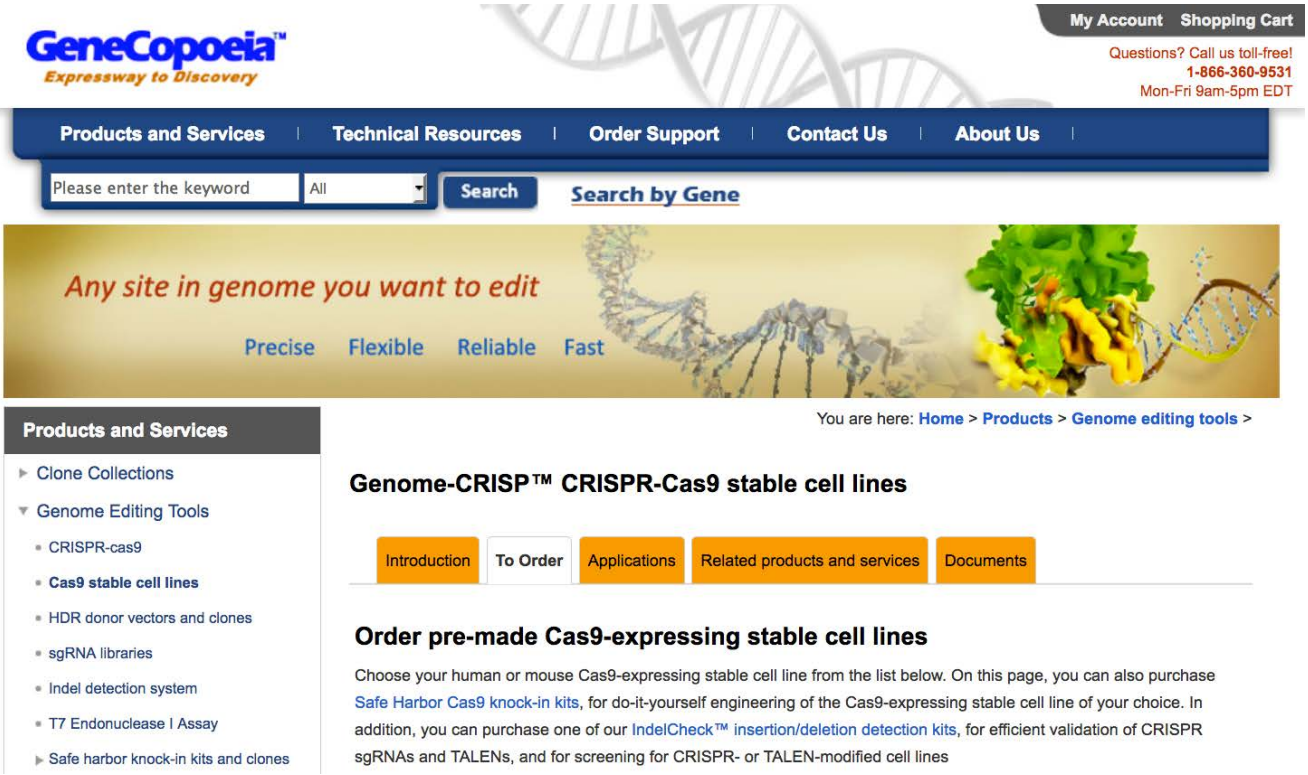


To be sure, this advertisement is a loss leader[5], but it illustrates that the cost of RNA manufacture has fallen dramatically. If the cost weren't low, a loss leader would hardly be possible.

Presently, the cost of manufacturing a small RNA sequence is in the range of $30.

STEP THREE: Get the appropriate Cas protein molecules.

Here is an advertisement from a Cas supplier (again chosen almost at random from an internet search):



[5] Advertising a product below cost to stimulate other sales.

Prices run a little above $1,000, but once you have your Cas molecules, you can probably produce more of them on your own.

STEP FOUR: All the pieces have to be put inside the targeted cells.

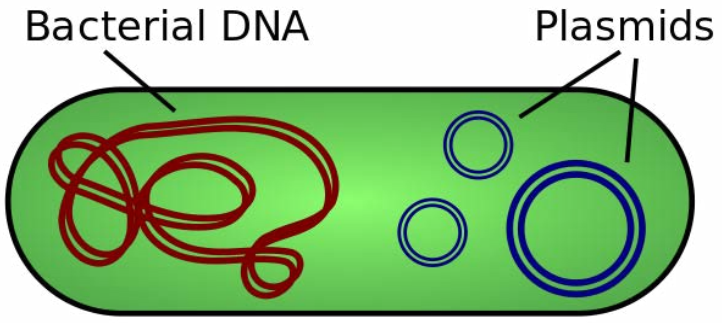
This is actually the hard part. Getting all of these molecules past cell membranes[6] can be complicated. For starters, cell membranes vary a lot from one type of cell to another. And human cell membranes, which are far more complex than those of lower lifeforms, make this particularly difficult.

This process of getting things past cellular membranes is called *transfection*[7]. You'll also see the term *permeable* applied to cell membranes. A permeable cell membrane is one that can be penetrated easily, and *permeability* refers to the ease with which this can be done.

A cell which is ready to be transfected will often be called *competent*. (If not ready to be transfected, it will be called *incompetent* or *not competent*.)

Several methods are used to transfect cells, that is, to get past the cell membranes and put these chemicals into the interiors of the cells:

> **Custom-designed viruses.** This method, while not used very often today, will almost certainly become the best way in the future. Viruses, as you saw in the illustrations above, are almost perfect "DNA inserting micro-machines."
>
> As harmful as viruses have been to mankind, they can be that beneficial and more, provided we learn how to tame them and use them… and we're well on our way.
>
> **Plasmids.** *Plasmids* are rings of DNA, and they are very common in living cells. While the primary strings of DNA carry all the cell's genetic information for normal conditions, plasmids (which are much smaller) carry supplemental information to be used in particular situations.
>
> What makes plasmids especially useful for CRISPR is that they can pass through many cell membranes fairly easily. The illustration below shows what plasmids look like in a bacteria cell.



(Source: Wikipedia)

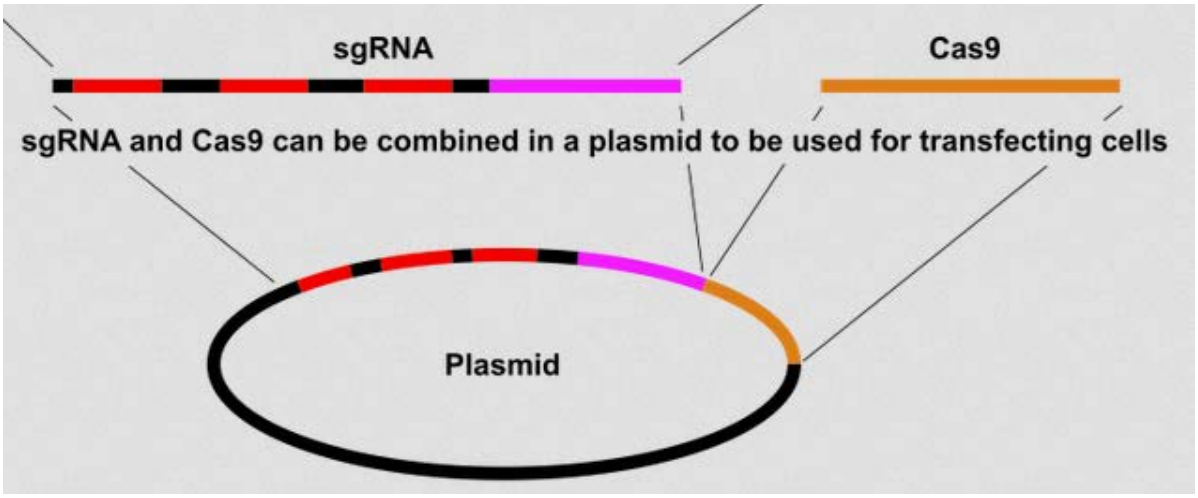
> Specially constructed plasmids are useful for getting CRISPR components through cell membranes.

---

6 If you went to school around the time I did, you'll remember the term *cell walls*. These days, however, that term is restricted to cells that have particularly hard exteriors, such as plant cells. For everything else, *cell membranes* or *cellular membranes* are the correct terms. And these really are better terms. Human cells, for example, have very complex exteriors, more like a skin than anything hard and smooth..

7 For viruses, it's called *transduction*.

This illustration shows how the components are wrapped together, making a plasmid ring. (*sgRNA* on the drawing is simply the *guide RNA*.)



(Source: Wikipedia)

There are many sources for plasmids, as you can see in this randomly chosen ad:

addgene
The nonprofit plasmid repository

Login | Create Account

Search for plasmids

Find Plasmids | Deposit Plasmids | How to Order | Plasmid Reference | About Addgene

Genome Engineering / CRISPR Plasmids

CRISPR/Cas9 Plasmids and Resources

Addgene is working with the leading scientists in the field to assemble the reagents and information you need to use the CRISPR/Cas9 technology in your own lab. Browse plasmids below or check out our CRISPR resources on how to start using CRISPR in your lab.

Addgene CRISPR Resources

- CRISPR Guide: Essential background information on CRISPR/Cas9 and the basics for planning your first CRISPR experiment.
- CRISPR Resources: Browse depositor protocols, find software for gRNA design and deep sequencing analysis, discover links to CRISPR forums, and more.
- CRISPR Pooled Libraries: Find more information about using gRNA pooled libraries and browse our current list of all pooled libraries.
- CRISPR History: Learn how CRISPR/Cas9 was modified from a bacterial immune system to a revolutionary tool for genome engineering.
- Validated gRNA Sequences: Search or deposit gRNA sequences that have been described in peer reviewed publications.
- Blog Posts: Want the latest news on CRISPR? Experts cover CRISPR/Cas9 topics on Addgene's blog.

Browse CRISPR Plasmids by Function

Cut — Wild type Cas9 efficiently generates double strand breaks (DSBs) at sequences homologous to co-expressed gRNA.

Nick — A mutated "nickase" version of the Cas9 enzyme generates a single-strand DNA break (Nick), instead of a double-strand DNA break (Cut).

You may also like...

CRISPR Guide
gRNA Design Tools
Depositor Protocols
Genome Engineering Kits

CRISPR Depositing Labs

Michalis Averof
Gang Bao
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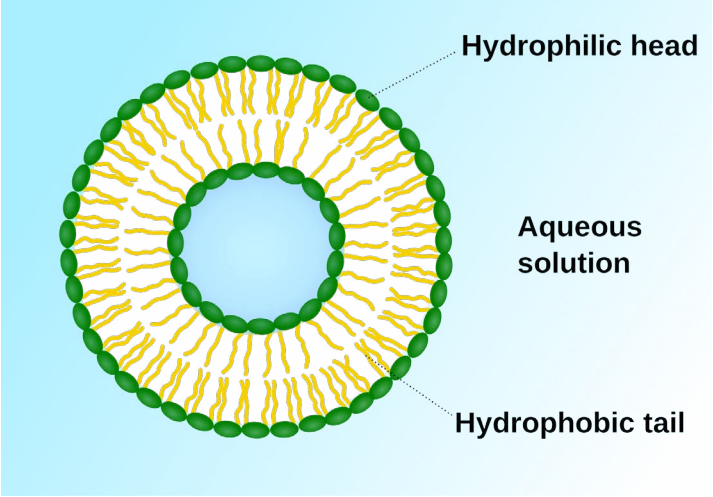
Chase Beisel
Mario de Bono
Mike Boxem
Marc Bühler
Simon Bullock
John Calarco
Jamie Cate
Qi-Jun Chen

Electrical stimulation of the cell membranes. This method, properly termed *electroporation*, applies an *electrical field* (that is, a *voltage*) to cells. This loosens up the cell membranes ("increases their permeability"), allowing CRISPR materials into the cell.

This is a laboratory technique and uses electrical fields in the range of 1,000 volts to 1,500 volts, exposing the cells to between 250 and 750 volts per centimeter. That's enough to open the membranes but not enough to cause significant damage.

Liposomes. Liposomes are small balloon-like structures inside of cells. They are filled with various fluids and encased with a double layer made of a waxy substance. (Technically this is called a *lipid bilayer*… lipids being the waxy substance and bilayer meaning two-layered.) Here's what they look like:



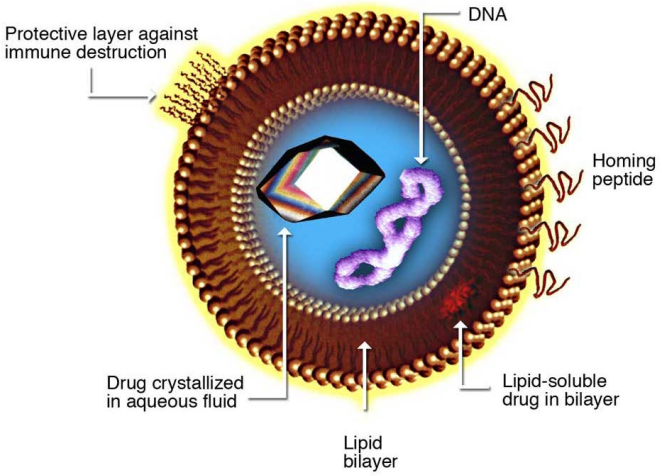
(Source: Wikipedia)

Hydrophobic, by the way, means "dislikes (or repels) water," and *hydrophilic* means "likes water."

Aqueous, as you may know, means *water-based*.

These tiny balloons are very good at getting things inside a cell (*transfecting* it). A liposome designed for transfection looks like this:

Liposome for Drug Delivery

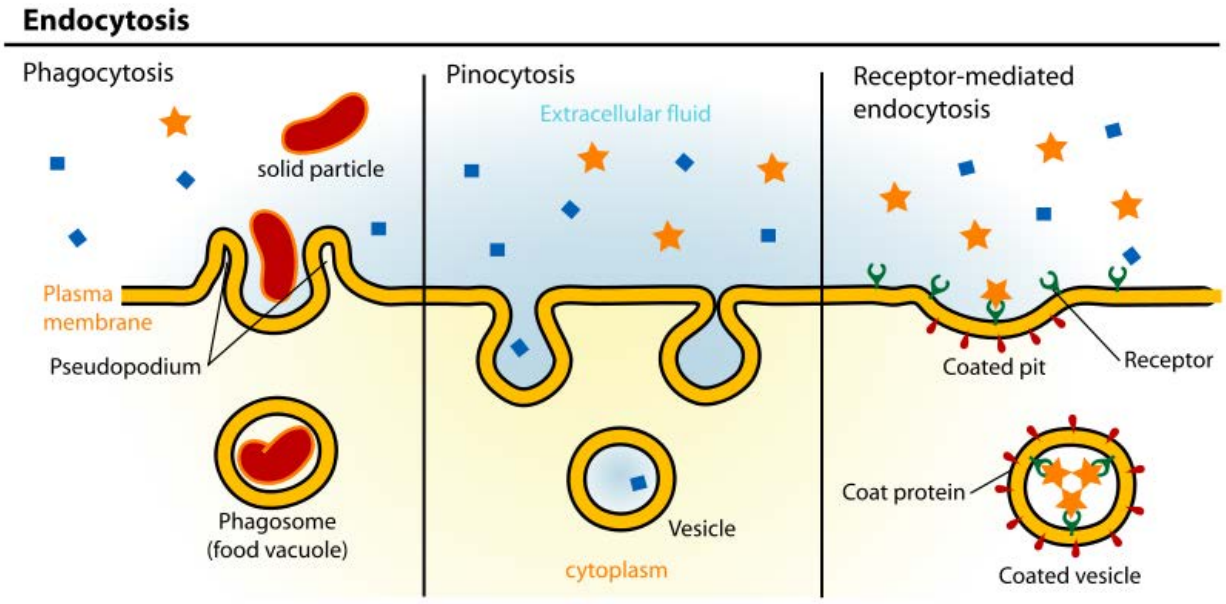


(Source: Wikipedia)

The *homing peptides* noted in the drawing are special protein-like chemicals (the *peptides*) that help the liposome reach its destination (the *homing* – finding home – function). As astonishing as it is that so many of these tiny things have such intricate sensing and locating systems, we see this consistently in biology. And it really is amazing.

Polyethylenimine. *Polyethylenimine* is an *ionic* chemical, "ionic" meaning that it has electrically positive and negative sides. In other words, it carries its own small electrical charge. (As opposed to the *electroporation* method we mentioned above, which uses an *externally* applied voltage.)

Polyethylenimine works for CRISPR by giving its electrical charges to the CRISPR-associated molecules. Many cellular membranes will respond to those charged molecules by "swallowing" them. The process looks like this:



(Source: Wikipedia)

Endocytosis simply means "into the cell." Its opposite term is *exocytosis*, which means "out of the cell."

A *vacuole* is simply a closed compartment within a cell. A *vesicle* is the same thing (the liposomes we covered above are types of vesicles) but structured a bit differently.

A *pseudopodium* is precisely what it looks like – a temporary projection of the cell. (In Greek it means "false foot.")

I think you can see from the diagram why "swallowing" the molecule is a good description. The terms used in the diagram, however, are *phagocytosis* ("cell eating") and *pinocytosis* ("cell drinking").

Transformation buffers. Special chemical mixtures are used to open cell membranes and allow CRISPR-associated molecules to get inside. These are called *transformation buffers* or *transformation mixes*. This was the method we used at the CRISPR party I mentioned in the Forward to this report.

The usual chemicals used for CRISPR transformation mixes are DMSO, calcium chloride (aka road salt), and polyethelene glycol (also referred to as *PEG* and used in skin creams). This is a rapidly evolving field. But again, it simply involves surrounding the cells we want to work on with simple chemicals. The hard parts are knowing which chemicals to use and getting them to the right places.

None of these methods are particularly expensive to use[8], but they have to be applied carefully, depending on the type of cell, whether it will be used in living tissue (*in vivo*, meaning "in life") or in a laboratory (*in vitro*, meaning "in glass").

STEP FIVE: Let the CRISPR process work.

CRISPR needs time to work. The various molecules must be delivered to the cell, and then they need time to react. And the cells need time to recover.

In our CRISPR experiment, it took some 12–24 hours for the process to work, then another few days before we had visible bacterial growth. (We could have seen it sooner with a microscope.)

In most cases, the results of the CRISPR process can be seen in a few days.

CRISPR has very high effectiveness rates. The usual range is that it works between 75% and 99% of the time. That 75% figure, however, typically relates to things like bacteria, meaning that each bacterium has a 75% chance of being altered as expected. But since you'll nearly always be dealing with millions or billions of bacteria, you can count on success; 75% of millions is still millions.

STEP SIX: Verify that it worked.

Verifying that your work was effective is part of every scientific process. Even when you can see positive results, it's important to measure those results. Without good measurements, improving the process is much harder.

There are a number of techniques used to verify that a CRISPR process has worked. Which method to use depends upon the process and the practitioner. One of the more interesting methods involves *optogenetic switches*, which give the finished cell extra pieces of DNA that absorb fluorescent dyes. You can literally see the cell glow.

Turning Genes Off

We've passed over one important application of CRISPR, that of turning specific genes off. (*Genes* are segments of DNA.)

Normally, CRISPR systems cut the DNA they're targeting. However, there are ways to prevent the cutting of the DNA.

So, to turn genes off, we get the proper CRISPR-associated molecules to their destination but without the cutting feature. And so, the Cas molecule binds itself to the desired segment of DNA and remains in place. By doing this, it blocks that gene (that segment of DNA) from expressing itself.

To be a bit more precise, locking a Cas molecule in place prevents that segment of DNA from being read by the cellular mechanism (RNA) that copies the DNA code.

Without that information, the cell cannot build proteins from it (which is what cells do with DNA). If those proteins are never built, entire processes are prevented from happening. This is, for example, how obesity genes can be turned off.

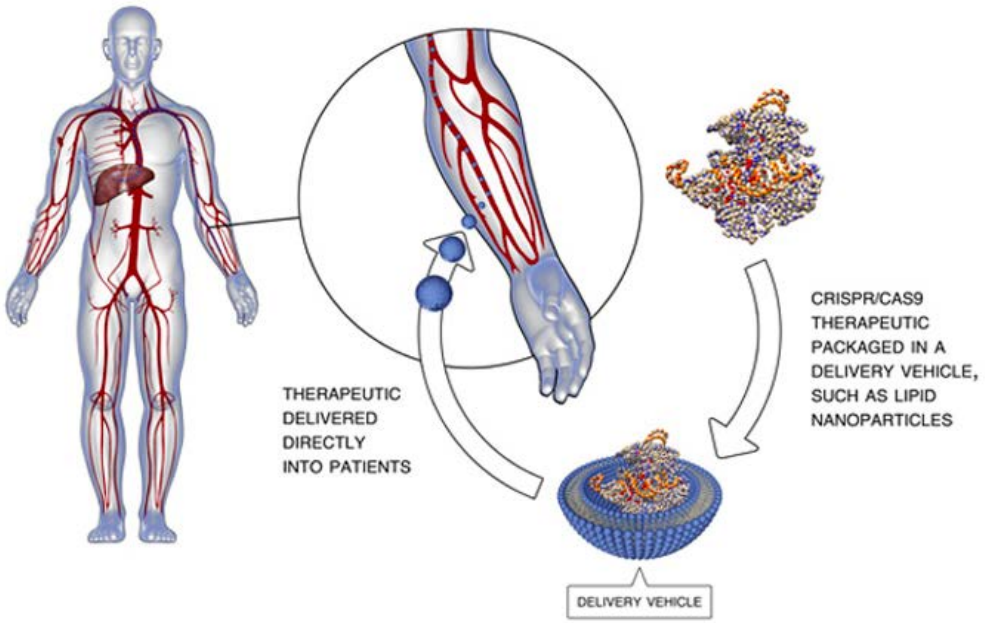
[8] Some of them have been expensive to develop.

Delivery into Large Organisms

Thus far we've discussed the delivery of CRISPR to individual cells, but in many cases we'll want to deliver the CRISPR system to cells inside a fully grown and functioning organism. Dealing with a full-grown human, for example, is more complicated than dealing with bacteria.

To deliver CRISPR materials into a full organism, there are two primary methods: *In vivo* – in the live body – or *ex vivo* – outside the living body.

In vivo delivery is illustrated here:

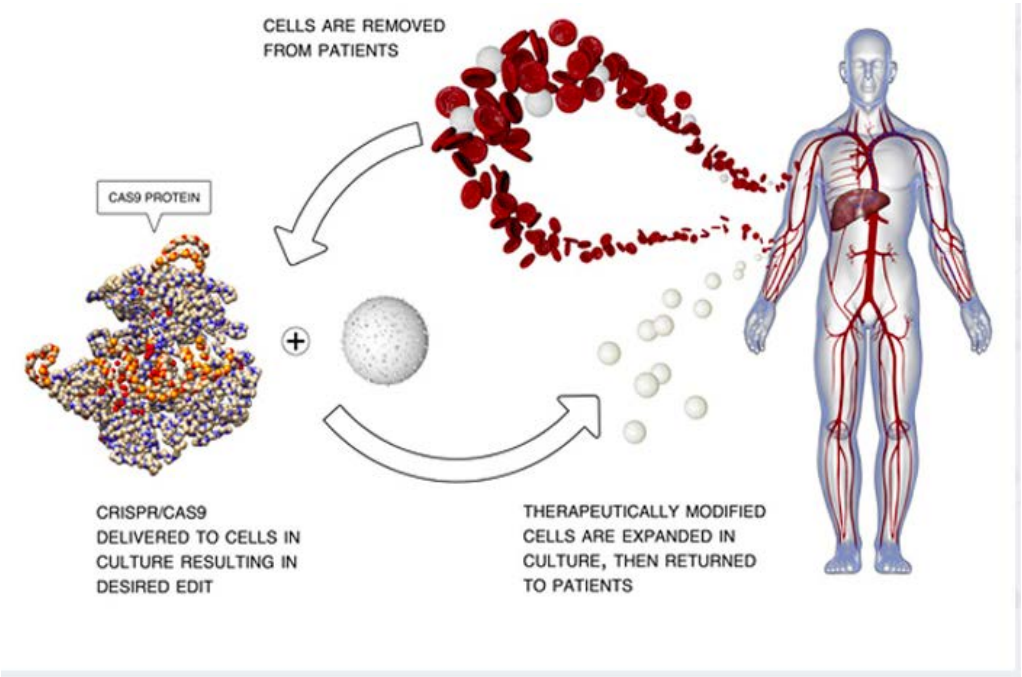


(Source: *Exponential Tech Newsletter*)

The "lipid nanoparticles" noted in the illustration would be the liposomes we covered earlier. In some cases these particles can be injected directly into the bloodstream. In others, the particles can be inhaled.

What is likely to become the primary method, however, is to use pre-engineered viruses to inject CRISPR materials (probably as *plasmids*, rings of DNA) into the cells.

Ex vivo delivery is illustrated here:



(Source: *Exponential Tech Newsletter*)

Ex vivo involves removing cells, altering them in a lab, and then returning them to the living organism.

Blood cells, shown in the illustration, are particularly easy to remove and return, but other types of cells can be removed also. And this can be done repeatedly if an entire organ (which can't be removed) must be treated.

Where the drawing above notes CRISPR-associated molecules "delivered to cells in culture," it's simply referring to them being modified in a laboratory. "In culture" is more or less the same as "in a test tube" or "on a plate."

Where We Are Now

Having gone through the basics of CRISPR's operation, I'd like to take a look at where we are currently[9] and where we seem to be going. And I'll begin with where we're going.

I've discussed this with CRISPR practitioners and researchers, and all of them agree that there are no apparent limits to this technology. Certainly some applications will be harder than others, and some of them are very hard at the present time, but those difficulties involve things like our Step 4 above, getting all the right pieces to the right places. Effective treatments may also require repetition, getting CRISPR materials into a mature organism enough times to affect a sufficient number of cells. (Imagine fixing a damaged human liver, composed of a billion cells. Affecting enough of them couldn't be accomplished with a single treatment… at least not yet.)

But difficulties aside, there doesn't seem to be any DNA that CRISPR couldn't edit. Perhaps some essential limitation will appear as we go, but thus far the only limitations we see are associated with particular applications, not with the DNA modification itself.

To make this point, below is a photo of two common beagles, one of which (on the left) has been genetically altered to have roughly twice the muscle mass of the other. This was accomplished merely by deleting the DNA instructions for building a protein called myostatin.

Source: *Science Magazine*

In another recent experiment, CRISPR restored production of a protein called dystrophin in mice, *curing their muscular dystrophy*.

[9] December 2017.

In a somewhat more mundane experiment, mushrooms that don't turn brown when sliced were developed (below), simply by removing the segment of their DNA that was responsible for the usual browning. Interestingly enough, this use escaped the US Department of Agriculture's regulatory processes, because no foreign DNA (or viruses) was added to the mushrooms. Only a segment of DNA was removed.

In another case hornless cows were created. This is actually an important thing for dairy farmers, as breading out the horns (which occasionally injure or kill other animals) in milk cows was very difficult to accomplish via crossbreeding.

Dalmatian dogs without the serious (and painful) uric acid issues common to the species have been bred with CRISPR, although the breeder has been warned not to sell them (or else be punished) by the US Food and Drug Administration.

All of these are things that have already been done, and it's highly likely that I've missed many dramatic cases.

Now let's move on to things that *can* be done:

- As mentioned earlier, more than 6,000 diseases are now known to be caused by genetic mutations, and there is no approved therapy or treatment for 95% of them. *All of these* can be cured with CRISPR, though some will be limited by side-effects. And as we noted earlier, some applications may be difficult.
- Alzheimer's disease can almost certainly be prevented.
- Down's syndrome can almost certainly be prevented.
- Allergens in peanuts can almost certainly be edited out.
- Sarah Richardson, a researcher at Ignition Genomics, says,

> Anything that can be polymerized to make plastic – succinic, fumaric, and malic acids – would be top of my list [of chemicals to develop]. They could be swapped in to make nylon and polyurethanes that are currently made entirely from benzene made from petrochemicals.

- A team at the University of California Riverside has been working with CRISPR to manipulate a type of yeast that transforms sugars into hydrocarbons. They hope to create the necessary building blocks for plastics, adhesives, and fragrances (rather than using oil-based processes). Eventually, they'd like to create new fuels.
- Any and all plants can be edited, for any number of uses. Crops that grow in a wider variety of locations and resist diseases are just a first thought. For every plant used in any type of industrial or medical process, important (and often game-changing) modifications are not just possible, but nearly certain over time.
- Every type of animal genome can be edited. Malaria-resistant mosquitoes are already in development, but again, this is merely a first case. Animals from which we get food can be modified – to produce leaner meat, better milk, more and better honey, etc. – in an immense number of ways.
- Microorganisms can be edited to do anything from improving industrial processes to breaking down plastics (already under development) to growing who knows what.

You can see from this list that CRISPR modification of organisms is a gigantic field and will be developing for decades at least.

And while it's certainly fitting for us to focus on the human health aspects of this technology, we shouldn't forget the industrial and commercial aspects. Aside from smelting and forming metals, nearly everything we do involves organisms as central components. And from now on, all of those organisms can be customized… easily, cheaply, and in unlimited quantities. That will affect nearly everything and in ways we can't yet imagine.

The opportunities here are stunning, and the scope is larger than we can yet see.

The Coming Storm

You'd think people would heartily welcome miracles, but that really isn't the case. Anything big enough to be called "miracle" is big enough to shake things up and displace the psychological supports that people lean upon. And it's all too easy to make anything new sound terrifying; humans have a weakness for that kind of manipulation.

On top of that, there are a lot of big, entrenched interests that stand to be hurt by CRISPR. What happens to the cancer industries (which are many and gigantic) if most cancers can be eliminated? Will these people simply walk away from their jobs, their retirement packages, and their limos? Or will they, rather, find ways to make CRISPR look dangerous?

I do hope for many scientists to join the CRISPR revolution, but I also expect that their bosses – the ones who keep politicians awash in donations – will find one scary possibility after another, paying the aforesaid politicians to open hearings, pushing newspapers and news channels to cover the horror stories and in general to scare the populace in any way they can.

So, one way or another (and probably in several), fear is coming. That being so, let's jump right into the big fear and answer it directly:

> *Fear:* People could use this to create freaks and monsters!
>
> *Response:* Doubtless some sick persons will do that. (There are always a few of them around.) But the worst uses will be pursued by the same people who make nerve gas and stockpile anthrax.

The larger dangers, truth be told, come from "authorized" people who make "approved" weapons, not from the garage tinkerer.

It is important to understand that "authorized" people are insulated from the results they create, while the tinkerer confronts them up close and personally. Thus, she or he becomes a more responsible actor.

The truth, however, is that CRISPR *could* be used to do bad things, just as any tool can be used badly. You can kill with a kitchen knife or a hammer, but those things don't bother people, since there's nothing new about them. A fear-seller must seize upon *new* threats and must cultivate *imagined* fears.

And imagined fears will be the primary tool of CRISPR's opponents: "This could happen!" and so on. Such fears are infinite, and so we can expect a long chain of them being thrown at us… while moving silently past all the good CRISPR can do.

And we should expect a few experiments to go badly. But sad as that may be, it's not the hopeless problem it may seem at first. One of the beautiful things about CRISPR is that more or less anything we *do* can be just as easily *undone*. If editing a particular gene produces a bad result, we can change it back with a reversed CRISPR process. Doubtless there will be some loss of life somewhere along the way, but despite what fear-mongering politicians and news readers may say, accidents are part of every new venture. We avoid them as best we can, but all big new technologies carry risks.

Now, please consider the genetic manipulations we already undertake without giving them a second thought:

- We've been crossbreeding plants for as long as we've purposely grown plants. Every time we do this, we are hacking genomes; we're just doing it slowly and haphazardly.

- The same goes for every case of breeding animals, as we've been doing from time immemorial.
- We hack genomes even when we pick mates. We prefer mates with certain characteristics, most of which (whether health centered or character centered) have a strong genetic component.

In all of these cases we are editing genomes. But these things don't scare us – because we've always done them.

Another factor in this is a false standard of perfection. As soon as something goes wrong with a CRISPR process, some fear merchant will be screaming about it, utterly ignoring the fact that CRISPR is saving thousands of lives and that "authorized" medical practices fail to save thousands of people every day. These are simply appeals to human weakness and the ridiculous assumption that anything new must be perfect or else be thrown out.

Big media of course can be counted upon to portray things the other way around, and it appears that they're already under way. It was reported in 2016 that NBC was working on a new show called *C.R.I.S.P.R.*, involving Jennifer Lopez. Each episode of the show, said the *Hollywood Reporter*, "will explore a bio-attack and crime – from a genetic assassination attempt on the president to the framing of an unborn child for murder."

So, the first attempts to scare people away from CRISPR have begun. Where they will go is unclear, but the Western news networks (and a lot of entertainment) feed on fear. As they used to say, "Good news doesn't sell papers." And the entertainment corps have sold themselves fully to that concept, as have their political and deep state partners.

Those of us who care about CRISPR should expect such assaults and be prepared to answer them. The better the fear storm works, the more CRISPR will be taken from us. The more people see through the fear, the better we'll be able to thrive with it.

Gene Drives

Some of the early CRISPR fears have centered around what are called *gene drives*. Gene drives are nothing more than editing the DNA of an embryo. Once that's done, the entire organism will carry the edited DNA and, by the usual reproductive processes, pass that adaptation to its offspring.

This is simply an application of genetic engineering, and even though it was fairly obvious that this could be done with CRISPR, it was proven to work in 2015 in both yeast and fruit flies and then in human embryos.

This gene drive (which you may also see described as *modifying a germline*) is able to permanently modify an organism, and that has triggered a lot of scary what-ifs from the control-loving class.

The Center for Genetics and Society in Berkeley, California, has gone out of their way to oppose CRISPR for any research on embryos. This desire of theirs has been backed up, not by facts, but by the usual fear and implying that someone like them should oversee CRISPR research. And the ability to control research is real power, especially in this area.

You can see the delivery of fear (or at least I do) in the organization's post on CRISPR:

> Human germline modification has for many years been widely considered off-limits, for both safety and social reasons. It is formally prohibited in more than 40 countries.

Notice that they never specify *why*. They appeal to lot of authority: "considered off-limits… formally prohibited in more than 40 countries." They do mention "safety reasons," but they never tell us what they are. They claim "social reasons" as well, but that has no clear meaning at all.

They continue:

> [T]o create genetically modified people… raises grave safety, social, and ethical concerns. These range from the prospect of irreversible harms to the health of future children and generations, to concerns about opening the door to new forms of social inequality, discrimination, and conflict.

This passage, full of implications, still lacks anything specific. Consider:

- They mention "ethical concerns," but *whose* ethical concerns? Who gets to be the ethics god? Them?
- "irreversible harms to the health of future children and generations" is simply fear in disguise. The purpose of editing is to eliminate disease. And as we noted earlier (and which these people either knew or should have known), CRISPR changes are reversible.
- "concerns about opening the door to new forms of social inequality, discrimination, and conflict." Being healthy will make us go to war? As for social inequality, do they really want to ration this out, deciding which family is "unequal" enough to be allowed treatment? That's what they're implying.

This letter is a lesson in elitist fear-mongering. And there's nothing specific in it.

As more of these come along, please remember to stop, get a written version of their fancy statements, and break them down piece by piece. It's an important skill to develop.

And make no mistake, groups like these want to decide which of our children can be treated and which can't. Don't let them lord it over human health.

A far more rational opinion, even if reserved, was expressed by the *Economist* in August of 2015:

> However much [healthy people] worry about the nefarious applications of gene editing, the needs of the sick will continue to drive science and medicine forward – as they should.

Projections

As CRISPR moves into the world, all the theoretical science needs to be applied, and that will require products and services, in other words, businesses. That requires examination as well. We'll start at the corporate and investment level and work our way forward from there.

Investments

CRISPR has become the subject of strong corporate interest. And it has spawned a major patent war. This war will probably not be decided until late 2018 and quite possibly later. The claims and counter claims are many and complex. What is fairly clear, however, is that there are five major corporate players:

- **Editas Medicine** (NASDAQ:EDIT), backed by Google Ventures, Fidelity, Khosla Ventures, Juno Therapeutice, and bng0.
- **Crispr Therapeutics** (NASDAQ:CRSP), backed by Mission Bay Capital, 5 Prime Ventures, and Novartis.
- **Intellia Therapeutics** (NASDAQ:NTLA), backed by New Enterprise Associates, Versant Ventures, Celgene, and Bayer.
- **Caribou Biosciences** (Held privately), backed by Atlas Venture, Fidelity, Janus Capital, Novartis, and OrbiMed Advisors.
- **ERS Genomics** (Held privately), backed by Emmanuel Charpentier and Bayer.

The play on these is to buy most or all of them with stops not far below major support levels. One or more of them will go wild once the patent war ends, but the rest of them are also likely to rise some time following. After all, there is far, far too much CRISPR business for any one company to handle, even for giants like Bayer and Novartis. Analysts expect, and I think reasonably, that all the major players – even the losers in the patent wars – will make licensing agreements soon enough; it's in everyone's interests.

The risk in investing in these firms, as I see it, is three-fold:

1. One or more of the companies will fail under the strain of the patent fights, their assets being purchased cheaply by others before the run up in price begins.
2. Governments and their regulators will shut down CRISPR treatments in the name of safety. This risk *should* be offset by the fact that many big players are involved in this and that they'll make sure the right senators make the right committee votes at the right times to prevent that from happening. But in the face of a large enough fear campaign, even that may fail. And if it looks like CRISPR will derail too many cash-cow medical treatments, the mega-corps may simply let CRISPR be regulated away… or at least mostly away.
3. Systemic risk: that markets will fail and investment houses close. Given the current debt situation nearly everywhere, this must be considered a possibility, even if not an imminent one. Please remember that shares held by a broker are legally owned by the broker and can be lost by the broker. This is why some people demand stock certificates directly from the companies they invest in.

There are, as well, numerous side businesses to CRISPR, as we noted and illustrated earlier in the report.

There are enormous areas of opportunity.

The Dark Scenarios

Humans have a real problem with over-focusing on darkness. Still, bad things do happen. So, while not obsessing on the bad, we should keep our eyes open to things that move in an ugly direction. (And then go back to building a world around our abilities, not around our fears.)

That said, here are the dark scenarios:

1. The patent battles that are currently under way result in a single winner (whose stock price will go through the roof), but outside-the-patent versions of CRISPR keep coming (more on that below), and armies of lawyers are kept busy (and legislatures awash in "donations") for a long time. With regulatory control maintained, Big Pharma will release CRISPR cures drip by drip, over decades. They will ignore the people who will unnecessarily suffer and die along the way. "It wasn't approved" will be the mantra, much like "I was only following orders" used to be.
2. The regulatory agencies, unable to keep up with all the small CRISPR operators, will turn to the FBI (and similar agencies) to organize and infiltrate biohacker[10] groups. Then they'll hold widely publicized show trials for a few hackers and send them to jail for ridiculous periods of time, thereby terrorizing other hackers and driving them out of the field[11].
3. In conjunction with #1 above, the elite classes will use CRISPR for their own families. Special clinics, whether secret or not, will operate for their benefit.

The Bright Scenarios

This refers to the general availability of treatments that eliminate human disease and suffering: more treatments reaching the masses faster.

1. Governments and big pharmaceutical companies put the good of humanity first and maximize benefit rather than seeking monopoly-juiced profits. (Hopefully this is at least as likely as the dark scenarios.)
2. Governments get busy with other matters (financial downturns, wars, rebellious populations), and the buyers of legislation have to wait for their enactments. Meanwhile, thousands of people are cured of major diseases, obesity genes are turned off, and the public comes to love CRISPR. That point passed, reigning it back in will be nearly impossible.
3. CRISPR, while regulated to near meaninglessness in the big Western countries, is left mostly alone in places like Costa Rica and Thailand. That way, the people of the West can still get treatment via medical tourism. Even if one country after another is shut down, there are a lot of options: Vietnam and Uruguay might follow, India, Hungary, and Belize after them, and so on.
4. CRISPR routes around censorship. John Gilmore's comment on the internet – that it routes around censorship[12] – hasn't held up over time (the entire infrastructure was captured), but CRISPR could route around censorship.

[10] I should add that when I use the term "hacker," I'm referring to people who use their skills and knowledge to overcome problems, not to malicious and destructive people.

[11] Though it should be noted that other bio-hackers will continue to provide cures in the same ways that marijuana dealers have operated for several decades.

[12] The actual quote was, "The Net interprets censorship as damage and routes around it."

CRISPR-Cpf1, which uses a different "pair of scissors," than Cas9, looks like it will *not* be part of the current patent wars. Only Cas9 is specified in them, and Cpf1 is clearly a different molecule. (It's smaller and simpler.) In fact, Cpf1 will be easier to *transfect* (to get into cells.)

And CRISPR-Cpf1 may be just the first of many successors to Cas9. The lawyers and regulators may have a hard time keeping up. As Feng Zhang, one of the patent holders, says, "I can't even begin to count how many there may be. There really is great diversity that we as a scientific community should go out and explore."

It should also be noted that CRISPR-Cpf1 wasn't found by examining bacteria, but by data mining… by digging through a database of DNA sequences for promising pieces.

5. Not bothering to wait for permission, local CRISPR operators (aka biohackers) start doing things like fixing Alzheimer's disease and turning off genes that control obesity on a neighborhood-by-neighborhood basis. Central information depots open in the darknet. An unregulated CRISPR economy develops, including supplemental services like genome sequencing, RNA synthesis, etc.

   Even if called "outlaws," these people will continue to do business on a person-to-person basis, with cash, with cryptocurrencies and via pseudonymous markets like Open Bazaar. Word gets around ("They cured my husband; maybe they can cure yours too") and soon enough it becomes unstoppable[13].

I prefer number five myself, but any of the above will suffice to help a lot of people.

What Does CRISPR Do to Our Cultures?

Generally, cultures change very slowly. Even when it seems otherwise – as during a "revolution" – a closer examination reveals that the culture had been shifting for a long time, and the revolution was merely a moment when things came to a head.

There is a difference, however, once science gets involved. Science does not depend upon people's feelings. If new scientific abilities offer enough advantages to people, they'll grab at those benefits and drag their psychology along for the ride.

This is precisely what happened during the Industrial Revolution. The new processes and tools (steam engines, iron, farm machinery, mass production) were irresistible. So, people changed their lives, abandoned their ancient ways of living, and dragged themselves into the modern world.

Might something akin to that happen with CRISPR? It's awfully hard to know at this early stage, but there are reasons to think that it might.

Throughout this issue we've mentioned one "irresistible" benefit after another: cancers cured, Alzheimer's and Down's Syndrome prevented, better and cheaper food, better and cheaper chemicals and fuels, materials that allow us to do previously unimagined things on the cheap.

Regardless of the fear storm that rages out of DC and Hollywood, people will ignore it all when it comes down to the choice between keeping the rules and preventing mental retardation in their unborn child. Even a Stalinist outlawing of CRISPR wouldn't stop people from using it in those circumstances, and we'd soon be seeing graffiti like this:

CRISPR Lives! Break the law and heal your neighbor!

[13] The interesting moment then comes when governments offer "standing" to the small operators if they'll accept registration and "a little bit of oversight."

Then there is the concept of changing our physical bodies by ourselves. Suffice it to say that this will not only bother many people, but it will upset a lot of institutionalized ideas, including religions.

Being able to fix ourselves casts us as powerful beings, and all our modern institutions – from governments to religions to corporations – rely upon us feeling small, weak, insecure, and needy. They couldn't exist as they do if we felt confident about ourselves.

And so, CRISPR, by placing us as masters of the physical world, threatens the belief that mankind is a dark, fallen, congenital failure of a species… and the existing powers will soon enough fight to retain those dark and degrading assumptions.

Furthermore, if CRISPR escapes the grasp of the mega-corps, average people will be using it and making choices on how to use it. That spawns individuality and character development. In the best scenarios, we'll see large numbers of people who, embracing science and responsibility, begin to mature and to trust themselves. That would be a big change and very strongly positive.

Specific Opportunities

As we've mentioned, there are an unknown and unknowable number of CRISPR opportunities coming at us. That being so, I'll address them by classes:

At the Official, Corporate Level.

First, obviously, are the investments noted above.

Next are jobs at the big pharmaceutical companies. I don't see those as tremendous opportunities, but some people do.

A better set of opportunities are as suppliers to the giant corps. Dealing with such corporations is not for the faint of heart, but if you're already working in such a field, there are a lot of CRISPR-related opportunities standing in front of you in materials, modifications, processing, research of many kinds (some of which they'll have to farm out), shipping, and much more.

At the Small Business Level.

More or less everything that can be done at the corporate level can also be done at the level of small businesses… and generally faster and more creatively.

Any number of CRISPR-related businesses will be spawned by the new biological and genetic developments we've covered here; there are already dozens at least. Many of these we might expect to be small to mid-sized laboratories and others almost like dental labs, filling orders for customized substances.

Some readers will have experience with small businesses, but for those who do not, a few words on the subject seem worthwhile.

Business is not science; those are two very different things. You can be the best lab technician in the world – the best scientist in the world – but you can still go broke (and quickly) as the operator of a business. It's a very different set of skills.

So, if you haven't run businesses before, get clear that the central virtues of commerce are reliability, honesty, and persistence. What matters in business are things like these:

1. Show up early, every day.
2. Deliver whatever you promise. (The secret is to never promise more than you can deliver.)
3. Listen sympathetically – understand what people mean, not only what they say.
4. Don't let people take advantage of you. Hold your ground: "We said $400, and that's what we expect." Do not become threatening and overly aggressive, but do not back down easily. And if you must back down for something unfair, never do business with that person again.
5. Turn down deals that aren't in your best interest. Say "No." Let some other sucker take the pain.
6. Give people better service and more value than they expect.

These things are not easy; they are hard. It will take you years to learn to do them well. You may also go for years without seeing a serious payoff. Things will go wrong. That's just the way it is: complex, difficult, and sloppy. Business is not like lab work.

Actually, if you haven't run a business of this type before, working six months beforehand at a testing lab (or any kind of lab) would be an excellent idea.

There is one complication to this opportunity, however: You'll have to keep abreast of regulations. That will vary, depending upon where you live and work, but the wild card in all of this is government meddling. You'll have to stay aware of it.

Starting at Home

Getting started in CRISPR isn't hard. As one of our interviewees below says, "Anyone who has the drive can do this. Money and degrees aren't needed… Just a brain, time, and passion."

That said – and please reread the section above on small business – here are the things you'll need to start from scratch:

- Learn to use the equipment. You must be proficient with pipettes, plates, and proper laboratory procedures. If not, your procedures *will* fail. You'll have to work with experienced practitioners before you get started doing anything, and you'll have to practice.
- Remember that the biggest challenge is maintaining a very clean working environment; contamination of your work is a big headache. Always keep an isolated backup.
- Learn the terms. We've given you a good start in this report, but you'll have to be familiar with all of these, as well as others related to your specialty.
- Learn more about the other parts of the process. In changing fields like this one, you're likely to start in one area, switch to another after a few years, and end up in a third or fourth over time. Stay informed so you are able to see and respond to the opportunities that present themselves.
- Spend your free time reading. Nothing can replace reading; if you're not willing to do that, forget this business. Earl Nightingale used to teach that if you'd spend just 30 minutes every day reading on one subject, you'd be an expert in six months. And he was right. So take his advice. The time will pass anyway, and if you do as he said, you'll become an expert.
- Keep good data and keep good notes.
- Publish both your successes and your failures. This may be business, but it's also science, and we need to be serious about that part of it. Share your results and keep the scientific process going.
- Be 100% honest with your clients. Tell them all the risks, as well as the level of those risks.
- Be prepared to be a professional or don't even start. Not merely as good as certified practitioners, but *better*… and *clearly* better. Your processes must be better. Your dealings with patients must be better. If you're not committed to excellence, stay home until you are. Certification mills have no magic that we lack. In fact, they make excellence harder for their students by focusing on memorization, arbitrary rules, and the fear of authority. We, on the other hand, can focus on the real facts and the people involved. We *should* be better.

- Learn to handle your business in ways that won't expose your patients' private information. Learn how to use encryption and use it as your default, learn how to use cryptocurrencies and learn how to use them anonymously. Again, we have to be *better*.
- Spend time developing, verifying, and updating your sources. This field changes all the time. New suppliers will arise continually.
- Remember to do pro bono work. People who are truly poor (not just cheap) need help too, and they need kindness. Stay away from dangerous people (some people are poor for a reason), but do help those who suffer unjustly, those who've had bad luck, and so on. Had your life gone a different way, you might have been in such a situation. Act like an exceptional man or woman and you'll become one.
- Remember that there are hazards. Germany has already been threatening biohackers. Regulators and enforcers in other places are trying to crawl in through back doors, particularly the FBI in the US. Keep your distance and be careful, but live the life *you* want to live. Bless your friends, your neighbors, and the world.

Interviews

David Ishee

I recently had a discussion with David Ishee, a life-long professional dog breeder, who is using CRISPR to improve his dogs. I think you'll also be interested to see his story about repairing the damage done to Dalmatians by excessive breeding, only to be shut down by the US Food and Drug Administration.

David started our discussion by surprising me:

How much do you use CRISPR?

I do use it some, but CRISPR is over hyped honestly. It's a great tool but it's just one tool in a whole toolbox. The real change is that the price of everything is crashing.

CRISPR wouldn't be cheap if DNA still cost what it did in 2007. Synthesis and sequencing costs are the main drivers.

Is there a particular reason they are falling?

Technology, automation, computer power.

Minion systems are doing amazing things. [Whereupon he sent me this link, which I urge you to see.] It fits in your pocket and plugs into your laptop.

Whoa! And does it perform as advertised?

There are limitations and they have issues of course, but I have friends who have them and use them, for whole genome projects.

That's exciting.

Yes, but CRISPR is only one of many new technologies. Some are here and some are on the horizon but very close.

Such as?

One thing is the amazing synthesis costs from Twist Bioscience. I just bought custom DNA for $.09 per base pair. That's by far the cheapest it's ever been. In a few years it'll be pennies per kilobase [one thousand base pairs]. It'll become reasonable to buy whole custom chromosomes. That's why this is all changing. There have been a lot of great ideas that for a long time were just too expensive to be realistic that are now.

And how do you see this all developing?

I can see a lot of ways this could go. It depends on so much and so few players. One disaster or great success… hell, the right story at the right time, and everything shifts.

Shifts how?

Regulations and public opinion are going to be strong players. I can see biotech being regulated into uselessness like in Europe. Or I can see it staying free enough for long enough to get public support.

Someone could make a killer robot in their garage, but no one is trying to regulate robotics. People used to be afraid of killer robots. Now people are hoping in a few years they won't have to fold laundry anymore.

If everyone wants it, no one will stand for shutting it down; if everyone is afraid they won't stand for its freedom.

Have the regulators given you a hard time?

Nothing terrible yet, but they're determined to claim that my dogs are drugs. [Ed: That's not a misprint.] And they changed the rules days after I asked them about my plans to fix a dog disease in a way that wasn't regulated. Talking to them is just going around and around to the same nonsense.

Currently I'm hoping a public commentary campaign we're building can squash this regulation. It's also politically complex with Trump's executive order.

Anything you'd like to add before we close?

Just that things are changing faster than anyone who isn't in the middle of it knows. There are things happening all over the world that people have never seen before. The next few years are going to be an explosion of biotechnology. This will change how we live and who we are, for the better I think.

It's important, though, that we don't let a handful of people decide for all of us how that's going to happen. If these benefits are going to be for the good, then everyone needs to get involved, and we don't need to make regulations so strict that only giant corporations and governments can afford to use this technology. We are all biological; this affects us all and belongs to us all.

For now the biohackers are holding open the door for everyone, but there aren't a lot of us.

Anyone who has the drive can do this. Money and degrees aren't needed to be a biohacker. Just a brain, time, and passion.

Biohacker X

I very much appreciate biohackers. All those I've met have been intelligent, articulate, passionate, and just plain nice people. To give you some flavor for them, here are some snippets from an email exchange with a biohacker whose name we'll leave out:

- I do have a friendly acquaintance who is working on CRISPing mice to remove a certain disease.
- So far it seems like using CRISPR on a friendly virus is the way to upload code to your human cells. I think it will be possible at some point to upload genetic resistance to your immune system using a hacked HIV or something similar that infects the immune system. People who have immunity can get their genome sequenced and then have it packaged in a virus. Actually I bet we could manufacture those immunity sequences using rabbits. (Though this would involve a lot of dead rabbits.) Just my speculations.
- I stayed up for an hour or two last night talking to a guy about how he's going to manufacture an HAC [human artificial chromosome… an actual tool, not speculative woo-woo] and inject it into his fat cells. One of the major takeaways is that the project is speculated to cost $3,000, and he's testing it on a small section of fat cells that can be cut out if something goes wrong.
- Another friend is using a tattoo gun to implant a non-replicating virus under the skin. So you can use the virus to modify certain cells and it may cost only $500 for a vial of "ink."
- A tendon-weakness thing happened to rats that got their myostatin gene completely knocked out… Humans would not have this problem, or to a much lesser degree. So no reason to suspect tendon weakness as a tradeoff in humans via this method. The tradeoff actually is that follistatin blocks signals that cause eggs to be released and sperm to be produced, so the mod is for people who do not want to create biological children. It is expected to have less of a hormonal impact than oral contraception, because there is no estrogen or progesterone added or removed.
- It also turns out, perhaps, the best method for both drug and DNA delivery is a particular subclass of liposomes. Liposomes are micro- to nano-sized phospholipid vacuoles with drugs/DNA inside them. Liposomes seem very easy to make. But most of the research is after 2010, so it's still new. You rub it on your skin and it puts the drugs/DNA directly into your cells and blood without dragging other things along with it (like DMSO, it allows anything that touches your skin to pass in). It's very safe and seemingly very easy to make. And you just rub it on the skin. We're going to start making it this coming week. Wild!
- It's still hard to use CRISPR to directly edit adult human cells, but what you can do is make DNA vaccines. A DNA vaccine is when you find somebody who is immune to a disease (like HIV) and identify what genes confer this trait. Then you can copy those genes into a bacterial plasmid that is designed to code for a minicircle [a circular loop of DNA that has only the human immunity gene on it] and inject the minicircle into your adult human fat cells. These fat cells have an average lifespan of 10 years, and as long as the minicircle is inside the fat cell, they will create the antibody (or the hormone) that you want it to. If you want to pull out the modification, you cut the fat cells out. Your chromosomal DNA is not edited. Minicircles allow you to add "scripts" to your body that produce more of individual hormones or proteins.
- Biohackers will produce a widespread cure for HIV before institutions will… and many more diseases including cancers. This much is proving to be clear.

Josiah Zayner

Josiah Zayner is the owner of a fascinating technology supply company, called The ODIN, and an experienced biohacker. I was very pleased when he took some time to answer my questions.

I'm interested in your webpages at the-odin.com. You sell some very interesting products. Tell me about them.

The ODIN was created to make science and technology research more accessible, with a focus on synthetic biology and genetic design. We believe that smaller groups of people, small labs, or even biohackers and nontraditional scientists on their own can do amazing things if they have access to resources that are normally available only to large, heavily funded labs and companies.

What is your the driving desire behind what you do? We all hope to get paid for our work, but there's very obviously more to The ODIN than just that. What do you hope comes from your efforts?

Our goals are to make synthetic biology and genetic engineering accessible and to unite synthetic biology with everyday life.

Synthetic biology has the ability to transform the world we live in through the eradication of genetic diseases, creation of crops that can survive in arid landscapes, and provision of nutrients to those who desperately need them.

Beyond necessities, synthetic biology can be used to create something beautiful – from flavours we have never tasted before to living art we have never seen.

What CRISPR milestones have you seen?

In 2016, we released a CRISPR kit for general audiences. It was the first-ever consumer genetic design kit and opened the door to modern biotechnology entering the home. Despite the continued success of our CRISPR kit, to us it left something to be desired. Its applications were limited for people without broad scientific knowledge. In 2017, we released new kits that allow people to create something tangible with their genetic design by engineering most any yeast DNA, which can then later be used for brewing or baking and maybe candlestick making.

How did you get involved with biohacking and CRISPR?

I started BioHacking during my Ph.D. in Molecular Biophysics at the University of Chicago, creating The Chromochord in my apartment, the world's first musical instrument that uses engineered protein nanotechnology. After that I received a prestigious fellowship to work with NASA's Synthetic Biology program. There I worked on engineering bacteria and yeast to help humans colonize Mars. I thought it was a dream.

Unfortunately, the government is like everyone says it is: no one gets anything done, and exciting, nontraditional ideas aren't supported. I saw firsthand the lack of availability for people to pursue and answer scientific questions that they found interesting.

So in early 2016, I left to run The ODIN full time by myself, a company that makes science more accessible by providing inexpensive resources to allow anyone to perform cutting edge genetic design at home. Since then, we released the first genetic design kit for home use, a DIY CRISPR kit.

We're in contact with about 25% of people who use the kits – it's a mix between schools, organizations, and people with no real experience with science who are ordering kits to do experiments at home just for the fun of it.

The CRISPR kit comes with example experiments, so you get the kit, do experiments, and learn how the technology works. You can also do more complicated experiments, but to do so requires somebody to go more in depth with how the technology works. It would be like if you ordered an Oculus Rift, and they had one demo game, but to play another, you had to program the game yourself. Our new kits are working to overcome this problem.

On the regulatory side, how do you see CRISPR developing over the next five or 10 years?

It is hard to predict what is going to happen on the regulatory side, especially with the new administration. One thing is that it seems the government is becoming more open to deregulation in spaces that can spur innovation and the economy. Fortunately, it seems that genetic engineering is one of these innovative spaces.

What do you tell someone who just realized how huge this field is and wants to become involved?

If someone wants to get involved I would tell them that they need to do experiments. Learning genetic engineering requires much more physical ability than people imagine. Just as you can't be an expert guitar player by just reading books or watching YouTube videos, the same is said for genetic engineering. Eventually, you need to pick up a pipette, and the sooner the better.

Final Words

So, now that you have a good grounding in the CRISPR technology, I want to ask you a serious question: *How do you want this to play out in the world?*

I ask this because I think it is supremely relevant. We're early enough in the game that you and I as individuals can make major differences in how this goes. Consider…

What if you had been part of the Homebrew Computer Club back in 1975? Out of this group came the designers of Apple computers and many other pioneers. To the right is the front page of one of their early newsletters.

NEWSLETTER

Homebrew Computer Club

Robert Reiling, Editor ☐ Post Office Box 626, Mountain View, CA 94042 ☐ Joel Miller, Staff Writer
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RANDOM DATA
By Robert Reiling

Computer clubs continue to form around the country…E. Brooner would like to have material to help him get started with the "Flathead Computer Society" in the Kalispell area. His Address is P.O. Box 236, Lakeside, Montana 59922.

Did you see the SOL terminal demonstrated by Bob Marsh at the Sept. 1st meeting? An excellent design that will interest hobbyists and commercial users alike. It's available from Processor Technology, 6200 Hollis St., Emeryville, CA 94608. Write them for prices and specifications.

The OSI Systems Journal has been sent to all OSI customers (free—at least for the time being). It's a bi-monthly magazine with plans to go monthly in the future. There are 28 pages in the first issue (August 1976, Vol. 1, No. 1) with a hardware feature covering the OSI 440 Video Graphics System and software, features concerning Tiny BASIC for the 6800 and a Graphics Editor for the 6502. It also includes OSI product and software catalog data. The BASIC is, of course, the 2K Tiny BASIC developed by Tom Pittman. Many of you have met Tom at the Homebrew computer Club meetings. The OSI Systems Journal is a good way to learn more about the OSI computer hardware and software along with helpful user information. The contact address is: The OSI Systems Journal, P.O. Box 134, Hiram, Ohio 44234.

KIM-1 users now have a newsletter. Eric Rehnke is producing the newsletter every 5-8 weeks, MOS Technology, Inc. helped get it started by sending copies to all known KIM owners. The user group, however, is independent of MOS Technology, Inc. The newsletter is devoted to KIM-1 support. Subscriptions are $5.00 for the next six issues. Contact "KIM-1 User Notes," c/o Eric C. Rehnke, Apt. 207, 7656 Broadview Rd., Parma, Ohio 44134.

The BAMUG club has a new contact address. It is BAMUG, c/o Timothy O'Hare, 1211 Santa Clara Ave., Alameda, CA 94501. Write Timothy for club information. I suggest you include a stamped, self-addressed envelope.

Beware of board snatchers! Glenn Ewing reports 11 boards were taken out of his IMSAI computer. The boards are: MPU, 4 RAM-4's, SIO-2, P10-4, PIC-8, PROM-4, IFM and FIB. Glenn suggests you consider providing good security for your computer and associated equipment. In his case the computer was in a locked office which was burglarized. In the event you have information on the above boards, write Lt. Glenn Ewing, Code 62EI, Naval Post Graduate School, Monterey, CA 93940.

For family and friends of people who always wanted to know about computers, but didn't want to ask them, four easy-going classes are available starting Oct. 19th on Tuesdays from 7 to 9 p.m. You can learn how computers work and what they can and can't do. You will also have some of the jargon deciphered, see what you can do with a computer, play some games and learn to program. The cost is $25. Contact the Community Computer Center, 1919 Menalto Ave., Menlo Park, CA 94025, phone (415) 325-4444.

A call for papers in personal computing has been issued by the 1977 National Computer Conference. The conference is scheduled for June 13-16, 1977. I have a few copies of the guidlines if you would like to submit a paper.

The First West Coast Computer Faire will be held April 16 and 17, 1977 at the San Francisco Civic Auditorium. This faire is shaping up rapidly. If you would like to lead a conference or participate in a conference session, please contact me. More information about the Faire is in the accompanying article.☐

THE FIRST WEST COAST COMPUTER FAIRE
A Call For Papers And Participation

The San Francisco Bay Area is finally going to have a major conference and exhibition exclusively concerned with personal and home computing—The First West Coast Computer Faire. And, it promises to be a massive one! It will take place in the largest convention facility in Northern California: The Civic Auditorium in San Francisco. It will be a two-and-a-half day affair, starting on Friday evening and running through Sunday evening, April 15-17.

It is being sponsored by a number of local and regional hobbyist clubs, educational organizations and professional groups. These include:

- The two largest amateur computer organizations in the United States—the Homebrew Computer Club and the Southern California Computer Society
- Both of the Bay Area chapters of the Association Of Computing Machinery—the San Francisco Chapter and the Golden Gate Chapter
- Stanford University's Electrical Engineering Department

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And CRISPR stands where the Homebrew Computer Club did in 1975. Those people *did* set the course of the computer industry, just as we now have the position to set the course for CRISPR's use in the world.

So again I ask: How would you like CRISPR to play out in the world?

This is the choice – the opportunity – that stands before us now. There will be few times in your life where such an opportunity beckons, and CRISPR needs as many good and decent people as we can find to move it in the right direction.

* * * * *

Resources

Here are several CRISPR resources. We can't guarantee anything, but this is a good list from which to start:

Oxford Nanopore Technologies

Helix Works

The ODIN

Twist Bioscience

AddGene

GenScript

Sequetech

Synthego

Genecopoeia

Encapsula

Prophase Biostudios

"How to Genetically Engineer a Human in Your Garage"

Conferences:

BioHack The Planet

BodyHacking Con

Glossary

Antibiotic: Substances (generally considered drugs) to kill or inhibit the growth of bacteria.

Aqueous: Water-based.

Base pair (bp): A unit consisting of two amino acids, forming the "rungs" of DNA and RNA.

Cas: CRISPR associated protein. These will be identified as Cas4, Cas9, etc.

Chromosome: A single DNA molecule, roughly X-shaped.

Competent (cells): A competent cell, in CRISPR applications, is one that is capable of being transfected.

CRISPR types: Many subtypes of CRISPR systems have been discovered, and more are being added all the time. You can find a current listing of them here.

DNA: Deoxyribonucleic acid. A molecule carrying the genetic instructions used in the growth, development, functioning, and reproduction of all known living organisms.

DNA Sequencing: Any process of determining the precise order of molecules within a DNA molecule.

Electroporation. The process of applying an electrical field (that is, a voltage) to cells in order to increase their permeability, thus allowing substances into those cells. (See also: Transfection.)

Eukaryote: A complex type of cell (more complex than prokaryotes), typical of multi-cellular life forms.

Genetics, genetic: The study of genes, genetic variation, and heredity in living organisms.

Gene: A long segment of DNA.

Genome: The genetic information of an organism. All the primary DNA of an organism.

Homeostasis: Organisms have many processes running together, each of which uses an *activator* system and an *inhibitor* system to remain inside a healthy range. This range is called *homeostasis*, and it's a fundamental operating principle of all advanced life forms.

in vivo: "In life," signifying "in the body."

In vitro: "In glass," signifying, "in the laboratory."

Liposomes: Small, balloon-like structures inside of cells. They are filled with various fluids and encased with a double layer made of fatty or waxy lipids.

Palindrome: Something that's the same backward and forward.

PAM: Protospacer adjacent motif. A 2–6-base-pair DNA sequence immediately following the DNA sequence targeted by a Cas9 molecule. PAM is an essential targeting component for the Cas9 system.

Phage: Short for *bacteriophage;* a virus that infects bacteria.

Plasmid: A ring of DNA, separate from chromosomal DNA.

Prokaryote: A single-cell organism.

RNA: Ribonucleic acid. A molecule essential in the coding, decoding, regulation, and expression of genes.

Transfection: The process of getting things past (that is, through) cellular membranes.

Transformation buffer (or transformation mix): A chemical mixture that opens cell membranes.

Vacuole: A closed compartment within a cell.