CRISPR

In order to simply explain to you what CRISPR is and what we can use it in or how does it solve a problem, we have to briefly tell you the headache that's been bothering us. Nowadays we have major medical therapies that we are using to manage disease processes and its pathogenic or mis-regulated proteins or molecules associated with the disease. Worth to mention, we know that these proteins are encoded and affected by changes in the genes and their sequences, yet we haven't got enough treatment therapies on that scale. So, we need to improve our impact on that molecular level by using genome (gene) editing tools such as TALENs, ZFN and of course our precious CRISPR/CAS9.

CRISPR, a new genome editing tool, but why CRISPR specifically rather than the other tools. In a matter of fact this question is answered plainly, because it fulfills the criteria that we are looking for. These criteria are that it edits the genomes with exceptional precision, efficacy and flexibility. Also we have to mention that it wasn't invented by scientists but that it's naturally occurring in bacteria ***(Streptococcus pyogenes)*** as way of self-defense against viruses that prey on bacteria.

When we undeclared the mechanism of defense of those bacteria we understood how CRISPR/CAS9 works and that it's a part of the bacteria's immune system. The CRISPR part in the bacteria would keep pieces and parts of those vicious viruses around it so that it can recognize them the next time they encounter. Not only that, CRISPER then ignites CAS9 which is one of its famous associated proteins to assault the violating virus by snipping parts of their DNA at specific sites. Usually the encoding genes for CAS are settled close to those of CRISPR.

Now that we understood what CRISPR is and how it works, we can put a simple map in our heads by imagining that CRISPR is a collection of DNA sequence that tells it associates (helpers) what to do. One of CRISPR'S loyal comrade is CAS9 that is responsible for snipping the DNA at specific sites that CRISPR assign's it to by guiding it by providing it by (logically called) guide RNA.

We start to wonder how we are going to apply it in medical therapy. It's really obvious that we are going to guide our tool to snip the unwanted DNA in order to regain the normal regulation of the genes and proteins and so that the disease is cured. That is going to happen by inserting a guide RNA to match the undesirable gene and let the CAS9 snip it off.

Always put in mind that the DNA is a long sequence of bases, we can't just insert all of the genome for CAS9 to detect and snip, it doesn't work like that. In fact, CAS9 can only take up to about 20 base long sequence to be recognized. Ordering the matching guide RNA after using an online tool to design the targeted sequence.

When scientists put the editing tools in trial the other tools were proven to be more specific yet, there was a huge downside, Scientists have to create a designed protein each time, and create several variations before finding one that might work. CRISPR saved all the time and is also more likely to work….BESIDES that it can be used in all kind of organisms.

In order to use such technique we had to totally understand how it works and we couldn't have done this unless for those who started using it and simplified the ideas for us like Genetic Home References. [i]

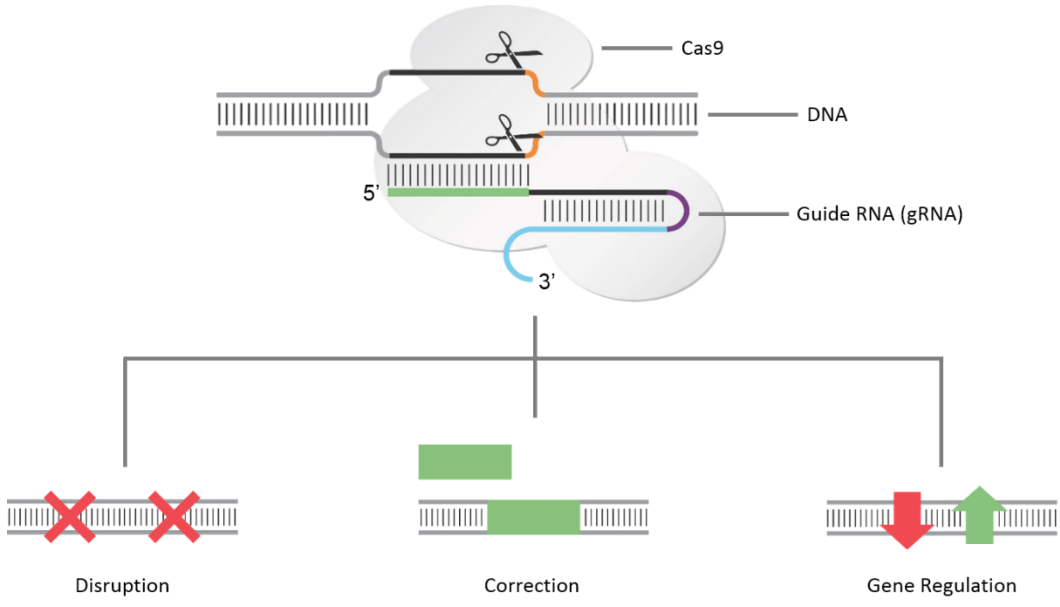
We can't neglect those who matured this technique and helped to enlighten our minds to carry on with our theory and experiments.

We first read that, Jie Wang et al. mentioned in their paper about using CRISPR/CAS9 to inhibit hepatitis B virus replication that, these results suggested that CRISPR/Cas9 system could efficiently destroy HBV expressing templates (genotypes A-D) without apparent cytotoxicity. It may be a potential approach for eradication of persistent HBV cccDNA in chronic HBV infection patients.[ii]

Also, we found that, Panpan Hou et al. published that they found two sites in *CXCR4* that can be targeted effectively and specifically by the CRISPR/Cas9 system, resulting in co-receptor *CXCR4* ablation. We also show that the modified cells are resistant to X4 type HIV-1 infection and this may provide us with an alternative approach of gene therapy for treating AIDS. Although lenti-CRISPR/Cas9 provides powerful means to disrupt *CXCR4*, the optimized delivery methods using adenovirus need to be explored as to further improve their specificity and minimize the concern for therapeutic safety. Due to the variation in viral

infection, co-disruption of *CCR5* and *CXCR4* should be tested using lenti- or adenovirus mediated CRISPR/Cas9 system in the future. Furthermore, the successful disruption of *CXCR4* in Rhesus macaque $CD4^+$ T cells may accelerate gene therapy studies for AIDS in non-human primate models.[iii]

As well as, **Kit-San Yuen et al.** CRISPR/Cas9-mediated genome editing of Epstein–Barr virus in human cells published that CRISPR/Cas9-mediated editing of the EBV genome in human cells provides a new technology platform for the genetic study of EBV. In particular, it will facilitate rapid analysis of the roles of individual EBV genes in viral replication, persistence and transformation. Compared with BAC clones, it will be more difficult to obtain large amounts of CRISPR/Cas9-edited viral DNA for the assessment of genome integrity by restriction mapping. However, the new method also has several advantages and is highly complementary to the existing BAC technology. First, CRISPR/Cas9 technology is applicable to any EBV strain, whereas EBV BACs are currently available for only three strains. Second, CRISPR/Cas9-mediated editing is performed completely in human cells, whereas EBV BACs are constructed and produced in *Escherichia coli*.[iv]



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