CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) has been creating a buzz in the scientific world for some time now. It has emerged as one of the most sought-after techniques of genetic manipulation that have revolutionized the gene editing methods, with the scientific community even referring to it as ‘future of medicine’. Even though the sequence repeats were identified in 1980’s, the field of CRISPR based gene editing really took off once the function of the repeats was demonstrated in a study showing acquired resistance against a bacteriophage in *S. thermophiles* [1]. It was found that CRISPR locus integrates the phage genome fragment to confer resistance against the infectious virus hence, opening up the several possibilities in the area of sequence-specific targeted genome engineering. Since then, it has been reprogrammed and repurposed and its function has been assessed in a variety of host organisms and model systems including fungi, fruit fly, mice and human cells and cell lines. Two classes of CRISPR systems with various subtypes within them have been identified so far [2]. Of those types, Class 2, type II CRISPR-Cas9 has been the best characterized and adapted for genome manipulation in eukaryotic models because of the least number of components required to carry out the editing [3,4]. It consists of three components (Cas9, the mature crRNA, and trans-activating CRISPR RNA, tracrRNA) that constitute the nuclease system essential for DNA cleavage. It has been further simplified as a two-component system by combining crRNA and tracrRNA into a single synthetic single guide RNA (sgRNA) [5]. This sgRNA (crRNA+tracrRNA) is capable of targeting Cas9 nuclease to perform targeted gene alterations [3,5]. Specifically, Cas9 makes use of the endogenous double-strand break (DSB) repair pathway to make desired mutations in the target DNA. One additional requirement for the successful identification of the target DNA is the presence of 3-bp protospacer-adjacent motif (PAM) immediately followed by the target DNA sequence [6,7]. Simply put, the CRISPR-Cas9 mechanism works similar to a search feature in a word document that can find a short string of RNA in a complex genome and allows for the modifications.

The reason that it is better than other conventional gene editing technique like RNA interference (RNAi) is that the effects are not temporary and the off-target effects are minimal [8]. It guarantees the genetic manipulation in a sequence-specific manner with high reagent consistency and validation rates. It is currently the most precise and efficient method of genome editing in eukaryotic cells that is providing a wealth of information about the gene function. CRISPR mediating gene editing is routinely used in labs worldwide for high-throughput functional genome screens, the creation of the models of inherited diseases, somatic cancers, and to study epigenetic relationships between genes [9-11]. The true potential of CRISPR-Cas9 in biomedical research and therapeutics has just begun to be discovered. In order to get a better control on CRISPR mediated genomic manipulation, both the Cas9 nuclease and sgRNA can be modified to get the desired effects. Variants of Cas9 nuclease have been engineered imparting the system differential functional specificity of gene editing [12,13]. Several regulatory elements and reporter probes have been fused on the CRISPR scaffold, leading to the ‘at will’ transcriptional regulation and visualization of the target genes, thus providing a never before understanding of several gene functions and phenotypes [14,15]. It can be said that sky is the limit, for the endless possibilities of utilization of the CRISPR system in the near future. Not only it could be used for designing treatments and therapeutics for rare genetic disorders, cancers, infectious diseases, and xenotransplantation in the medical field, but could also advance agriculture and bio-manufacturing capabilities in form of disease-resistant crops and biofuels respectively.

With great power comes great responsibility, a famous quote that is an apt reminder for the researchers and biotech companies racing towards commercial exploitation of the CRISPR technology. Very recently, scientists utilized CRISPR to edit DNA in viable human embryos in order to correct a genetic mutation affecting the heart [16]. It once again has triggered a debate among the different strata of society about the ethical and moral implications of genetically altering human embryos. It would require rigorous rules and regulations on part of governments, scientists and regulatory bodies worldwide to come up with a
mutually agreed plan for CRISPR applications involving humans. Not only that, the potential benefits of CRISPR has also instigated a race among different companies and organizations, claiming the sole right to the technology for commercial interests. Several have already filed patents with different agencies and battling it out amidst the ongoing social and ethical implications associated with CRISPR research. Constant monitoring by all responsible parties would ensure uninterrupted research advancement that only allows for ‘must have’ rather than ‘like to have’ genetic manipulations.

Nothing in the world is perfect. Even though CRISPR has emerged as a versatile and effective technique of genome editing, it too is not foolproof, at-least not yet. Several constraints still exists that need more research and study before this system becomes perfect. Some of the factors and criterions that still need attention or further studies include the delivery method of CRISPR-Cas system into the cells or tissue, efficiency of synthesized sgRNAs, time taken for the editing post delivery, interaction with cellular machinery for toxic effects, screen readout methods and statistical tools for data interpretation to name a few [17-19]. Even though the roadmap for CRISPR research looks complex and full of uncertainties, but rest assured the movement forward seems to be in the right direction.

Who would have thought that something that was once identified as a microbial adaptive immunity mechanism against the invading viruses would rock the scientific community and become ‘THE TOOL’ for genetic manipulations in living mammals with the potential of other applications. The journey of CRISPR development is an inspirational, motivational and one that teaches all of us researchers how discoveries are made in science. It reminds us about the importance of serendipity, collaboration, and perseverance for scientific success. It equips the humanity with a powerful means, with plethora of uses but also makes us all responsible to see that it is never abused.

References