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Important Impact of PTEN tumor suppressor Gene has using in CRISPR-CAS9 Gene Editing Technology of ChopChop Version 3 Method with IDT Technology Analysis that *In-Silico* approach detects in the Endometrial Cancer.

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Abstract: The CRISPR-associated protein 9 (Cas9) system is primarily a defensive mechanism utilized by bacteria to protect themselves from phage invasion. Endometrial cancer, a particular type of gynecological cancer, poses a serious hazard to people all over the world because to the high occurrence of the PTEN gene mutation. It has, however, been purposely exploited as a powerful foundation for RNA-guided DNA targeting, making genome editing, transcriptional disruption, epigenetic alteration, and genome imaging more manageable. When using a fusion protein of nuclease-deficient Cas9 and effector domain, this technology allows for precise manipulation of any genomic sequence specified by a short stretch of guide RNA, allowing for the elucidation of gene function involved in disease development and progression, correction of disease-causing mutations, **a**ctive therapeutic approaches include rectifying disease-causing mutations, inactivating oncogenes, and activating deactivated cancer suppressor genes. The CRISPR-Cas9 in-silico operation was performed using the ChopChop technique version 3. CHOPCHOP is suitable for a variety of CRISPR applications, including gene knock-out, sequence knock-in, and RNA knock-down. Using CHOPCHOP's visualization, gRNAs may be chosen through knowledge and experimentation. CHOPCHOP v3 broadens the variety of DNA targeting methods available. Among these are (i) mechanisms for nanopore enrichment. Targeted sequencing is the process of obtaining high-quality sequencing reads in a specified area of interest. CHOPCHOP v3 is limited to sgRNA searches inside each isoform, which necessitates knocking off the PTEN gene with IDT Technology. The gRNA had to be selected for usage since "Molecular Scissor" is a procedure that uses primer design to assist the UCSC Genome Browser in measuring the variation of the intron and exon variation in the targeted gene by target sequences. Furthermore, this programmable endonuclease technique allows scientists to investigate the roles of multiple gene sequences concurrently by targeting multiple genomic loci in a single experiment, greatly accelerating our understanding of pathological processes involving large sets of genes or mutations, such as tumor development. CRISPR-based genome-wide screens using single-guide RNA (Sg RNA) libraries can be used to identify drug-target or disease-resistance genes, such as novel tumor suppressors or oncogenes, as well as to rapidly assess therapeutic targets. Drug-target or disease-resistance genes, such as novel tumor suppressors or oncogenes, can also be exploited to quickly identify therapeutic targets. The CRISPER-CAS9 gene editing technique, as a novel therapeutic impact method, emphasizes the need of understanding the mechanism of endometrial cancer therapy. CRISPRs, or clustered regularly interspaced short palindromic repeats, were developed as a gene editing tool. CRISPR-associated (CAS) nucleases, which are guided by single guide RNAs (sgRNAs), can be used to fix mutations in disease-associated genes and delete specific genes. When it comes to creating genetic alterations or mutations, the CRISPR/Cas9 system outperforms earlier methods.

Index Terms - CRISPER-CAS9, Endometrial cancer, PTEN gene, ChopChop method, IDT Technology, guide RNA.

I. INTRODUCTION

Jennifer Duodena and Emmanuelle Charpentier have received the 2020 Nobel Prize in Chemistry for their groundbreaking work in this sector. CRISPR/Cas9 is a bacterial defense system that involves cutting genomic DNA at a specified site, resulting in the removal of old genes and the insertion of new genes. On the other hand, we are well aware that cancer is a genetic condition in which certain tumor cells have genetic mutations that affect growth-regulatory genes, leading them to differ from their normal progenitors. Certain genes may include unique instructions required for our bodies' cells to function and make certain proteins. However, some genetic mutations may result in cells that resist the regulators' normal growth process before progressing to cancer. Endometrial carcinoma, also known as Endometrial cancer (EC), is one of the many forms of cancer that affect women, and as mortality and morbidity rates rise, this illness has emerged as a serious threat to the general population's health. Scientists have uncovered various genetic variations that increase a person's chance of acquiring certain cancers, including breast, ovarian, uterine and endometrial, colorectal, prostate, and others. Genetic testing of malignant malignancies has indicated that a patient's cancer may be linked to Hereditary Cancer Syndrome; tumor DNA sequencing of cancerous cells can detect the existence of hereditary mutations. These cancer-causing genes are classified into two types: tumour suppressor genes, which act as negative growth regulators, and oncogenes, which act as positive growth regulators. On the other hand, we must guarantee that it has been intentionally employed as an effective framework for RNA-guided DNA targeting, allowing for genome editing, transcriptional disruption, epigenetic modification, and genome imaging. When a fusion protein of nuclease-deficient Cas9 and effector domain is used, this technology allows precise manipulation of any genomic sequence specified by a short stretch of guide RNA, allowing for the elucidation of gene function involved in disease development and progression, correction of disease-causing mutations, and inactivation of activated oncogenes or activation of deactivated cancer suppressor genes. Furthermore, this programmable endonuclease approach allows scientists to examine the functions of several genes. By targeting numerous genomic loci in a single experiment, researchers may explore the function of multiple genes at the same time, dramatically increasing our understanding of pathological processes requiring vast sets of genes or mutations, such as tumor growth. CRISPR-based genome-wide screens with single-guide RNA (Sg RNA) libraries can be used to uncover drug-target or disease-resistance genes, such as new tumor suppressors or oncogenes, as well as to quickly evaluate therapeutic targets. There are in-silico or bioinformatics approaches that help to examine the target gene (here PTEN) associated to cancer, as well as research CRISPR-Cas9 editing technology. A crRNA array at a specific CRISPR site contains the 20-base-long target sequence. The Cas9 protein selects the appropriate base pair bonding site for the target sequence. Cas9 recognizes the host genome's PAM sequence (5'-NGG-3'). PAM-related sequences may be constructed into a plasmid, and target sequences can be transfected into cells, proving that Cas9, employing crRNA, can detect the correct sequence within a host cell. This solved the genetic cut problem and acted as a molecular scissor by causing a single or double strand break at the proper location in the DNA. The 20-base target sequence may be discovered on a crRNA array at a specific CRISPR site. The Cas9 protein selects the appropriate base pair bonding site for the target sequence. Cas9 recognizes the host genome's PAM sequence (5'-NGG-3'). PAM-related sequences may be constructed into a plasmid, and target sequences can be transfected into cells, proving that Cas9, employing crRNA, can detect the correct sequence within a host cell. This resulted in a single or double strand break at the proper spot in the DNA, resolving the genetic cut issue and acting as molecular scissors. Mutations in the tumor suppressor gene or any of the checkpoints can alter the flow of events and throw a person's life off course in malignant clinical manifestations. In this situation, genome editing using CRISPR/Cas-9 technology can aid in replacing the mutant base pair with the proper base pair. In summary, Cas-9 identifies target sequences of 20 base pairs (bp). The protospacer Adjacent Motif (PAM) sequence and target determine the genetic cut's fidelity, accuracy, and precision. A mutation in the tumor suppressor gene or one of the checkpoints can interrupt the flow of events and throw a person's life off course, resulting in malignant clinical signs. In this situation, CRISPR/Cas-9 technology may be used to replace the mutant base pair with the proper base pair. To summarize, Cas-9 finds target sequences of 20 base pairs (bp). The protospacer Adjacent Motif (PAM) sequence and target determine the genetic cut's fidelity, accuracy, and precision. Using in-silico or bioinformatics methods, research may be conducted on the CRISPR-Cas9 editing technology and the targeted gene (in this case, PTEN) associated with Endometrial Cancer.

II. ORGANIZATION OF CRISPR-CAS9 IN ENDOMETRIAL CANCER:

However, in Endometrial Cancer Development, the mechanism and causes of Cancer where Cancer cells' widespread poor control over their growth is produced by abnormalities across several species. This keeps it from spreading to other parts of the body by attacking nearby healthy tissues. Malignant Tumor: Cells from the Lymphatic or Circulatory systems can infect adjacent healthy tissue and spread throughout the body via metastases. Because "cancer cells" only refer to malignant tissues that evolve into tumors, their primary characteristic is "tumor clonality." Cancer cells' tendency to form tumor clones where malignancies arise, as well as the fact that cancer is frequently resistant to such targeted therapy due to its hazard to distant physiological sites, may be reflected in cancer cells' cell regulatory systems. Because only malignant tissues and tumors are recognized to as cancer cells, cancer is exceedingly destructive in remote body sites and frequently grows resistant to such focused treatment. In the Cancer Pathophysiology: Where the cell regulatory systems of malignant cells may resemble cancer pathogens. The tumors are aberrant cell growths that can be benign or malignant. It can usually remove surgically. Benign tumor: A growth that remains in its original place. It has been taking up the surrounding healthy tissues without going on to other body parts. After overrunning the surrounding normal tissues, it does not spread to other parts of the body. In addition to exhibiting mutations, its cells have the capacity for unchecked growth, multiplication, survival, invasion, and dissemination. The two probable reasons of tumor initiation are the rapid multiplication of progeny cells owing to genetic alteration or a mutation that eventually becomes dominant in the tumor population through clonal selection. The principal endometrial cancer cell types in which clonal selection is always active throughout tumor development, causing tumors to grow quicker and become more malignant over time. The many functions that different cell, tissue, and organ types have can cause some variation in the structures and types of modifications that cause an abnormal single cell to proliferate, and this abnormal proliferating cell allows the population of tumor cells established through clones to expand and sometimes mutations increase a cell's selective advantage. For example, a mutation may allow the cell's children to multiply rapidly, or the mutation may eventually become dominant in the tumor population through clonal selection. The primary cancer cell types in which clonal selection is ongoing during tumor growth and tumors continue to grow faster and become more aggressive. Different cell, tissue, and organ types can perform a range of functions, resulting in variations in their kinds and architectures. There are five primary kinds of cancer cells: 1) Cancer. 2) Sarcoma. 3) Leukemia. 4. Lymphoma 5) Myeloma. 6) Cancer of the brain or spinal cord.

III. THE CRISPR/CAS9 MECHANISM:

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), a prokaryotic immunological defense mechanism, has paved the way for nucleotide-level targeting of hereditary disorders such as cancer. A collection of nucleotides known as repeat sequences, which are found upstream of the Cas9 gene operon, are homologs or identical to "tracrRNA" or trans-activating CRISPR RNA. Emmanuelle Charpentier was the first to discover this novel noncoding RNA, which is a little trans-encoded RNA. The CRISPR/Cas9 locus is related with the Cas9 operon and consists of repetitions separated by non-repetitive sequences known as spacers. The CRISPR array-associated Cas9 operon, which generates the juvenile section known as pre-crRNA from previously inserted spacers and host DNA, allows for co-transcription of the whole spacer area. So, to bind the guide RNA (gRNA) and form a Cas9/gRNA complex, the CRISPR/Cas9 system requires Cas9 endonuclease. RNase III is involved in the co-processing of both tracrRNA and pre-crRNA, or long main transcript. For tracrRNA to anneal to pre-crRNA and generate the mature segment known as crRNA, short CRISPR-RNA is produced. Cas1 and Cas2 nucleases are used to break each segment of crRNA into a 20-nucleotide sequence, which results in guide RNA for enzymes that target either DNA or RNA. This process matures crRNA into gRNA. To guarantee target specificity, two nuclease domains—the HNH domain and the RuvC-like nuclease endonuclease domain—cause breaks in double strands in DNA with three base pairs upstream inside the PAM region. Endonuclease Cas9 is inactive until connected by gRNA. The ensuing double strand breaks can be repaired either error-free or mistake-prone that using non-homologous end-to-end joining (NHEJ), which allows the insertion or deletion of a specified sequence and homologous directed repair (HDR) without any abnormalities.

IV. PTEN IS A TUMOR SUPPRESSOR GENE FOR ENDOMETRIAL CANCER:

Phosphatase and tensin protein, commonly known as PTEN homolog protein, is located on chromosome 10 and degrades lipids by removing phosphate groups. The PTEN gene's mechanism involves the PTEN enzyme attaching to another PTEN enzyme (dimerizing) and then attaching to the cell membrane, where the phosphatidylinositol-(3,4,5)-triphosphate is broken down and produced by the phosphatidyl-inositol-3-kinase (PI3-K) pathway, which primarily catalyzes cell survival via protein kinase (PKB/Akt). In addition to attaching to the cell membrane, which may be altered by the body's other proteins and lipids, as well as oxygen and

phosphorus atoms, the single PTEN enzyme dimerizes PTEN. The phosphatase enzyme hydrolyzes the substrate variant because PTEN is a tumor suppressor gene with low processor activity. The phosphatase binding P loop in the nuclear binding properties of GTPase and ATPase controlled the dephosphorylation of membrane-bound PIP3 that resulted from the loss of the PTEN gene.

1. RESEARCH METHODOLOGY

1.1. gRNA can cure Endometrial Cancer by the *in-silico* technique:

Using a guide RNA, the Cas9 enzyme locates the targeted DNA sequence, and then it double-strand breaks PTEN. A template DNA with a corrected/mutated region is also required to edit/repair the target DNA using endogenous homology-directed repair (HDR). But in the absence of the DNA template, the damaged DNA can re-connect by a process called non-homologous end joining, or NHEJ mutations, which can add or remove base pairs to change the structure. Then, in order to create a nick in double-stranded DNA and increase the number of bases that are particularly identified while reducing off-target cleavage, a Cas9 Nick-ase creation was designed. The PTEN gene in the human genome is the particular target of gRNA sequences generated by the Endometrial Cancer Gene Mutation Resistance region in these individual gRNA sequences. These gRNA sequences can be coupled with WT SpCas9 or used as crRNA with the WT SpCas9 enzyme to create a DSB and alter the genome. These single-stranded RNA sequences were validated in genome-wide libraries in preparation for CRISPR investigation.

1.2. Materials and Methods:

Good internet facilities, CRISPR-CAS9 Gene Editing Technology by ChopChop Version 3 Method with IDT Technology, PTEN gene mutation (83%) information in Endometrial cancer.

1.3. Web tool CHOPCHOP Method:

This is a type of prokaryotic adaptive immune system that has developed CRISPR/Cas9, a cutting-edge gene editing technology that guards against foreign DNA invasions and phage/virus invasions. It is frequently employed in mammalian genome editing to control gene expression, eliminate genes, fix disease-related genes, and create endometrial cancer models based on animals. All must, however, do so via internet-based accessibility. It is possible to identify the CRISPR-Cas9 single guide RNA (sgRNA) targets by employing a web-based program named "CHOPCHOP" that was used online. It can act with either TALEN proteins or CRISPR/Cas9. Among the several input types and formats that CHOPCHOP may provide are genetic IDs, genomic coordinates, and concatenated event sequences. Together, they gathered data and presented it as a visual representation. CHOPCHOP can be utilized in complex environments or with only a few basic input choices. The research's evolving list of distinct species, a gene's name, genomic dimensions or a pasted sequence (including RefSeq and ENSEMBL gene IDs), and the decision to use CRISPR/Cas9, CRISPR/Cas9Nickase, CRISPR/Cpf1, or TALEN mode are all essential inputs.

2. Procedure :

The editing technology used the CRISPER-Cas9 genome editing methodology, and instead of using the In-Vivo method, the CHOPCHOP method was used. Instead, it is the sole basis for an in-silico computerized online method. They also perform similar roles, such as producing gRNA, sgRNA, or guide RNA to match the intended target gene. PTEN, for instance, is the desirable gene in this situation. Furthermore, Cas 9 breaks double-stranded DNA in the same way as a DNA endonuclease does at the time, allowing CRISPR to correct errors and turn genes on or off using amazingly fast and affordable gene-editing technology.

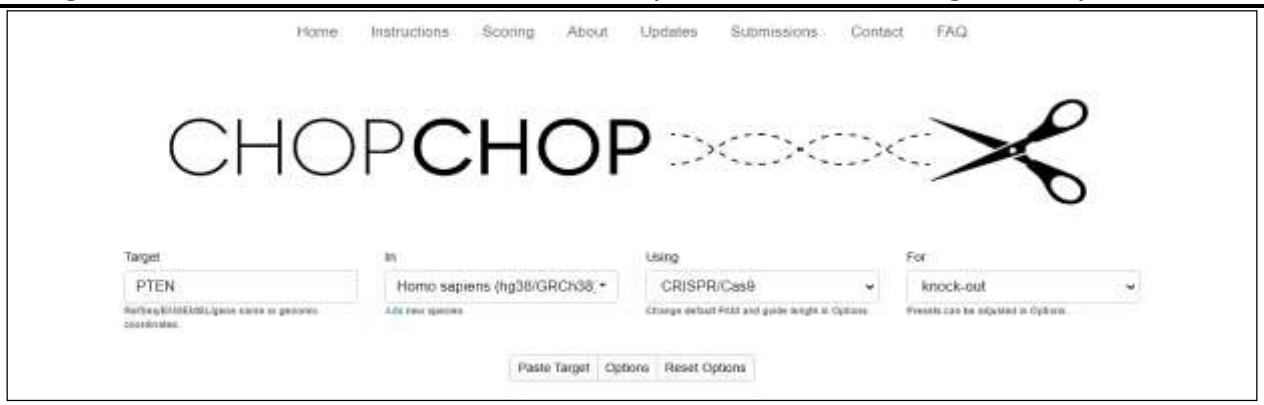


Figure 1: This illustration below shows the "Home" page for the CRISPR/Cas9 genetic editing system, which is formally referred to as "CHOPCHOP." Users can enter or paste their genomic coordinates, RefSeq, ENSEMBL, or gene name as the "Target" here. "In" means adding the species' name; "For" means utilizing CRISPR/cas9 and other PAM or guide length; and "Knock-out" or "Knock-in" means recombination homologues or non-homologues end-joining, either in addition to preset activation methods.

Figure 2: The next step is to assess the likelihood of the best desired results. To check options for the best "Target results". For the design, we have set the primer parameters, including primer size, primer design, primer Tm, and the minimum primer distance to the target site.

CHOPCHOP has corrected the following: Fasta/genome input switch history, Fasta input scoring of off-targets, Fasta input bug, repeating sgRNAs, shift in results view, rare strand problem, summary of off-targets at the PAM N location. CHOPCHOP has absolutely new features: CRISPR/Cpf1 mode, CRISPR/Cas9 Nickase mode, targeting to promoter regions, support for truncated sgRNAs, off-target search up to 3 miss-matches, self-complementarity scoring for sgRNAs, support for orthologous type II CRISPR/Cas PAMs, new efficiency scoring metrics based on large-scale studies, scoring of complementarity of sgRNAs against backbone regions It is now mobile friendly, has many more species, cookies that remember your last query, downloadable table with created primers, and results integrated with the UCSC genome browser. Restricting to 3' UTR, fixing ensemble identifiers for the mm10 genome, updating hg38 to the most recent gencode supported by the UCSC, small fixes for the website whole chromosome instead of 1bp position, adding -no Overlapping option and forcing it on website users searching for nanopore enrichment (large area, relaxed off-target requirements), and isoform targeting the permissive PAM results in a lot of unnecessary overlaps.

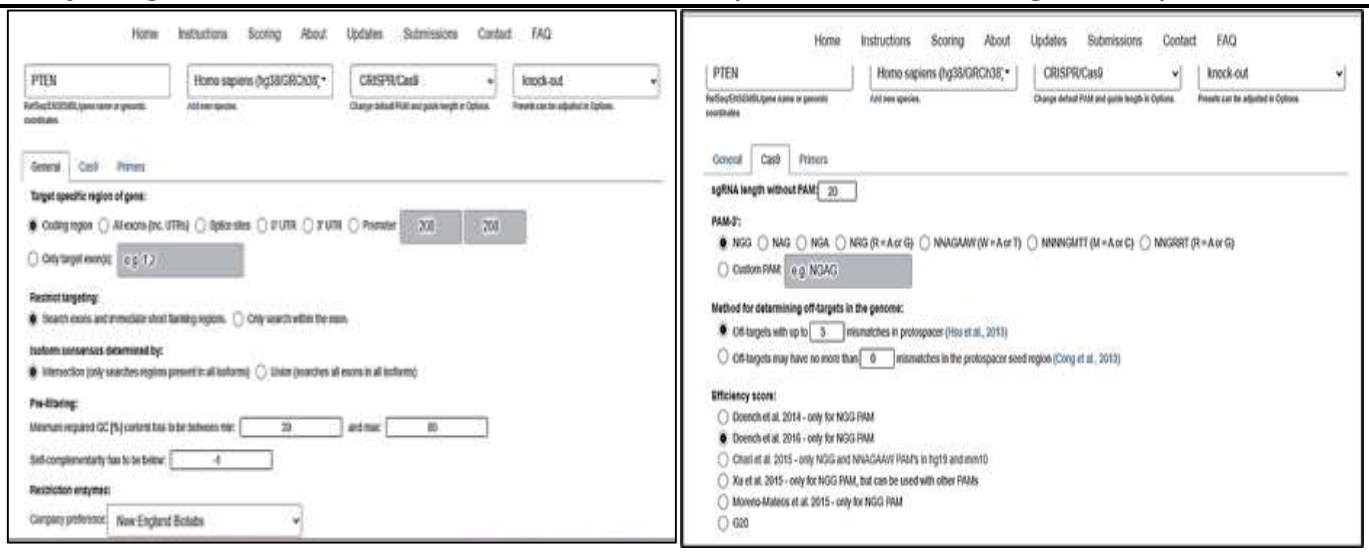
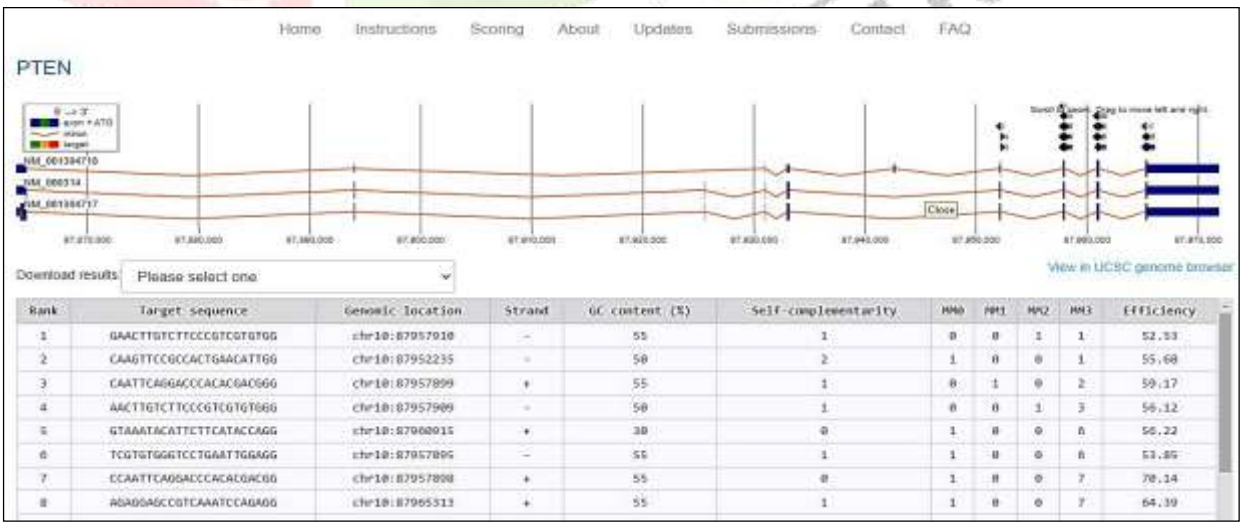


Figure 3: To get a good result, we generally need to take into account specific gene regions to target, restriction targeting, isoform consensus, prefiltering, and restriction enzymes. Cas9 detects sgRNA length without PAM, PAM-3", off-target genome, and efficacy score. Anything may be adjusted by users to get a precise outcome. The sgRNA length detection without PAM, PAM-3," off-target genome, and efficacy score are determined in option cas9. Users can alter any of these to achieve precise results.

Figure 4: After pasting all of the relevant Find Target coordinates, click the appropriate button to be notified that the procedure was accomplished. You must paste all of your desired coordinates for Find Target in order to receive the notification that the operation was completed successfully.

3. Results:



Rank	Target sequence	Genomic location	Strand	GC content (%)	Self-complementarity	MM0	MM1	MM2	MM3	Efficiency
1	GAACTTGTCTTCCCGTCGTGTGG	chr10:87957010	-	55	1	0	0	1	1	52.53
2	CAAGTTCCGCCACTGAACATTGG	chr10:87952235	-	50	2	1	0	0	1	55.68
3	CAATTCAGGACCCACACGACGGG	chr10:87957899	+	55	1	0	1	0	2	59.17
4	AACTTGTCTTCCCGTCGTGTGGG	chr10:87957909	-	50	1	0	0	1	3	56.12
5	GTAAATACATTCTTCATACCAGG	chr10:87960015	+	30	0	1	0	0	6	56.22
6	TCGTGTGGGTCCTGAATTGGAGG	chr10:87957895	-	55	1	1	0	0	6	53.85
7	CCAATTCAGGACCCACACGACGG	chr10:87957898	+	55	0	1	0	0	7	70.14
8	AGAGGAGCCGTCAAATCCAGAGG	chr10:87965313	+	55	1	1	0	0	7	64.39

Figure 5: Now, we may receive the many "Targets" for the PTEN gene, which is located at Chr10. There are some '+' and '-' strands. The efficiency ranges from 52.53 to 70.14, varying between in-vivo and in-silico. The GC content is +/- 55%. The self-complementary region of the target sequence is very similar. The results must include the Left primer coordinates (Left primer, left primer Tm, Left primer off-targets), as well as the Right primer coordinates (Right primer, Right primer Tm, Right primer off-targets, and so on). Also, the product sizes different. There are various distinctions here.

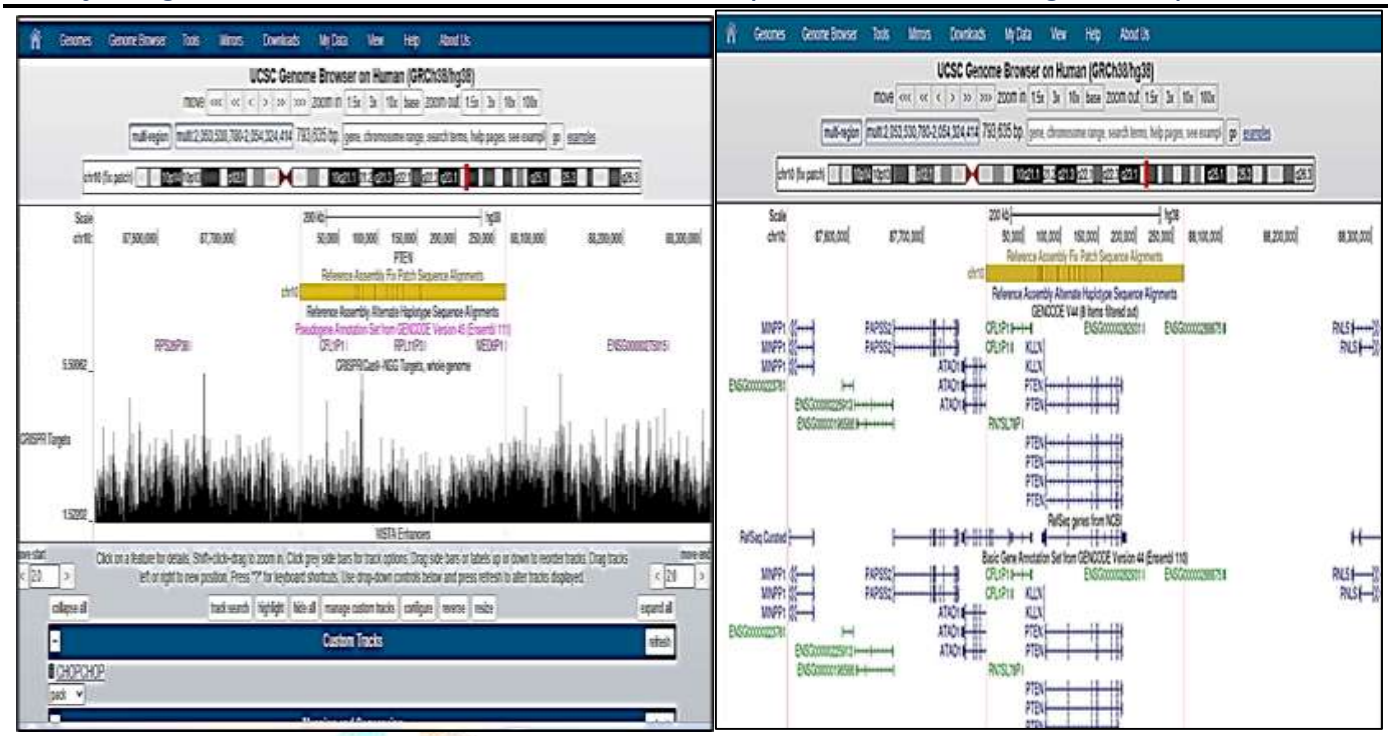


Figure 6: The CRISPR/Cas9 target of the whole PTEN genome is visible in the UCSC Genome Browser (of Human GRCh38/hg38); based on the unique track created by CHOPCHOP, this target gene may be an enhancer.

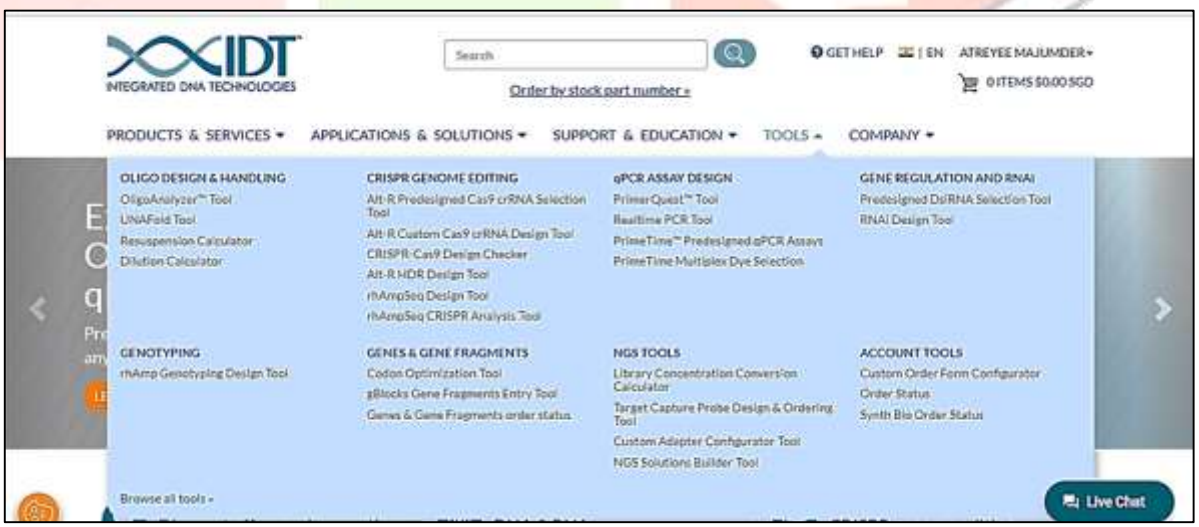
Figure7-10: Here are several examples: 1) Chr10:87957990-87958012: GCATCTTGTTCTGTTTGTGGAA60.2:1;0:161; or 2) Chr10:87957851-87957873:AGATCCTCAGTTTGTGGTCTGC:60.7:0; or 3) Chr10:87952432879524 54:CCATTGTTTGGCTTTGGTTCTTAG we can see here . These mismatches may

be noticed in off targets, and for gRNA or guide RNA, we must utilize either the left or right primer sequences.

4. Another Web tool is Integrated DNA Technologies has been using:

Users may do all kinds of molecular foundation *in-silico* procedures in genetic engineering or gene-editing technologies using the web-based program from Integrated DNA Technologies. Integrated DNA Technologies, Inc. (IDT) produces specific nucleic acids for academic research, biotechnology, clinical diagnostics, and pharmaceutical development. IDT's principal focus is on the development of novel DNA and RNA oligonucleotides (oligos) for scientific purposes. Joseph A. Welder, M.D., Ph.D. (Northwestern University) created Integrated DNA Technologies, Inc. in 1987 at the University of Iowa's Technology of Innovation Center business incubator. IDT's research goals in biology and medicine include enhancing nucleic acid synthesis technologies and creating new uses for DNA and RNA-based molecules.

Figure11: In the Integrated DNA Technologies where after getting the CRISPR-Cas9 Target sequence for the PTEN gene using the CHOPCHOP approach, we must apply IDT Technology to synthesize CRISPR-Cas9 gRNA that accurately targets the DNA segments.



There are three methods included in IDT Technology for CRISPR-Cas9 gRNA that targeting the DNA segments. These 3-method are:

(A) CRISPR-Cas9 gRNA design Checker:

(B) Search for predesigned gRNA:

(C) Design Custom gRNA:

: In these **3 methods** we have known that:

(A) CRISPR-Cas9 gRNA design Checker:

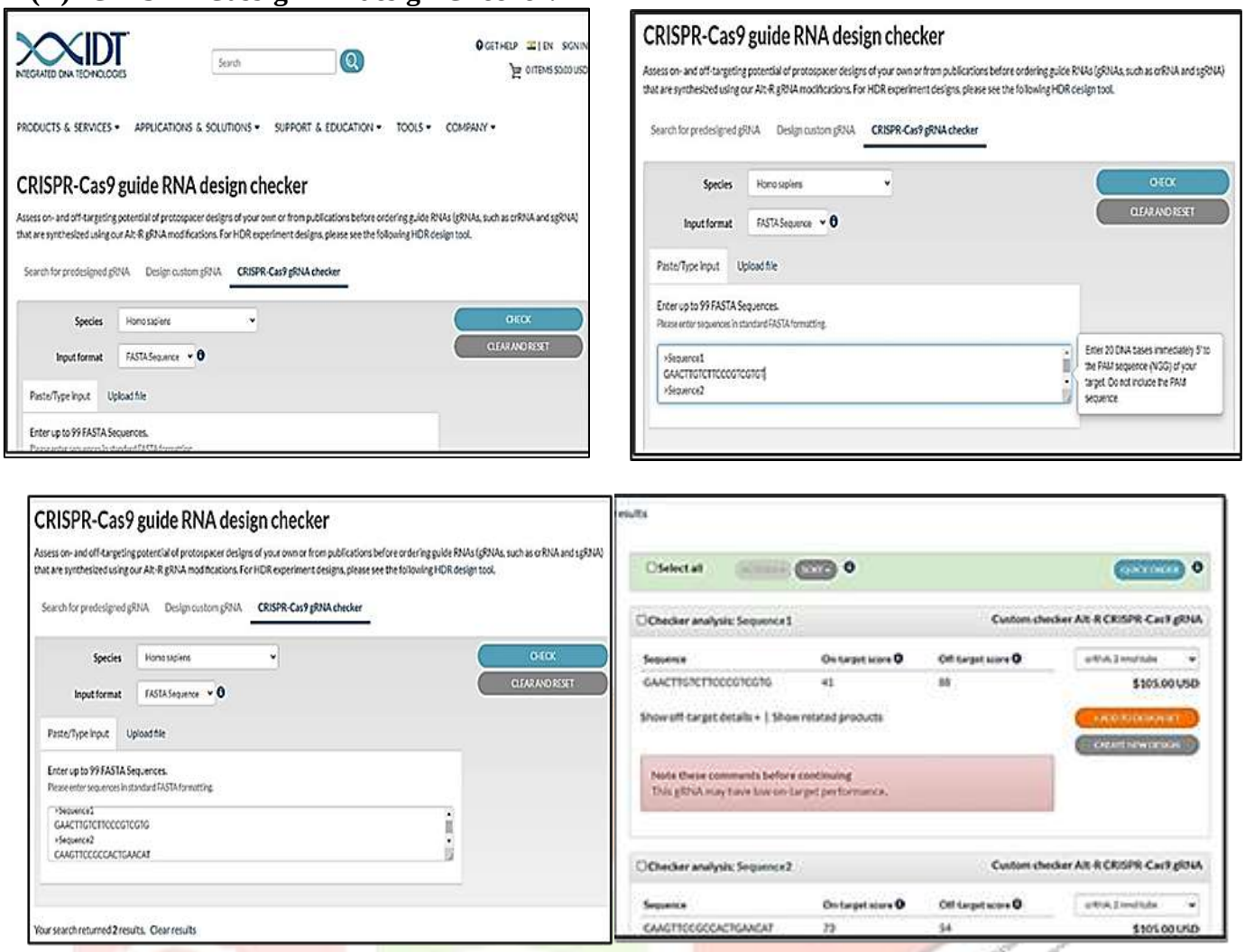


Figure 12-15: In IDT, here. Predesigned ALT-RTM CRISPR-Cas9 guide RNA; here, gRNA may be checked using the FASTA sequence or the Target Sequence from the CHOPCHOP technique for the PTEN gene. Only twenty DNA bases can be provided. Two together. Product Design Size: " [1chr10: (Locus range)87957851879578 73AGATCCTCAGTTTGTGGTCTGC60.7" "0chr10:87957990879580 12GCATCTTGTTCTGTTTGTGGAA 60.2" 1 0 161-"2 chr10:87957868-87957890 TCTGCCAGCTAAAGGTGAAGAT 60.4"-"1chr10:87958133-87958155-CAATGCCAGAGTAAGCAAAACA 60.3" 0 0 287 3 "chr10:87957868-87957890 TCTGCCAGCTAAAGGTGAAGAT 60.4" "1chr10:87958131-87958153-ATGCCAGAGTAAGCAAAACACC 60.5" 0 0 285 4 "chr10:87957851879578 73AGATCCTCAGTTTGTGGTCTGC 60.7" "0chr10:87957991-87958013-AGCATCTTGTTCTGTTTGTGGA 59.8 1 0 162" "5 chr10:87957847-87957869]. ATGCAGATCCTCAGTTTGTGGT 60.9 0chr10:87957990–87958012 GCATCTTGTTCTGTTTGTGGAA 60.2" 1 0 165 Left primer coordinates Left primer Left primer Tm. Left primer off-targets Correct primer coordinates The right primer Right primer, Tm. Right primer off-targets Pair targets with off-target sequences. In the input device format sequence, both sequences should be compared, and the sgRNA and crRNA gRNAs were synthesized by Alt-R gRNA using the HDR design tools in the experiment. We obtained two findings by comparing two sequences, and we must use those sequences to determine the basic relevance of gRNAs and whether they are good in target sequences. The gRNA may have low on-target performance at the PTEN gene sequence, with an on-target score of 73 and an off-target score of 54, as do many other gRNAs.

- The Alt-R Prime editing enzymes, Cas9, Cas12a, Cas13, and other CRISPR nucleases may all be found in custom CRISPR gRNA libraries. These libraries were created in response to the demand for improved CRISPR screening tools. These chemically altered guide RNAs (gRNAs) are produced using IDT's own high-fidelity RNA manufacturing technology to generate dependable, high-quality gRNA libraries quickly. 1. A novel approach provided by a world authority in RNA synthesis and

CRISPR innovation, 2. Delivery that is dependable, consistent, and quick, with customized formulas to meet a range of project requirements, 3. Adaptable to several CRISPR systems, including primary editing, Cas12a, and Cas13, 4. Increased nuclease resistance for maximum editing using ribonucleoproteins (RNP) or Cas9-expressing cells, 5. Enhanced RNA manufacturing procedures to reduce the possibility of cross-contamination

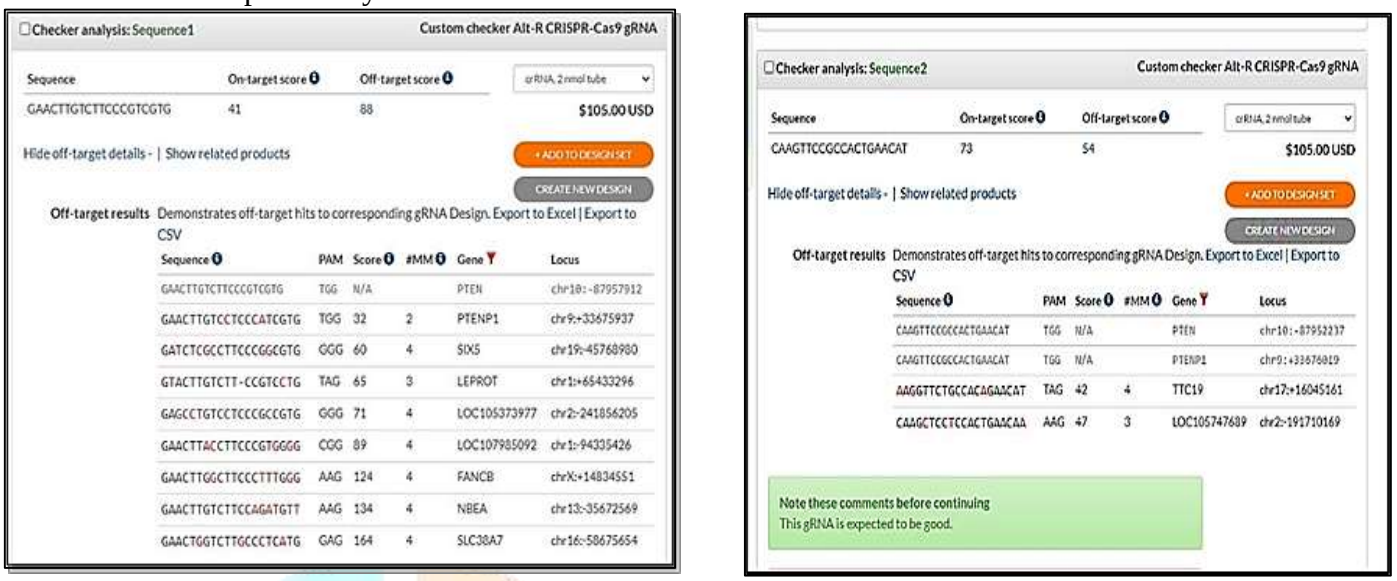


Figure16-17: (a) In Checker analysis, sequence no.1 on target score 41 and off target score 88 gRNA differs from another distinct no. in 'TGG' PAM score. (b) In this case, sequence 1 has 32 points whereas sequence 2 has N/A, meaning there is no score difference between the on-target and off-target scores in the checker analysis of PTEN and PTENP1. So, this gRNA was predicted to be good.

(B) Search for predesigned gRNA:

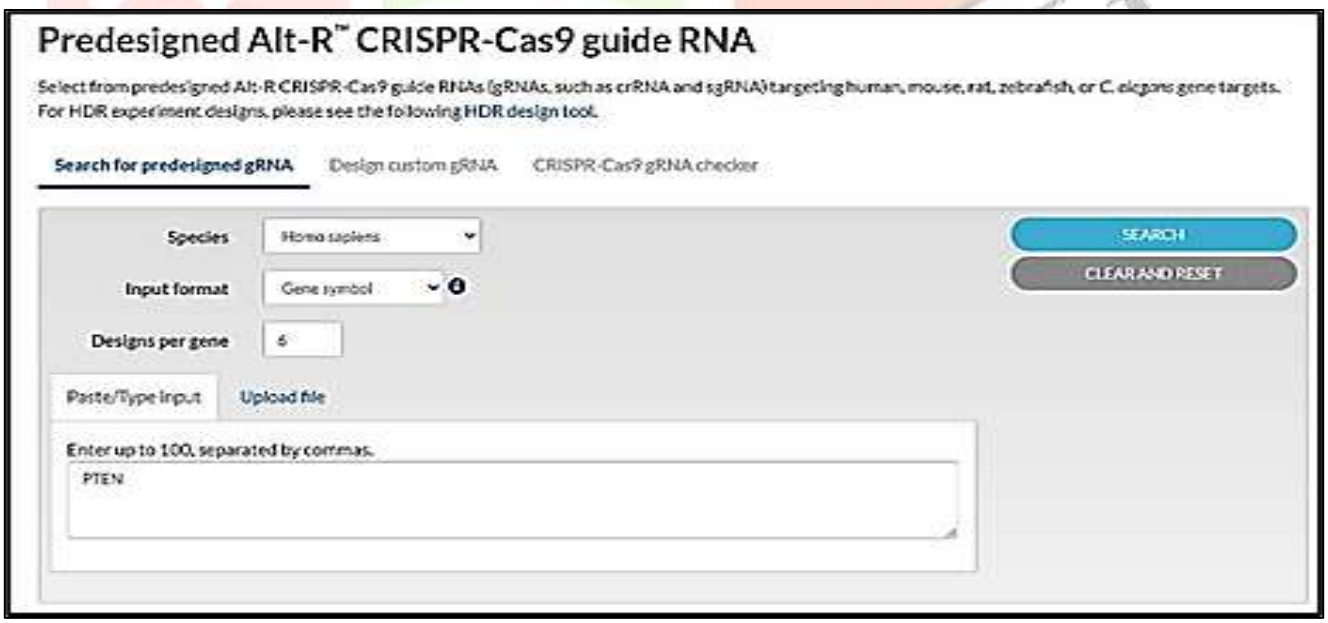


Figure 18: To find predesigned gRNA processing, enter PTEN (desired gene name) in the box, locus difference 87,900,000-87,920,000. crRNA, and search specifically for the PTEN gene on those pages.

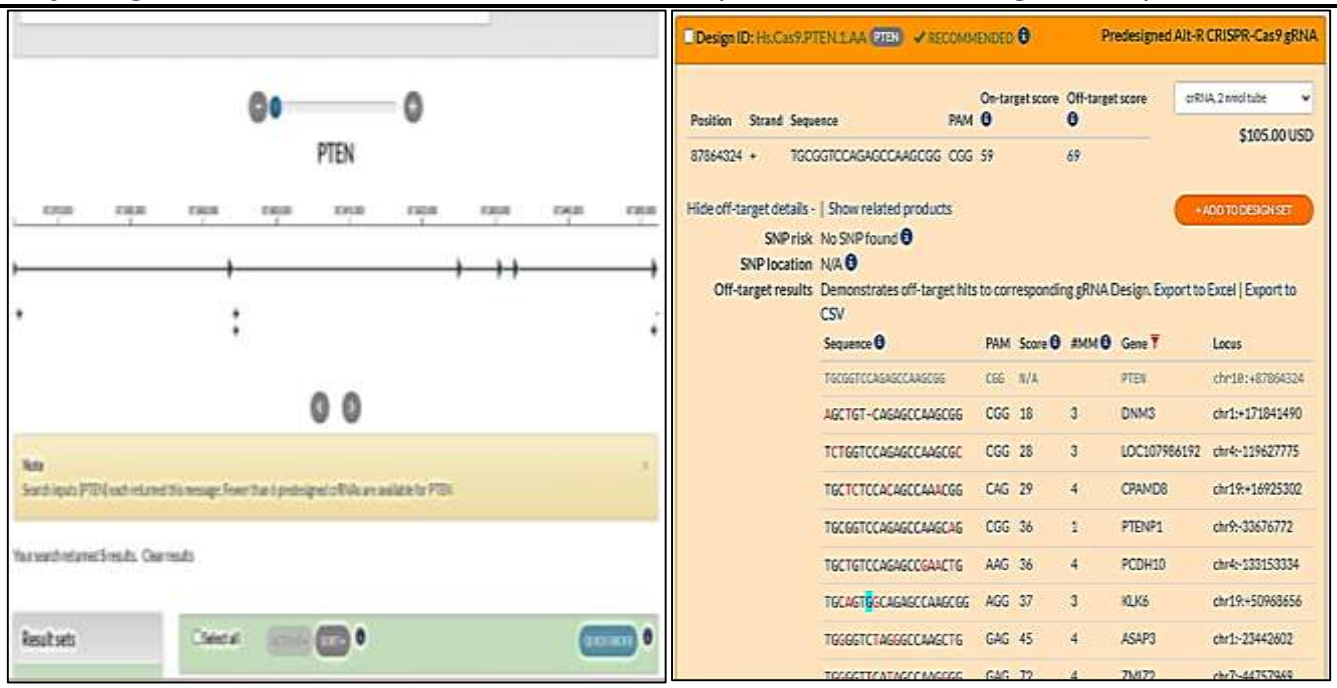


Figure 19-20: (a)This sequence formation scale contains inputs (PTEN) relevant to gRNA. There were 6 types of PTEN pre-design cRNAs in that range. (b)This predesigned Alt-R CRISPR-Cas9 gRNA has a successful Design ID-HS. Cas9.PTEN.1AF in the PTEN, demonstrating off-target gene hits to the appropriate gRNA design. Between the PTEN gene and other gene targets, hits occurred, such as PTENP1, where T arrived via polymorphism and modified the C.

(C)Design custom gRNA:

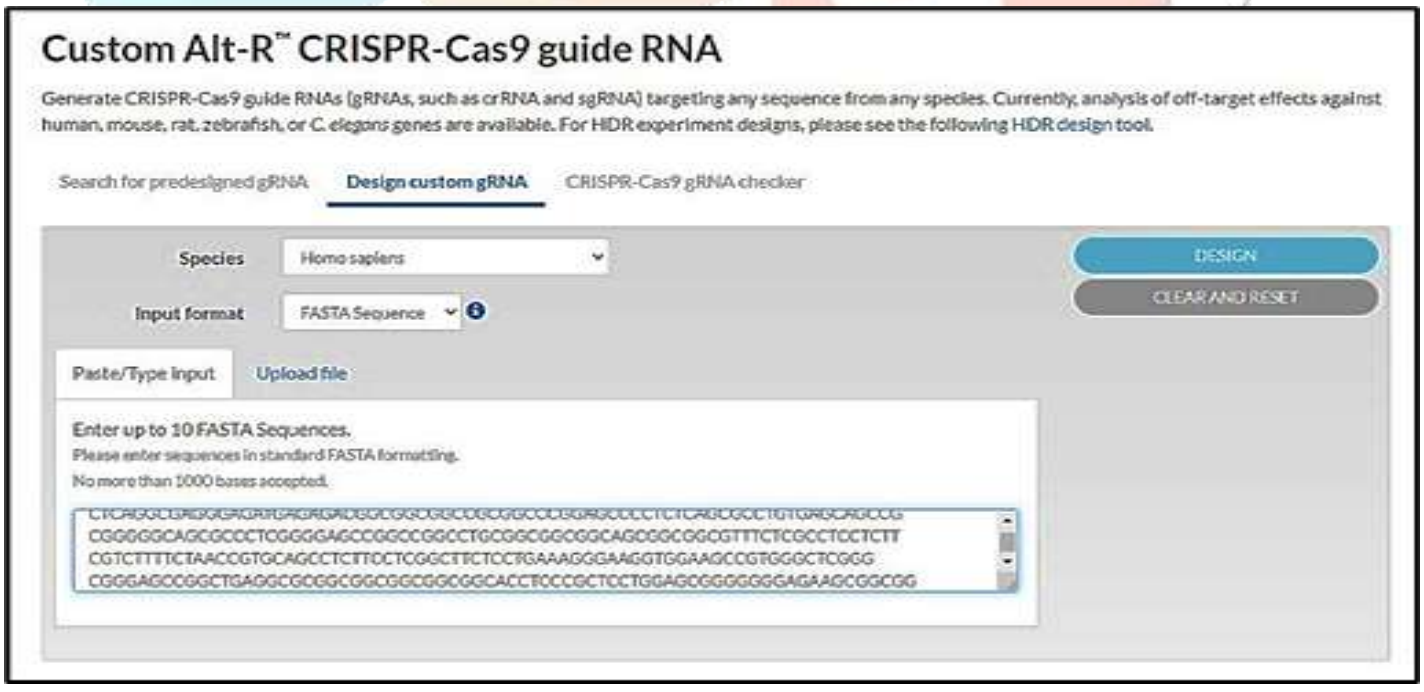


Figure 21: To develop a custom gRNA, use the Custom Alt-R CRISPR-Cas9 guide RNA HDR tool from the Design custom gRNA section. Input format: FASTA sequence of the Homo sapiens PTEN gene. Enter up to ten FASTA sequences.

For Cas9, you may choose between the IDT Alt-R S.p. Cas9 Nuclease V3 (available with or without glycerol, and in fluorescent-labeled—GFP or RFP—or unlabeled formats) and the Alt-R S.p. HiFi Cas9 Nuclease V3 for targeting genomic areas with NGG sequences. The Alt-R S.p. Cas9 Nuclease V3 adequately delivers efficient genome editing for the majority of investigations. For the most accurate editing, utilize the HiFi Cas9 enzyme if you are worried about off-target consequences.

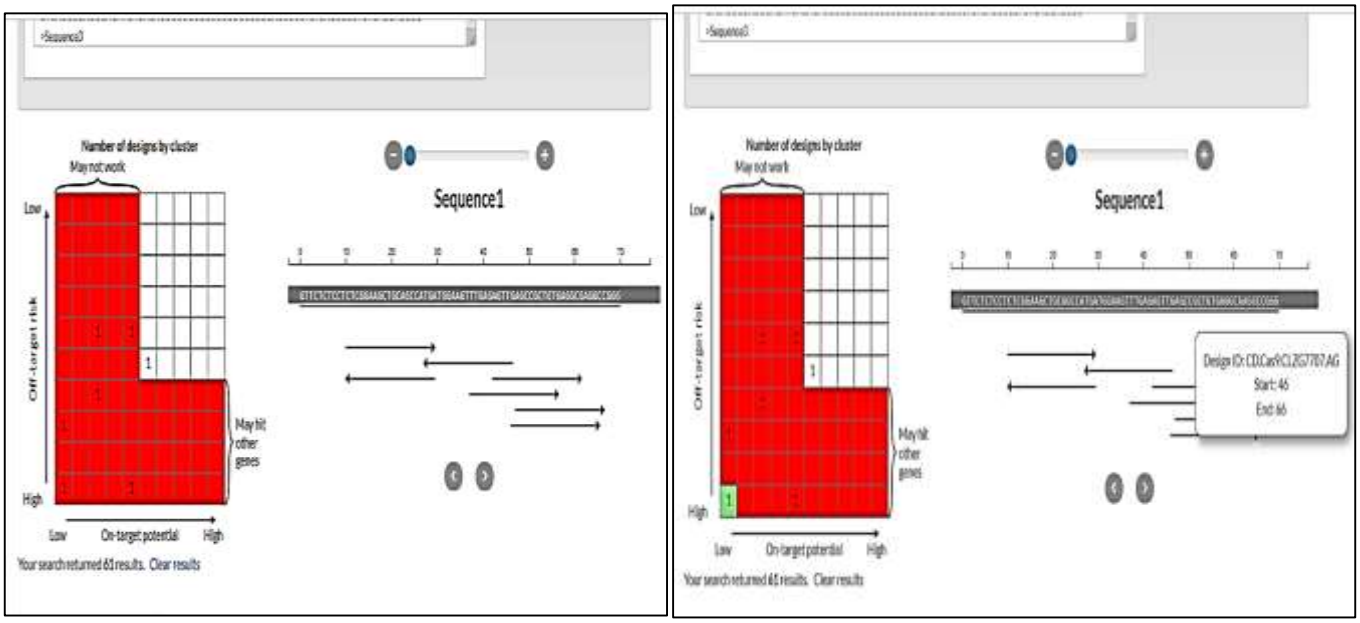


Figure 22: This CRISPR plate has a donor template design and Cas9-guided RNA selection. We then received the graphical estimates. Here on target potential in the lower end and the off-target risk may heat the genes in the sequence of PTEN gene scale.

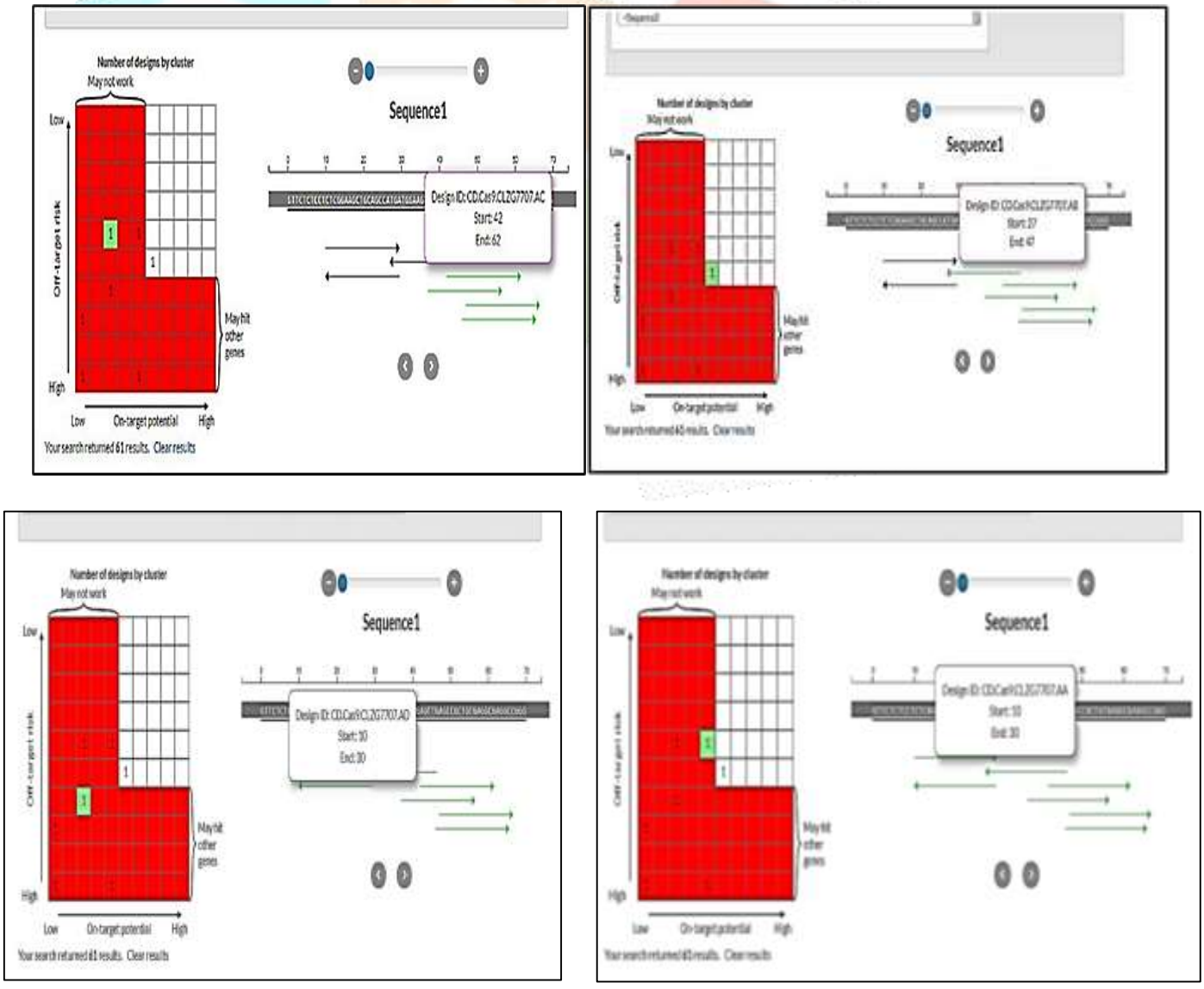


Figure 23: The CRISPR Sequence CD: Cas9 where the 'green' area in all red area has been shown where the DNA break started and ended somewhere in the graph may work or may not. The gRNA has been counted in the Integrated DNA Technology scale.

5. Applications of the CRISPR/Cas9 Technique in Endometrial Cancer :

Gene-targeted treatment for EC has been shown to increase local effectiveness and decrease systemic adverse effects by controlling gene expression in tumor cells and the biologic activity of tumor cells at the genetic level. Lentivirus particles were created by transiently co-transfecting 1 μg of Cas9 or MUC1 gRNA expression vectors (with 250 ng pMD2.G, 750 ng psPAX2, and 6μL Lipofectamine 2000 into HEK293 cells). Multiple drug resistance (MDR) in endometrial cancer hinders therapeutic drugs from passing across the plasma membrane, reducing the efficacy of traditional cancer treatments. Chimeric antigen receptors (CARs) are synthetic immunoreceptors that have recently been employed in endometrial cancer immunotherapy. They function as controllers of malignant cell adhesion to external membrane proteins. The key to gene-targeted treatment is to identify carriers for genetic material that can shield medications or genes from destruction by serum enzymes. They also have biodegradable lipid shells and are safe, efficient, and non-toxic. Ultrasound cavitation boosts the efficiency of gene transfer.

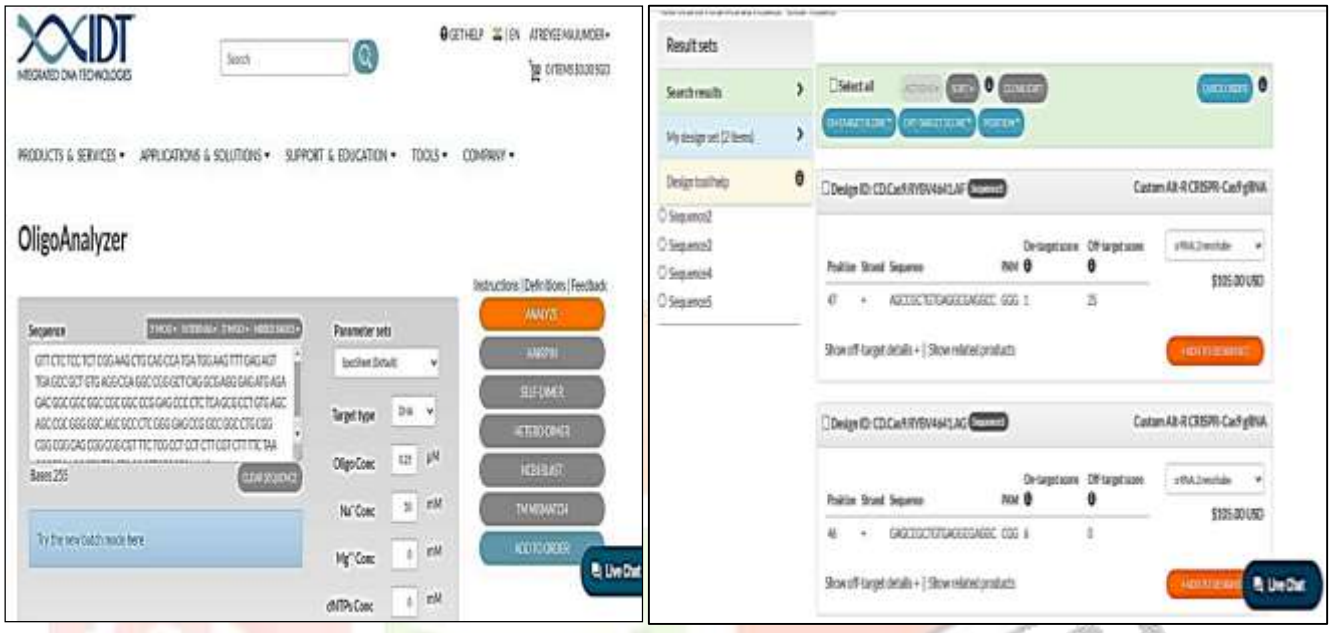


Figure 27: After sequencing done, we put the FASTA sequence in the OligoAnalyzer to analyses the CRISPR-CAS9 gRNA.

This is the analyses result to design the we have to post that are not for the develop.

Conclusion: In the cancer immunotherapy, the overall cancer management approach is critical for diagnosis and treatment. Its major goal is to cure endometrial cancer patients or greatly prolong their lives while maintaining an acceptable standard of living in isolation. It must be linked to an early detection system in order to detect cases at an early stage, when treatment is more effective and cure rates are greater. CRISPR/Cas9 holds enormous potential for treating drug-resistant for endometrial cancers and fixing tumor-causing mutations in human genomes. CRISPR is chosen over other genome editing technologies due of its inexpensive cost, as well as its specificity and simplicity of synthesizing gRNA to match particular targets. Moreover, as immunotherapy becomes more successful in a variety of tumors due to its link with the genome editing system, several ongoing clinical studies are looking into CRISPR/Cas9 in the context of endometrial cancer-resistant immunotherapeutic drugs to help the immune system attack malignancies. CRISPR systems are also used in animal models to identify novel mutations and candidate genes. Incorporating CRISPR into endometrial cancer treatment and research has demonstrated great promise both *in-vitro* and *in-vivo*. However, various constraints must be addressed in the future to identify the full potential of this approach of target site mutations, such as generic limits on DNA upstream of the Protospacer Adjacent Motifs (PAM), a lack of ethical agreements, and no scientific consensus. If the CRISPR/Cas9-C-erbB-2 in plasmid has been shown to significantly reduce C-erbB-2 protein synthesis in HEC-1A cells. It might give a novel target for gene therapy's frightening risk assessment and EC prognosis. All forms of endometrial cancer where the CRISPR/Cas9 gene-editing approach of gene therapy may be developed can greatly increase survival by treating curable the endometrial cancers. The overall cancer management plan is vital and beneficial for diagnosis and treatment. Its major goal is to cure cancer patients or greatly prolong their lives while providing

an acceptable quality of life in isolation. It should be linked to early detection. Additionally, IDT offers two Cas9 Nickase: Alt-R S.p. Cas9 D10A Nickase V3 cuts a single strand of DNA, while Alt-R S.p. Cas9 H840A Nickase V3 makes a single-strand cut in the non-targeted strand of DNA. In genome editing studies, a single Nickase is combined with two guide RNAs to generate a double-strand break important in these Endometrial cancer protection treatment.

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