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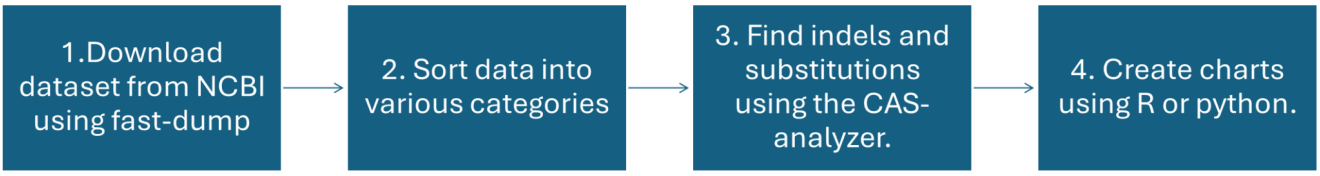
A CRISPRi-Seq screen for functional assessment of BRCA1 mutants.

Background/Introduction: The BRCA1 gene plays a crucial role in maintaining genome stability within cells. Mutations affecting this gene have been associated with potential health issues such as breast, ovarian, prostate, and pancreatic cancer due to the loss of its normal function [1]. Despite progress in identifying various BRCA1 mutations, there remains a lack of comprehensive pathogenicity studies due to various constraints [2]. Consequently, the CRISPR-Cas9 system was devised to facilitate precise genome editing within living cells and organisms, aiming to address this limitation [3].

Significance: This study assesses BRCA1 variant pathogenicity using CRISPR-Cas9 BE3, analyzing genetic impact on cell viability. BE3's targeted substitutions offer a precise evaluation [4,5], particularly for large genes like BRCA1. This study identifies pathogenic mutations in BRCA1's 5′-UTR, affecting gene expression. Findings offer insights for improved diagnostics and therapies for hereditary cancers.

Aim: The aim of this project is to utilize a CRISPRi-Seq screen to comprehensively assess the functional consequences of various BRCA1 mutants implicated in breast and ovarian cancers. By leveraging bioinformatics tools and experimental techniques, we aim to identify which mutations of BRCA1 have the most significant impact on DNA repair and genomic integrity, thereby elucidating key molecular mechanisms underlying cancer susceptibility.

Pipeline: Datasets were retrieved from the NCBI utilizing fastq-dump, and the accession numbers were obtained through the go to option on the NCBI platform. Subsequently, the dataset names were gathered and compiled into a txt file containing the sample accession numbers. A while loop incorporating the fast-dump command with the split-files option was created. The cas-analyzer was then employed to analyze these datasets, enabling the identification of respective samples by referencing the NCBI database. This process facilitated the search for indels and substitutions within the data.



Results in Brief /Discussion: Our work revealed that a BRCA1 gene responsible for cancer suppression with loss of function is harmful due to gene mutation. However, with the introduction of the CRISPR cas-9 - BE3 system, BRCA1 function was restored. It is also a potent tool for reclassifying variants of uncertain significance (VUSs) in BRCA1. This study opens avenues for further research in functional assessment of BRCA1 variants. Future investigations could explore the application of CRISPR-based base editing in larger cohorts to validate the pathogenicity of identified variants and elucidate their roles in cancer.

References

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