CRISPR gene editing technologies enable DNA to be altered at precise locations

A complex of a single guide RNA (sgRNA) and a nuclease (here Cas9) is introduced into a cell. The sgRNA contains a region of ~20 nucleotides which binds to the complementary DNA region.

The target DNA must contain a protospacer adjacent motif (PAM). CRISPR nucleases differ in their PAM requirements and cutting location. Cas9 recognises PAMs with the sequence "NGG", downstream of the target region and cuts both strands of DNA ~3 bases upstream of the PAM.

Our R/Bioconductor package CrispRVariants allows researchers to evaluate and visualise sequences resulting from gene editing experiments. We have also developed a Shiny web application CrispRVariantsLite which implements a complete analysis pipeline for small, standard data sets.

CRISPR technologies are evolving rapidly. Our ongoing work focuses on detecting and removing sources of error in CRISPR sequencing analyses as well as allowing users increased flexibility to specify custom variant counting and filtering methods.

One way researchers assess the outcome of a CRISPR gene editing experiment is by sequencing the targeted DNA region.

Above: sequenced reads from an edited CRISPR target region aligned to the original genome sequence.

Recent developments in CRISPR technology enable repeated mutation at a single locus. In applications such as cell line banking, researchers aim to determine the order of repeated mutational events. To date, studies have focused on the location of insertions and deletions in manipulating the order of events. However, sequencing errors and naturally occurring variation can affect how sequence aligns place insertions and deletions. The figure to the right shows four sequences which are identical within the region of interest despite differences in alignment. The figure below shows the complete sequences of the first two of these, which differ only in the presence of a single "R" outside the region of interest.

In the latest version of CrispRVariants, we added support for visualising subregions of interest from within a large target region. This figure shows results from a paired CRISPR experiment where two separate sgRNAs were introduced simultaneously. Alignments that do not span the entire target region are indicated with "-".