CRISPR or RNAi Screening helps to uncover key therapeutic candidates

Identifying Genes that Control Functional Changes: Scientists often use RNA interference (RNAi) and CRISPR to identify candidate genes required for various biological responses such as differentiation, drug sensitivity or resistance, and other phenotypes.

**Design and Introduce Libraries:** Scientists design short RNA sequences—either short-hairpin RNA (shRNA) or single-stranded guide RNA (sgRNA)—to target and perturb any set of genes. DNA encoding these short RNA sequences are then packaged into a library of different viral particles that is introduced into a population of cells. Different gene perturbations cause different responses in some cells to therapeutic treatments, disease activation, or other manipulation. The candidate genes that drive these responses provide putative targets for drug therapies and treatments.

**RNAi (Knock-down):** After the lentivirus with the sequence coding for the shRNA is reverse-transcribed and integrates into the genomic DNA, it begins producing the short-hairpin (shRNA) sequence. The product is then processed by the Dicer protein which cleaves the hairpin to create two hybridized RNA sequences (siRNA). The RNA-Induced Silencing Complex (RISC) then degrades the antisense strand and the sense strand guides RISC to the complementary target mRNA sequence where it rapidly degrades the target mRNA transcript.

**Screen Results:** For a large-scale screen of thousands of genes, the library lentiviral vectors integrate randomly into cells across the population, introducing a different shRNA or sgRNA to each cell. Scientists can then expose these cells to a particular drug or other treatment and observe which cells remain viable post-treatment.

Some gene modifications may confer an advantage, others a disadvantage. Next-generation sequencing (NGS) analysis of the shRNA or sgRNA in the remaining viable cells allows scientists to identify which genes are required for the response of interest.

**CRISPR (Knock-out):** The lentivirus encoding the sgRNA integrates into the genomic DNA and produces the sgRNA sequence. The sgRNA sequence then hybridizes with the genomic DNA target. To disrupt its target, CRISPR requires the Cas9 nuclease. The Cas9 protein binds to the sgRNA hybridized to its genomic target. This CRISPR-Cas9 complex then cuts the genomic DNA. Faulty repair of this lesion results in an insertion or deletion (an “indel”) that silences the targeted gene. As a result, CRISPR-sgRNA can completely eliminate or “knock out” the target gene’s expression in the cell.

**Identify Candidates:** High-throughput sequencing of pooled sgRNA or shRNA sequences isolated from the screened populations of cells provides a way to quantify changes in the fractions of cells harboring each sgRNA or shRNA. This data can be used to assess which sgRNA or shRNA associated to individual genes affect cell viability or other cellular response used for the screen.

Many scientists now couple screening experiments with single-cell sequencing technologies such as CRISP-seq, Perturb-seq, and CROP-seq. By barcoding shRNA or sgRNA libraries before transduction, scientists can identify the causal gene alteration from a given treatment and track the transcriptomic changes that result. Combining these technologies helps reveal how a specific gene acts to alter a cell’s biology and produce the response. This facilitates high-throughput functional assessments of complex biological pathways to reveal a plethora of candidate genes and therapeutic targets.