A Validation Process for Putative Target Genes Identified by Genome-wide CRISPR-Cas9 Screening

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Abstract

CRISPR-Cas9 technology has revolutionized genetic screening allowing genome-wide screening for loss of function mutations. This is achieved by using delivery of pooled lentivirus expressing guide RNAs that are barcoded to facilitate deconvolution. Screening then utilizes phenotypic selection to enrich the desired pool of knock out cells identifying genes that have an effect of the observed phenotype. Consequently, such screens identify many genes which impact the observed phenotype resulting in lists of potential genes modulating that can be quite large (even as many as 1000 genes).

Previously this challenge had been addressed by cloning individual guides from the putative hits for initial validation. To confirm the genetic ablation of the targeted gene, the knock out clone needs to be expanded and genomic DNA purified to allow Sanger sequencing and TIDE analysis.

This report describes a streamlined process that utilizes RNP delivery of Cas9 protein and two guides, which target a putative hit approximately 100bp apart (within the same exon) and in different reading frames. This brings about deletion and downstream frame shift. Deletions are easily detected with a simple PCR amplification of the target region. In addition, blunt ended oligonucleotides that encode stop codon in all reading frames are introduced to ensure ablation of the target gene. This process allows gene knock out without the need for cloning, lentivirus packaging and Sanger sequencing to characterize the resultant cells. Surprisingly, the ablation of the gene targets was often more successful than would be expected from the relative abundance of the deletion. Direct colony sequencing shows that in addition to deletions, single editing events and the introduction of indels help to achieve the extremely efficient ablation of the target genes.