

A CRISPR/CAS9 BASED ENGINEERING TOOL TO ACTIVATE EXPRESSION OF MULTIPLE GENES INDIVIDUALLY OR IN ANY SPECIFIC COMBINATION

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Engineering of cells by overexpression or knock-down/out of individual genes has demonstrated that in most cases the manipulation of single genes is not sufficient to alter a cellular phenotype. Rather, multiple genes involved in a pathway need to be manipulated. Especially in mammalian cells such as CHO, where clonal variation is large, it has been difficult to unequivocally assess whether the observed change in phenotype is due to such clonal variation or the engineered gene. This can in part be overcome by testing multiple subclones, however, once it comes to engineering multiple genes and combinations thereof, the required workload quickly becomes prohibitive.

We here present a simple technology for successive and/or specific activation of multiple genes integrated into a single genomic locus, which presents a potential solution to this problem.

The technology consists of a vector containing multiple genes to be engineered or copies of the same gene. The promoters of these genes/gene copies are separated from the translation start site by repressor elements, flanked by individual guide RNA (gRNA) target sites. After integration of the construct into the genome and clone selection, these repressor elements can be removed by transfection with Cas9 and the corresponding pair of gRNAs that target the repressor of the gene(s) to be activated. Efficiency of target gene activation was in the range of 20-30% of the population for individual genes. Using 4 different fluorescent genes, the success of the technology was shown by activation of different combinations of these genes, followed by sorting of cells with the correct combination of required target genes activated.

For pathway engineering studies, the selected genes can be expressed linked to these fluorescent genes e.g. via an IRES or a 2A self-cleaving peptide and cells with the desired co-expression pattern sorted, thus obviating the necessity to subclone for subsequent phenotypic characterization of the engineered cells. The technology provides a rapid procedure to assess the effect of gene combination on cellular behavior.

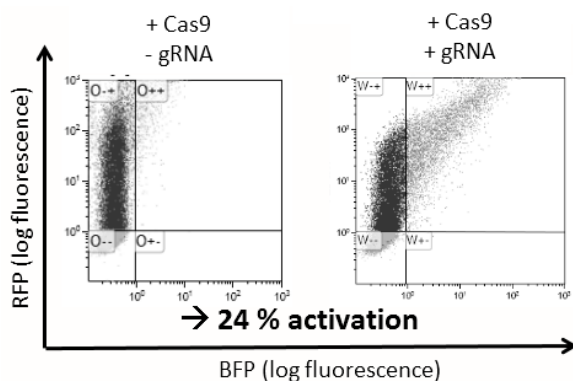


Figure 1 – Flow cytometry analysis of transfected CHO cells.

Y-axis: log fluorescent signal of the RFP

x-axis: log fluorescent signal of BFP

Left: CHO cells transfected with RFP, BFP, where the BFP gene is repressed.

Right: CHO cells as on the left after transfection with Cas9 and the respective gRNA