**Activation of pluripotency genes using CRISPR/dCas9 system**

**M. Vilarino1, J. Meckler2, D.J. Segal2 & J.P. Ross1**

1Department of Animal Science, University of California, Davis, CA.
2Genome Center, University of California, Davis, CA.

Successful reprogramming of differentiated human somatic cells to a pluripotent state can be accomplished by ectopic expression of reprograming factors and includes reactivation of endogenous pluripotency related genes. In order to bypass the need for overexpressing exogenous factors we used CRISPR/dCas9 to induce reactivation of endogenous reprogramming genes. To accomplish this, a mutated Cas9 (dCas9) without endonuclease activity was fused to the transcriptional activation domain VP64.  dCas9-VP64 and gRNA constructs targeting upstream promoter sequences of OCT4, SOX2, NANOG, c-MYC and KLF4 were co-transfected using Lipofectamine 2000 into HEK293T cells. For each of reprogramming gene, we used five gRNAs within 1kb of the transcription start site. Two days following transfection the expression of the target genes was evaluated by real-time RT-PCR analysis. Transfection efficiencies were determined by fluorescence microscopy after delivery of a control GFP expression plasmid. The results are expressed as fold increases in mRNA expression of the gene of interest normalized to HPRT expression. We observed gene activation in all cases when each gene was individually targeted (4.9, 25.7, 40.7, 55.8 and 3.4 fold changes for OCT4, SOX2, NANOG, KLF4 and c-MYC, respectively). Then, we performed multiple gene activations by co-transfecting all gRNAs simultaneously (5 genes, 25 gRNAs). We again observed gene activation in all cases (13, 4.0, 36.8, 12.9 and 2.1 fold changes for OCT4, SOX2, NANOG, KLF4, and c-MYC, respectively). These results demonstrate that activation of multiple endogenous genes simultaneously is possible, and support the hypothesis that CRISPR/dCas9 system could be used to reprogram somatic cells to a pluripotent state.