Cloning Grnas to Enable Crispr-Mediated Human Gene Knockouts

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The human genome encodes over 80 members of the tripartite motif (TRIM) protein family. Many of the TRIM proteins are upregulated in cells responding to interferon-beta (IFNβ). Interestingly, screens have shown that approximately half of the TRIM proteins act on interferon signal transduction pathways in positive and negative feedback loops. Cell lines are being created that can be used as tools for identifying the step of IFN signaling acted upon by TRIM proteins. To create tools for this goal, we have cloned guide RNAs targeting IFNB, IFNAR1, IRF3, and IRF7 genes into the pSpCas9(BB)-2A-EGFP vector using recombination techniques. Success of cloning was confirmed by restriction digests and DNA sequencing. Human cells (293T) were successfully transfected and are currently being cultured to attain monoclonal EGFP+ lines. Screening for loss of gene expression in clonal lines will assessed by immunoblotting as well as tested functionally using luciferase reporter assays.