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Technical Data Sheet: MCF7 dCas9-KRABCell Line

ATCC® Number	HTB-22dCas9-KRAB™
Organism	*Homo sapiens,* human
Tissue/Disease Source	Breast, adenocarcinoma
Product Description	MCF7 dCas9-KRAB was created by knocking-in a KRAB-dCas9 (from *S. pyogenes*) expression cassette into the safe harbor AAVS1 locus using CRISPR/Cas9 gene editing technology. This cell line stably expresses KRAB-dCas9, RFP.
Application	Functional evaluation of HTB-22dCas9-KRAB shows greater than 50% gene repression can be achieved for p53 and SETD9 genes when their respective gRNAs were delivered into the cells. MCF7 dCas9-KRAB can be used as a tool for loss-of-function genetic studies.

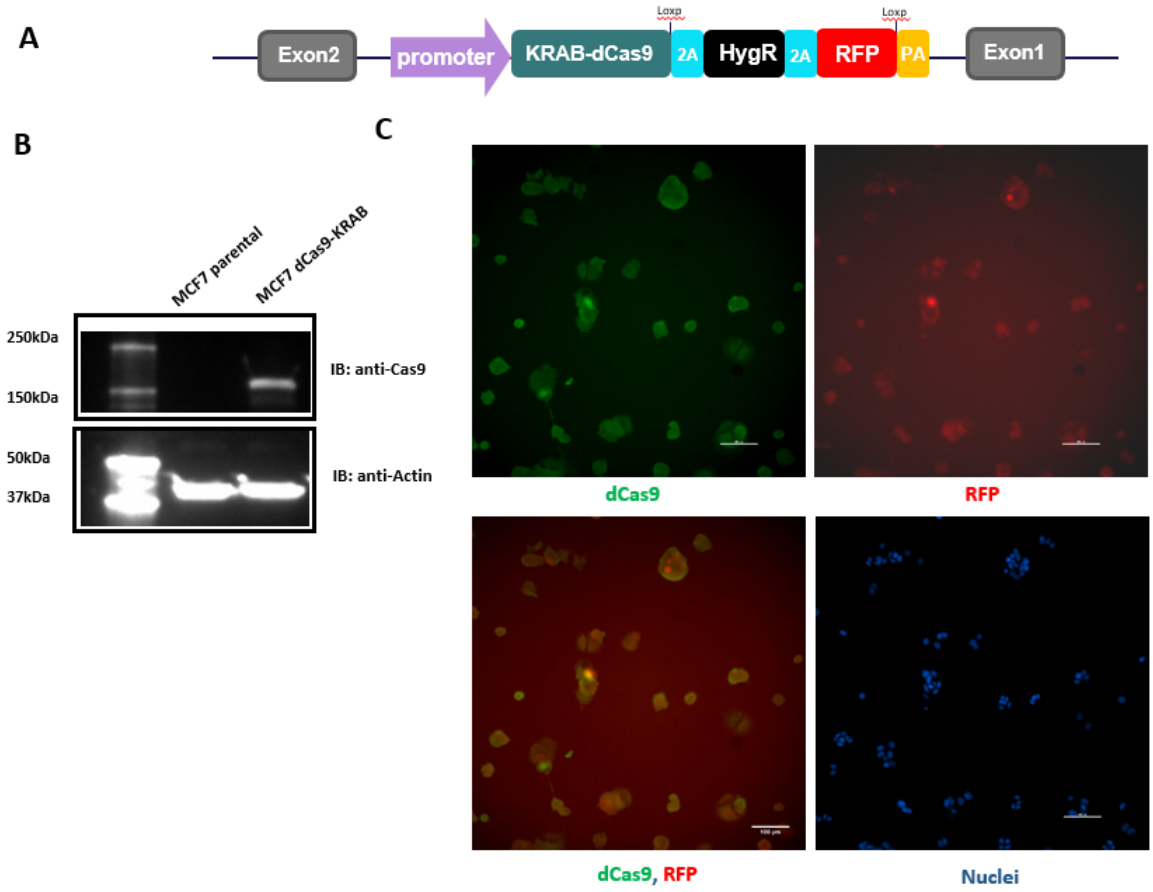

Figure 1. Generation of MCF7 dCas9-KRAB (ATCC® HTB-22dCas9-KRAB™). (A) Schematic of AAVS1 dCas9-KRAB expression knock-in cassette, showing RFP gene and hygromycin selection marker. (B) Detection of dCas9 protein expression by Western blotting in MCF7 dCas9-KRAB cells, but not in parental cells. (C) Co-localization of dCas9 protein (green; top left) and RFP protein (red; top right) in 293[HEK-293] dCas9-KRAB cells. The overlay image (bottom left) indicates dCas9 and RFP are expressed in the same cells. The nuclei of cells were stained with DAPI (blue, bottom right).

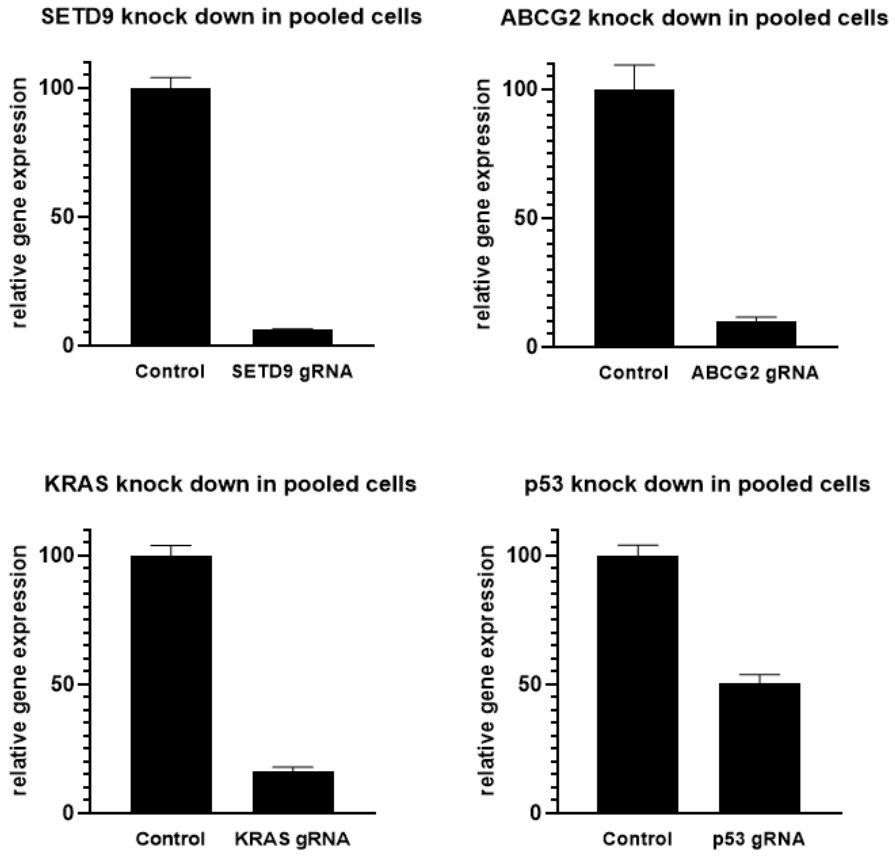

Figure 2. Validation of gene expression knock down in MCF7 dCas9-KRAB cells (ATCC® HTB-22dCas9-KRAB™). Repression of SETD9, ABCG2, p53, and KRAS gene expression. Lentivirus expressing gRNAs targeting SETD9, ACBG2, p53, and KRAS gene were used individually to infect MCF7 dCas9-KRAB cells. Lentivirus without gRNA expression was used as the control. 24 hours after infection, antibiotics was added to the culture media to enrich antibiotics resistance cells. Cell pellets were collected after 5 days selection and subject to ddPCR gene expression quantification analysis. The expression of SETD9, ABCG2, p53, and KRAS genes were significantly repressed in cells infected with gRNAs.