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Technical Data Sheet: 293[HEK-293] Cas9Cell Line

ATCC® Number	CRL-1573Cas9™
Organism	*Homo sapiens*
Tissue/Disease Source	Carcinoma
Product Description	293[HEK-293] Cas9 Cell Line
Application	293[HEK-293] Cas9 cell line was created by knocking-in Cas9 (from *Streptococcus pyogenes*) expression cassette into the AAVS1 safe harbor locus using CRISPR/Cas9 gene editing technology. This cell line stably expresses Cas9, red fluorescent protein (RFP), and the neomycin resistance gene. In addition, this cell line also contains Loxp, which was introduced to flank the entire knock-in sequence, and FRT, which was introduced to flank the neomycin expression cassette. CRISPR/Cas9 mediated DNA cleavage relies on the co-expression of a Cas9 protein and gRNA(s). While Cas9 is a DNA cutting protein, the cutting specificity is largely determined by sequence specific gRNAs. Since HEK-293 Cas9 cells stably express Cas9 protein, high efficiency DNA cleavage can be achieved when gRNAs alone are delivered into the cells. HEK-293 Cas9 cells can be used as a tool for evaluating gRNA activity, creating knock-out and knock-in alleles, and CRISPR based loss-of-function screening.

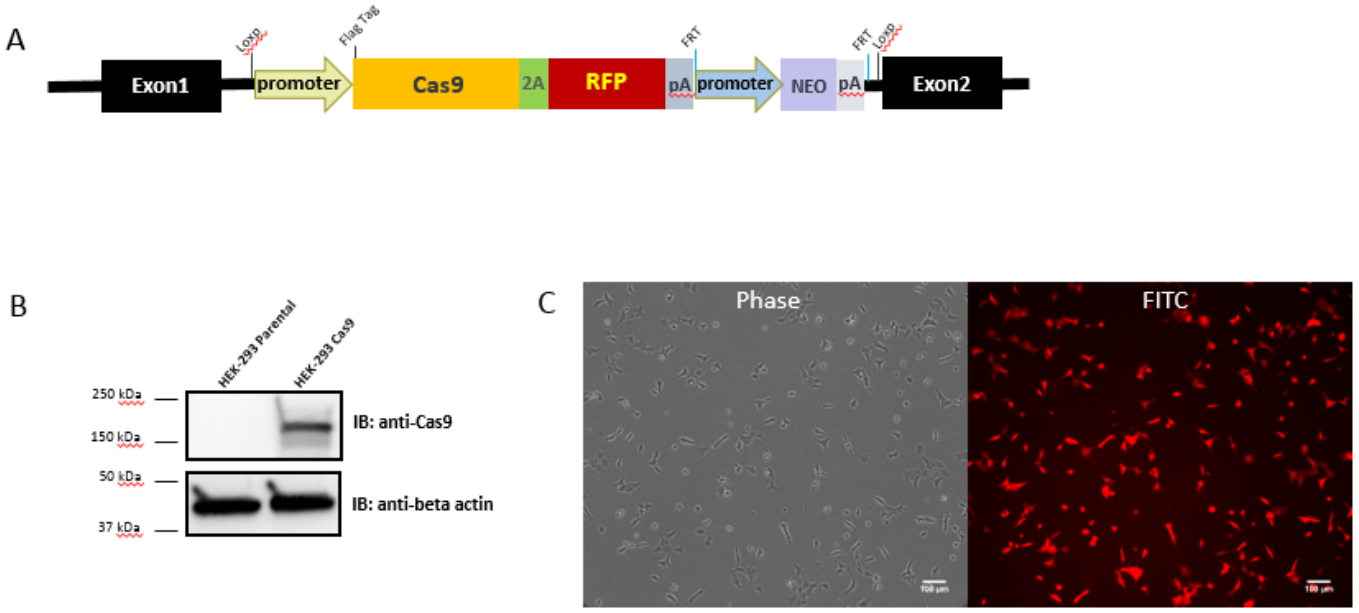

Figure 1. Cas9 expression cassette knock-in allele at AAVS1 locus in HEK-293 Cas9 cell line (ATCC® CRL-1573Cas9™). A) Schematic of Cas9 expression knock-in cassette at AAVS1 locus, showing RFP gene and neomycin selection marker. B) Detection of Cas9 protein expression by Western blotting in HEK-293 Cas9 cells, but not in HEK-293 parental cells. C) Comparison of phase image (left) and TRITC image (right) showing HEK-293 Cas9 cells expressing the RFP gene.

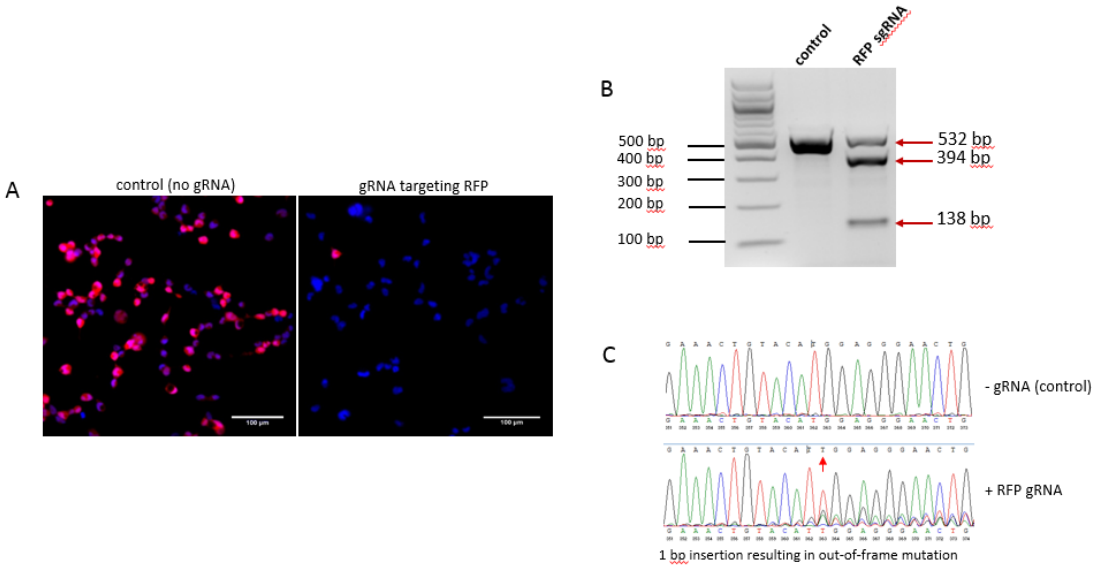

Figure 2. Confirmation of Cas9 activities in HEK-293 Cas9 cells. A) HEK-293 Cas9 cells were transduced with lentiviruses that do not express gRNA (left, as a no gRNA control) or express gRNA targeting RFP (right, gRNA expressing cells). 6 days after transduction, RFP expression in gRNA expressing HEK-293 Cas9 cells was significantly reduced compared to control cells. The nuclei of cells were counterstained with DAPI (blue). B) T7E1 assay showed the expected DNA cleavage patterns in gRNA expressing HEK-293 Cas9 cells, but not in control cells. C) Sanger sequencing of amplicons from (B) revealed that a 1 bp insertion (indicated by red arrow) was introduced into the RFP gene of gRNA expressing HEK-293 Cas9 cells, resulting in an out-of-frame mutation of the RFP gene.