Enhancement of Viral Titers for Vectors Encoding shRNA^{miR}s via *DROSHA* Knockout

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RNAi-based gene therapy using shRNA^{miR} is a powerful approach to modulate gene expression. However, we have observed low viral titers with shRNA^{miR} vectors and hypothesized that this could be due to cleavage of viral genomic RNA by the endogenous microprocessor complex during virus assembly. We designed gRNA CRISPR/Cas9 constructs targeting *DROSHA* and successfully generated knockout (KO) HEK293T cells. We produced lentiviral vectors containing Venus with or without shRNA hairpins and generated virus using *DROSHA* KO packaging cells. We observed an increase in the hairpin-containing Venus transcripts in *DROSHA* KO consistent with reduced microprocessor cleavage of encoded mRNA transcripts, and recovery in the viral titer of hairpin vectors. We confirmed the absence of shRNA^{miR} processing by Northern blot and this correlated with an increase in the full-length vector genomic RNA. From rescue experiment, re-expression of WT DROSHA in *DROSHA* KO cells led to reduction in viral titer. These findings may have important implications in production of viral shRNA^{miR} vectors for RNAi-based therapy.