Optimal CRISPR gene editing relies on effective Cas9 nuclear localization [1]. Previous work in our lab has shown that the SV40 promoter is poorly recognized in an Oreochromis mossambicus cell line [2] (Omb cells) compared to endogenous promoters, leading us to suspect that the SV40 NLS is also poorly recognized (Figure 1). Honda et al. showed distinct nuclear localization with the tilapia Neu4 using IHC [3].

We hypothesize that the endogenous Neu4 NLS will impart more efficient nuclear localization than the conventional mammalian SV40 NLS.

METHODS

To test this hypothesis, we cloned the SV40 and three versions of the Neu4 NLS to the C-terminus of EGFP in a custom expression plasmid (Figure 2), transfected into Omb cells, using viafect, and imaged after 16 hours using fluorescent microscopy. We applied DAPI to define nuclear boundaries.

RESULTS

Figures 3a-3c. Results of the initial transfection with just GFP. The images show that the GFP not only worked, but strongly implies that there is localization using the SV40 NLS. The experiment was then repeated using DAPI to confirm the GFP enrichment is within the nucleus. A red pseudo color was used in place of the blue DAPI filter to better show any overlap of the DAPI and GFP filters.

Based on our imaging results the SV40 is clearly the superior NLS for nuclear localization over the Neu4 NLS variations in the OMB cell line.

Downstream application using the SV40 NLS showed it imparted sufficient nuclear localization to support efficient CRISPR/Cas9 gene editing in Omb cells when fused to Cas9 (Figure 7).

Sequencing of CRISPR treated OmB cell DNA using a validated Nanos3 gRNA for proof of principle, showed 17 out of 21 mutations at the targeted genomic loci.

REFERENCES

