# Rewiring PAM Specificity of SpdCas9 for Gene Repression

Li, Michelle (School: North Oconee High School)

The CRISPR Cas9 Ribonucleoprotein (RNP) complex is well known for its precise gene editing abilities. Catalytically dead Cas9 (dCas9) also has diverse applications, including base editing, CRISPR interference (CRISPRi), and CRISPR activation (CRISPRa). Unfortunately, one stringent limitation in the most widely used Cas9, SpCas9 (derived from Streptococcus pyogenes), is its strict 5'-NGG-3' PAM requirement that restricts targeting range on genomic DNA. In order to target 5'-CAT-3' PAM, the reverse complement of ATG, variants of deactivated SpdCas9 (SpdCas9) were engineered and verified to be a universal gene repressor targeting the start codon. Using the already PAM-expanded SpdNG variant (5'-NG-3' PAM), stepwise structure-guided mutations of the PAM interacting and proximal residues residues yielded a variant which retained 5'-NGG-3' PAM specificity and had new 5'-CAT-3' PAM specificity, both with and without nuclease activity. In addition, this variant was tested in various genetic contexts with the added advantage of customizable sgRNA design and tunability. The variant described here is also relevant to other Cas9 or dCas9 based applications, and is a promising asset in the future of gene editing.

**Awards Won:**
Second Award of $2,000