

**GRADUATE SCHOOL OF BIOMEDICAL SCIENCES**

**BIOCHEMISTRY AND MOLECULAR PHARMACOLOGY**

**Ph.D. THESIS DEFENSE**

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MENTOR: Scot Wolfe, PhD

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"DEVELOPMENT OF CHIMERIC CAS9 NUCLEASES FOR ACCURATE AND FLEXIBLE GENOME EDITING"

Type II CRISPR-Cas9 bacterial adaptive immunity systems have recently been engineered as RNA-guided programmable nucleases. Native CRISPR-Cas9 nucleases have two stages of sequence-specific target DNA recognition prior to cleavage: the intrinsic binding of the Cas9 nuclease to a short DNA element (PAM) followed by testing target site complementarity with the programmable guide RNA. The ease of reprogramming CRISPR-Cas9 nucleases for new target sequences makes them a favorable genome-editing platform for many applications including gene therapy. However, wild type Cas9 nucleases have limitations: (i) the PAM element requirement restricts the targeting range of Cas9; (ii) despite the presence of two stages of target recognition, wild type Cas9 can cleave DNA at unintended sites; (iii) there is lack of control over the mutagenic editing product that is generated.

In this study, we developed and characterized chimeric Cas9 platforms to provide solutions to these limitations. In these platforms, the DNA-binding affinity of Cas9 protein from *S. pyogenes* is attenuated such that the target site binding is dependent on a fused programmable DNA-targeting-unit that recognizes a neighboring DNA-sequence. This modification extends the range of usable PAM elements and substantially improves the targeting specify of wild type Cas9. Furthermore, one of the featured chimeric Cas9 variants developed in this study has both robust nuclease activity and ability to generate predictable uniform editing products. These superior properties of the chimeric Cas9 platforms make them favorable for various genome editing applications and brings programmable nucleases one step closer to therapeutic application.

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