GUEST SPEAKER SEMINAR

Molecular Genetics
UNIVERSITY OF TORONTO

Delving into the diversity of Cas9 orthologs for biotechnology applications

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Cas9 nucleases are abundant in microbes. To explore this largely uncharacterized diversity of orthologs for biotechnology and genome editing applications, we established a phylogeny-guided bioinformatic approach to select Cas9s from sequenced organisms and sampled identifiable CRISPR systems in metagenome sequence to select candidates. We developed and applied biochemical screens for the rapid identification and characterization of the protospacer adjacent motif (PAM) and guide RNA (gRNA) requirements of new Cas9 proteins. Using this approach, we characterized over 80 Cas9 orthologs with more than 50 distinct PAM sequence requirements. The set contained nucleases that generate staggered-end breaks and nucleases that require longer spacers to function robustly *in vitro*. In addition, it contained Cas9s that are active at narrow and broad temperature ranges. To establish broad applicability for use as genome editing tools, cellular activity was examined in both plant and human cells. Of those examined, several were capable of generating targeted chromosomal mutations and some exhibited robust mutational activity approaching that of *Streptococcus pyogenes* Cas9. Our results demonstrate that the diversity of Cas9 orthologs provides a rich source of PAM recognition and other potentially desirable properties that may be mined to expand the biotechnology toolbox with new programmable nucleases.

Host: Dr. Julie Claycomb

Date: Friday, February 28th, 2020
Time: 3:00 PM
Place: Donnelly Centre, Red Room