

Enhancing the efficiency of CRISPR-Cas9 based gene editing in *Chlamydomonas reinhardtii*

Nidhi Kulkarni¹ and David J. Vinyard¹

¹*Department of Biological Sciences, Louisiana State University, Baton Rouge, Louisiana 70803, USA. nkulka1@lsu.edu.*

Gene editing in *Chlamydomonas reinhardtii* using the CRISPR-Cas9 system is still an emerging field. Although several successful CRISPR-Cas9 mediated mutations have been done, editing efficiency varies widely among target genes with reported efficiencies of 0.17-96%. The gene editing efficiency depends on several factors – those that inherently make certain genes difficult to target due to chromatin inaccessibility and topology, and those that can be externally modulated like recruitment of enzymes involved in double strand break repair, maintaining ion balance and recovery post electroporation. Among many factors that affect the latter, we show that magnesium is critical. We added different concentrations of magnesium to electroporated cells and tested resulting transformation efficiencies. We report that supplementing electroporated cells with 1 mM to 5 mM magnesium can increase the efficiency of CRISPR-Cas9-based gene editing up to 50%.