GERG Study 2019-2020: Reproducibility of indel formation rates by comparing guideRNA format and delivery method

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Abstract

Recent advances in genome engineering are allowing scientists to better understand biology by precisely deleting, editing, or tagging genomic DNA. The clustered regularly interspaced palindromic repeats (CRISPR)/CRISPR-associated (Cas) system was first used to edit mammalian cells in 2013 and has grown in popularity ever since. Multiple guideRNA and Cas9 reagents formats can be used for editing cells. In this study, we compared three popular methods: 1. a plasmid expressing both the guideRNA and Cas9, 2. Cas9 protein combined with a synthetic single guideRNA, and 3. Cas9 combined with a 2-part guideRNA. In addition, the CRISPR/Cas system can be delivered to cells via lipofection or nucleofection transfection methods. This study aims to compare the efficiency of gene editing outcomes at 3 different genomic targets, 3 unique guideRNA reagent formats, and 2 delivery method across multiple labs. For the 2018 GERP study, the group performed a pilot study and found that the results varied considerably across the 4 sites. Three possible sources of the variation are: 1. researchers had different levels of experience with the different methods, 2. the provided protocols (from the companies) were challenging to understand, and 3. each researcher only performed one replicate. In 2019, we wrote a standard protocol and repeated the experiments multiple times to more accurately evaluate the reproducibility of these methods. Determining which CRISPR reagent format is the most reproducible and has the highest gene editing outcomes will be beneficial for core facilities or research labs getting started with genome editing.

Study Design

Plasmid Lipofection vs. Nucleofection

Figure 2. For this experiment, we performed transfections with three guides that were previously determined to have low, medium, or high efficiency. A one-part gRNA that is comprised of both the crRNA and tracrRNA was obtained from Synthego. A RNP was formed with Cas9 protein. The plasmids were delivered into 293 cells via either lipofection (LipoD293) or nucleofection (Lonza nucleofector IIB or 4D). We used 1ug of plasmid for lipofection and 0.5ug for nucleofection (4D).

1-Part sgRNA Lipofection vs. Nucleofection

Figure 4. For this experiment, we performed transfections with three guides that were previously determined to have low, medium, or high efficiency. A one-part gRNA that is comprised of both the crRNA and tracrRNA was obtained from Synthego. A RNP was formed with Cas9 protein. The plasmids were delivered into 293 cells via either lipofection (LipoD293) or nucleofection (Lonza nucleofector IIB or 4D). We used 3 pmol sgRNA and 3 pmol Cas9 for lipofection and 180 pmol sgRNA and 20 pmol Cas9 for nucleofection (4D).

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ABRF GERG website: https://abrf.org/research-group/genome-editing-research-group-gerg