# 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 reagent 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 GERG 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

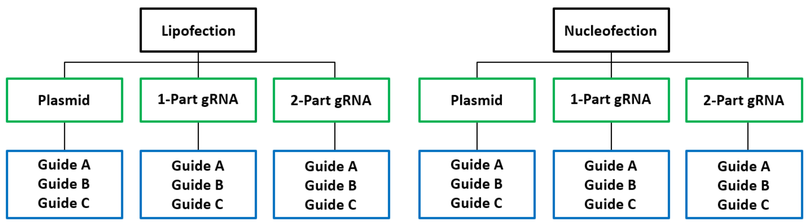


Figure 1. For this study, we performed the same experiments in three sites using three different guides, three formats , and two transfection methods in order to compare both reproducibility and function. They guides were previously determined to have either low, medium, or high efficiency. The guides were delivered in either plasmid format (PX330), a 1-part sgRNA ribonucleoprotein (RNP) or a 2-part gRNA ribonucleoprotein. The guides were transfected into the cells via either lipofection (LipoD293) or nucleofection (Lonza nucleofector IIB or 4D). All experiments were done in 293 cells and performed in triplicate.

## Acknowledgments

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## Plasmid Lipofection vs. Nucleofection

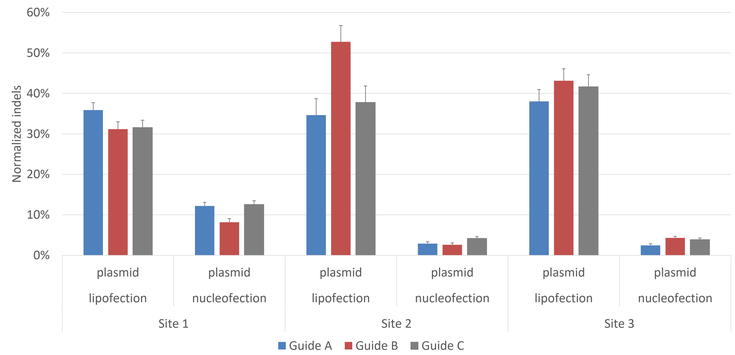



Figure 2. For this experiment, we performed transfections with three guides that were previously determined to have low, medium, or high efficiency. The guides were cloned into PX330, a plasmid that also expresses Cas9. The plasmids were delivered into 293 cells via either lipofection (LipoD293) or nucleofection (Lonza nucleofector IIB or 4D). We used 1ug of plasmid for lipofection and 0.5ug for nucleofection (4D).

## 2-Part gRNA Lipofection vs. Nucleofection

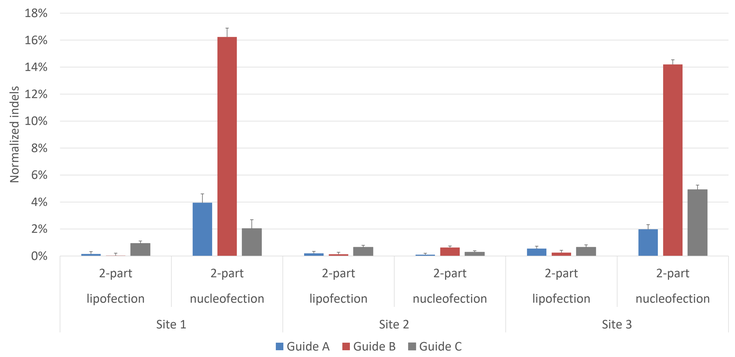



Figure 3. For this experiment, we performed transfections with three guides that were previously determined to have low, medium, or high efficiency. A two-part gRNA that contains both the crRNA and tracrRNA in complex was obtained from IDT. A RNP was formed with Cas9 protein. The plasmids were delivered into 293 cells via either lipofection (LipoD293) or nucleofection (Lonza nucleofector IIB or 4D). We used 6 pmol gRNA and 6 pmol Cas9 for lipofection and 120 pmol gRNA and 104 pmol Cas9 for nucleofection (4D).

## 1-Part sgRNA Lipofection vs. Nucleofection

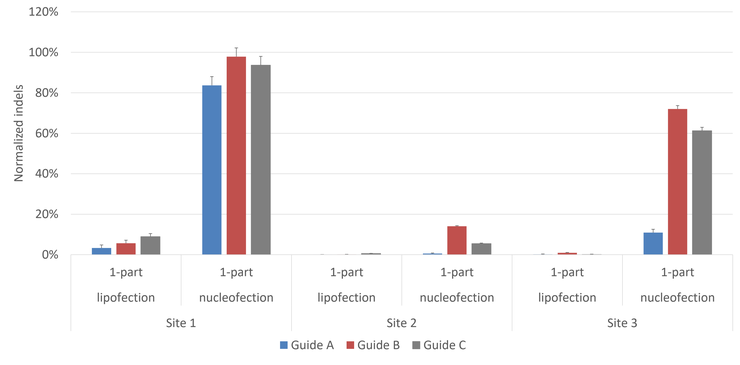
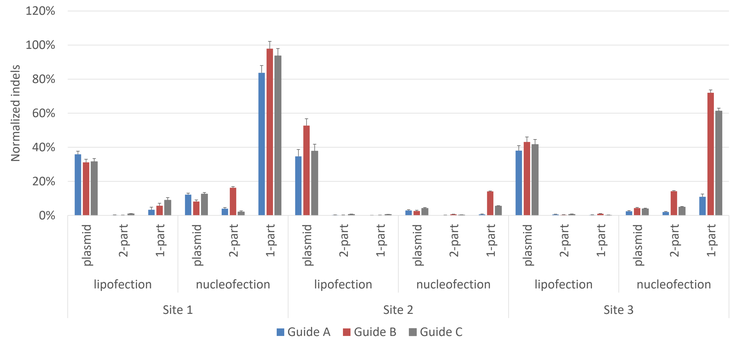



Figure 4. For this experiment, we performed transfections with three guides that were previously determined to have low, medium, or high efficiency. A one-part gRNA that is comprised of both the crRNA and tracrRNA was obtained from Synthego. A RNP was formed with Cas9 protein. The plasmids were delivered into 293 cells via either lipofection (LipoD293) or nucleofection (Lonza nucleofector IIB or 4D). We used 3.9 pmol sgRNA and 3 pmol Cas9 for lipofection and 180 pmol sgRNA and 20 pmol Cas9 for nucleofection (4D).

## Reproducibility




| | Site 1 | Site 2 | Site 3 |
|---|---|---|---|
| Delivery method with highest indel rate | nucleofection | lipofection | nucleofection |
| GuideRNA format with highest indel rate | sgRNA | plasmid | sgRNA |

Figure 5. The reproducibility between two of the three locations was high, with one location differing from the others. Although the editing efficiency varied between locations, overall nucleofection with a one-part sgRNA seems to be the best method to use for reproducible, efficient indel formation.

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