Reproducibility of indel formation rates by comparing guideRNA format and delivery method

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Introduction

The clustered regularly interspaced palindromic repeats (CRISPR)/CRISPR-associated (Cas) system is a powerful genome editing technique that has grown in popularity since it was first used to edit mammalian cells in 2013. Multiple configurations of guideRNA and Cas9 components can be used for editing, including: a plasmid expressing both the guideRNA and Cas9, Cas9 protein combined with a synthetic single guideRNA, and Cas9 combined with a 2-part guideRNA. In addition, delivering the components to cells can be done using lipofection or nucleofection transfection methods. In the GERG 2017 survey, plasmid format and lipfection delivery were favored among cell culture users. But, RNP format is gaining in popularity in combination with nucleofection delivery. This study aims to compare cutting efficiency at 3 different guideRNA targets based on the guideRNA format and delivery method across multiple labs. Determining which method or format is the most reproducible will be beneficial. for developing standard operating procedure. Also, core facilities or research labs getting started with genome editing could use these results as a benchmark for optimizing their own protocols.

Study Design

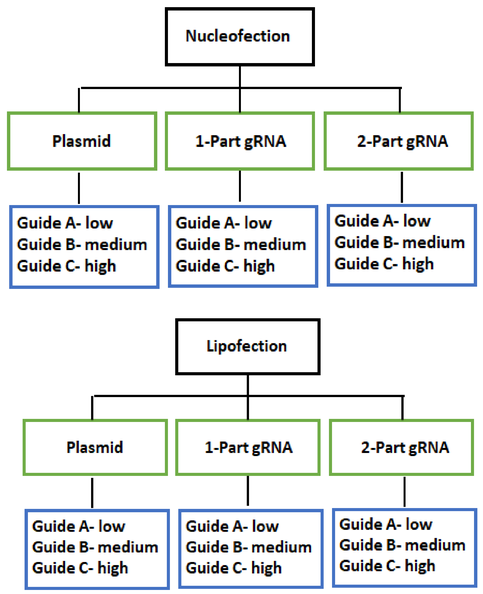
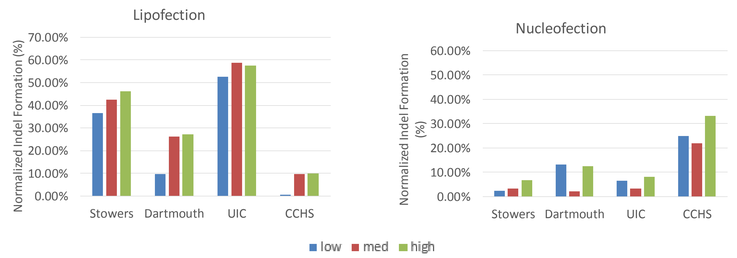


Figure 1. For this study, we performed the same experiments in 4 sites using three different guides, three formats , and two transfection methods in order to compare both reproducibility and function. The three guides were ones previously determined to have low, medium, or high efficiency. The guides 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.

Plasmid Lipofection vs. Nucleofection

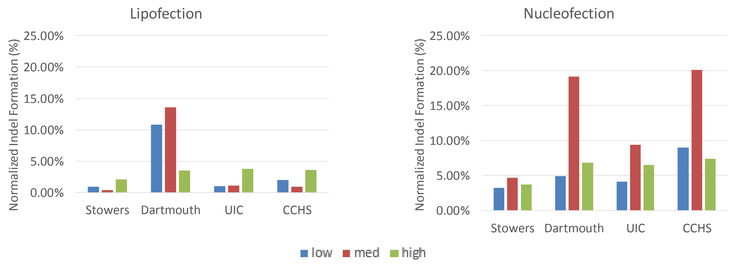


	Rxn volume	plasmid [ug]	Cells/rxn
Stowers	----	1	500,000
Dartmouth	----	1	500,000
UIC	----	1	500,000
CCHS	----	1	500,000

	Rxn volume	plasmid [ug]	Cells/rxn
Stowers	100ul	2	1,000,000
Dartmouth	100ul	2	1,000,000
UIC	100ul	2	1,000,000
CCHS	20ul	0.5	250,000

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).

2-Part gRNA Lipofection vs. Nucleofection

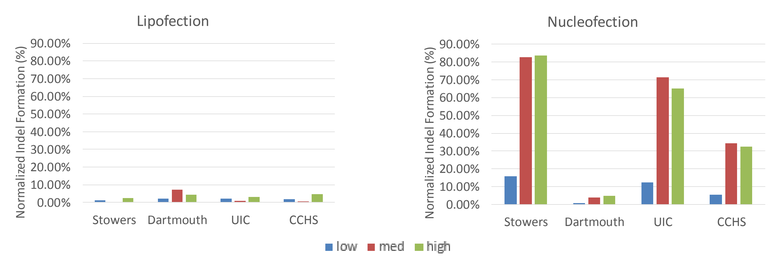


	Rxn volume	Guide RNA:Cas9 Protein [pmol]	Ration of RNA:protein	Cells/rxn
Stowers	----	24:24	1	500,000
Dartmouth	----	6:6	1	160,000
UIC	----	6:6	1	160,000
CCHS	----	6:6	1	160,000

	Rxn volume	Guide RNA: Cas9 protein [pmol]	Ratio of RNA:protein	Cells/rxn
Stowers	100ul	160:139	1	460,000
Dartmouth	100ul	480:416	1	1,400,000
UIC	100ul	160:139	1	460,000
CCHS	20ul	120:104	1	350,000

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).

1-Part sgRNA Lipofection vs. Nucleofection



	Rxn volume	Guide RNA: Cas9 protein [pmol]	Ratio of RNA:protein	Cells/rxn
Stowers	----	15.6:12	1.3:1	500,000
Dartmouth	----	3.9:3	1.3:1	120,000
UIC	----	3.9:3	1.3:1	120,000
CCHS	----	3.9:3	1.3:1	120,000

	Rxn volume	Guide RNA: Cas9 protein [pmol]	Ratio of RNA:protein	Cells/rxn
Stowers	100ul	720:80	9:1	600,000
Dartmouth	100ul	720:80	9:1	600,000
UIC	100ul	720:80	9:1	600,000
CCHS	20ul	180:20:00	9:1	200,000

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).

Reproducibility

	Stowers	Dartmouth	UIC	CCHS
Delivery method with highest indel rate	nucleofection	lipofection	nucleofection	nucleofection
GuideRNA format with highest indel rate	sgRNA	plasmid	sgRNA	sgRNA

Table 1. The reproducibility between three of the four 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.

Acknowledgments

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