Controlling Rearrangement Frequencies in the Context of Multigene Genome Editing

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Chief Technology Officer

Genome Engineering: From Mechanisms to Therapies, February 23, 2019

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VM is an employee and shareholder of Editas Medicine, Inc.

Understanding Consequence of Editing is Important

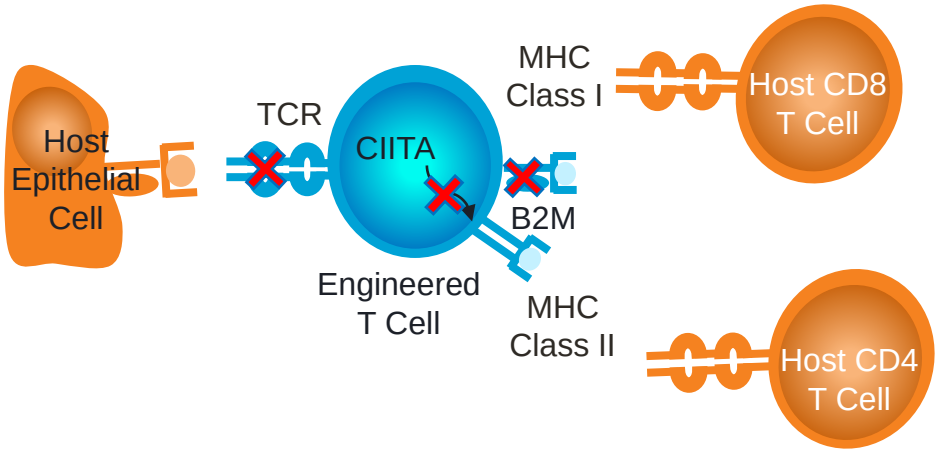
Human Genome Editing Safety Concerns

- Off-target genome editing
  - Sensitivity of off-target screening methods
- Unintended biological consequences of on-target editing
  - Mutagenesis as a result of imprecise DNA repair following on-target editing
- Additional adverse effects due to genomic DNA cleavage at on- and off-target sites
  - Chromosomal translocations, inversions, etc.
- Immunogenicity
- Adverse impact of the delivery system
- In the case of *in vivo* genome editing, off-target cell/tissue editing

Anna Kwilas, Ph.D.
CMC Reviewer, Gene Therapy Branch
Division of Cellular and Gene Therapies
Office of Tissues and Advanced Therapies
Center for Biologics Evaluation and Research
Food and Drug Administration

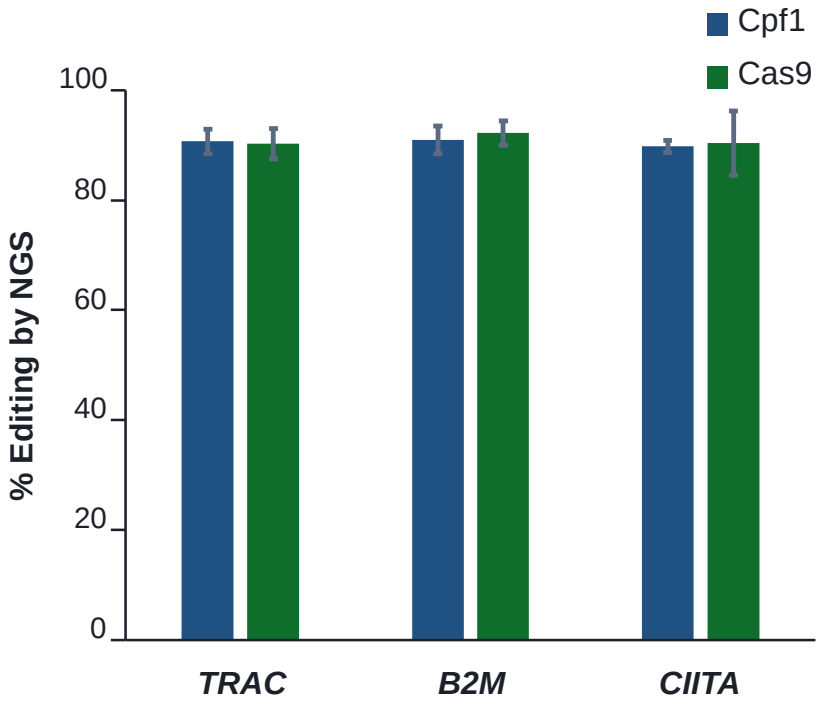
NIST-FDA Genome Editing Workshop
April 23, 2018

Allogeneic Therapies Will Require Multiple Edits



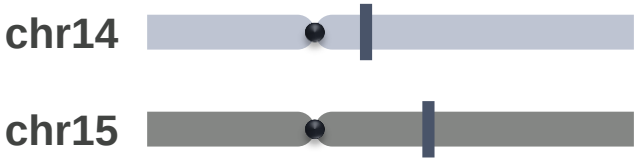
Multiple edits to eliminate the T cell receptor, Class I, and Class II presentation may be required

Highly Active Single Molecules For Cas9 and Cpf1

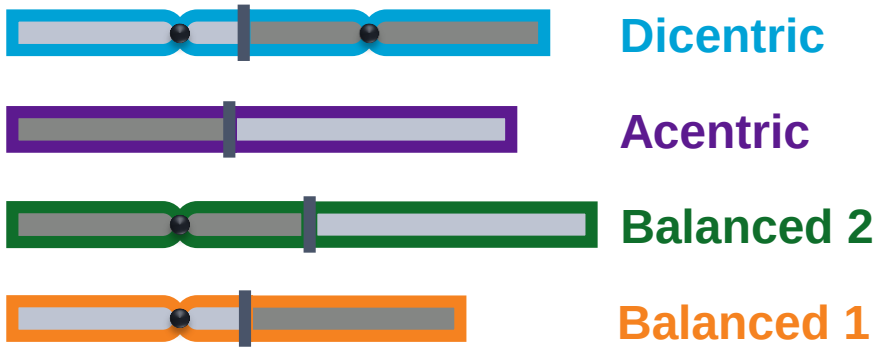


Translocation Detection in the Context of Multiplexing

Develop detection methods for accurate quantitation of genome editing induced structural rearrangements.



Simultaneous edit in primary human CD4+ T cells at TRAC and B2M



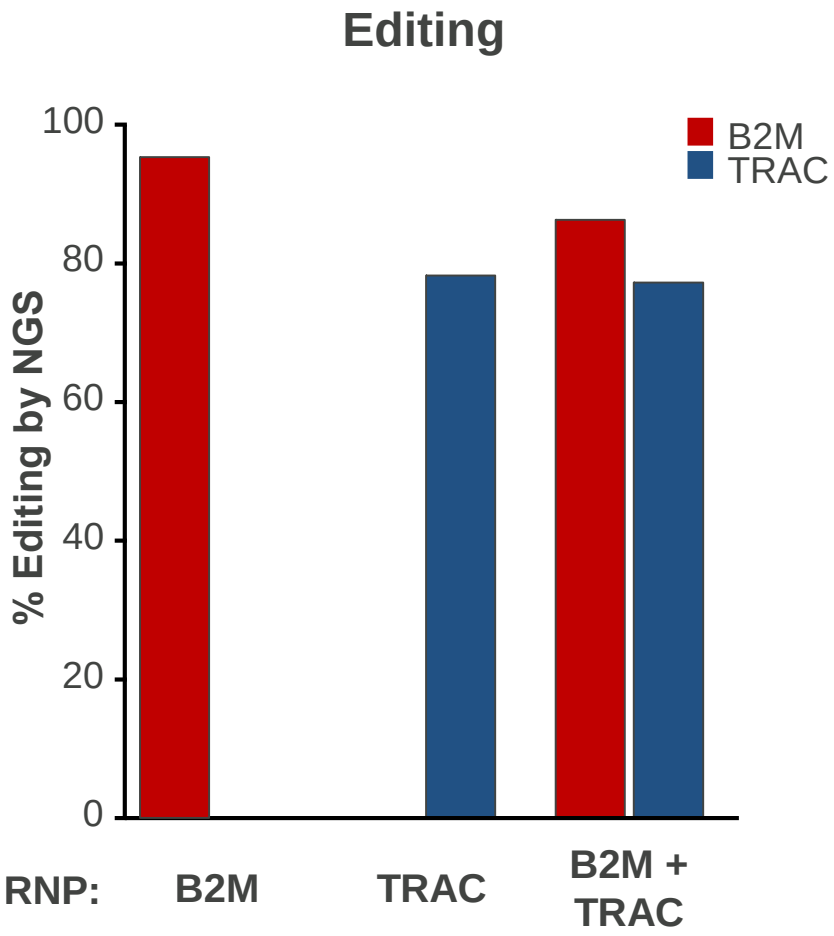
High Resolution (Targeted)

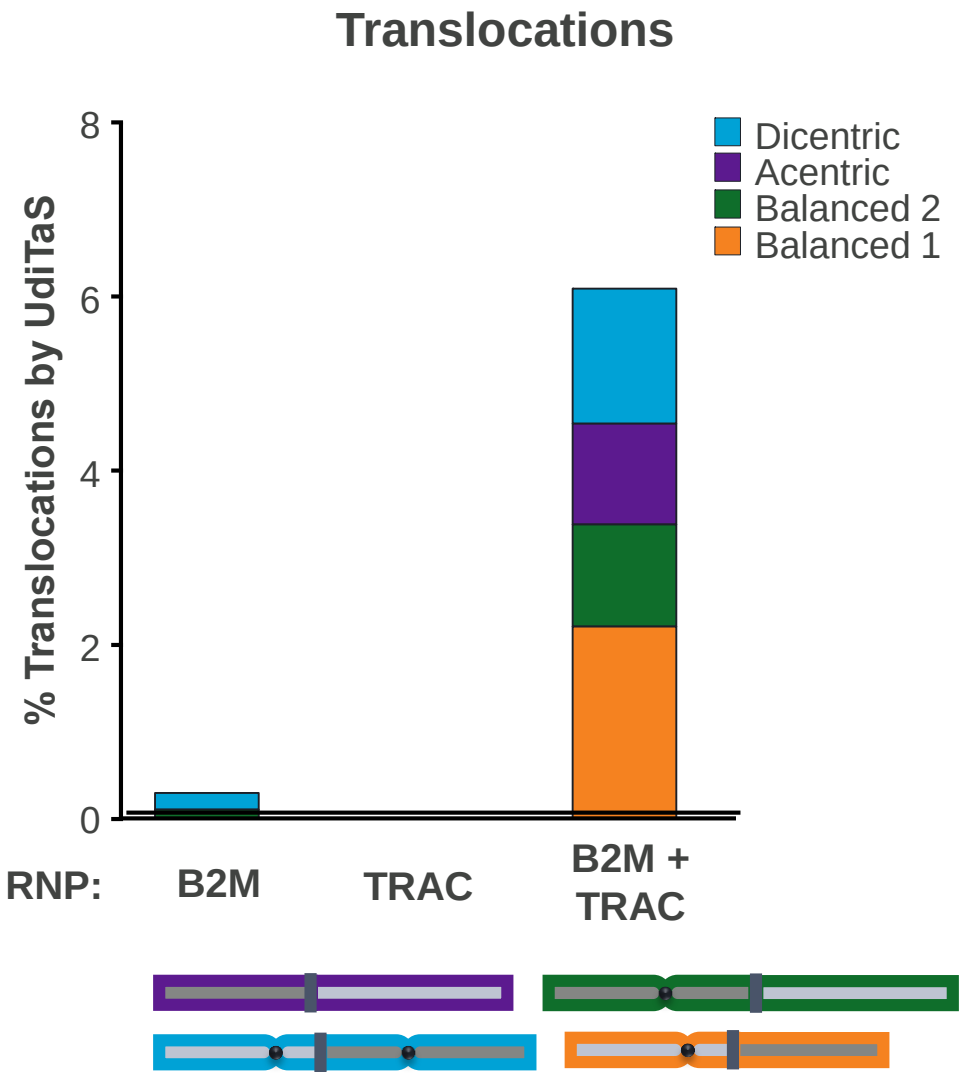
UDiTaS **ddPCR**

Low Resolution (Genome-wide)

FISH

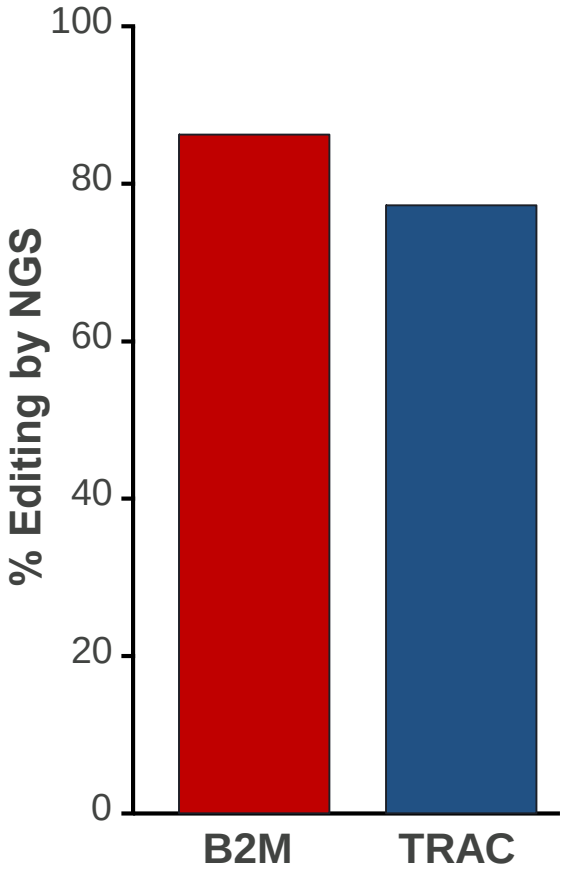
Multiplexing Leads to Translocation Formation

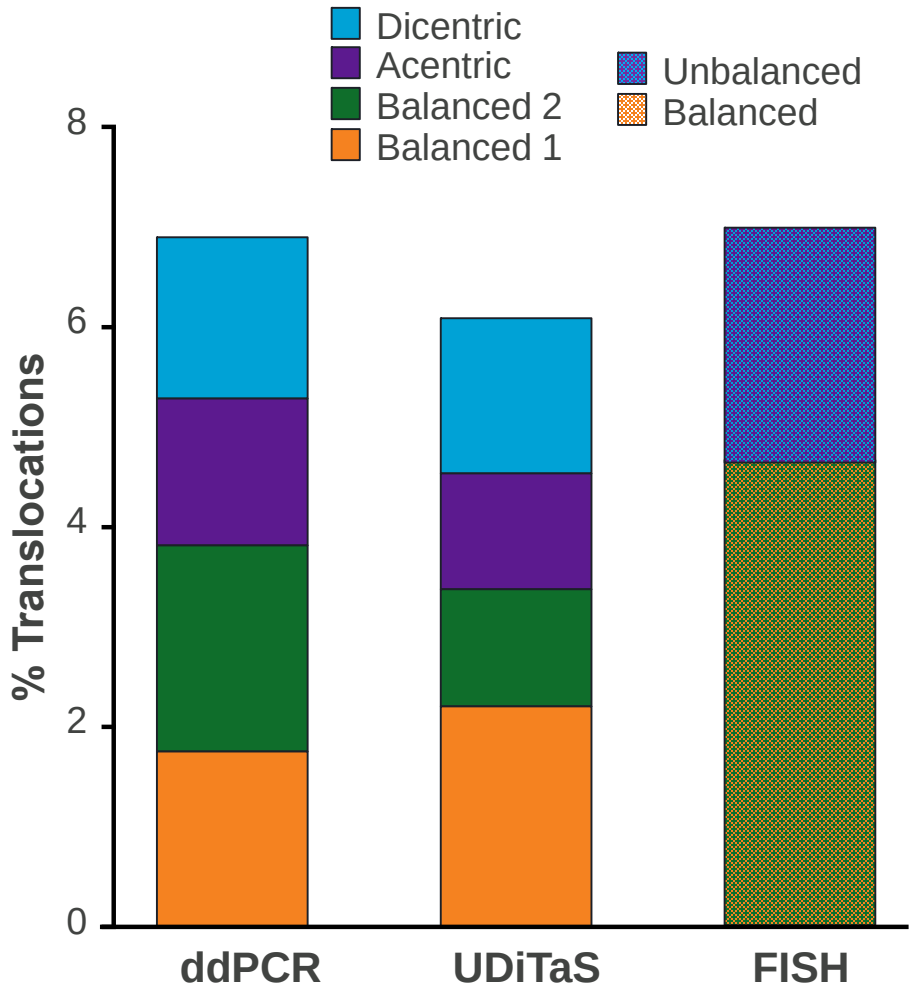




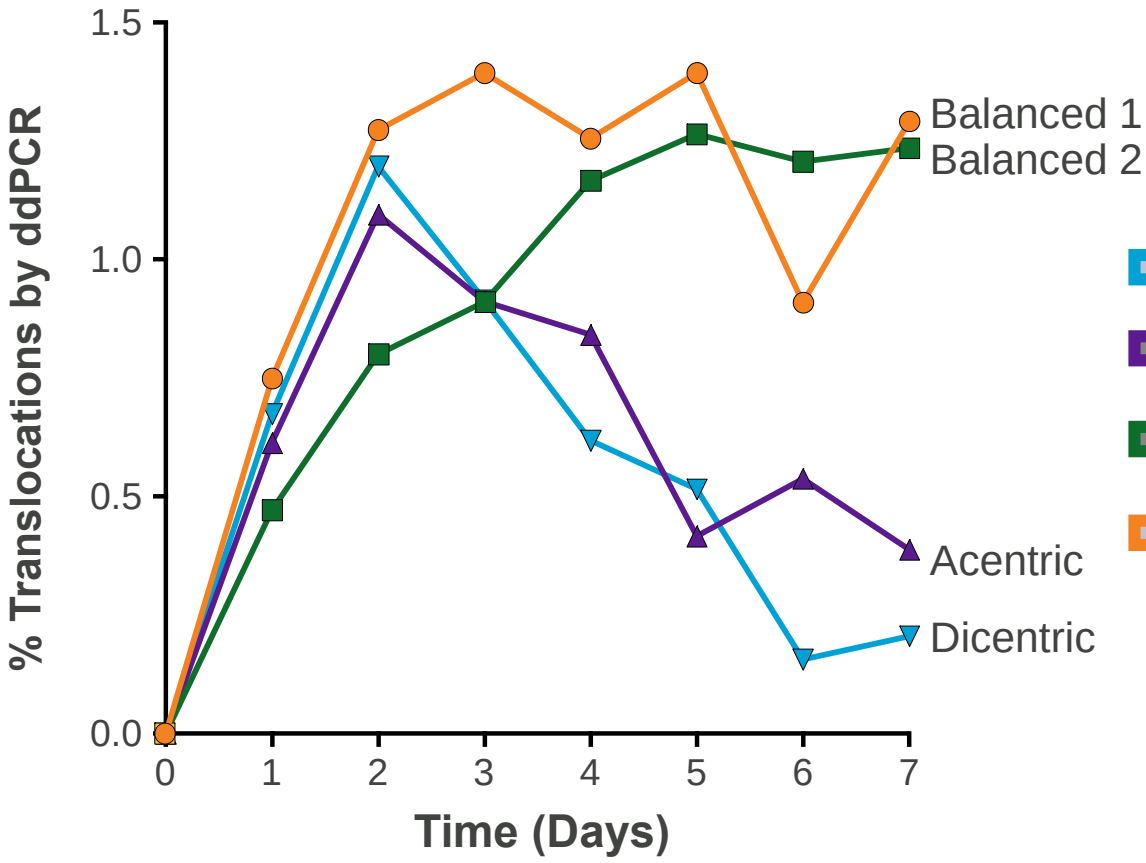
Day 3 Post-nucleofection, Primary human T cells, SpCas9

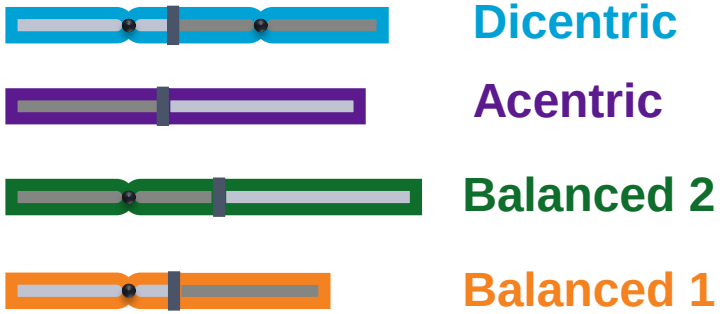
Targeted and Genome-wide Methods Yield Comparable Translocation Frequencies





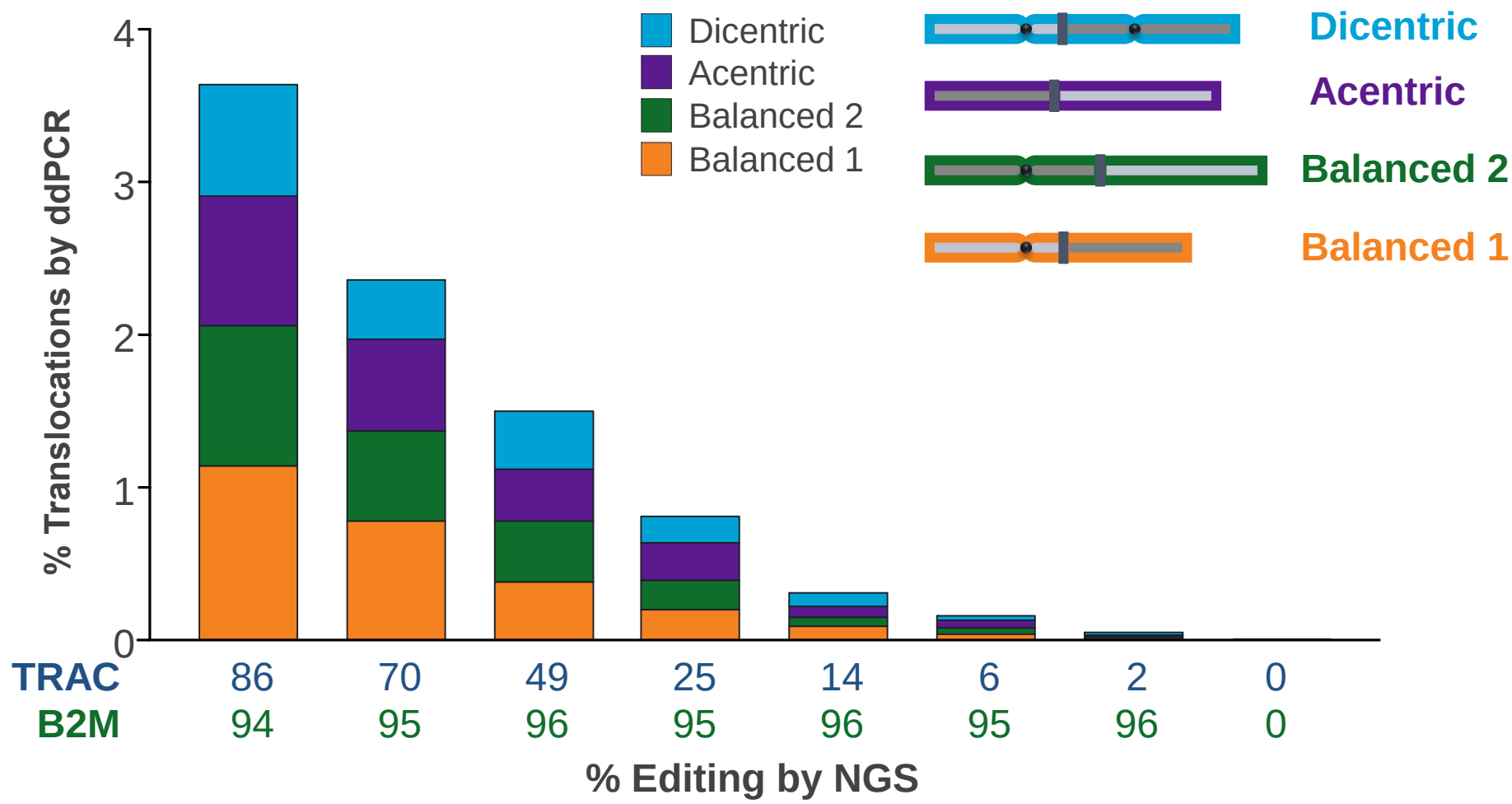
Balanced Translocations Persist, Unbalanced Do Not





CD4+ Human Primary T Cells, B2M and TRAC, SpCas9

Translocation Rates are Dependent on Editing Rates



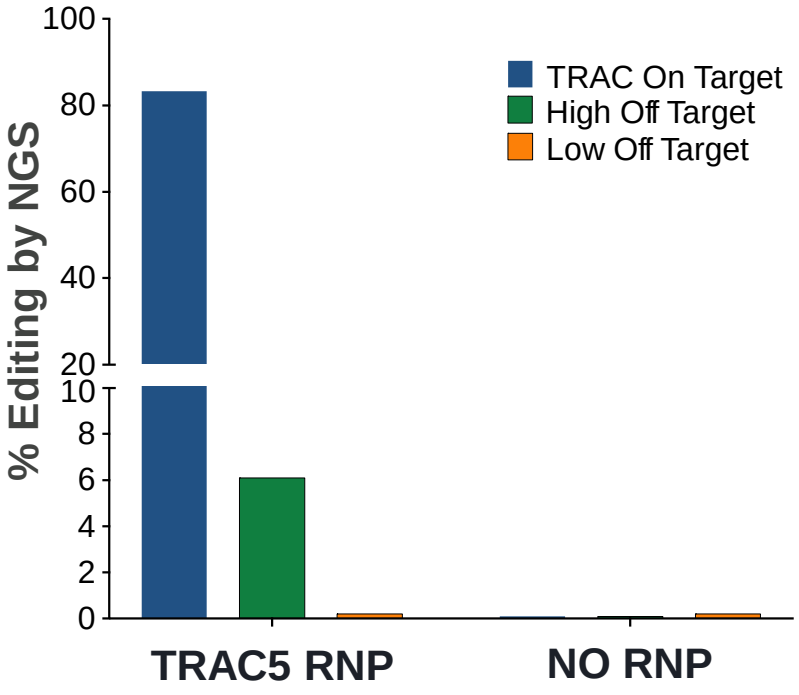
- Titrated down TRAC RNP while holding B2M constant

LLOD of ddPCR assay: 0.01%; CD4+ T cells, SpCas9

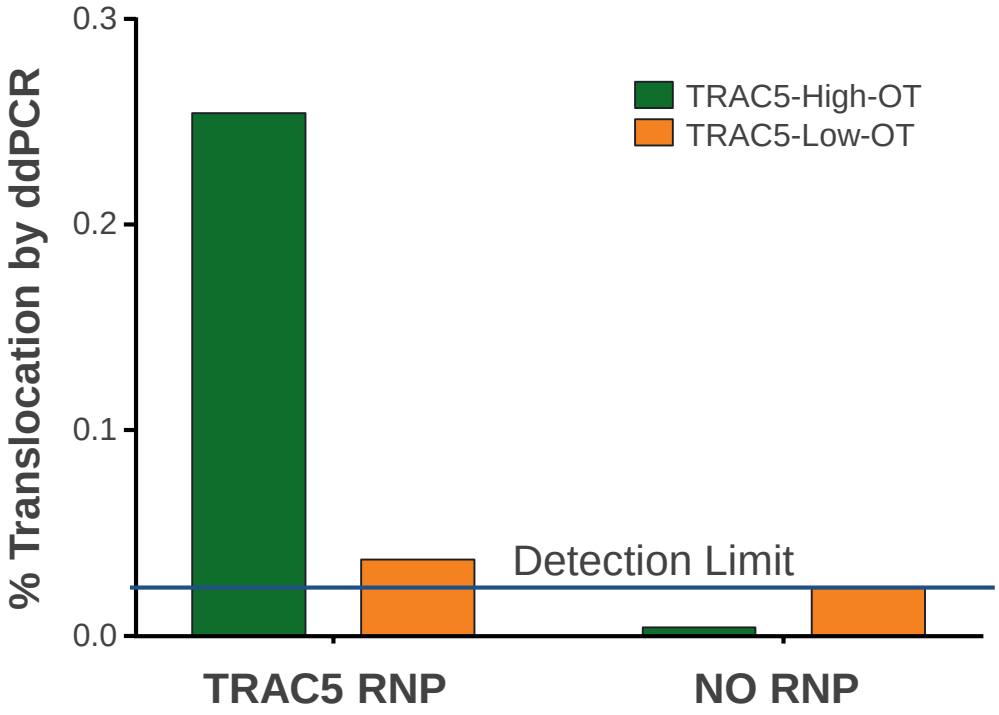
Translocations to Off-Targets for a Dirty Guide

TRAC5 gRNA: Many off targets

Targeted Sequencing



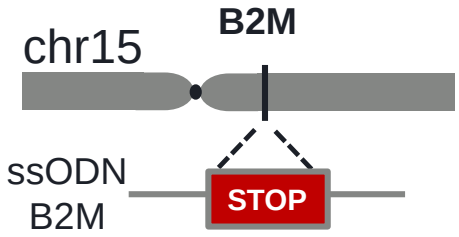
Translocation Detection



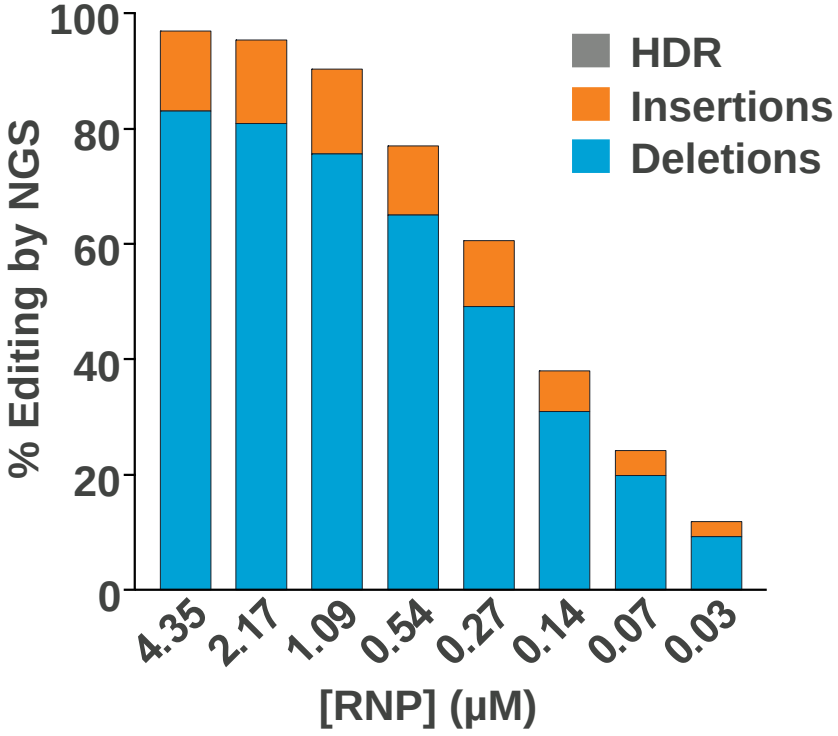
Translocations: So What?

- Highly efficient, multiple edits are achievable but:
  - Translocations happen
  - The rate of translocation is proportional to:
    - # cuts
    - Editing efficiency
- Translocations are not dangerous per se. They become a concern when they drive a phenotype that is unexpected. They *may* reduce your efficacy and/or cellular viability
- Ideally, you want to identify them, track them, understand them, and (if possible) reduce them.
- How would you do that?
  - Modulate repair pathways?
  - Combine enzymes?

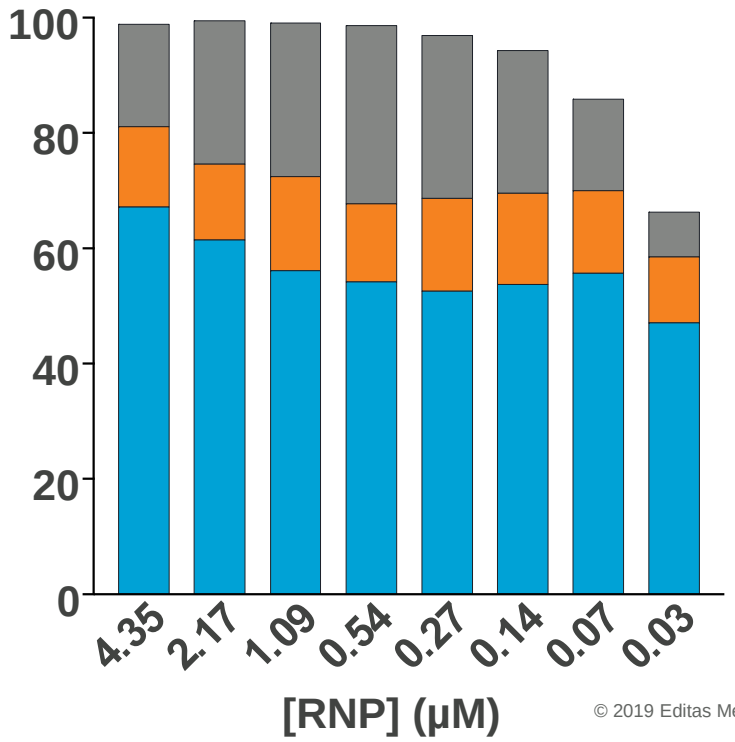
Modulating Repair Pathways: HDR with a DNA Oligo



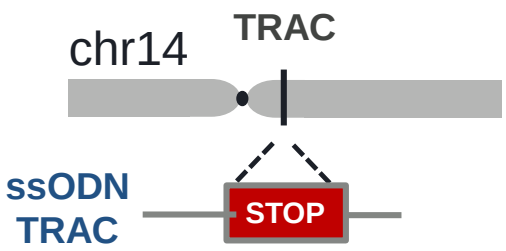
No Oligo

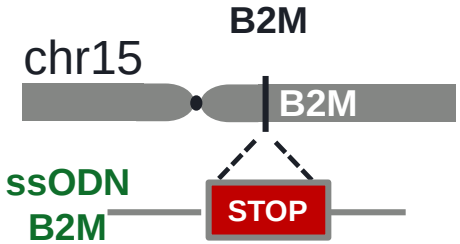


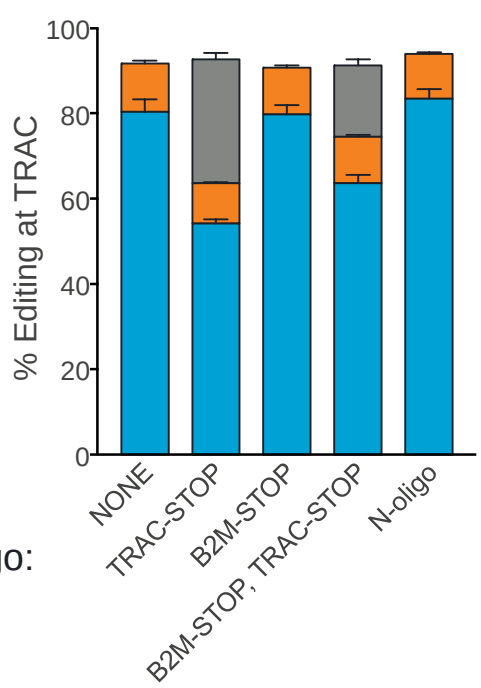
+ Donor Oligo

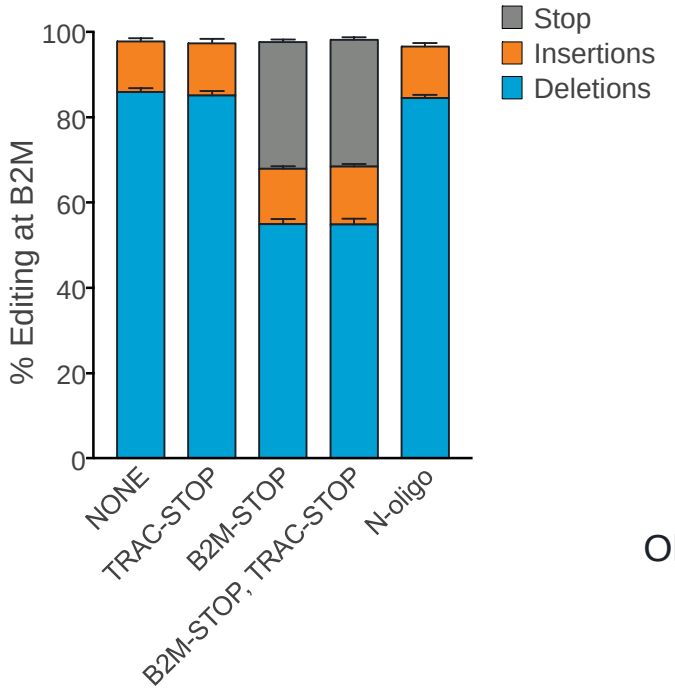


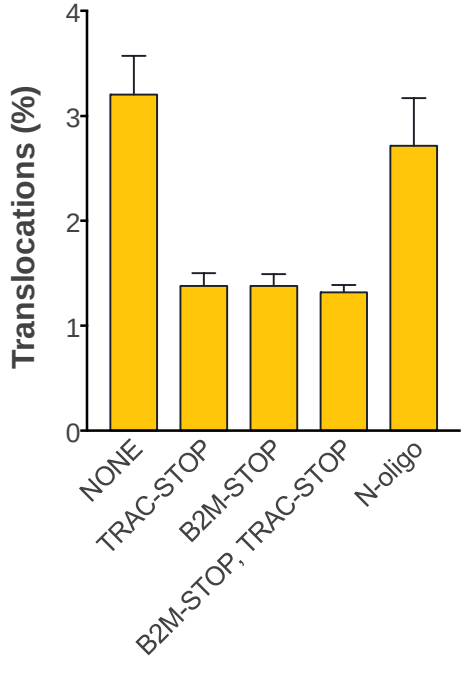
Single Stranded Homologous DNA Oligo Reduces Translocations





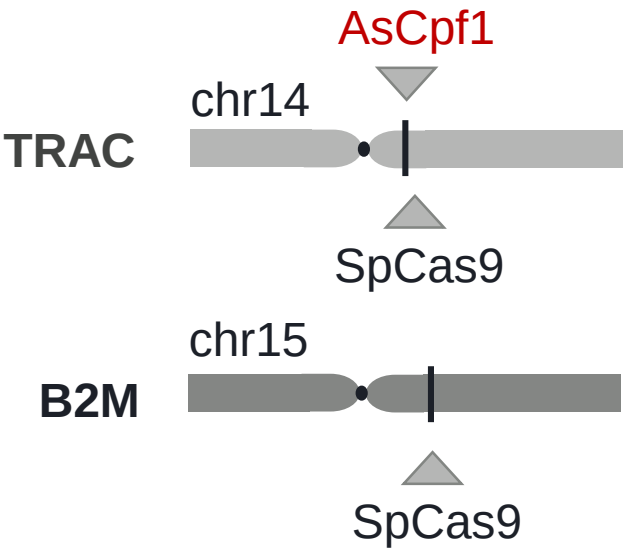






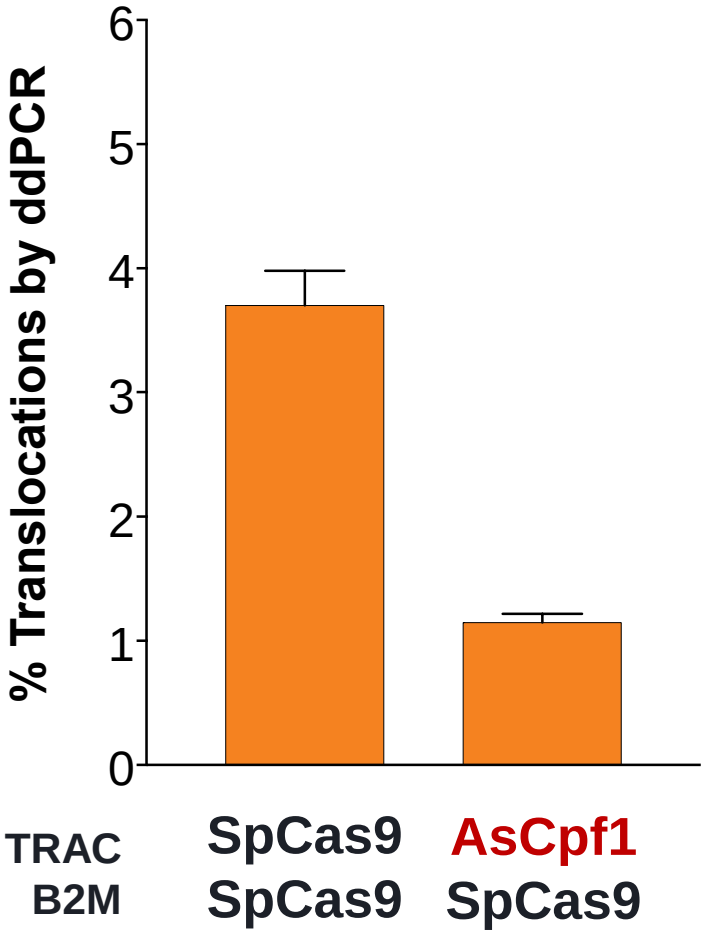
- **ssODN homologous to either cut site results in repair pathway balance shift from c-NHEJ to HDR with 2-fold decrease in translocations**
- **ssODN administration enables 2-4 fold reduction of RNP dose**

Using Cas9/Cpf1 (Cas12a) for Multiplexing Reduces Translocations



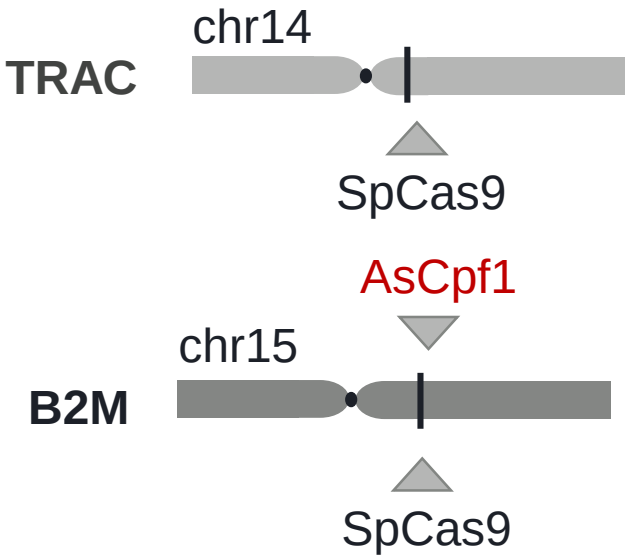
On Target Editing (% by NGS)

Locus	Enzyme		Percent
	B2M	TRAC	
TRAC	Cas9	Cas9	87
	Cas9	Cpf1	94
B2M	Cas9	Cas9	90
	Cas9	Cpf1	94



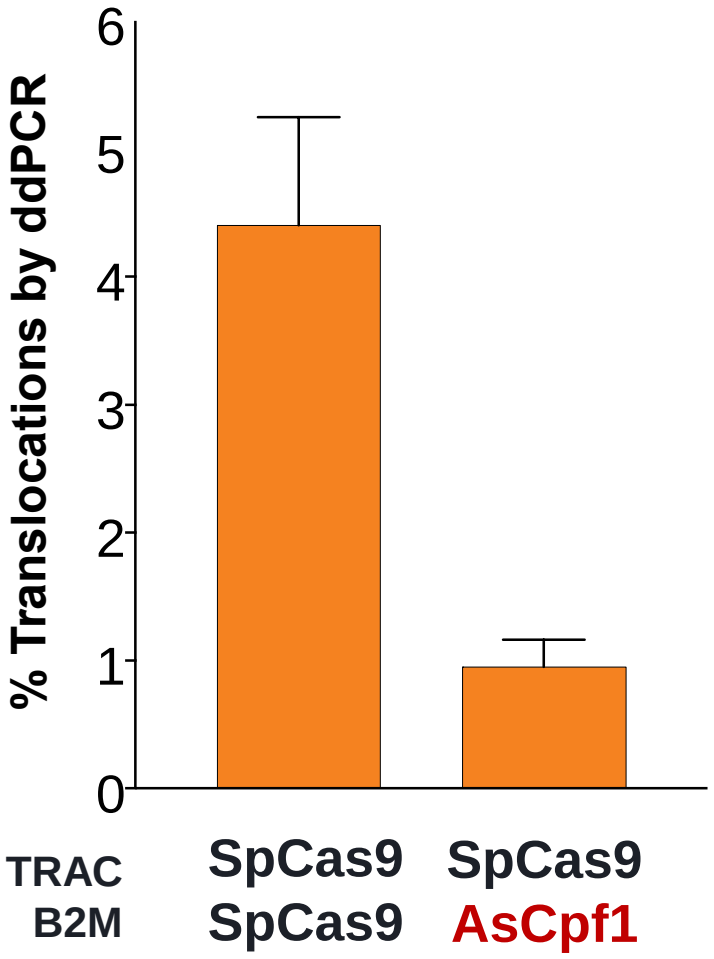
Day 4

Using Cas9/Cpf1 (Cas12a) for Multiplexing Reduces Translocations



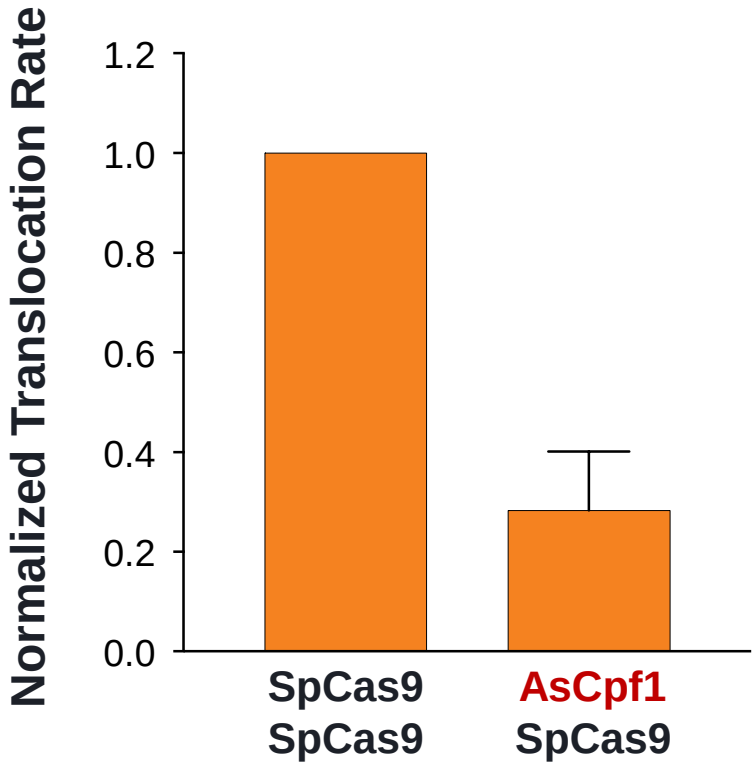
On Target Editing (% by NGS)

Locus	Enzyme		Percent
	B2M	TRAC	
TRAC	Cas9	Cas9	86
	Cpf1	Cas9	89
B2M	Cas9	Cas9	92
	Cpf1	Cas9	88



Day 4

Using Cas9/Cpf1 (Cas12a) for Multiplexing Reduces Translocations



n=13
p<0.0001
(Student's two tailed t test)

- Multiplexing with **SpCas9 (WT)/AsCpf1(WT)** significantly reduces translocation rates
- Reduction in translocation rates is independent of assay (UDiTaS/ddPCR), locus, and gRNA

Summary

- Highly efficient, multiple edits are achievable but:
  - Translocations happen
  - The rate of translocation is proportional to:
    - # cuts
    - Editing efficiency
- Translocations are not dangerous per se. They become a concern when they drive a phenotype that is unexpected. They *may* reduce your efficacy and/or cellular viability
- Ideally, you want to identify them, track them, understand them, and (if possible) reduce them.
- Translocations *can* be reduced by modulating repair pathways and mixing enzymes appropriately