Zinc finger fusions and synthetic DNA donor engineering improve the performance of reprogrammed modular integrases at the TRAC locus

Jessica E Davis, Friedrich Fauser, Sebastian Arangundy-Franklin, Lifeng Liu, Nicola J Schmidt, Luis Rodriguez, Danny F Xia, Nga Nguyen, Nicholas A Scarlott, Yuanyue Zhou, Lynn N Truong, Rakshaa Mureli, Irene Tan, Satria Sajuthi, Sarah J Hinkley, Bhakti N Kadam, Stephen Lam, Bryan Bourgeois, Emily Tait, Mohammad Qasim, Vishvesha Vaidya, Adeline Chen, Andrew Nguyen, Yuri R. Bendaña, David A. Shivak, Patrick Li, Andreas Reik, David E Paschon, Gregory D Davis and Jeffrey C Miller

Sangamo Therapeutics, Inc.

ASGCT, May 11-16, 2026

Disclosure

I am a full-time employee of Sangamo Therapeutics

Talk Overview

- We developed a platform to retarget Large Serine Integrases (LSIs) to therapeutically-relevant sites
- We used this platform to retarget Bxb1, an LSI, to the TRAC locus
- We achieved ~29% TRAC integration and up to 44% GFP expression in primary human T cells

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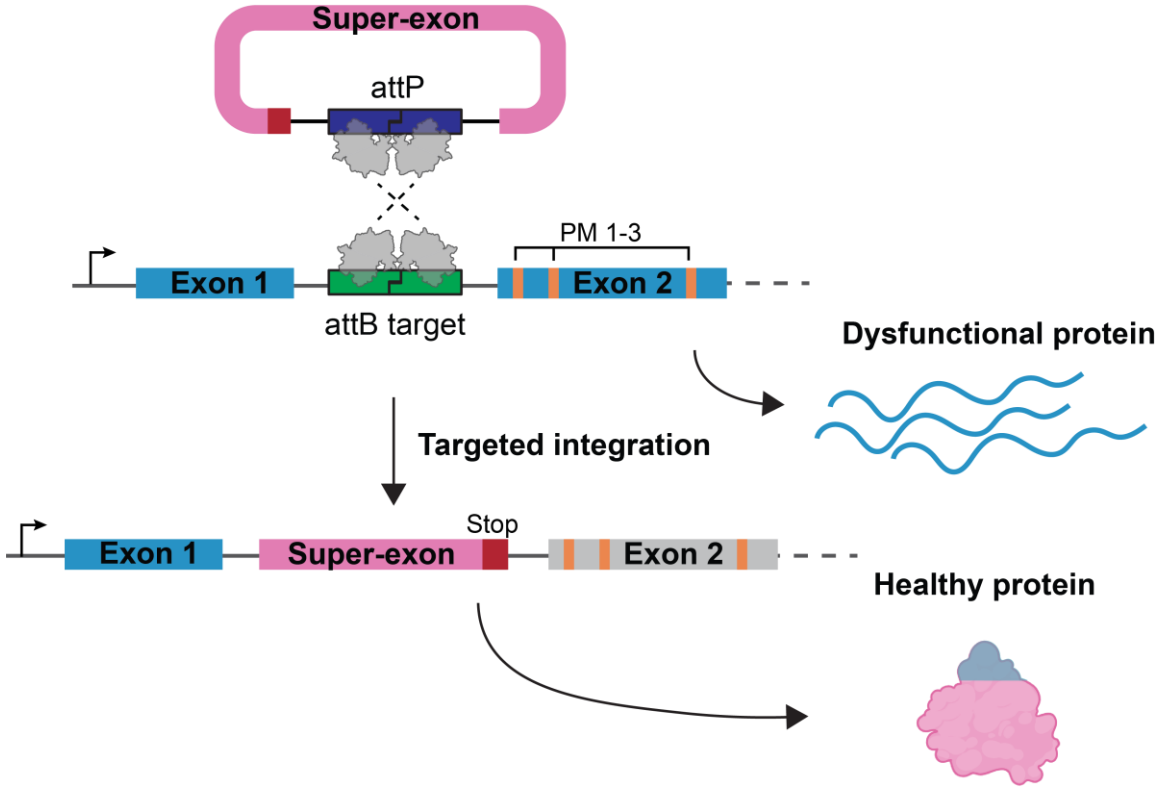
Fully programmable gene integration: the ideal genomic medicine approach

- **Targeted integration promises a single therapeutic approach to many Patient population Mutations (PM)**
- **LSIs are strong therapeutic candidates for targeted integration**
  - **Large payload delivery**
  - **Irreversible integration**: Integrates via **att**achment sites (**attP** → **attB**)
  - **DNA break-free**
  - **Cell type independence:** Does not depend on DNA repair machinery
- **Reprogramming existing LSIs to target therapeutically-relevant sites with high precision remains a challenge in the field**



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Images by Biorender

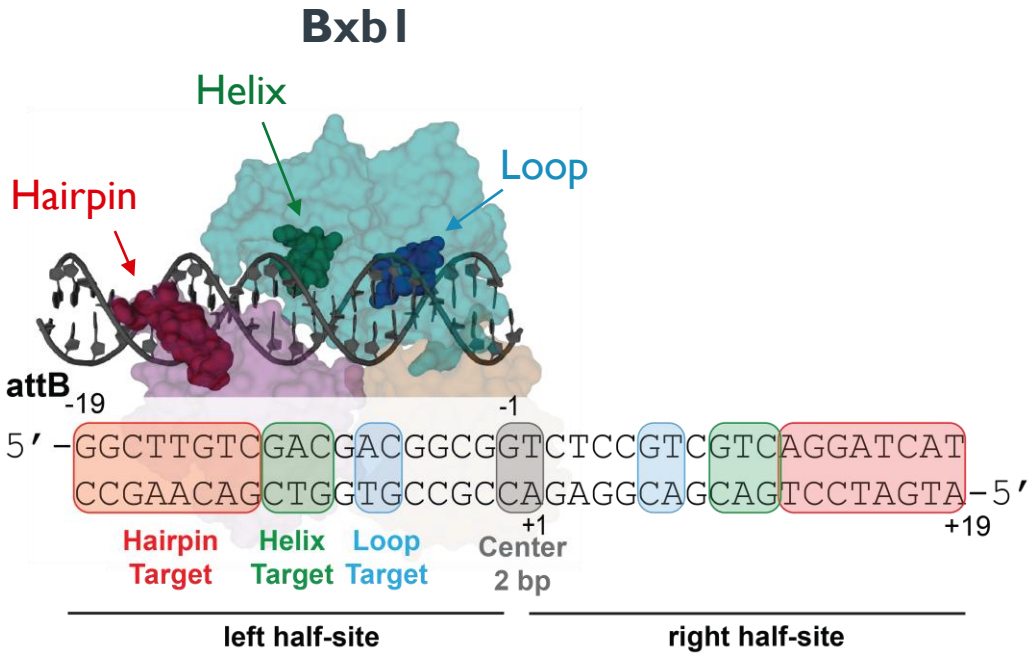
Bxb1 is a Large Serine Integrase with promising therapeutic potential

High activity in human cells, and *in vivo* mouse and NHP studies

High fidelity of cargo integrity after integration via cut and paste mechanism

Low off-target integration levels in human genome

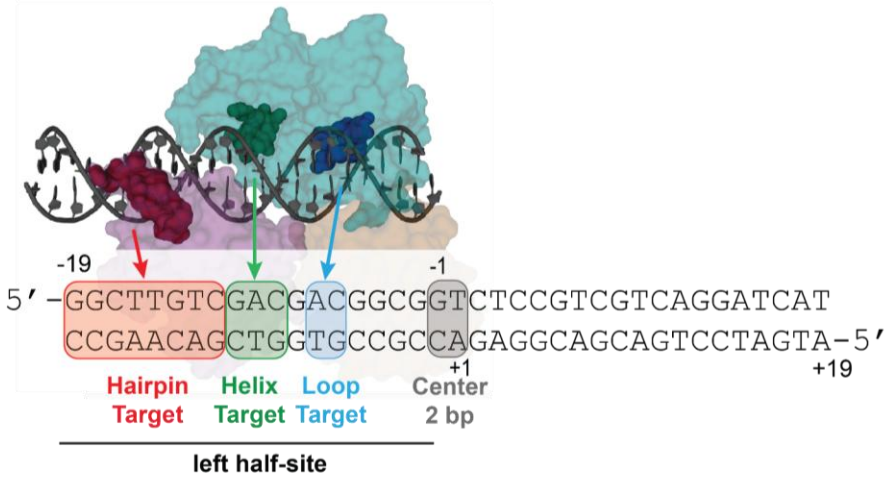
- Structural analysis and scanning mutagenesis studies predicted likely DNA-binding domains and locations of their target sites within attB
- DNA binding by each domain hypothesized to occur in a modular fashion, guiding our approach to retargeting



Our Modular Integrase (MINT™) platform retargeting strategy

1. Bacterial selections to generate each DNA-binding domain

- Built archive of domains for ease of retargeting



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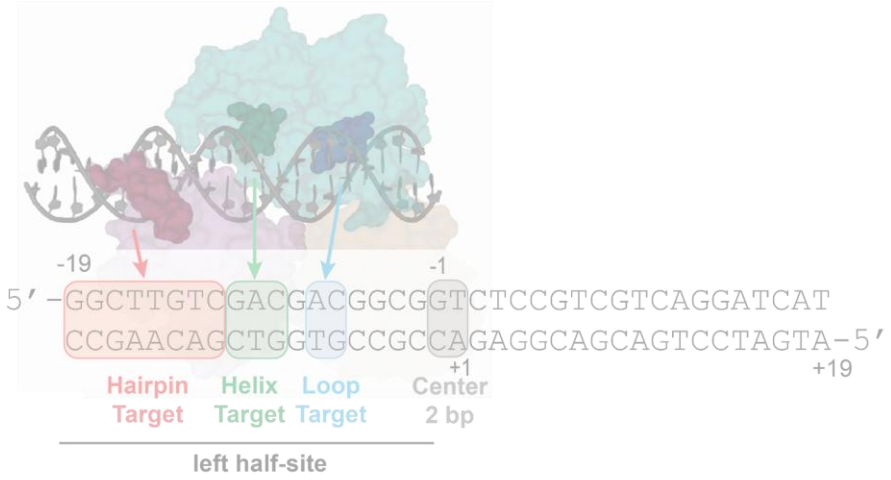
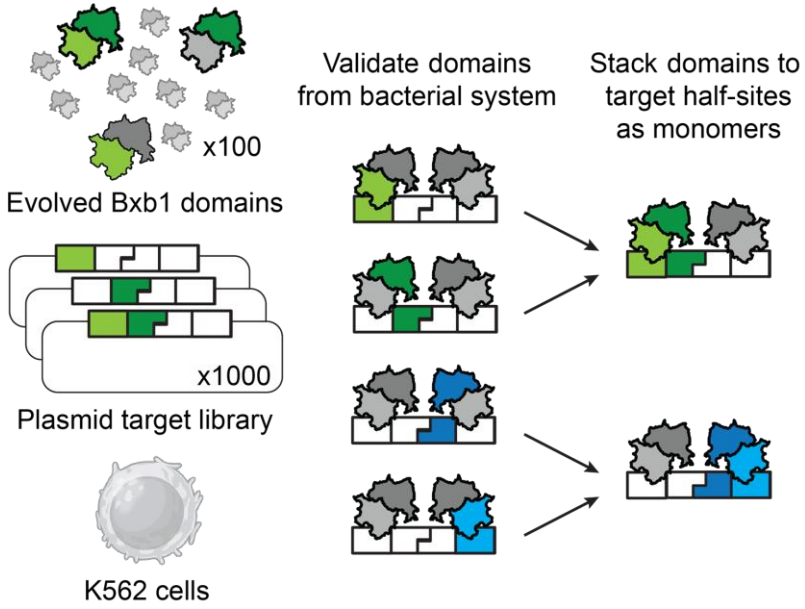


2. Stack domains as monomers to target attB half-sites



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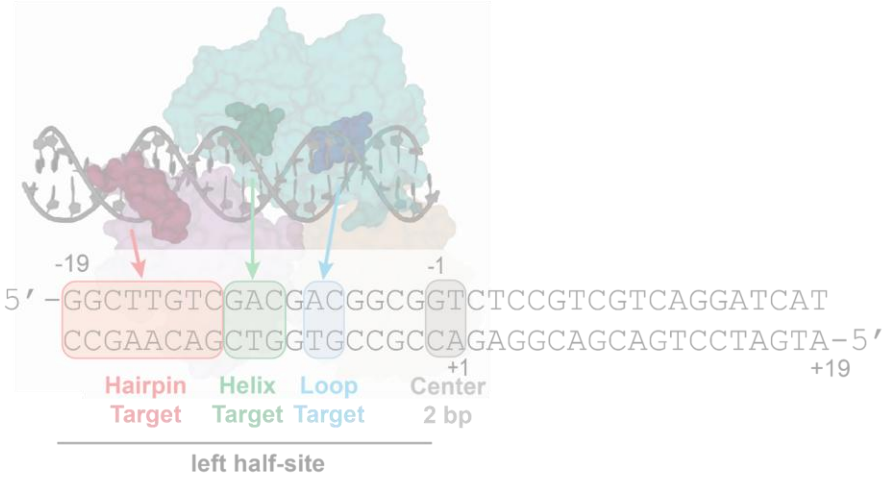

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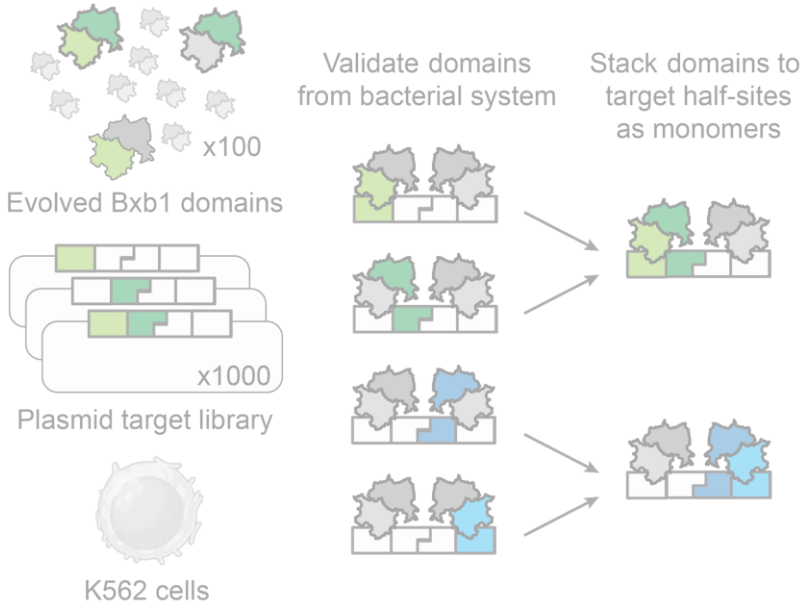
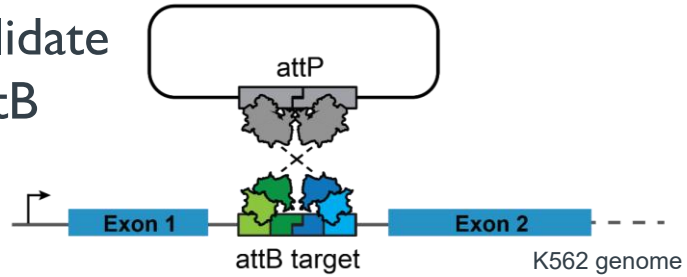


3. Pair monomers and validate activity at full genomic attB target site

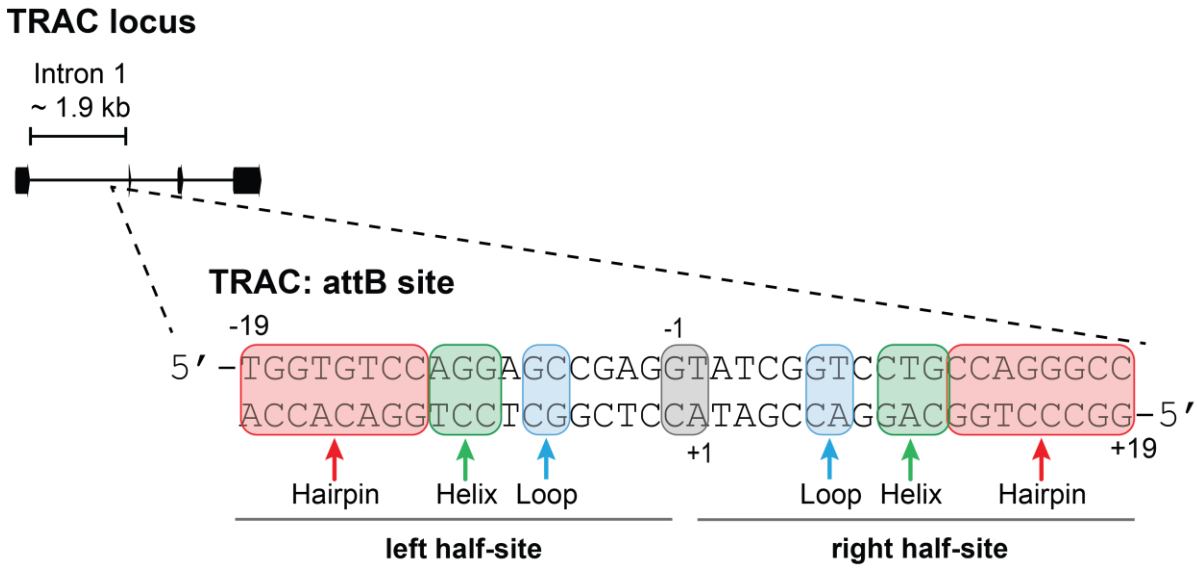


Images by Biorender

We fully retargeted Bxb1 to the human TRAC locus

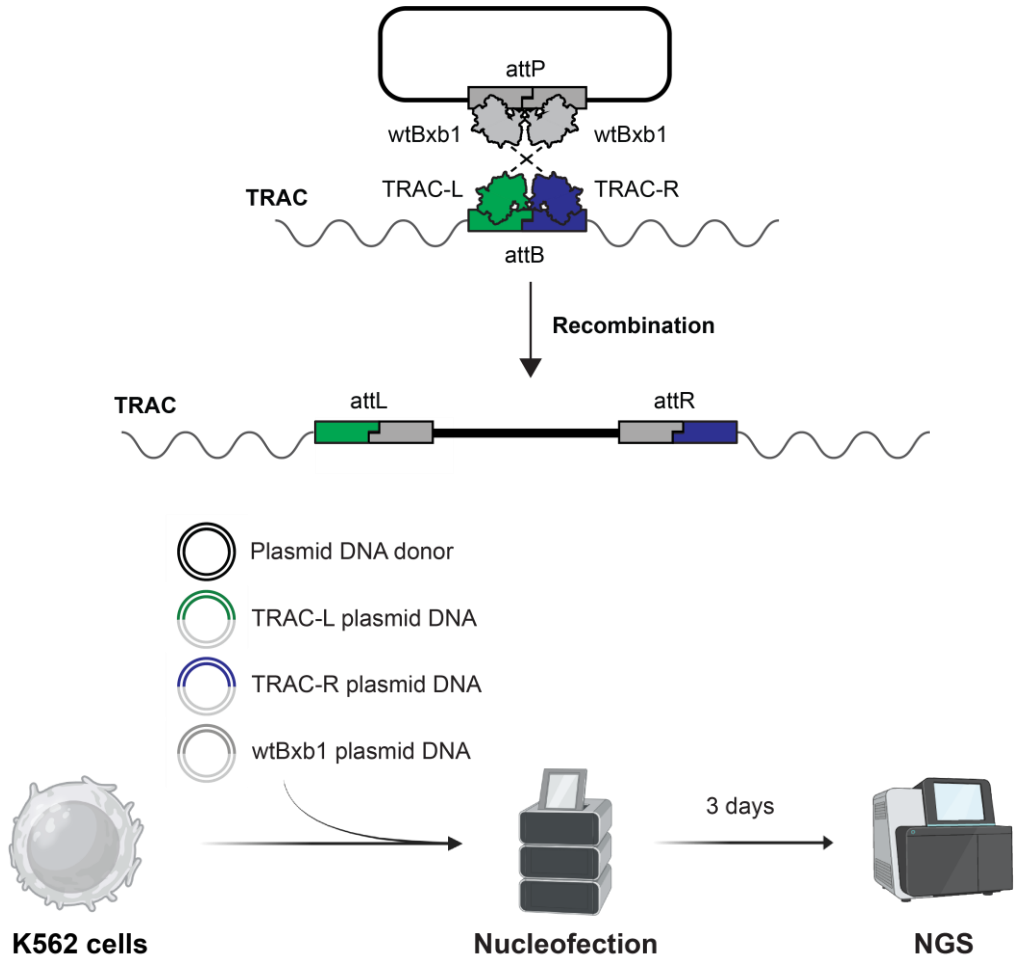
- TRAC intron 1 is an appealing retargeting challenge due to:
  - Small search window (1.9 kb) pushes capabilities of platform
  - Relevance for CAR-T engineering therapies

- Using our MINT platform, we evolved Bxb1 DNA-binding domains that targeted a novel attB site here



We fully retargeted Bxb1 to the human TRAC locus

- Domains stacked to make two Bxb1 monomers targeting the left and right TRAC attB half-sites
- Genomic integration assayed in K562 cells



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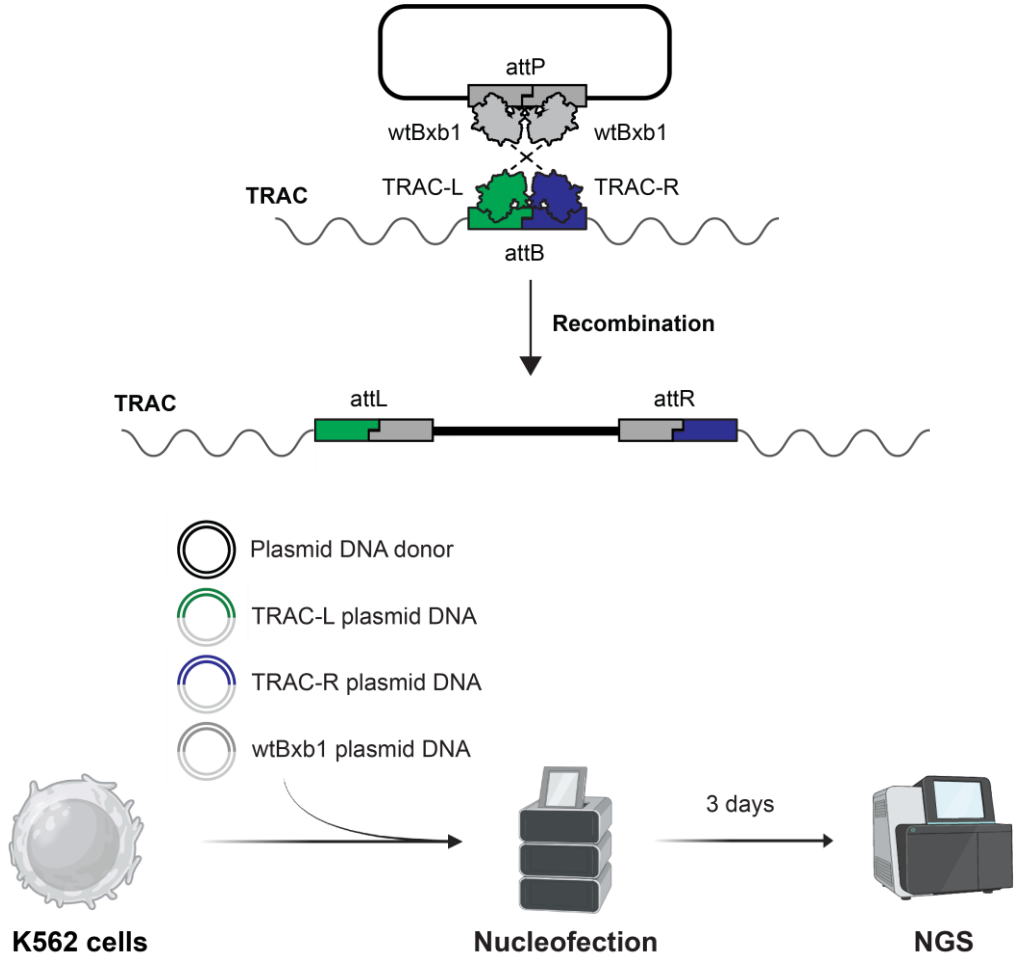

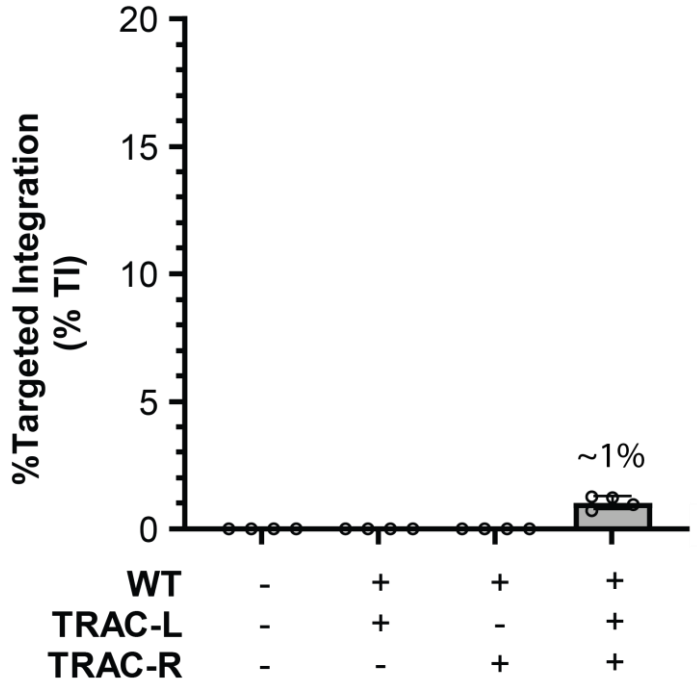

We achieved full re-targeting of Bxb1 to TRAC, achieving 1% TI

Next step: increase activity further

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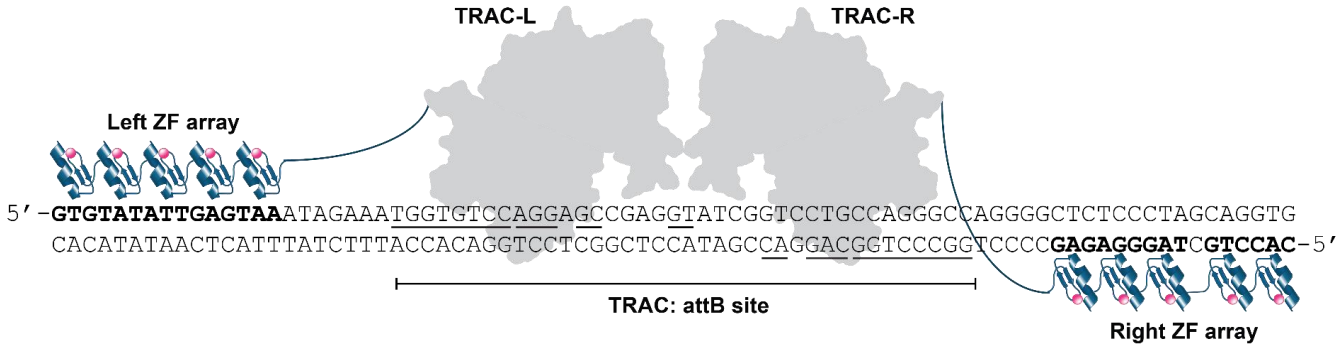
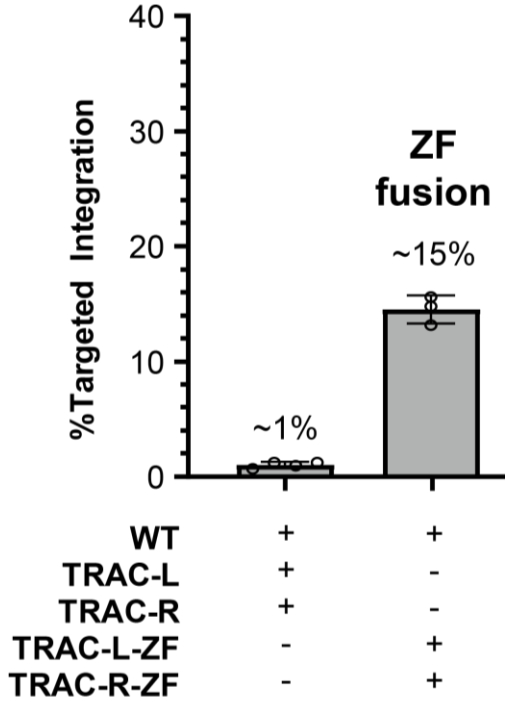
ZF fusions give a 15-fold increase to the on-target activity of MINT reagents

- **Zinc Finger (ZF) arrays increase binding affinity** by localizing MINT reagents to their intended genomic target site

- Screened a panel of ZF arrays and linkers with TRAC reagents

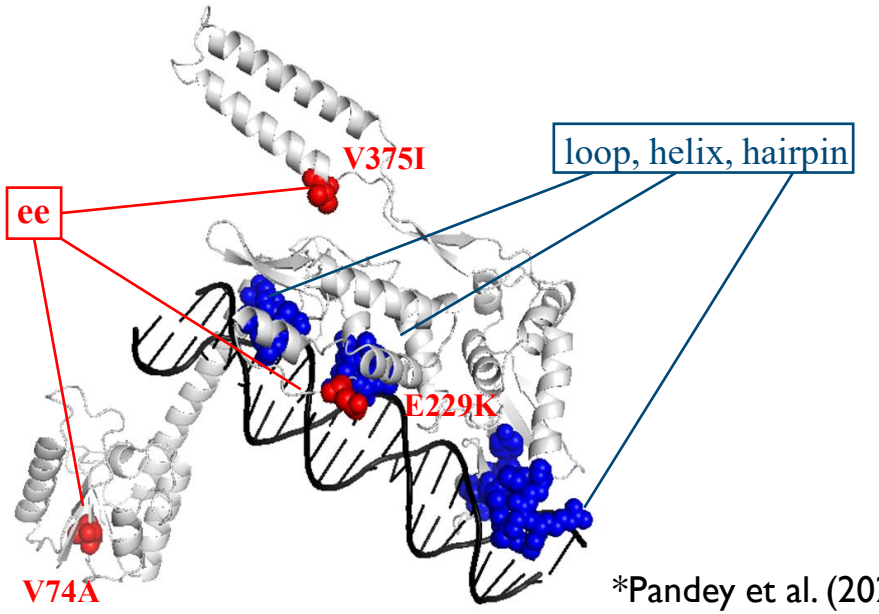




Best set of ZF fusion and linker combinations achieved
15% TI at the TRAC locus in K562 cells

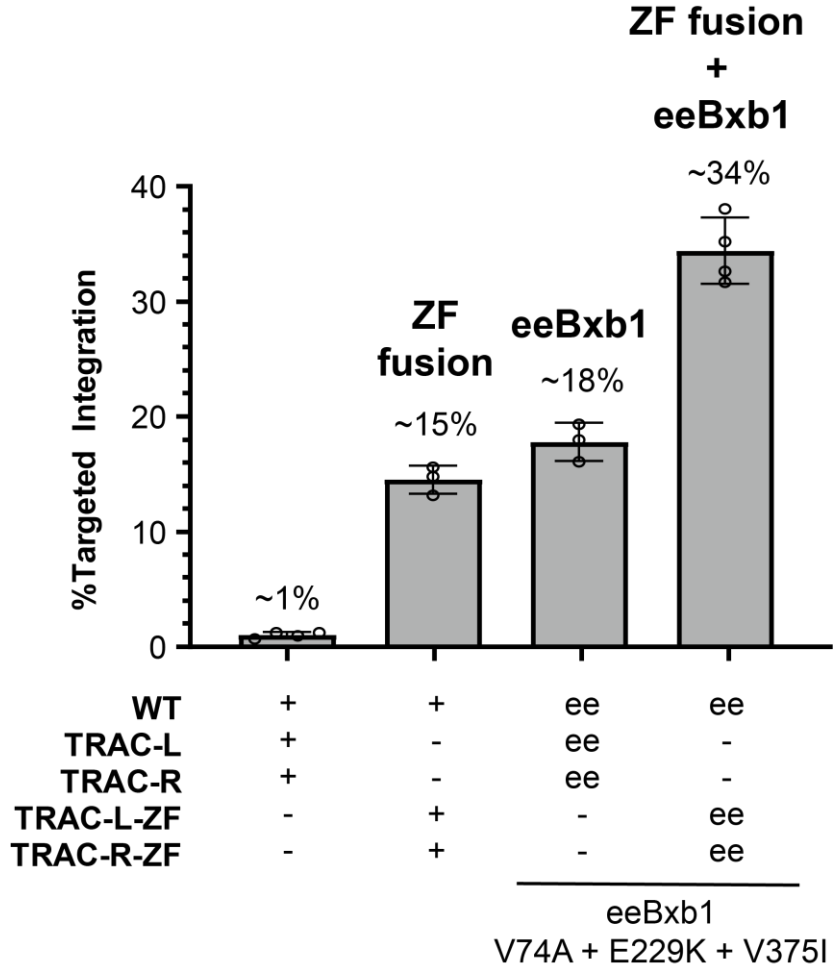
Combining ZF fusions and activity-increasing mutations yield therapeutically-relevant activity with TRAC reagents

- eeBxb1 is an activity-increasing Bxb1 variant developed by David Liu's team at Harvard*



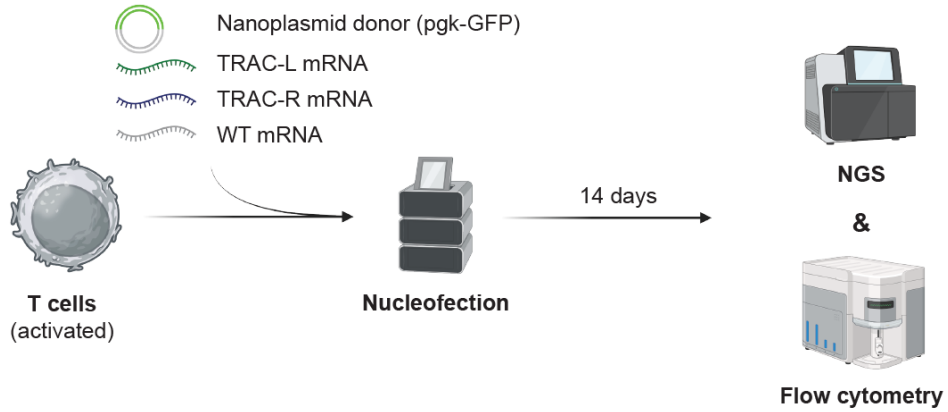
*Pandey et al. (2024) Nature

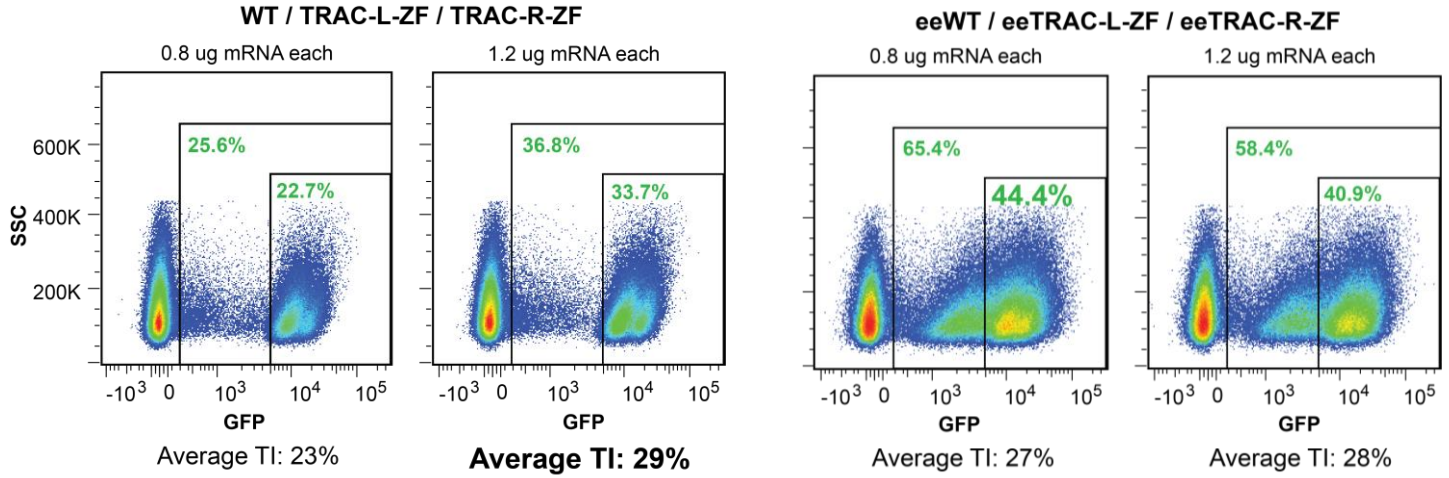
- Combining eeBxb1 with our TRAC reagents drove comparable improvements as ZF fusions



Combining ZF fusions with activity-increasing mutations achieved **~34% TI** at the TRAC in K562 cells

We successfully targeted the TRAC locus in primary human T cells





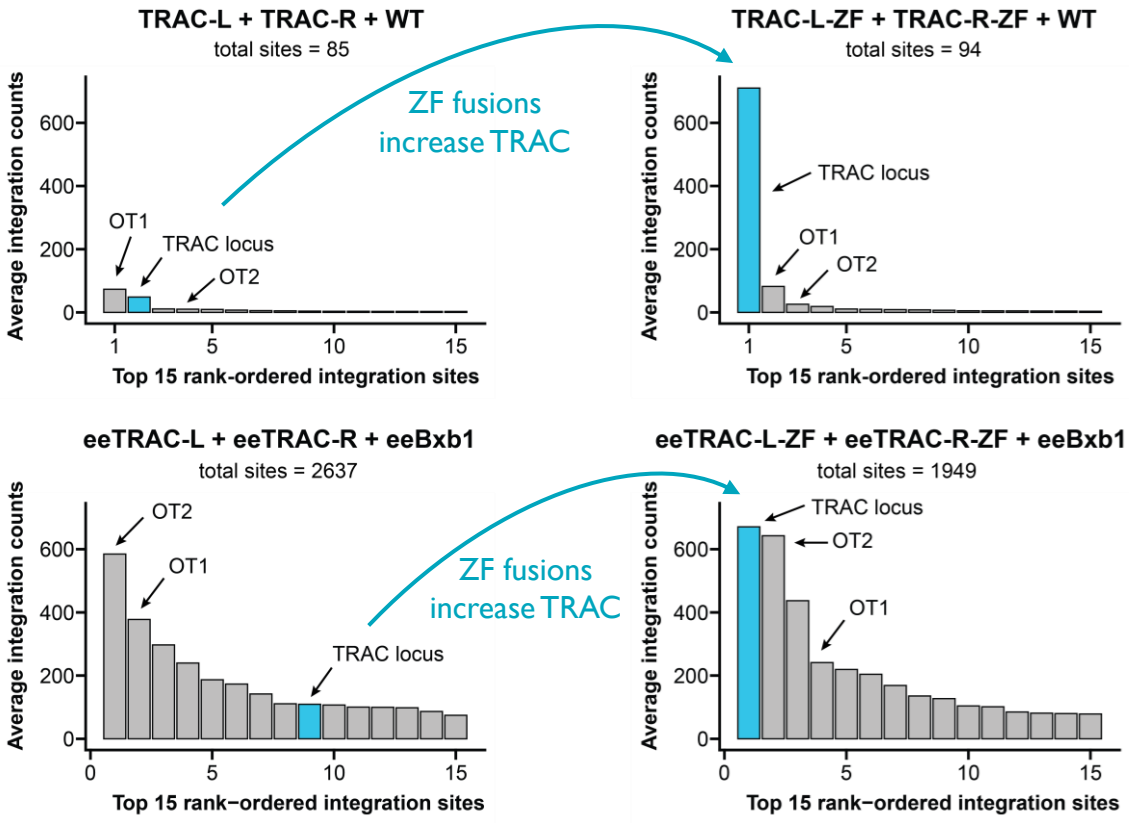
Achieved **~29% TI** at the TRAC locus and up to **44% GFP expression** in primary human T cells using ZF fusions

Images by Biorender

ZF fusions improve MINT reagent specificity and on-target activity

Unbiased genome-wide assay nominated off-targets

- Activity-increasing mutations reduced specificity
- ZF-fusions selectively increased the on-target signal
- Validated top two off-target sites in K562 and T cells
  - Relative TI followed similar trends as nomination assay
- No translocations observed across samples
  - Did observe a weak, local genomic inversion only with activity-increasing mutations



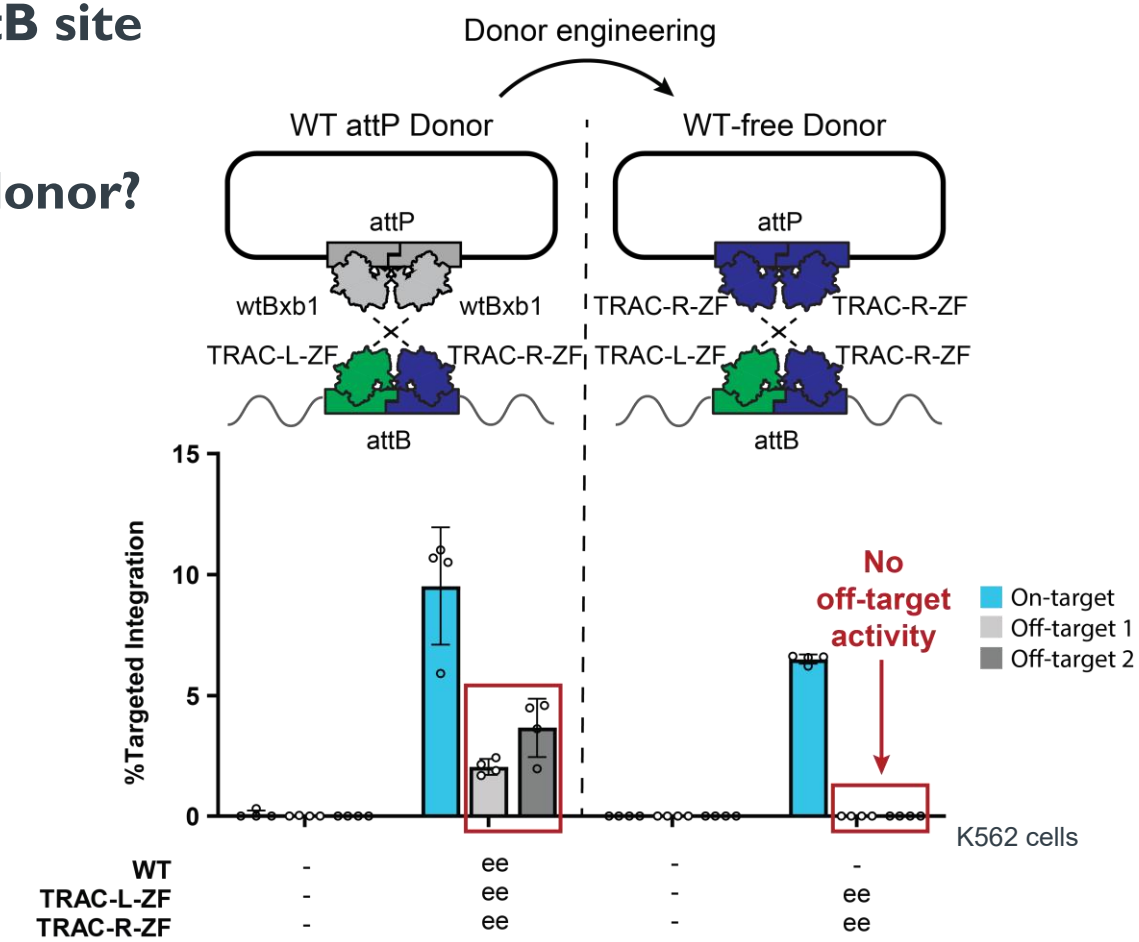
All assays performed in K562 cells

ZF-fused MINT reagents indicate a superior specificity profile

WT-free donor architecture further improves MINT reagent specificity

- **We retargeted Bxb1 to the genomic TRAC attB site**
- **Can we engineer a TRAC-like attP site in the donor?**
  - Only requires retargeted MINT reagents for activity
  - Removes need for wild-type Bxb1
  - **Reduces off-targets**

Donor engineering **removed off-targets** 1 and 2 for the most active TRAC reagents



Summary

- **MINT platform enables retargeting Bxb1 to therapeutically-relevant sites**
  - 34% TRAC TI and 29% AAVS1 TI in K562 cells
  - Used archive of pre-characterized Bxb1 DNA-binding domains to target two additional sites
- **Achieved high activity in T cells**
  - 29% integration at TRAC locus
  - 44% GFP-expressing cells
- **ZF fusions improve activity & specificity**
  - WT-free donor engineering further increases specificity

Accepted at Nature Biotech

Full preprint of work presented here:

Reprogrammed Serine Integrases Enable Precise Integration of Synthetic DNA

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doi: https://doi.org/10.1101/2024.05.09.593242

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May 12
Tuesday

Single-cell characterization of ST-506, a BBB-penetrant epigenetic repressor of Prion protein expression, in the nonhuman primate brain

Victoria Chou, **#1469**
Poster presentation

ST-503 nonclinical safety studies evaluating zinc finger repressors regulating the expression of the Nav1.7 gene for treatment of small fiber neuropathy

Kathleen Meyer, **#1309**
Poster Presentation

- Neurology Epigenetic Regulation

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Thank You

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Scientist, Design & Technology

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