Defining Genome Editing Technologies for Therapeutics

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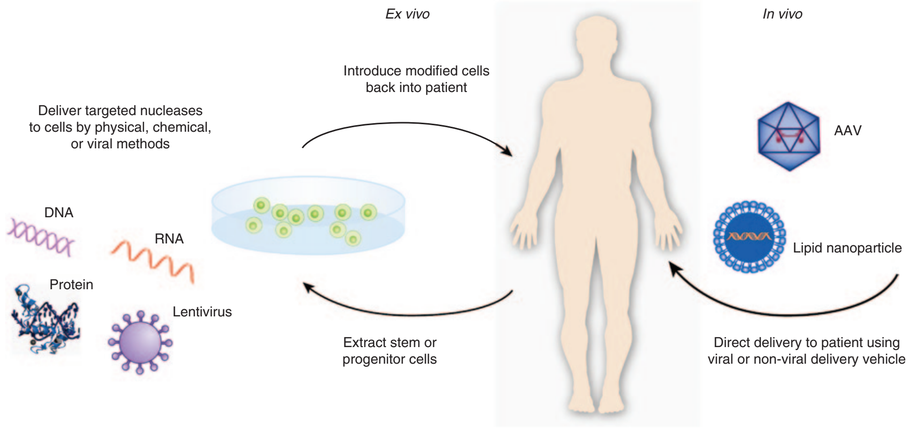
April 23, 2018
National Institute of Standards Technology

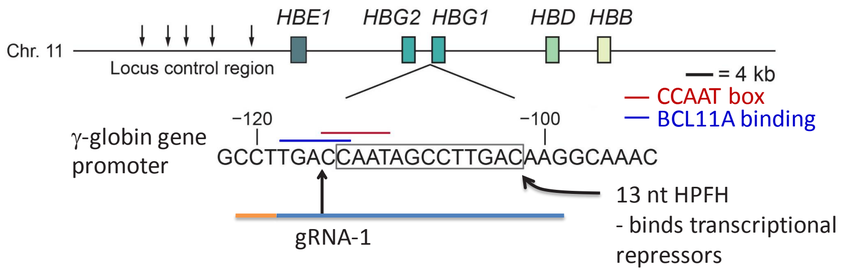
Potential Conflicts of Interest Disclosure

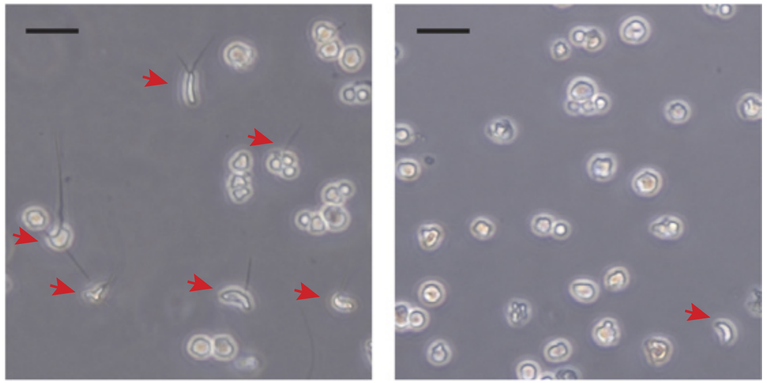
Shengdar Q. Tsai is a scientific co-founder of Monitor Biotech.

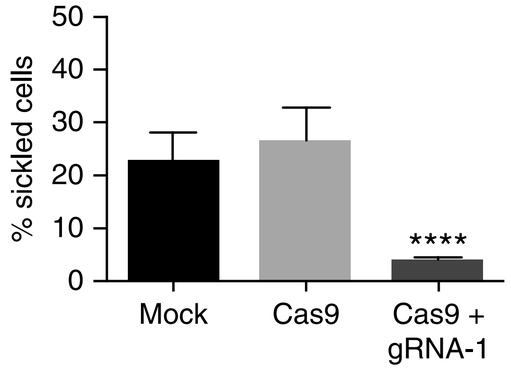
1. **Introduction**
2. State-of-the-art
3. Challenges
4. Summary

Genome editing for therapeutics









Exciting promise of genome editing is the potential to develop curative genetic therapies.

Maeder & Gersbach *Mol Ther.* 2016
Traxler *Nat. Med.* 2016.

Safety considerations: What about off-target effects?

- Typically, hundreds of millions or more cells modified by therapeutic gene editing
- Even low-frequency **off-target mutations** may be relevant if they induce a cellular growth advantage
- Important to address, particularly for **therapeutic applications**

Defining **where** off-targets may occur enables critical safety monitoring

- Unlike gene therapy, no vector integrations in gene editing for easy tracking
- **Defining off-target locations is important**, even if we cannot currently interpret the function of many off-target sequences
- Methods for defining off-targets as comprehensively as possible enable **monitoring for clonal expansion** of cells harboring specific unintended edits
- However, new methods for assessing functional impact (**risk**) are urgently needed.

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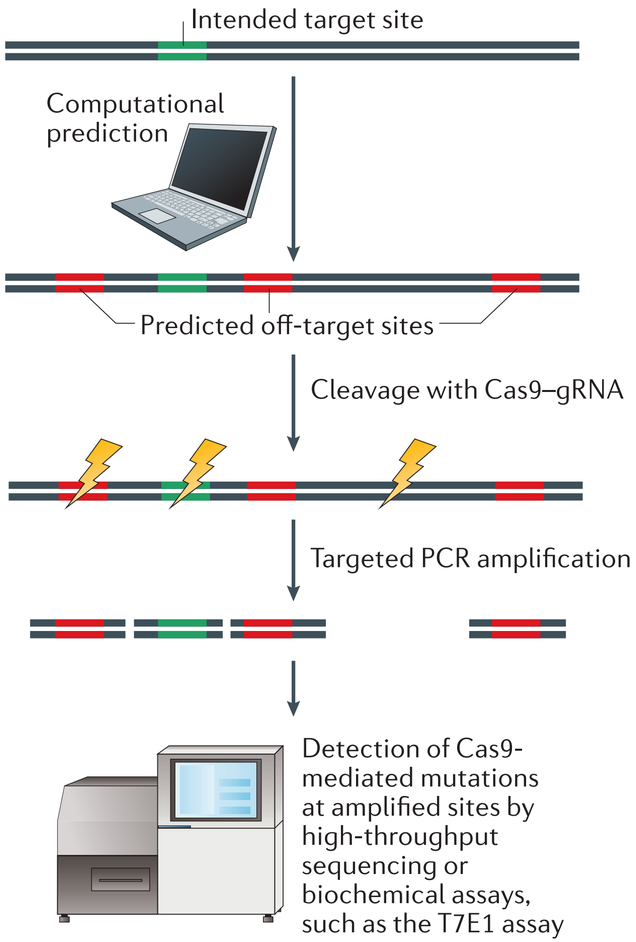
In silico prediction



Principle

- Computationally predict sites based on sequence similarity, etc.

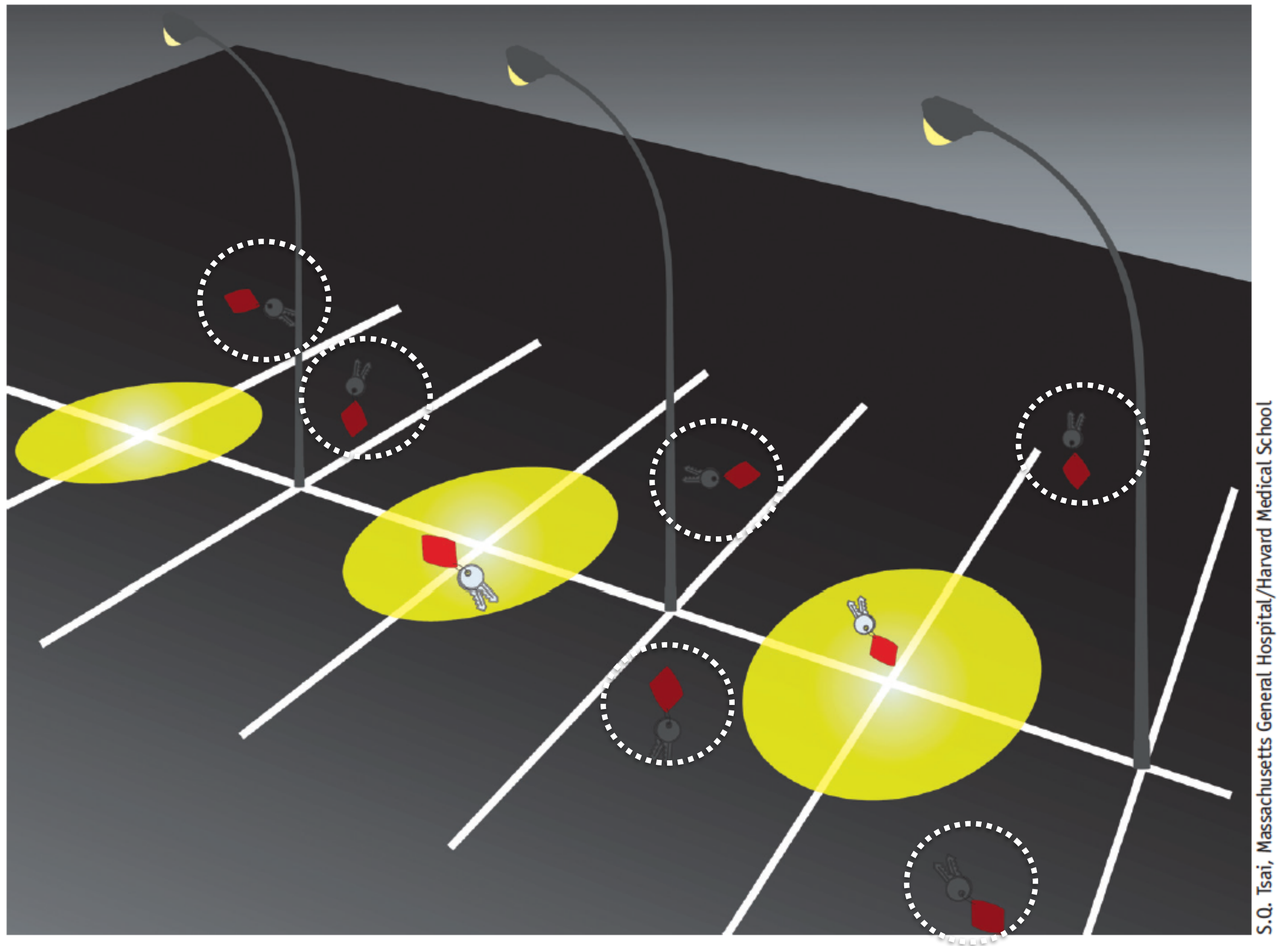
Advantages

- Easy

Disadvantages

- Biased by assumptions

The Streetlight Effect



S.Q. Tsai, Massachusetts General Hospital/Harvard Medical School

Whole genome sequencing is broad but shallow

- not practical to sequence large numbers of genomes
- significant limitation: it will miss off-target effects with low-frequency (i.e. sensitivity is poor)

Strategies for Defining Gene Editing Nuclease Genome-wide Activity

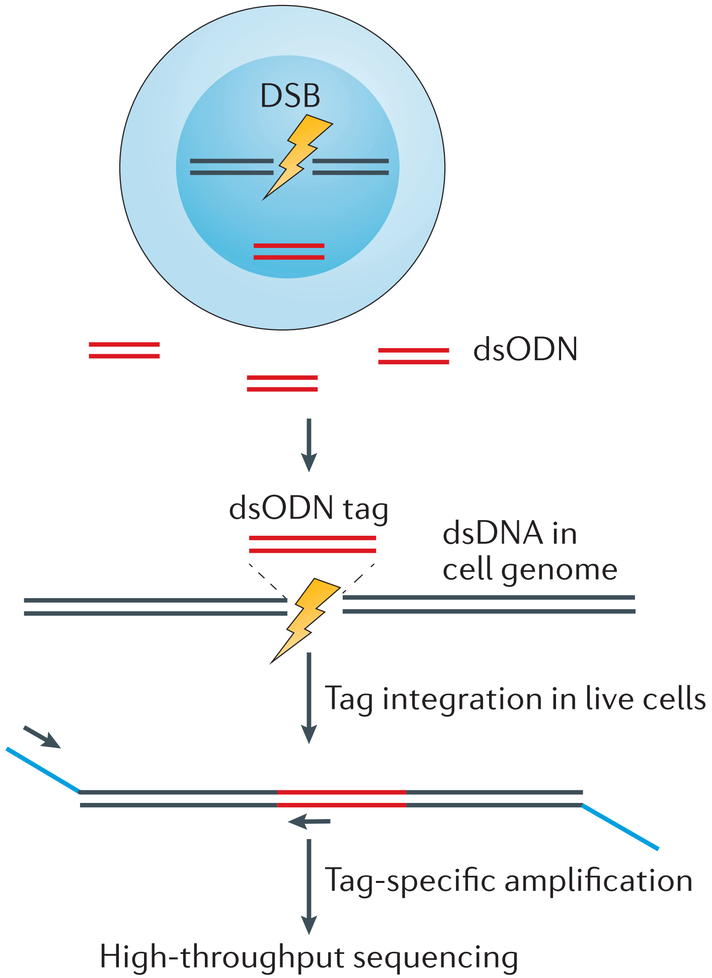
Cell-based Methods

- Integration deficient lentiviral (**IDLV**) capture
- High-throughput genome-wide translocation sequencing (**HTGTS**)
- Breaks labeling, enrichment on streptavidin and next-generation sequencing (**BLESS**)
- Genome-wide unbiased identification of DSBs enabled by sequencing (**GUIDE-seq**)

In vitro Methods

- Digested genome sequencing (**Digenome-seq**)
- selective enrichment and identification of adapter-tagged DNA ends by sequencing (**SITE-Seq**)
- Circularization for *in vitro* Reporting of Cleavage Effects by Sequencing (**CIRCLE-seq**)

GUIDE-seq: Genome-wide unbiased identification of DSBs enabled by sequencing



Principle

- Optimized tag integration into DSBs followed by tag-specific amplification and sequencing

Advantages

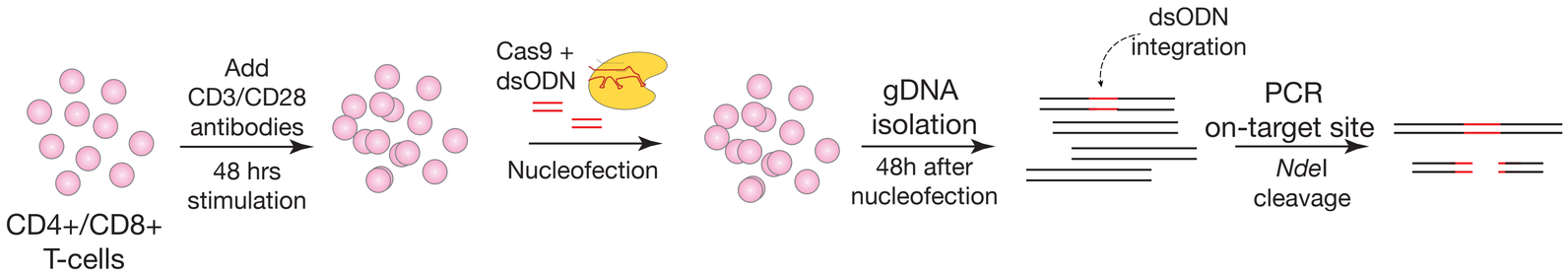
- Quantitative and unbiased method
- Can identify background or fragile sites

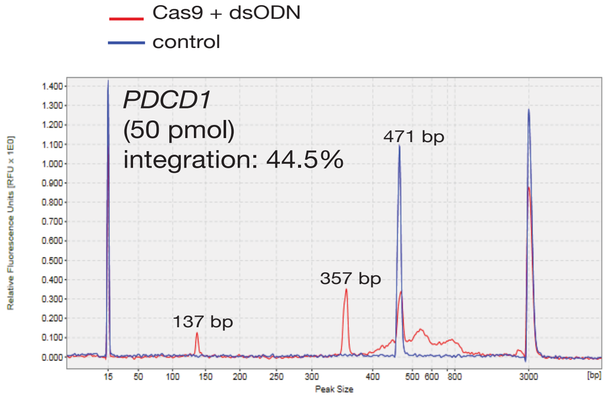
Disadvantages

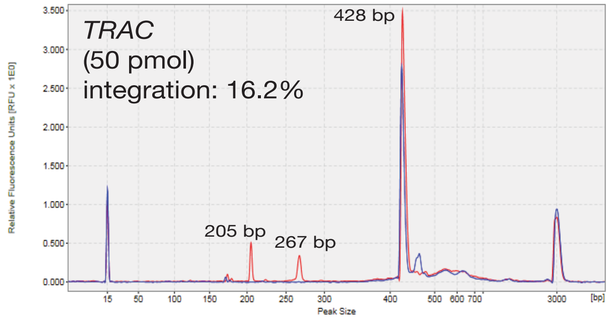
- Requires transfection of DNA tag, limiting use in some primary cells

Tsai et al. *Nat Rev Genetics* 2016

GUIDE-seq in Human Primary T-cells







GUIDE-seq

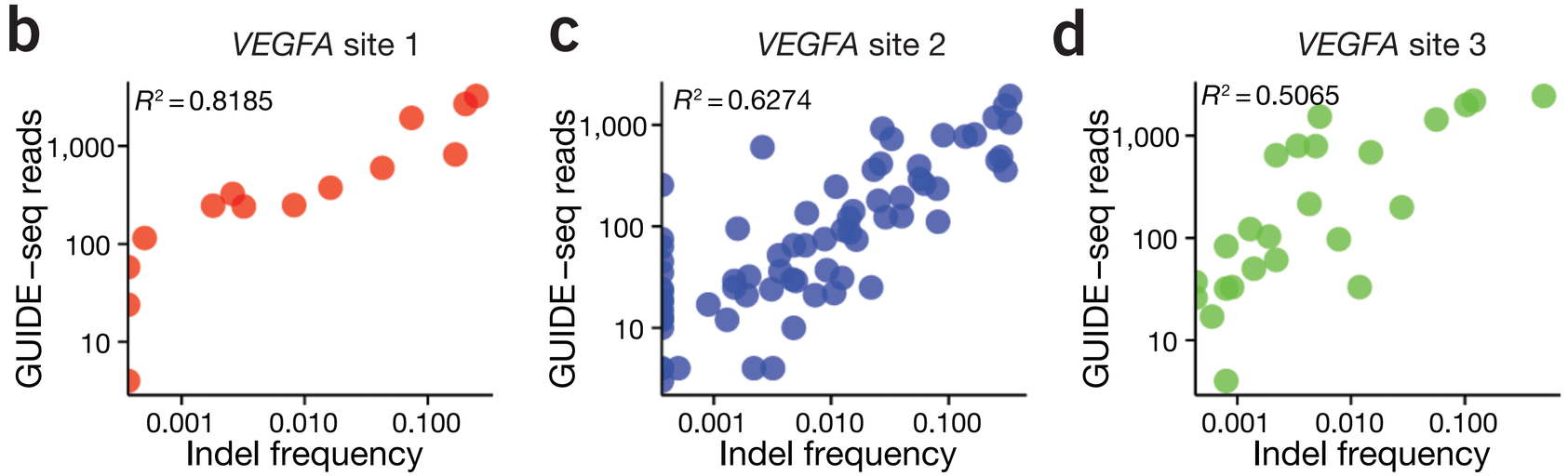
PDCD1

1									10										20		23	
G	G	G	C	G	G	T	G	C	T	A	C	A	A	C	T	G	G	G	C	N	G	G	Reads
·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	5098
A	A	·	G	·	C	G	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	17

TRAC

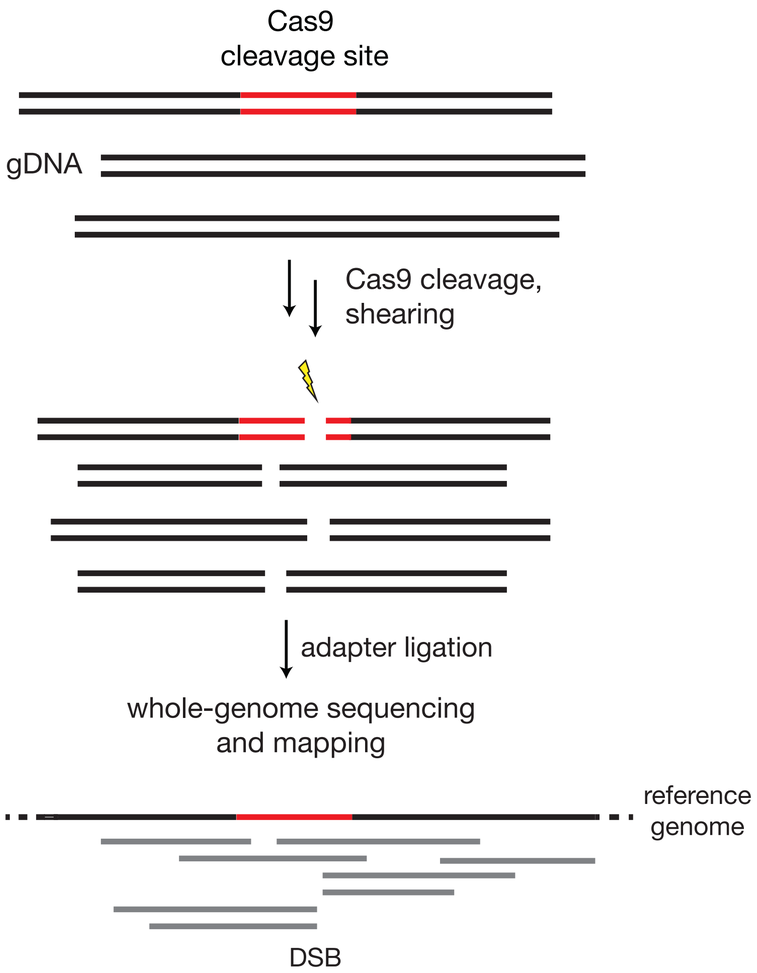
1									10										20		23	
C	T	T	C	A	A	G	A	G	C	A	A	C	A	G	T	G	C	T	G	N	G	G	Reads
·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	1506
T	·	G	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	234

GUIDE-seq is quantitative but has limits to sensitivity



- Tag integration proportional to mutagenesis frequency
- Increasing sensitivity will require linear scaling of input genomes and sequencing

Digenome-seq: Digested Genome Sequencing



Principle

- *In vitro* cleavage of genomic DNA, whole genome sequencing, and identify sites with uniform ends

Advantages

- Simple, PCR-free

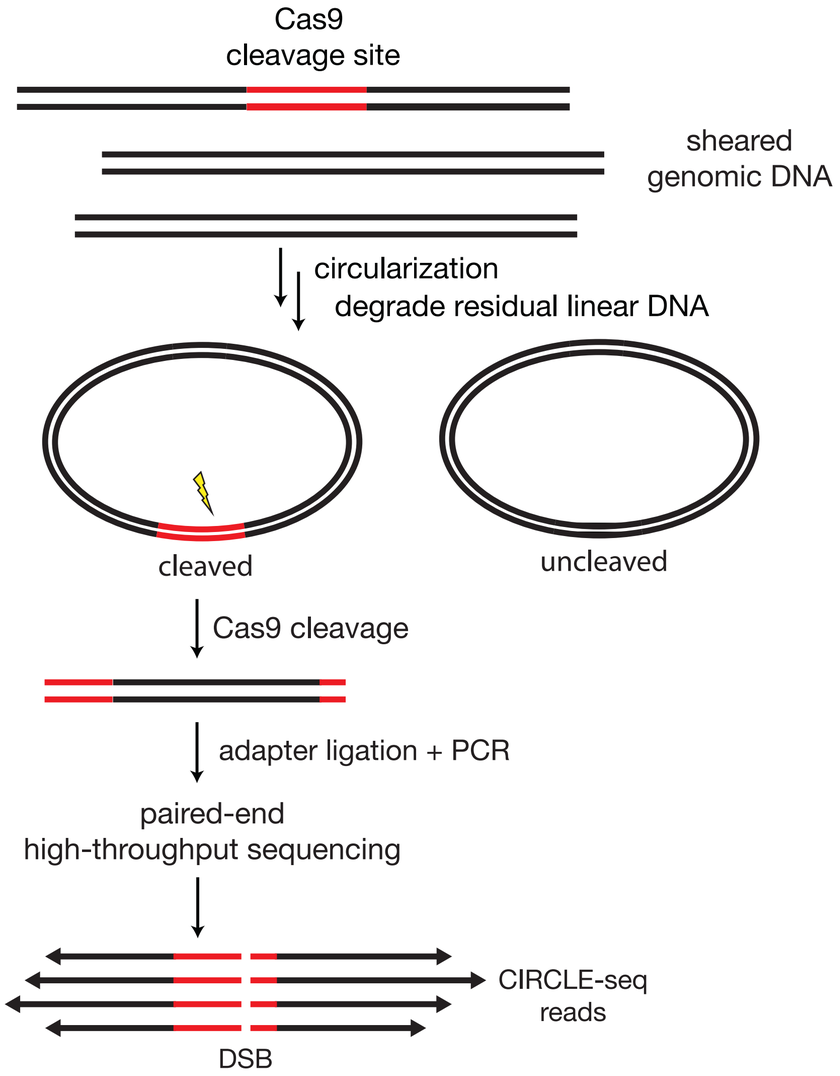
Disadvantages

- High number of sequencing reads required and high background
- Candidate sites need to be confirmed in cells

Tsai et al. *Nat Rev Genetics* 2016.

Standing out from a crowd

CIRCLE-seq: Circularization for *in vitro* reporting of cleavage effects by sequencing



Principle

- Selective sequencing of nuclease-cleaved genomic DNA

Advantages

- Low background
- High sensitivity
- Reference-free

Disadvantages

- Candidate sites need cellular confirmation

Tsai et al. *Nature Methods 2017*

CIRCLE-seq improves signal to noise while using 100-fold less reads

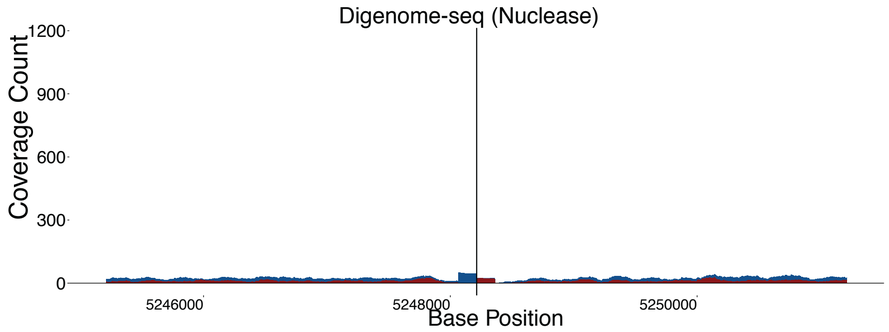
Digenome-seq

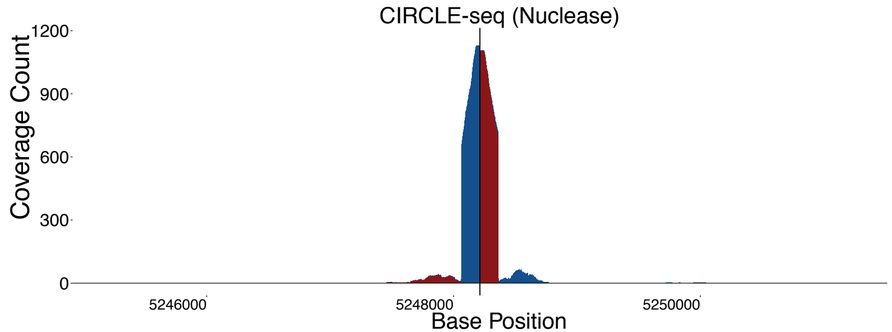
CIRCLE-seq

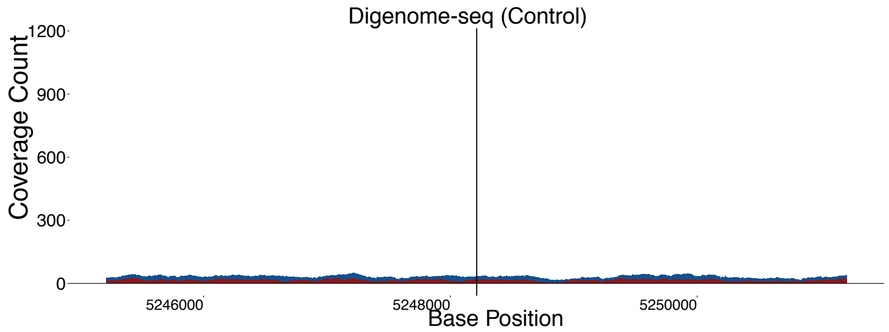
HBB

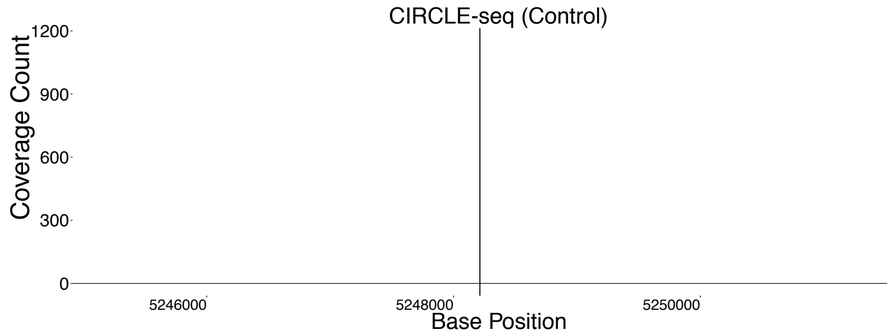
Nuclease

Control









400 million reads

4 million reads

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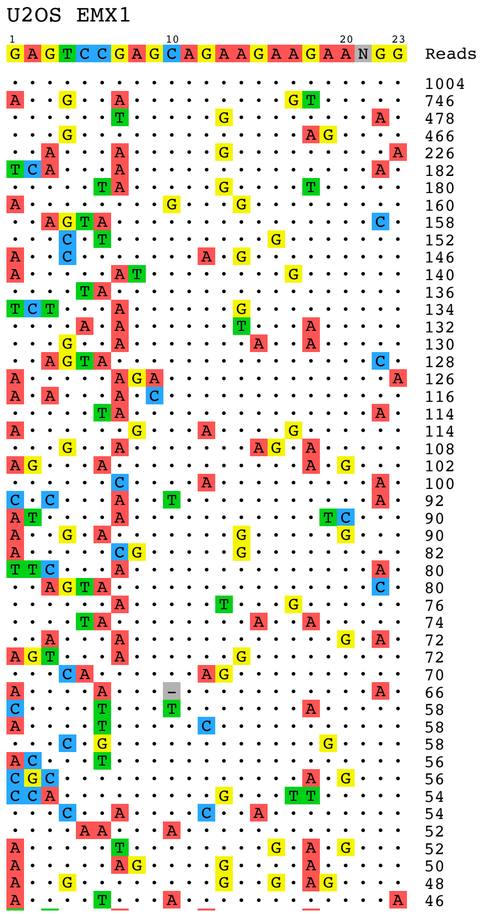
Tsai et al. *Nature Methods 2017.*

CIRCLE-seq genome-wide nuclease activity profiles



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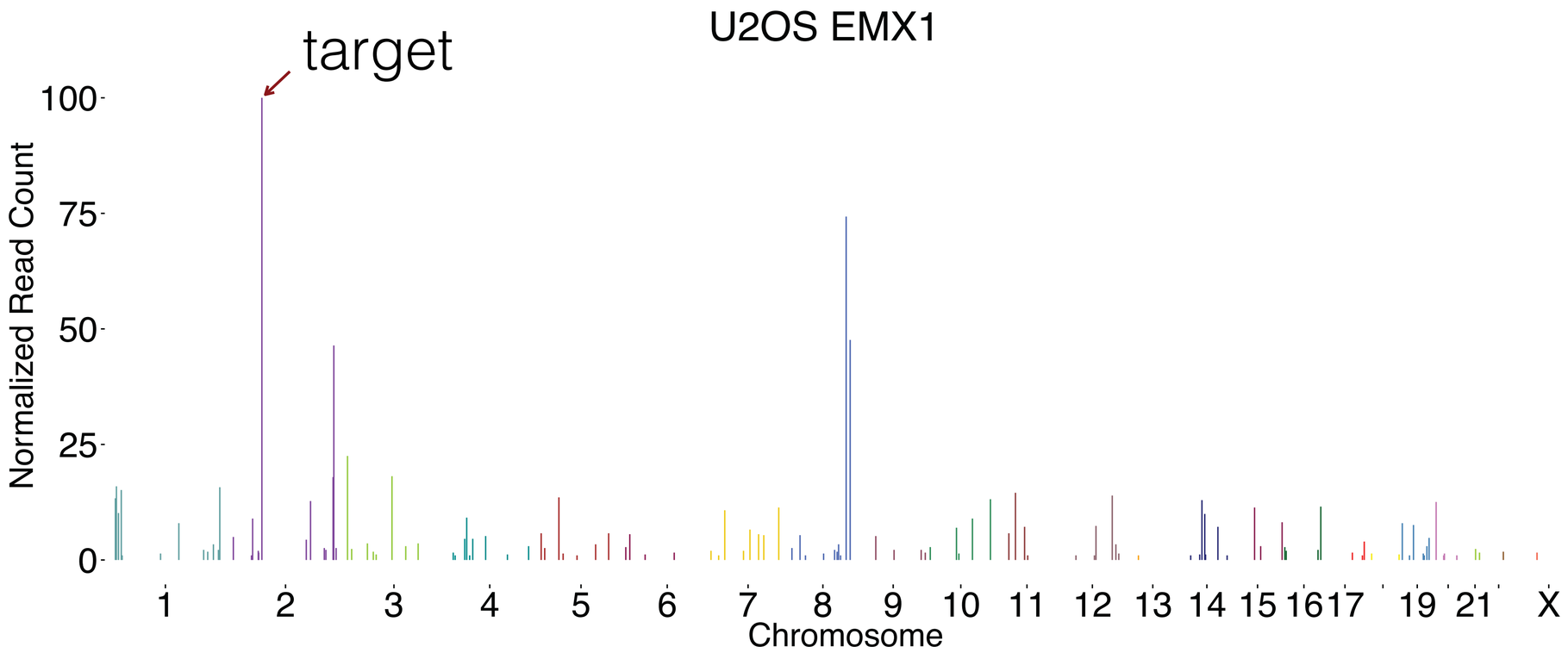

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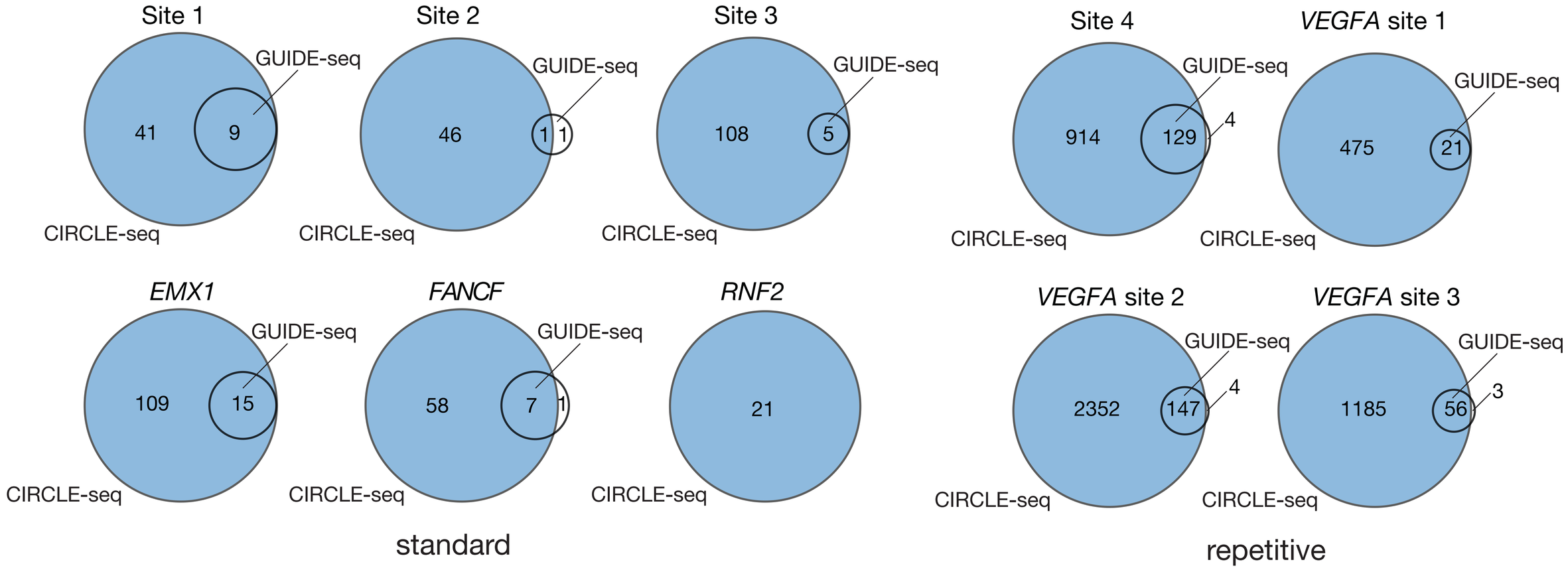
CIRCLE-seq detects nearly all sites previously detected by GUIDE-seq



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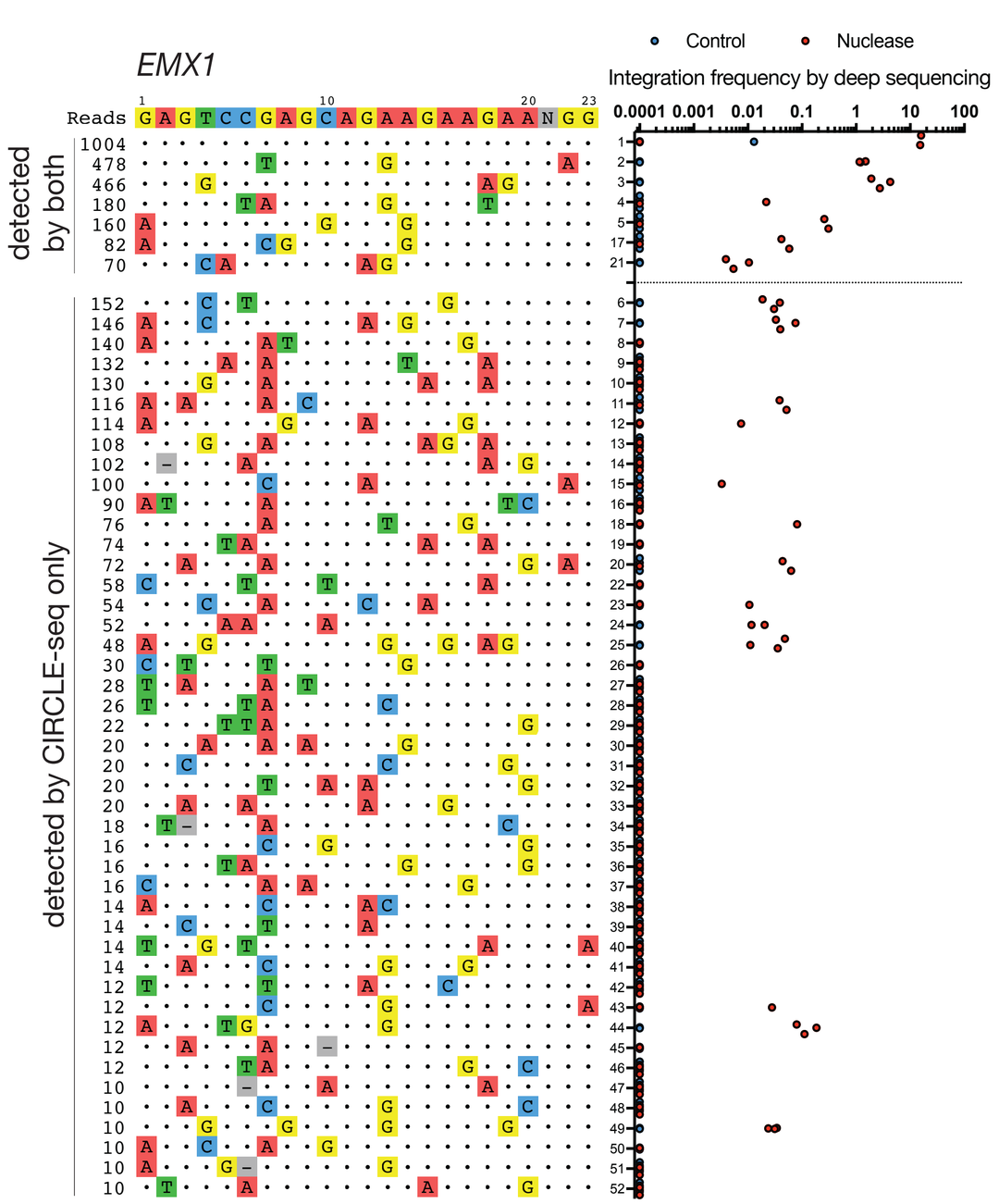
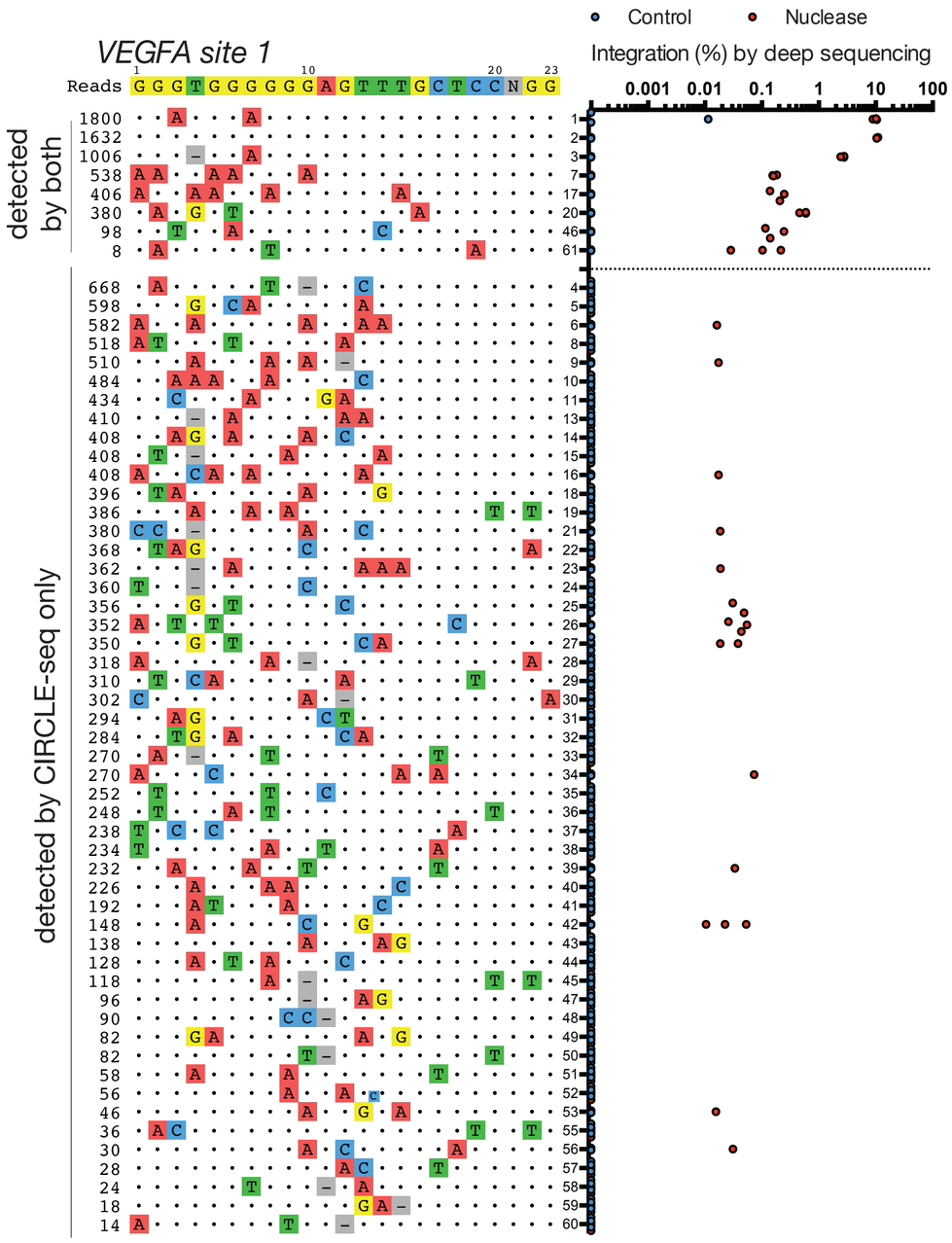
Targeted tag sequencing strategy can overcome standard NGS limits of detection

- Background indel rate of modern high-throughput sequencing around 0.1%
- Tag integration is unambiguous evidence of DSB repair
- Proxy for mutagenesis frequency

Tsai et al. *Nature Methods 2017.*





~25% of sites detected only by CIRCLE-seq confirmed as *bona fide* cellular sites.

Tsai et al. *Nature Methods 2017*

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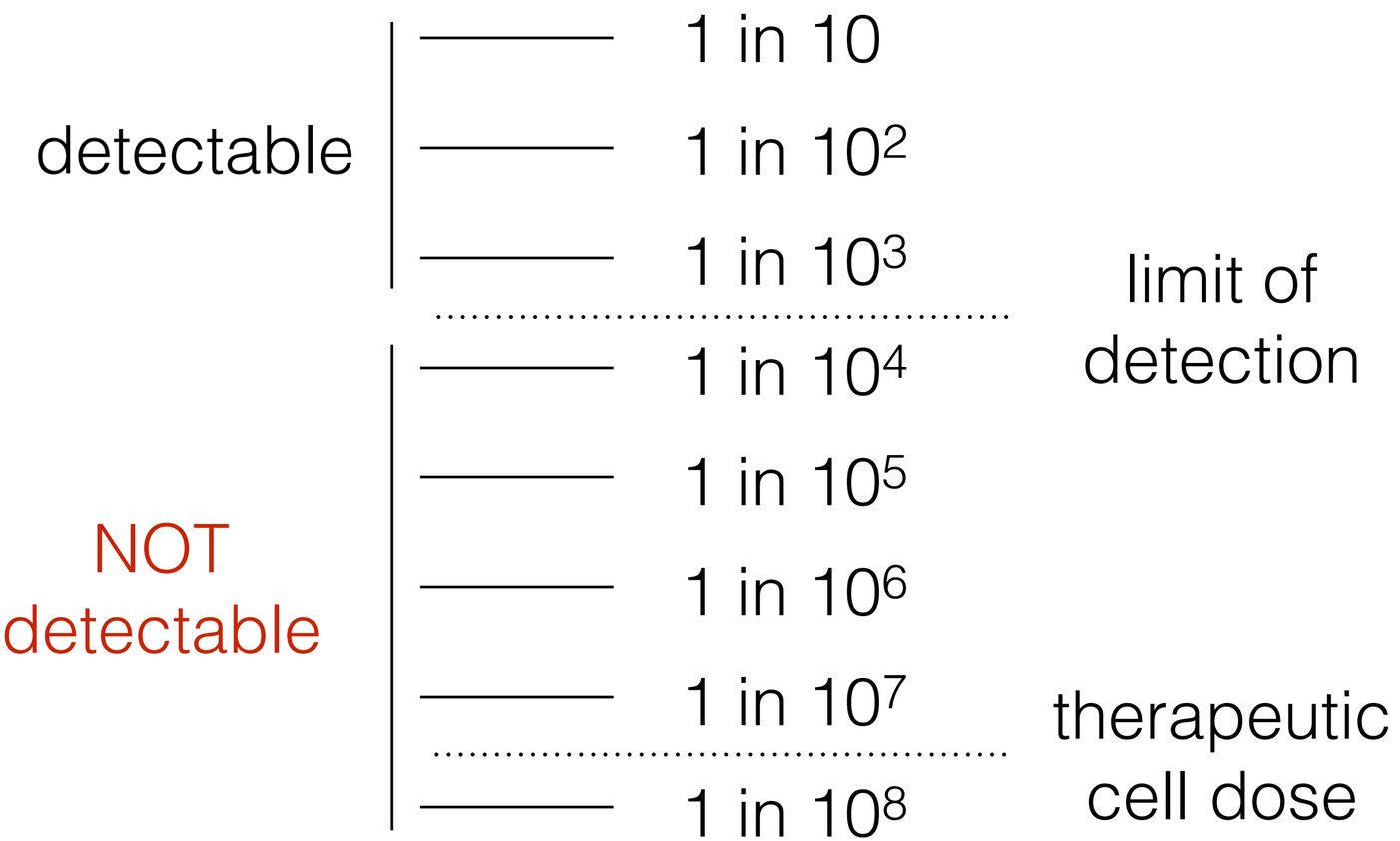
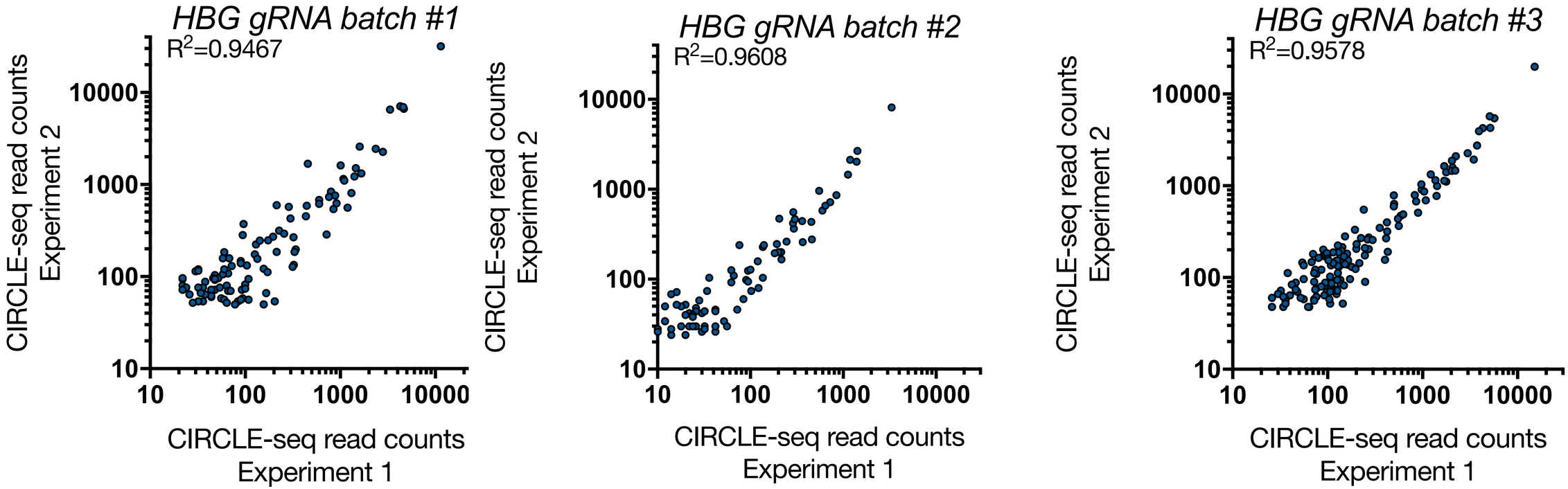
Technical limitations of standard high-throughput sequencing

- Error-rate obscures low-frequency mutations
- Short read length prevents mapping through repetitive regions



What is technical reproducibility of measurements?



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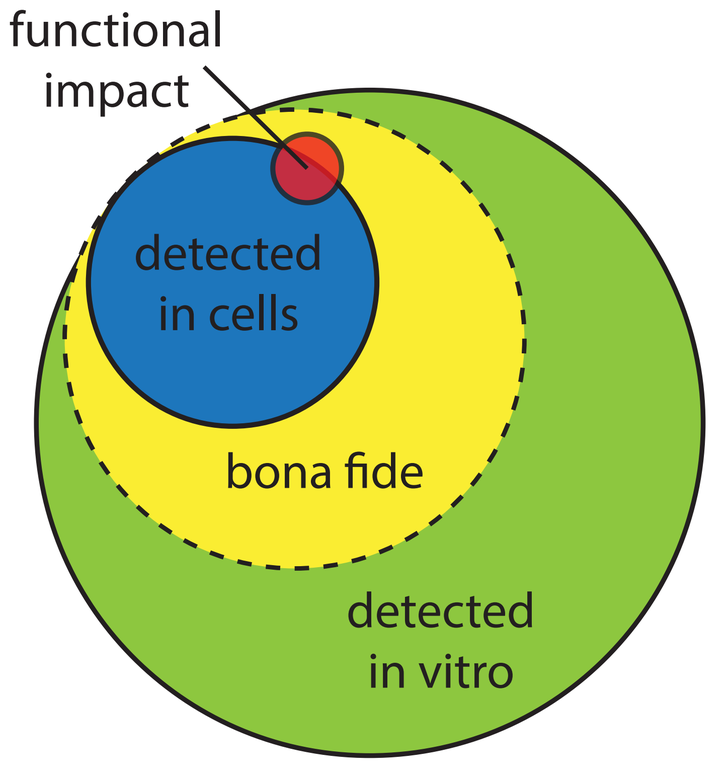
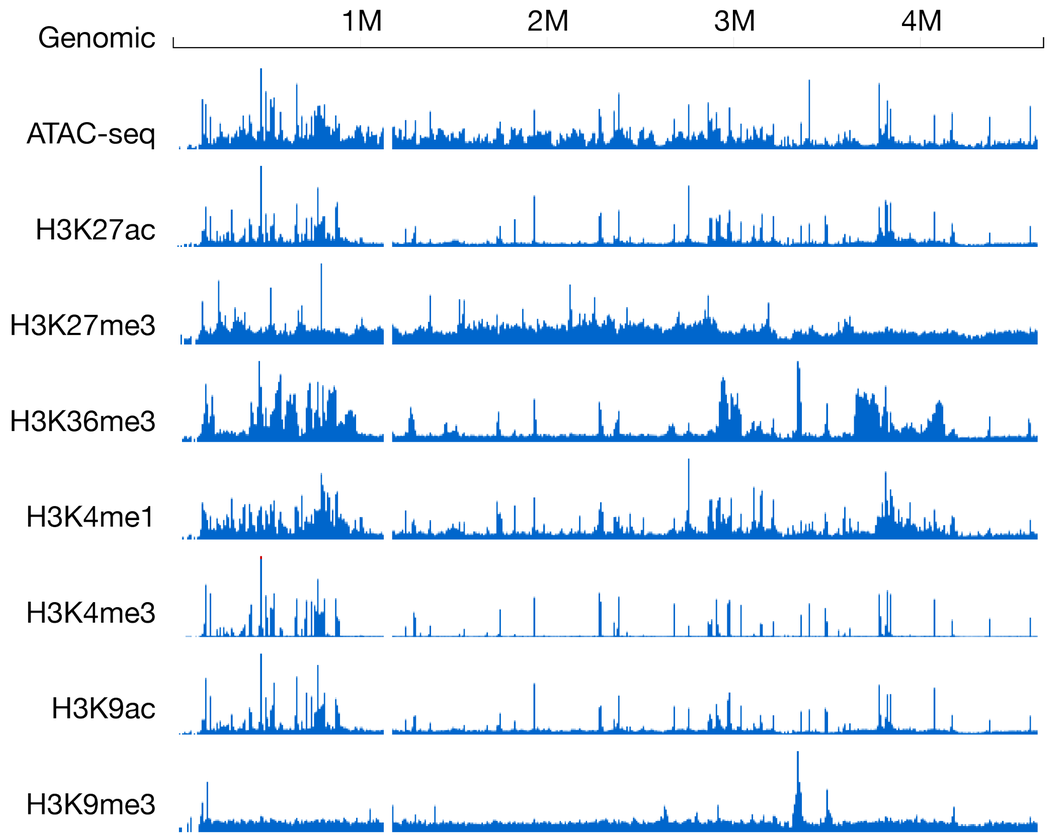
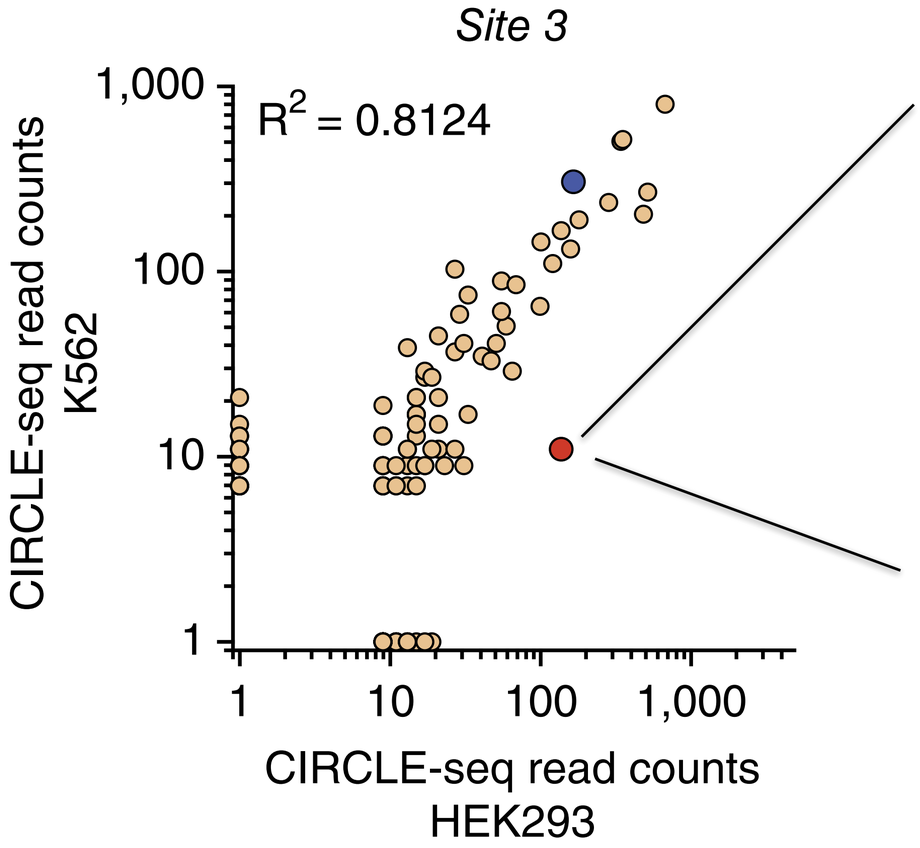
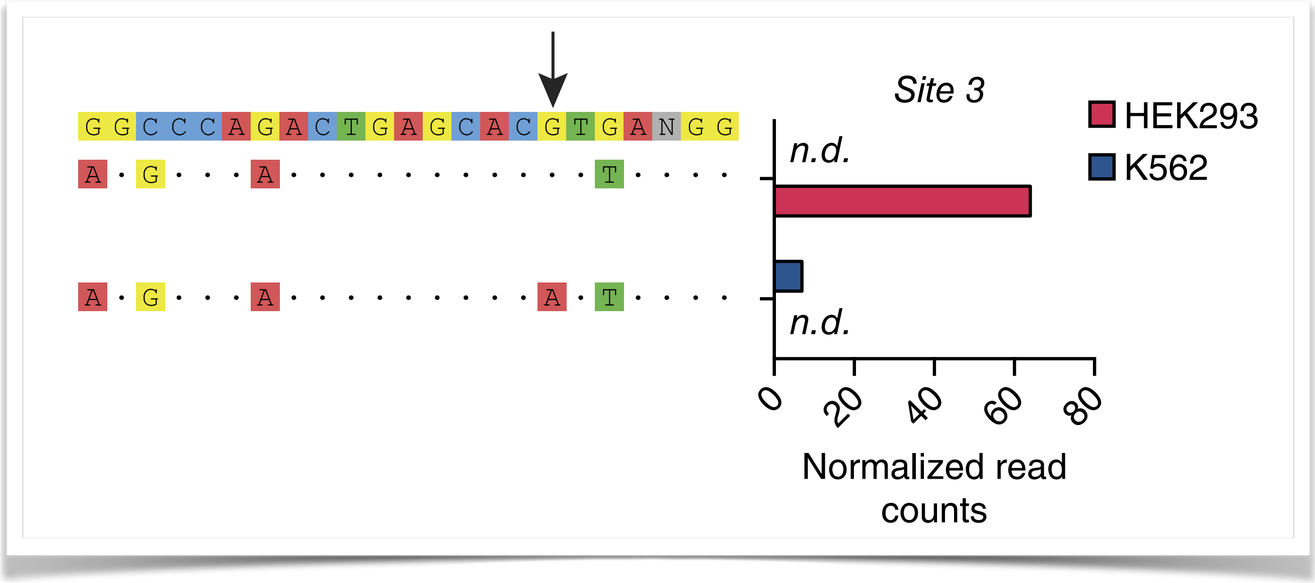
What determines relationship between cellular and *in vitro* measurements?





What is the impact of human genetic variation on genome editing activity?





Could be tested in genome-in-a-bottle reference cell lines.

Tsai et al. *Nature Methods 2017.*

Towards Developing Genome Editing Measurement Standards

- Reference standards for validating low-frequency mutation detection.
- Challenge and benchmark detection methods at common targets/nucleases/cells.
- Define reproducibility and limits of detection.

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Summary

- Important to empirically define genome-wide activity of gene editing for therapeutics
- GUIDE-seq and CIRCLE-seq are two complementary methods to define genome-wide genome editing activity
- Glass half full: fortunate to have many sensitive and unbiased genomic methods to define nuclease specificity
- NIST consortium has opportunity to develop standards to define genome editing measurements and increase confidence in safety of promising genome editing therapeutics

Thank you

Tsai Lab*
Cicera Lazzarotto
Nikolay Malinin

Weiss Lab
Jean-Yves Metais
Yu Yao
Sagar Keriwala

Joung Lab
Yanfang Fu
Nhu Nguyen
Ved Topkar
Jeff Sander

Aryee Lab
Jose Malagon-Lopez
Vishal Thapar