Genome-wide Identification of Gene Editing Off-target Effects

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Targeted Genome Editing

• capability to introduce precise targeted alterations into ANY specific DNA sequence with high efficiency in ANY living cell or organism . . .

• . . . ideally, without making any other undesired changes
Genome Editing is a Broadly Applicable Technology

- “Breakthrough of the year” 2015 in Science
- Robust in many living organisms, plants, animals, cells, and bacteria
- Correct human genetic disorders
- Improve biotechnology
Repair of nuclease-induced double-stranded breaks

NHEJ

Variable indels

“gene knockout”

Nuclease-induced DSB

HDR

Donor template

Precise sequence modification

“gene correction”

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Why Define Off-targets?

• For therapeutic applications, even low-frequency events (that could lead to increased oncogenic potential) are important.

• For research, important to exclude or minimize possibility that off-targets confound biological results.

• For biotechnology, defining off-targets could reduce unintended consequences of genetic engineering
**Early Targeted Approaches**

1. **Intended target site**
   - Computational prediction

2. **Predicted off-target sites**
   - Cleavage with Cas9–gRNA

3. **Targeted PCR amplification**
   - Detection of Cas9-mediated mutations at amplified sites by high-throughput sequencing or biochemical assays, such as the T7E1 assay

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Tsai et al. Nat Rev Genet 2016
observational bias
There are assays to predict and assess off-target effects, but for now they offer only partial illumination of the genome.
The Streetlight Effect

There are assays to predict and assess off-target effects, but for now they offer only partial illumination of the genome.
Cell-based Genome-wide Off-target Detection

The most sensitive cell-based methods can detect off-target mutations with frequencies down to ~0.1% or 1 in 1000.

IDLV
GUIDE-seq
HTGTS
BLESS

Tsai et al. Nat Rev Genet 2016
In Vitro Genome-wide Off-target Detection

**Digenome-seq**

- Cas9 cleavage site
- Genomic DNA
- Cas9 cleavage, shearing
- Adapter ligation
- Whole-genome sequencing

**CIRCLE-seq**

- Cas9 cleavage site
- Sheared genomic DNA
- Circularization degrade linear DNA
- Cleaved uncleaved
- Adapter ligation + PCR
- Paired-end high-throughput sequencing
- DSB
- CIRCLE-seq reads
Strategies to reduce CRISPR-Cas nuclease off-target effects

Tsai et al. Nat Rev Genet 2016
## Recommendations for Off-target Effects

<table>
<thead>
<tr>
<th>Research</th>
<th>Therapeutics</th>
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<tbody>
<tr>
<td>• Two orthogonal gRNAs targeted to same site can exclude possibility of confounding off-target effects</td>
<td>• Dependent on therapeutic dose, low-frequency off-targets are important to consider</td>
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<td>• Genetic complementation and reversion of a phenotype</td>
<td>• Need to do best job possible to identify off-target effects using sensitive, unbiased, genomic methods</td>
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For research, may not need genome-wide methods.
Take-home Messages

• Unintended off-target sites can be cleaved by gene editing nucleases

• Sensitive, unbiased, genome-wide methods enable careful, empirical consideration of gene editing off-target effects

• Specific application will determine level of scrutiny required