Genome Editing with Precision and Accuracy

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Gene Editing: Sequence Changes to DNA Code

Genome editing is the method to correct typographical errors.

Adapted from Matthew Porteus (Stanford), nationalacademies.org/gene-editing/Gene-Edit-Summit/Slide-Presentations/index.htm
**Genome Editing Components**

**Challenge:** Precise nanoscale delivery of all CRISPR components

DNA: 2.5 nanometer diameter

Q2: How does CRISPR work?

[YouTube Video](https://youtube.com/watch?v=O360cFGDbfE)

Max-Planck Gesellschaft
Precision & Accuracy Matter: Embryo Editing Case

Precision & Accuracy Matter: Embryo Editing Case

Before Genome Editing

Unedited CCR5

CRISPR cut site

TM1

CCR5Δ32 (HIV Protective)

IKDSHLGAGPAACHG
HLLGNPKNSAVS

Desired Goal

Human Embryo

CRISPR machinery

Ryder, *CRISPR Journal*, 2018

Normille, *Science*, 2018
Precision & Accuracy Matter: Embryo Editing Case

Before Genome Editing

Desired Goal

Ryder, CRISPR Journal, 2018

After Genome Editing
In the clinic, creating therapeutics for:

- Blood Disorders – *in vivo* and “ex vivo” editing
- Blindness
- Cancer
- Heart Disease…

Builds on momentum of approved gene therapies

Four Ways to Achieve Precision

1) DNA cutting precision
2) On-target, scarless knock-in of sequence variants
3) Transcriptional control of edited genes
4) Specificity in delivery to a specific cell or tissue

Precise Insertion of New Sequences (2)

DNA Repair Pathways After Cas9 DNA Cleavage

- Genomic DNA
  - error-prone end joining
  - homology directed repair

- ssODN
  - Precise gene editing

- Imprecise gene editing

Nucleus
Outcomes After 18 Nucleotide Insertion

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Sequence</th>
<th>Type</th>
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<tr>
<td>WT</td>
<td>CACCCTGACCGACTACAAAGACGATGACGACAAGAATTCTTAC</td>
<td>Precise</td>
</tr>
<tr>
<td>HDR w/mutation</td>
<td>CACCCTGACCCTACAAAGACGATGACGACAAGAATTCTTAC</td>
<td>Imprecise</td>
</tr>
<tr>
<td>HDR w/mutation</td>
<td>CACCCTGACCGCTACAAAGACGATGACGACAAGAATTCTTAC</td>
<td>Imprecise</td>
</tr>
<tr>
<td>Incorrect HDR</td>
<td>CACCCTGACCGACTACAAAGACGACAAAGAATTCTTAC</td>
<td>Imprecise</td>
</tr>
<tr>
<td>NHEJ</td>
<td>CACCCTGACCGACTACAAAGACGACCAAGAATTCTTAC</td>
<td>Imprecise</td>
</tr>
</tbody>
</table>
CRISPR Nanoparticle for Co-delivery of Components

All necessary components (Cas9, sgRNA, single stranded oligonucleotide DNA template) for homology directed repair assembled *in vitro* and delivered as complex

**Is co-delivery possible and could it make editing more precise?**

Base Editing to Avoid DNA Cleavage Altogether

- Engineer Cas9 protein to avoid DNA cutting on one strand

The “base editor” variant of Cas9 for C to T change:

Komor et al. _Nature_, 2016
Precise Cellular Targeting (4)

Issues with Viral Delivery

Viral Gene Therapies, 2018:

- Difficult to control amount and targeting of Cas9 using viral vectors
- Viral vectors difficult to use and expensive
- Viral DNA could be integrated into the genome
- Potential of immune response to viral vectors
- Off-target effects due to wide distribution and expression of Cas9
- Imprecise edits when attempting scarless editing

Solution  Novel nonviral strategies could be produced as off-the-shelf reagents
Redox responsive polymer composition to transflect cells with Cas9-sgRNA ribonucleoproteins

Advantages:
• No viral components

• Tunable and targetable
  • Cell/tissue targeting
  • Modify circulation
  • Evade immune response
  • Loading within biomaterials and scaffolds

• More biocompatible than commercial agents

Stable in the Bloodstream…But Degrades Within Cells

Transmission electron microscopy image of the RNP nanocapsules

Dynamic Light Scattering

Testing Gene Editing *In Vivo* Within Reporter Mice

Gene Editing Within Muscle
Decorated Nanocapsules To Target The Eye

Decorated Nanocapsules To Target The Eye

Gene Editing in vivo with Decorated Nanocapsules
Applications in the Eye: Diseases of the Retina

• Pursuing applications in the eye, brain, muscle, blood, and liver for blindness, neurodegenerative diseases, newborn disorders, cardiovascular disease and cancer

• **Off-the-shelf reagents**
  • Quality controlled, easy to manufacture
  • Biologic product without any viral components
Design Principles for Precise Genome Editing


INCCREASE

- (2) On-target, scarless knock-in of sequence variants
- (4) Progenitor cell targeting

- Genome Editor
  - TARGETED DELIVERY
  - ON-TARGET LOCUS
    - End-joining
    - Precise Insertion
  - OFF-TARGET LOCUS
    - DNA REPAIR TEMPLATE
    - Imprecise Indels
  - mRNA
  - ENDOGENOUS PROMOTER
    - PRECISE TRANSCRIPTIONAL CONTROL
  - IMPRECISE TRANSCRIPTIONAL CONTROL
  - Random integration
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