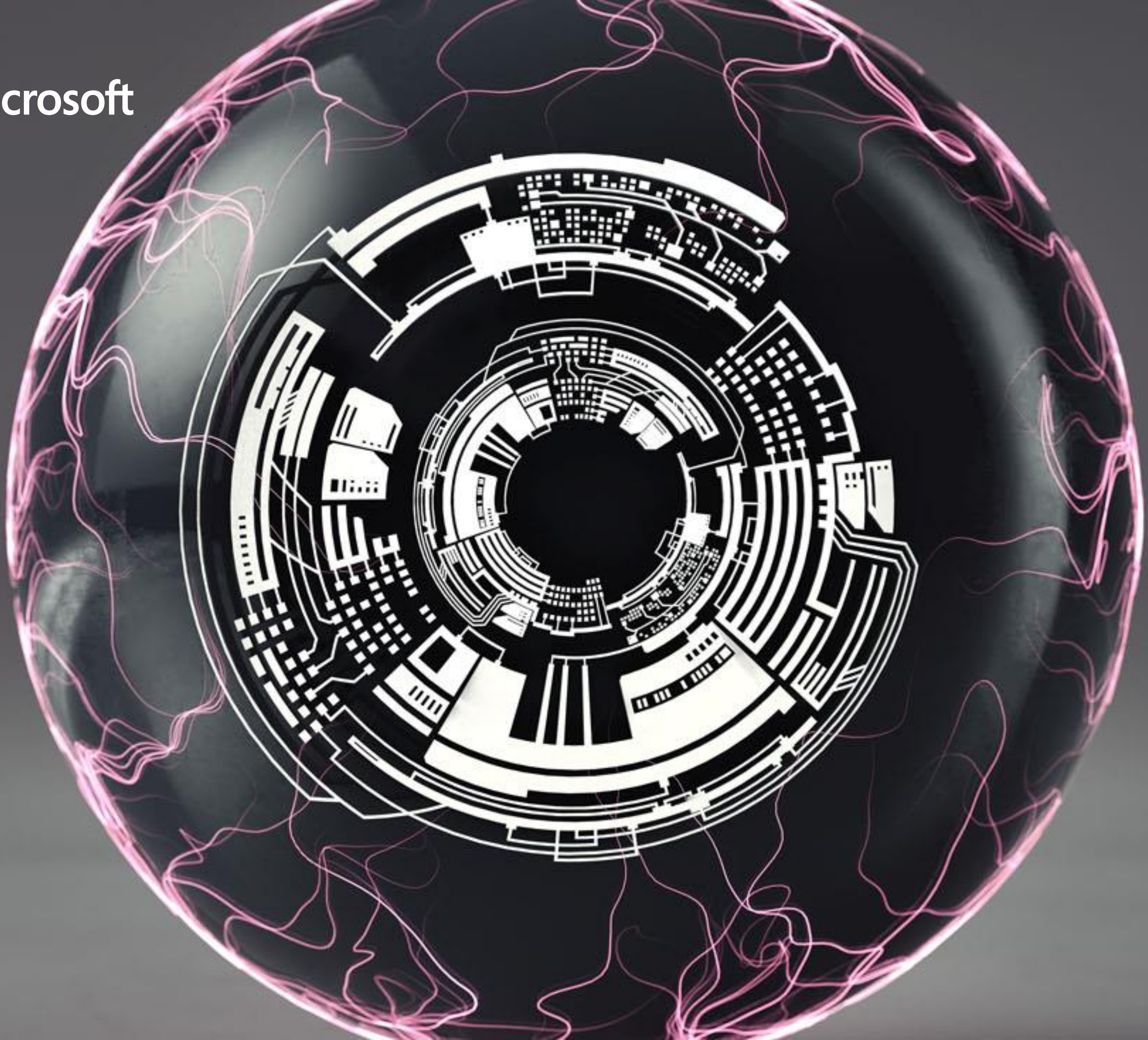
Microsoft



# Microsoft Research Faculty Summit 2016

# Machine learning for CRISPR gene editing

**Nicolo Fusi**

Microsoft Research

# Microsoft Research, New England


Source: MIT

# Acknowledgements


## Broad Institute of MIT and Harvard


**John Doench**

Meagan Sullender
Mudra Hegde
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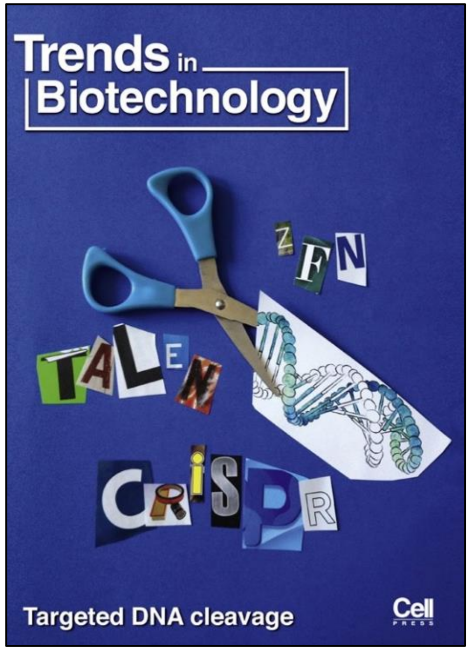
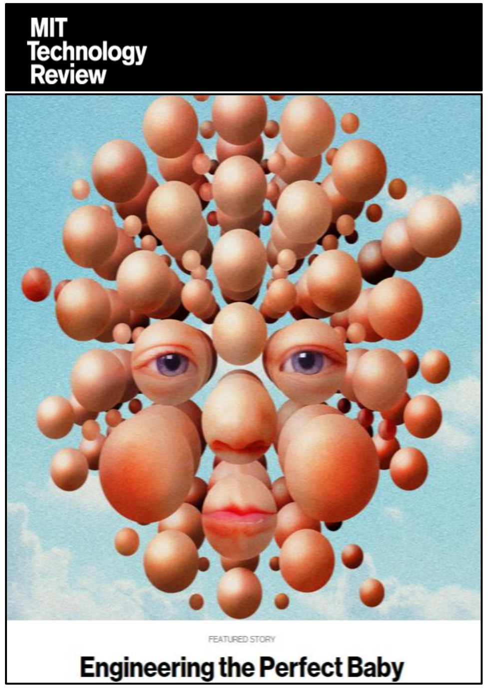




**HEALTH** The New York Times

# *A Powerful New Way to Edit DNA*

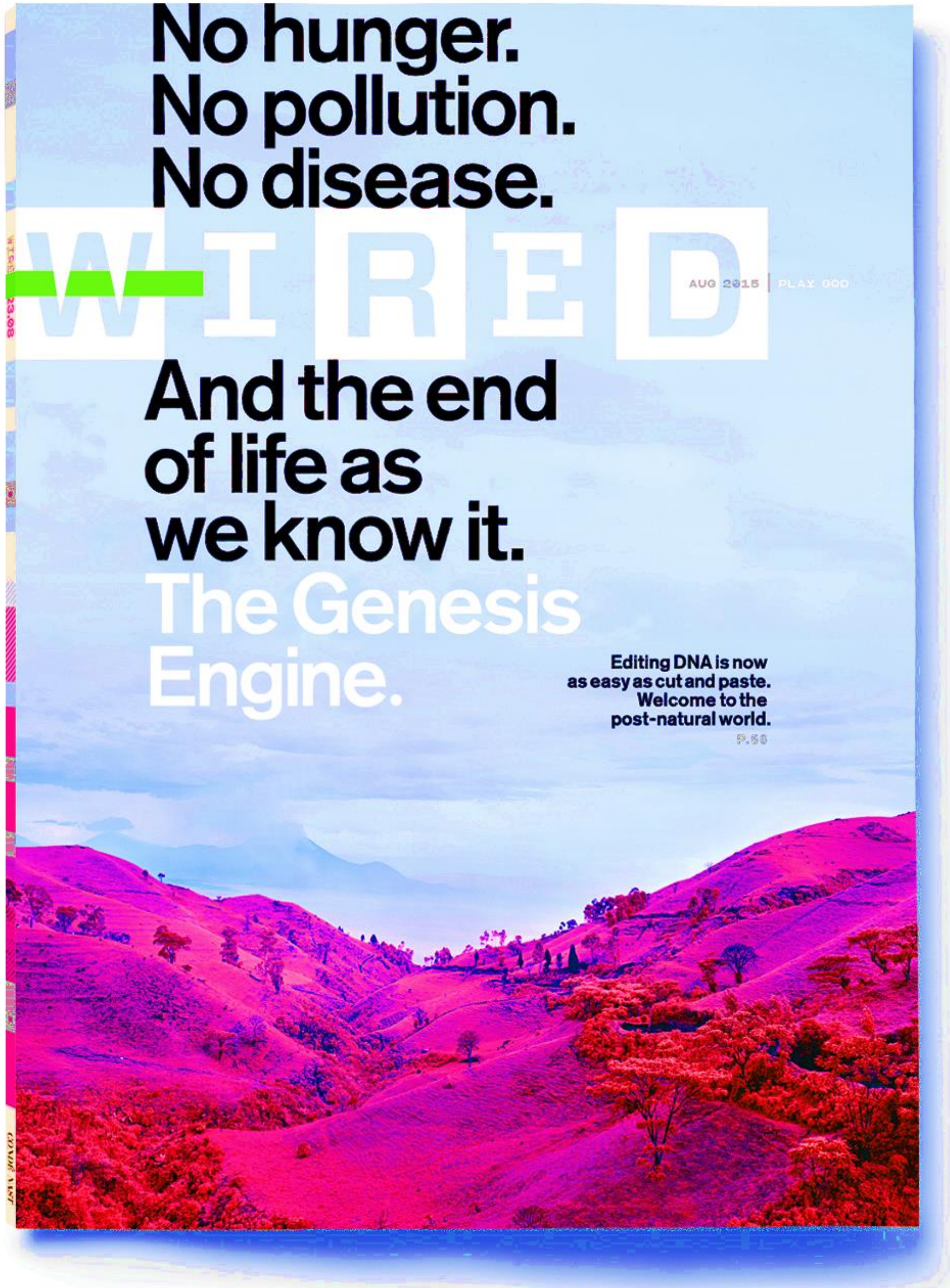
By ANDREW POLLACK MARCH 3, 2014

# Could the DNA-editing CRISPR revolutionize medicine?

**By Carina Storrs, Special to CNN**

Updated 12:22 PM ET, Wed August 12, 2015







HEALTH

*A Powerful New Way*

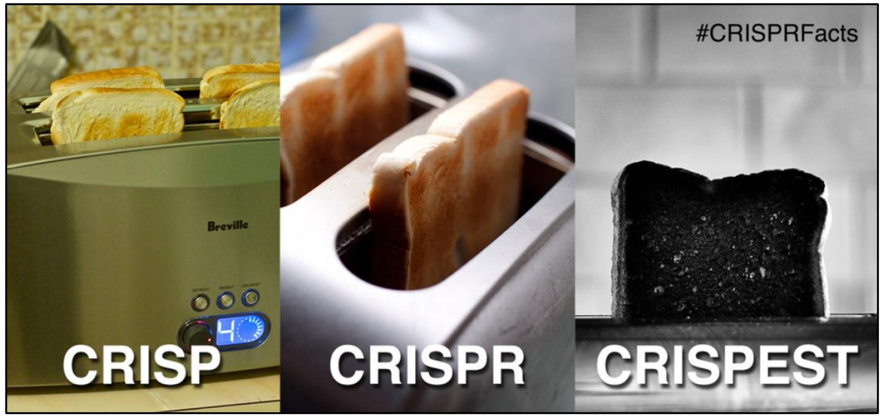
By ANDREW POLLACK MARCH 3, 2014

ting CRISPR
cine?

CNN

**Stephen B Montgomery** @sbmontgom · 2h
With CRISPRs my lab is picking and choosing what X-men they want to be @wired #crisprfacts @dgmacarthur

**smarf dos** @smarfdoc · Aug 20
CRISPR can turn you into a baby ALL OVER AGAIN #crisprfacts #late




Matthew Cobb
@matthewcobb

CRISPR is both gold AND blue #crisprfacts

**Chris Dwan**
@fdmts
Follow

@dgmacarthur @EricTopol CRISPR cannot be overhyped. CRISPR proves P = NP. #crisprfacts


Henry Scowcroft
@oh_henry

If you genetically edit the lettuce genome, you can make it CRISPR #crisprfacts

Terry D. Johnson
@terrydjohnson

Peter Jackson worked with CRISPR to edit The Lord of the Rings. CRISPR was unavailable for The Hobbit. #crisprfacts

# Promising results for translational medicine

Proof of principle in stem cells/model organisms:

- Remove CCR5 receptor used by **HIV**.[1]
- Correct a CFTR defect associated with **cystic fibrosis**.[2]
- Corrected **muscular dystrophy** gene to produce cured mice.[3]


1. Mandal *et al*, Cell Stem Cell 2014
2. Schwank *et al*, Cell Stem Cell 2013
3. Long *et al*, Science 2014

# Not quite ready for prime time

Want

Have

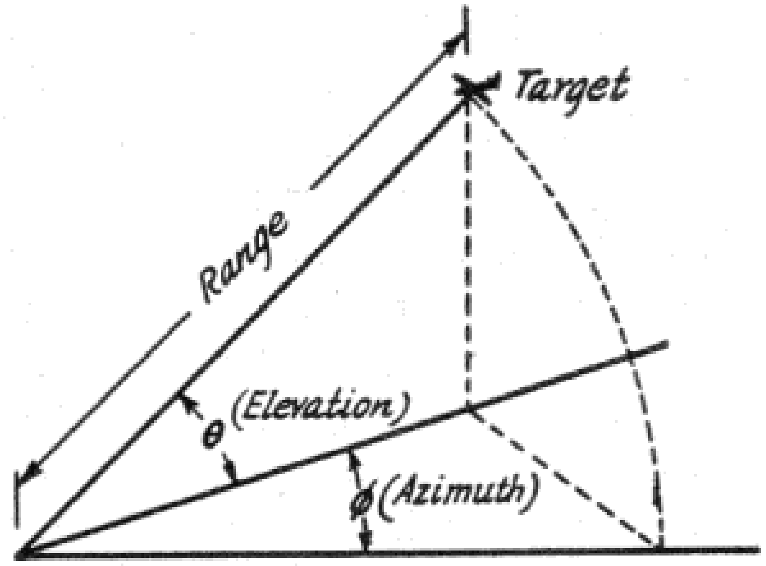
# Not quite ready for prime time

Two problems and two solutions:

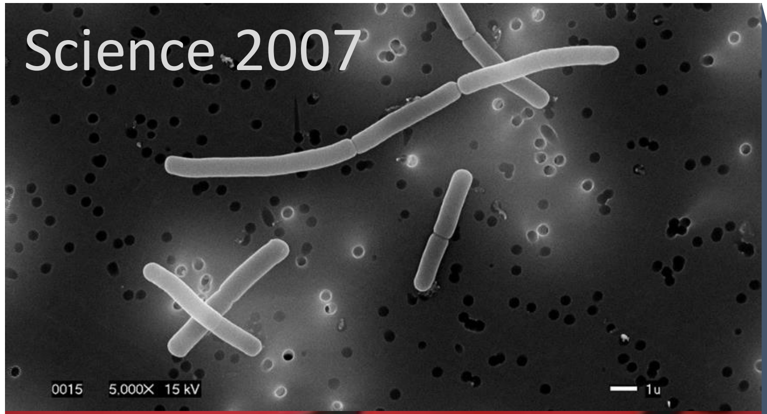
1. Better "on-target" efficiency needed: *Azimuth*.
2. Elimination/reduction of "off-target" effects: *Elevation*.

Solution paths:

- Smarter/improved lab protocols.
- Machine learning.



# A short intro to CRISPR for gene editing




naturally-occurring bacterial immune defense mechanism


co-opted for generic editing in any organism

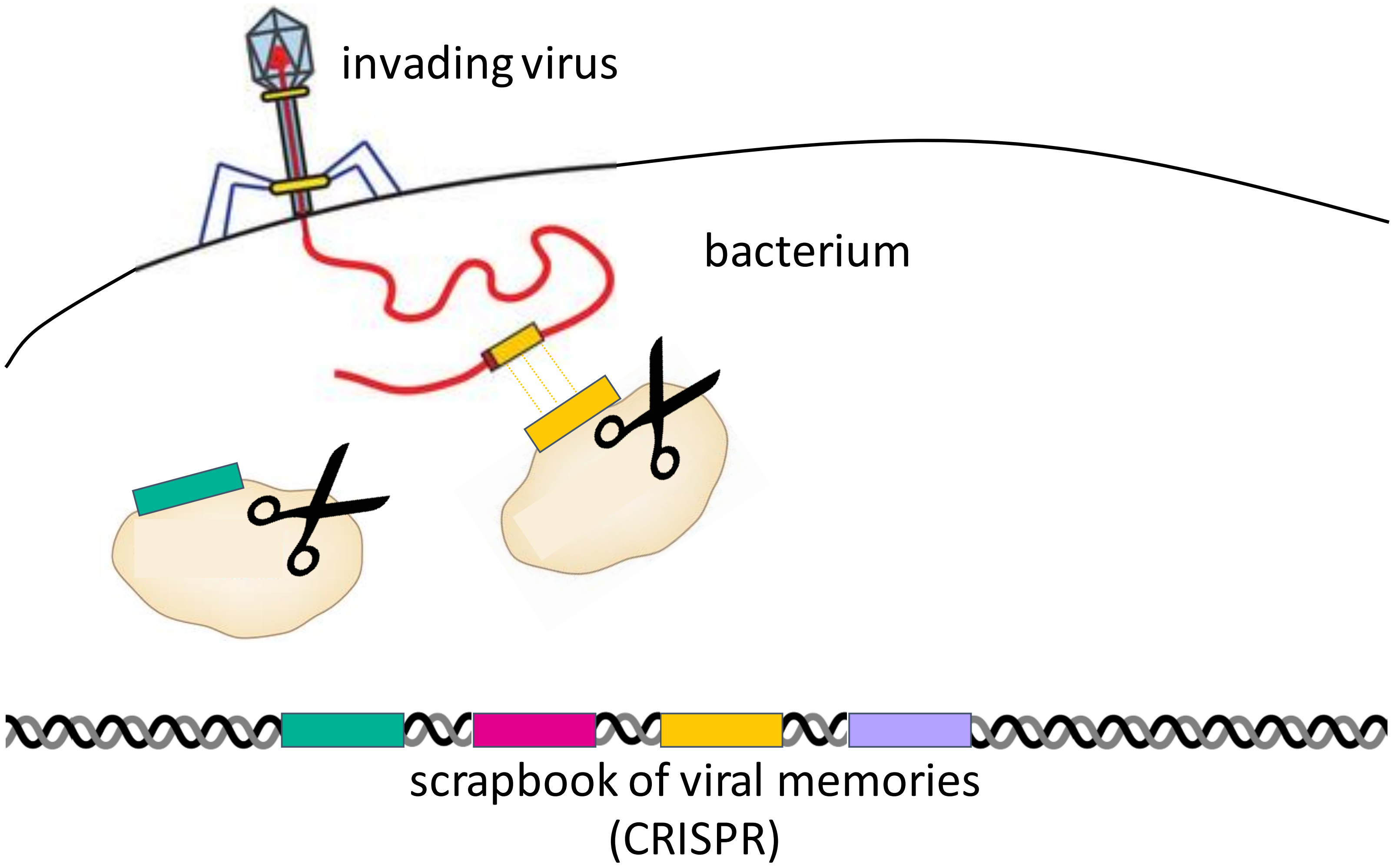
human disease

drug development

agriculture

**CRISPR** = **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats

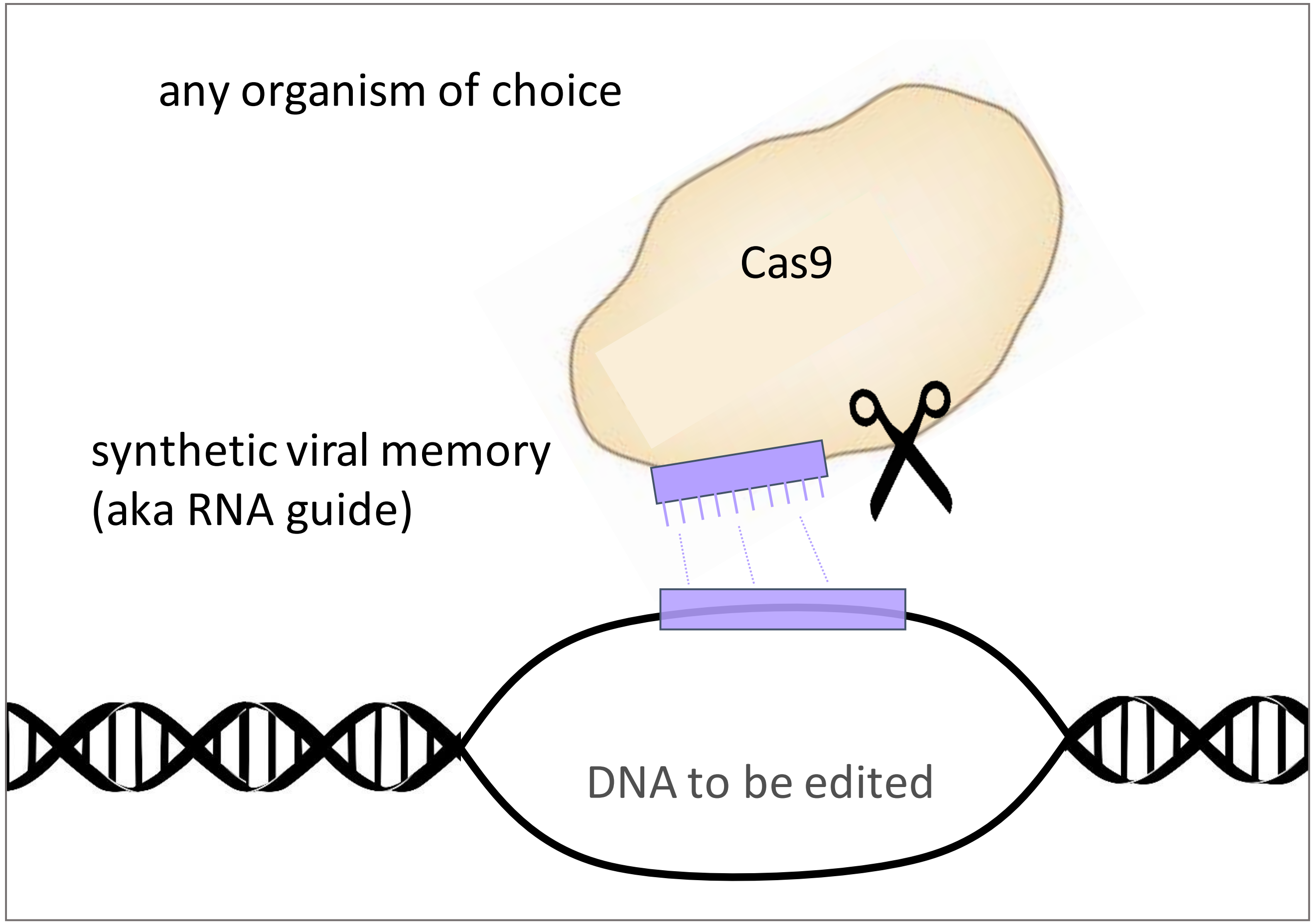
# Originates from two-part bacterial defense mechanism




2 Cut & paste mechanism

1 Viral scrapbook

# Gene editing using CRISPR



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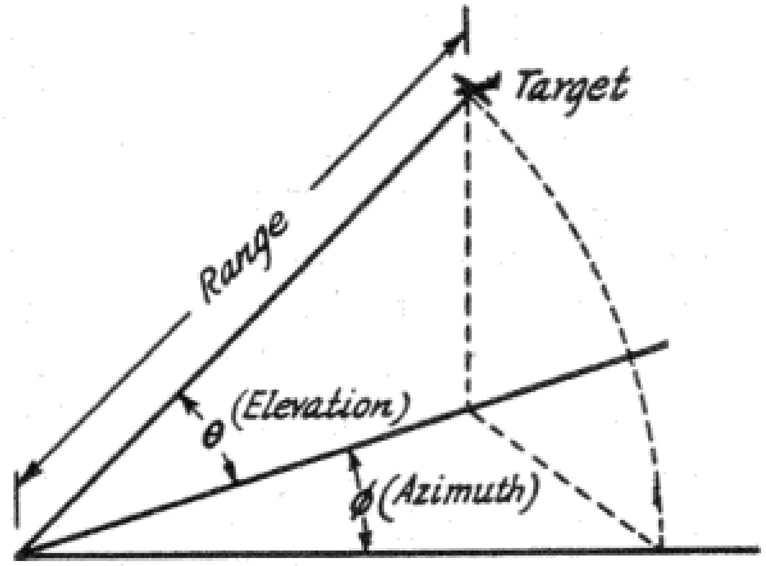
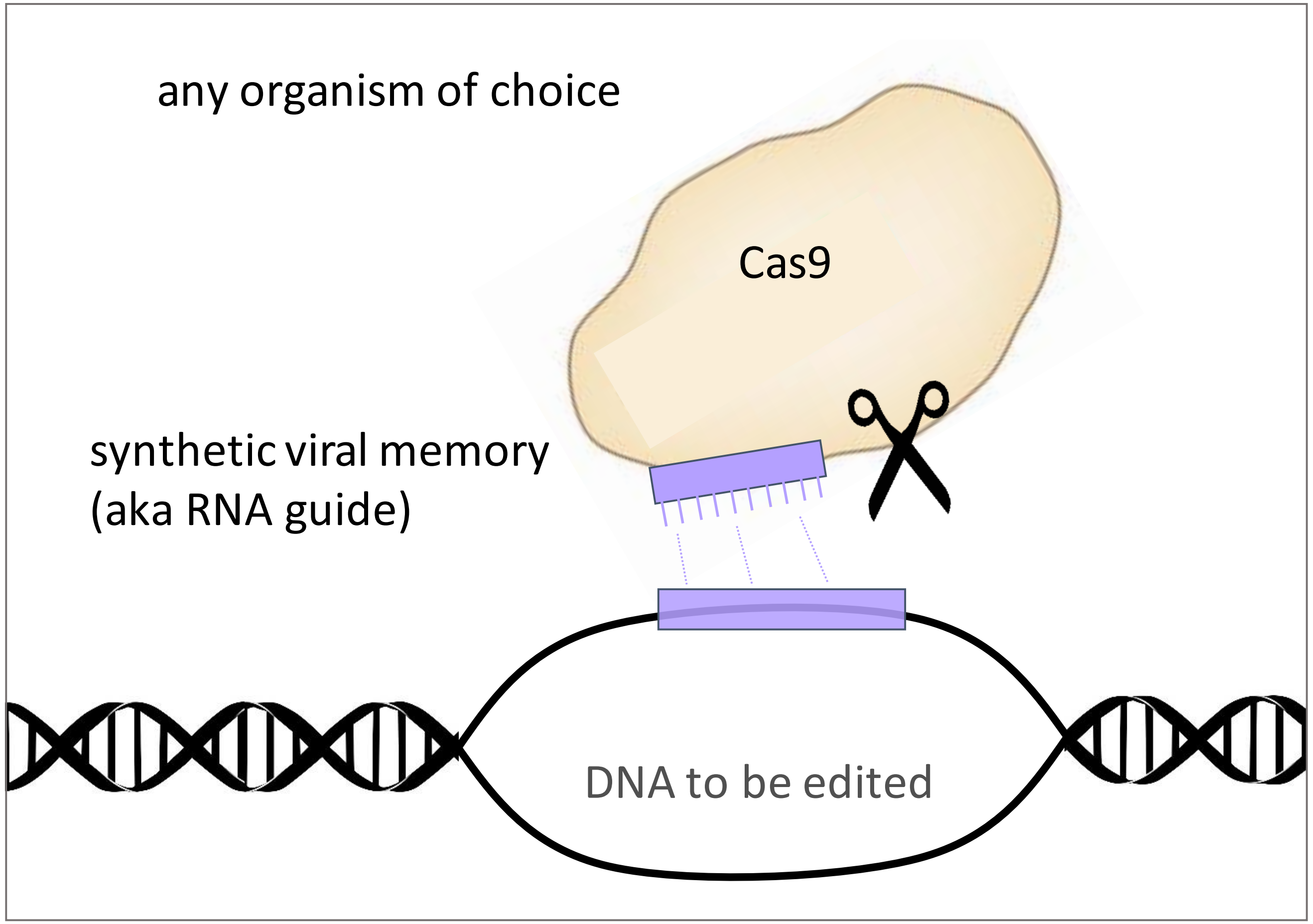


# Machine learning predictive modelling for CRISPR




| RNA guide | | gene edit success |
| --- | --- | --- |
| $[x_1, x_2, \ldots, x_M]$ | $\xrightarrow{f(\vec{x})}$ | $y =$ **not effective** |
| $[x_1, x_2, \ldots, x_M]$ | $\xrightarrow{f(\vec{x})}$ | $y =$ **effective** |
| $[x_1, x_2, \ldots, x_M]$ | $\xrightarrow{f(\vec{x})}$ | $y =$ **very effective** |
| $[x_1, x_2, \ldots, x_M]$ | $\xrightarrow{f(\vec{x})}$ | $y =$ **not effective** |

# *In silico* prediction of guide efficiency

$[x_1, x_2, \ldots, x_M]$

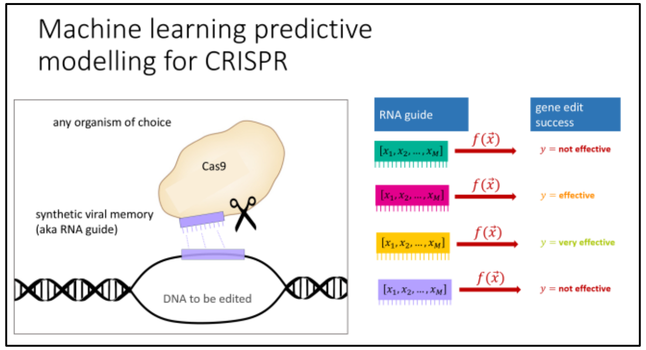
**Input features**
(e.g. guide sequence,
GC content of target gene)

$y =$ **effective**

**Measured guide efficacy**
(e.g. “working” vs “not working”)

$f(\vec{x})$

**Model**
(e.g. Logistic
Regression)



# Azimuth: our state-of-the art approach

- Investigate and use richer features of the RNA guide.
- Removed information bottlenecks to the supervised signal.
- Investigate richer model classes.

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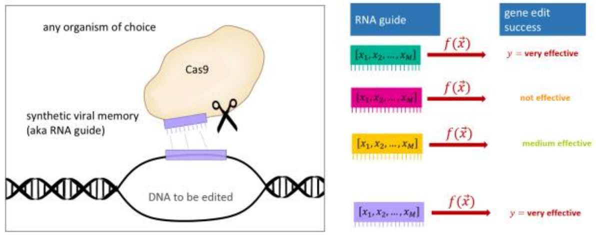
# Featurization of a guide




20mer guide

NGG PAM

TGGAGGCTGCTTTACCCGCTGTGGG**GG**CGC

4mer extra context

3mer extra context

$$\vec{x} = [x_1, x_2, \dots, x_M] = [0,1,1,0, \dots 3.4, 0,1,0,0,0,9.8, 0,0,0.1]$$

# J.A.J.

# Just Ask John

# Melting temperatures

**temperature** at which half of the DNA strands are in the random coil or single-stranded (ssDNA) state.

TGGAGGCTGCTTTACCCGCTGTGGGGGCGC

30mer

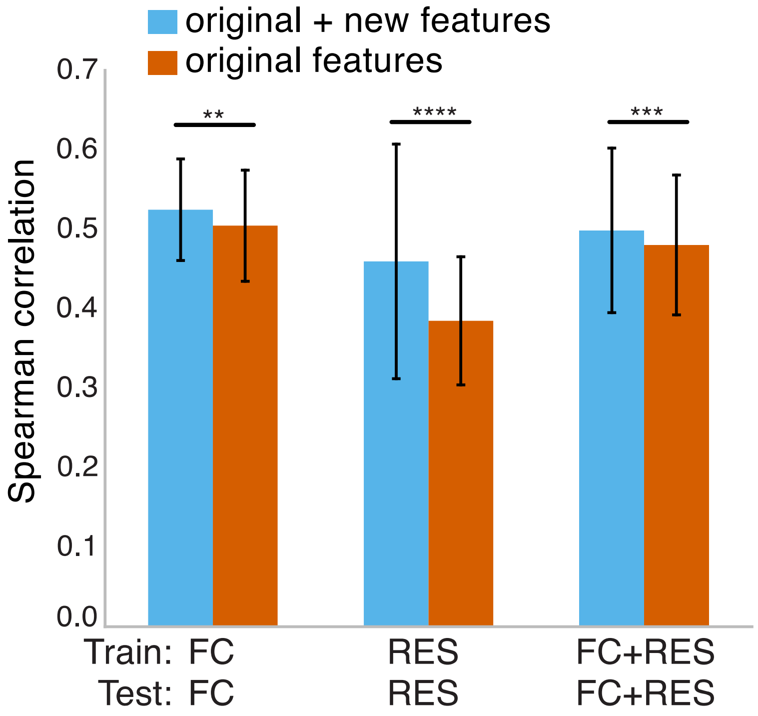
5mer proximal to PAM

8mer in position 8-15 of 20mer guide

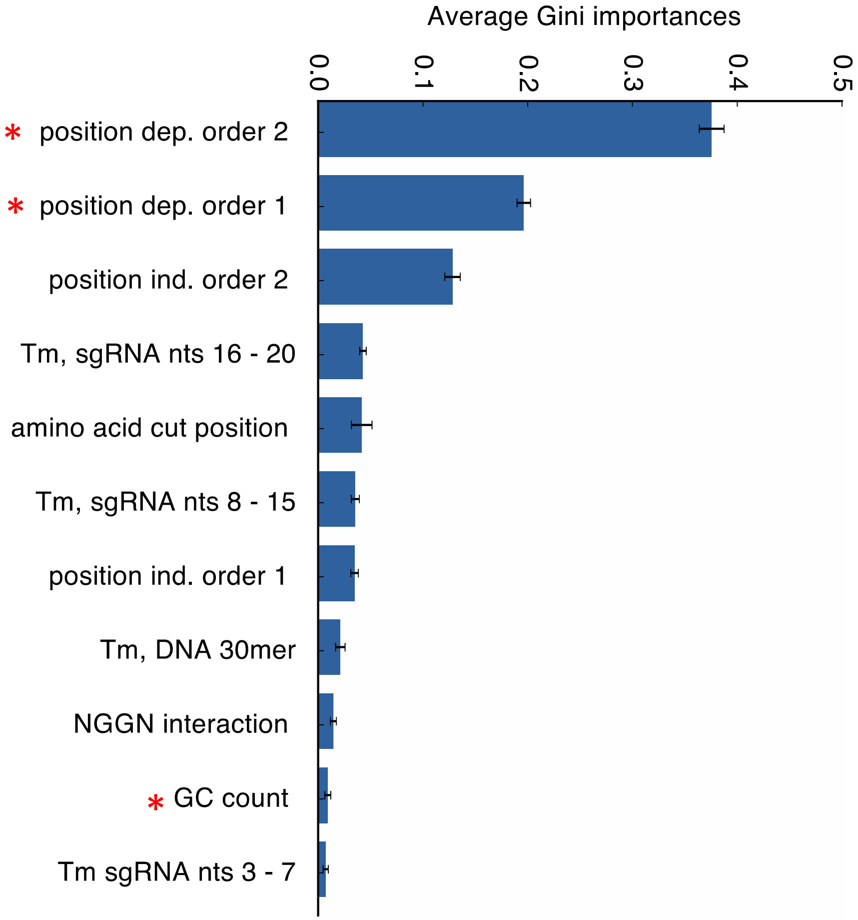
5mer in position 3-7 of 20mer guide

[credit: McGovern Institute for Brain Research at MIT]

# Additional features improve performance




evaluated using L1 regression



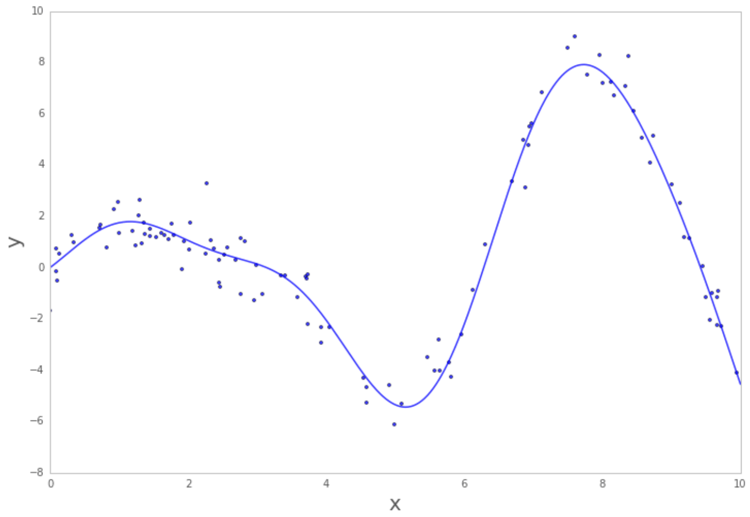

*old feature

# Azimuth: our state-of-the art approach

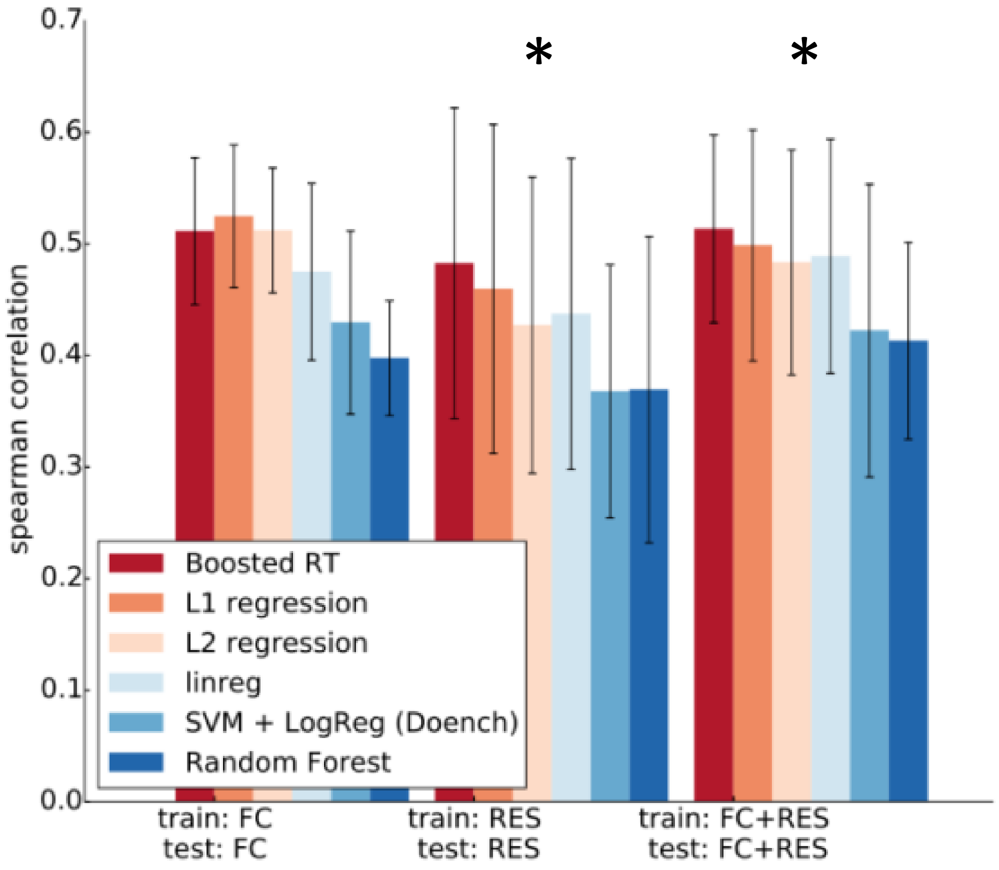
- Investigate and use richer features of the RNA guide.
- Removed information bottlenecks to the supervised signal.
- Investigate richer model classes.

# Non-linear modelling

- Simple linear models are incapable of representing or capturing complex interactions between the variables.
- For the final model we use **Gradient-Boosted Regression Trees** (GBRTs)
- An ensemble of weak predictors (regression trees).
- Each RT is trained on the residuals of the previous one.
- GBRTs can easily handle non-homogeneous data (mix of categorical and continuous).



# Systematic comparison of models



# Impact of our Azimuth model

- *Nature Biotechnology* 2016.
- Recommended by independent studies (Haeussler et al. 2016).
- **Adoption** by two startups and academics/researchers worldwide.
- Azure ML service **~1000 requests/day**, doubling every 3 months
- Web service **~300 requests/day**.
- Over **1000 open-source software downloads**.

http://research.microsoft.com/en-us/projects/azimuth

# Not quite ready for prime time

<u>Two problems and two solutions:</u>

1. Better "on-target" efficiency needed: *Azimuth*.
2. Elimination/reduction of "off-target" effects: *Elevation*.

<u>Solution paths</u>:

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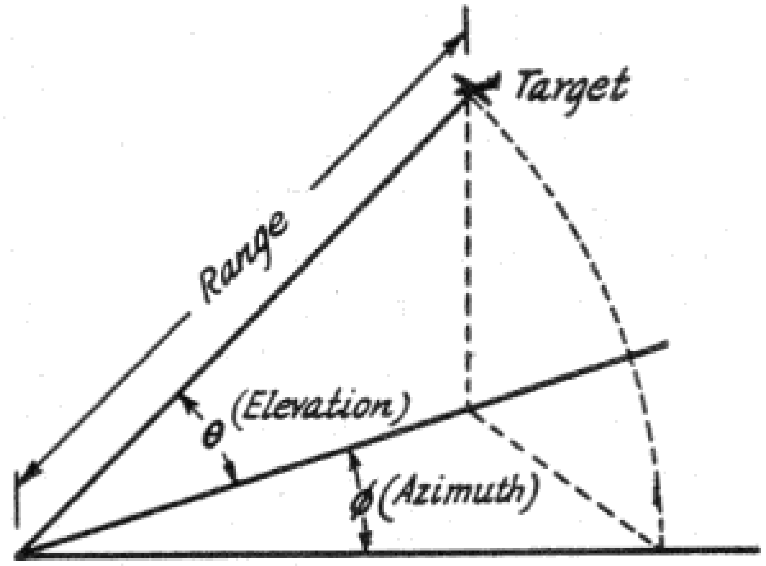


# *Elevation*: prediction of off-target effects

Much more challenging than on-target:

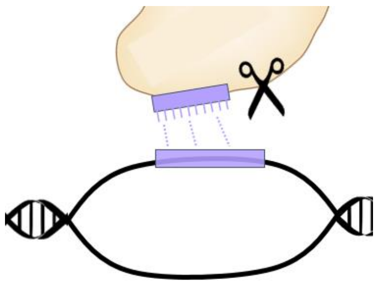
- For just one single guide need to check for imperfect matches genome-wide.
- Combinatorial explosion of mismatches, hard to get enough training data.

intended target

GGCTGCTTTACCCGCTGTGGG

...CTATAACTGGCAGCTCTACCCGGTGTGGGACAAG...

whole genome—potential off-targets



# Combinatorial explosion (for 1 guide in 1 gene)

1 mismatch: **69** sites

2 mismatches: **2277** sites

3 mismatches: **47,817** sites

4 mismatches: **717,255** sites

5 mismatches: **8,176,707** sites

1 full example

**very** sparsely sampled across different genes

# Previous state-of-the-art approach: *CFD* (Doench et al 2016)

intended target

GGCTGCTTTACCCGCTGTGGG

...CTATAACTGGCAGCTCTACCCGGTGTGGGACAAG...

*1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20*

**featurization**

T:C,8

*categorical (i.e. one-hot) encoding of single mismatch and position*

# Previous state-of-the-art approach: *CFD (Doench et al 2016)*

$$CFD \approx \prod_i P(Y = 1 | X_i = 1)$$

- Measured off-target activities (on a continuous scale) are **discretized** in **present** (1) vs **not present** (0).
- CFD computes probability of off-target given mismatch.
- Probabilities are aggregated assuming conditional independencies.

# *Elevation*: generalizations of *CFD*

$$CFD \approx \prod_i P(Y = 1|X_i = 1)$$

1. Change from classification to regression for $P(Y = 1|X_i = 1)$.
2. Augment the feature space from T:C,8.
3. Use non-linear regression model for $P(Y = 1|X_i = 1)$, in particular Boosted Regression trees.
4. Refine predictions with a second model layer using the multi-mismatch data.

# *Elevation*: generalizations of *CFD*

Goal 1: make better use of the better-sampled 1 mismatch data

$$CFD \approx \prod_i P(Y=1|X_i=1)$$

1. Change from classification to regression for $P(Y=1|X_i=1)$.
2. Augment the feature space from T:C,8.
3. Use non-linear regression model for $P(Y=1|X_i=1)$, in particular Boosted Regression trees.
4. Refine predictions with a second model layer using the multi-mismatch data.

# *Elevation*: generalizations of *CFD*

Goal 2: relax independence and other assumptions using sparsely-sampled data

$$CFD \approx \prod_i P(Y = 1|X_i = 1)$$

1. Change from classification to regression for $P(Y = 1|X_i = 1)$.
2. Augment the feature space from **T:C,8.**
3. Use non-linear regression model for $P(Y = 1|X_i = 1)$, in particular Boosted Regression trees.
4. Refine predictions with a second model layer using the multi-mismatch data.

# Cascading from single mismatch to multi-mismatch

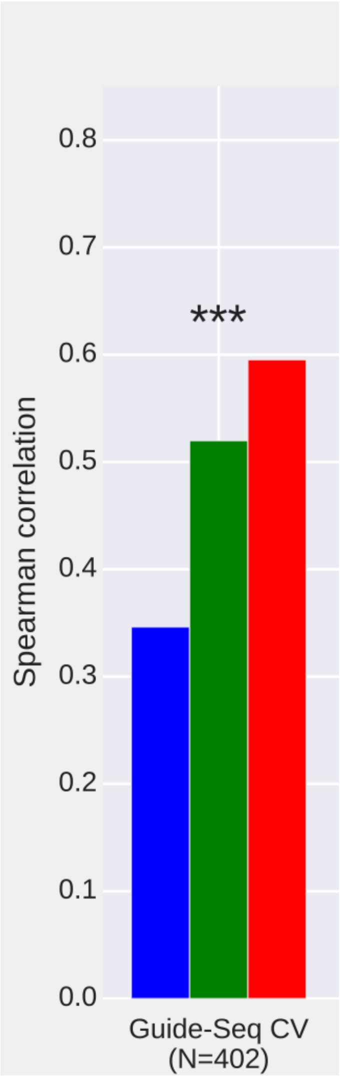
1. **Non-linear** regression model trained on **1-mismatch data**.
   - Complex model capturing interactions
   - Can only compute predictions for 1 mismatch at a time

Elevation-naive

2. **Linear** model trained on scarce **multi-mismatch data**
   - Relatively simple model
   - Trained on individual and aggregated predictions (e.g. product, sum) from layer 1

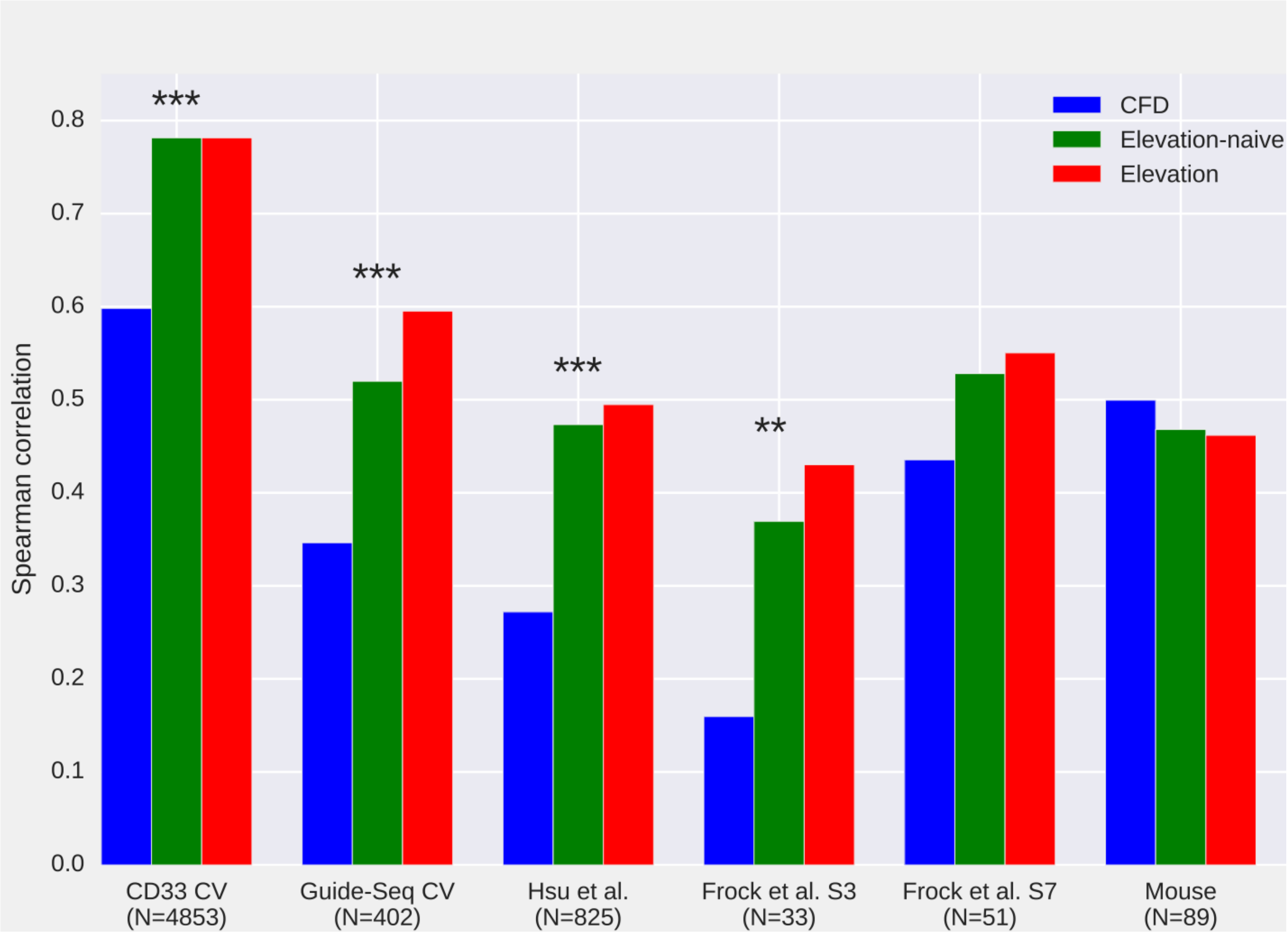
Elevation

# *Elevation* outperforms *CFD* by 64%



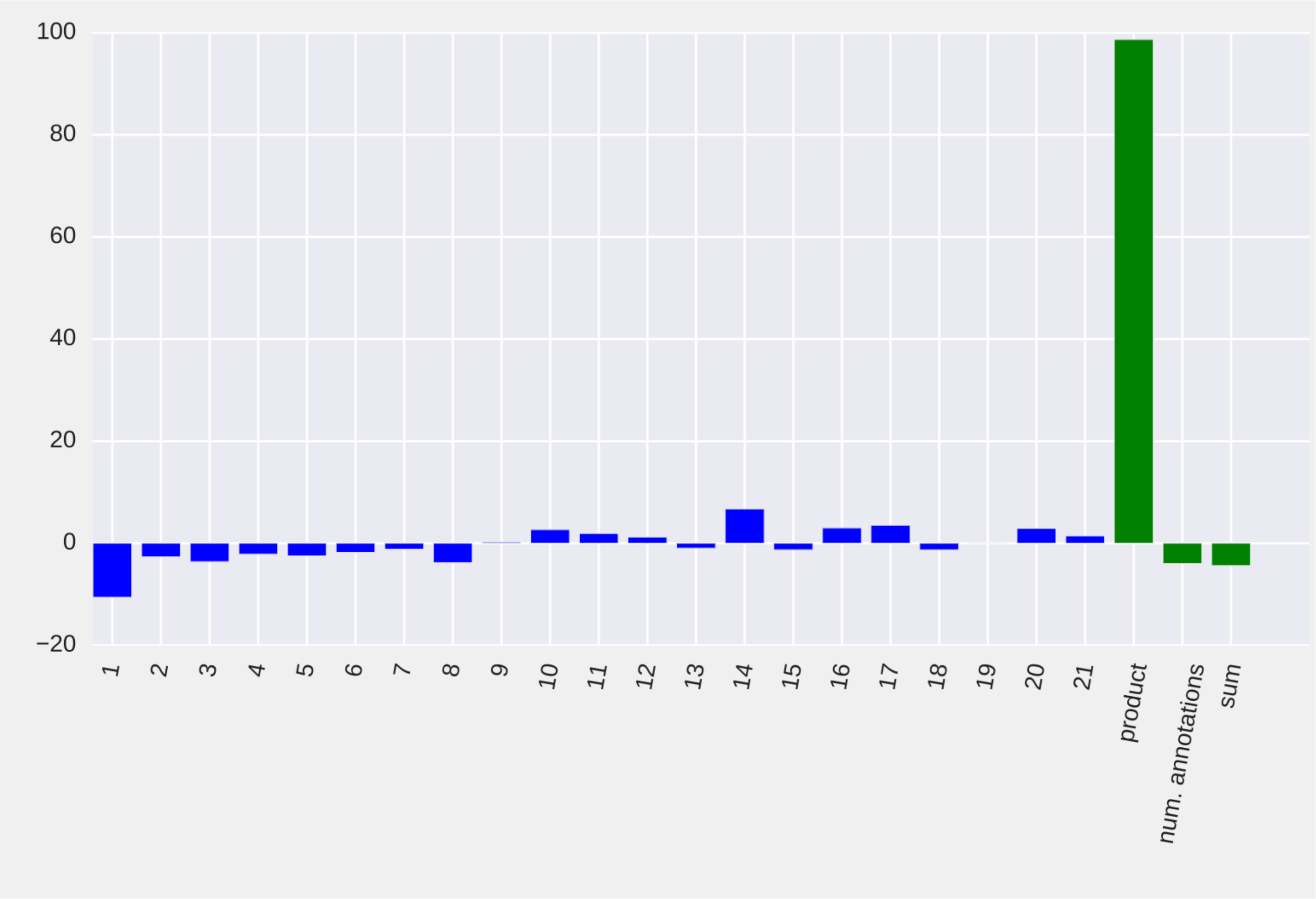

- Elevation spearman $\rho = 0.59$
- CFD spearman $\rho = 0.36$
- 64% improvement $(p = 5.5 \times 10^{-5})$

# *Elevation* performs best on 4/5 other data sets



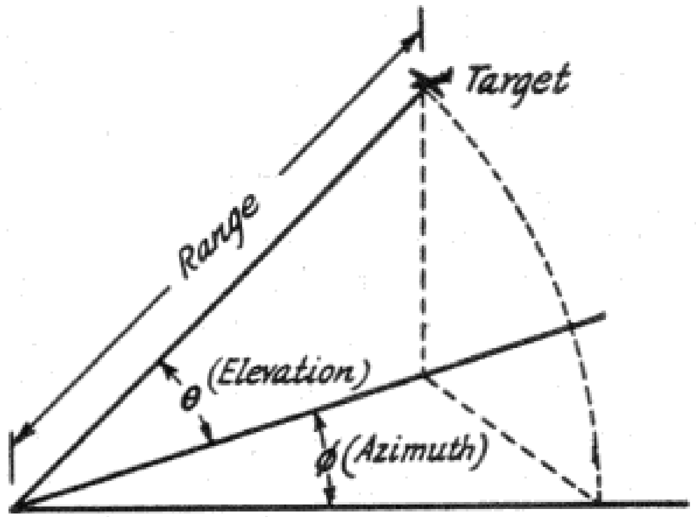
# Mitigation of assumptions

Quantitative correction from the full-assumptions model



# Putting it all together

- Elevation cloud prediction server.
- Open source code.
- Framework to efficiently search genome-wide for mismatches and call **Azimuth** & **Elevation**.



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