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Machine learning for CRISPR gene editing

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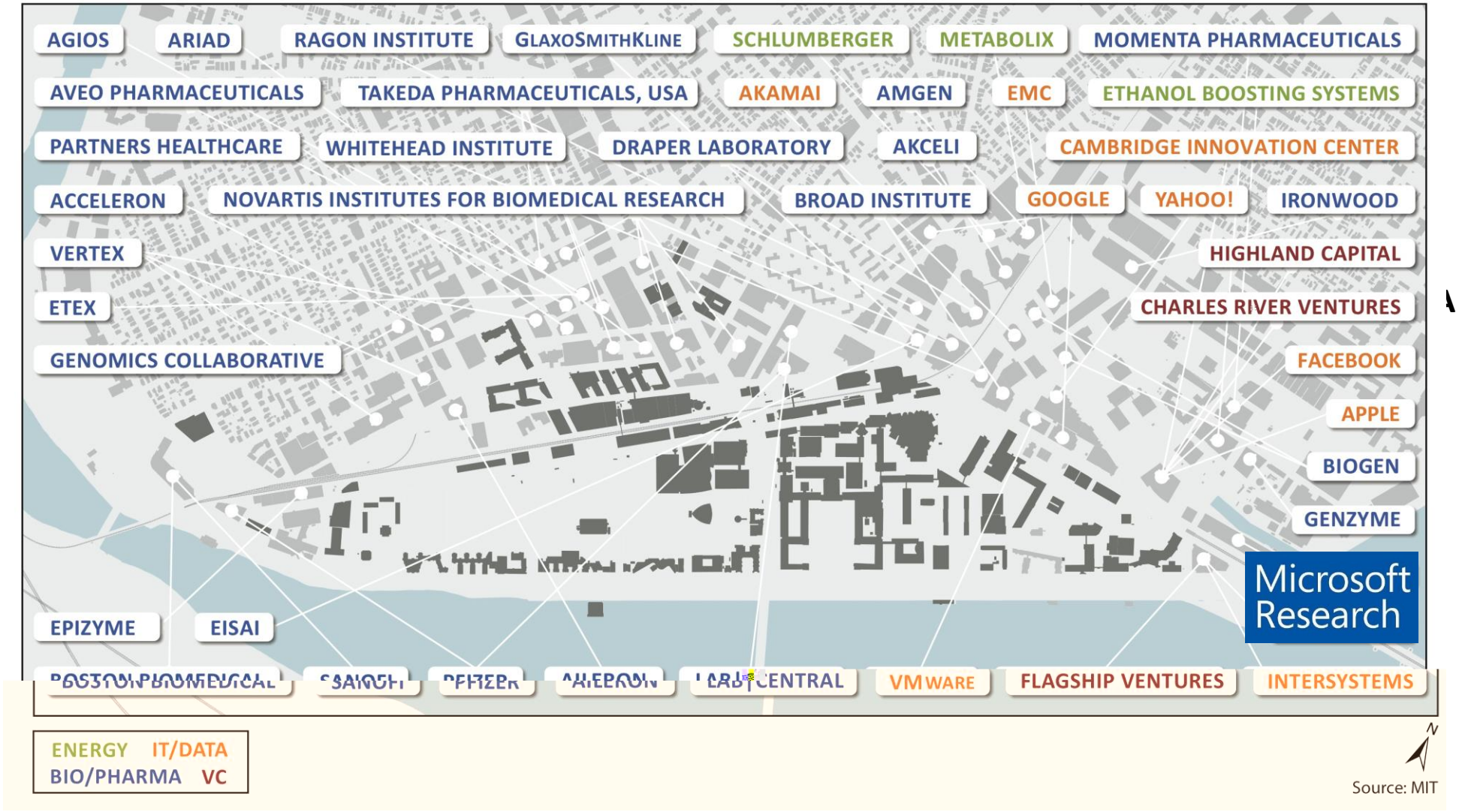

Source: MIT

Acknowledgements

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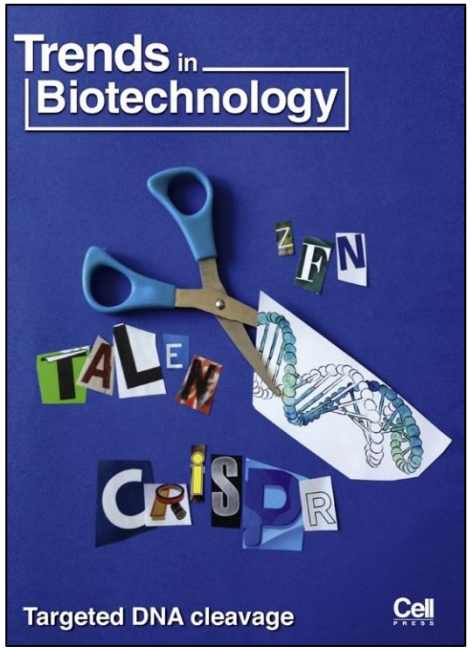
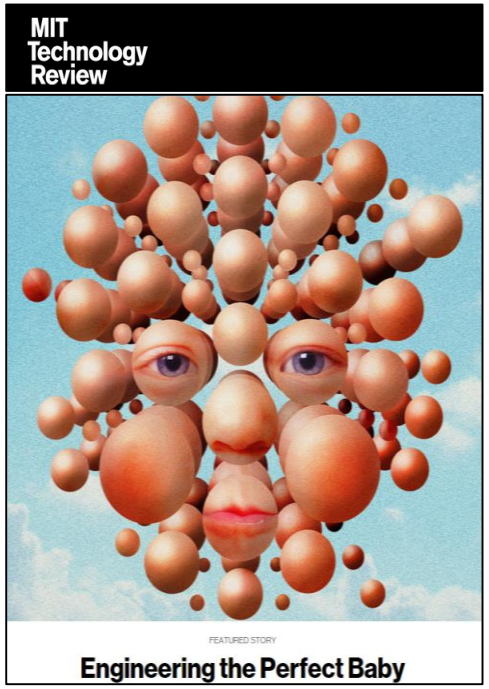




HEALTH

The New York Times

A Powerful New Way to Edit DNA

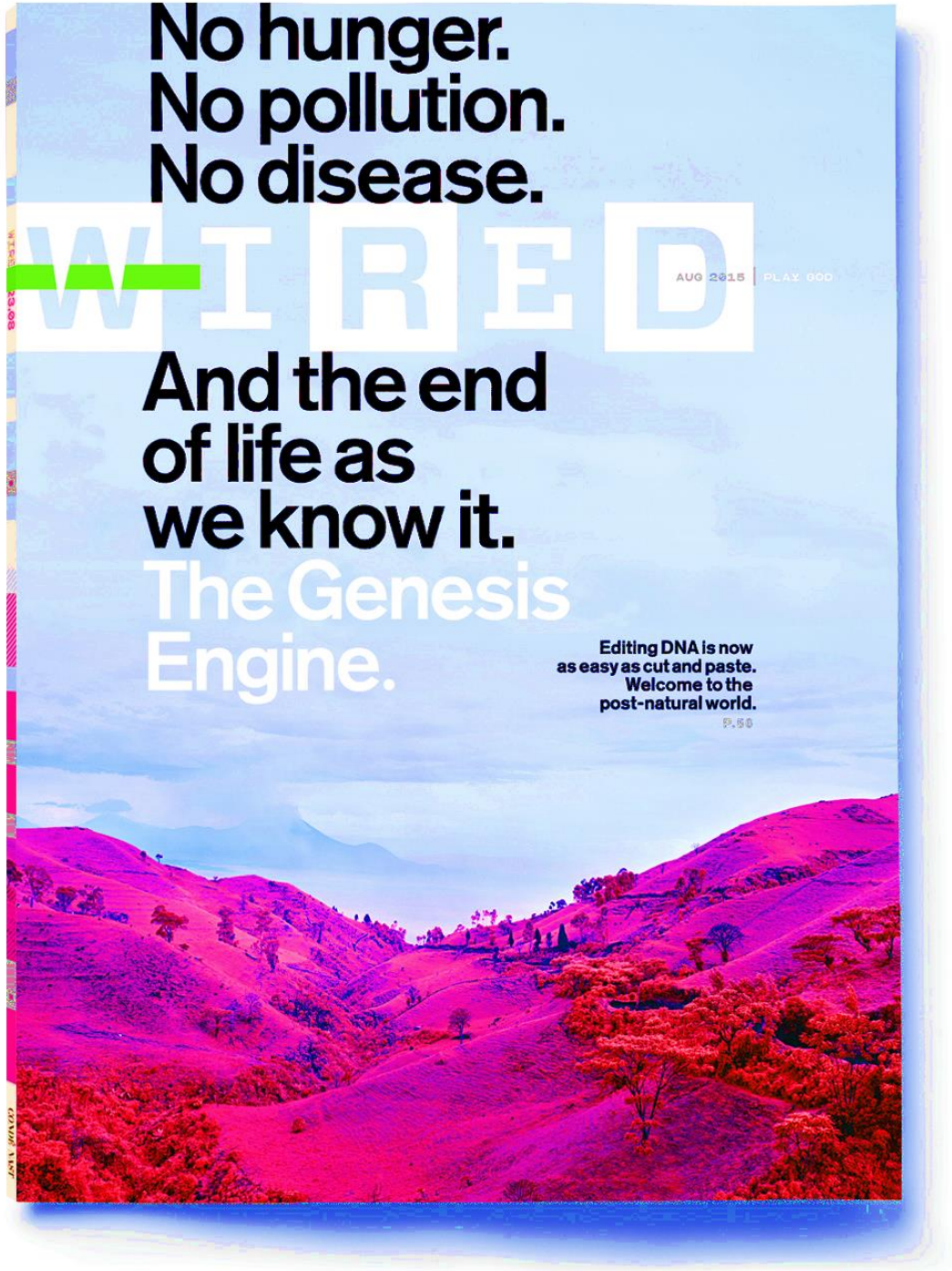
By ANDREW POLLACK MARCH 3, 2014

Could the DNA-editing CRISPR revolutionize medicine?

CNN

By Carina Storrs, Special to CNN

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



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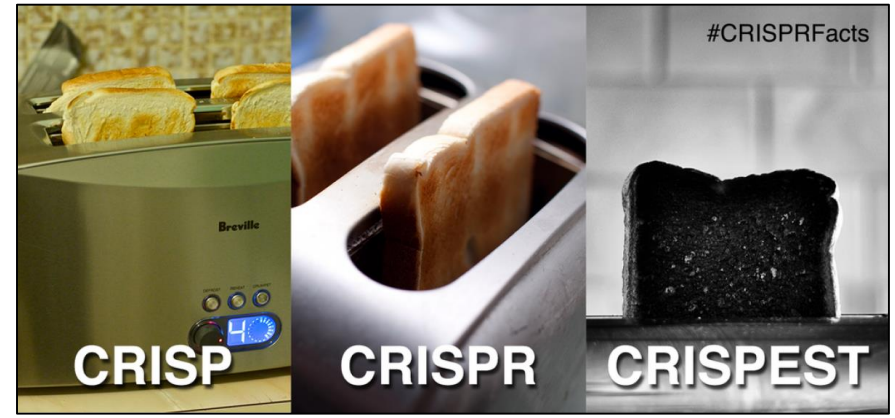

Stephen B Montgomery @sbmontgom · 2h

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

Henry Scowcroft
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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

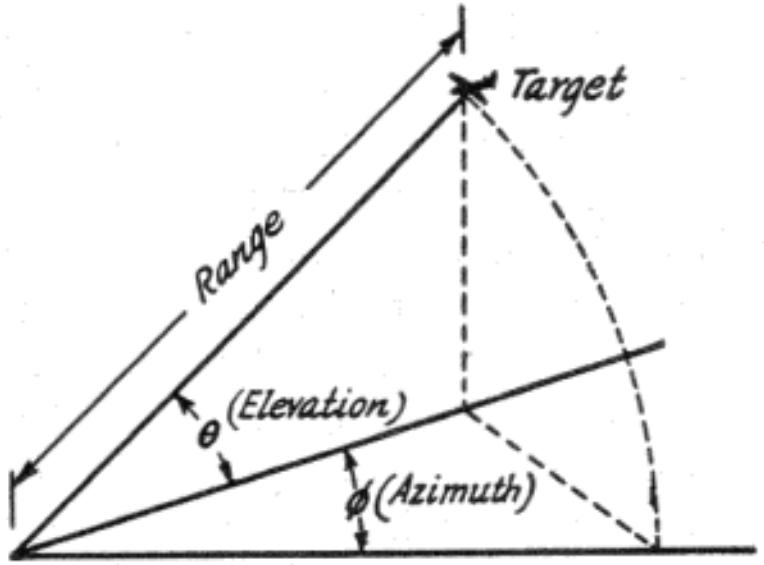
Not quite ready for prime time

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.



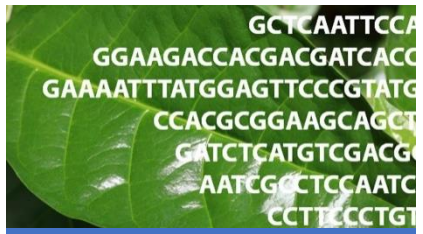
A short intro to CRISPR for gene editing

naturally-occurring bacterial immune defense mechanism

co-opted for generic editing in any organism

human disease

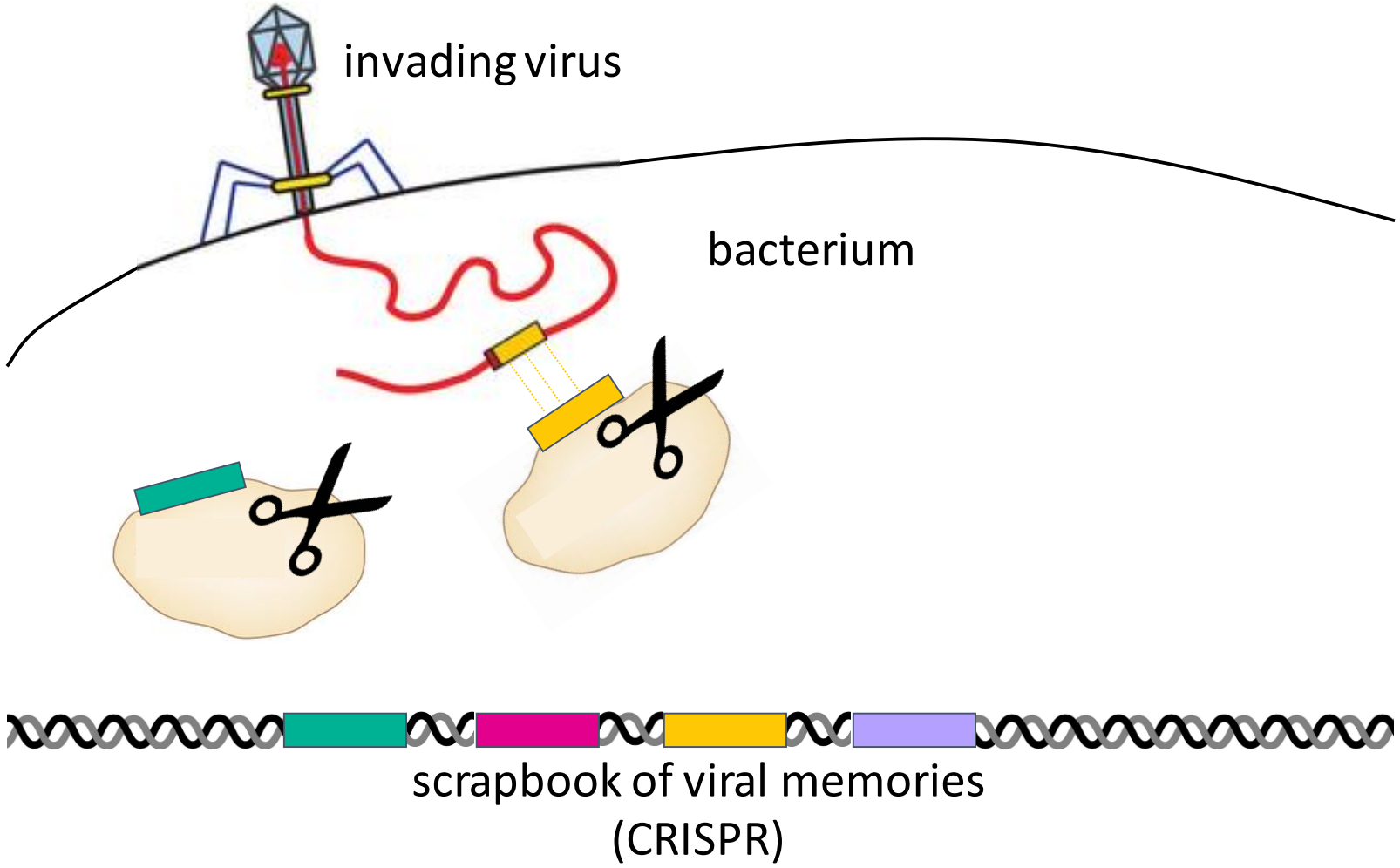
drug development



agriculture

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

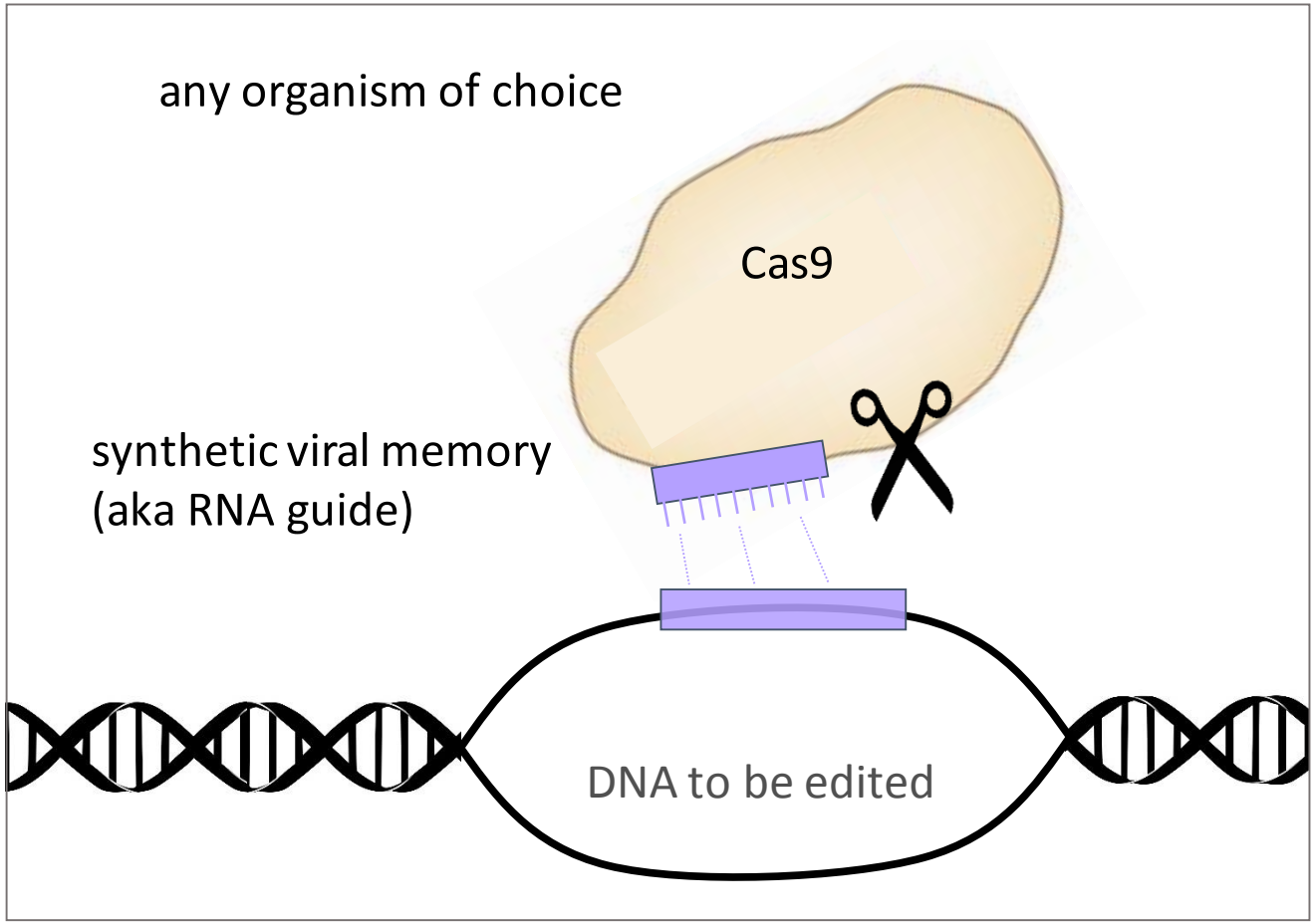
Originates from two-part bacterial defense mechanism



2 Cut & paste mechanism

1 Viral scrapbook

Gene editing using CRISPR



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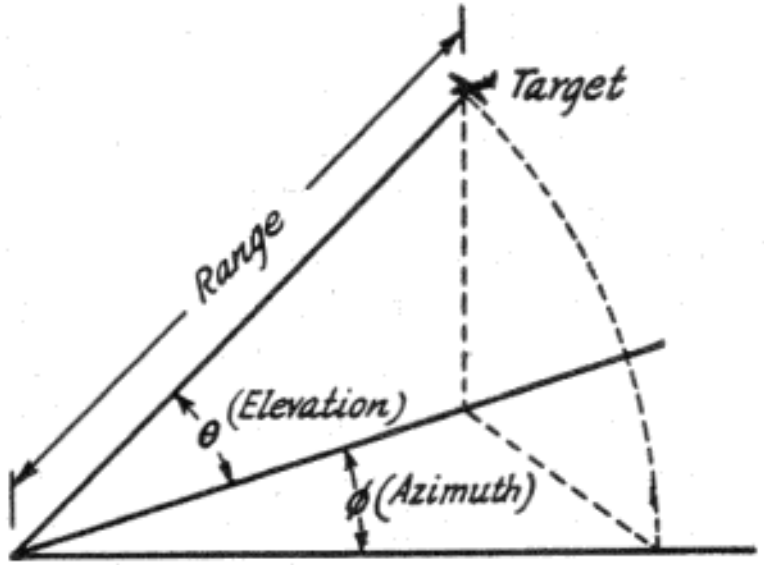
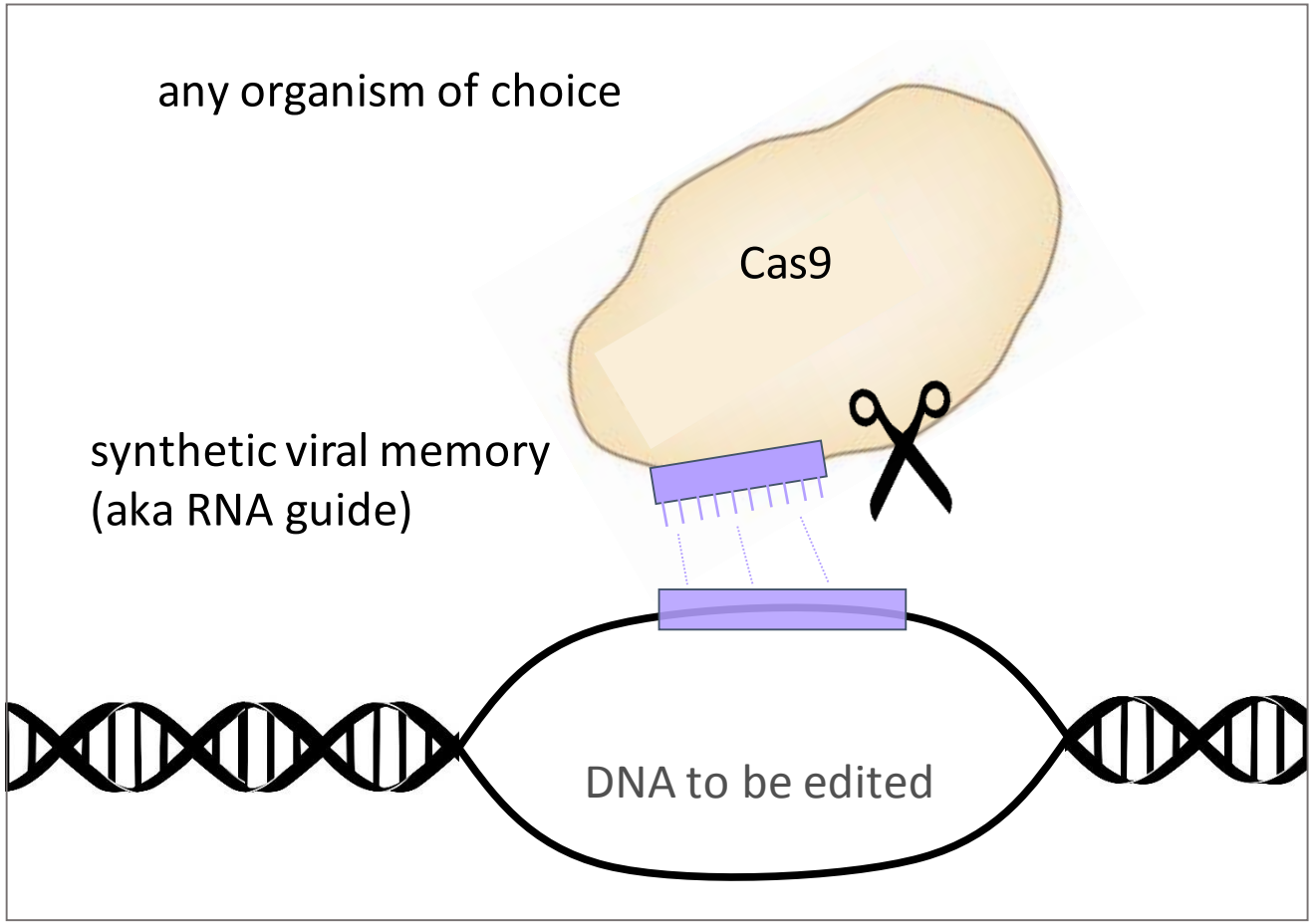


Machine learning predictive modelling for CRISPR



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

In silico prediction of guide efficiency

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

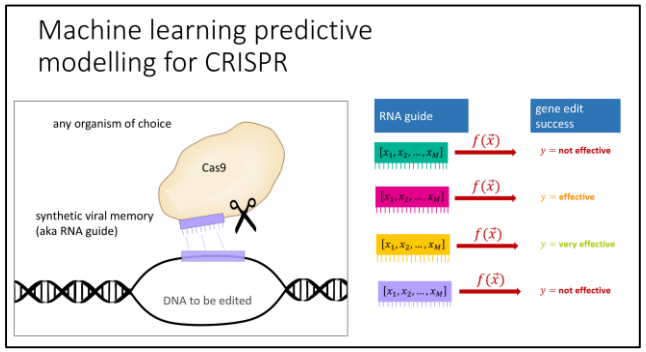
Input features
(e.g. guide sequence,
GC content of target gene)

$y =$ **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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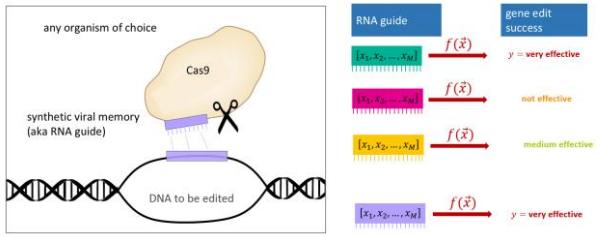
Featurization of a guide



20mer guide NGG PAM

TGGAGGCTGCTTTACCCGCTGTGGG**GG**CGC

4mer extra context 3mer extra context

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

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

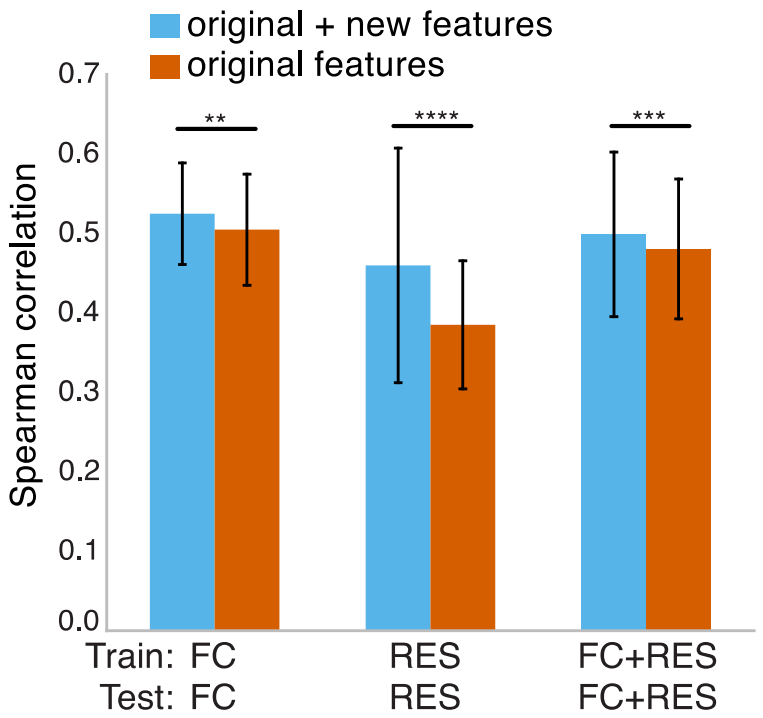
5mer proximal to PAM

8mer in position 8-15 of 20mer guide

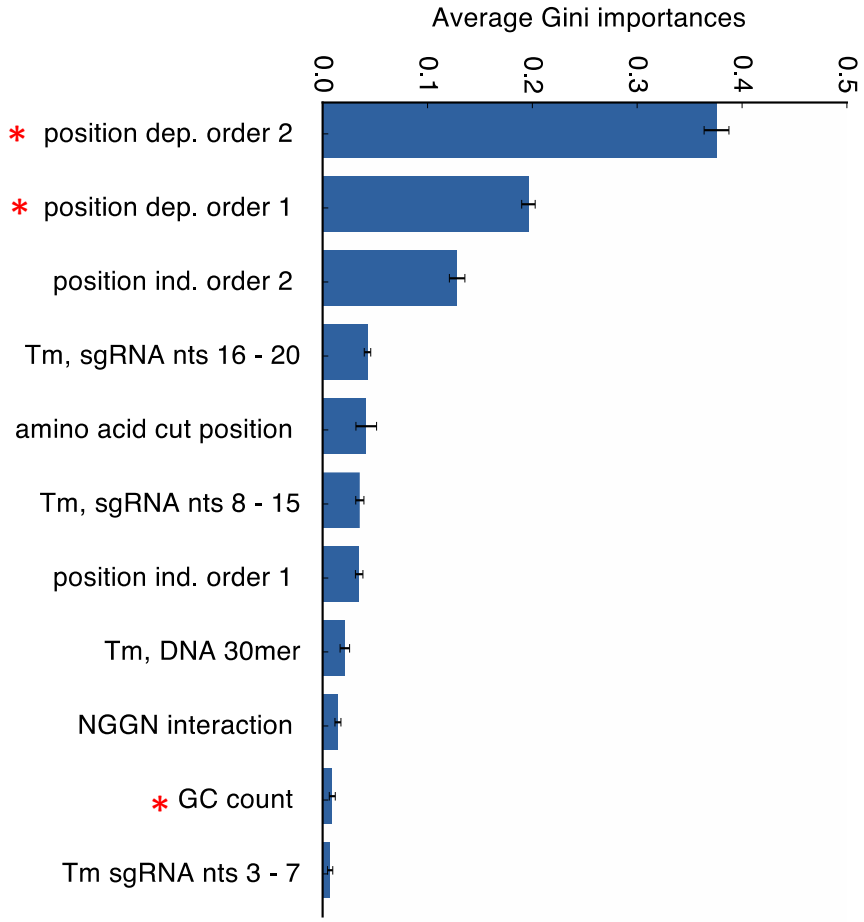
5mer in position 3-7 of 20mer guide

[credit: McGovern Institute for Brain Research at MIT]

Additional features improve performance



evaluated using L1 regression



*old feature

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- Removed information bottlenecks to the supervised signal.
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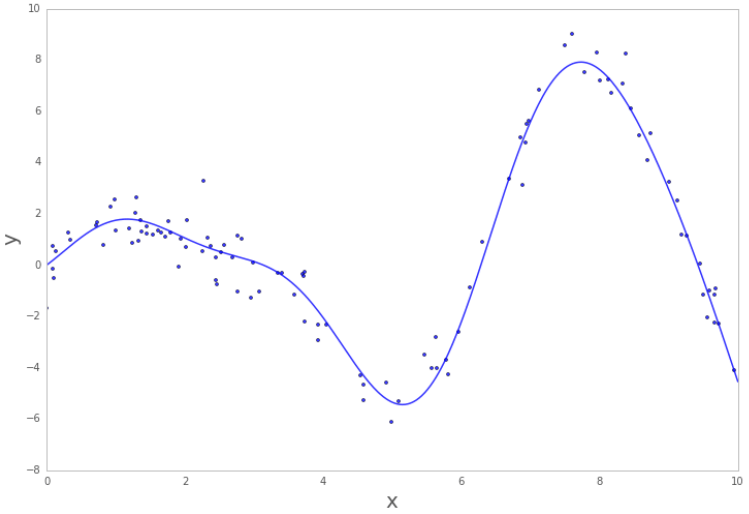
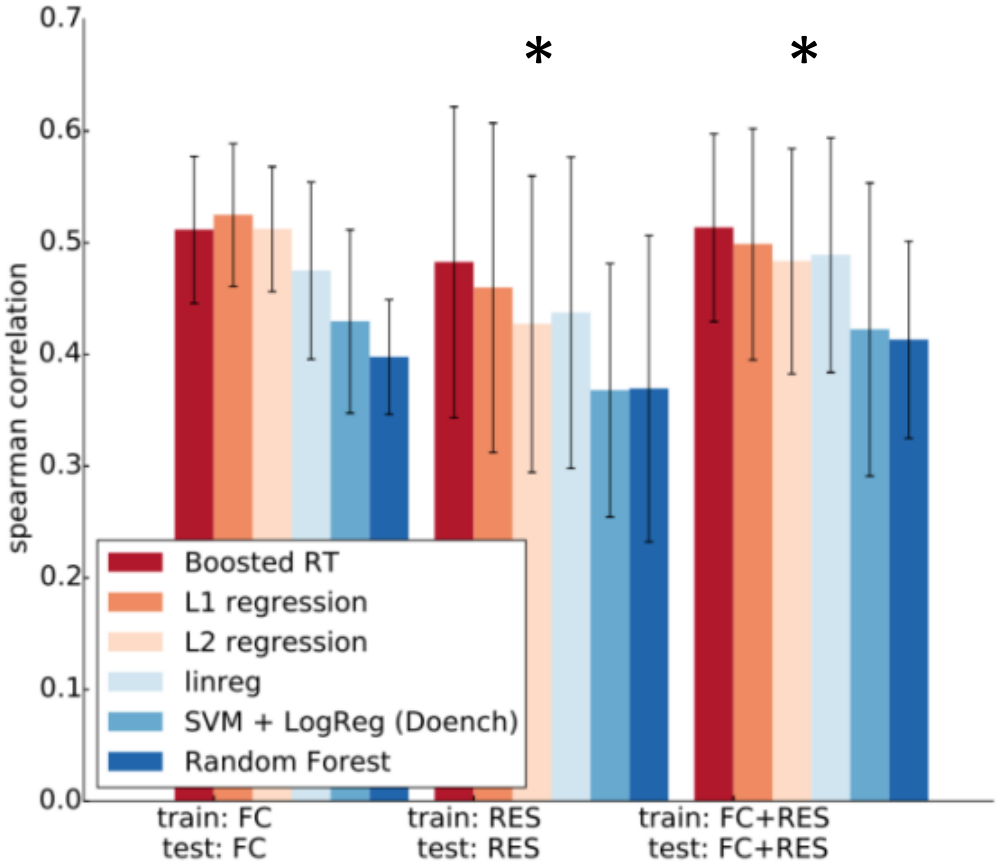
Non-linear modelling

- 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

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Two problems and two solutions:

1. Better "on-target" efficiency needed: *Azimuth*.
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Solution paths:

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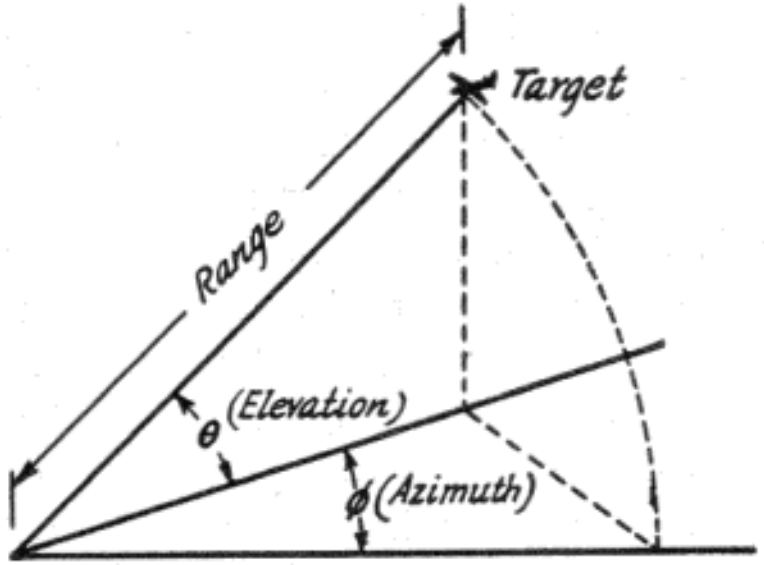


Elevation: prediction of off-target effects

Much more challenging than on-target:

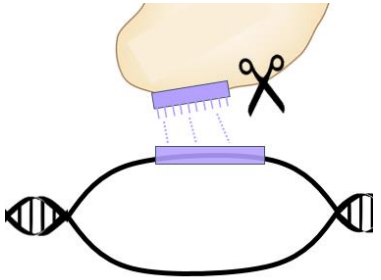
- For just one single guide need to check for imperfect matches genome-wide.
- Combinatorial explosion of mismatches, hard to get enough training data.

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

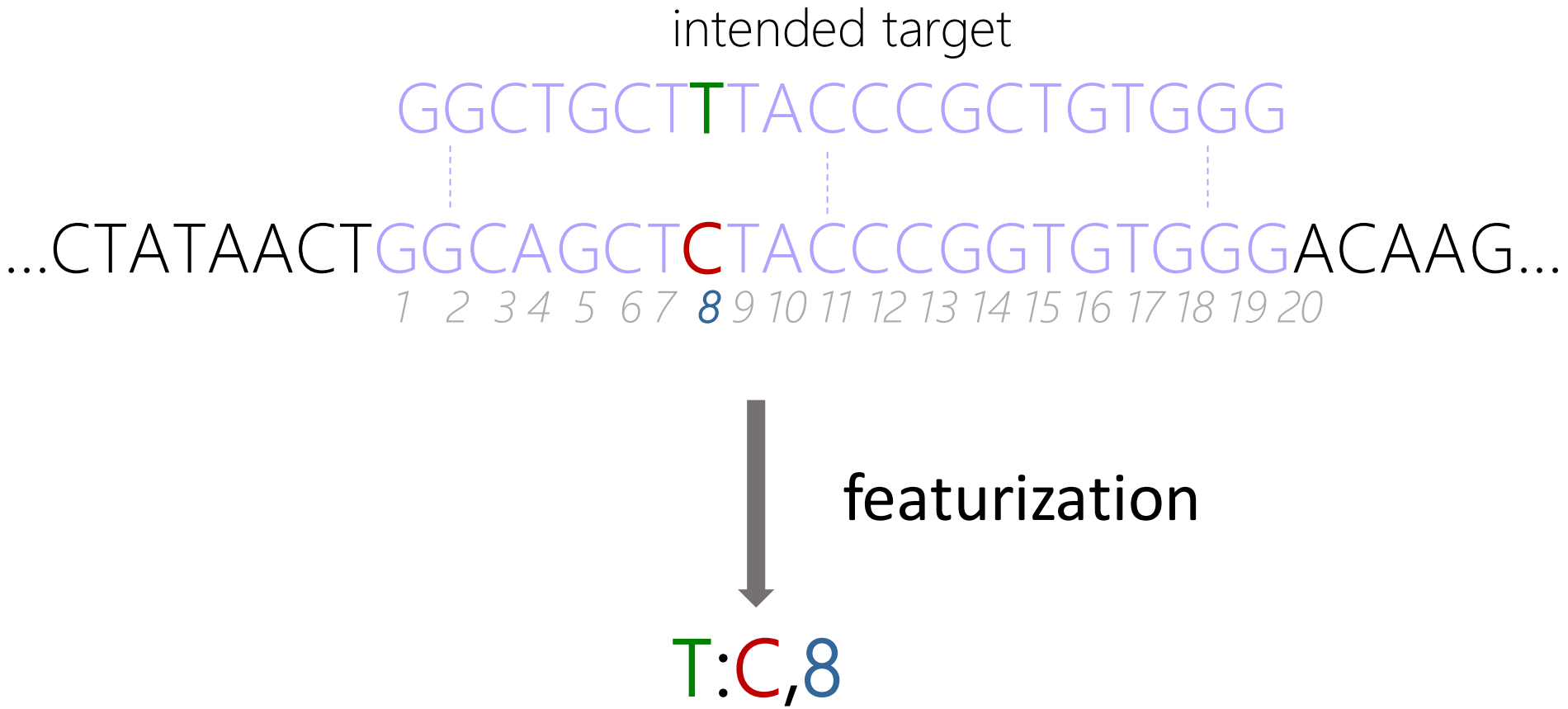
4 mismatches: **717,255** sites

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)



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*

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

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

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

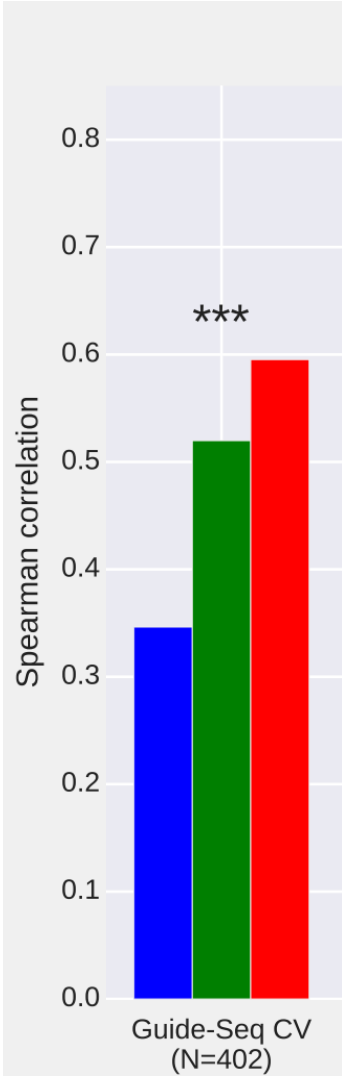
1. **Non-linear** regression model trained on **1-mismatch data**.
   - Complex model capturing interactions
   - Can only compute predictions for 1 mismatch at a time

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

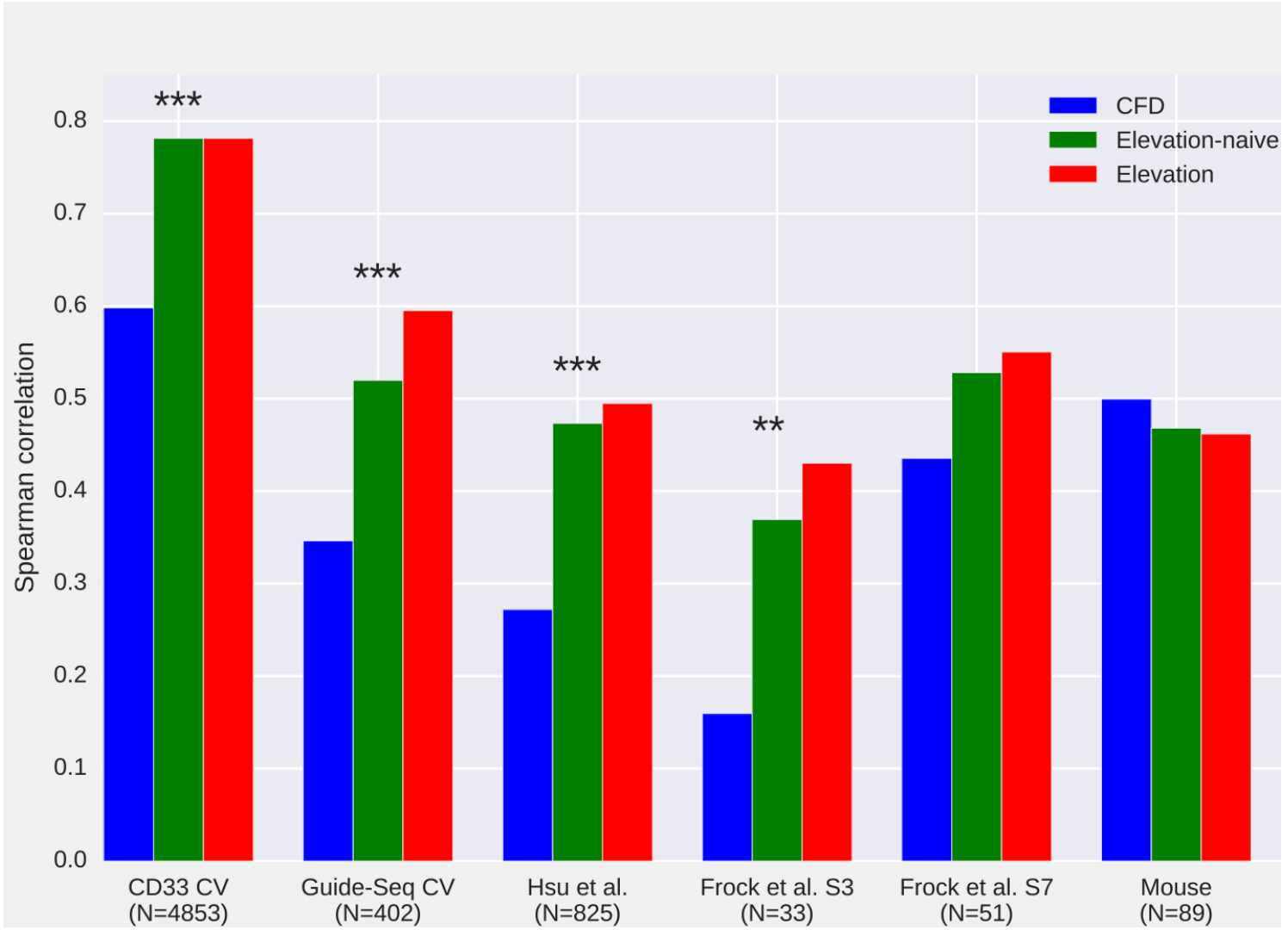
Elevation

Elevation outperforms *CFD* by 64%



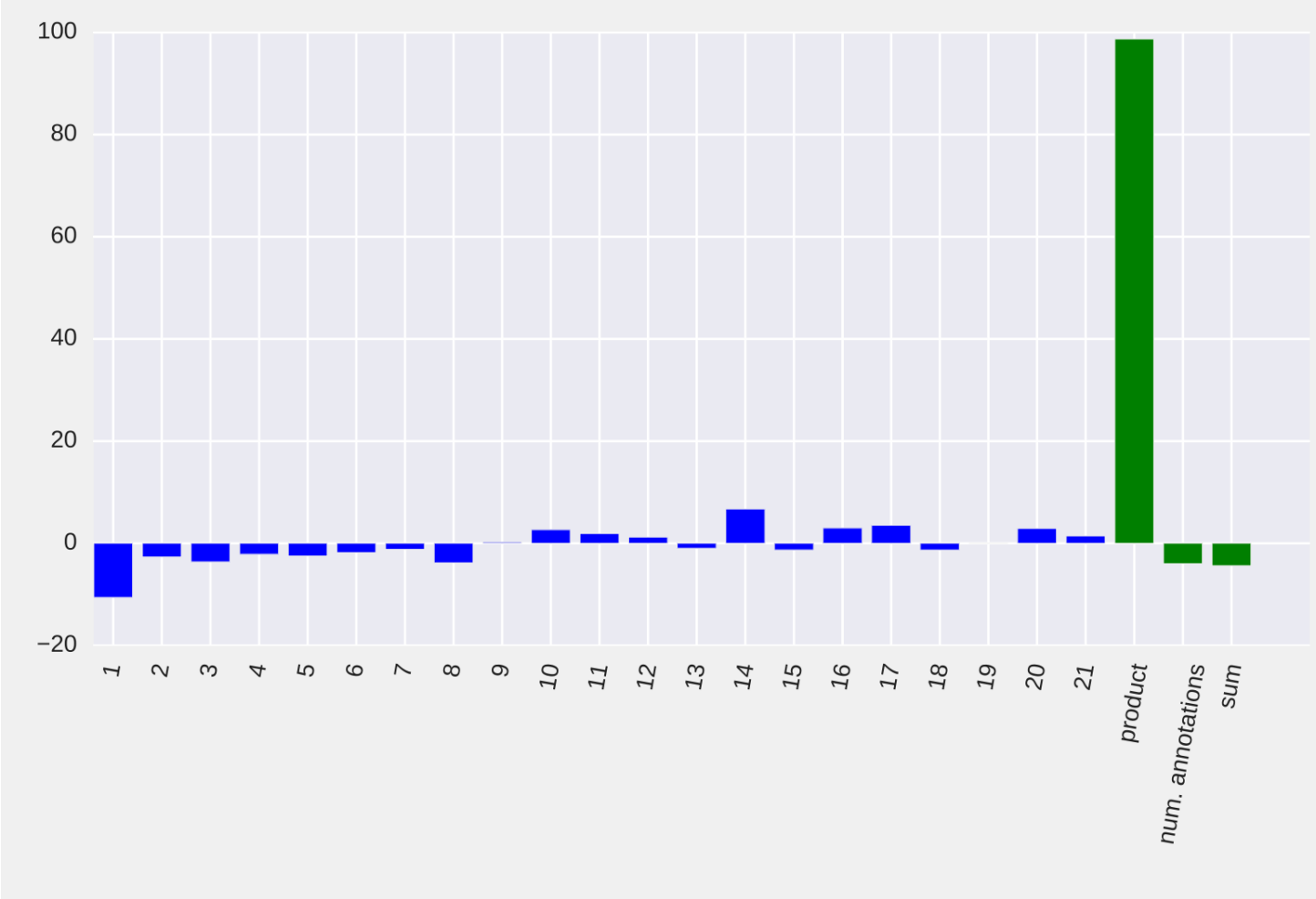
- Elevation spearman $\rho = 0.59$
- CFD spearman $\rho = 0.36$
- 64% improvement $(p = 5.5 \times 10^{-5})$

Elevation performs best on 4/5 other data sets



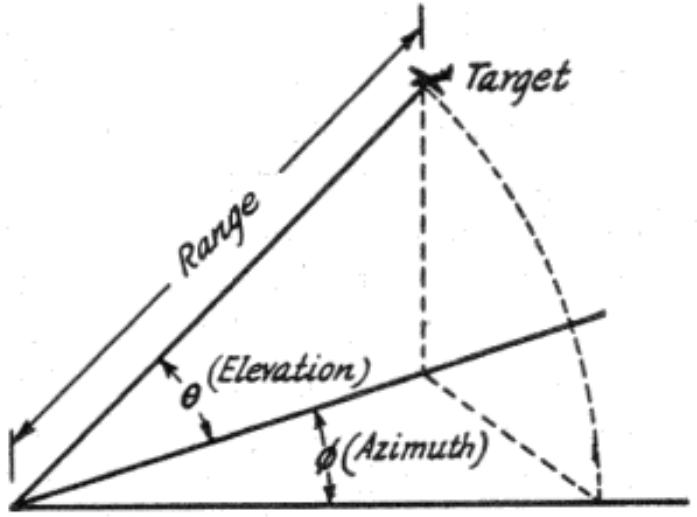
Mitigation of assumptions

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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