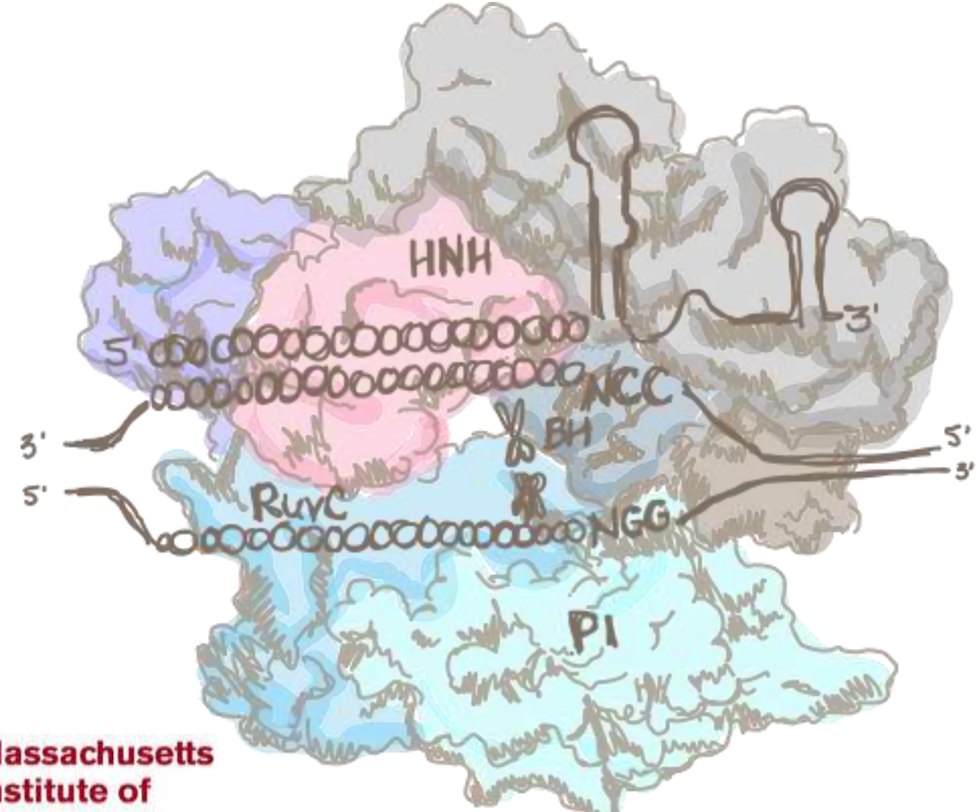
# CRISPR
## Editing the Human Genome

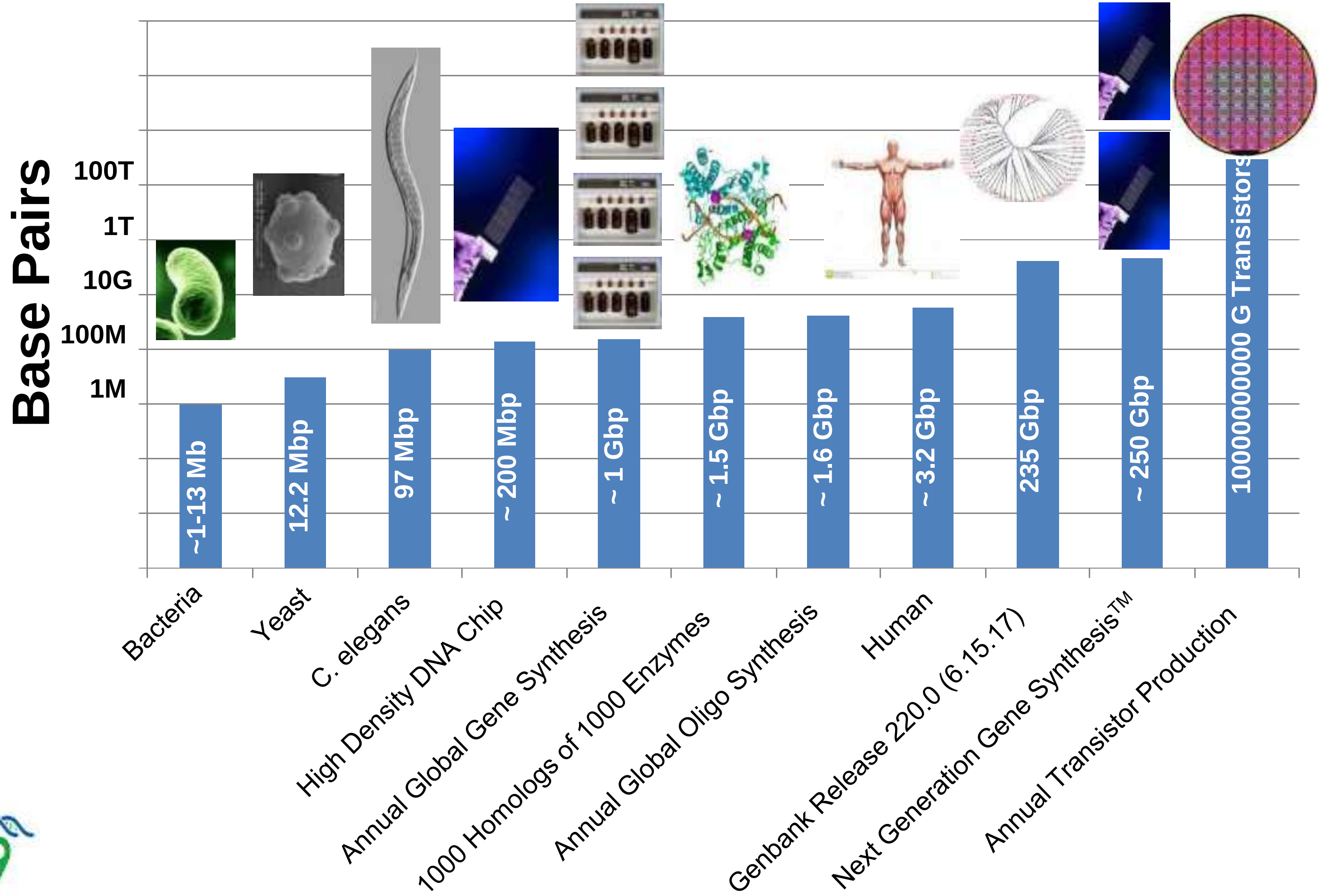



Molecular Machines (Jacobson) Group

mit media lab

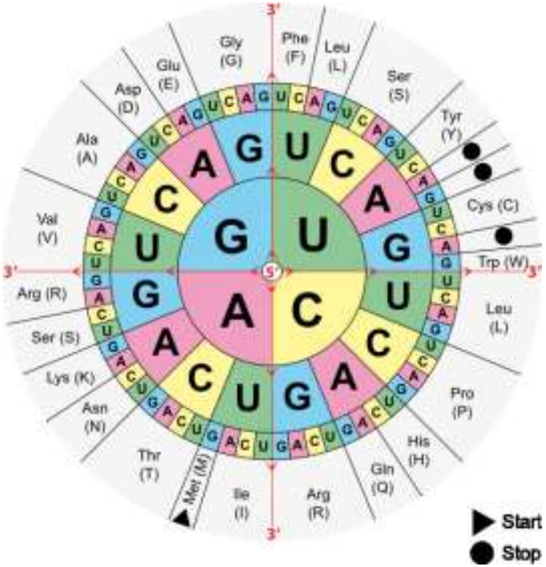
Massachusetts Institute of Technology

# Sizes of Genomes

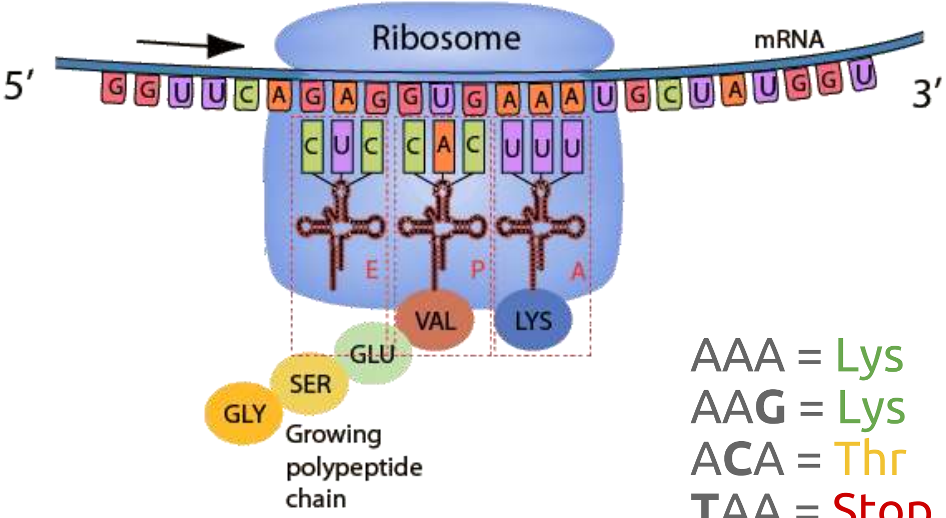



mit media lab Gen9

# Miscoded Mutations:
# Single Nucleotide Polymorphisms (SNP)

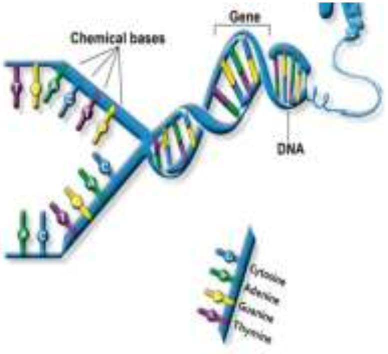



DNA/RNA -> Protein Lookup Table

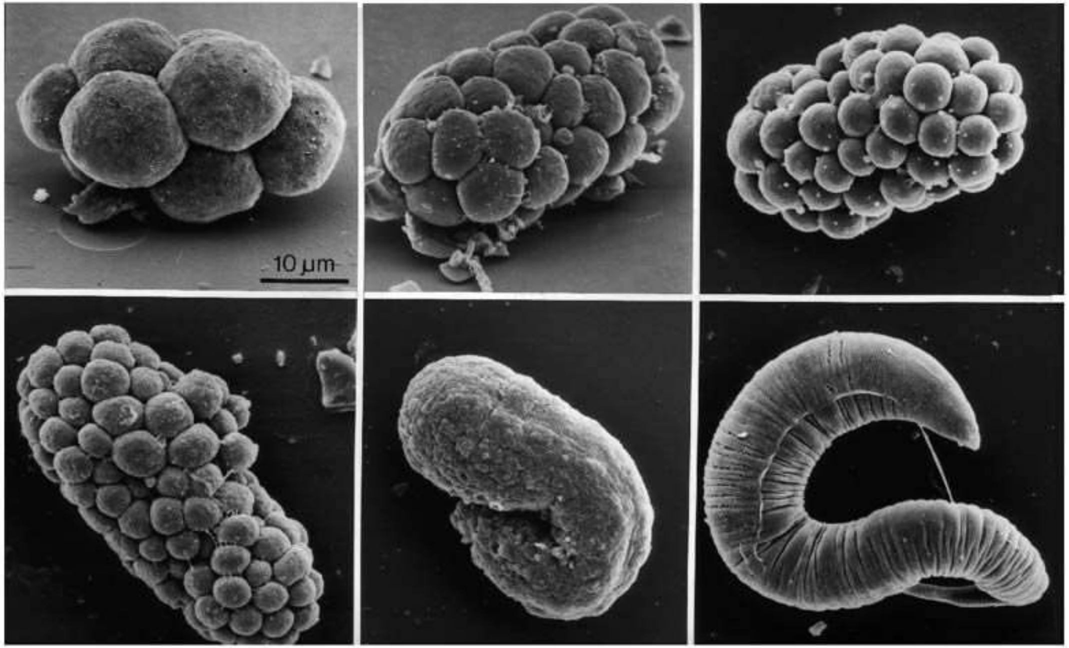



AAA = Lys
AAG = Lys
ACA = Thr
TAA = Stop
CAA = Gln

# Biology is a Program



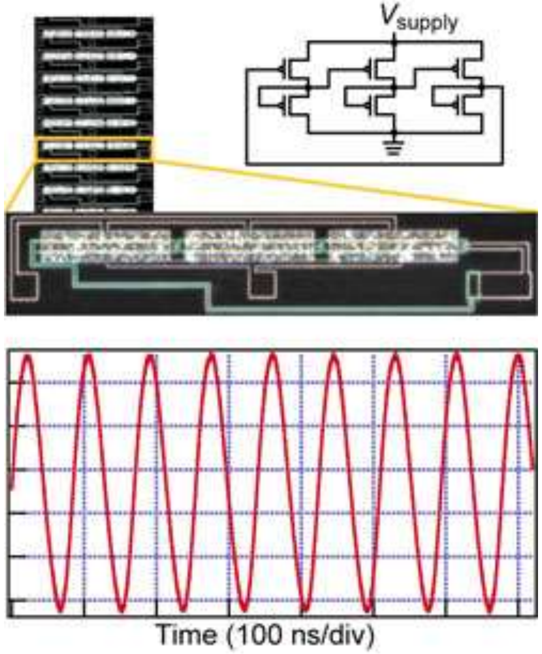

Genome (~100Mb)



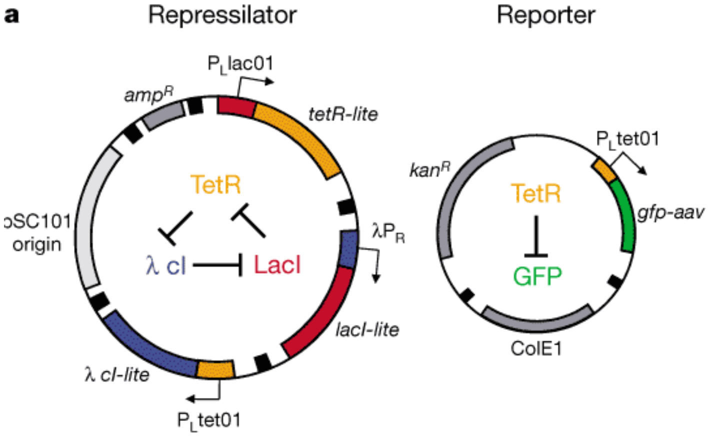

C. Elegans

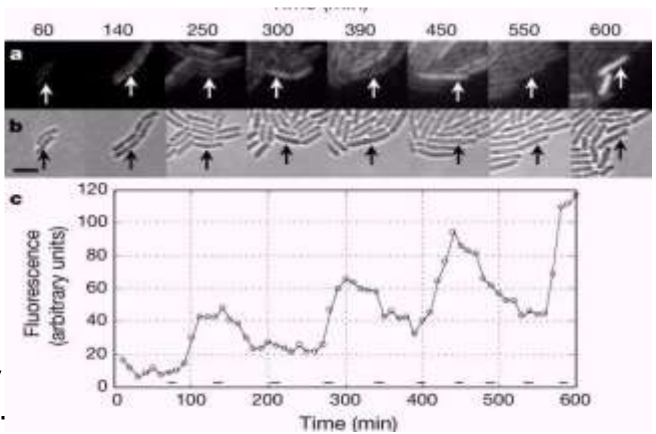
# Cells as Computers and Logic

## Ring Oscillators from Transcriptional-Translational Logic




http://echinacea.harvard.edu/research/image-gallery/

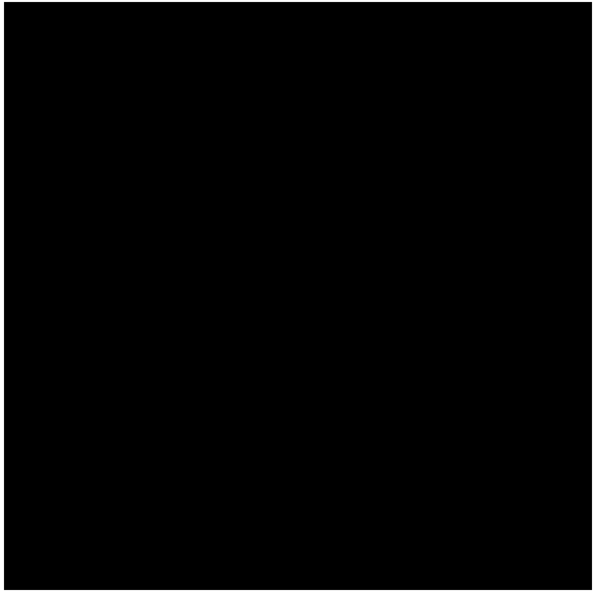


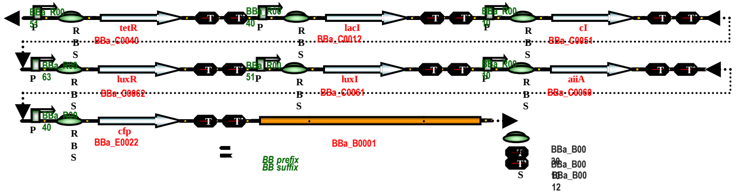

MB Elowitz MB and S Leibler (2000) A synthetic oscillatory network of transcriptional regulators.Nature.403 :335 - 338.

# Bacterial Ring Oscillators

Jeff Hasty - UCSD



http://elowitz.caltech.edu/



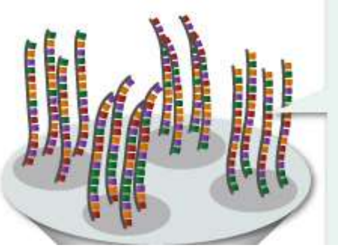
**הלכות יסודי התורה 2:2**

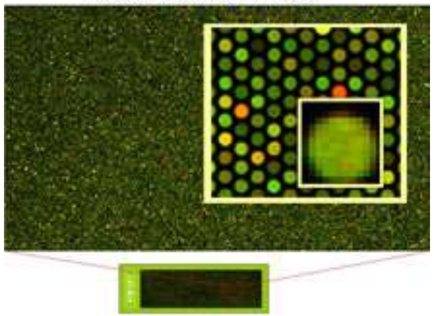
בשעה שיתבונן האדם המעשיות
ובראיו הנפלאים, הגדולים,
ויראה מהן חכמתו, שאין לה ערך
ולא קץ-מיד הוא אוהב ומשבח
ומפאר ומתאוה תאוה גדולה לידע
השם הגדול.
כמו שאמר דוד: "צמאה נפשי לא-
להים ל-א-ל חי"

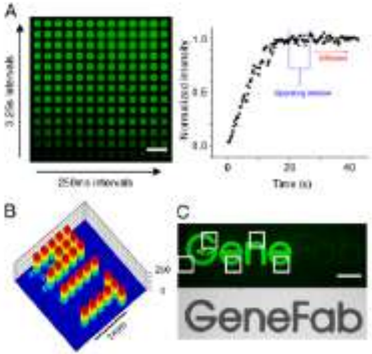
When a person contemplates His wondrous and great deeds and creations and appreciates His infinite wisdom that surpasses all comparison, he will immediately love, praise, and glorify[Him], yearning with tremendous desire to know [G-d’s] great name.

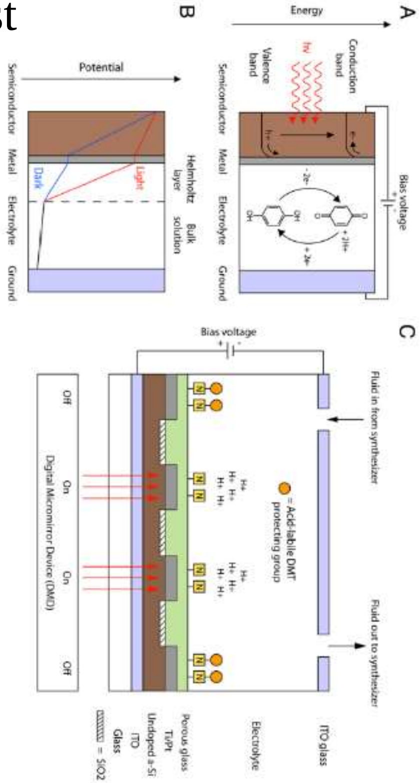
# Next Generation (Chip Based) DNA Synthesis

## ~1000x Lower Oligonucleotide Cost






## ~ 1M Oligos/Chip

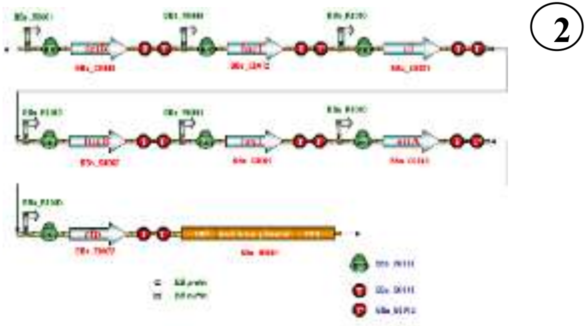
**Chow, Brian Y., Christopher J. Emig, and Joseph M. Jacobson. "Photoelectrochemical synthesis of DNA microarrays."** ***Proceedings of the National Academy of Sciences*** **106.36 (2009): 15219-15224.**

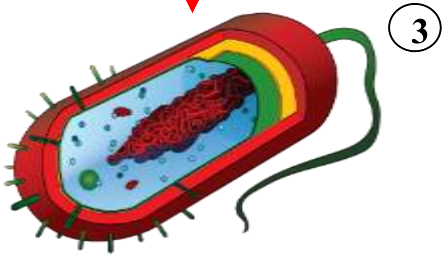
**http://www.technologyreview.com/biomedicine/20035/**

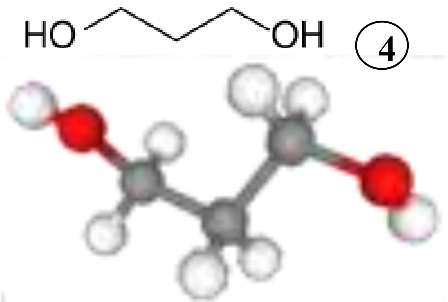
http://learn.genetics.utah.edu/content/labs/microarray/analysis

**Accurate multiplex gene synthesis from programmable DNA microchips**
Jingdong Tian, Hui Gong, Nijing Sheng, Xiaochuan Zhou, Erdogan Gulari, Xiaolian Gao and George Church
*Nature* **432**, 1050-1054(23 December 2004)

# Synthetic Biology

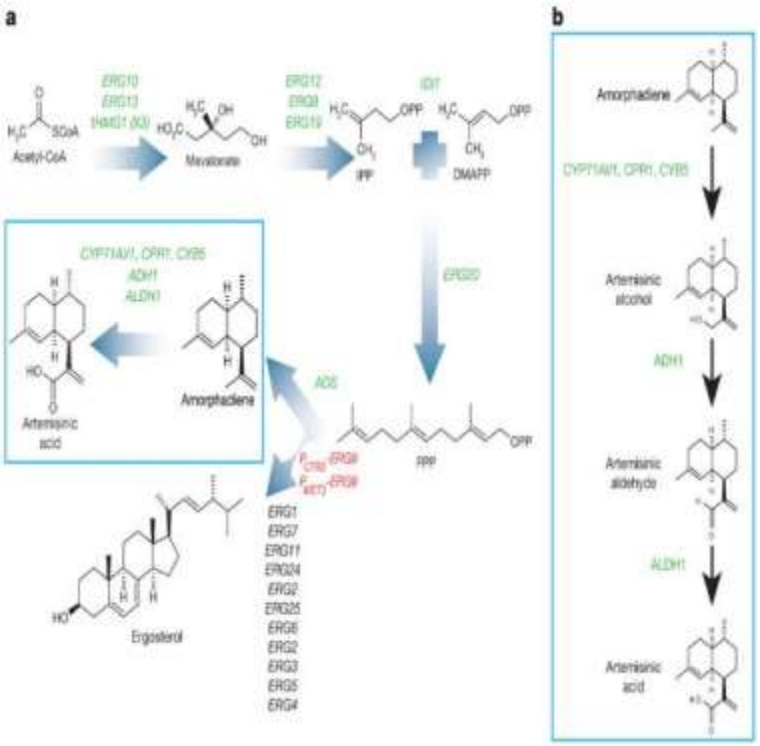

*to Make High Value Products (Biofuels, Chemicals, Pharmaceuticals)*

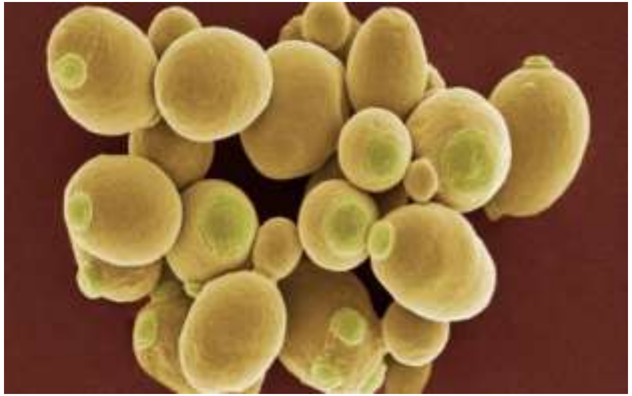
*to Program Cells to Become Cellular Factories, Sensors …*

# Cells as Small Molecule Pharmaceutical Factories

## ~6 Gb Synthesis For top 1000 small molecules drug intermediates

**Artemisinic Acid (Antimalarial)**



Paddon, Christopher J., et al. "High-level semi-synthetic production of the potent antimalarial artemisinin." *Nature* 496.7446 (2013): 528-532.

http://www.latonkorea.com/Plant.html

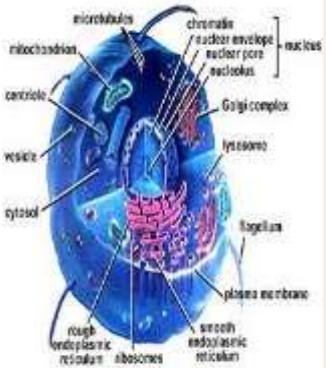
http://neweuropeans.org/eurowine-3-yeast-europe-and-

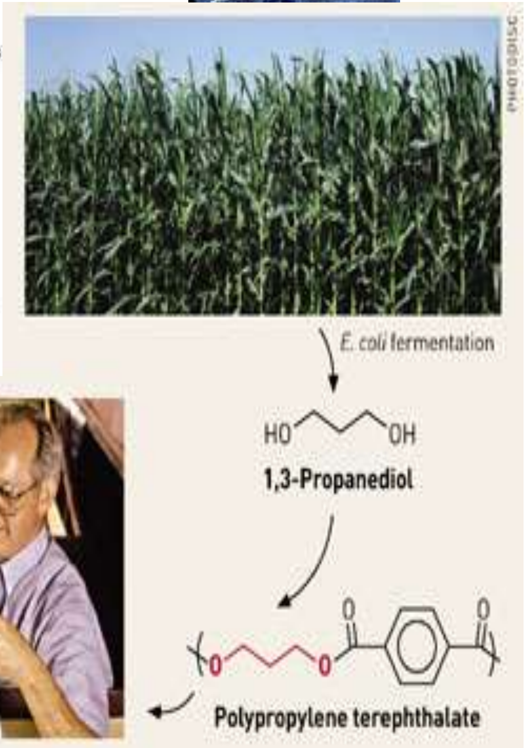
DUPONT
The miracles of science

DuPont Sorona
renewably sourced polymer

# Cells as Chemical Factories

## 1,3 Propanediol



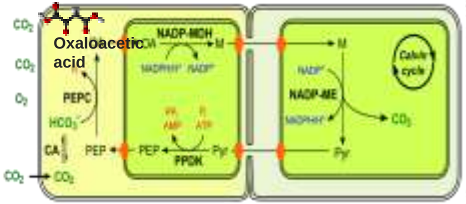
http://www.latonkorea.com/Plant.html

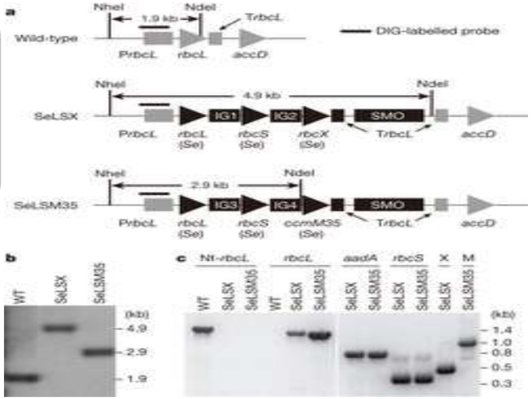
http://3rdpartylogistics.blogspot.com/2011/10/genetic-bacteria-genetic-modification.html

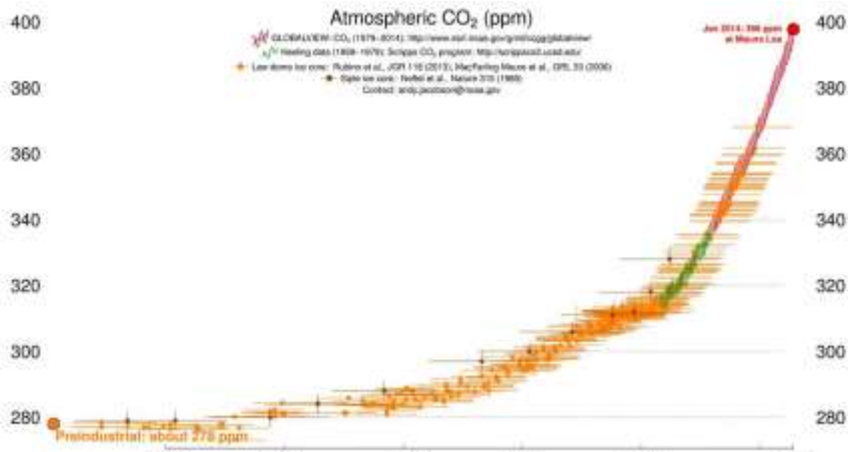
# C4 Photosynthesis

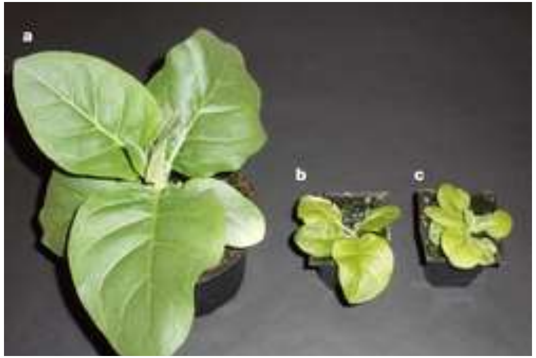
## *Feeding the World / Capturing CO2 at Scale*

**$C_4$ plants:**
5% of Earth's biomass 30% of terrestrial carbon fixation (6X more efficient)



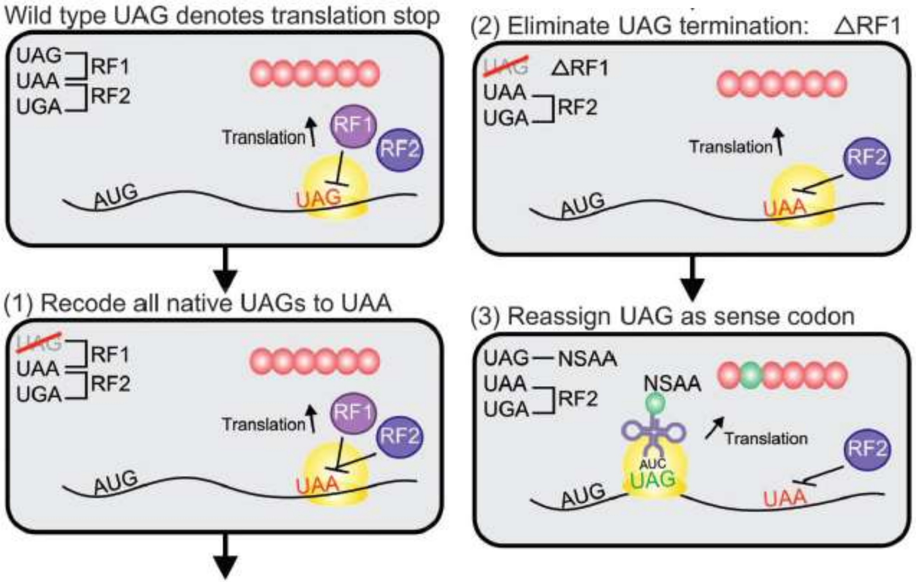

Lin MT, Occhialini A, Andralojc PJ, Parry MA, Hanson MR. A faster Rubisco with potential to increase photosynthesis in crops. Nature. 2014 Sep 25;513(7519):547-50.
Osborne CP, Beerling DJ. Nature's green revolution: the remarkable evolutionary rise of C4 plants. Philosophical Transactions of the Royal Society of London B: Biological Sciences. 2006 Jan 29;361(1465):173-94.

Science

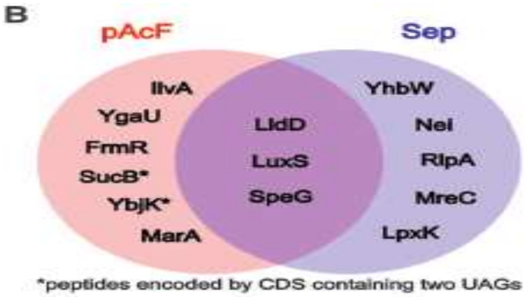
# Genomically Recoded Organisms Expand Biological Functions

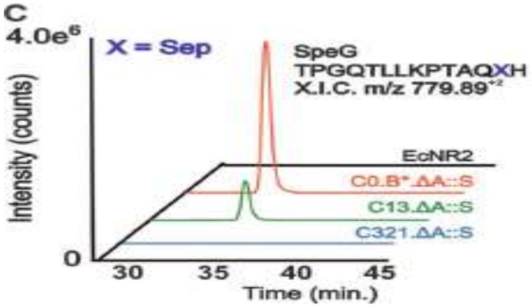
Marc J. Lajoie,[1,2] Alexis J. Rovner,[3,4] Daniel B. Goodman,[1,5] Hans-Rudolf Aerni,[4,6] Adrian D. Haimovich,[3,4] Gleb Kuznetsov,[1] Jaron A. Mercer,[7] Harris H. Wang,[8] Peter A. Carr,[9] Joshua A. Mosberg,[1,2] Nadin Rohland,[1] Peter G. Schultz,[10] Joseph M. Jacobson,[11,12] Jesse Rinehart,[4,6] George M. Church,[1,13*] Farren J. Isaacs[3,4*]

www.sciencemag.org SCIENCE VOL 342 18 OCTOBER 2013

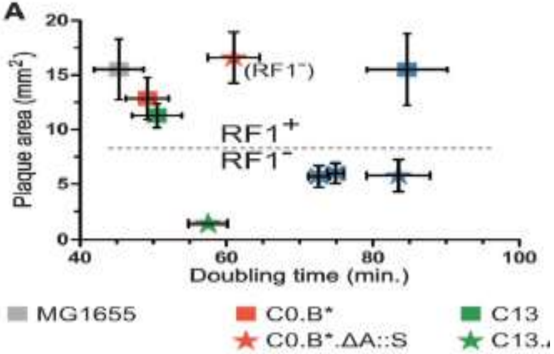



## Conferring New Function



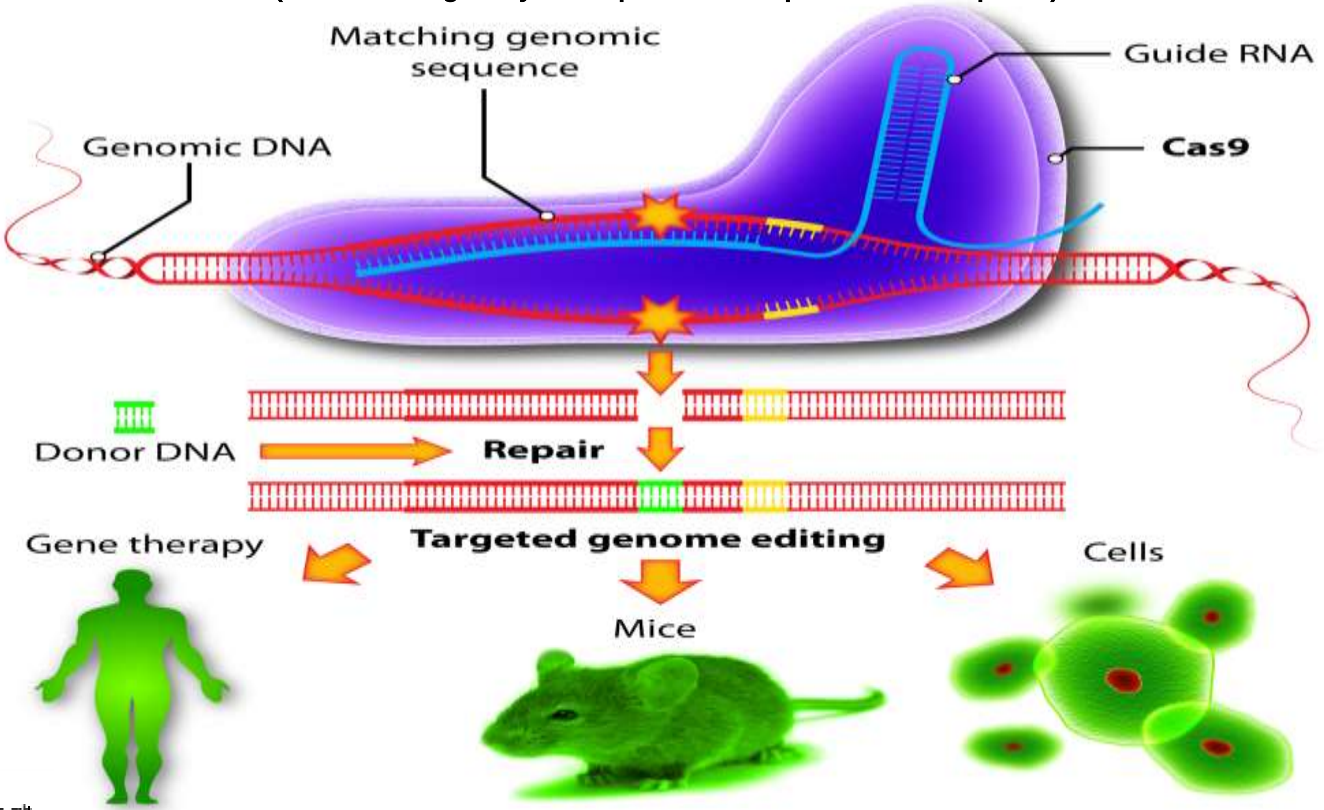

## Phage Resistance



# Programming the Genome

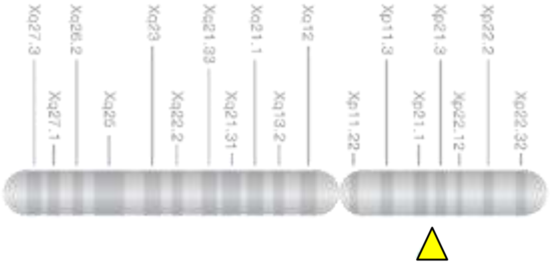
# CRISPR

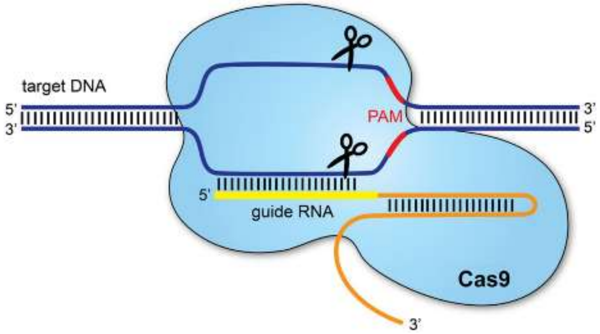
**(clustered regularly interspaced short palindromic repeats)**



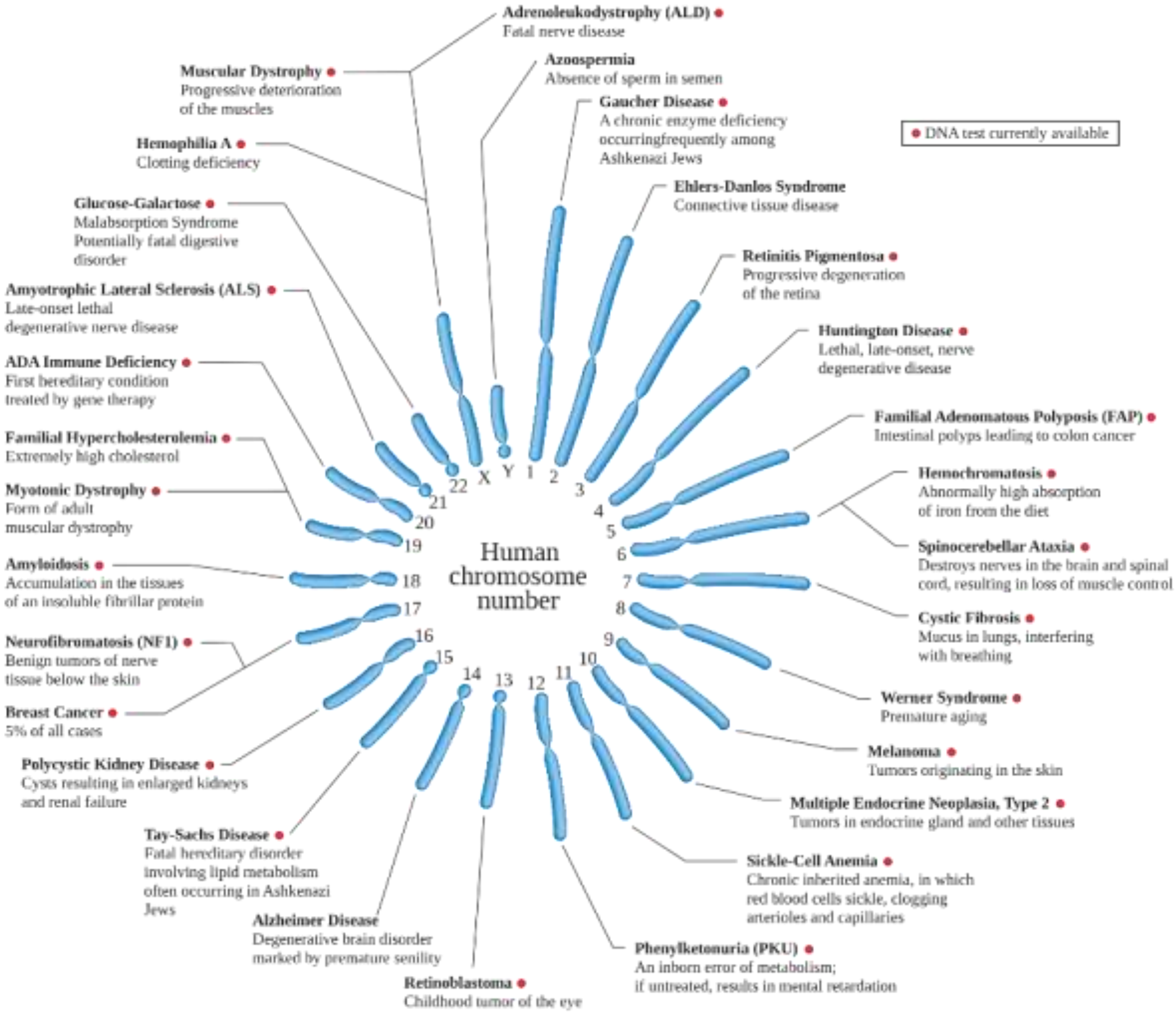

http://igtrcn.org/wp-content/uploads/2014/06/Cas9_green-01-1024x1024.png

# CRISPR-Cas9:
# A Genomic Search Bar



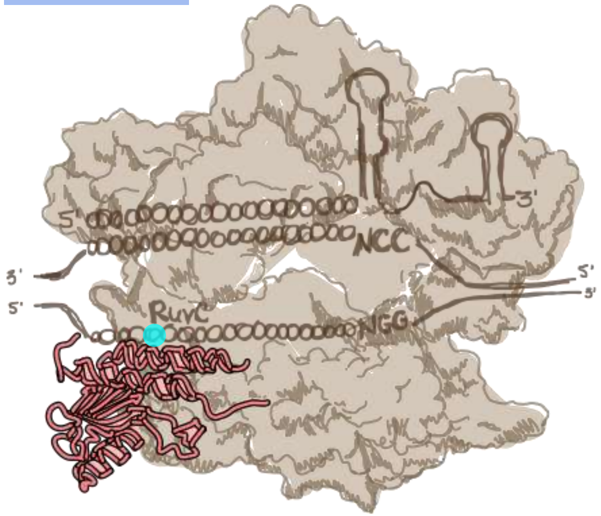

ACCCTGGATATATAACG



# CRISPR

## Base Editing

BASE-EDITING



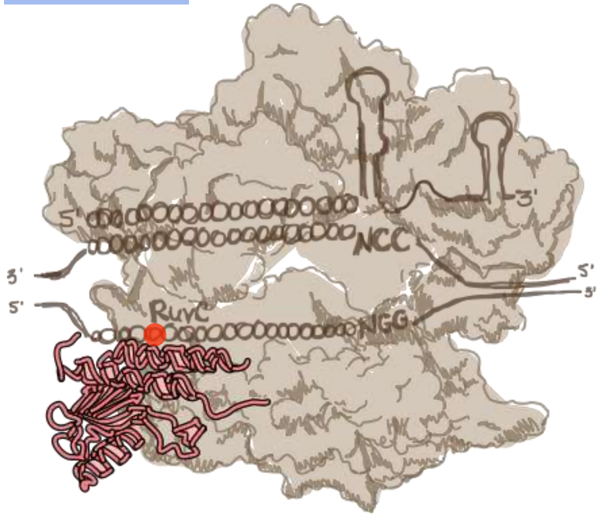

COMMON TARGET LIMITATIONS OF CRISPR SYSTEMS

| Enzyme | Species | PAM (5' to 3') | # Specific Bases | % Genome Addressable |
|---|---|---|---|---|
| SpCas9 | Streptococcus pyogenes | NGG, NGA*, NGD* | 2 | 12.5% |
| AsCas12a | Acidoaminococcus sp. | TTTV, TYCV*, TATV* | 3 | 2.3% |
| SaCas9 | Staphylococcus aureus | NNNRRT | 3 | 12.5% |
| CjCas9 | Campylobacter jejuni | NNNNRYAC | 4 | 3.13% |
| NmeCas9 | Neisseria meningitidis | NNNNGMTT | 4 | 1.56% |
| StCas9 | Streptococcus thermophilus | NNAGAAW | 5 | 0.39% |

# CRISPR

## Base Editing

BASE-EDITING

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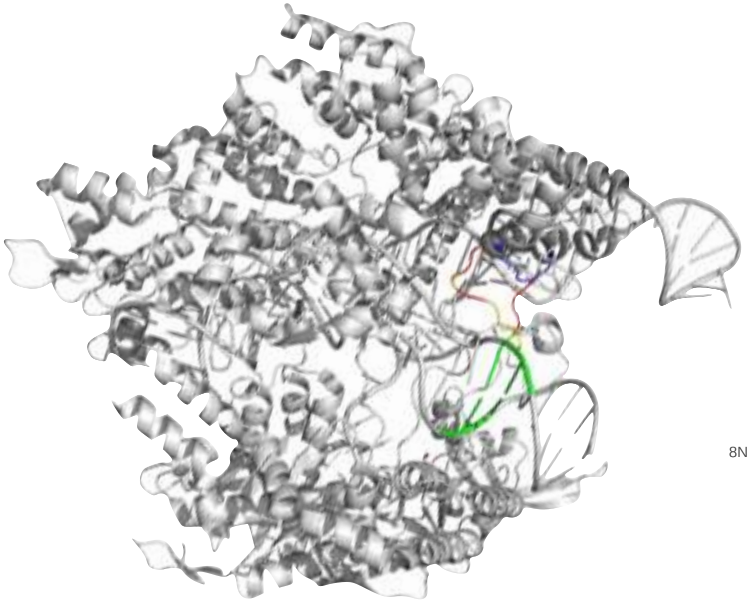
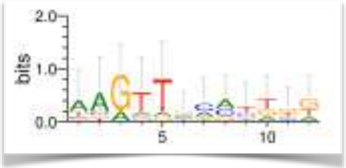
| StCas9 | Streptococcus thermophilus | NNAGAAW | 5 | 0.39% |

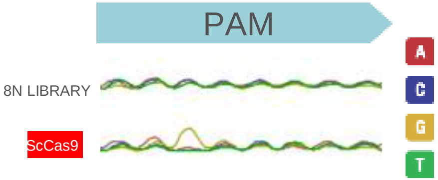
# CRISPR

## Expanding the Genomic Target Space

FROM BIOINFORMATICS COMPUTATIONAL PIPELINE



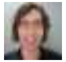


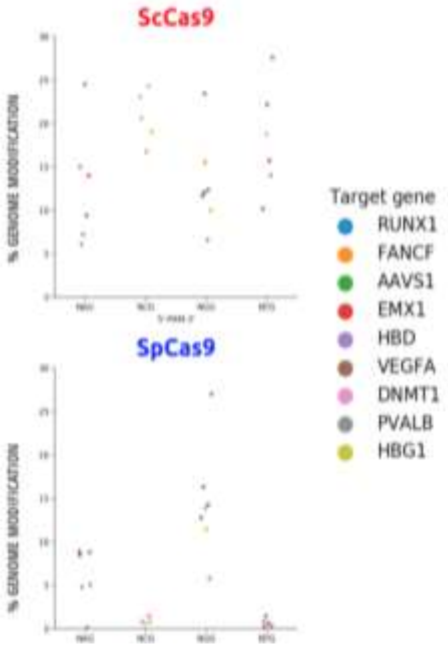

Alignment
Pos 1-1240: 93.5%
Pos 1241-1368: 52.8%

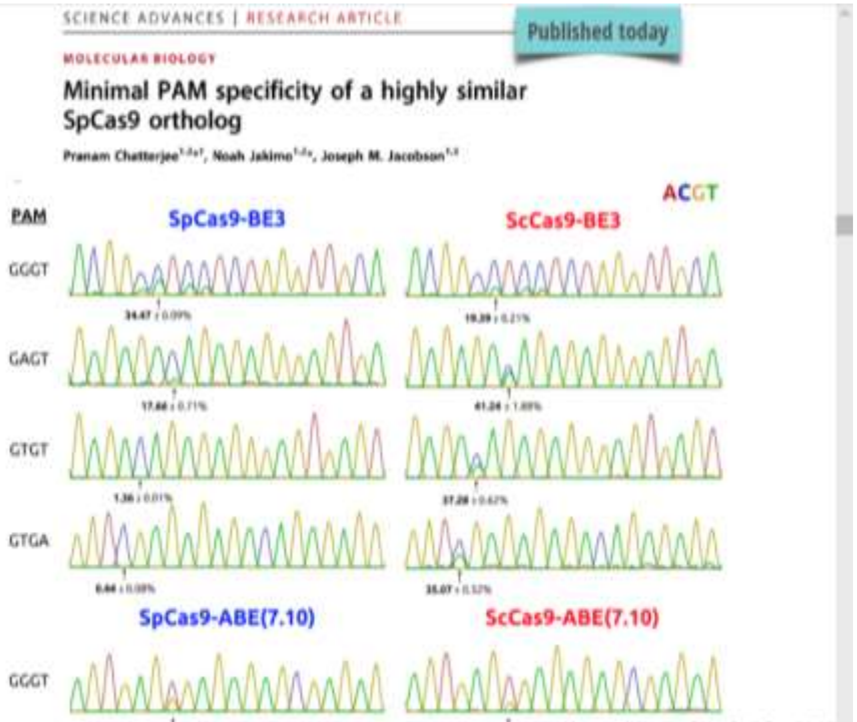
Chatterjee et al., Sci. Adv. 2018

 Pranam Chatterjee

 Noah Jakimo

# ScCas9: THE ONE-BASE EDITOR




Chatterjee et al., Sci. Adv. 2018

## EXPANDING ADDRESSABLE GENOMIC SITES

SpyCas9: ~27.5% pathogenic SNPs

ScCas9: ~72.4% pathogenic SNPs

SpyMacCas9: ~18.4% pathogenic SNPs

## DISEASES NOW TARGETABLE: 90.8%

**ScCas9**

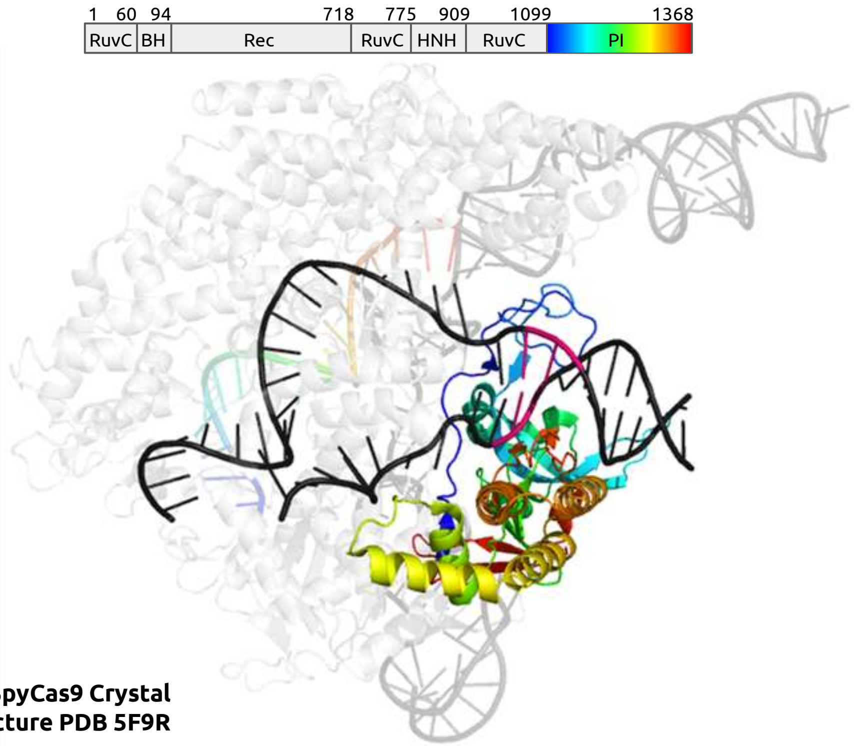
Ablepharon macrostomia syndrome
Muscular dystrophy-dystroglycanopathy
Fanconi-Bickel syndrome
Gordon Holmes syndrome

**SpyMacCas9**

Ventricular tachycardi


mit media lab

# Streptococcus Macacae (Smac) Cas9  NAA PAM




SpyCas9 Crystal Structure PDB 5F9R

8N PAM Library


PAM-SCANR Sorted (GFP+)

Spy dCas9

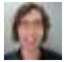
Spy-mac dCas9

Jakimo*, Chatterjee*, Nip*, Jacobson, *bioRxiv* (2019)

# EXPANDING ADDRESSABLE GENOMIC SITES

SpyCas9: ~27.5% pathogenic SNPs

2
1

 Pranam Chatterjee  Noah Jakimo  Lisa Nip

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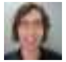
Pranam Chatterjee


Noah Jakimo


Lisa Nip

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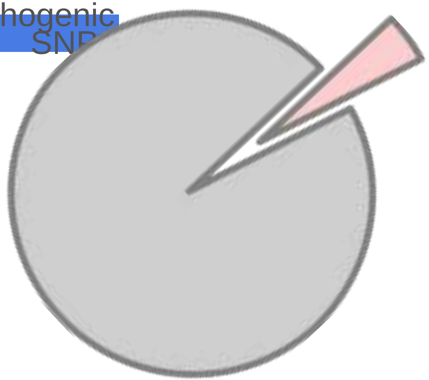
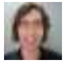



Pranam Chatterjee

Noah Jakimo

Lisa Nip

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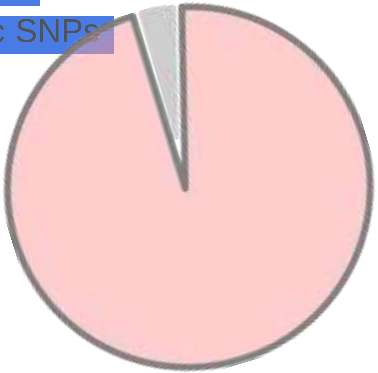


## DISEASES NOW TARGETABLE: 90.8%

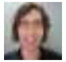
ScCas9

Ablepharon macrostomia syndrome
Muscular dystrophy-dystroglycanopathy
Fanconi-Bickel syndrome
Gordon Holmes syndrome

SpyMacCas9

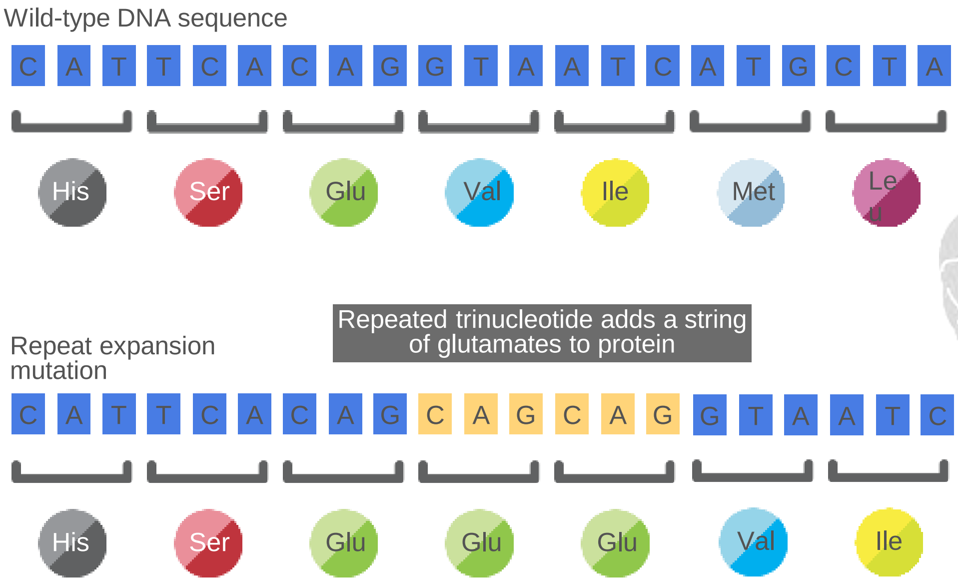
Ventricular tachycardi


Pranam Chatterjee


Noah Jakimo

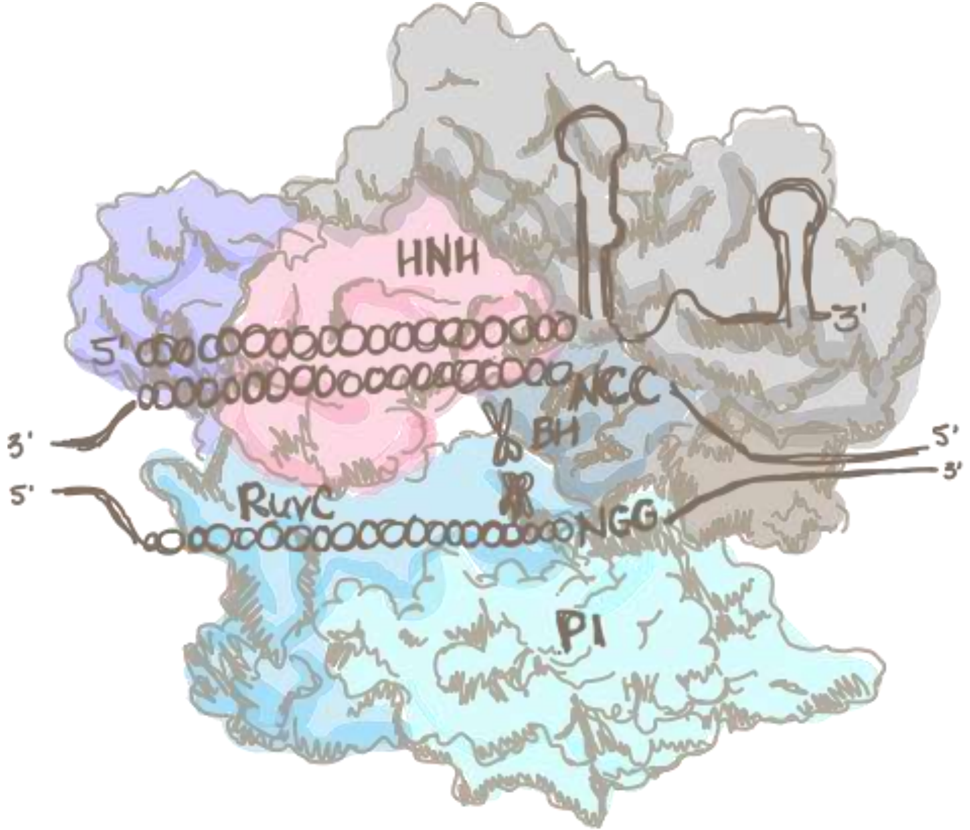

Lisa Nip

# TARGETING HUNTINGTON'S DISEASE

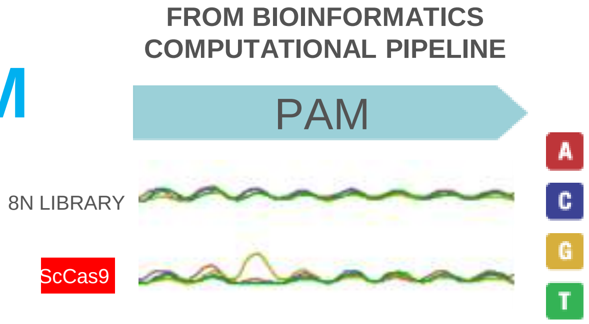



# CRISPR

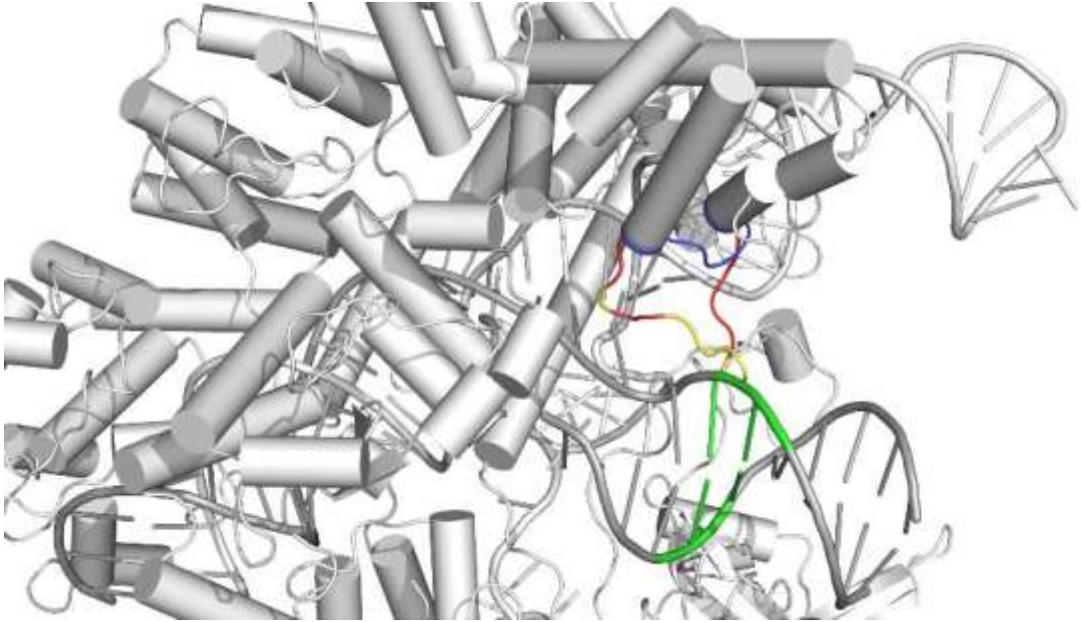
## Editing the Human Genome



# New Cas9 CRISPR Discovered
# ScCAS9 with Single Base NNG PAM

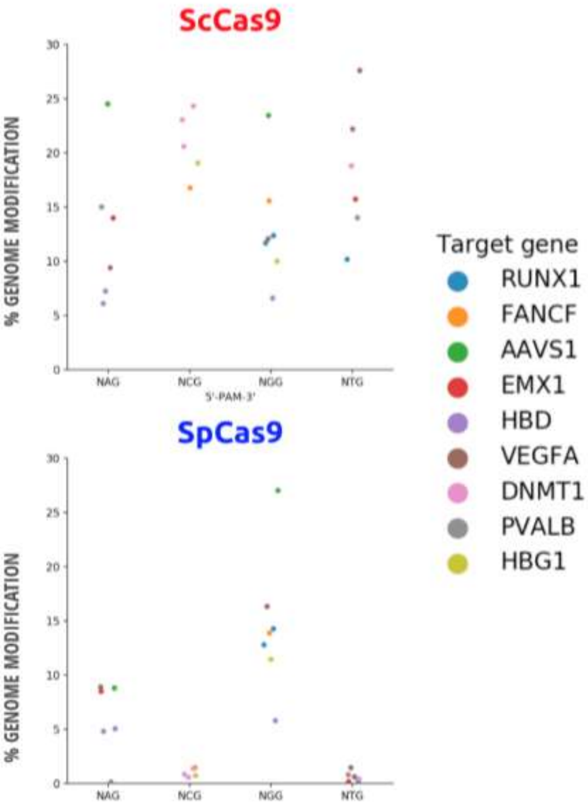



**Loop Modeling in PDB 4UN3 (Sp/Sc Loop and PAM)**



Chatterjee*, Jakimo*, Jacobson, *Science Advances* (2018)

# ScCas9: THE ONE-BASE EDITOR



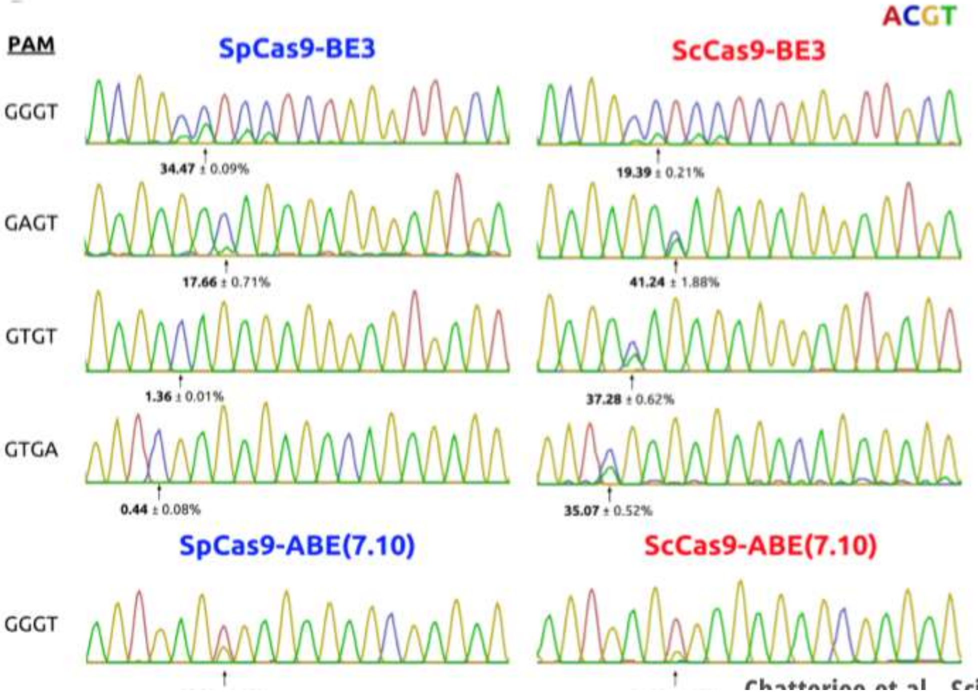

SCIENCE ADVANCES | RESEARCH ARTICLE

Published today

MOLECULAR BIOLOGY

## Minimal PAM specificity of a highly similar SpCas9 ortholog

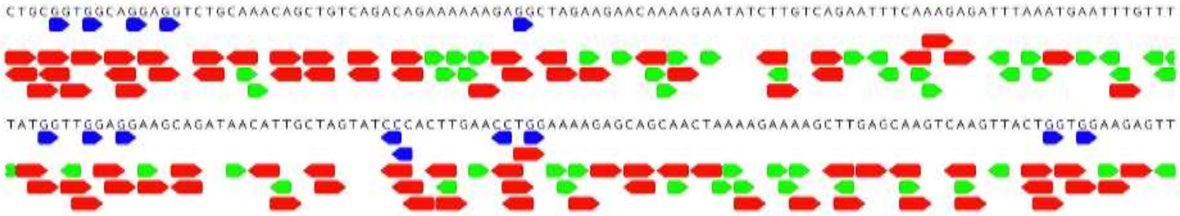
Pranam Chatterjee[1,2*†], Noah Jakimo[1,2*], Joseph M. Jacobson[1,2]




Chatterjee et al., Sci. Adv. 2018

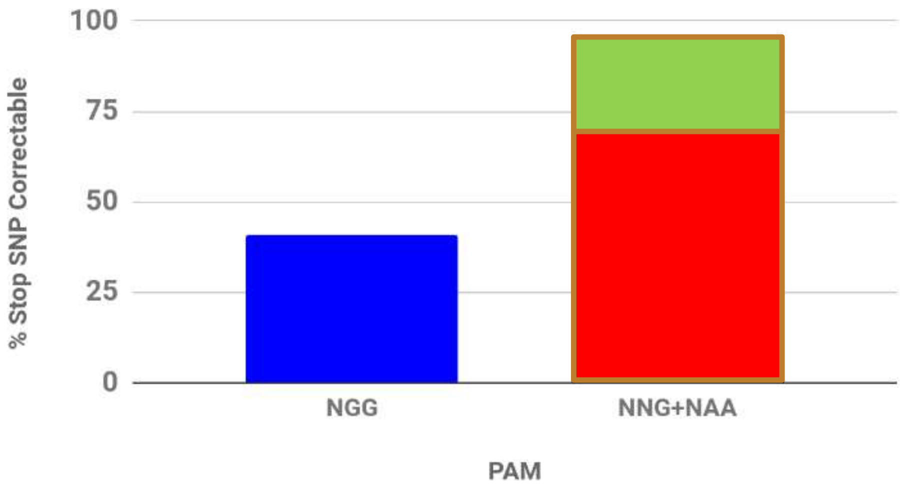
mit media lab

# Target Limitations in Disease-Associated Regions



Streptococcus pyogenes PAM Targets
Streptococcus canis PAM Targets
Streptococcus macacae PAM Targets

% Correctable Mutations



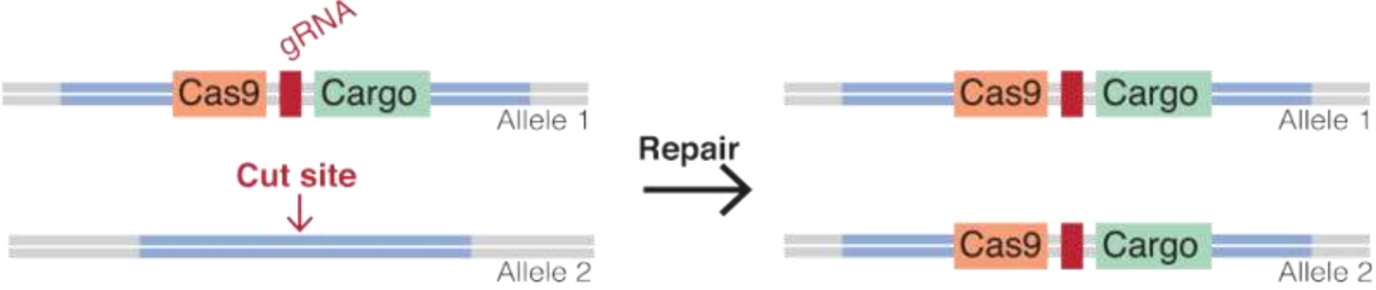

**EXAMPLES of NEW DISEASES NOW TARGETABLE**

ScCAS9

Ablepharon macrostomia syndrome
Muscular dystrophy-dystroglycanopathy
Fanconi-Bickel syndrome
Gordon Holmes syndrome

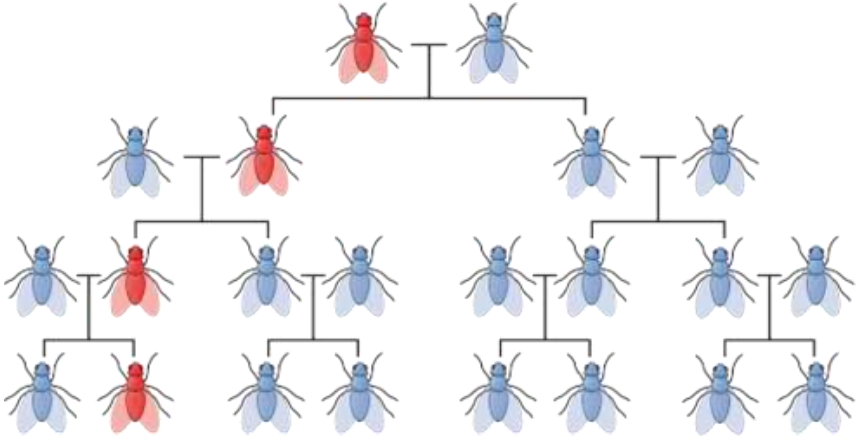
SpyMACCas9

Ventricular tachycardi



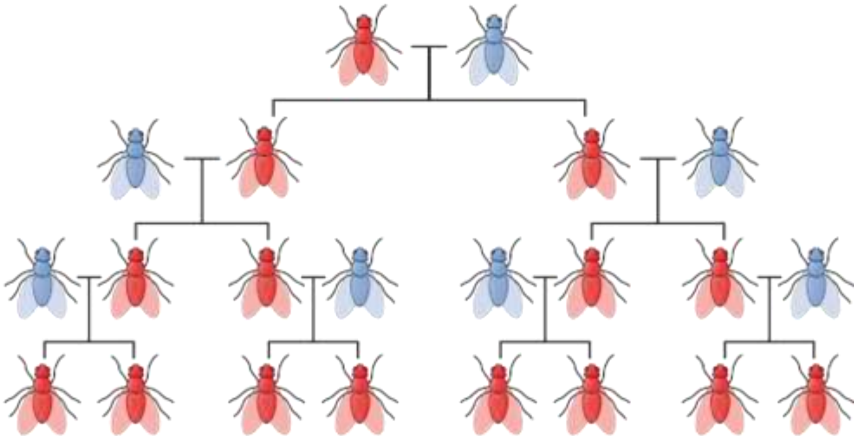

# GENE DRIVE

## Normal inheritance



Altered gene does not spread

## Gene drive inheritance



Altered gene is always inherited