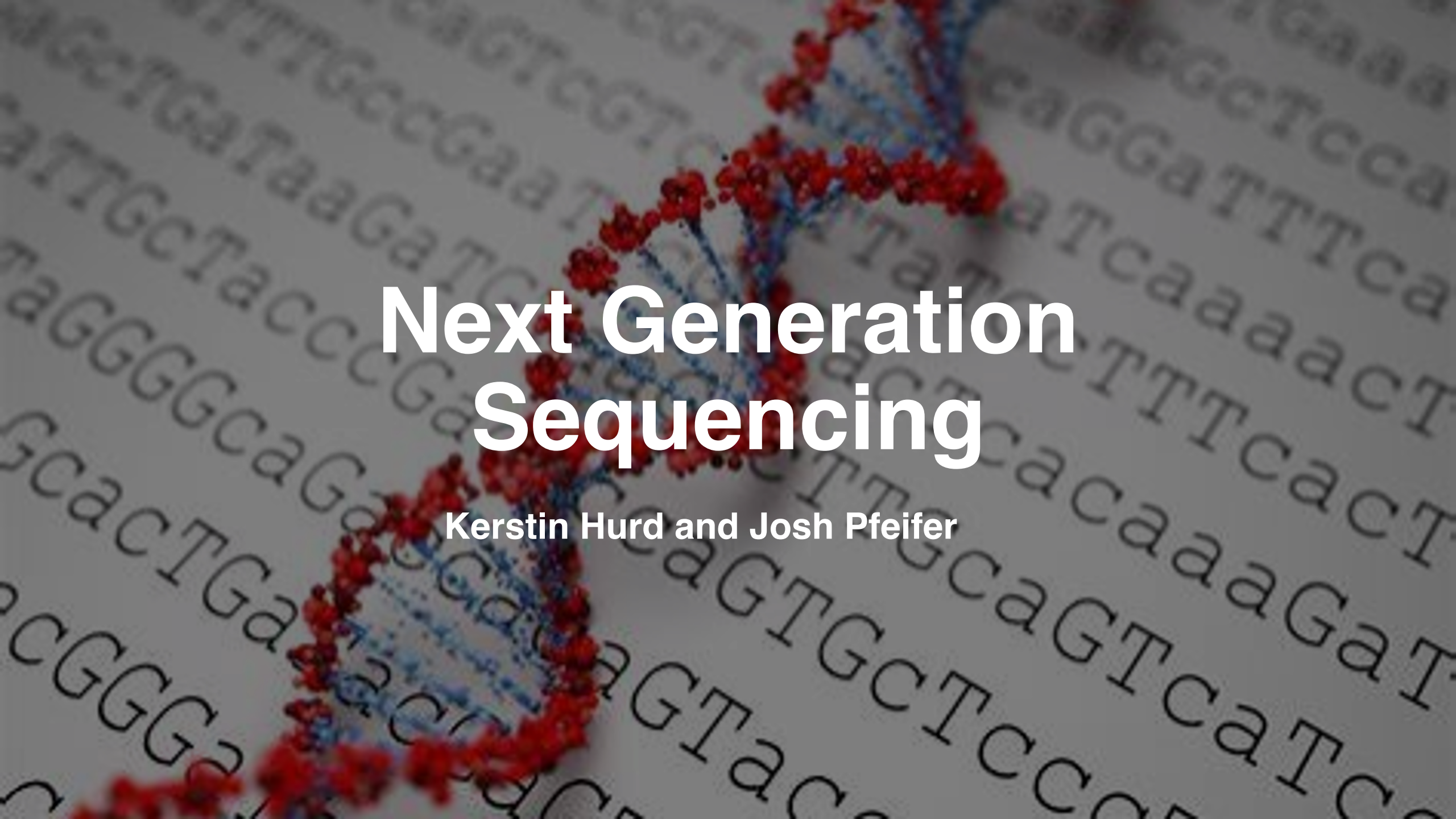


Next Generation Sequencing

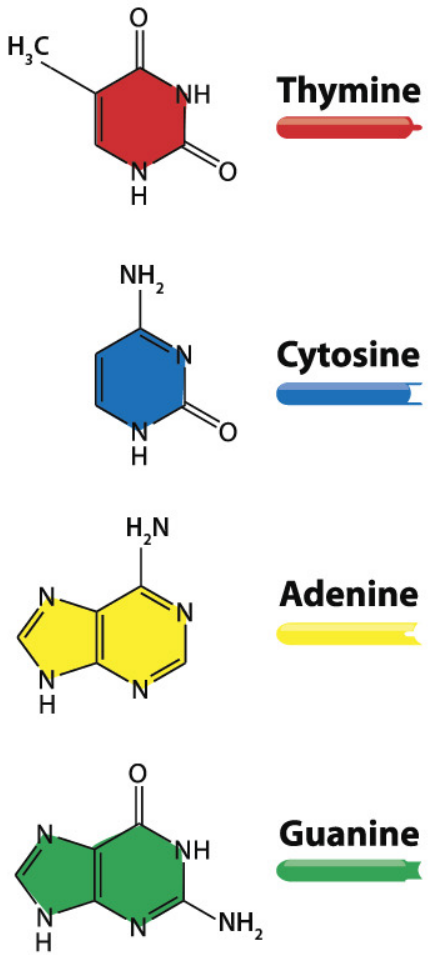
Kerstin Hurd and Josh Pfeifer

REVIEW

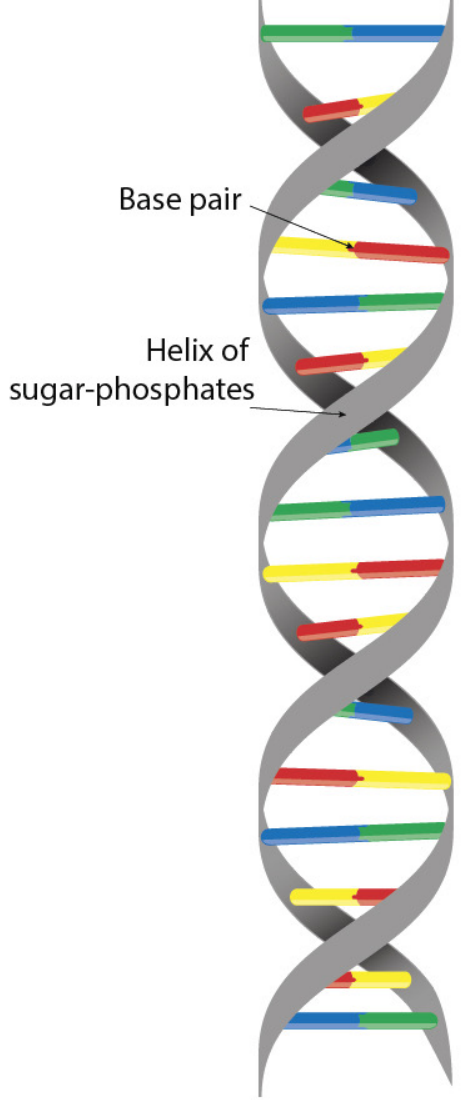
Next-generation sequencing: impact of exome sequencing in characterizing Mendelian disorders

Bahareh Rabbani[1], Nejat Mahdieh[1], Kazuyoshi Hosomichi, Hirofumi Nakaoka and Ituro Inoue

What is DNA?



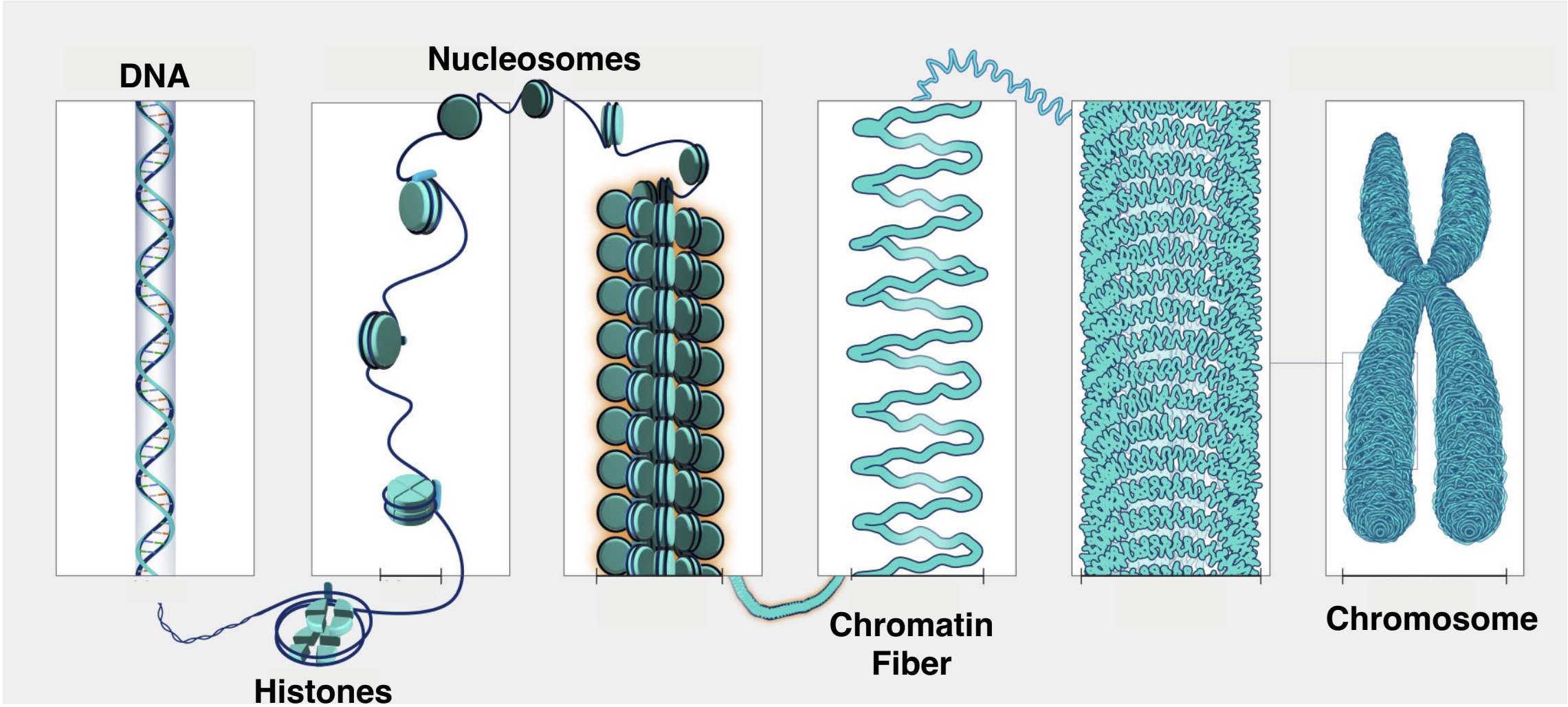
Nucleobases of DNA



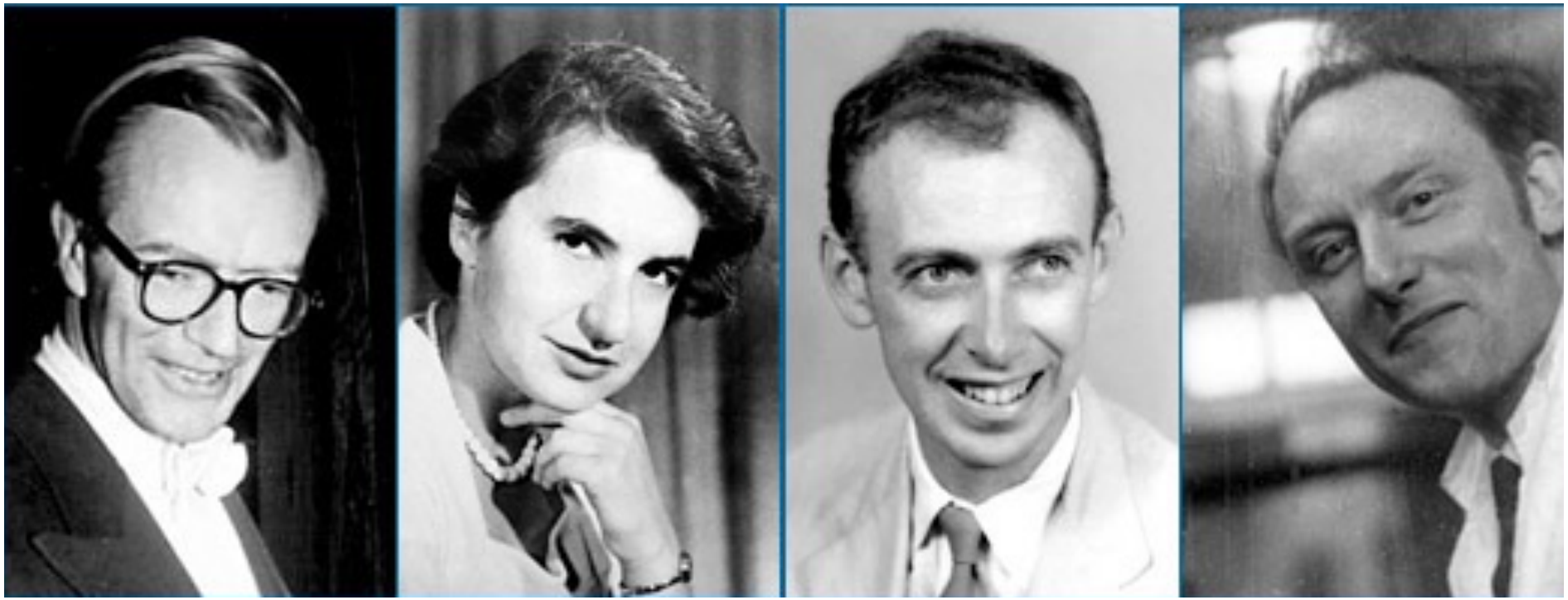
DNA
Deoxyribonucleic acid

Genetic material essential for the growth and development of organisms

How do we go from DNA to a whole genome?



Who discovered DNA?



Wilkins Franklin Watson Crick

Timeline for genomic sequencing

Sanger method

1977

Human Genome Project

1990

Complete eukaryotic genome

1996

Second generation sequencer：454 GS20

2005

Research Human Microbiome Project

2008

Nanospace sequencing

2014

1981

1995

2001

2007

2011

2019

Human mitochondrial genome sequence

Complete cell genome

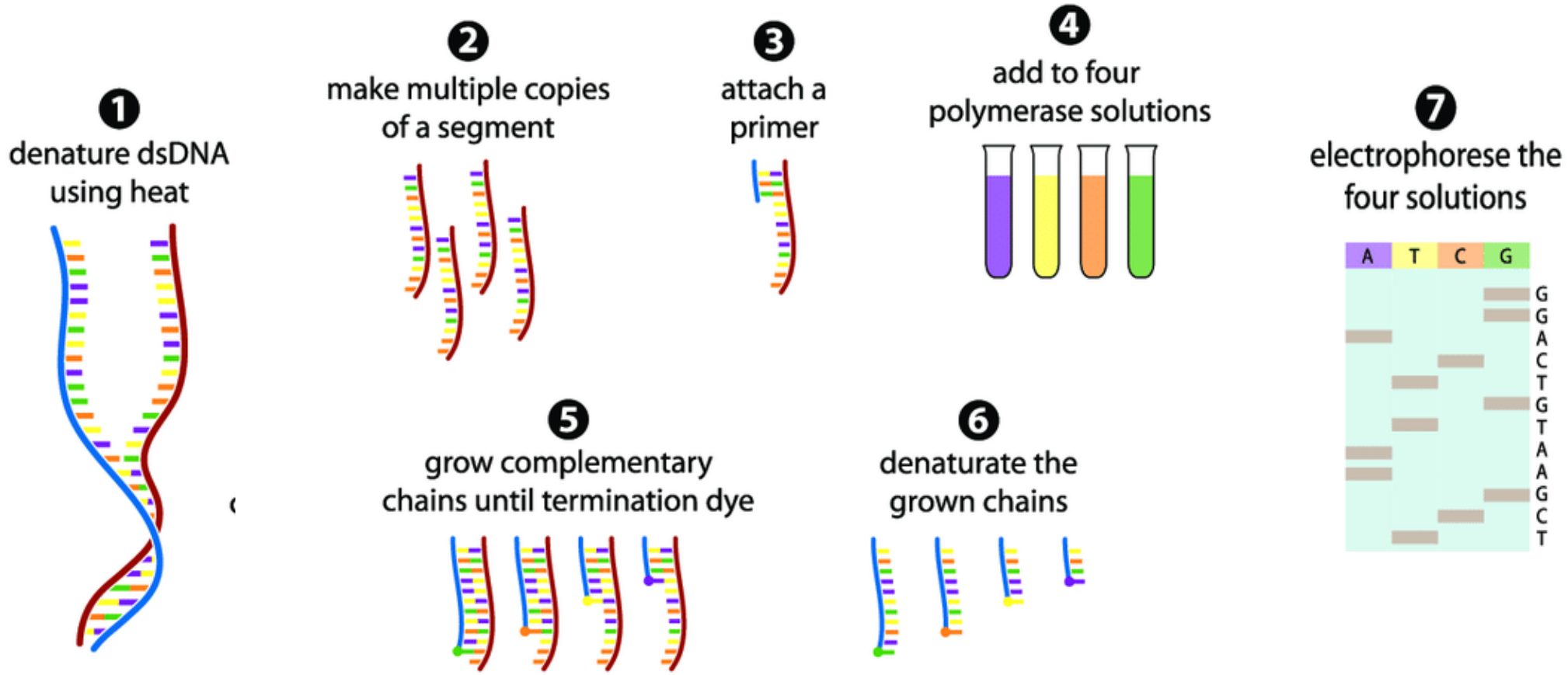
Complete the Human Genome Project

Second generation sequencer：Genetic Analyzer 2

Third generation sequencer：PacBio RS

The third stage Human microbiome project

What is Sanger sequencing?

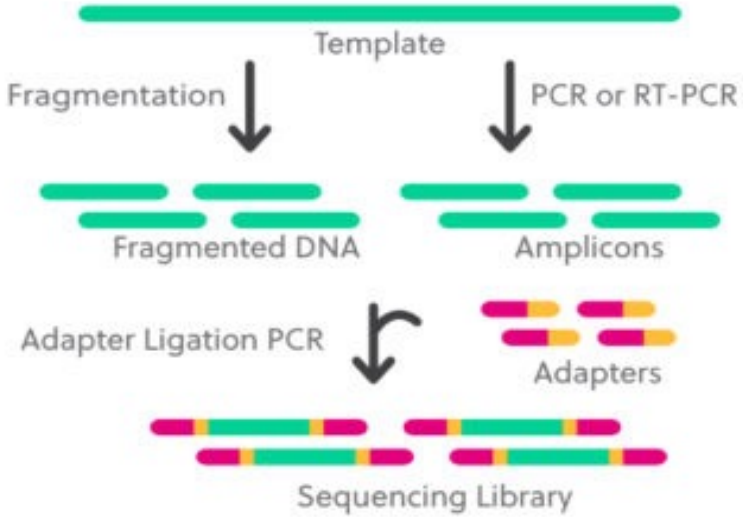


Method for determining genetic sequence through "chain termination"

What is Next Generation Sequencing?

STEP 1:
Extraction

STEP 2:
Library Prep

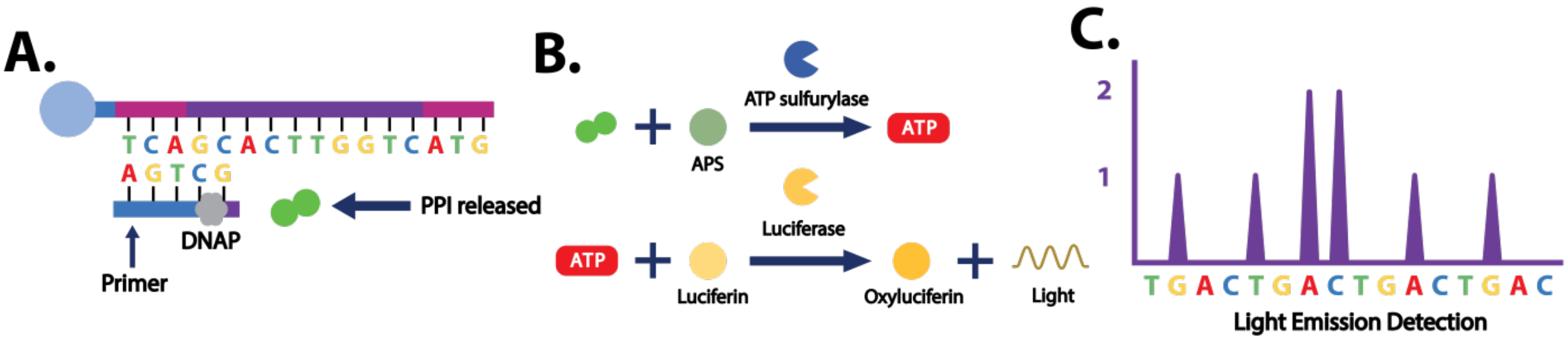


STEP 3:
Sequencing

STEP 4:
Analysis

Method that sequences millions of fragments simultaneously

How does Pyrosequencing (454) work?



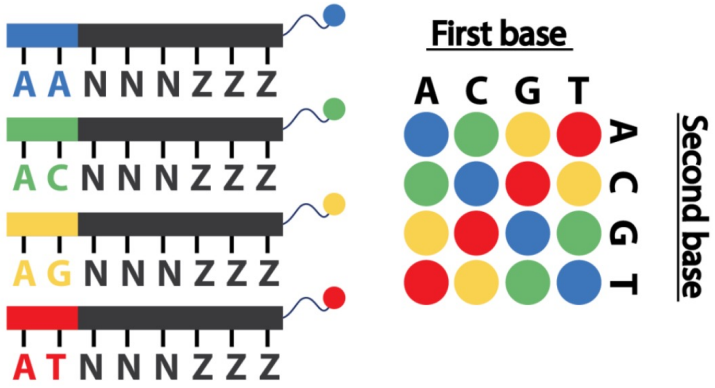
Pyrophosphate (PPI) released

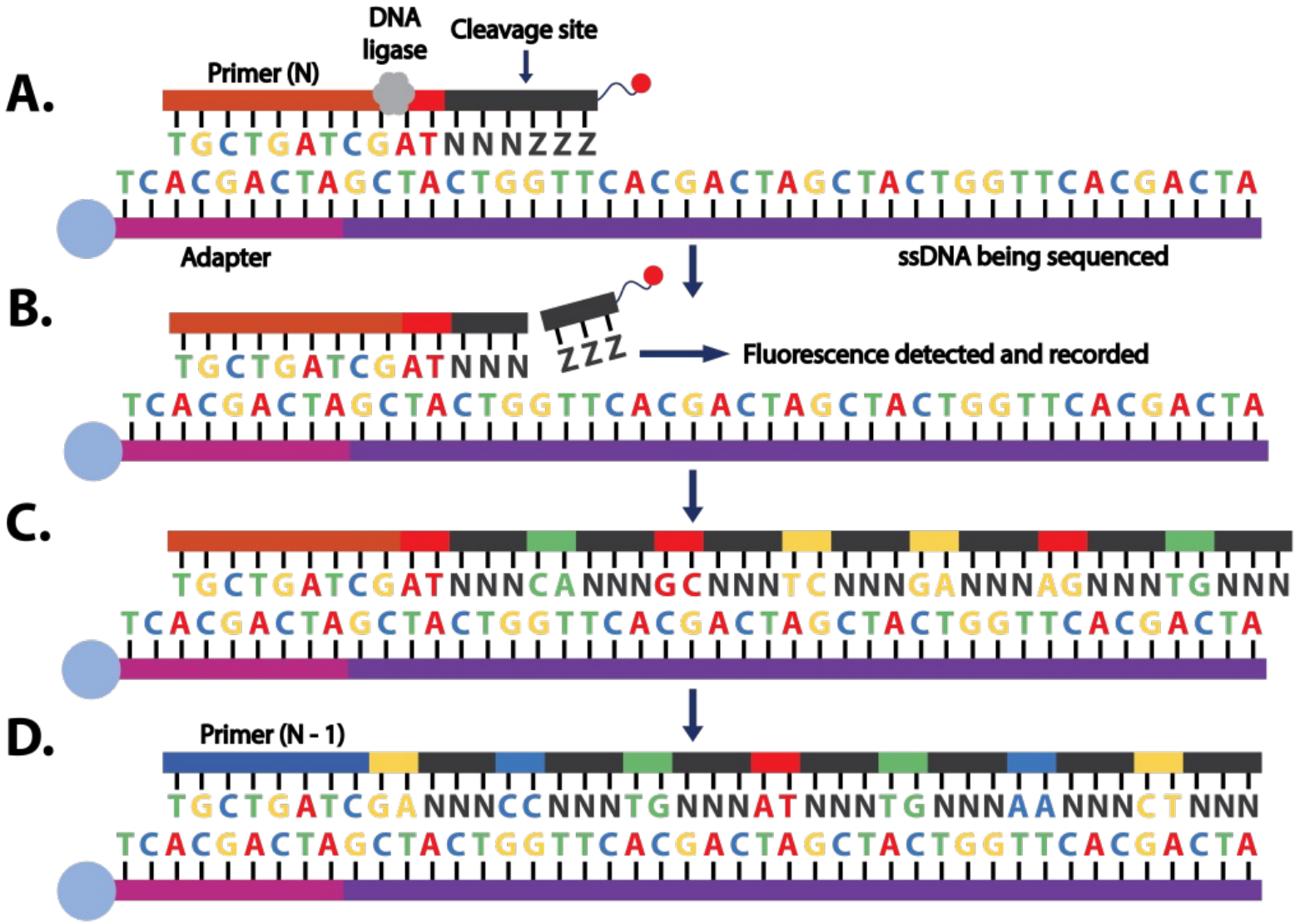
PPI and adenosine 5' phosphosulfate (APS) make ATP

ATP and luciferin make oxyluciferin and light

Light produced when the nucleotide matches the template

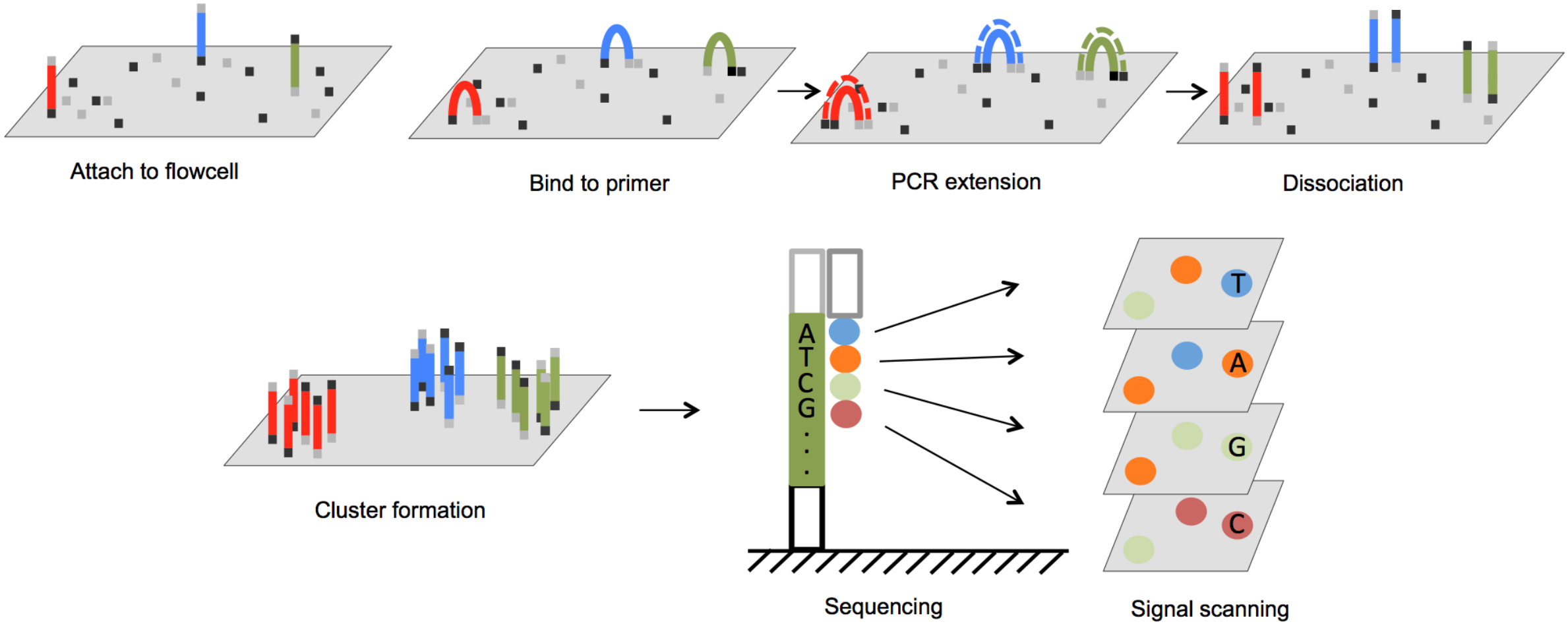
How does sequencing by ligation (SOLiD) work?





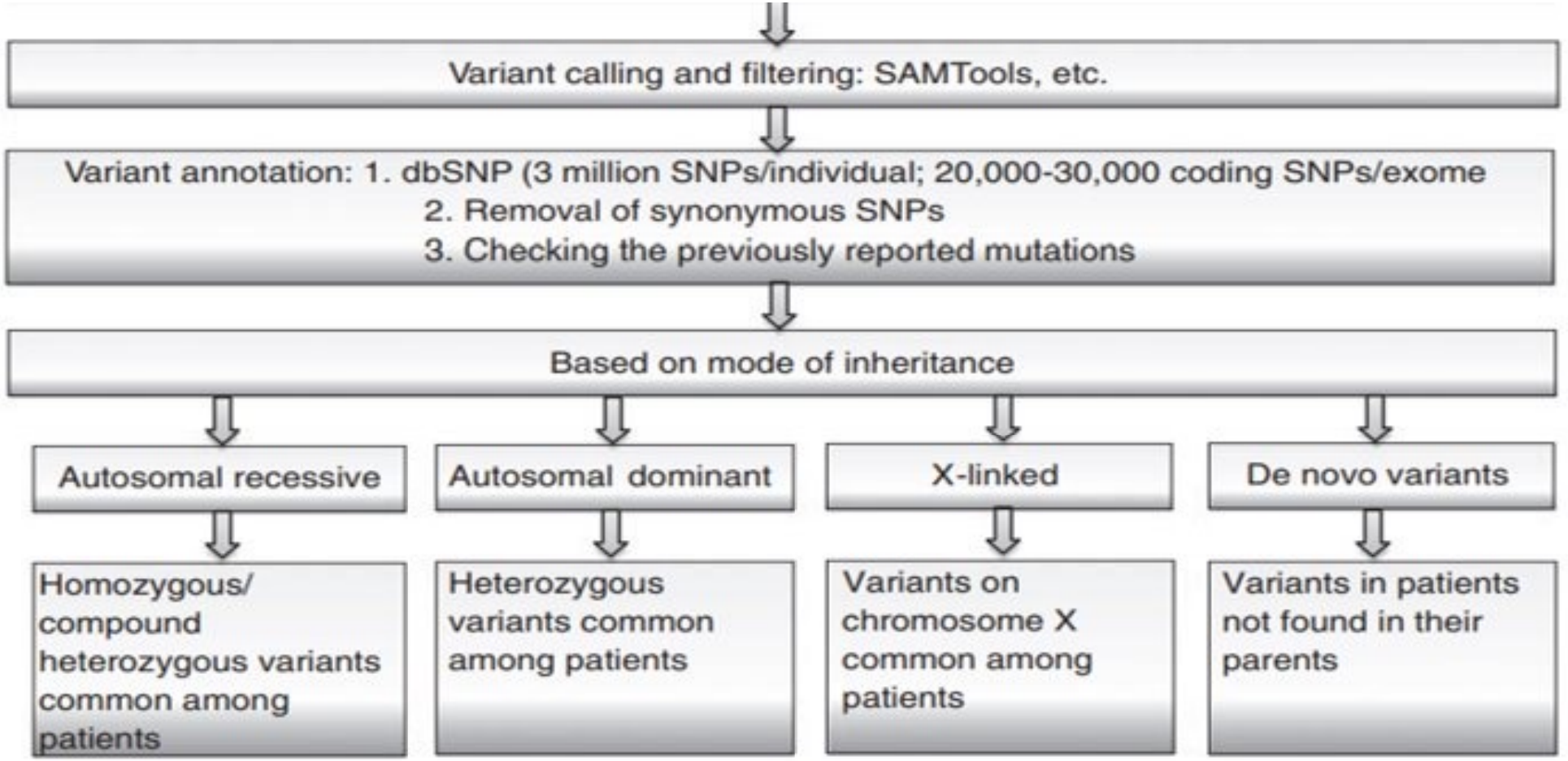
Fluorescent labeled molecules used to determine sequence

How does sequencing by synthesis (Illumina/Solexa) work?



A polymerase adds fluorescently tagged dNTPs to determine the sequence

What can we learn from sequencing?

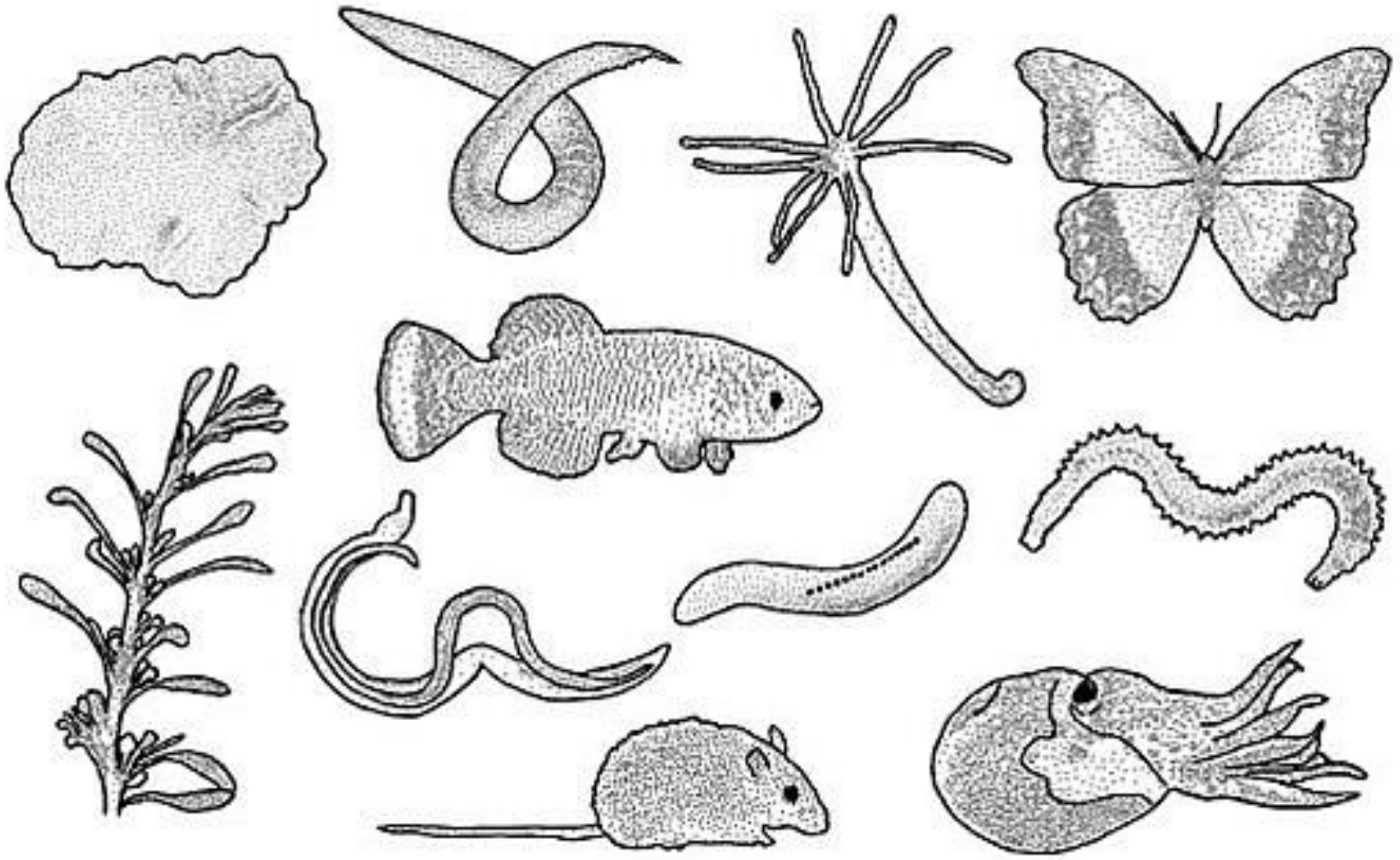


Identify genetic variants with a link to disease

Model Organisms Facilitate Rare Disease Diagnosis and Therapeutic Research

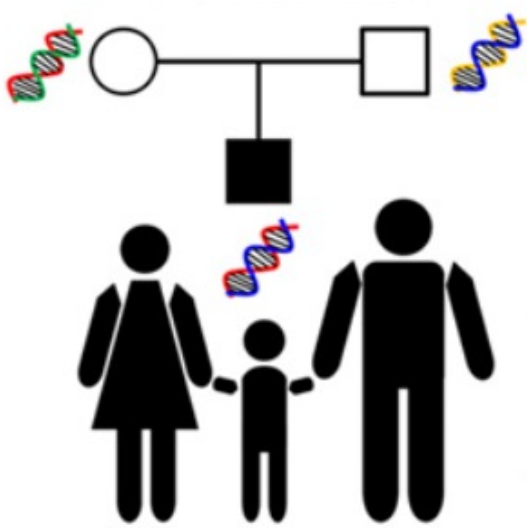
Michael F. Wangler,*,†,‡,§,1 Shinya Yamamoto,*,‡,§,**,1 Hsiao-Tuan Chao,†,‡,†† Jennifer E. Posey,* Monte Westerfield,‡‡ John Postlethwait,‡‡ Members of the Undiagnosed Diseases Network (UDN),[2] Philip Hieter,§§ Kym M. Boycott,*** Philippe M. Campeau,††† and Hugo J. Bellen*,‡,§,**,‡‡‡,3

What are model organisms?



Important part in determining the molecular mechanisms of disease

How do model organisms facilitate disease diagnosis?

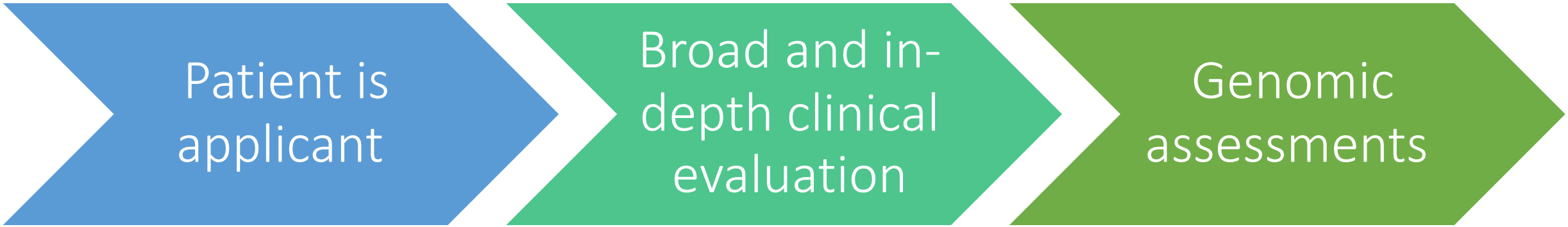


- Deep clinical phenotyping
- NGS
- Matching cases

- Phenotyping of mutant fish
- Similarities with humans
- Screens for potential drug targets

- Gene function conservation
- Functional effects of variants
- Uncover molecular mechanisms

What is the Undiagnosed Disease Program model?

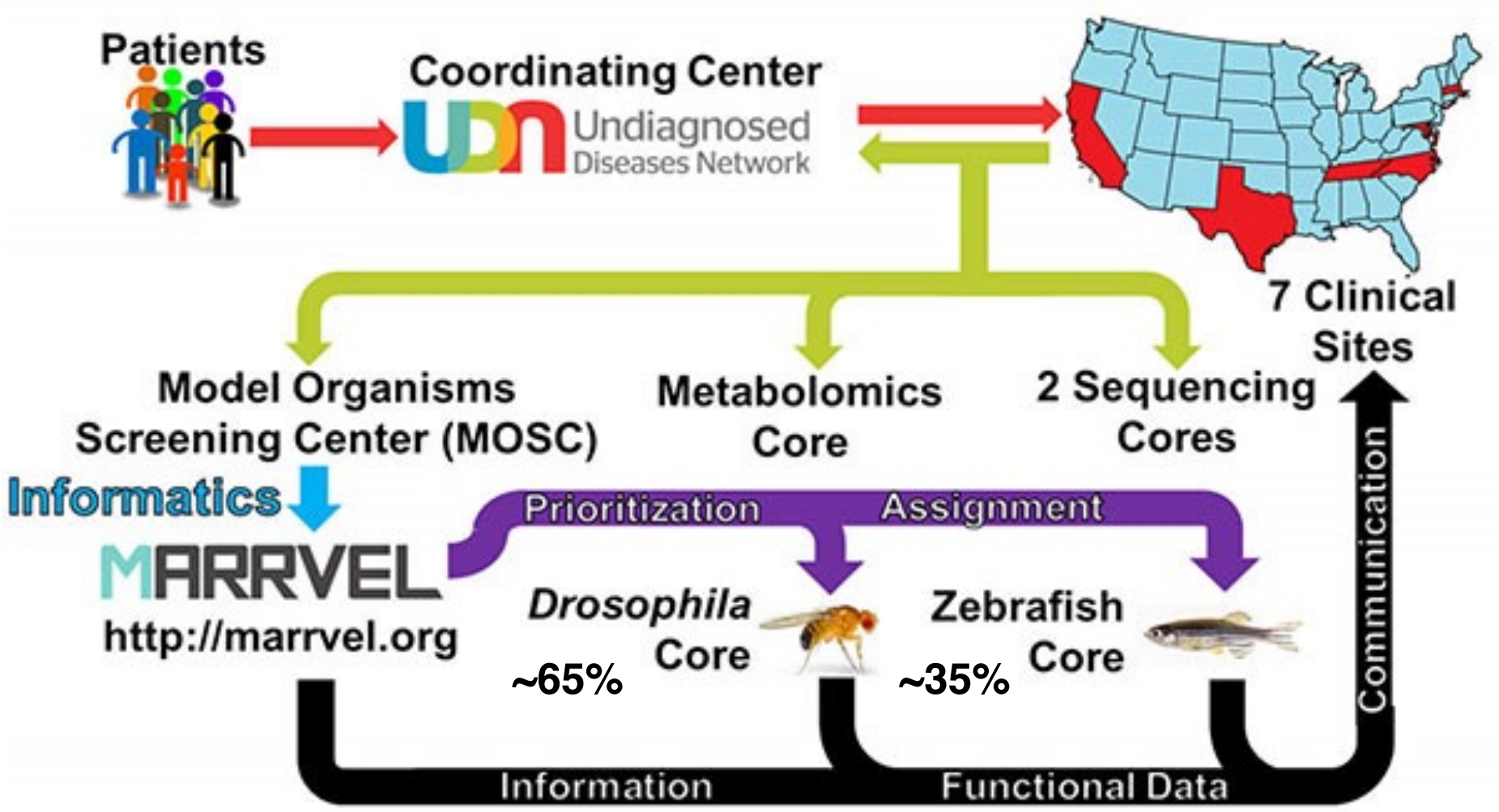


Providing diagnoses for patients that have exhausted other diagnostic measures

What is the Undiagnosed Disease Network?

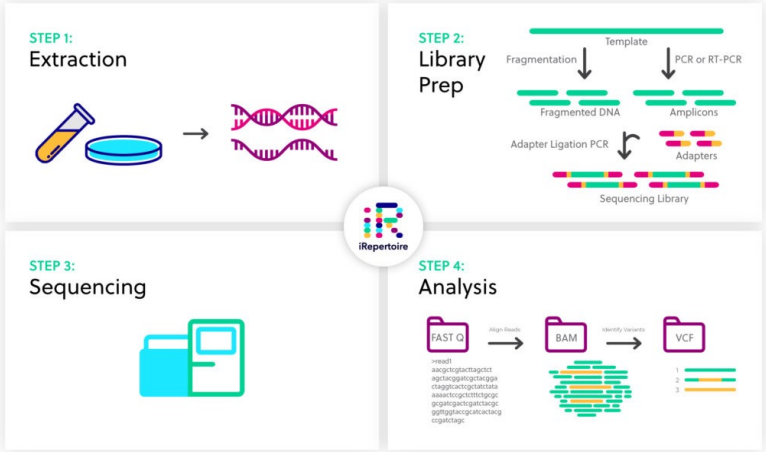
7 Clinical Sites
2 Sequencing Cores
Model Organism Screening Center
Metabolomics Core
Coordination Center

How does the UDN use model organisms to facilitate disease diagnosis?

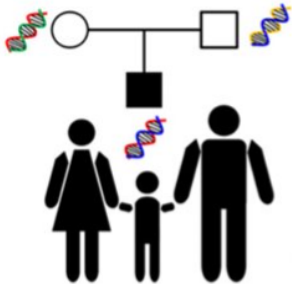


Model organisms are used to study relationship between gene, function, and variant

Summary



Next generation sequencing is an important tool in characterizing disease



Model organisms play an important role in elucidating molecular mechanisms

The UDN uses both NGS and model organisms to help diagnose and treat undiagnosed diseases

Article

In vivo base editing rescues Hutchinson–Gilford progeria syndrome in mice

https://doi.org/10.1038/s41586-020-03086-7

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Check for updates

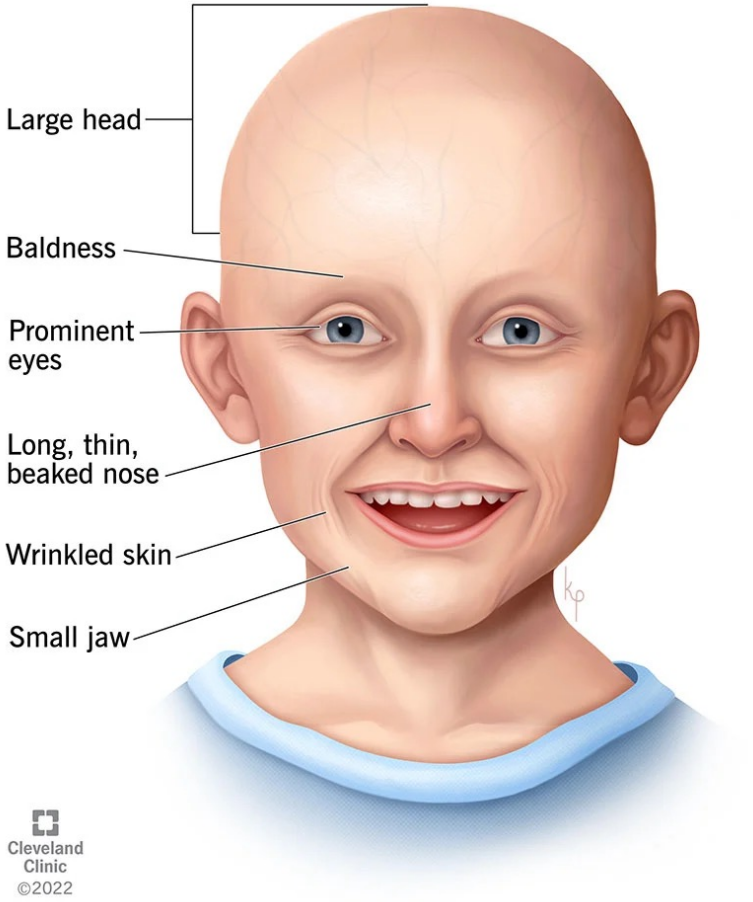
Luke W. Koblan[1,2,3,13], Michael R. Erdos[4,13], Christopher Wilson[1,2,3], Wayne A. Cabral[4], Jonathan M. Levy[1,2,3], Zheng-Mei Xiong[4], Urraca L. Tavarez[4], Lindsay M. Davison[5], Yantenew G. Gete[6], Xiaojing Mao[6], Gregory A. Newby[1,2,3], Sean P. Doherty[5], Narisu Narisu[4], Quanhu Sheng[7], Chad Krilow[4], Charles Y. Lin[8,9,12], Leslie B. Gordon[10,11], Kan Cao[6], Francis S. Collins[4]✉, Jonathan D. Brown[5]✉ & David R. Liu[1,2,3]✉

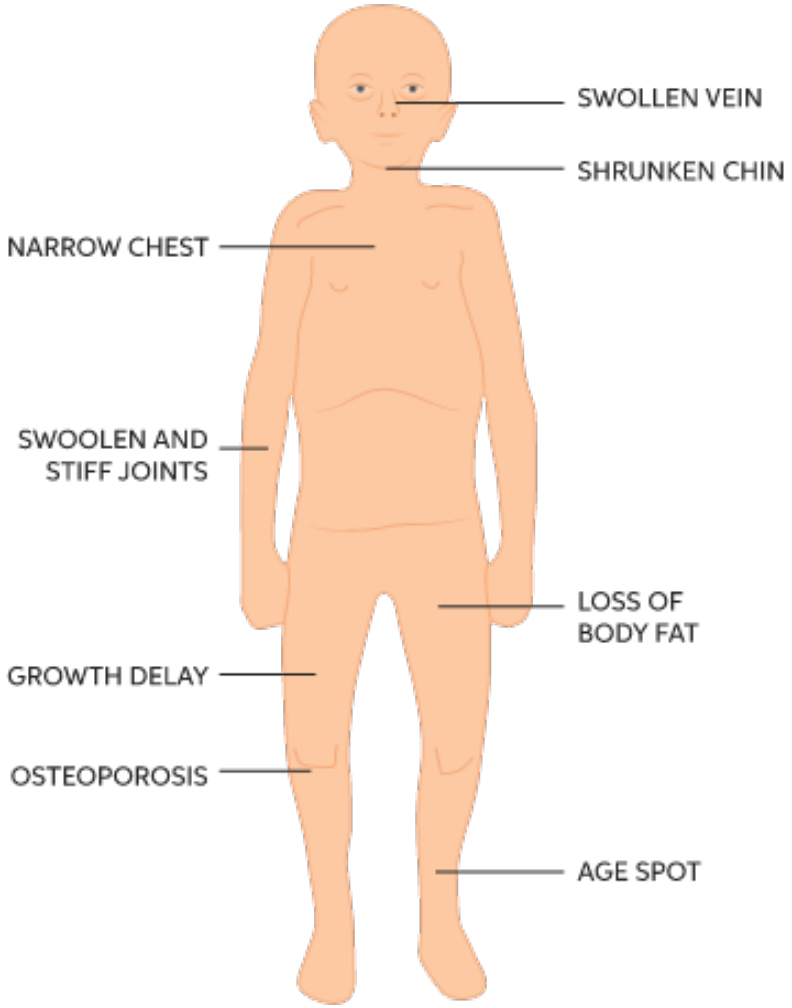
What is progeria?

Hutchinson-Gilford progeria syndrome (HGPS)

Progressive genetic disorder caused by random mutation

How does progeria affect you?



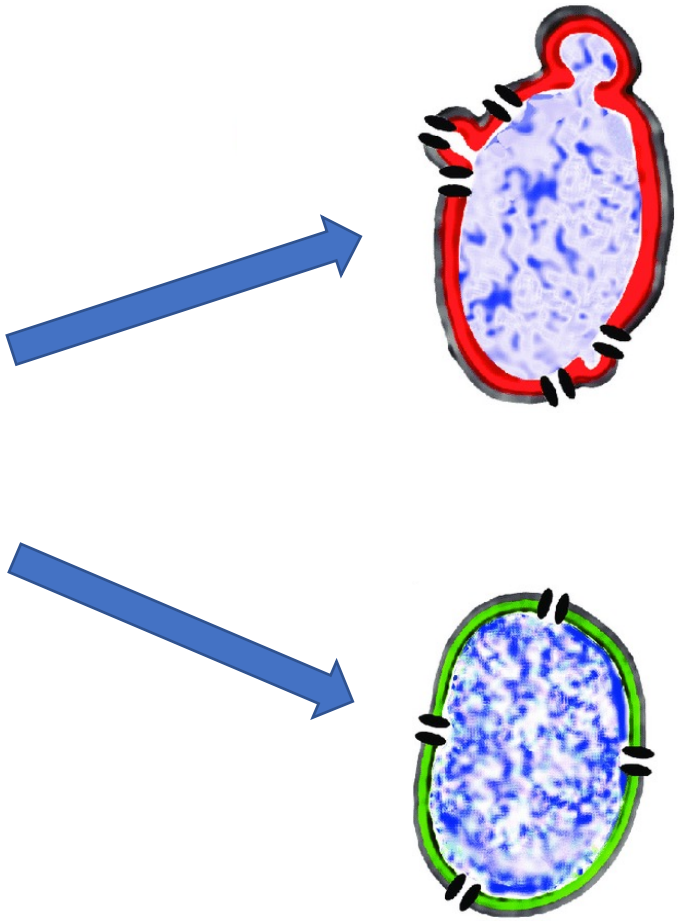


Characterized by accelerated aging

What causes progeria?

Progeria: ....GGAGCCCAGGGTGGG**T**

Normal: ....GGAGCCCAGGGTGGG**C**



Lamina thickening
Nuclear pore complex clusters
Loss of heterochromatin

C>T mutation causes 50bp mis-splicing that loses 50 amino acids in Lamin A protein

What labs are studying progeria?

David Liu, PhD

LIU GROUP

Michael Erdos, PhD

National Human Genome Research Institute

A to G base editors (ABE)
C to T base editors (CBE)

Identifying and validating potential therapeutics

References

Koblan LW, et al. In vivo base editing rescues Hutchinson-Gilford progeria syndrome in mice. Nature. 2021 Jan 6. doi: 10.1038/s41586-020-03086-7.

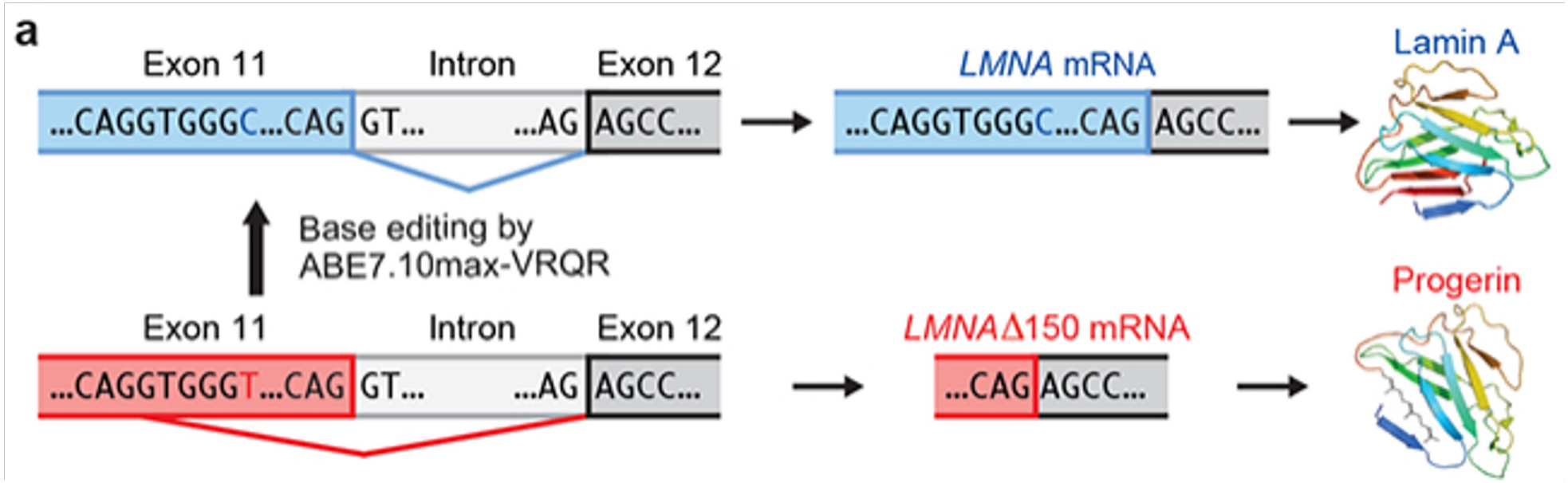
Next-generation sequencing: impact of exome sequencing in characterizing Mendelian disorders. Rabbani B, et al, J Hum Genet. 2012 Oct;57(10):621-32. doi: 10.1038/jhg.2012.91. Epub 2012 Jul 26. Review. PMID: 22832387 •

Model Organisms Facilitate Rare Disease Diagnosis and Therapeutic Research. Wangler MF, Y et al., Genetics. 2017 Sep;207(1):9-27. doi: 10.1534/genetics.117.203067. Review.

In Vivo Base Editing Rescues Hutchinson-Gilford Progeria Syndrome in Mice

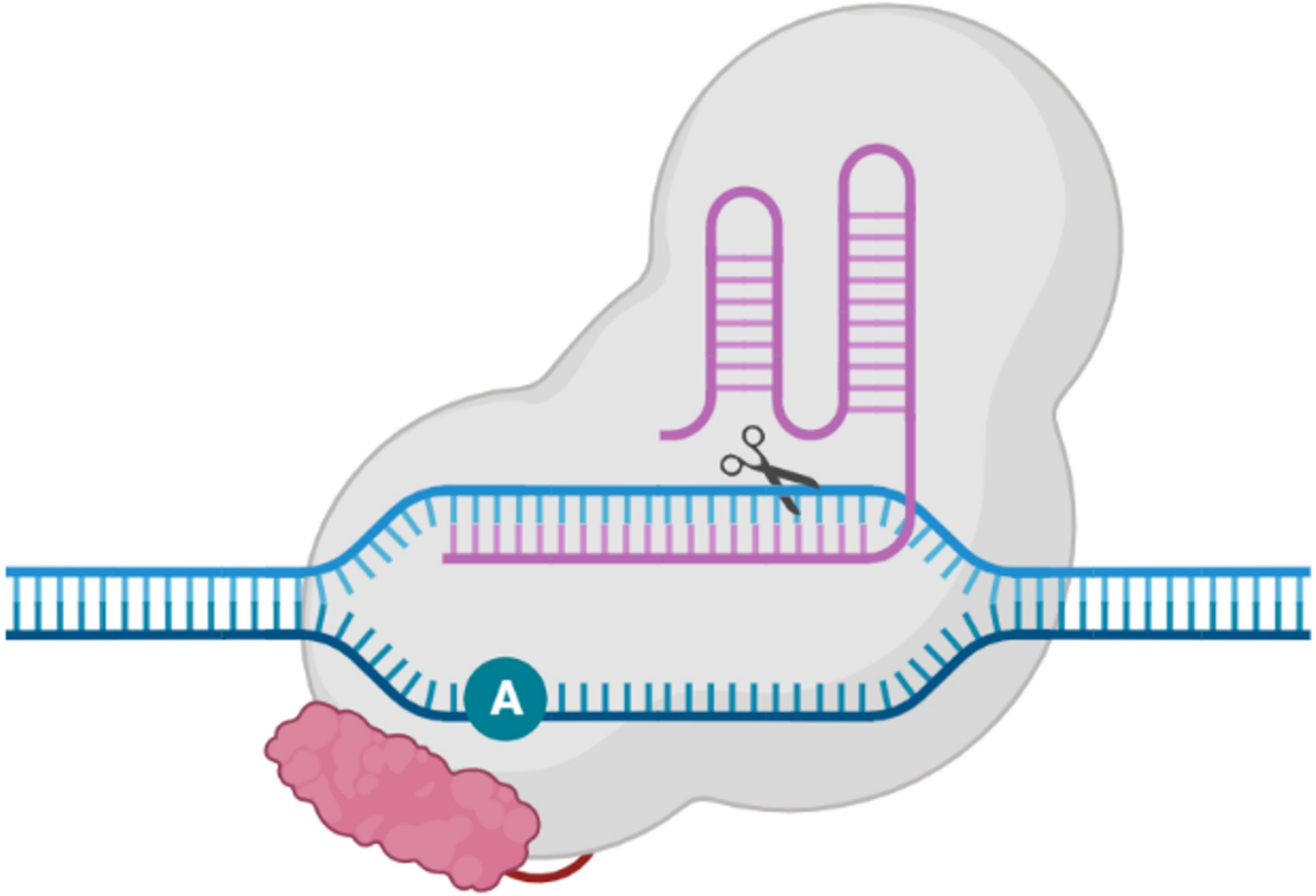
Koblan et al., 2021

The mis-splicing of the LMNA ultimately causes progeria



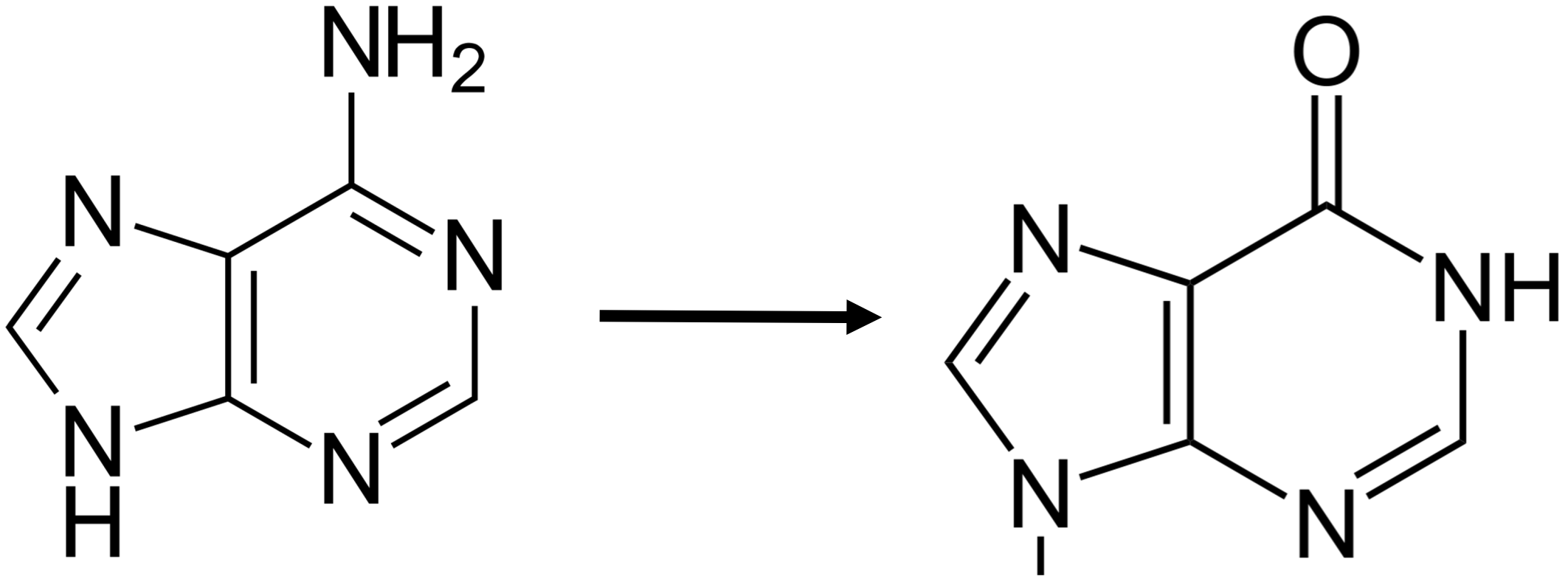
Targeted single C->T point mutation that causes mis-splicing

How was Lamin A targeted for base editing?



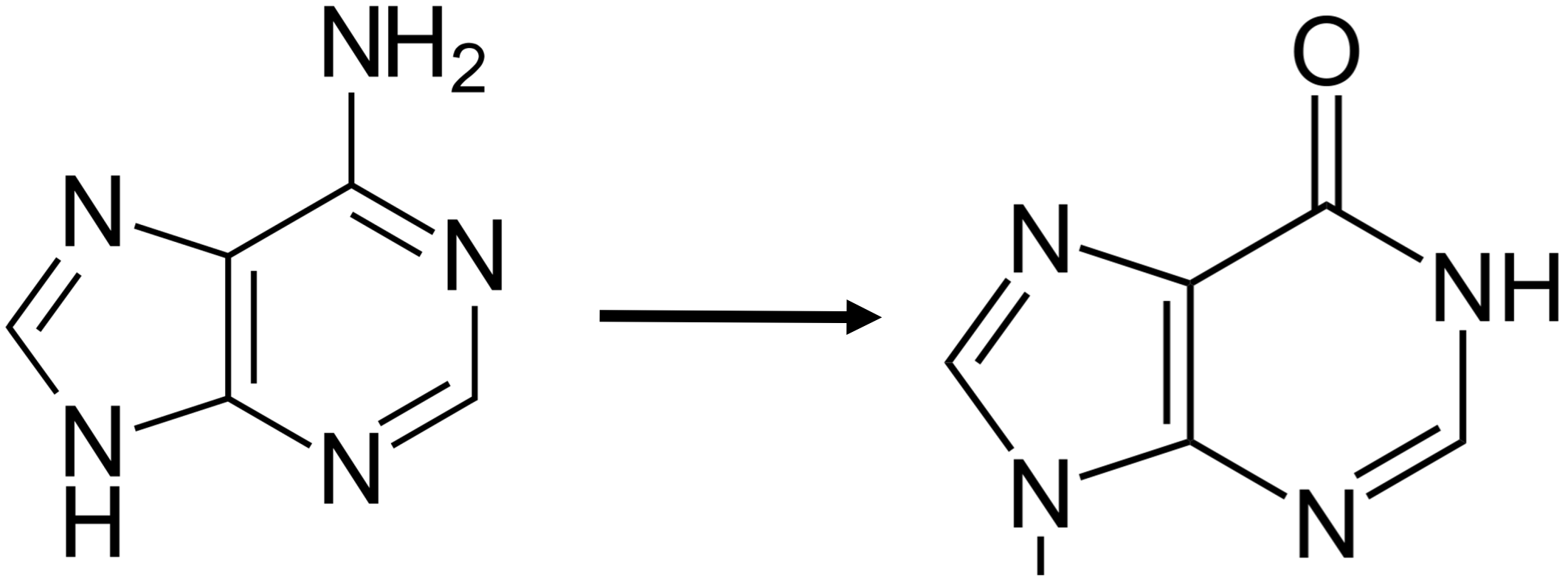
Adenosine Base Editors (ABEs) convert A-T base pairs to G-C base pairs

How do ABEs convert A-T base pairs to G-C?



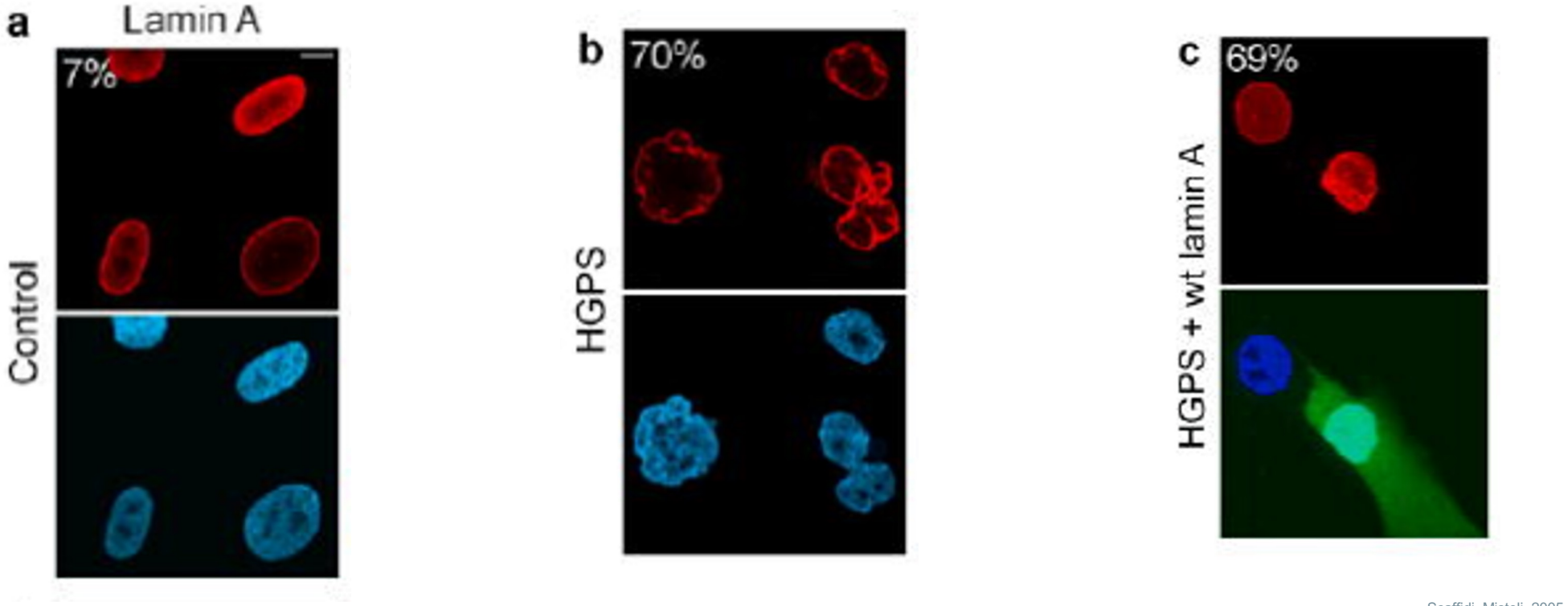
Adenine is converted to Inosine (pairs like Guanine)

How do ABEs convert A-T base pairs to G-C?



Adenine is converted to Inosine (pairs like Guanine)

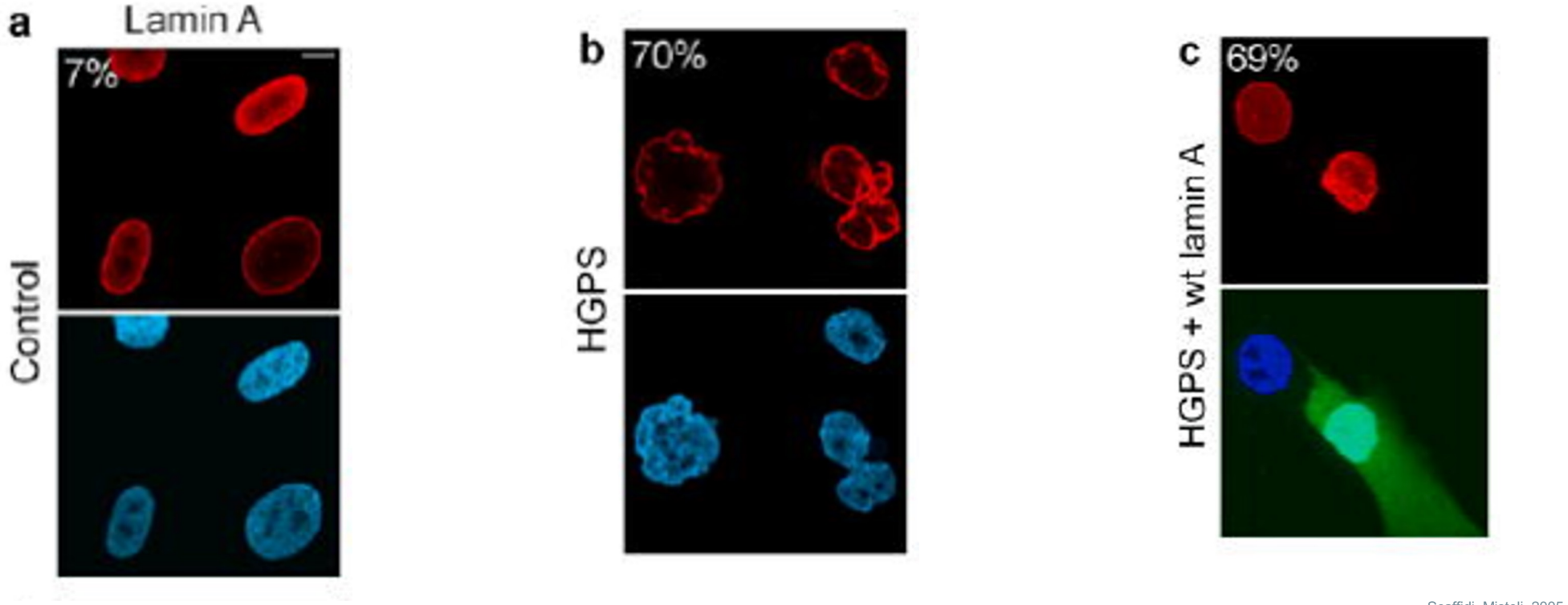
Why would ABEs be effective?



Scaffidi, Misteli, 2005

Overexpression of wild-type does not rescue cell

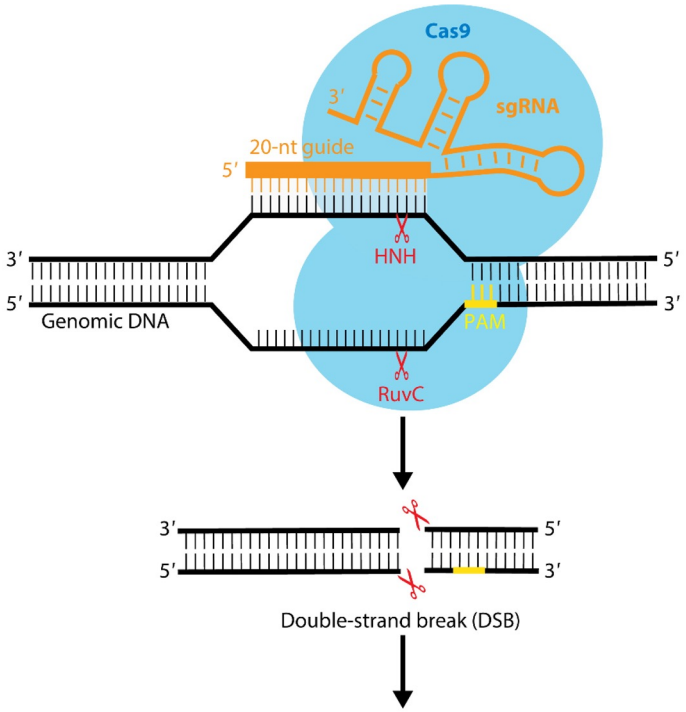
Why ABEs vs. wild-type rescue?

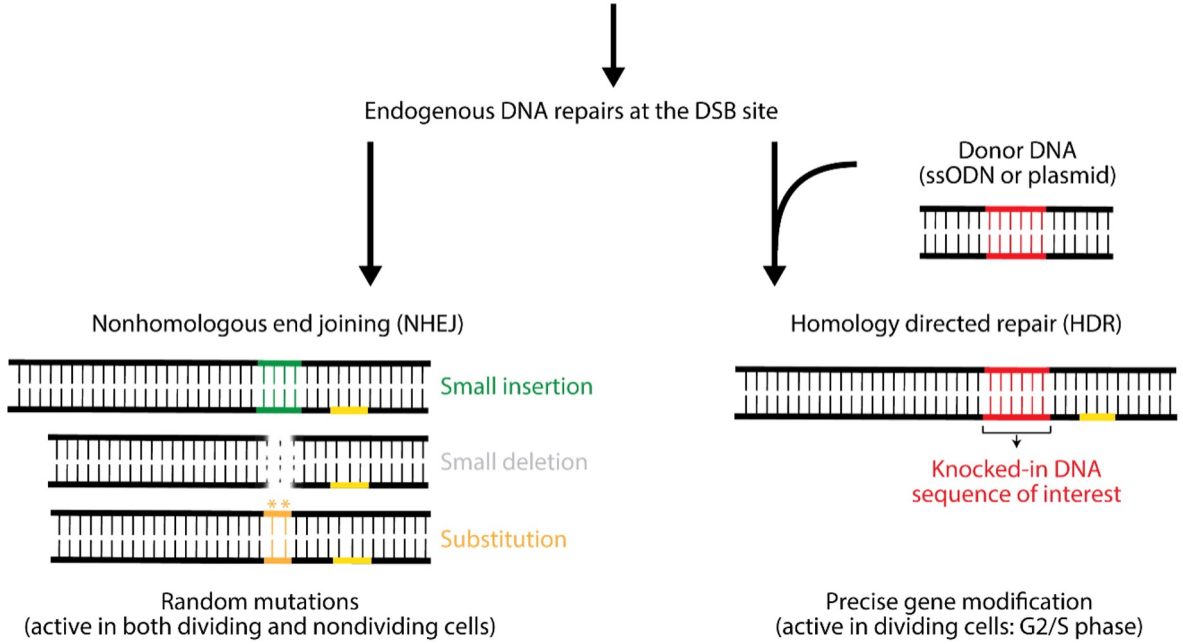


Scaffidi, Misteli, 2005

Overexpression of wild-type does not rescue cell

Why ABEs vs. CRISPR?

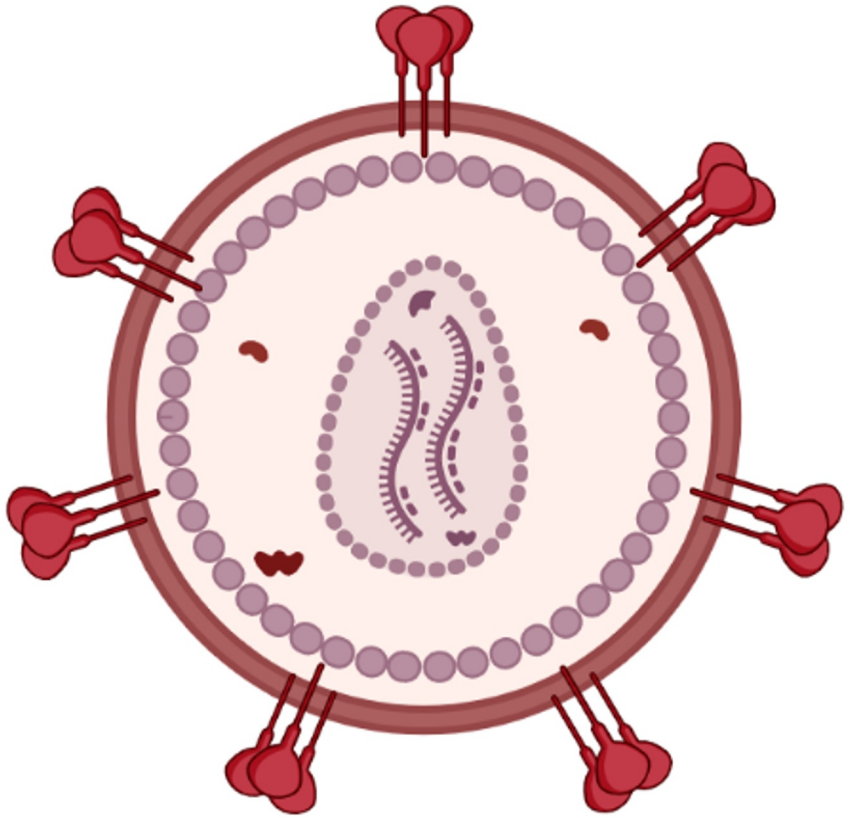




Jiang, Doudna, 2017

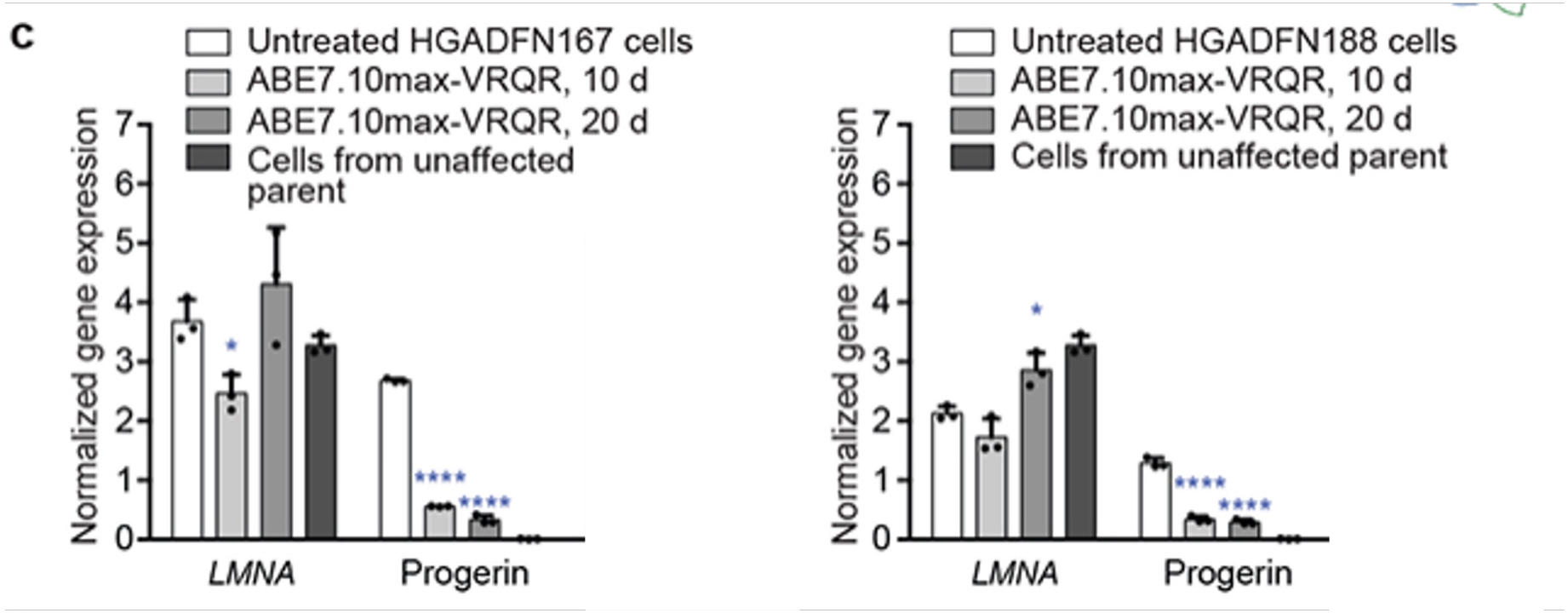
CRISPR too random (indels), too non-specific

How were the ABEs tested in vitro?



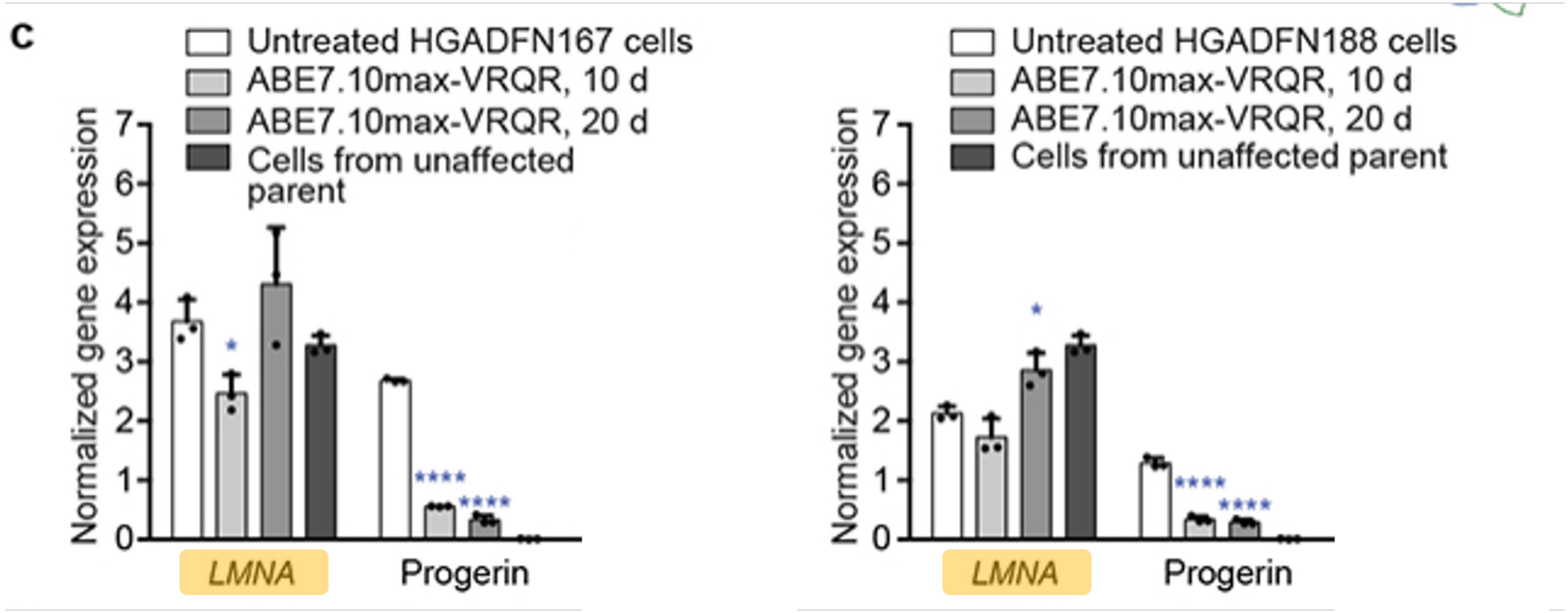
Two developed fibroblast lines treated with ABEs using Lentivirus delivery

How effective were the ABEs in vitro in reducing mis-splicing?



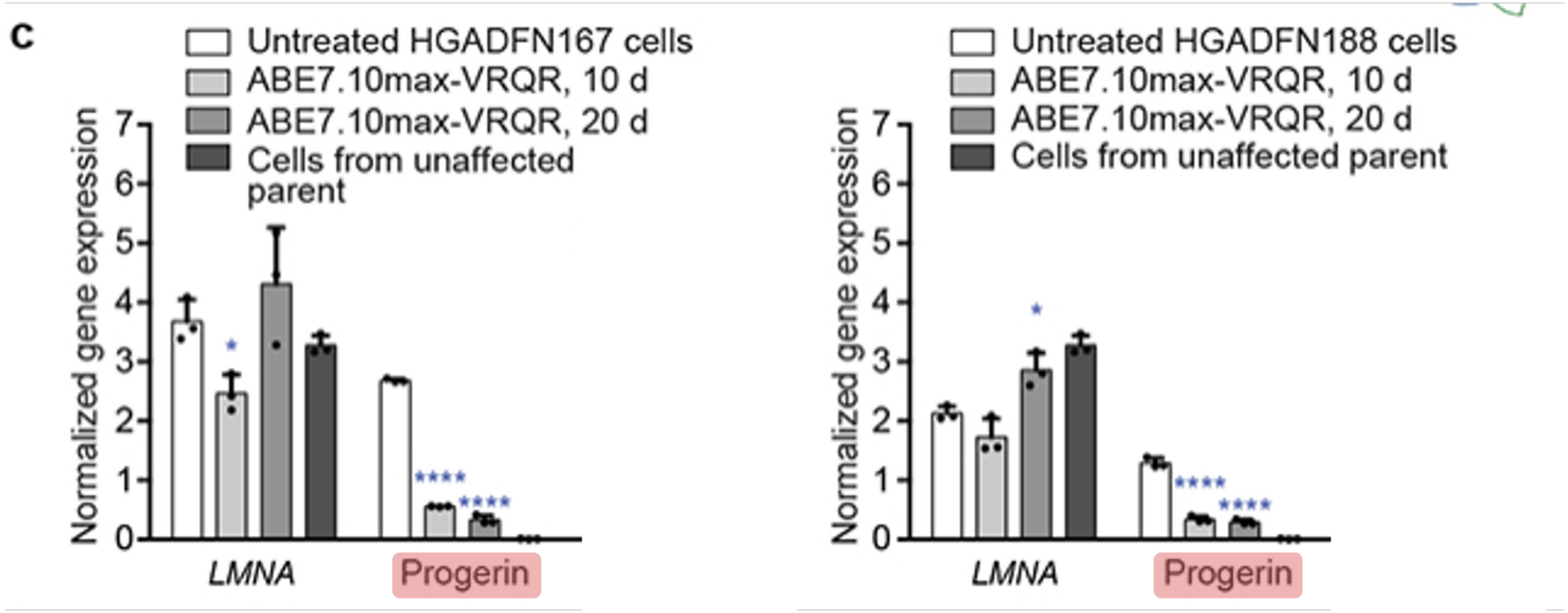
8.1/4.4x reduction in mis-spliced LMNA transcripts

How effective were the ABEs in vitro in reducing mis-splicing?



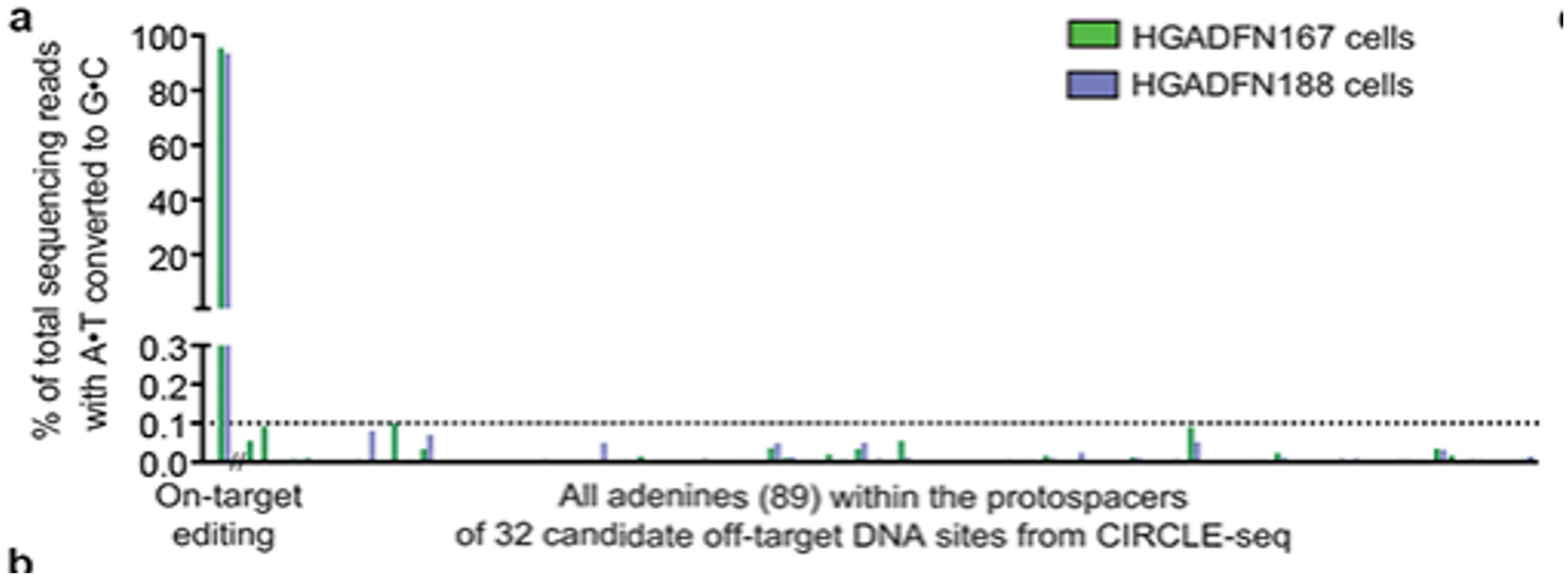
8.1/4.4x reduction in mis-spliced LMNA transcripts

How effective were the ABEs in vitro in reducing progerin levels?



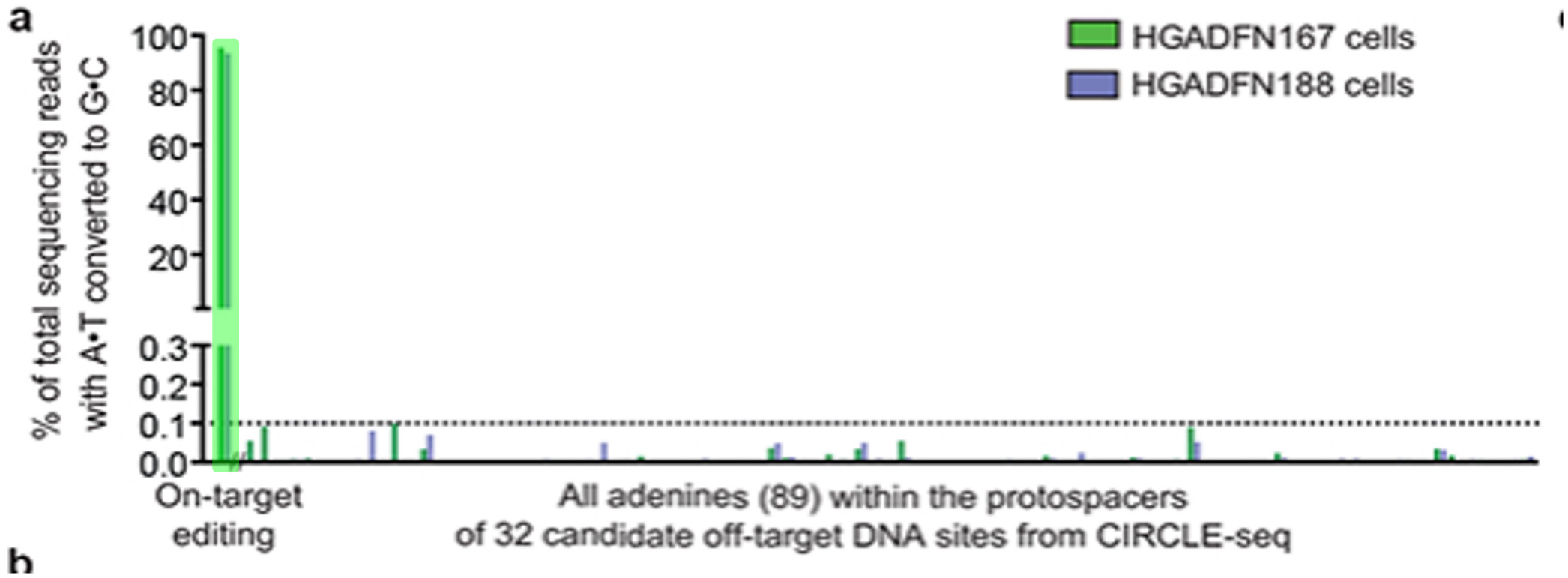
6.1/15x reduction in progerin levels

How accurate were the ABEs in vitro?



87-91% on target editing, >0.1% off target editing

How accurate were the ABEs in vitro?



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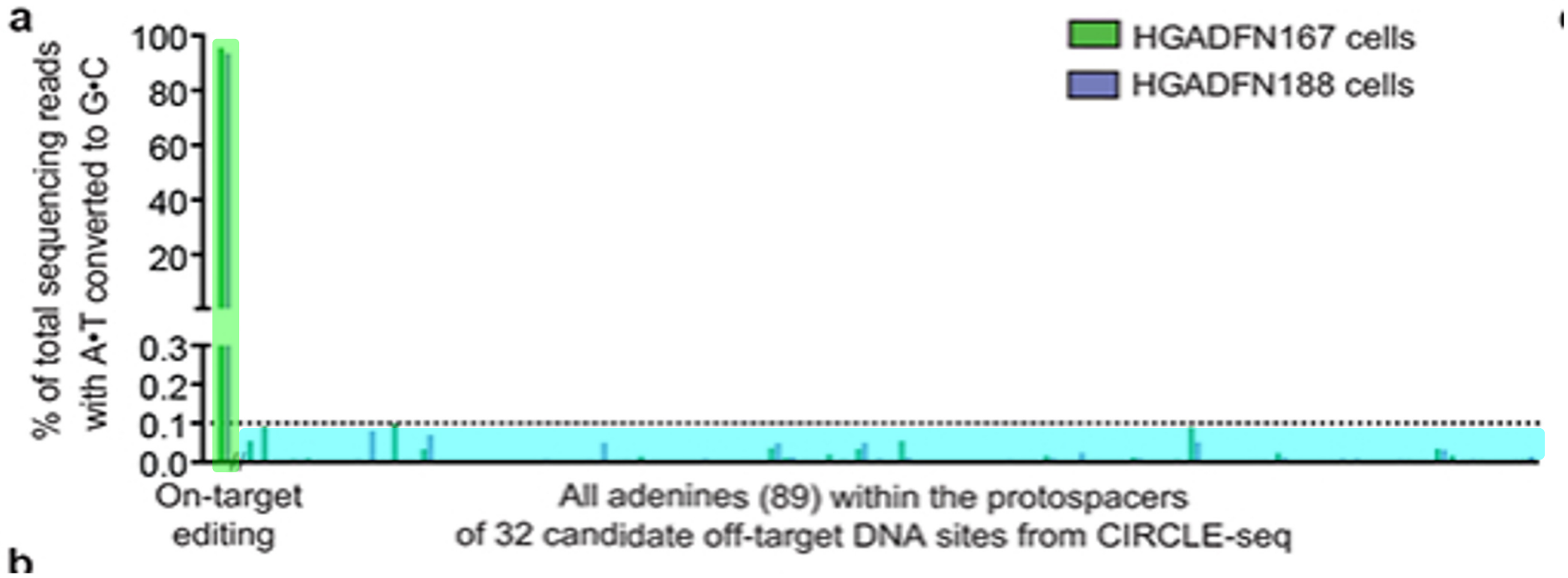
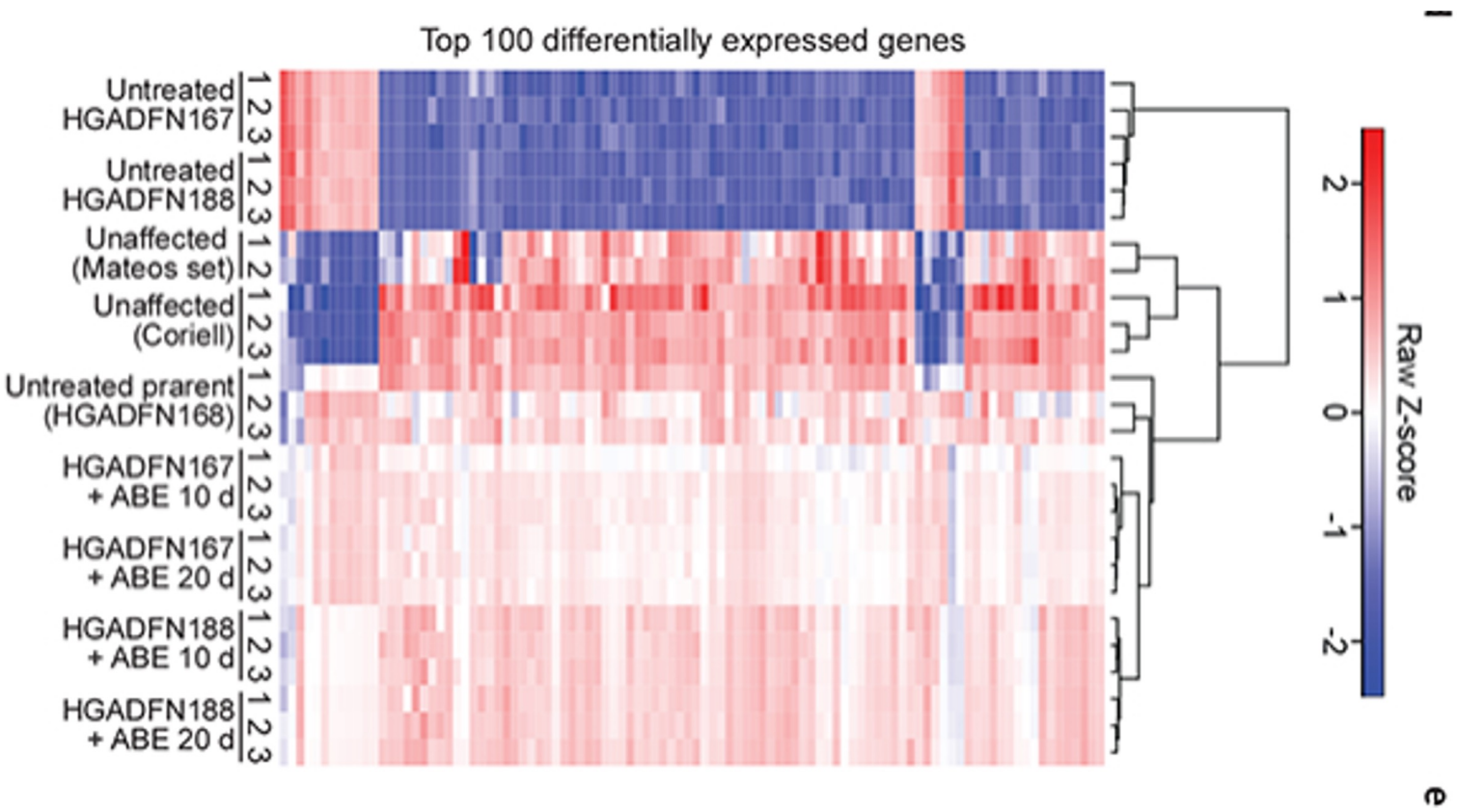


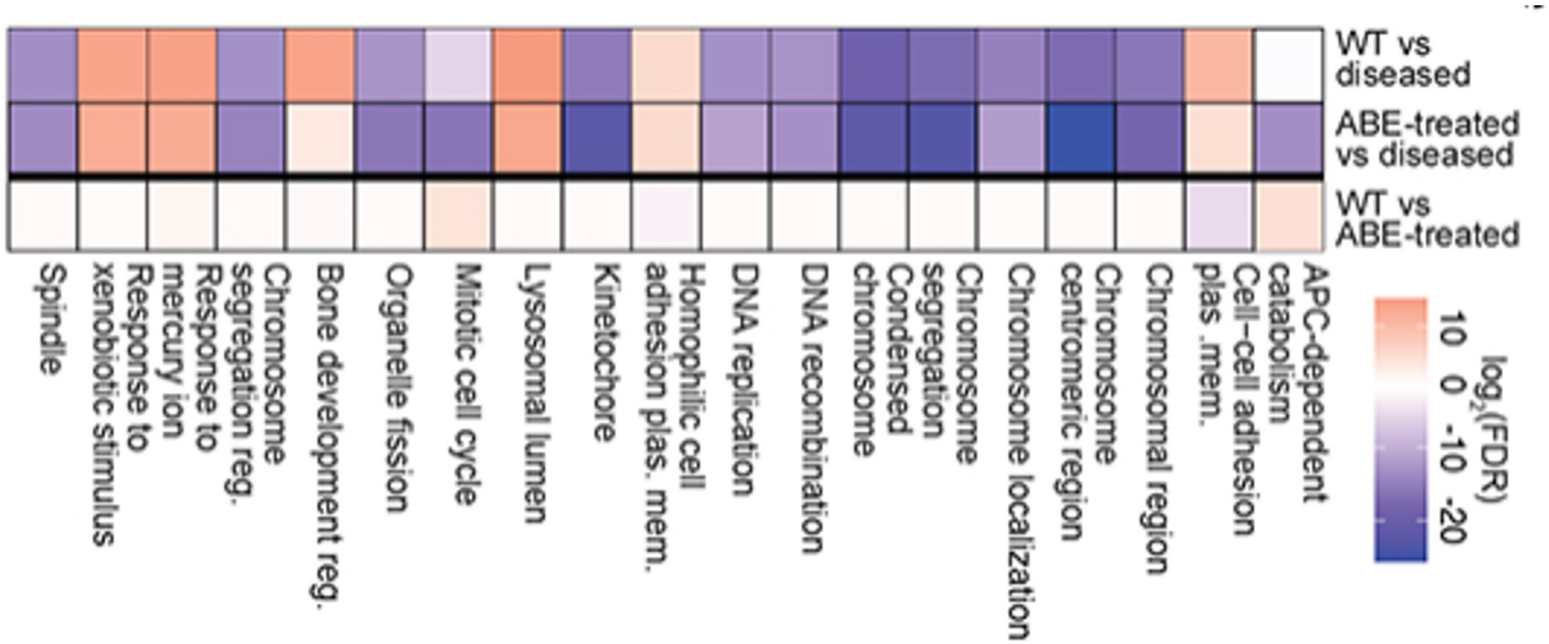
87-91% on target editing, >0.1% off target editing

How did the edited genomes resemble wild type cells?



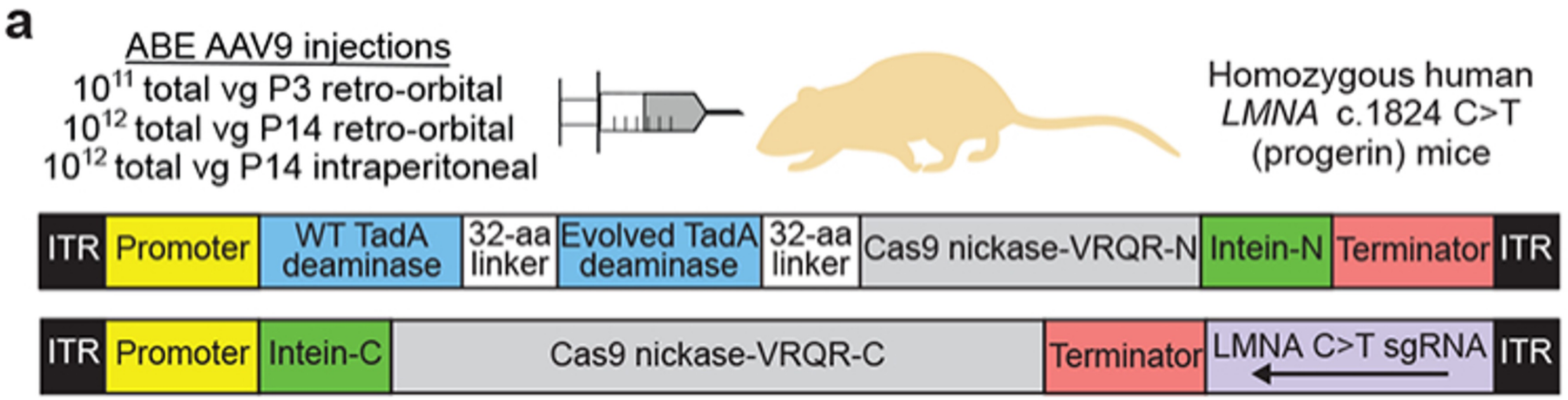
Treated cells scored more positive Z-scores

How did the edited genomes resemble wild type cells?



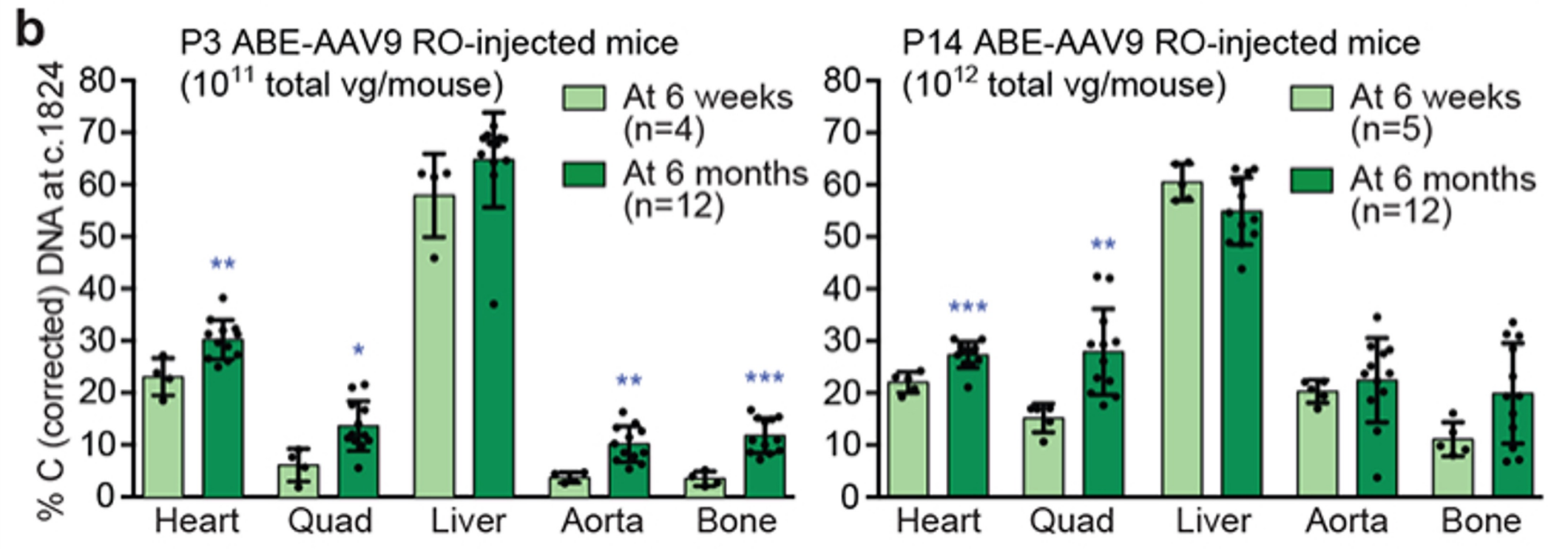
Treated cells had $\log_2$ scores closer to zero

How were the ABEs tested in vivo?



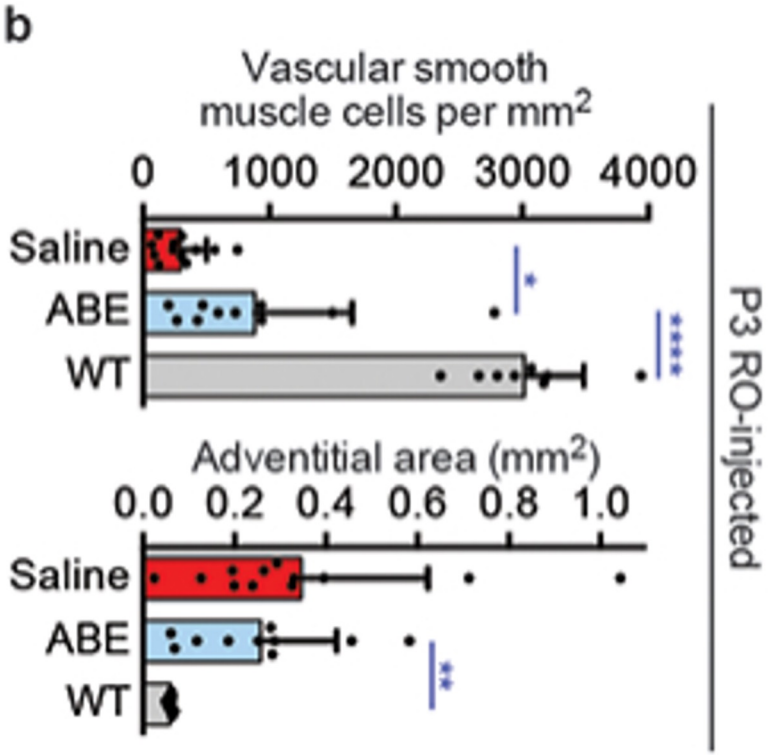
Transgenic progeria mice treated with ABEs via Adeno-associated Virus (AAV)

How effective were the ABEs in vivo over time?



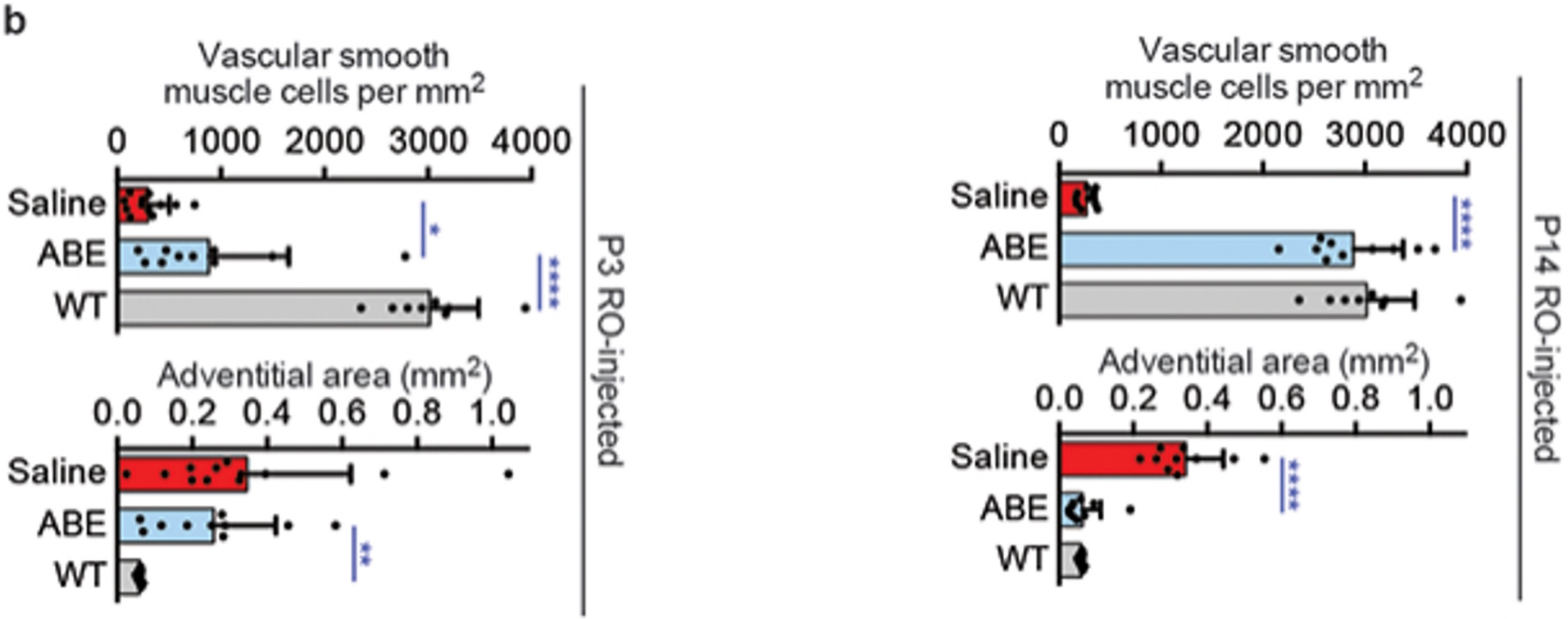
Increased DNA-editing efficiency over time

How effective were the ABEs in vivo in reducing key progeria symptoms?



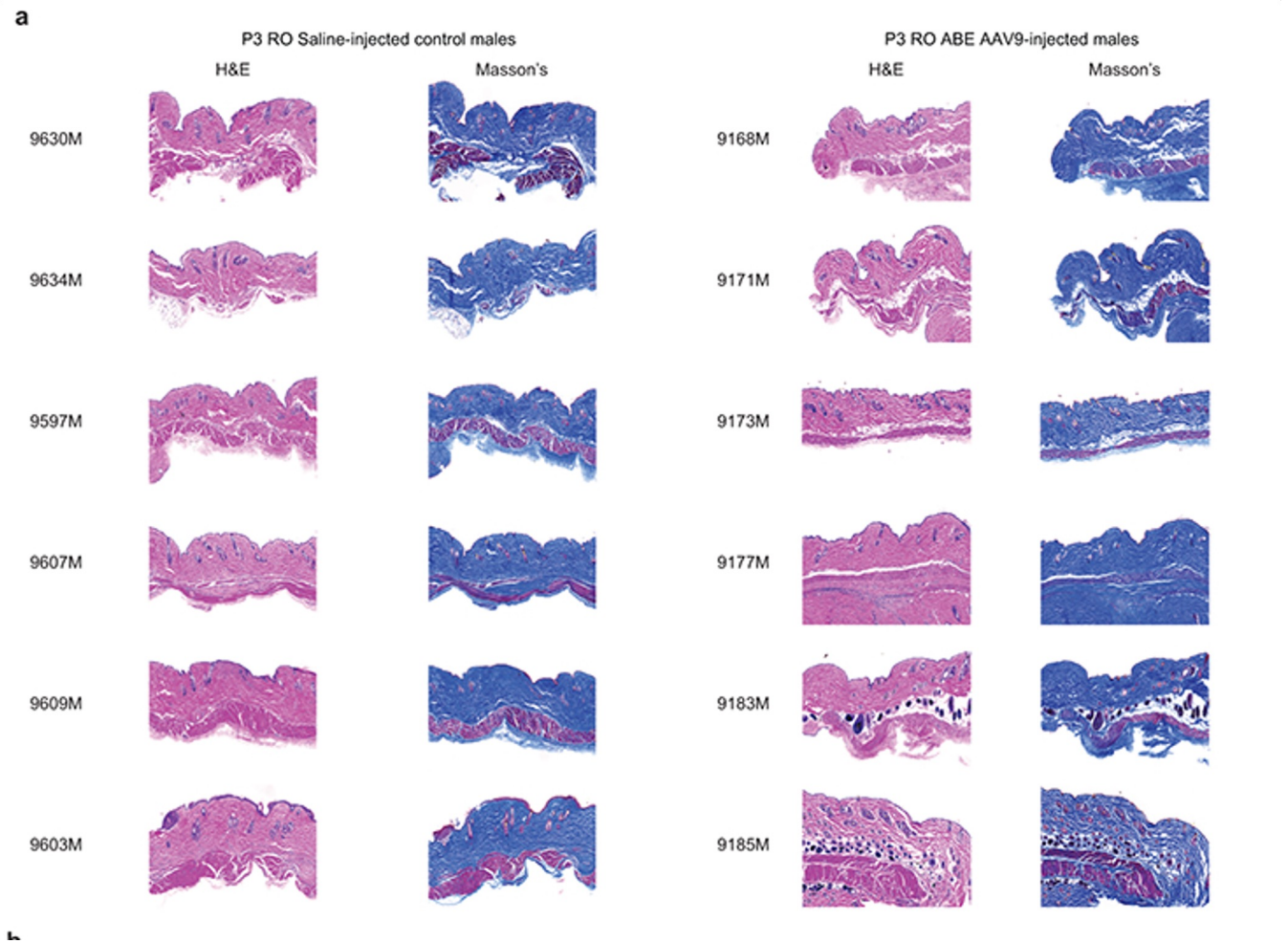
Rescued vascular smooth muscle cell (VSMC) loss and aortic periadventitial fibrosis

How effective were the ABEs in vivo in reducing key progeria symptoms?



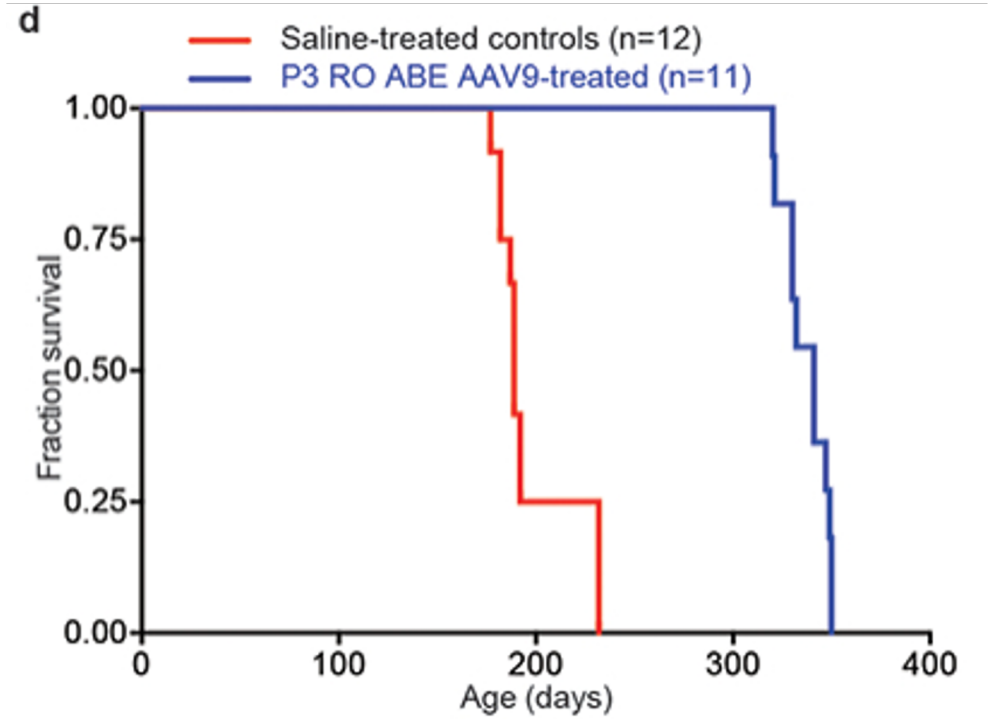
Rescued vascular smooth muscle cell (VSMC) loss and aortic periadventitial fibrosis

How effective were the ABEs in vivo at reducing fat loss?



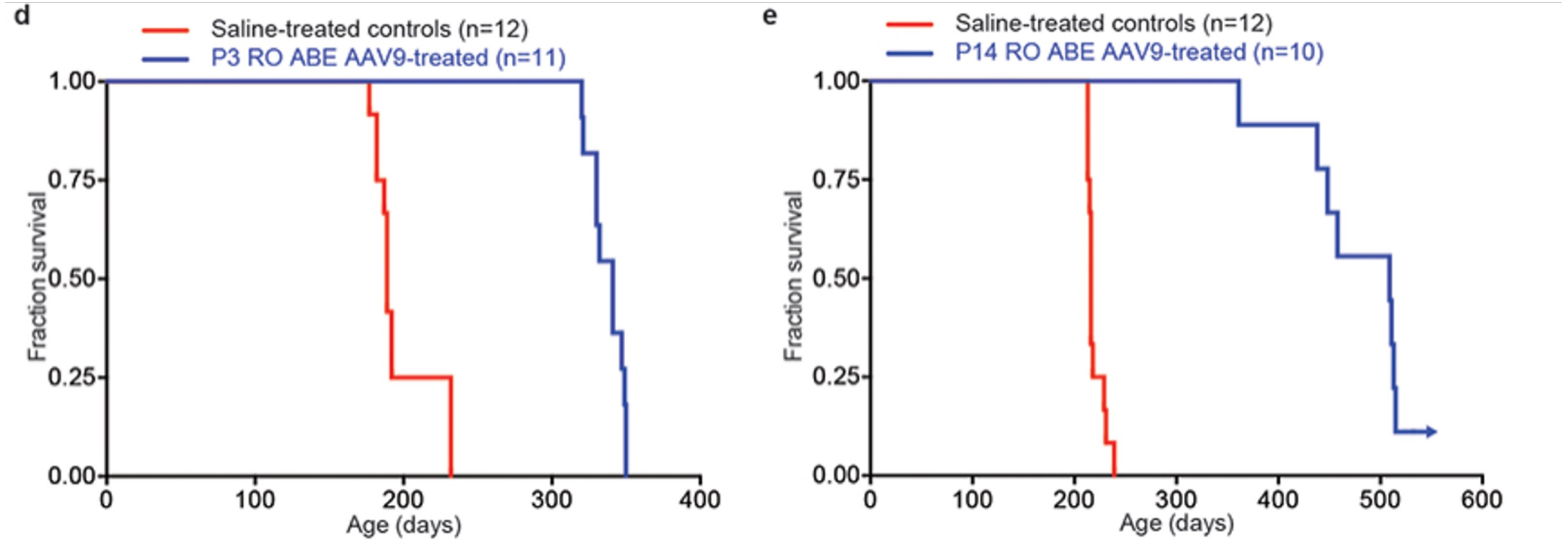
Reduced loss of adipose tissue

Was ABE treatment enough to significantly increase lifespan in vivo?



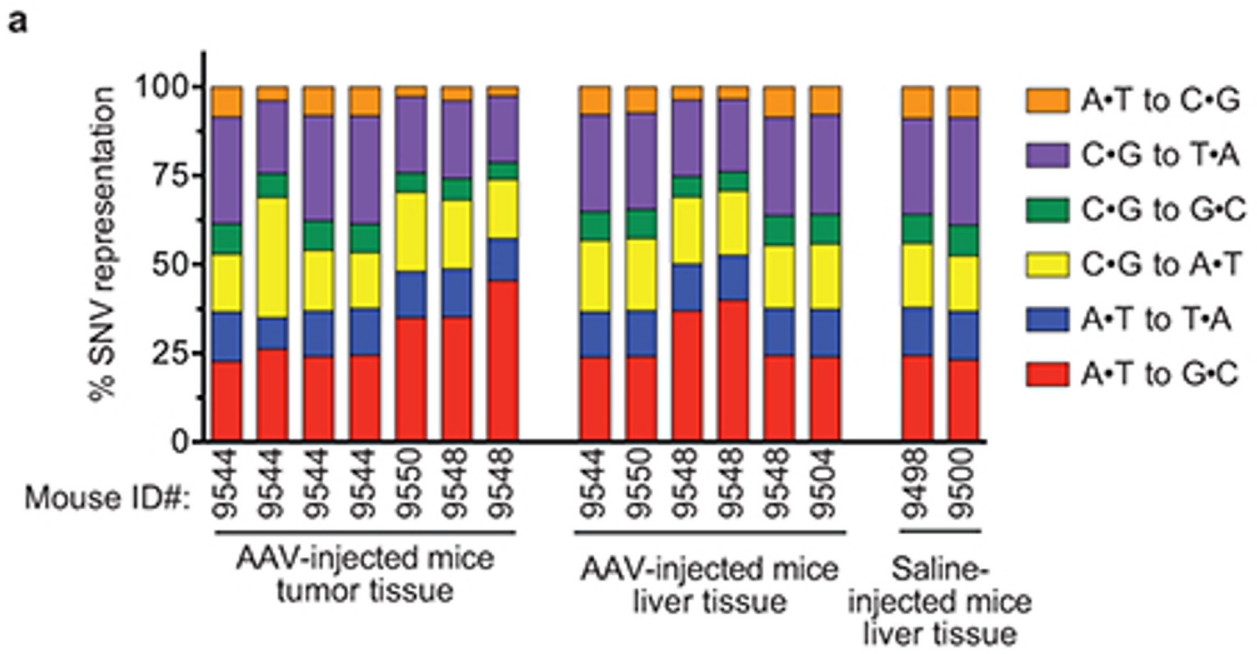
Massive increase in overall lifespan

Was ABE treatment enough to significantly increase lifespan in vivo?



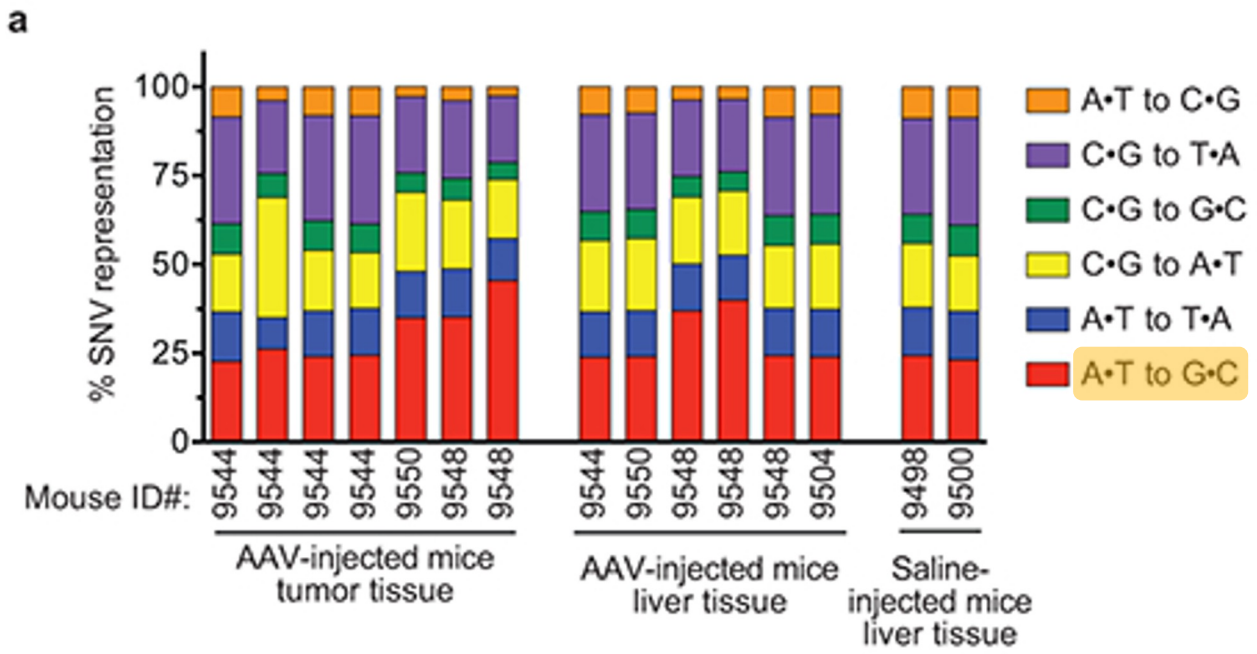
Massive increase in overall lifespan

What were the concerns from in vivo ABE use?



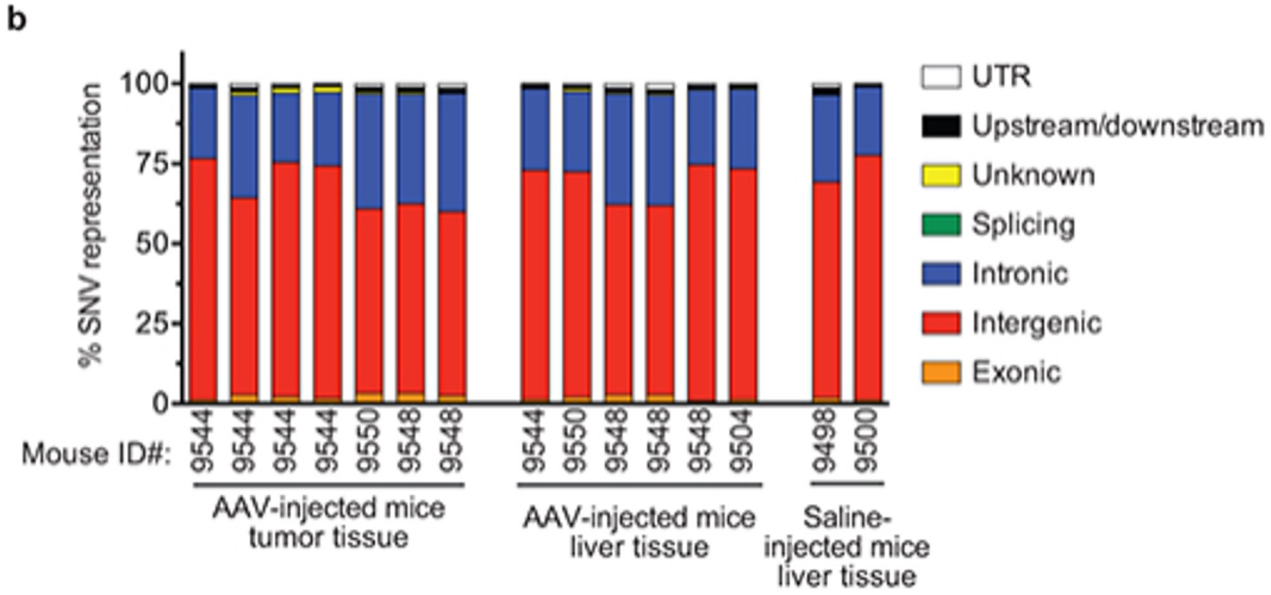
Potential cause of tumors in five mice

What were the concerns from in vivo ABE use?



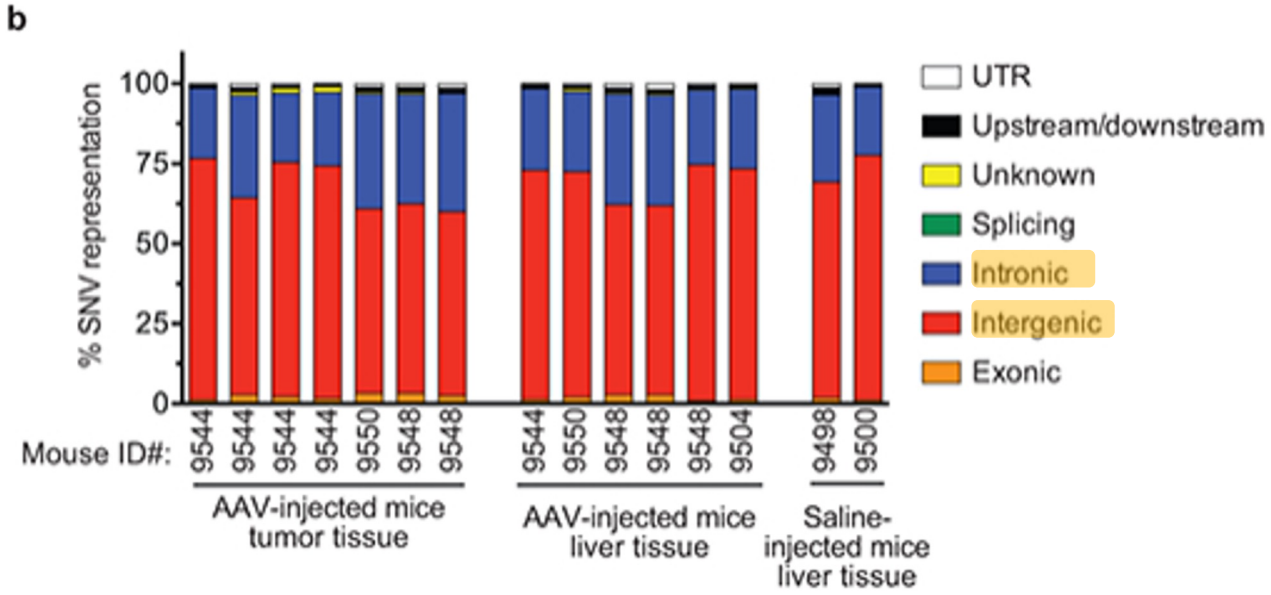
Potential cause of tumors in five mice

Were these concerns warranted?



Extensive testing showed otherwise (likely caused by trans-gene)

Were these concerns warranted?



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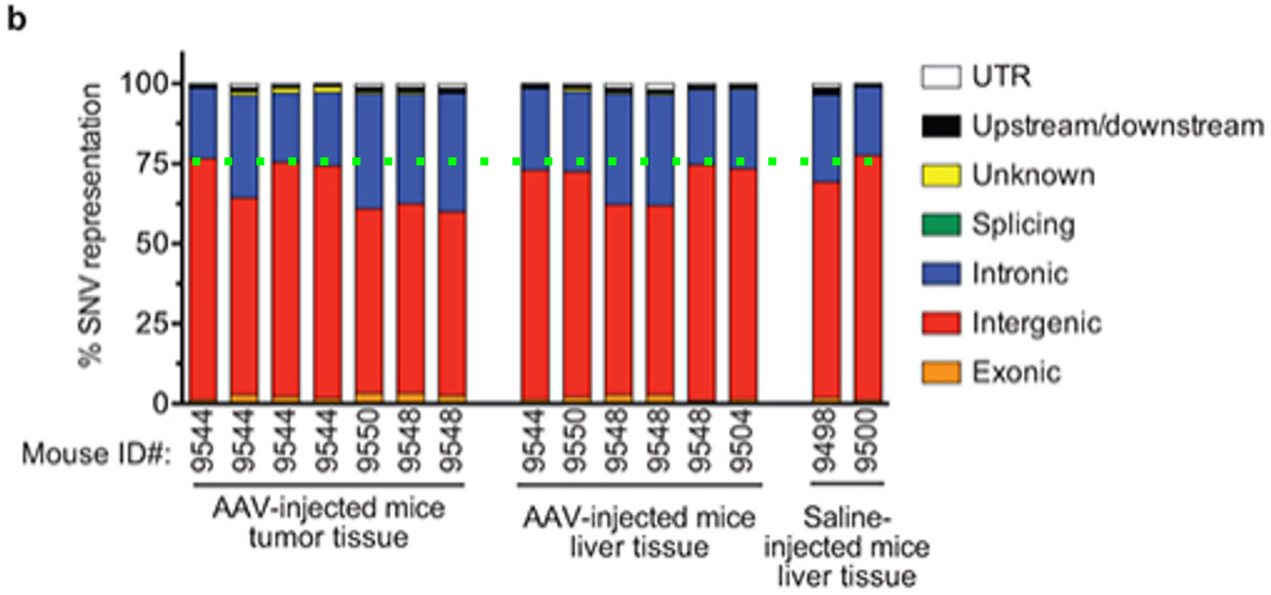
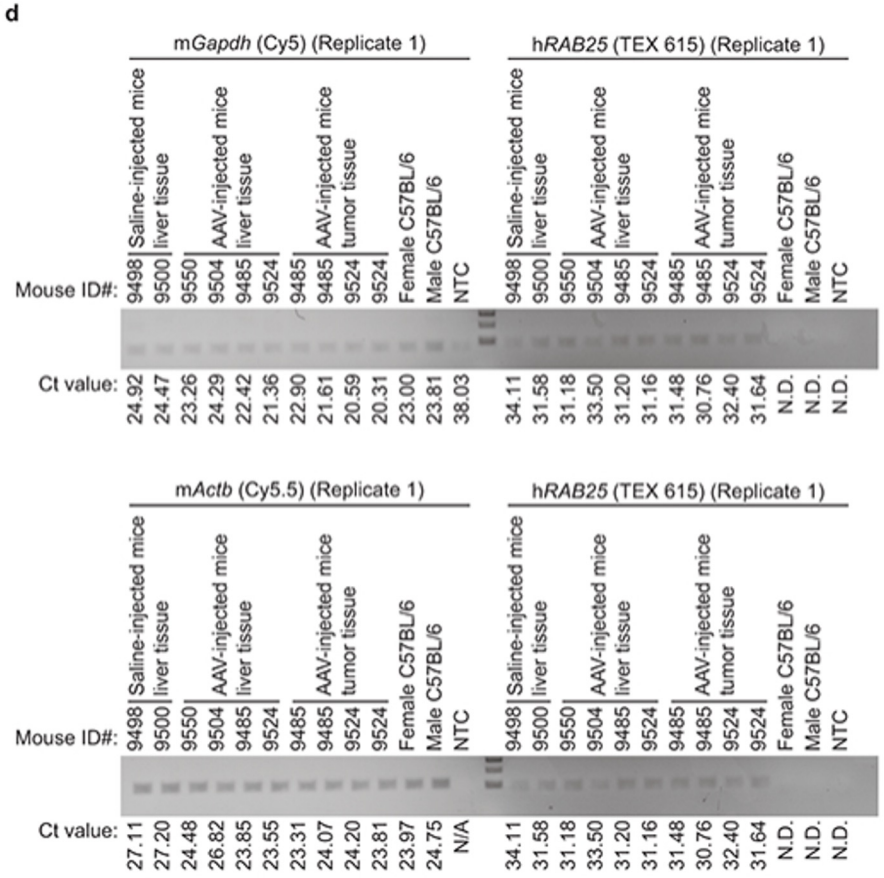


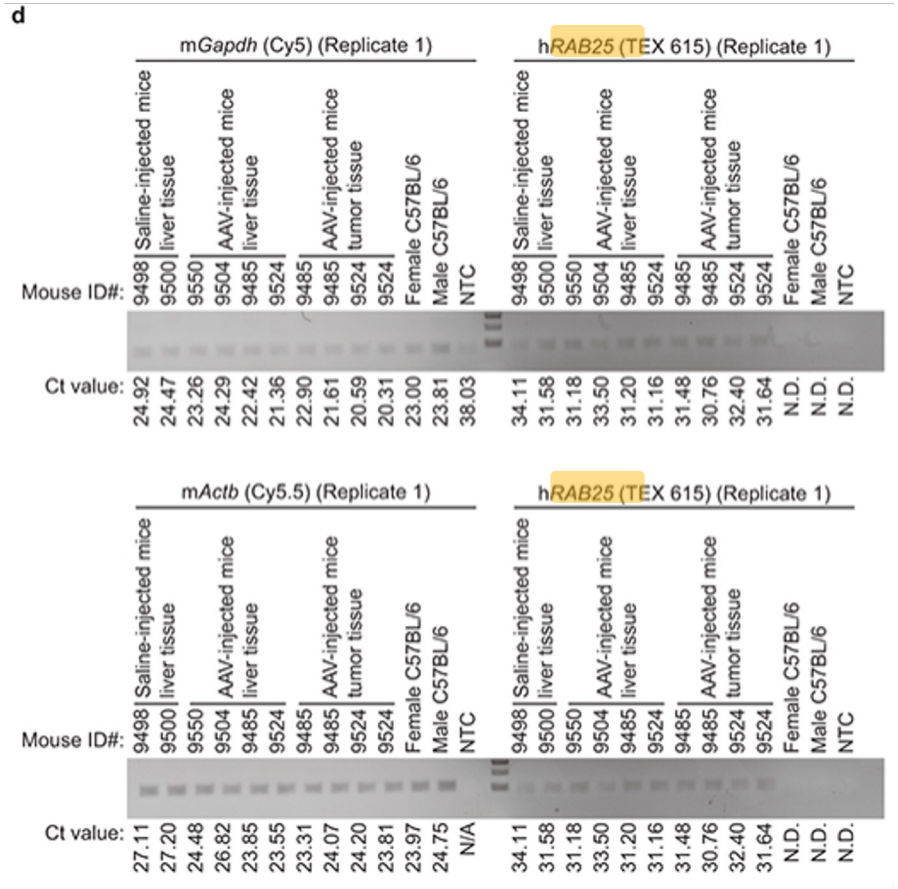
Extensive testing showed otherwise (likely caused by trans-gene)

What research should be done moving forward?



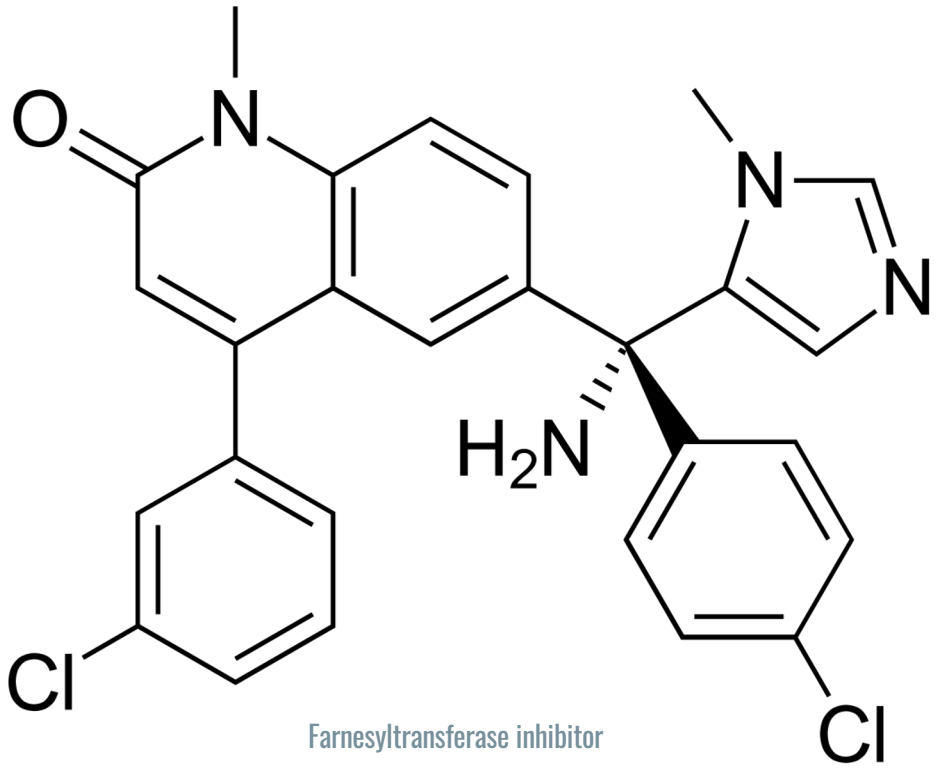
Further investigation into mouse-specific testing (trans-genes, vector, health)

What research should be done moving forward?



Further investigation into mouse-specific testing (trans-genes, vector, health)

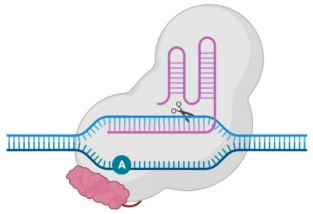
How can these findings be applied?



Farnesyltransferase inhibitor

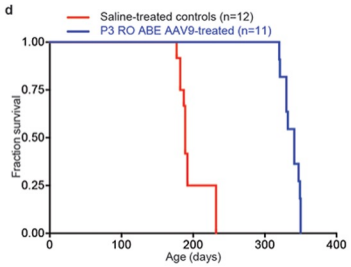
Further investigation into clinical application (more efficient ABEs, treatment timing, conjunctive treatments)

Looking forward

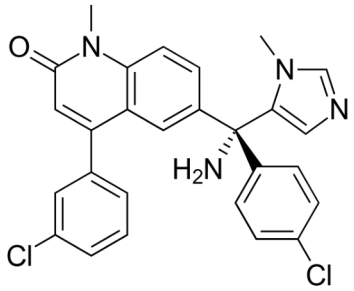


Use of ABEs to target point mutation

Science News, 2020



Successful in reducing key symptoms, improving lifespan



Hope for clinical application, pairing with other treatments

Figure/Photo References

https://en.wikipedia.org/wiki/Adenine

https://en.wikipedia.org/wiki/Inosine

https://www.annualreviews.org/doi/10.1146/annurev-biophys-062215-010822?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub++0pubmed

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1351119/

https://en.wikipedia.org/wiki/Farnesyltransferase_inhibitor

https://www.sciencenews.org/article/fda-approved-first-drug-treat-rapid-aging-disease-progeria

Various Biorender images