
The background of the slide is a dense grid of horizontal, multi-colored lines in shades of blue, purple, cyan, green, yellow, and red, representing DNA sequencing data. The lines are slightly blurred and vary in length and intensity, creating a sense of depth and movement.

Next Generation Sequencing


Kate Stack and Zoe Zwick

What is DNA?




 = Adenine

 = Thymine

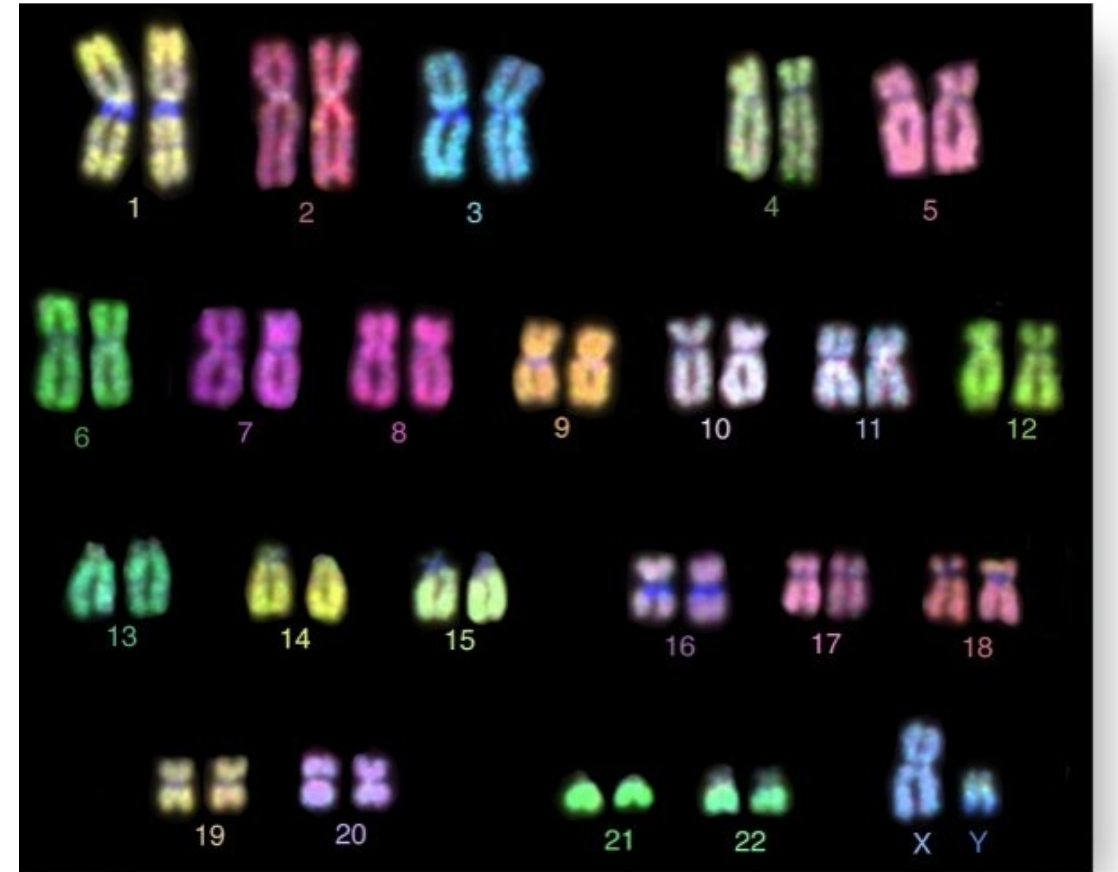
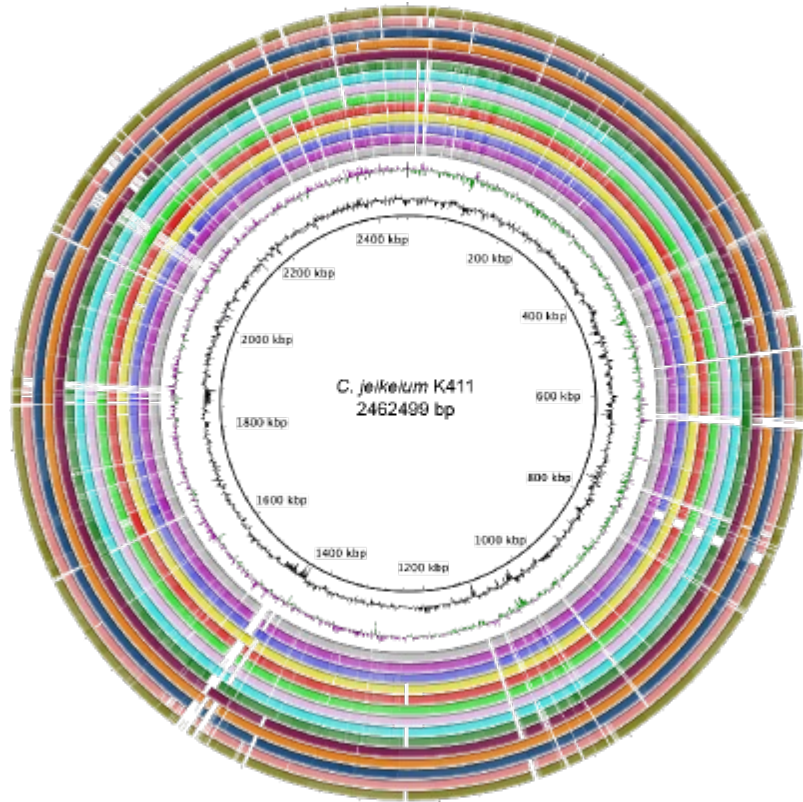
 = Cytosine

 = Guanine

 = Phosphate
backbone

DNA, is the “blueprint” for what makes up life, making up the genome

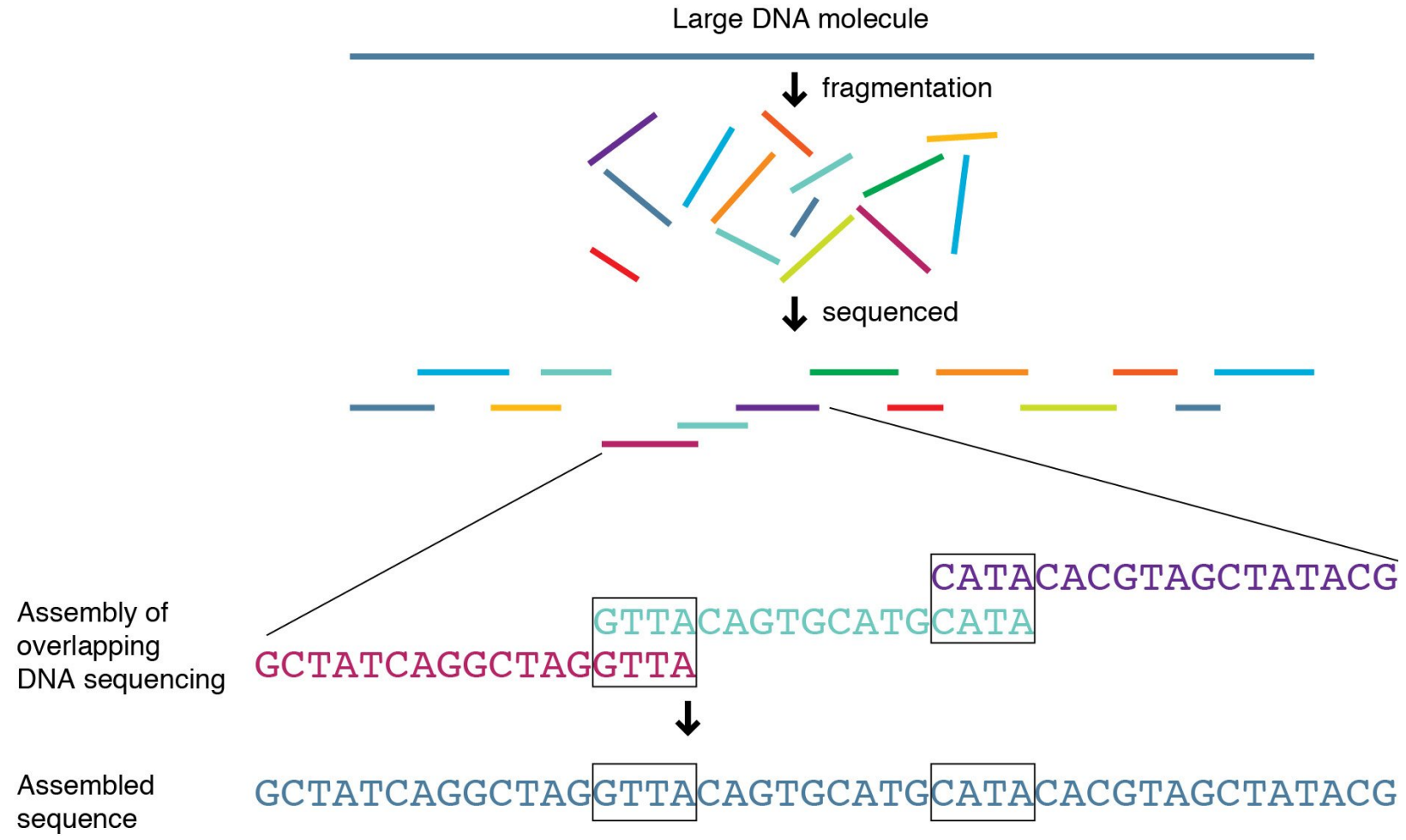
What is a genome?



The genome is the total DNA of an organism, providing all the information needed for function

(Maiti, 2022)

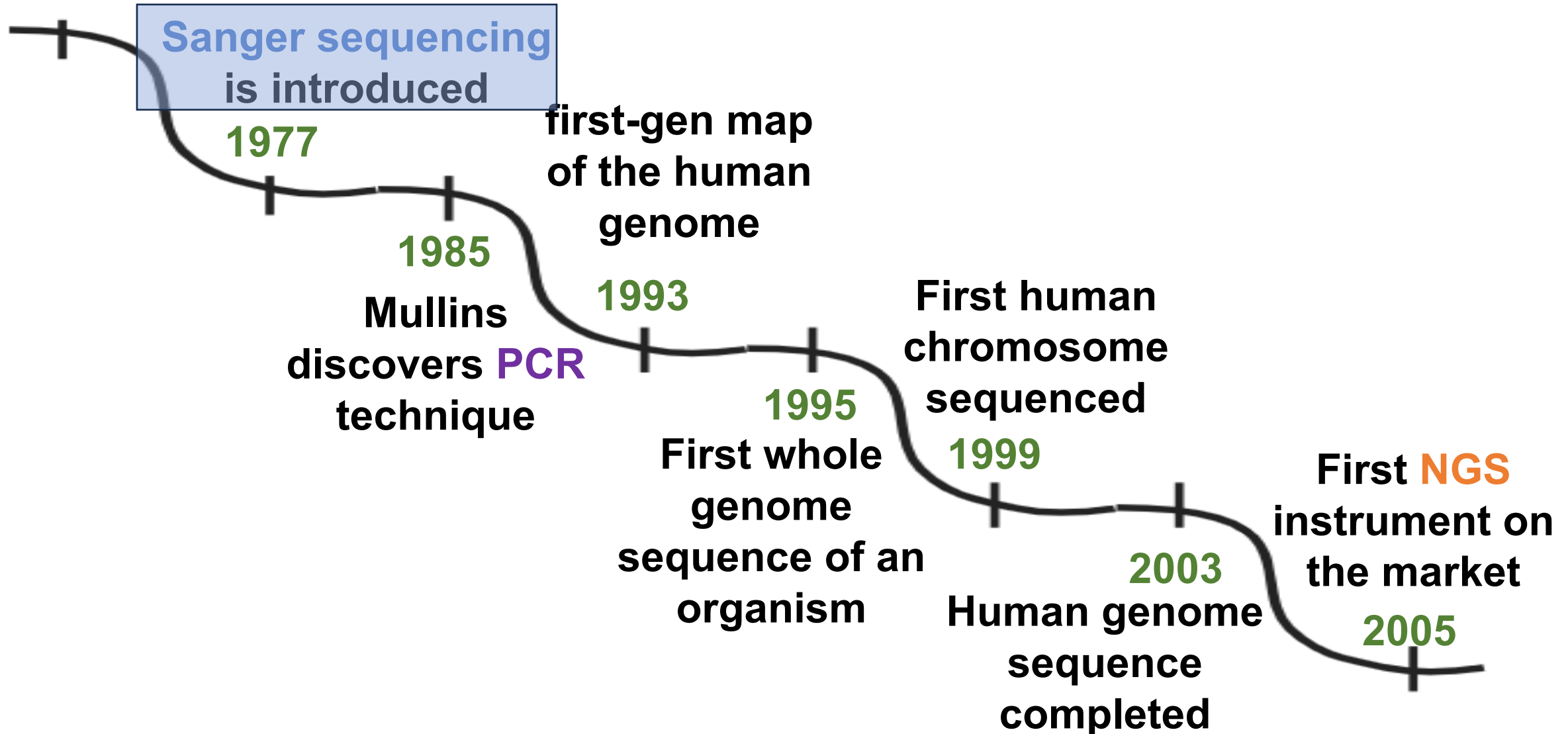
How can we access the genome's information?



We can sequence the DNA of an organism by breaking it up into smaller pieces

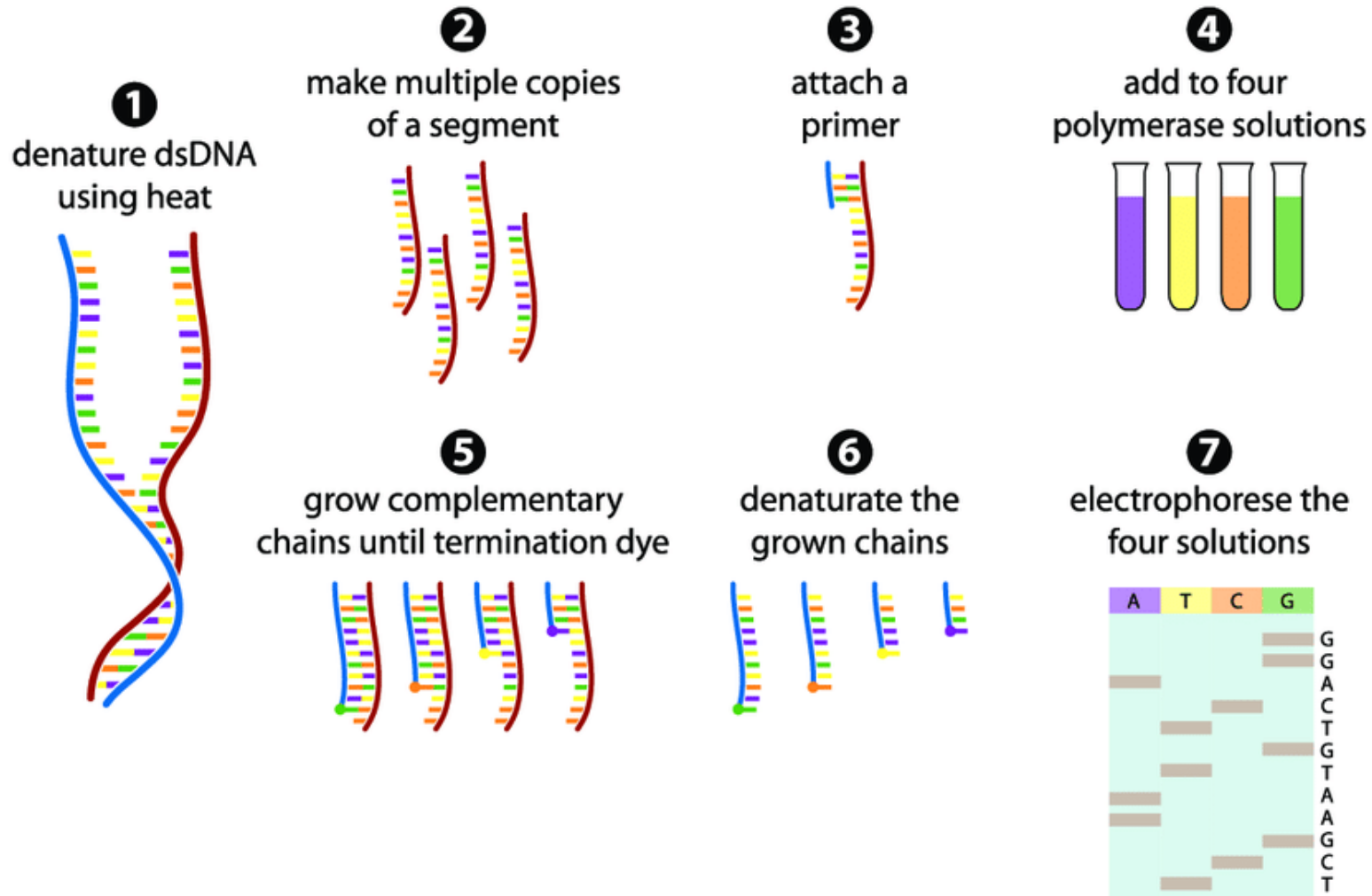
How has sequencing evolved?

Let's start here



(Rabani, 2012)

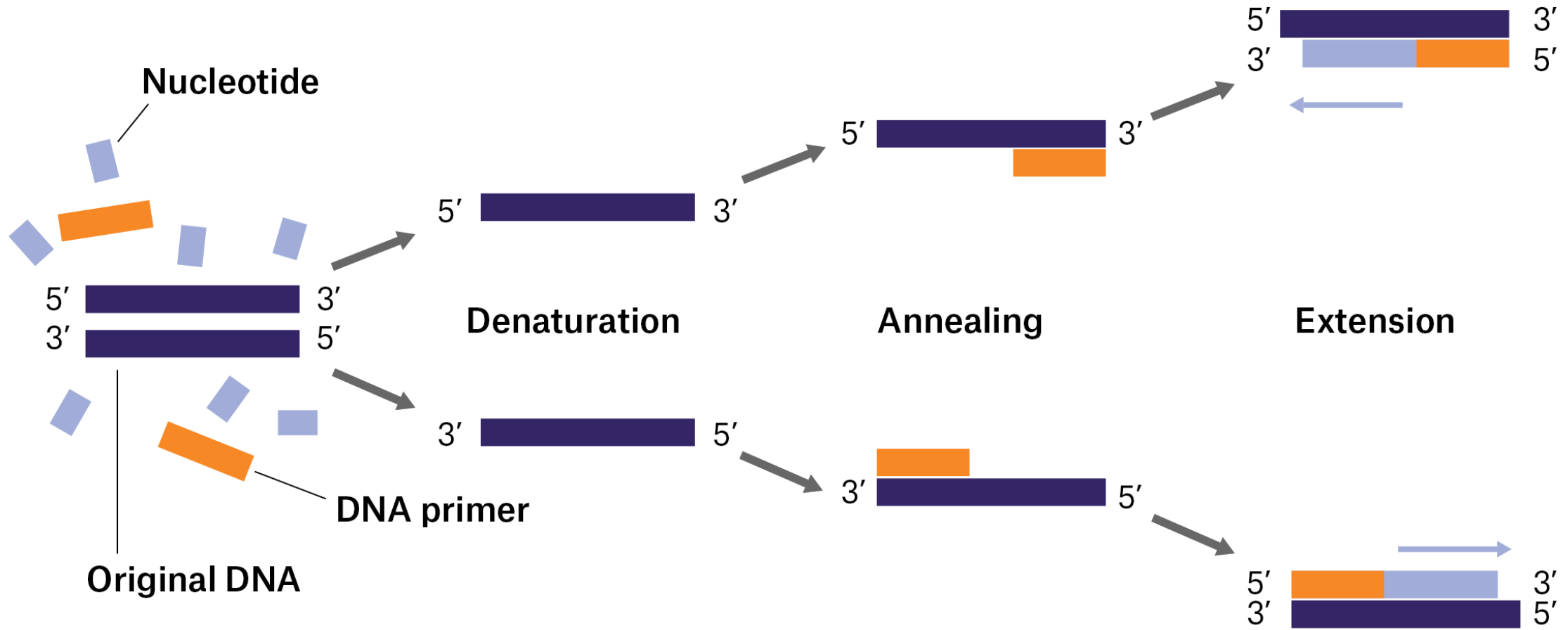
What is Sanger Sequencing?



Sanger Sequencing involves chain-terminating fluorescent dNTPs

(Gauthier, 2007)

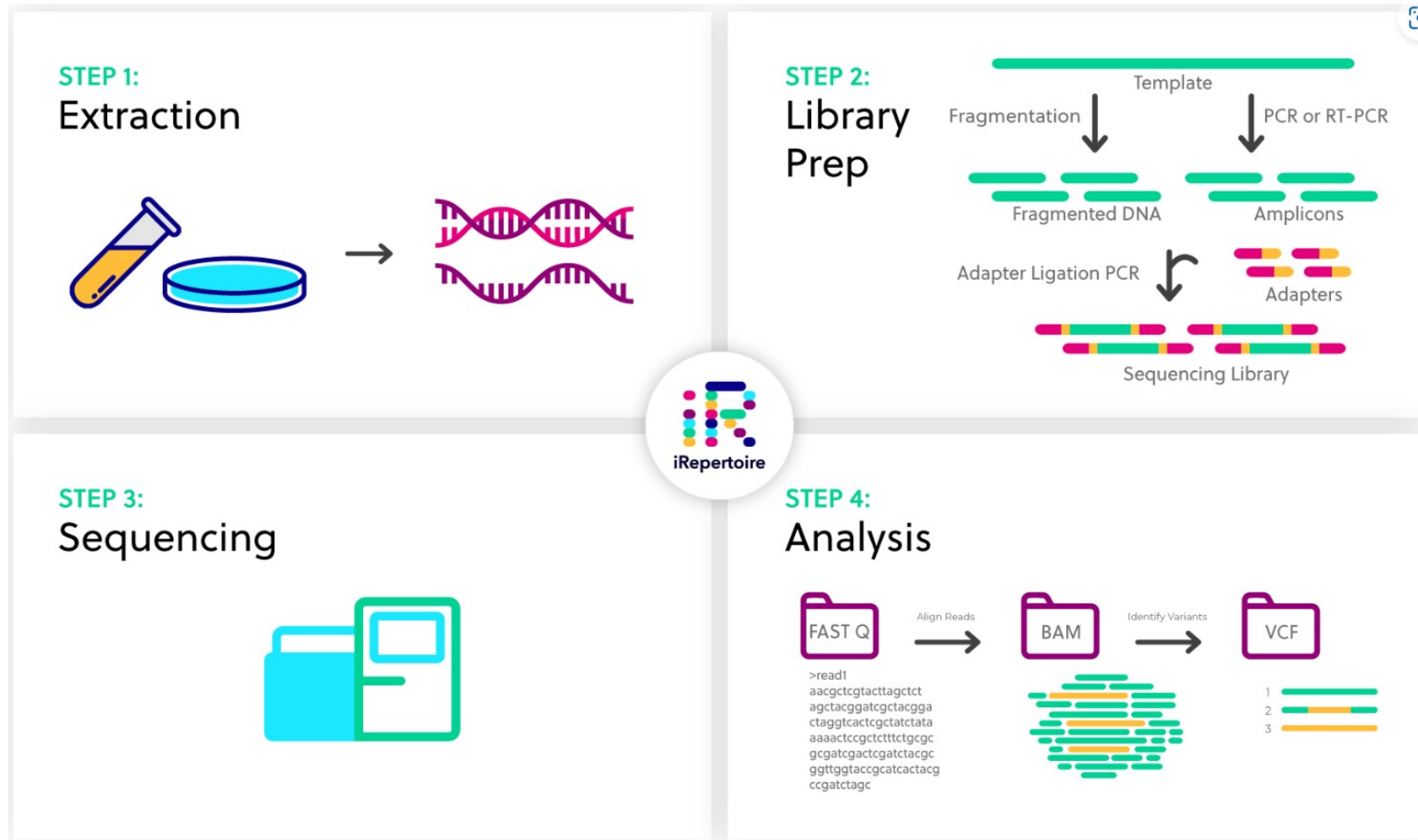
What is PCR?



©GA International

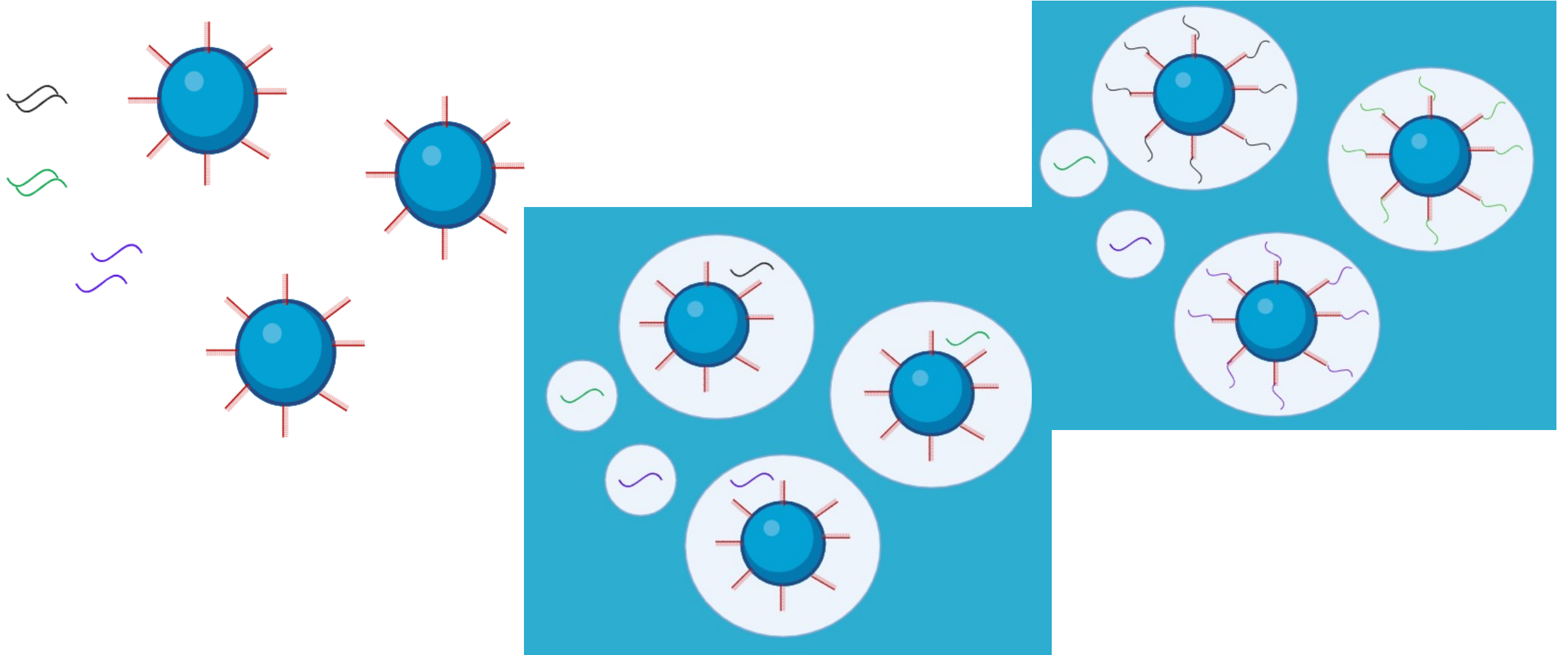
As opposed to **Sanger Sequencing**,
PCR uses both forward and reverse primers to replicate DNA

How is PCR used in Next Gen Sequencing?



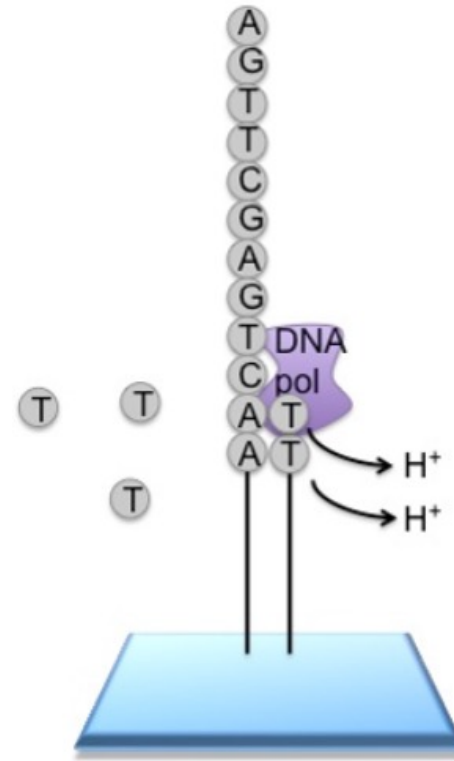
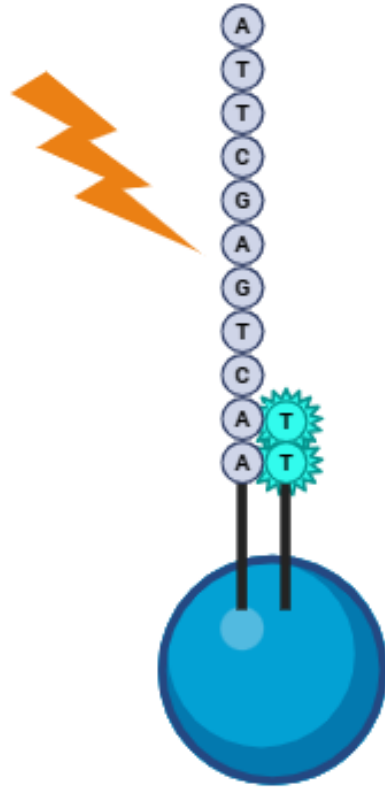
Next Generation Sequencing uses emulsion and bridge PCR to prepare samples

How does emulsion PCR work?



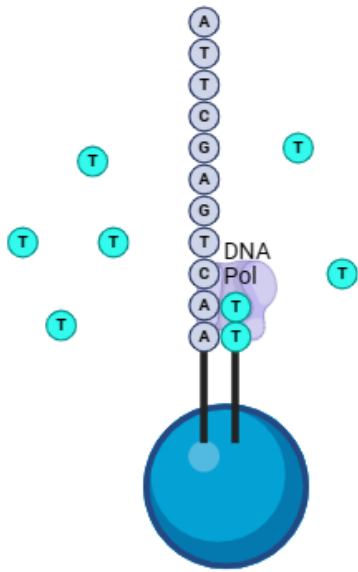
Emulsion PCR uses agarose beads with complementary adapters

What NGS technology uses emulsion PCR?



454 sequencing and Ion Torrent sequencing

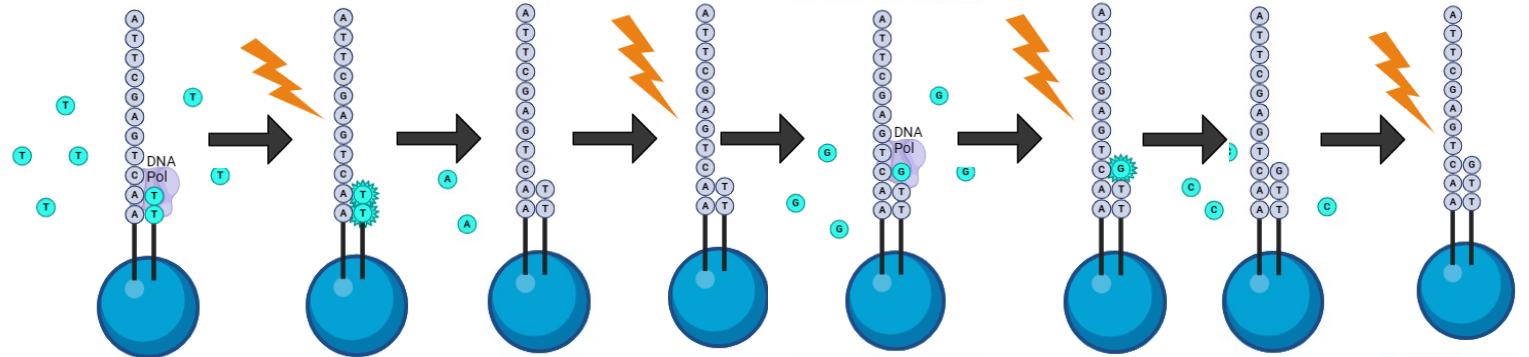
How does 454 Sequencing work?



One **nucleotide** is flooded into each well

When binding, **light** is emitted

nucleotides are changed out, and this process is **repeated many times**

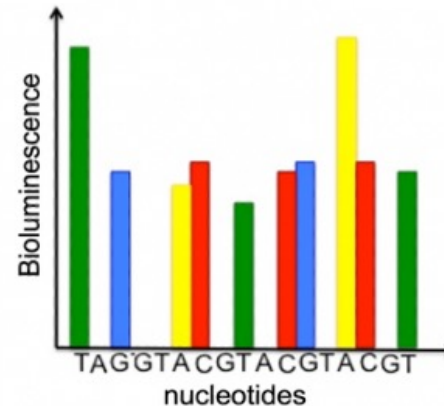


T

A

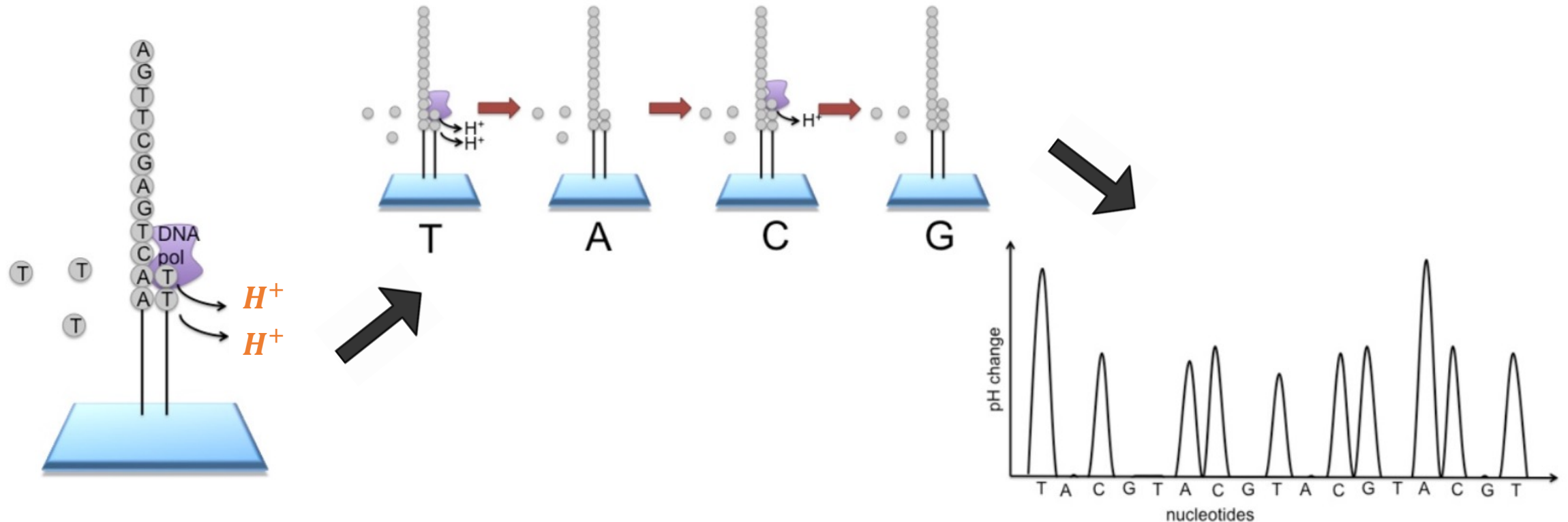
G

C



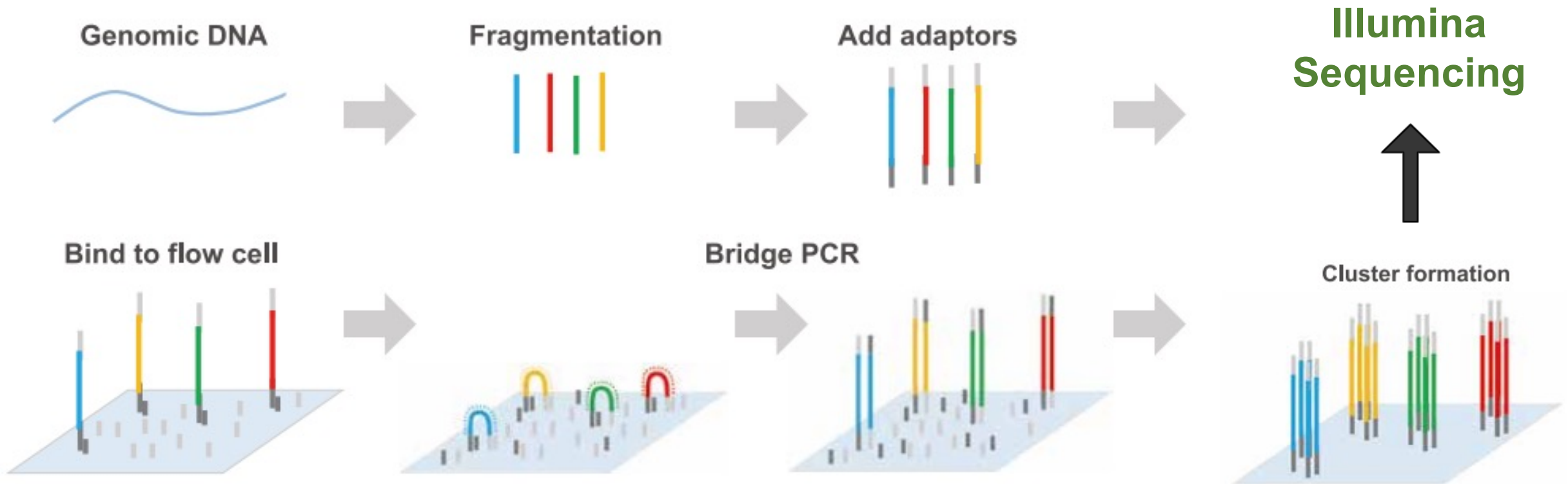
Reads for each **nucleotide** will reveal the **sequence**

How does Ion Torrent Sequencing work?



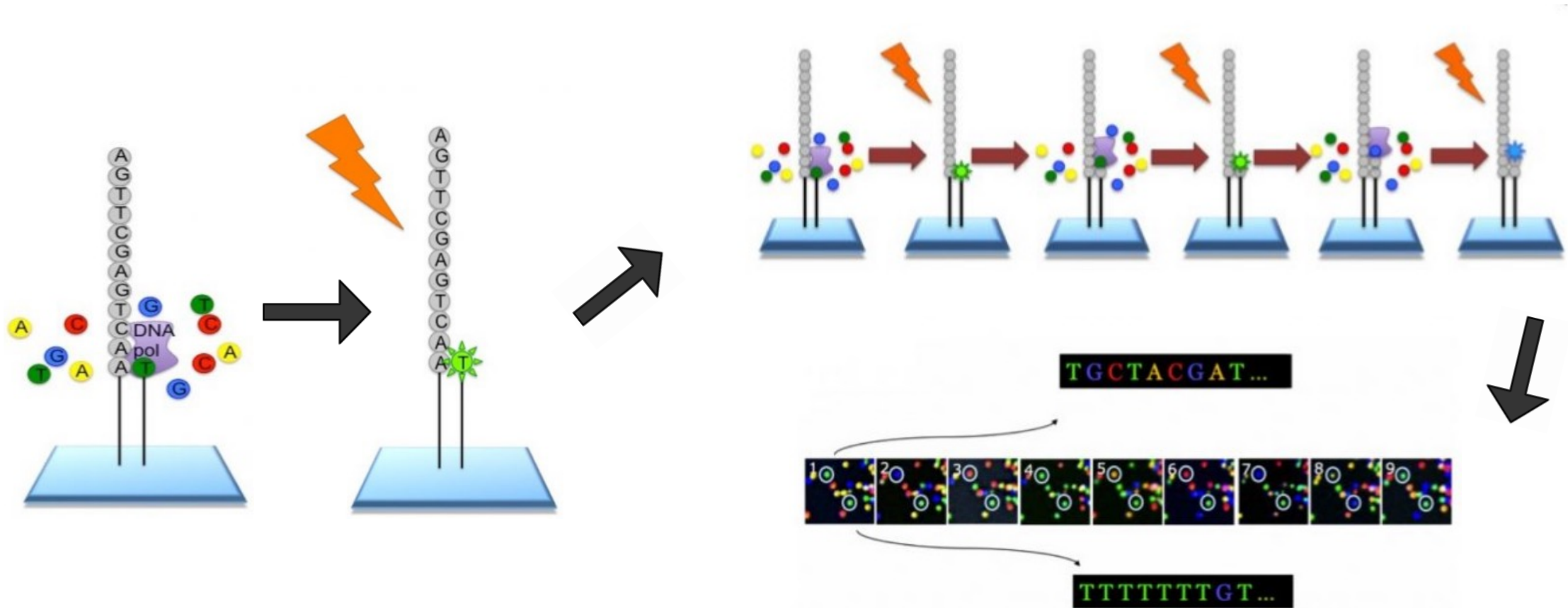
When a nucleotide is added to the new strand, a **Hydrogen ion (H^+)** is released, causing a pH change

How is bridge PCR used in Next Gen Sequencing?



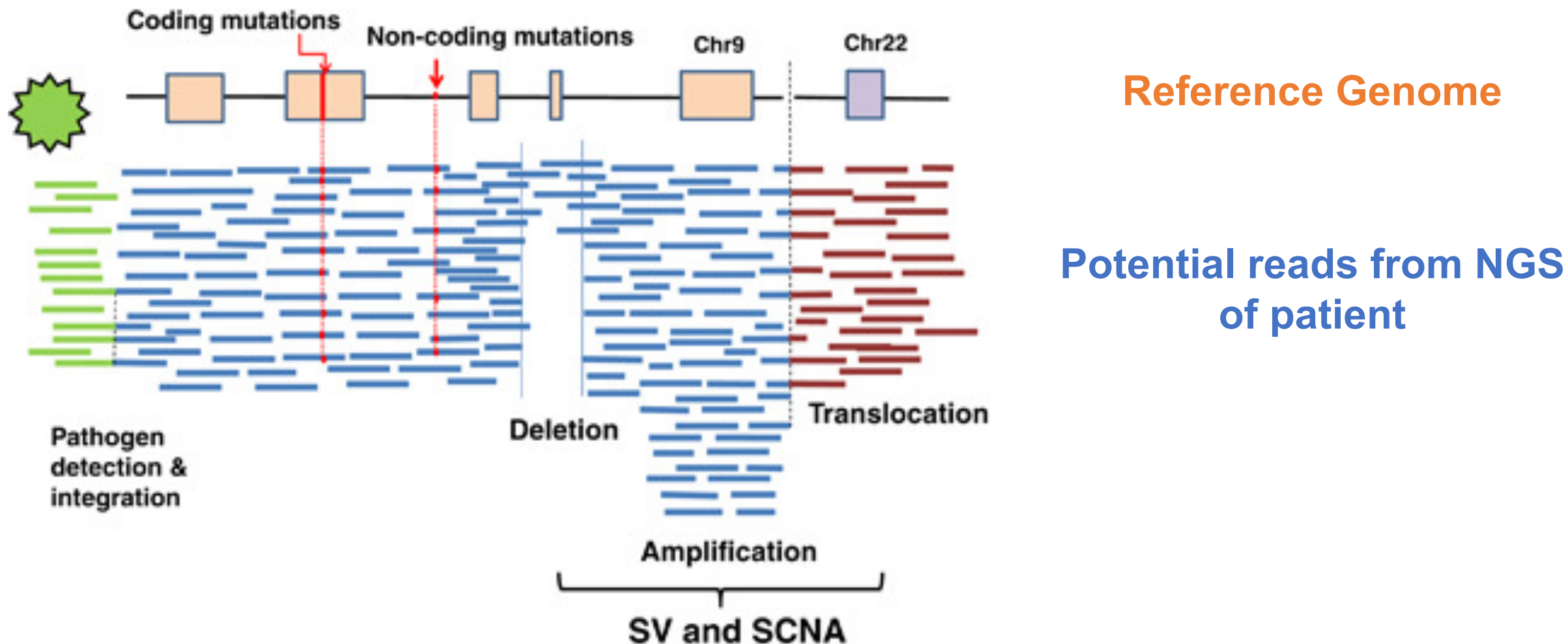
DNA fragments are bound to flow cell by adaptors, form bridges, then are cut and extended to form clusters

How does Illumina Sequencing work?



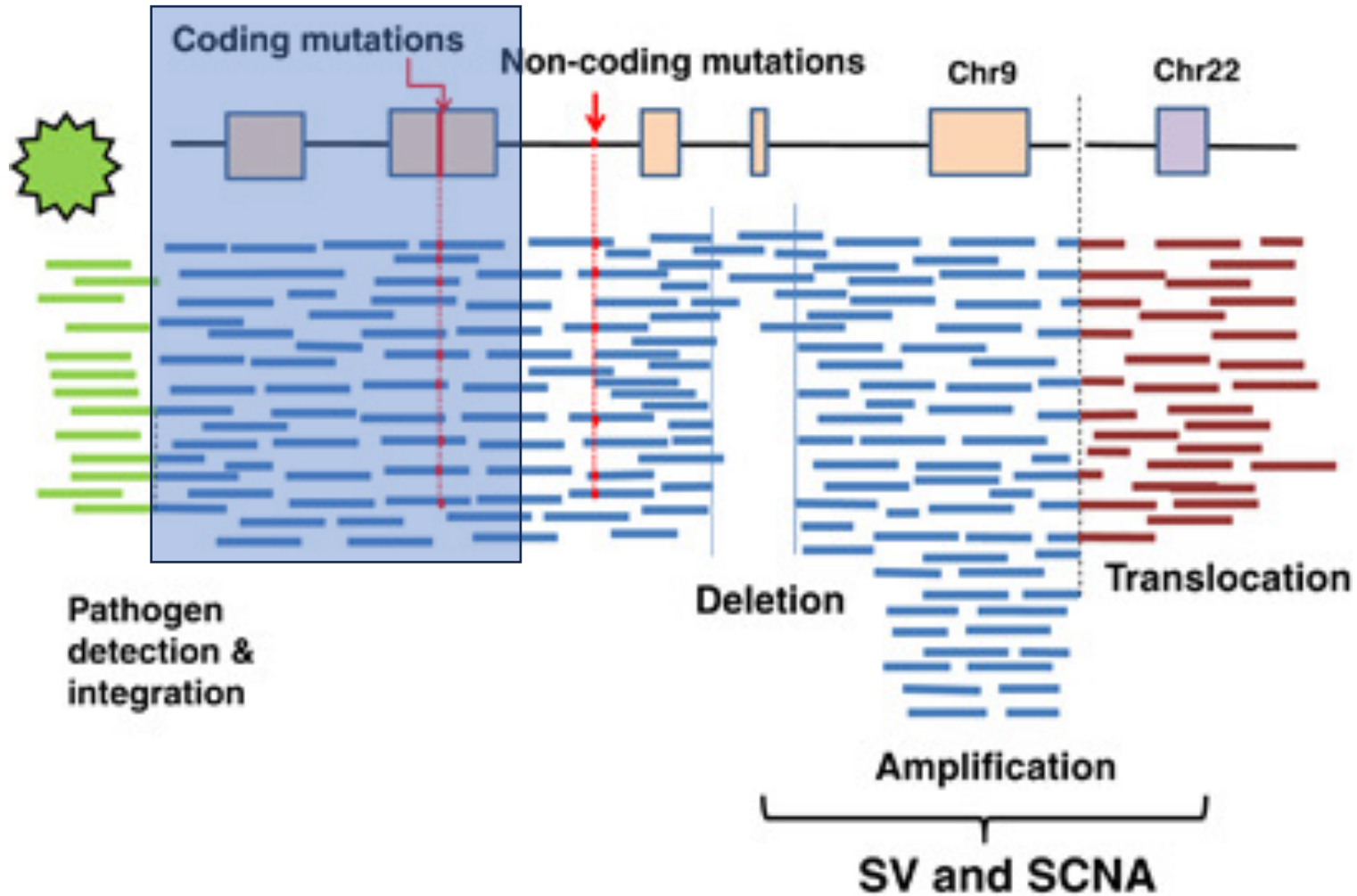
Nucleotides with removable fluorescent markers are added to plate, and when added, are able to be imaged

How can **NGS** help identify variants?



When lined up against a **reference genome**, potential disease-causing **variants of patients** can be identified

What if we are only interested in coding mutations?



We can also just sequence coding DNA by **whole exome sequencing**

What are the **pros** and **cons** of **whole exome sequencing**?

less expensive

faster turn around time

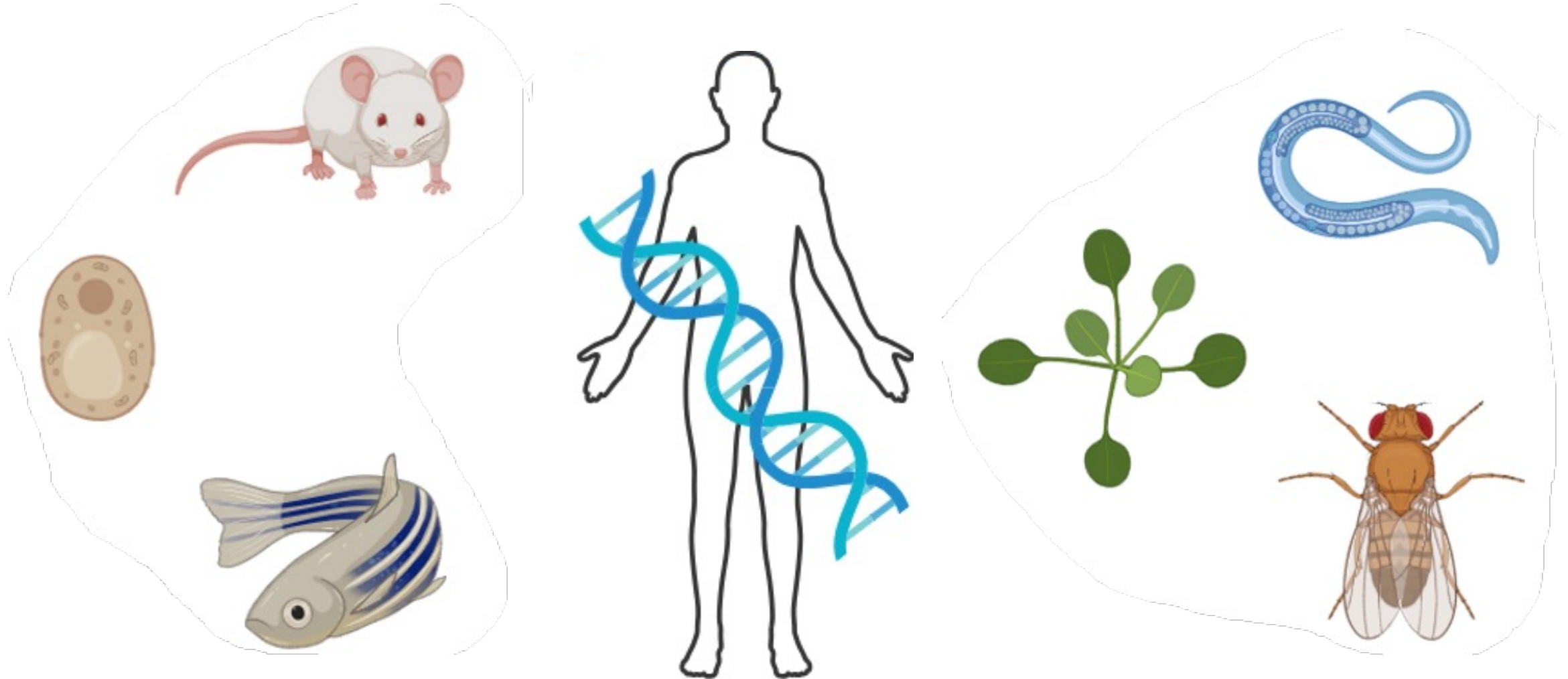
**many disease-causing
variants are in coding
regions**

**May miss important
variants in non-coding
regions**

**Less resolution around
exons themselves**

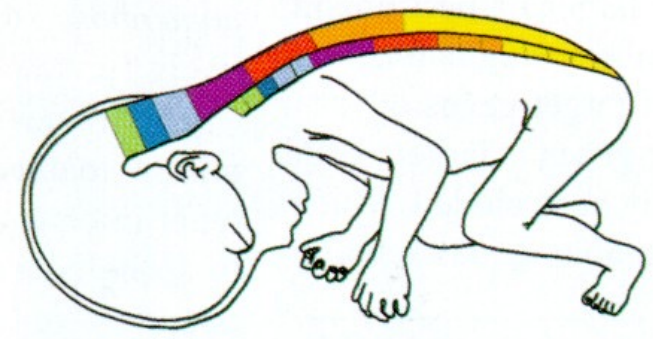
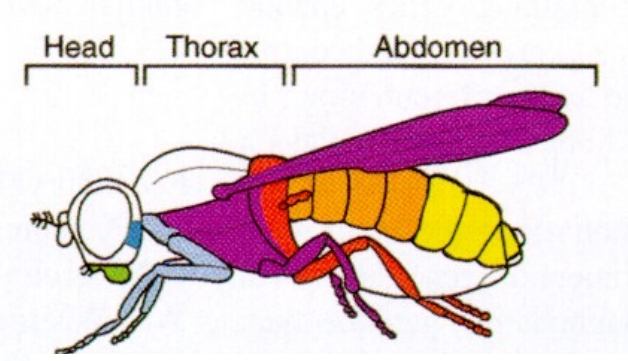
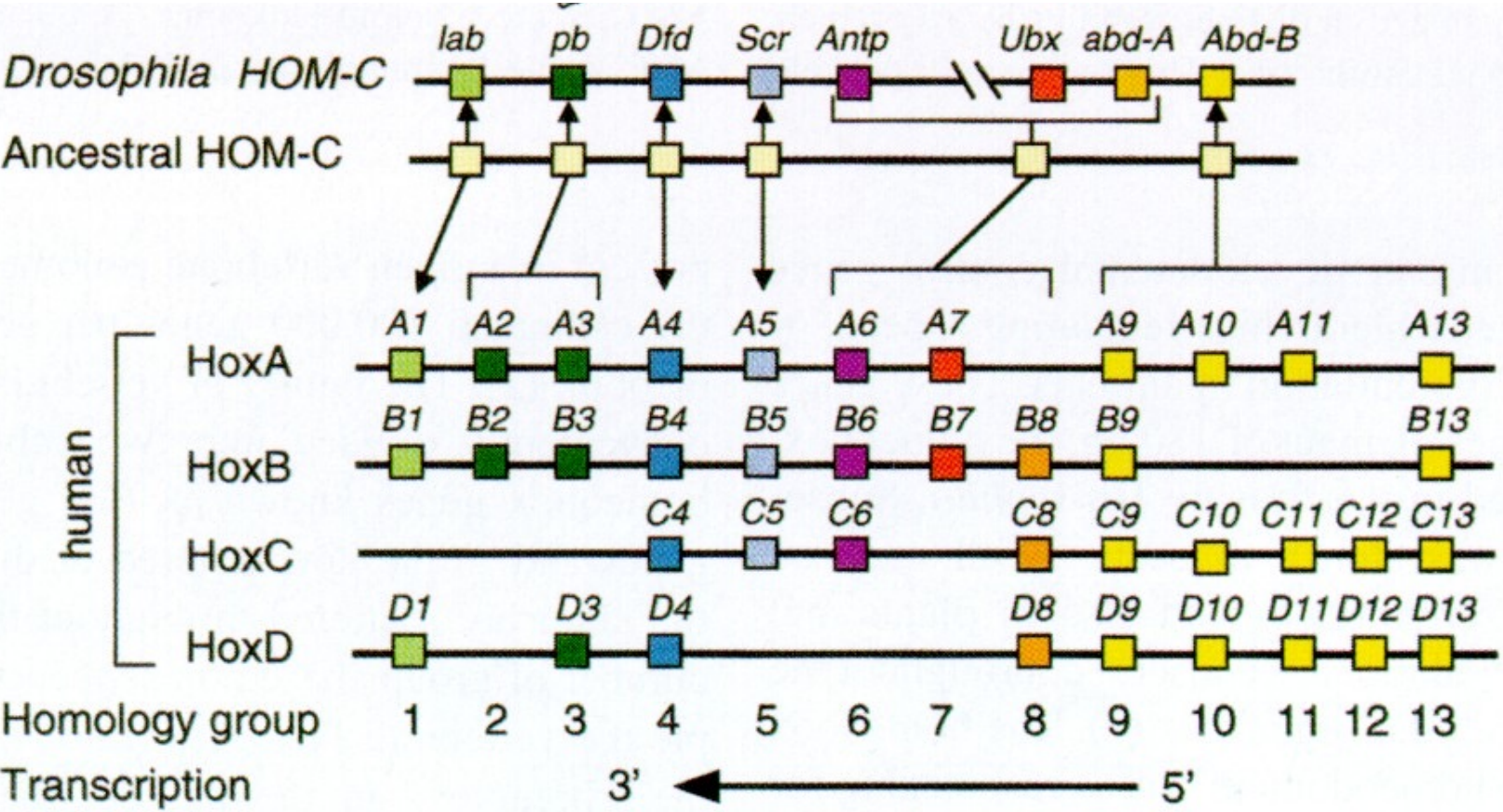
Model Organisms

What are Model Organisms?



non-human organisms used to study biology based on previously understood characteristics

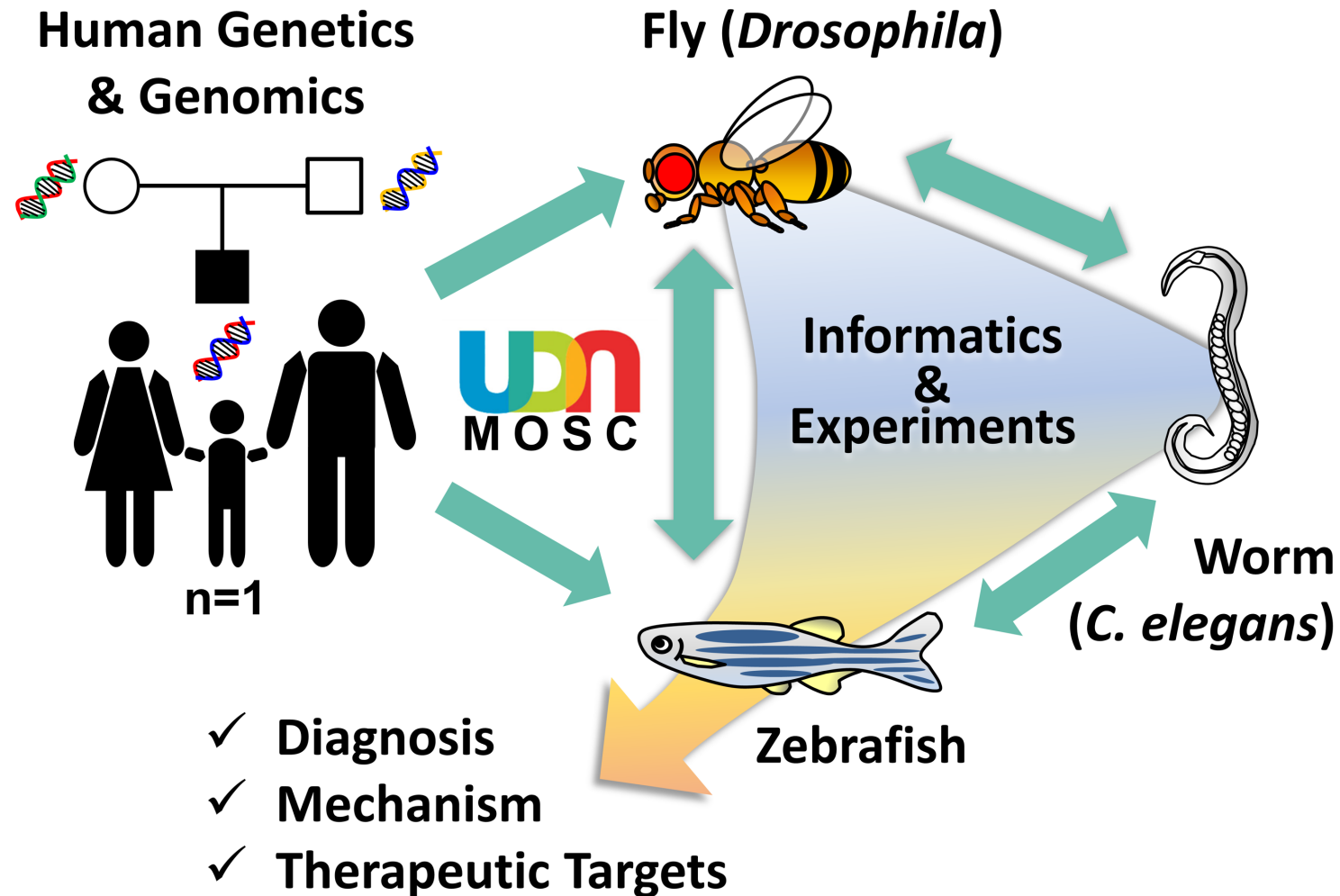
What is an ortholog?



evolutionarily conserved genes across speciation – if function of gene is known in a model organism it can be predictive of that of humans

(Mark, 1997)

How can we use model organisms to diagnose patients?



Patients are sequenced then rare variant information found is sent to animal facilities to further research

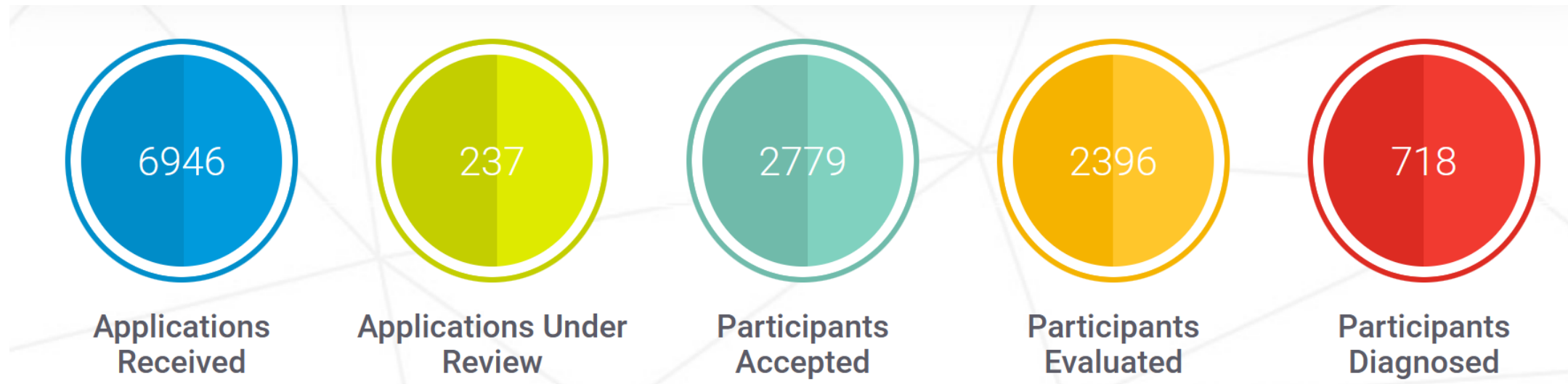
What is the Undiagnosed Disease Network?



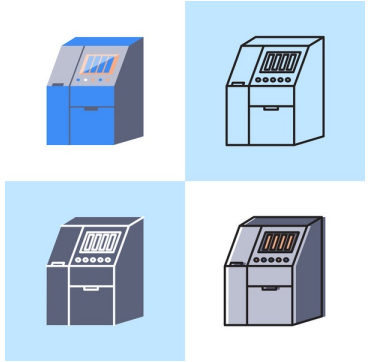
By combining digital resources, model organism communities and information from patients, diseases and their molecular causes are better understood

(Wrangler, 2017)

How has the UDN improved outcomes?



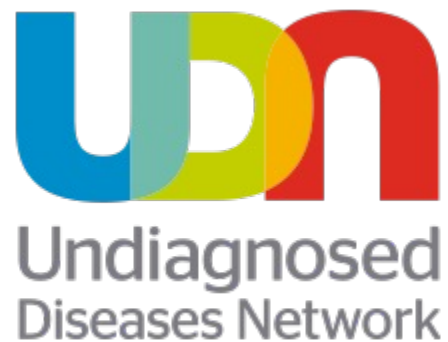
Summary



Next generation sequencing techniques can be used to understand our genomes



Model organisms help us research human diseases due to their evolutionarily conserved genes



The Undiagnosed Disease Network allows for the connection of patients with data that can help them better understand their conditions

Article | [Published: 06 January 2021](#)

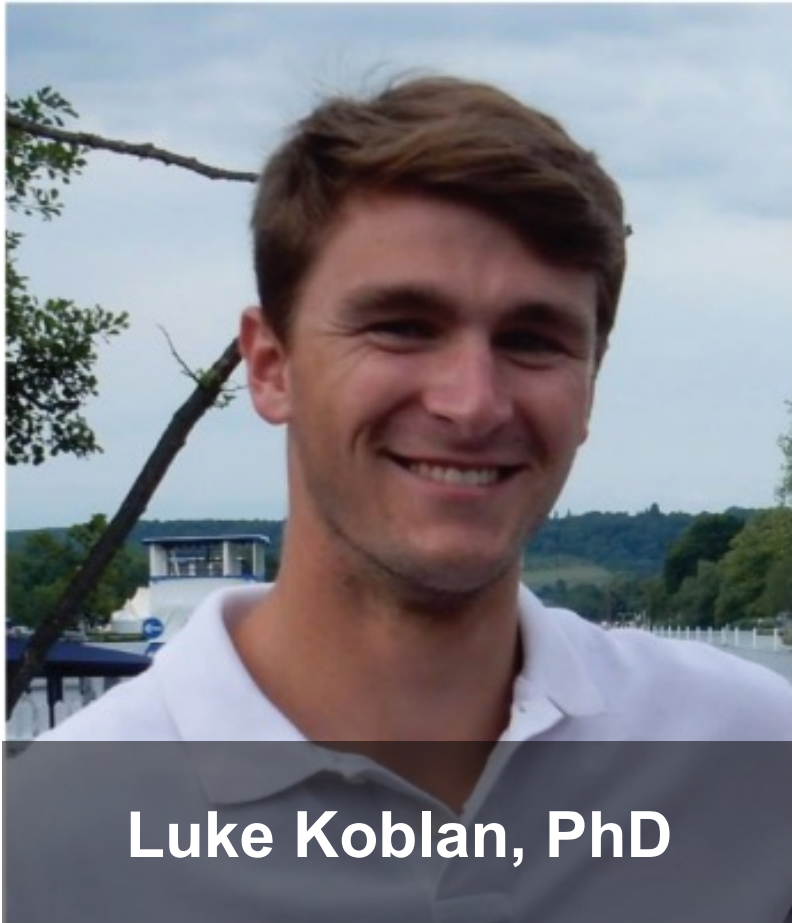
In vivo base editing rescues Hutchinson–Gilford progeria syndrome in mice

[Luke W. Koblan](#), [Michael R. Erdos](#), [Christopher Wilson](#), [Wayne A. Cabral](#), [Jonathan M. Levy](#), [Zheng-Mei Xiong](#), [Urraca L. Tavares](#), [Lindsay M. Davison](#), [Yantenew G. Gete](#), [Xiaojing Mao](#), [Gregory A. Newby](#), [Sean P. Doherty](#), [Narisu Narisu](#), [Quanhu Sheng](#), [Chad Krilow](#), [Charles Y. Lin](#), [Leslie B. Gordon](#), [Kan Cao](#), [Francis S. Collins](#) , [Jonathan D. Brown](#)  & [David R. Liu](#) 

[Nature](#) **589**, 608–614 (2021) | [Cite this article](#)

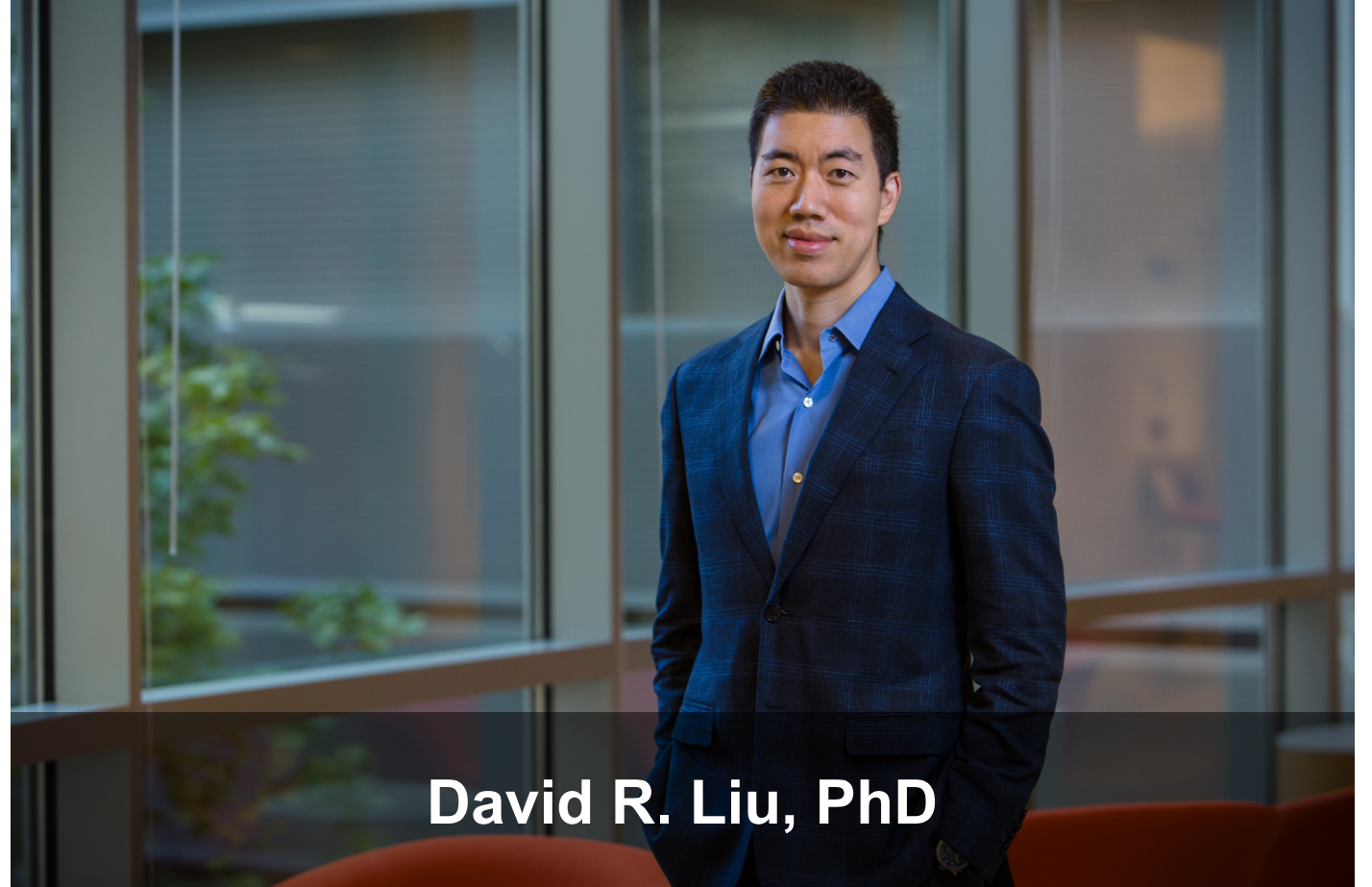
51k Accesses | **228** Citations | **1065** Altmetric | [Metrics](#)

Who are our scientists today?



Luke Koblan, PhD

First Author



David R. Liu, PhD

Principle Investigator

What has Luke Koblan worked on?



Luke Koblan, PhD

First Author

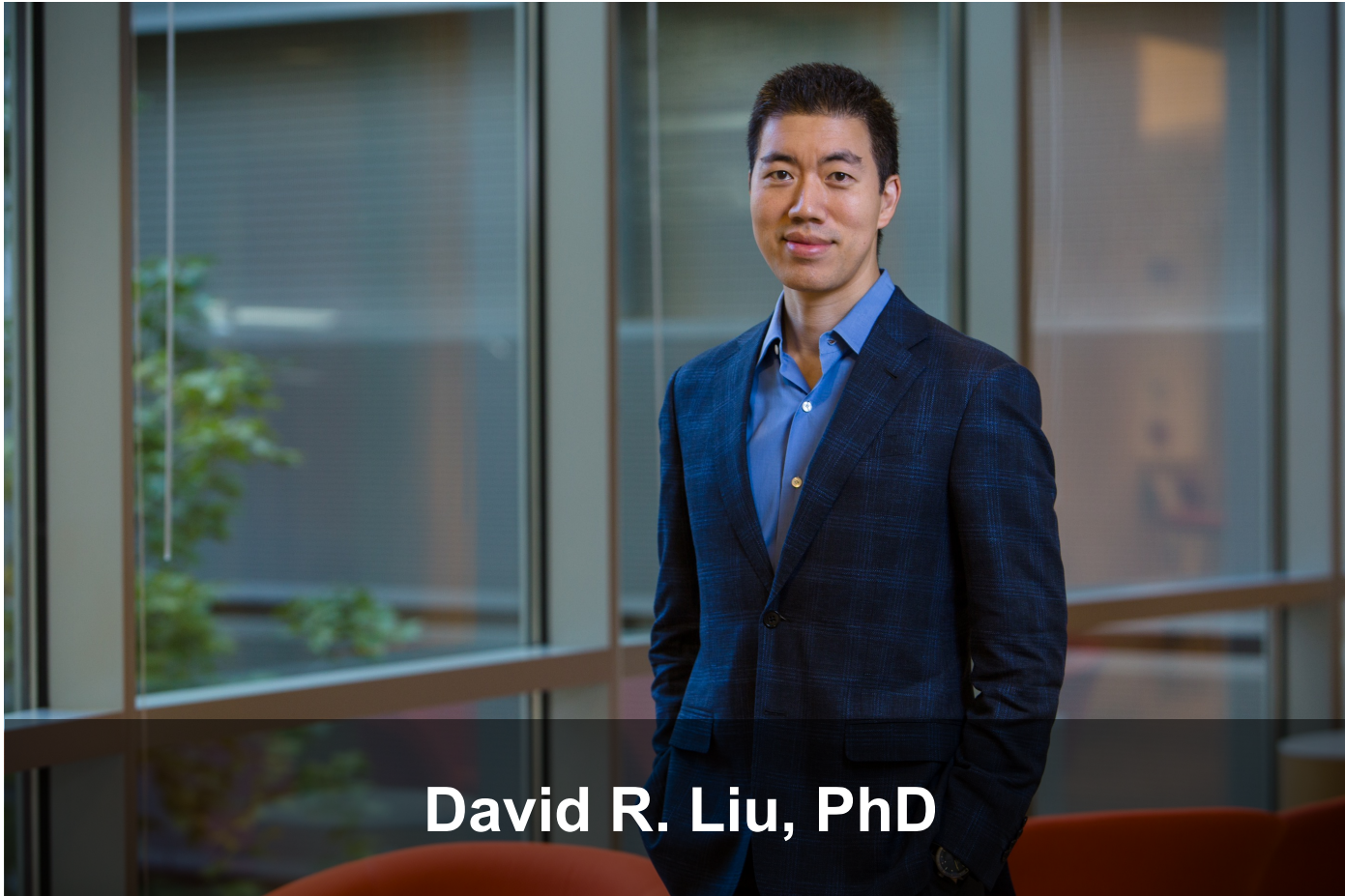
Was named in Forbes' 30 under 30 in healthcare in 2022



Received his PhD in chemical biology, working in David Liu's Lab at Harvard University

Now is working on Post-Doctoral Research at MIT

What does David R. Liu do?



David R. Liu, PhD

Principle Investigator

Head of Liu Group where they research 3 areas:



DNA-Templated Synthesis

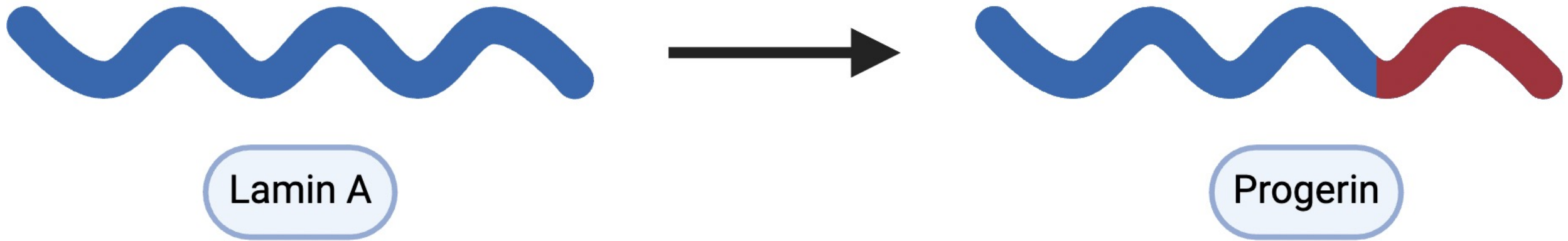


Protein Evolution & Delivery



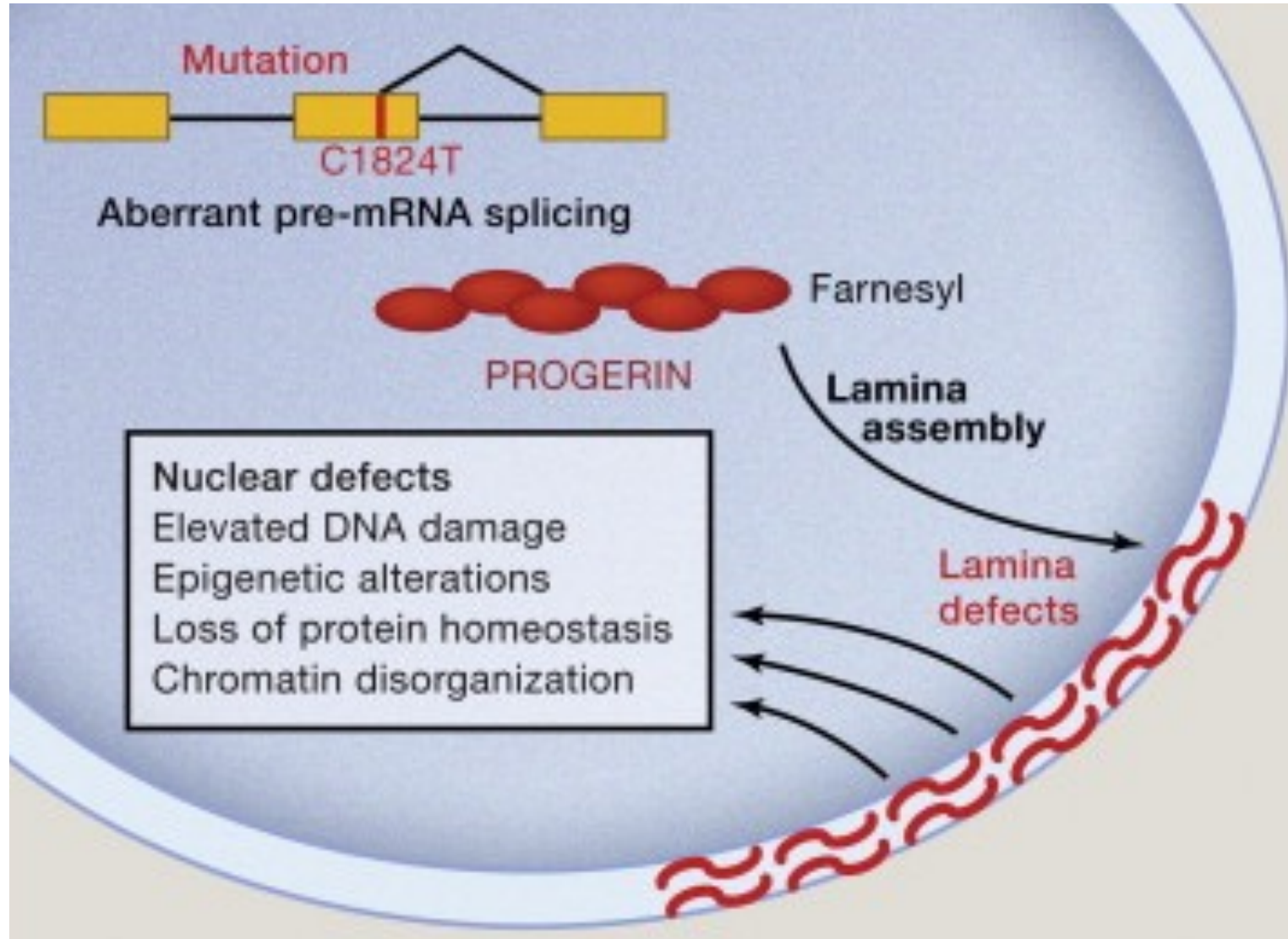
Genome Editing

What causes Progeria?



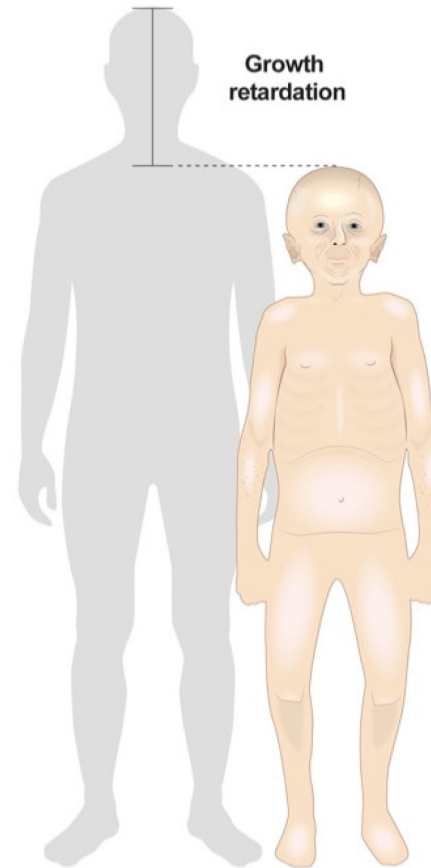
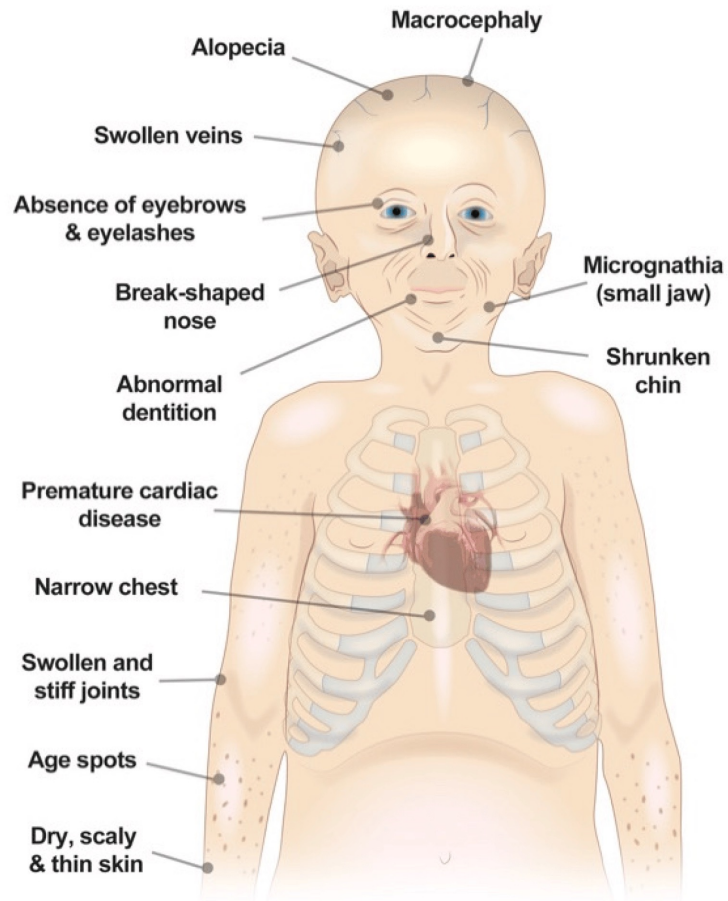
A denovo point mutation causes a mis-splice in the Lamin A gene, which ultimately impacts nuclear function and structure

How does Progeria affect the nucleus?



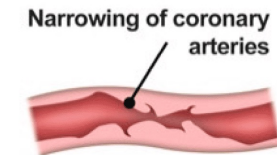
How does Progeria manifest?

HGPS patient features



Shared phenotypes with normal aging

- Abnormal gait
- Altered hearing
- Atherosclerosis
- CVD and stroke
- Hypertension
- Limited range of motion
- Low bone mineral density
- Loss of subcutaneous fat

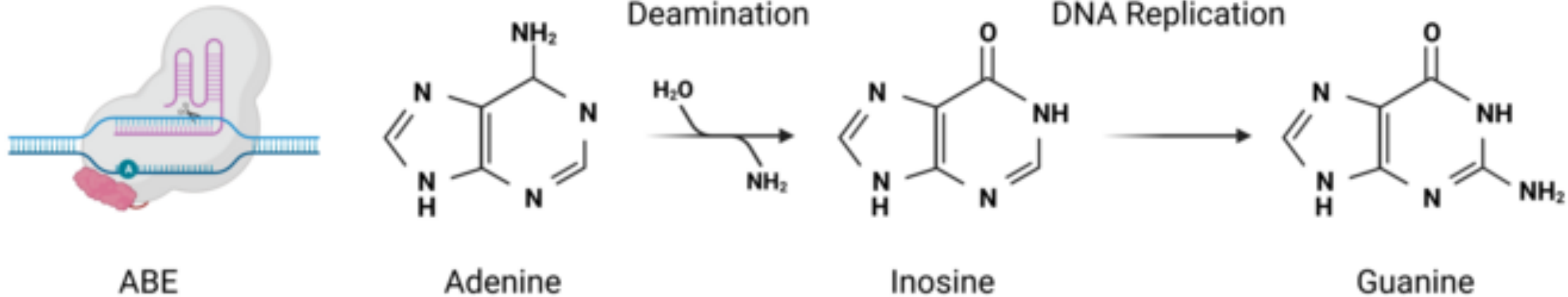


- Narrowing of coronary arteries
- Osteolysis
- Skin changes
- Vascular calcification

Aging phenotypes absent in HGPS

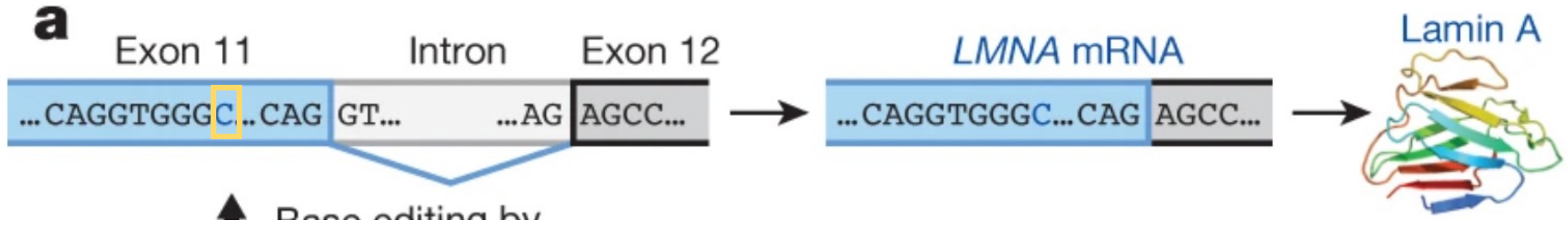
- Cancer
- Cataract
- Increased abdominal fat
- Neurodegeneration

How does base editing work?



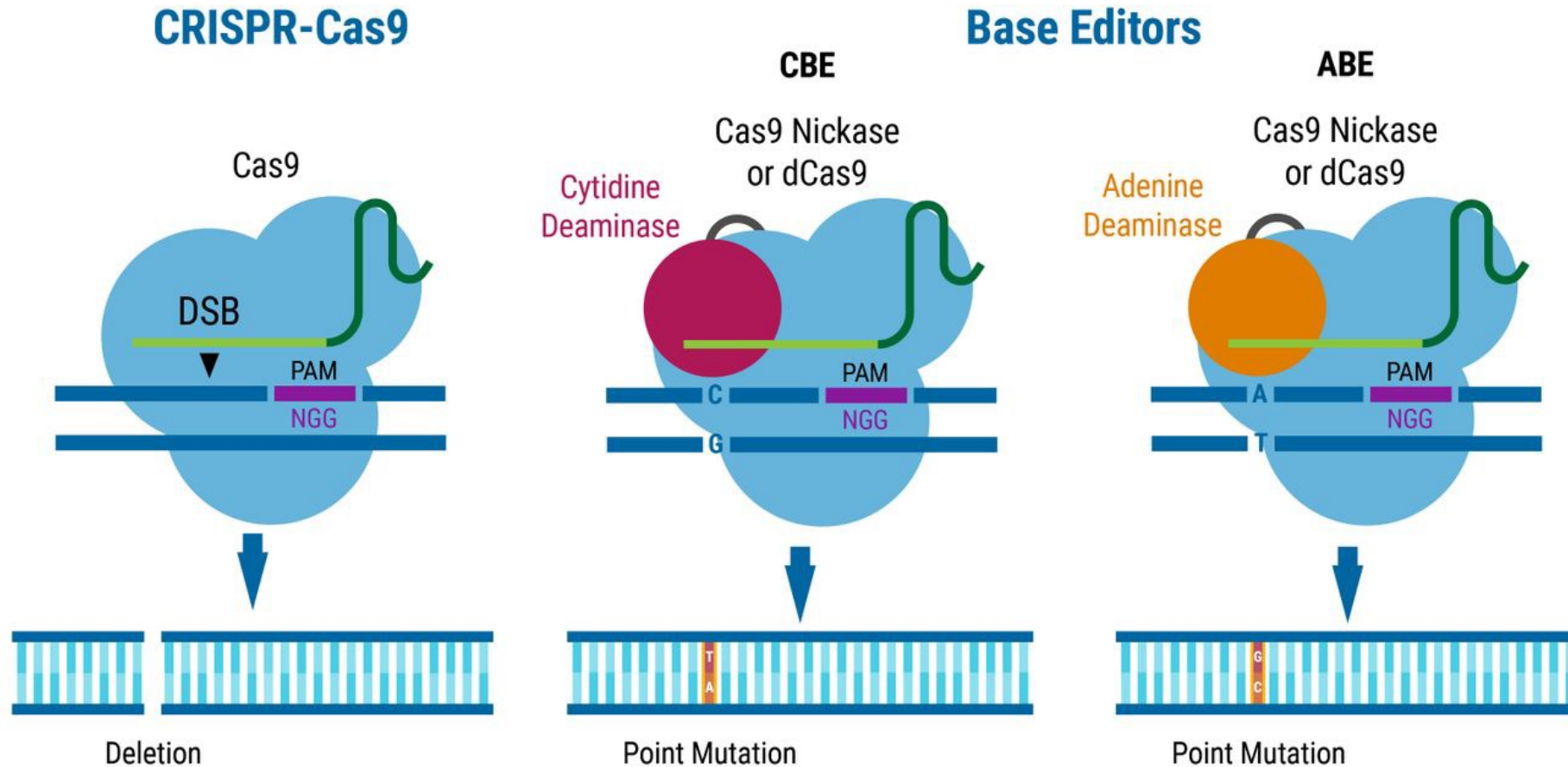
ABEs converts **A/T** to **G/C** by using a lab derived deaminase to convert adenine to inosine which can base pair like guanine

Why use an ABE system to correct Progeria?



Progeria is caused by the *LMNA* C>T mutation

Why **not** use the CRISPR-Cas9 system?



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(Labroots, 2022)

CRISPR-Cas9 generates too many **indels** and **lacks efficiency**

What is the gap in knowledge?



How was ABE used for in-vitro study?

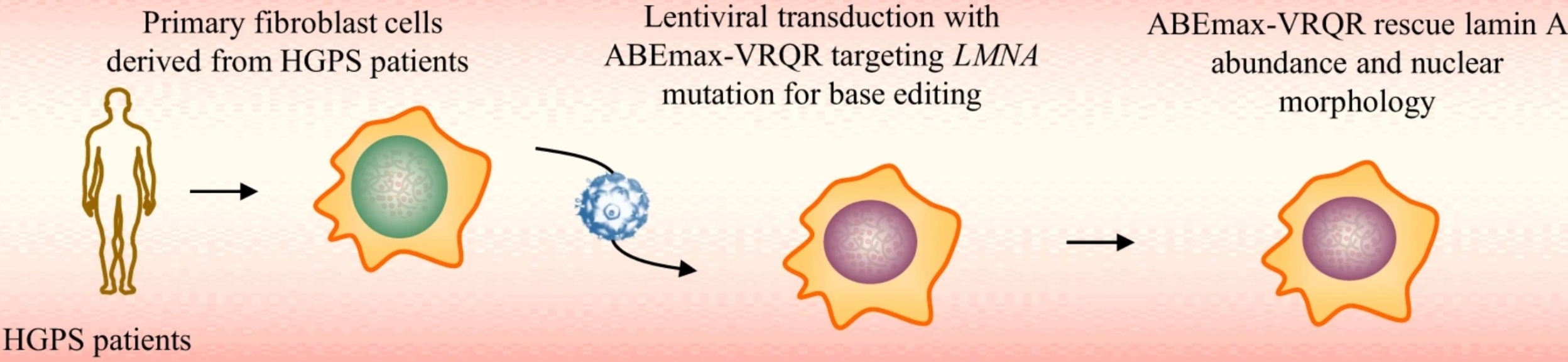
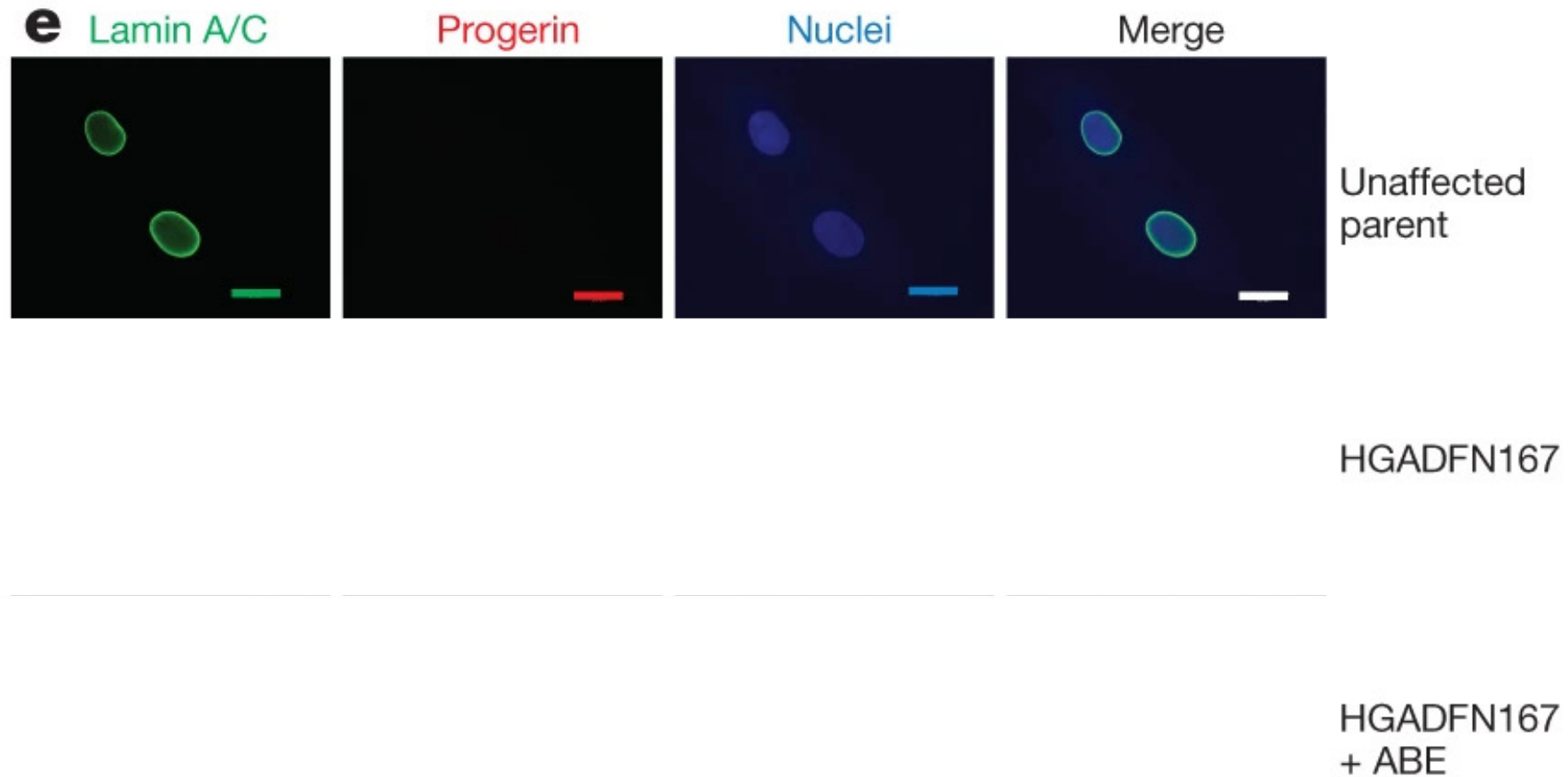
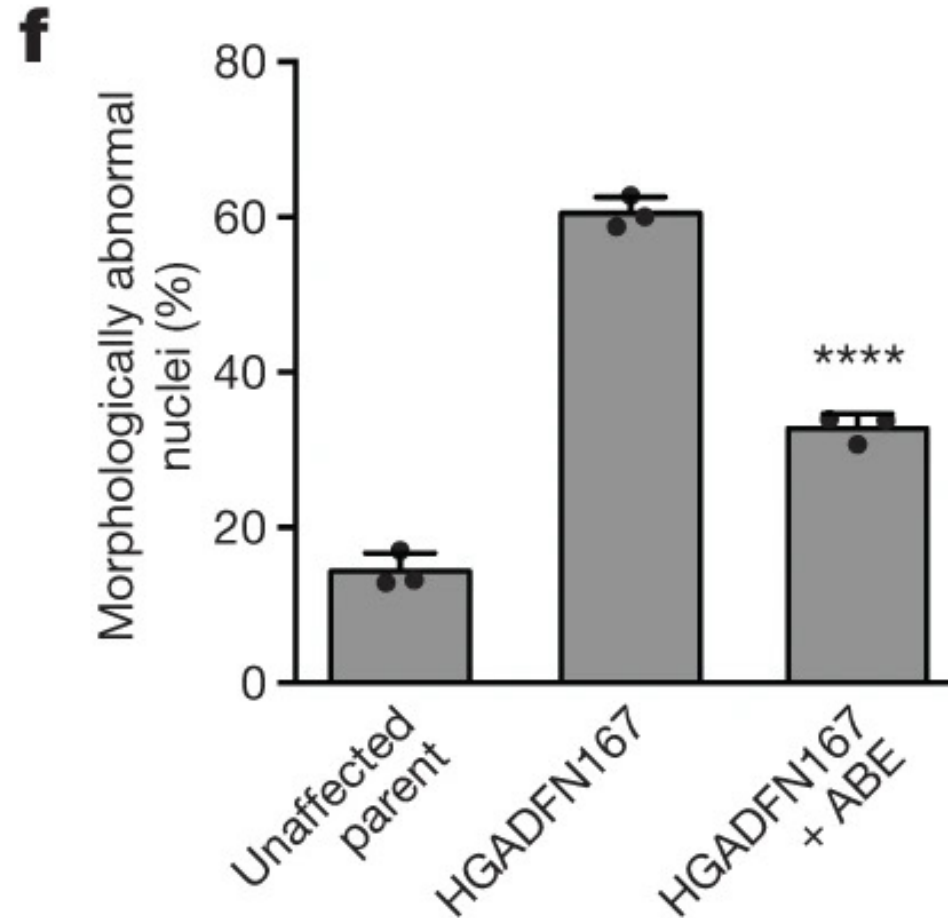


Fig 1E: Does ABE rescue **Lamin A/C** nuclear morphology?



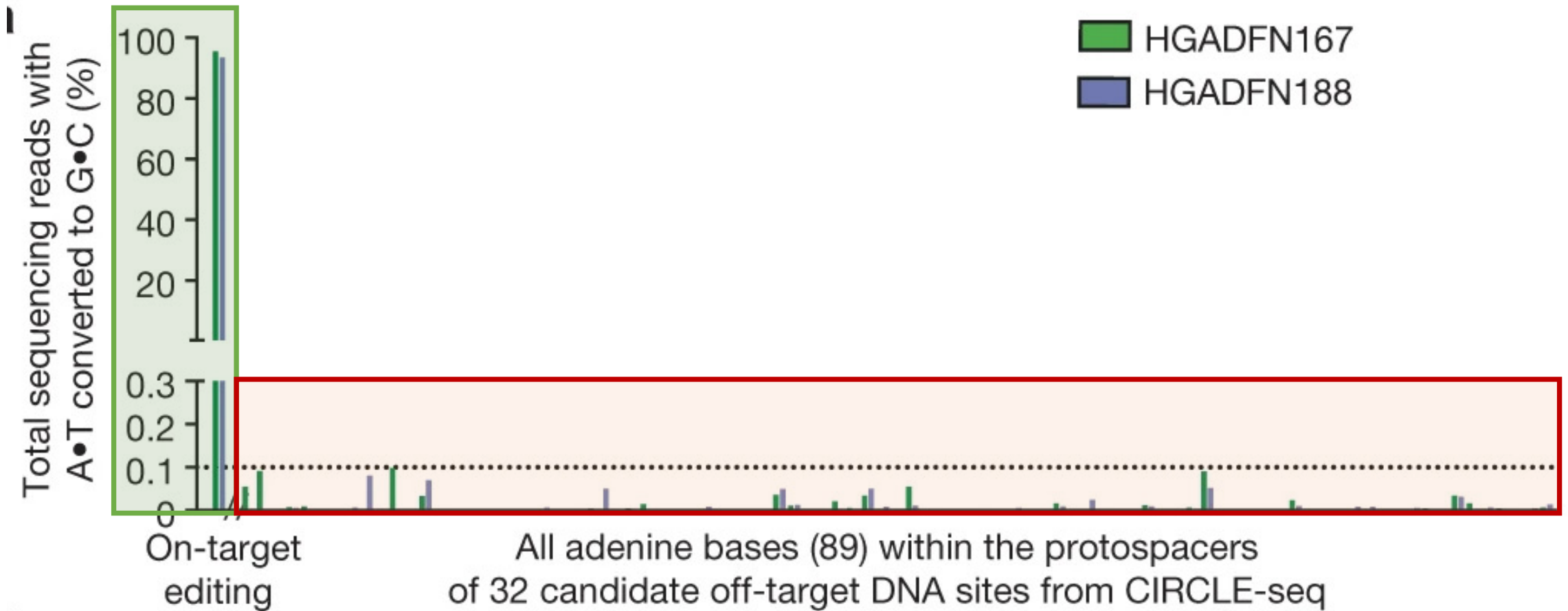
Visually showed **improvement** in nucleus structure

Fig 1F: Does ABE rescue nuclear morphology?



Compared to untreated patients, ABE treated nuclei had 1.8x less nuclear abnormalities

Fig 2A: What is the accuracy of ABE for DNA editing?



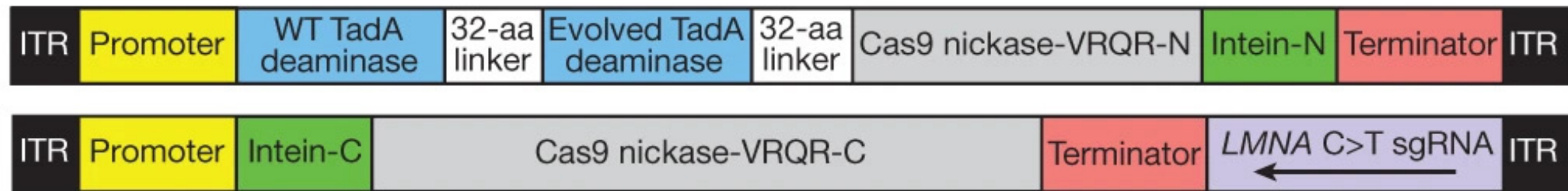
Exhibited 87-91% **on target** editing and <0.1% **off-target** DNA editing

Fig 3A: How was ABE delivered in-vivo?

ABE-AAV9 injection
P3 retro-orbital
P14 retro-orbital
P14 intraperitoneal

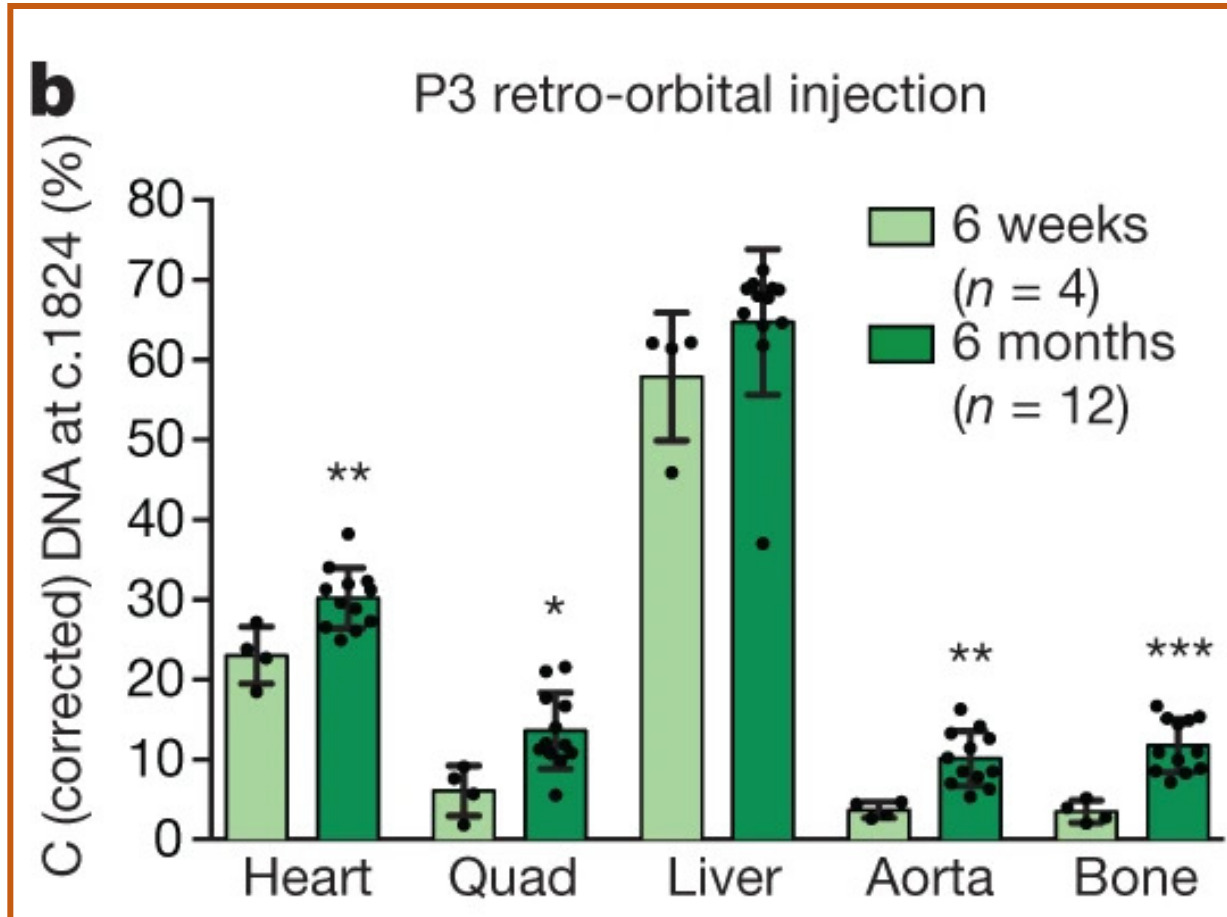


Homozygous
LMNA c.1824 C>T
(progeria) mice



Use of adeno-associated virus (AAV) to introduce sgRNA and Cas9 nickase

Fig 3B: Can ABE improve disease in various tissues?



Increased the amount of **corrected** point-mutation DNA by 10-60%

Fig 4A: Effects of ABE on tissue histopathology in females



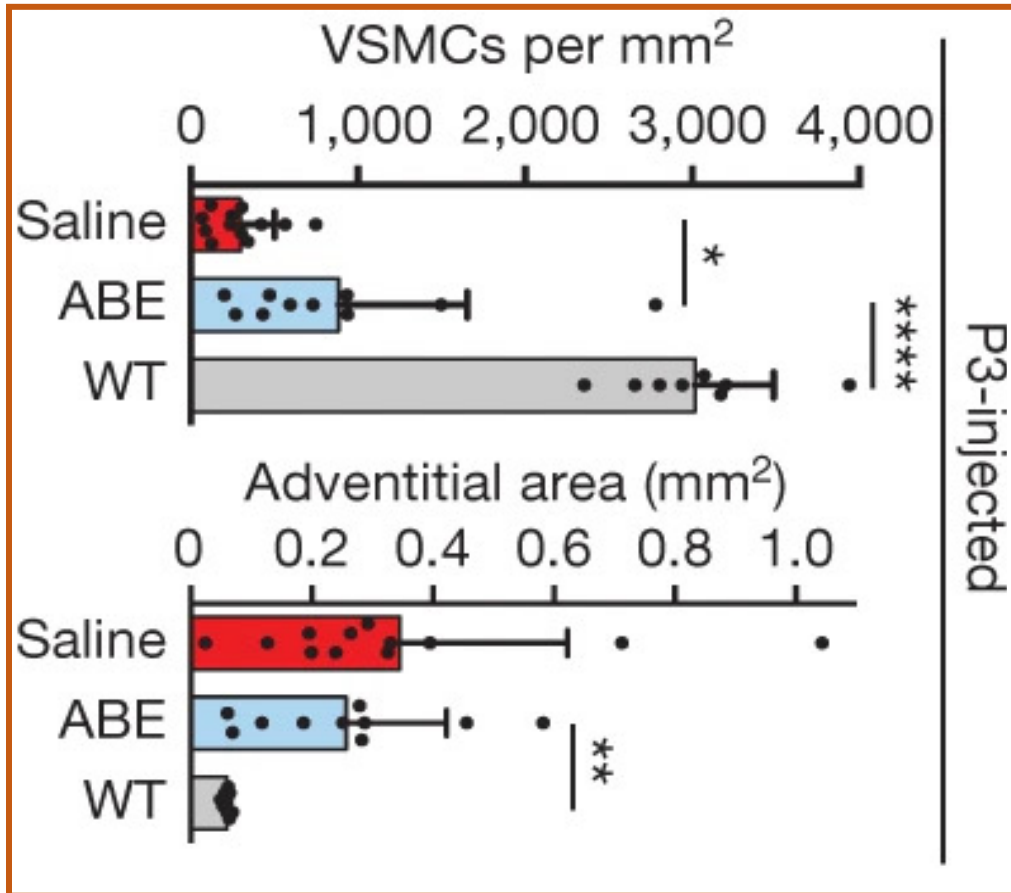
Shown **preserved VSMC counts and less adventitial fibrosis** in ABE mice

Fig 4A: Effects of ABE on tissue histopathology in males



Shown **preserved VSMC counts and less adventitial fibrosis** in ABE mice

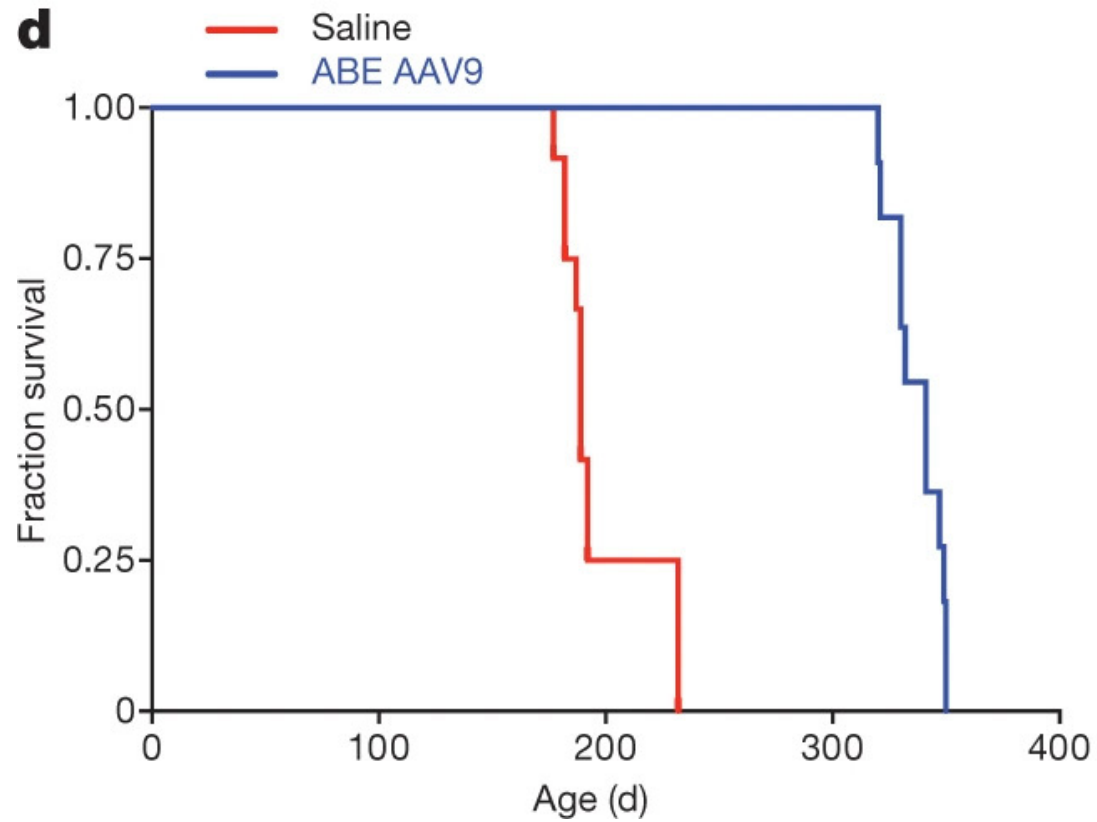
Fig 4B: What effect does ABE have on VSMCs?



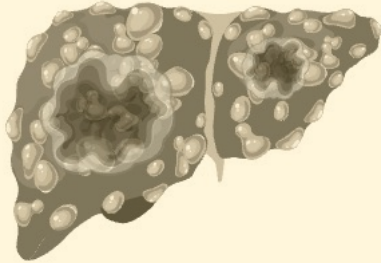
Exhibited an almost complete return to WT phenotype in **P14 mice**

Fig 4D: Can ABE-mediated correction increase lifespan in mice?

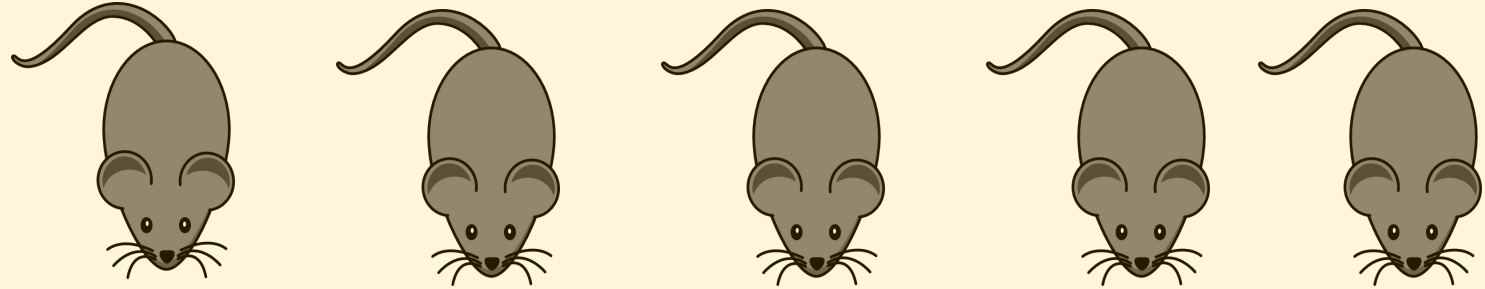
P3 Retro-orbital mice (n=12)



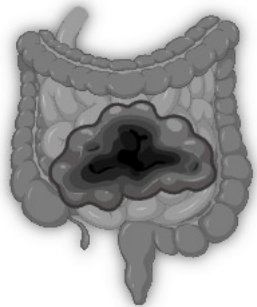
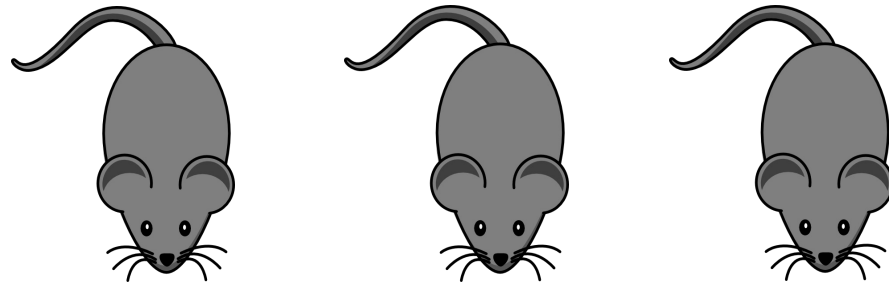
Cause of death in P14 ABE mice



Liver Tumors



Natural Causes



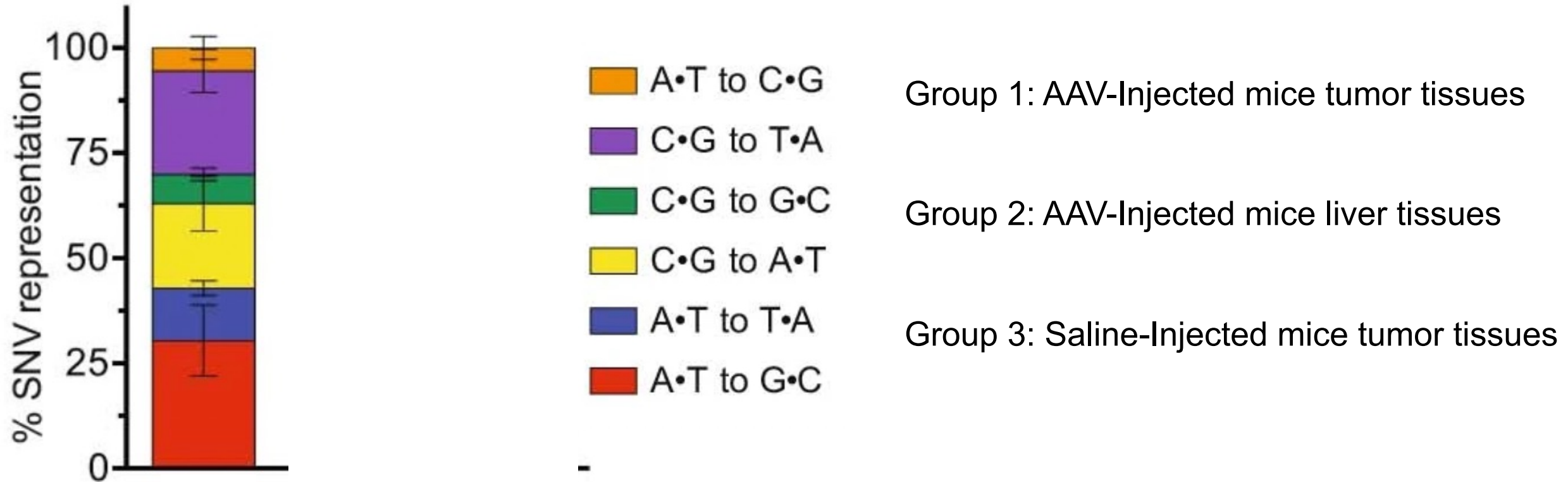
Gastrointestinal Necrosis



P14 Retro-orbital mice (n=9)



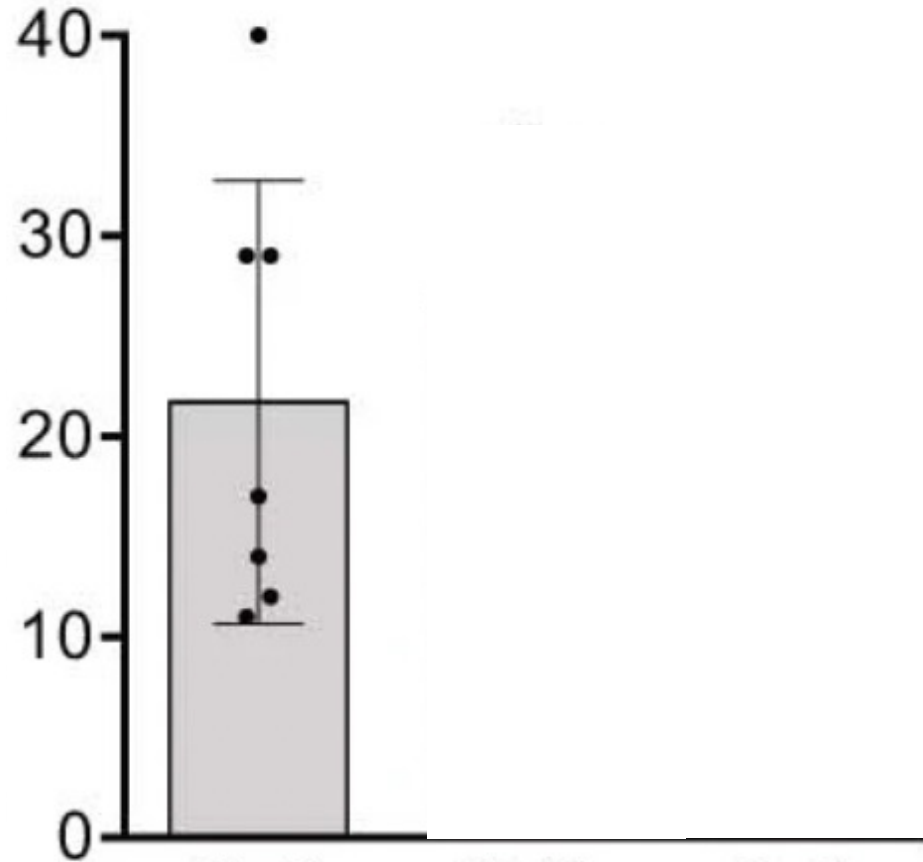
Fig 12A: Were the liver tumors potentially caused by ABE?



Similar trend in single point mutations exhibited between ABE and control groups

Fig 12C: Were the liver tumors potentially caused by ABE?

of A·T-to-G·C SNVs and indels found
in or near liver cancer-associated genes



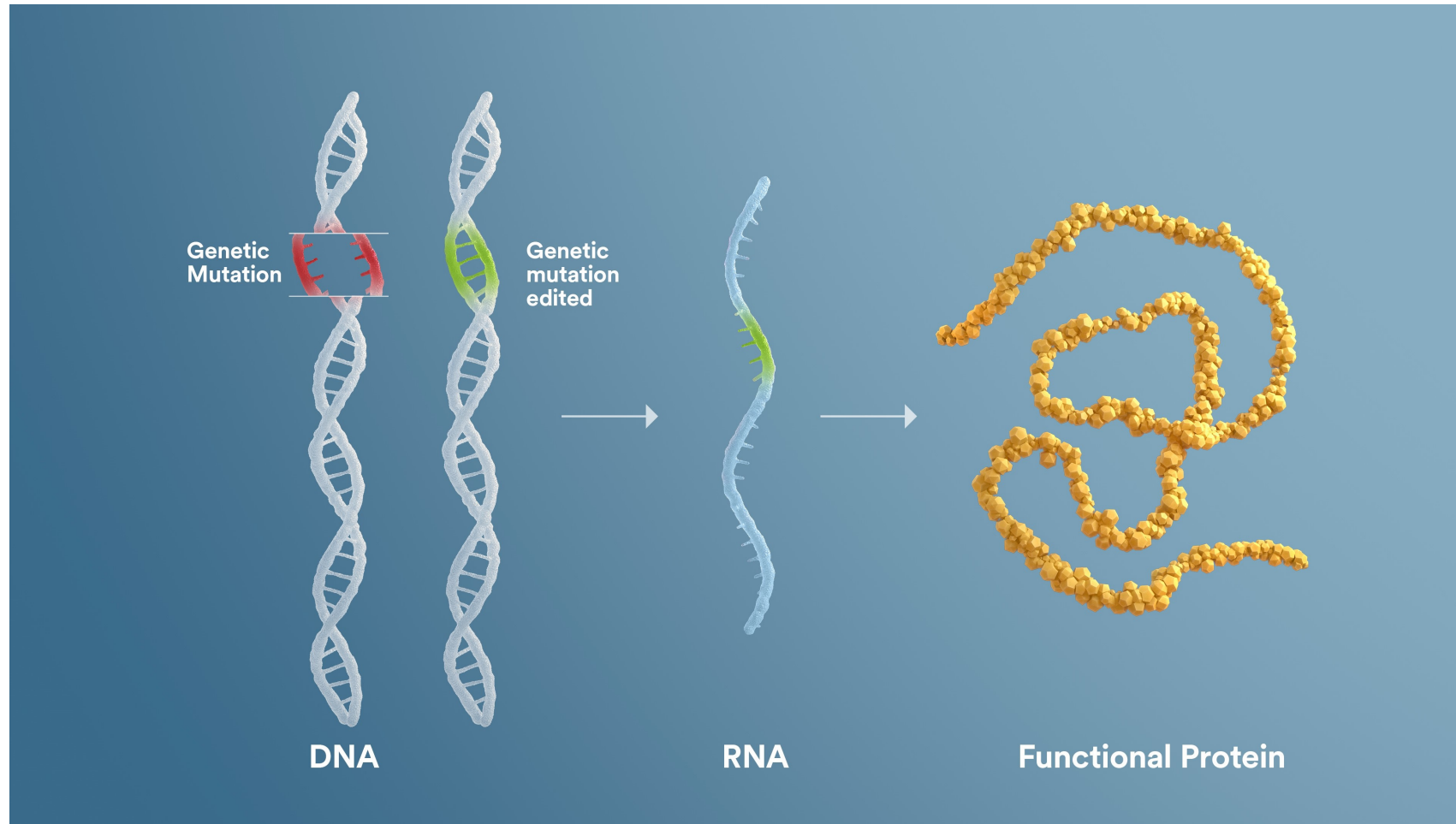
Group 1: AAV-Injected mice tumor tissues

Group 2: AAV-Injected mice liver tissues

Group 3: Saline-Injected mice tumor tissues

Similar trend in SNVs between groups in 84 recurrent liver cancer genes

Future Directions



Goals include optimizing system delivery and eventual human trials

(Sarepta Therapeutics)

Phenotype of ABE mice during the study



Who are some activists with Progeria?



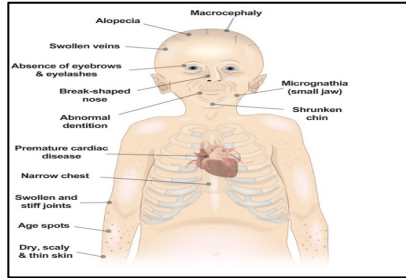
Youtuber Adalia Rose (2007-2022)



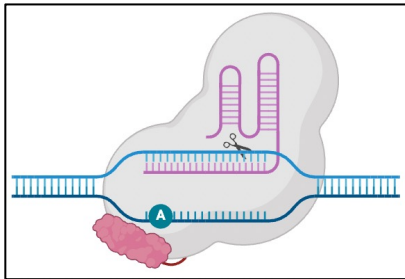
Activist Cláudia Amaral (age 23)

Summary

Progeria has a significant impact on quality of life



ABE mediated repair can effectively change the single point mutation C>T resulting in Progeria



Improvement in vascular and tissue pathology leading to a lengthened lifespan in-vivo

