Alternative CRISPR systems and CRISPR-diagnostics

Charlie Gilbert

Demonstrating CRISPR-Cas pathogen detection

- Both tubes contain Cas12 protein programmed to recognize a short target DNA sequence
- One tube with ‘on-target‘ DNA sequence
- One tube with water only
- Mix ‘em up and stick ‘em in my pocket

i. Alternative CRISPR-Cas systems

ii. CRISPR-diagnostics – a new tool for identifying and detecting organisms

iii. Applying CRISPR-diagnostics to hepatitis B virus (HBV) viral load tests

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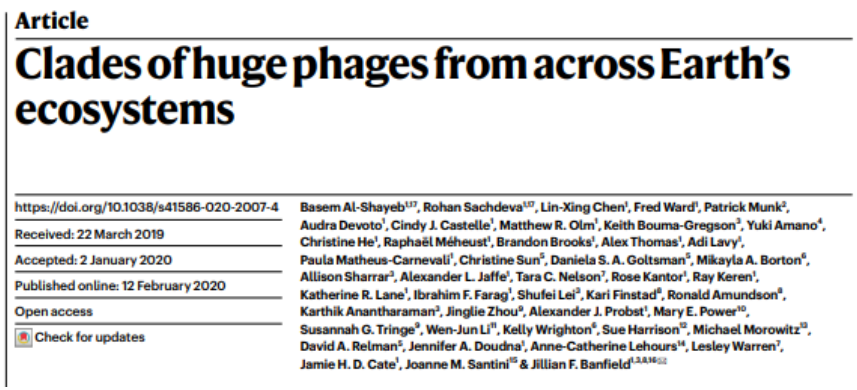
Alternative CRISPR-Cas systems

- Massive expansions in the known types of CRISPR-Cas system
- Most of this comes from ever-expanding DNA sequence databases

  → "What lives in this sludge?"

- Newly-discovered bacteriophages encode their own CRISPR systems



Article

Clades of huge phages from across Earth's ecosystems

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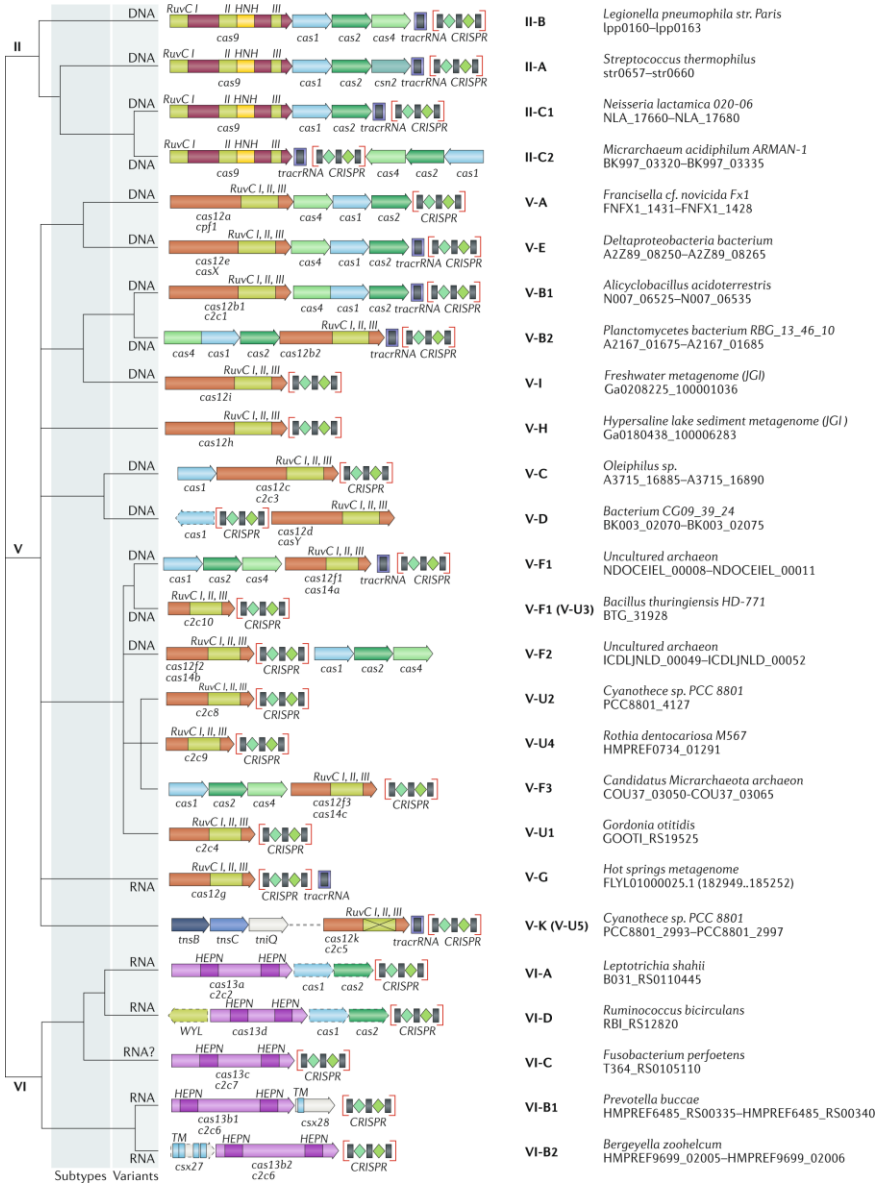

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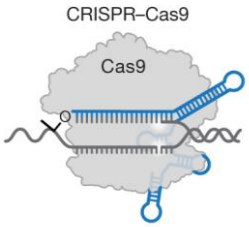

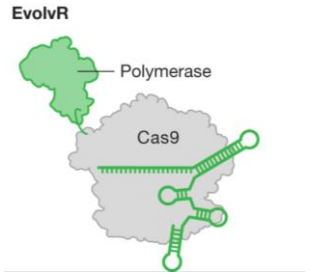

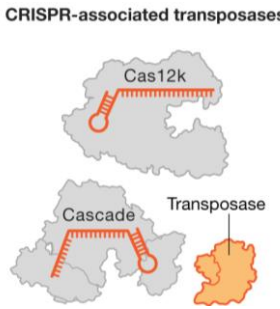

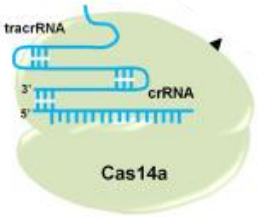

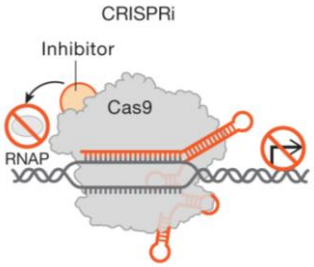

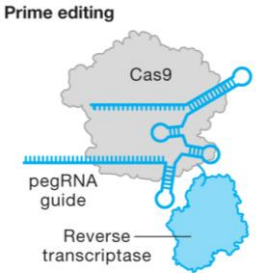

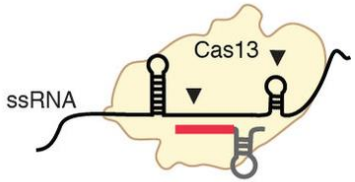

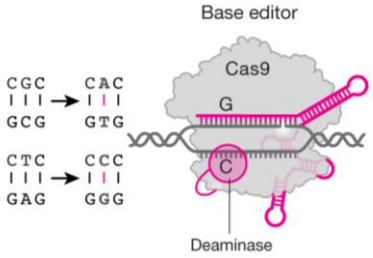

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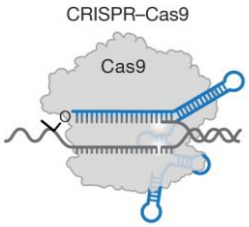

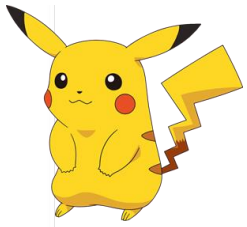

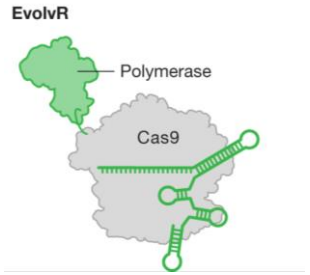

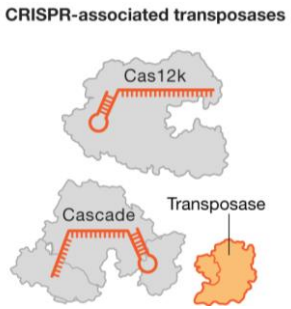

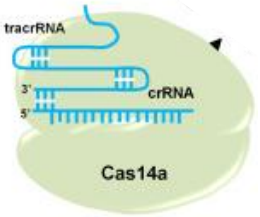

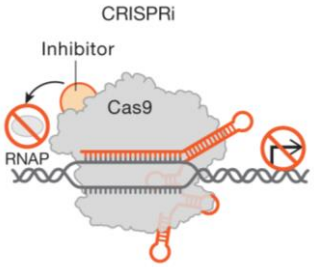

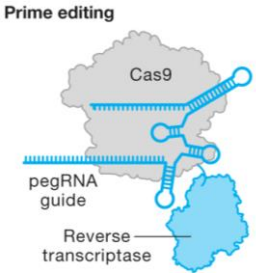

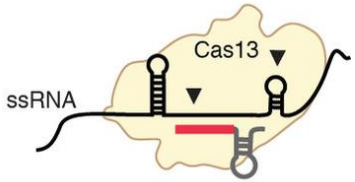

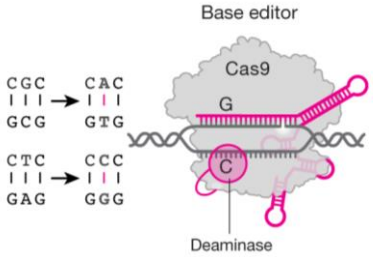

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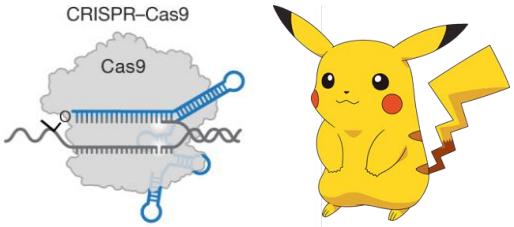

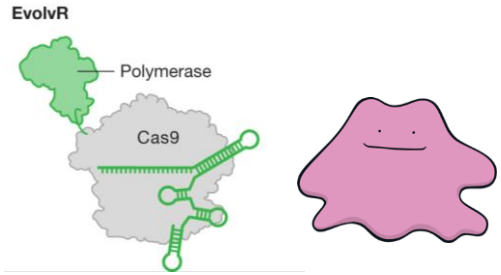

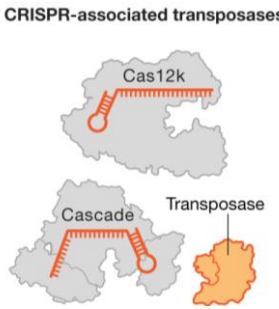

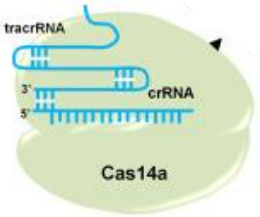

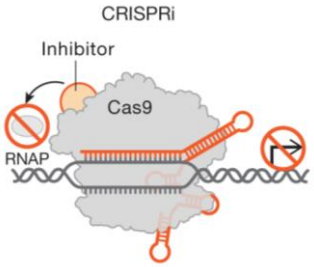

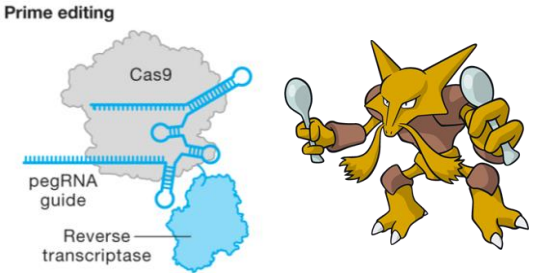

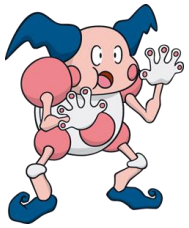

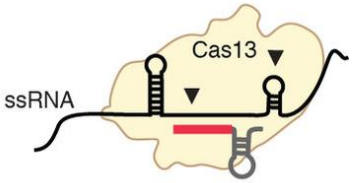

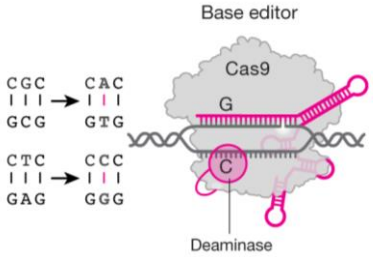

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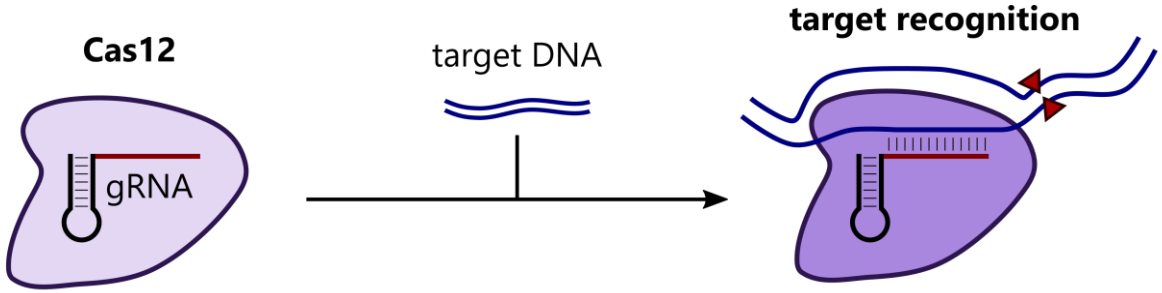
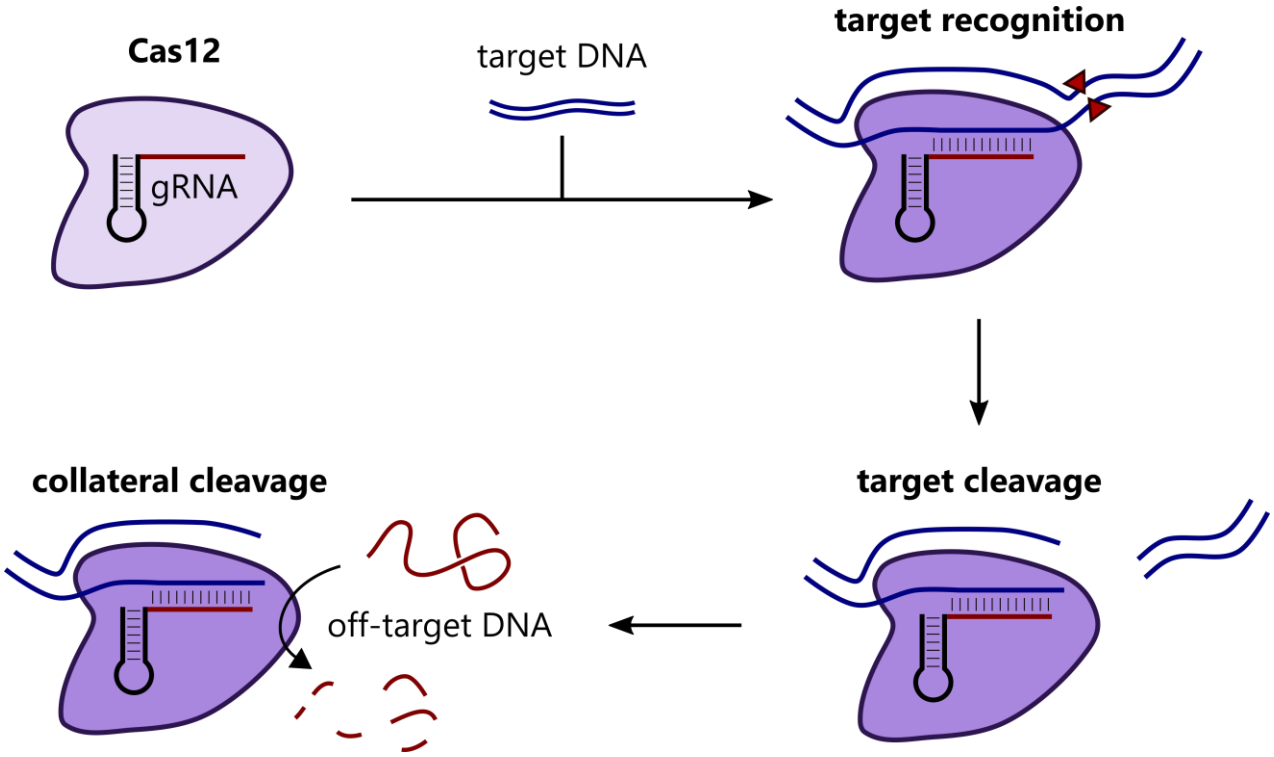
Some Cas proteins perform *collateral cleavage*



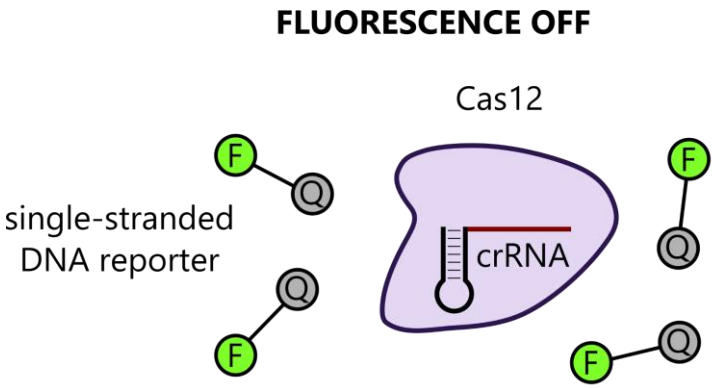
– Cas12 works like Cas9 – target what you want with guide RNA

Some Cas proteins perform *collateral cleavage*



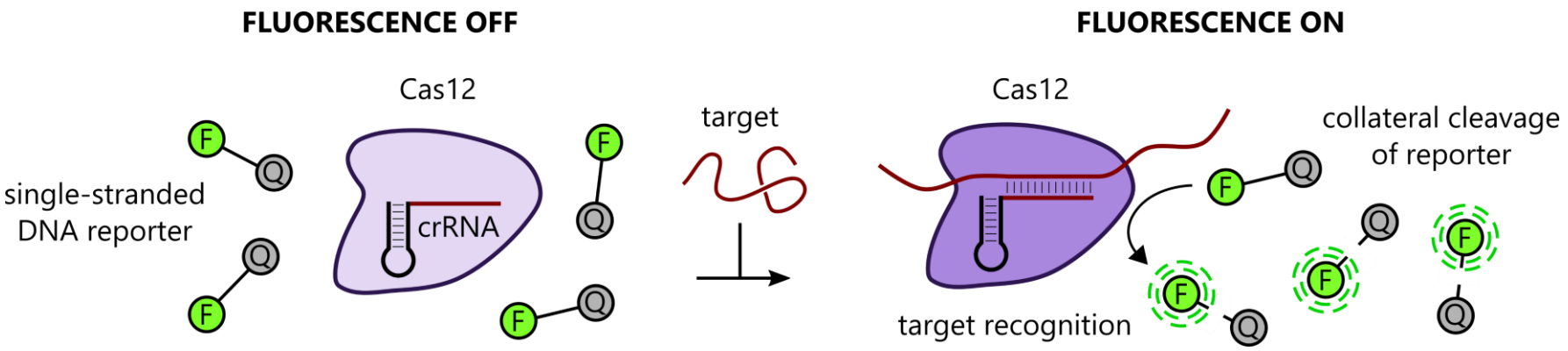
- Cas12 works like Cas9 – target what you want with guide RNA
- But, once target is cleaved, Cas12 enters ‘collateral cleavage’ state and goes berserk

CRISPR-Cas diagnostic systems



– Fluorescent molecule is linked to a quencher molecule by DNA

CRISPR-Cas diagnostic systems



- Fluorescent molecule is linked to a quencher molecule by DNA
- When target DNA is added, Cas12 collateral cleavage activity releases the fluorescent molecule from the quencher
- Cas12 is a programmable DNA sensor
- Several systems use this approach: SHERLOCK, DETECTR, HOLMES

sensitivity too low to detect many pathogens, amplification step required

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Hepatitis B virus (HBV)

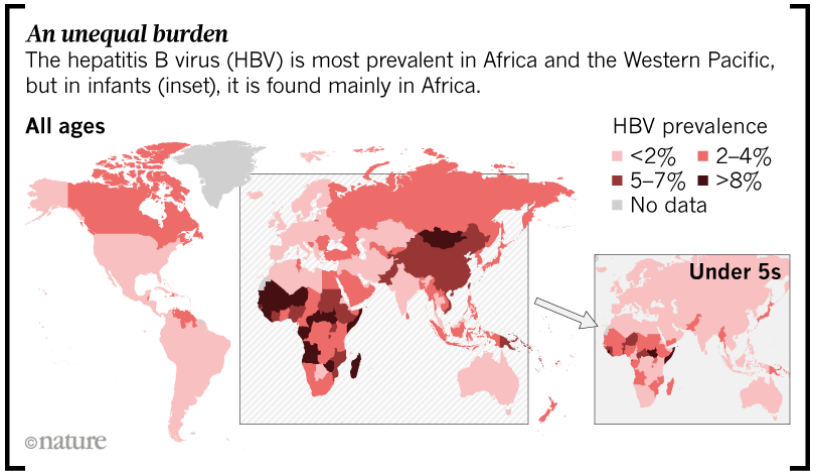
- In 2015, of 257 million people with chronic HBV infection – 3% of world population

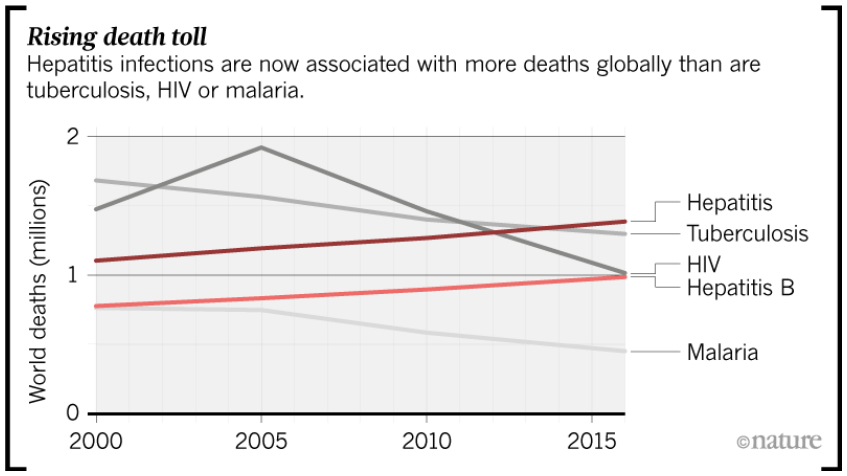
  → Of which 9% knew their status

- Highly effective vaccine and antiviral therapy available

- But there is still a high global mortality – estimated 887 000 deaths in 2015

- Many of the barriers to effective treatment are for socioeconomic reasons





Hepatitis B virus (HBV) viral load tests

- Tell you how much virus is in the blood of a patient, affects treatment decisions
- Currently qPCR-based: costs ~$200, not point-of-care
- The need is for: semi-quantitative, cheap, point-of-care tests for use in low-resource settings

Research Article
Viral Hepatitis

JOURNAL
OF HEPATOLOGY

The WHO guidelines for chronic hepatitis B fail to detect half of the patients in need of treatment in Ethiopia

Hanna Aberra[1], Hailemichael Desalegn[1], Nega Berhe[2,3], Bitsatab Mekasha[1], Girmay Medhin[2], Svein Gunnar Gundersen[4,5], Asgeir Johannessen[3,6,*]

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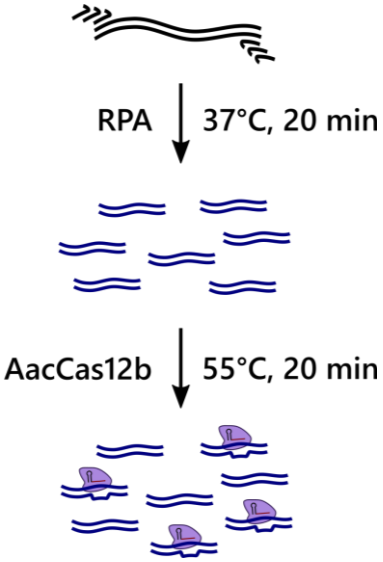
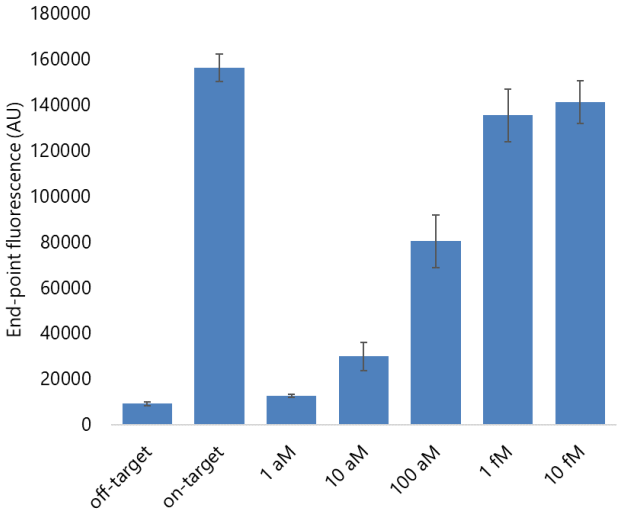
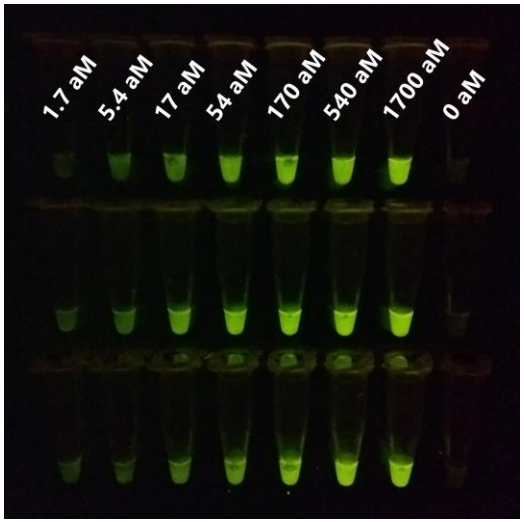
Developing a quantitative test for HBV DNA

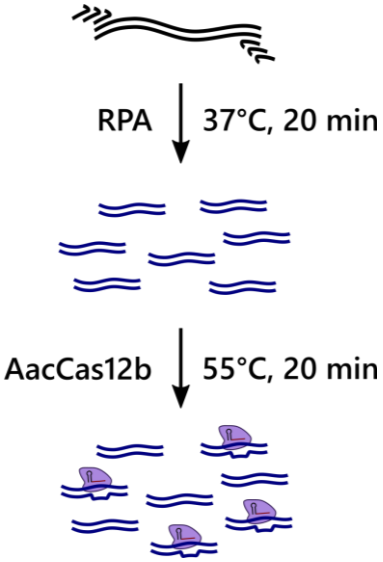


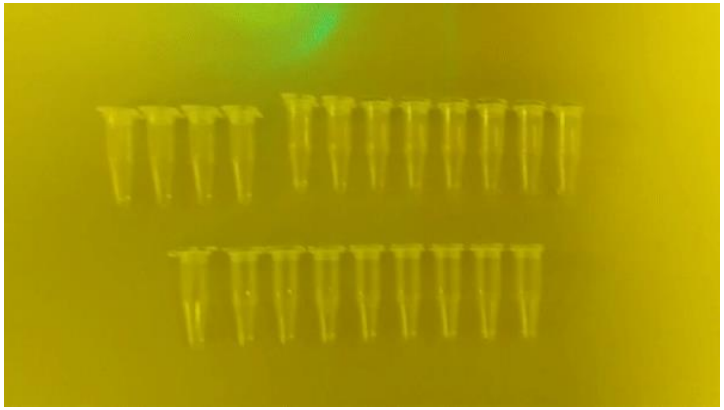


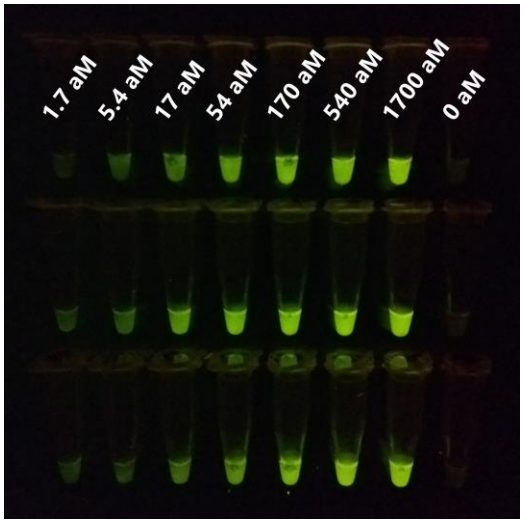


– We developed a semi-quantitative method to detect HBV DNA

Developing a quantitative test for HBV DNA

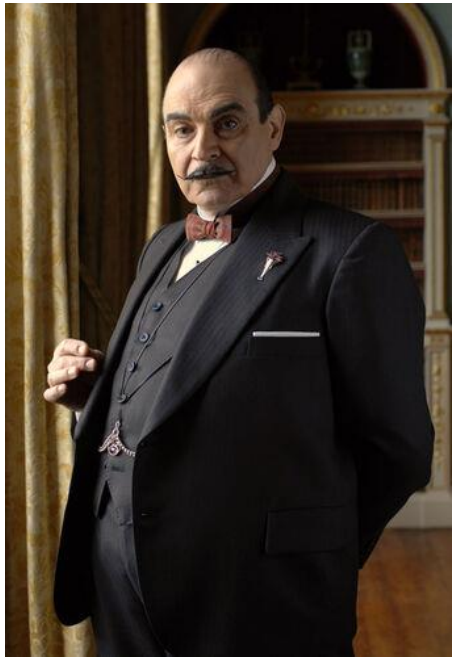


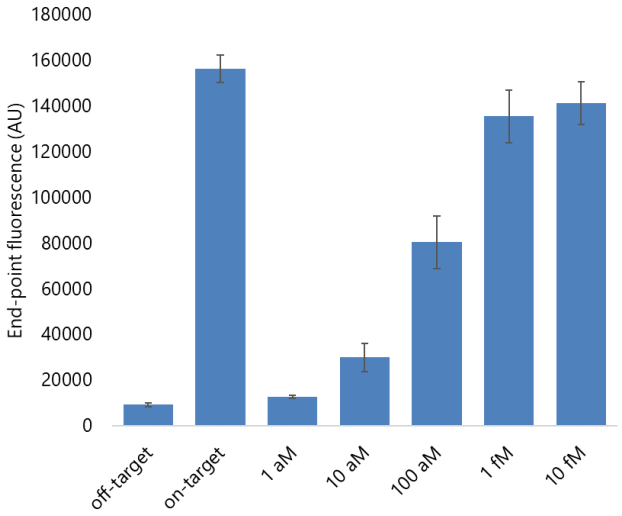


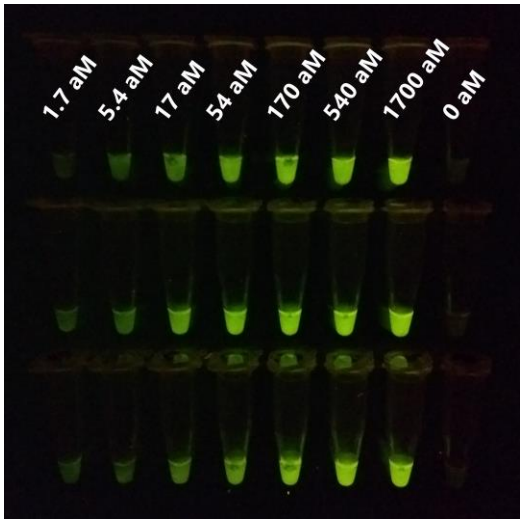


- We developed a semi-quantitative method to detect HBV DNA
- Fluorescence can be visualized with simple equipment

Developing a quantitative test for HBV DNA







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HERCULE – Heat-**e**xtinguished **R**PA for **C**RISPR-Dx **u**tilisation in **l**ow-resource **e**nvironments

Applying CRISPR-diagnostics to other diseases

- CRISPR-diagnostics for numerous other targets under development:

  → Zika, Dengue, HIV, typhoid, antimicrobial resistance, African swine fever virus, shrimp white spot syndrome

- Multiple SARS-CoV-2 diagnostics already developed

CRISPR-based surveillance for COVID-19 using genomically-comprehensive machine learning design

Hayden C Metsky, Catherine A Freije, Tinna-Solveig F Kosoko-Thoroddsen, Pardis C Sabeti, Cameron Myhrvold

doi: https://doi.org/10.1101/2020.02.26.967026

This article is a preprint and has not been certified by peer review [what does this mean?].

An ultrasensitive, rapid, and portable coronavirus SARS-CoV-2 sequence detection method based on CRISPR-Cas12

Lucia Curti, Federico Pereyra-Bonnet Sr., Carla Gimenez

doi: https://doi.org/10.1101/2020.02.29.971127

This article is a preprint and has not been certified by peer review [what does this mean?].

- Other than commercially and medically important pathogens, what else?

  → Which mushroom safe to eat?

Who know what other CRISPR systems are yet to be discovered?

Thank you

[check your pocket]