Rapid helicase-SSB assisted isothermal amplification of long DNA fragments

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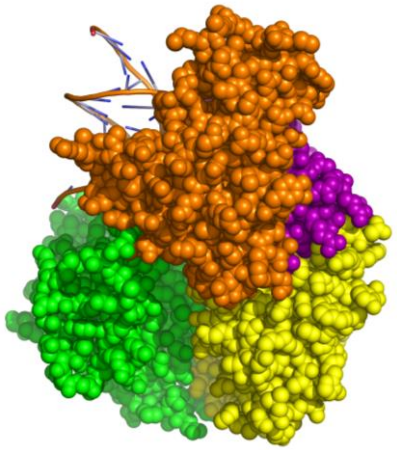
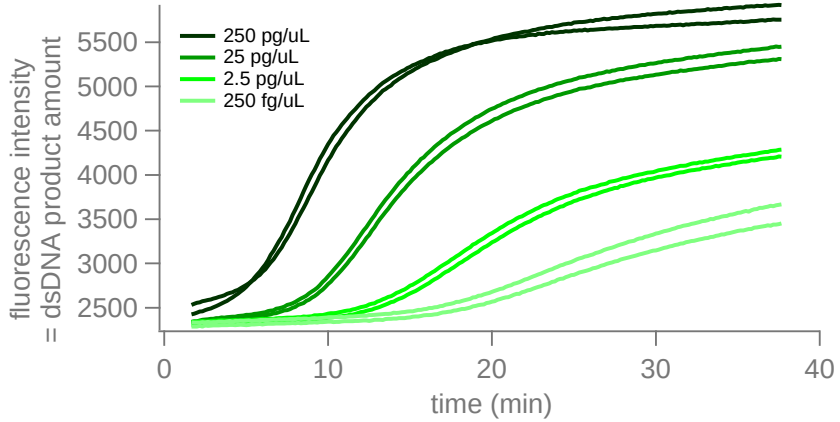
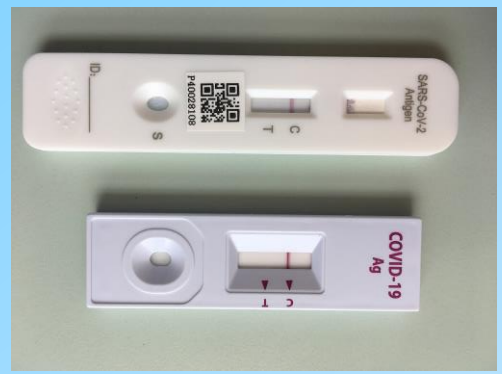




Motivation: Rapid PCR Covid-19 test

- Antigen test



Low sensitivity
Low specificity

Instant result
Home test

- PCR test

High sensitivity
High specificity

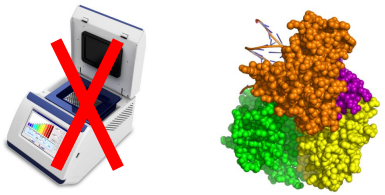
No instant test
No home test

We want to enable instant and home-based “PCR” tests!

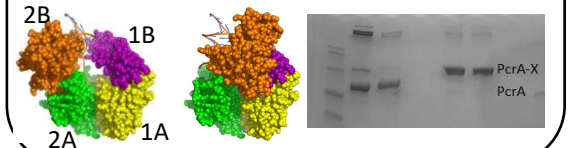
Rapid helicase-SSB assisted isothermal amplification of long DNA fragments

- We introduce isothermal PCR (Polymerase Chain Reaction)
- Our method is called SHARP (**S**SB-**H**elicase **A**mplification for **R**apid **P**CR).
- Key advantages of SHARP over traditional PCR or other isothermal DNA amplification methods:

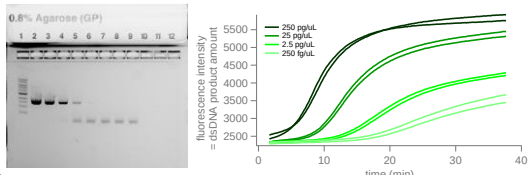
1. SHARP eliminates thermal cycler from PCR



2. SHARP's secret sauce ingredient is an engineered superhelicase



3. SHARP is faster than PCR with a cleaner product



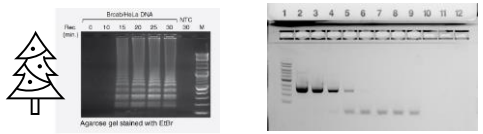
4. SHARP amplifies different template sizes equally well

155 bp 1436 bp

3245 bp 6816 bp

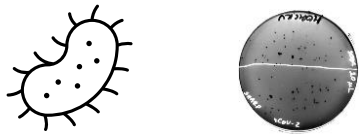
5. SHARP outputs a clean single-band product

LAMP* SHARP



*Loop-Mediated Isothermal Amplification

6. Living cells can take SHARP-made plasmid and survive



7. SHARP can amplify complex (CAG)n , (TA)n, and G4 repeats.

(preliminary result)

8. Acknowledgement:

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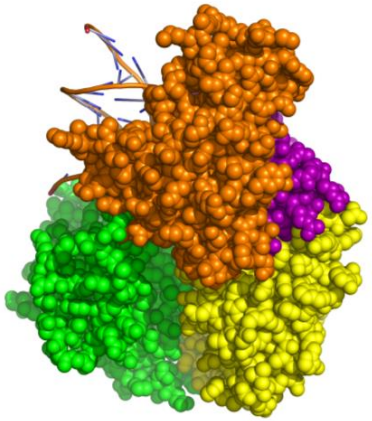
*More details about each subpanel are available on separate slides. Ask me!

Traditional vs isothermal method

PcrA
+ ATP



Traditional PCR
(**P**olymerase **C**hain **R**eaction)

DNA duplex
(1 template)

2 copies

Melting
95°C

Thermal cycling

Primers
extension

Single strands

Cooling
to 65 °C

Polymerase

Isothermal SHARP
(**S**SB-**H**elicase **A**mplification for **R**apid **P**CR)

DNA duplex
(1 template)

2 copies

helicase opens
the duplex

DNAP extends primers
and displaces any SSB,
duplex or helicase

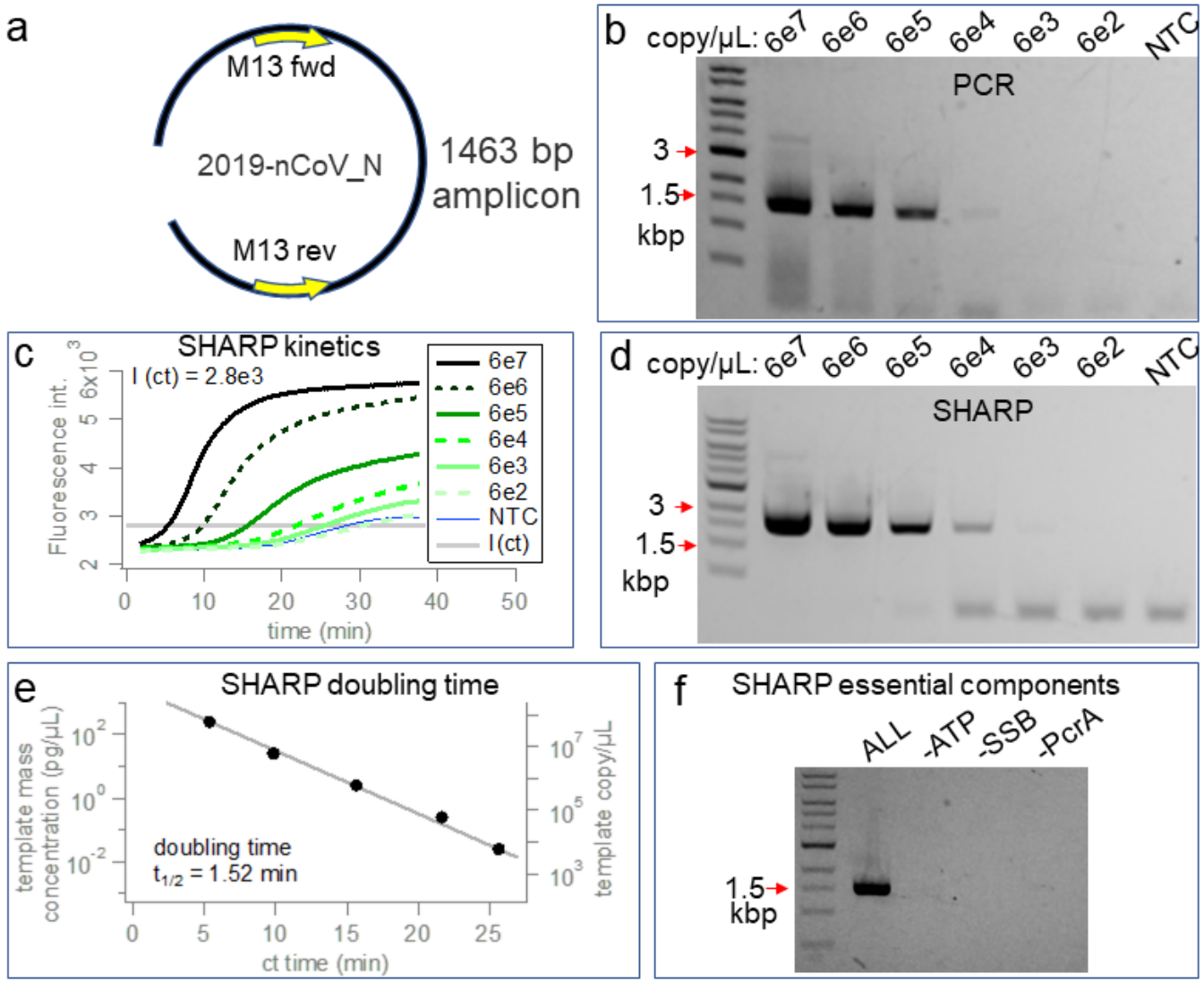
5’

SSB

primers

SSB and primers bind

PCR product vs SHARP product



PCR and SHARP amplification product. a) 2019 coronavirus nCoV-2 N protein sequence between M13 primers. b) PCR product and detection limit for the template-primer set in a. c) Kinetics of the SHARP reaction for the template-primer pair in a. d) SHARP product and detection limit enables the direct comparison with PCR. e) SHARP doubling time estimate. Ct time is the intercept of the intensity curve with the threshold in c. f) SHARP essential components. In the absence of ATP, SSB, or PcrA the reaction fails.

Other amplicons:

Other SHARP products. a) 3 kbp SHARP amplicon. b) 6 kbp SHARP amplicon c) 200 bp SHAR amplicon using lambda DNA as a template. d) SHARP doubling time estimate for 200 bp product. e) Detection limit versus the amplicon length. For short amplicons, SHARP can detect 1 molecule in a test tube. f) Doubling time shows that short amplicons replicate at the highest speed.