New method of developing diagnostic tests could help tackle future pandemics

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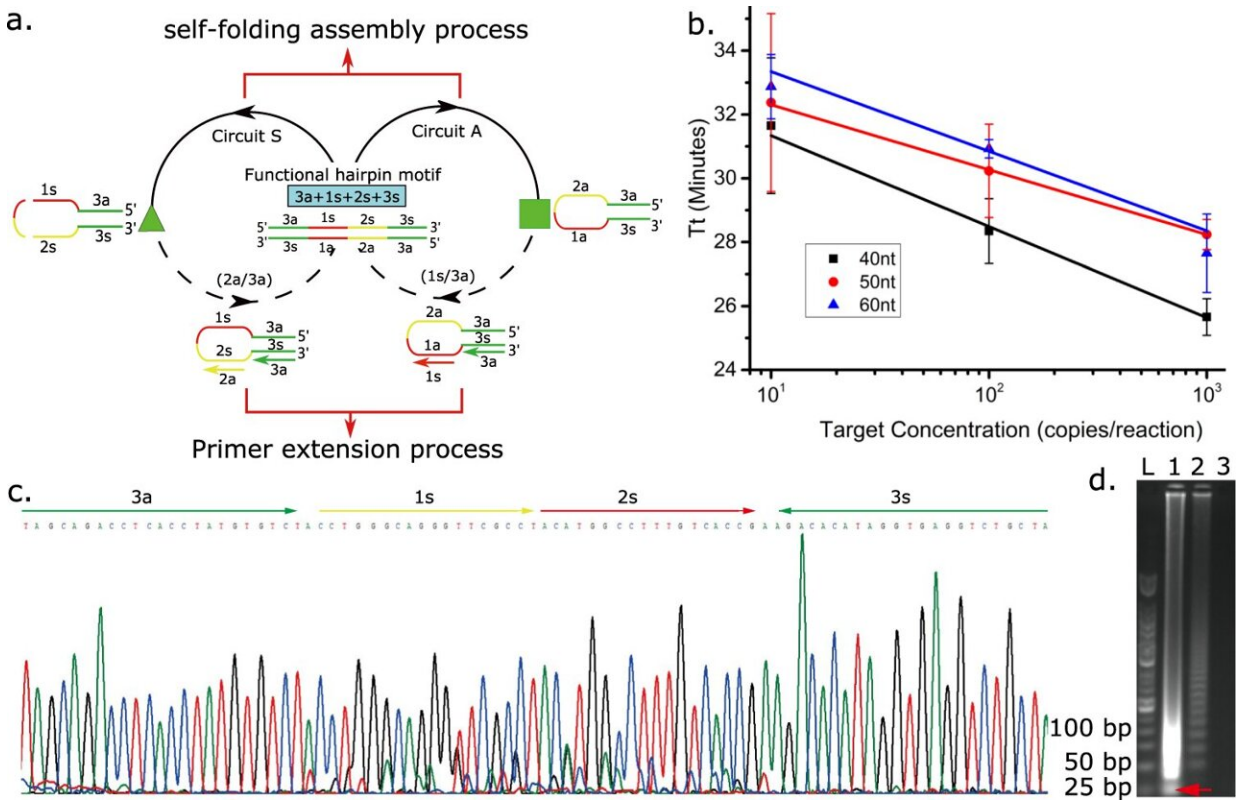

Fig. 1: Programming a nucleic acid isothermal amplification pathway as a reaction graph. Programming a nucleic acid isothermal amplification pathway as a reaction graph in which the hairpin complex acts as the initiator and encodes two priming sites in the loop region: (a) A schematic for generating the secondary structure mechanism and reaction graph from the functional hairpin motif-mediated isothermal amplification. The letters a/s denote the complementarity of the nucleic acid sequence and the numbers represent specific fragments in the functional hairpin structure (e.g. 2a is complementary to 2s). During their amplification, the functional hairpin motif double strand

product (blue box) disassembles (solid arrows) to form sense (green triangle) and anti-sense (green square) strand products. The formation of self-folding products exposes the priming sites which leads to the hybridisation of the primers and extension of the sequence by the strand-displacement DNA polymerase (dashed arrows) to generate a double strand amplicon. The amplicon then becomes the target (or initiator) for further amplification cycles via circuit S and A; b The reaction kinetics of the mechanism described in Fig. 1a with different lengths of loop fragment from 40 nt (black squares), 50 nt (red disk) and 60 nt (blue triangle). Data are presented as mean values; error bars are standard deviation, N = 3 independent experiments; c Sequencing of product (3a + 1 s + 2 s + 3 s) where the amplification products were digested (lane 1 in (d)), cloned and sequenced, identifying as tandem repeats; d Agarose gel electrophoresis of the amplification products. Lane L: 50 bp ladder; Lane 1: amplification product digested by the restriction enzyme Xba I. Lane 2: amplification product (with initiator); Lane 3: amplification product (without initiator). The gel is representative of experiments repeated independently three times with similar results. Credit: *Nature Communications* (2022). https://www.nature.com/articles/s41467-022-29101-1

Software that helps speed up the process of creating new diagnostic tests could help combat future pandemics, its developers say.

A team of bioengineers and chemists in Scotland and China have developed a system that suggests new reaction pathways to accelerate the design and development of new diagnostic assays.

The system, which is now freely available for other researchers around the world to investigate, adapt and use, can also be used to identify the early stages of non-infectious diseases like cancer, which could help patients receive more timely treatment.

In a new paper published today in the journal *Nature Communications*, researchers from the University of Glasgow in the UK and Shanghai Jiao

Tong University in China describe how they developed and demonstrated the effectiveness of their system.

They began by developing reaction graphs—representations of the biomechanical processes that enable rapid diagnostic tests like cross priming amplification (CPA) and loop-mediated DNA amplification (LAMP).

Unlike polymerase chain reaction, or PCR, tests, which require access to sophisticated labs operated by trained staff, isothermal tests like LAMP can offer quick, accurate results by creating interactions between chemicals and the DNA strands contained in patient samples and delivering rapid results at the point of care.

However, in many cases, those rapid tests are designed and developed for a specific purpose, which can introduce unnecessary complexity and make it difficult for one test to be easily adapted for use in a different diagnosis.

The researchers developed a more generalizable approach to the creation of new tests, building a software tool capable of turning the reaction graphs into suggestions as to how chemical primers and reactions could be used to create the desired diagnostic results.

In the paper, the research team describe how they probed the effectiveness of the software by using it to design the chemical primers and reactions for four different diagnostic tests—three for infectious diseases and one for cancer, a noncommunicable disease.

They successfully created a multiplexed test for a form of HIV with high levels of sequence variations, a highly sensitive test for tuberculosis, and a study for analyzing patient clinical samples for the presence of hepatitis B.

They also developed an assay to detect short miRNA sequences relevant in the diagnosis and prognosis of cancers including oral squamous cell carcinoma, breast cancer and glioma.

They used their newly-designed diagnostic assays to test patient-derived samples from clinical laboratories in China. Then, they confirmed their results using separate PCR tests. They tested their results against LAMP diagnostics for the same diseases, finding that their results were more specific and reproducible than the LAMP tests.

Professor Jon Cooper, of the University of Glasgow's James Watt School of Engineering, is the paper's lead author. Professor Cooper said: "We've been working for a number of years now on developing isothermal tests for diseases like malaria and hepatitis C for use in parts of the world where reliable access to PCR testing is limited.

"Over the course of building those diagnostic systems, it became clear that we were also building an understanding of how we could make a more generalizable approach to testing for specific biomarkers.

"Our programmable system automates a lot of the early trial-and-error work that goes into the development of new tests, and we've shown that it can be used to reliably diagnose a useful cross-section of communicable and non-communicable diseases. It's an exciting discovery, and suggests many potential applications in medicine."

Dr. Julien Reboud, a co-author of the paper from the University of Glasgow, added: "The COVID-19 pandemic has taught the world about how vitally important it is to quickly develop accurate, sensitive, specific diagnostics to track new diseases and direct treatment.

"Our programmable system offers one new route to supporting that kind of fast diagnostic development. We're keen to make it as accessible as

possible to other researchers around the world, so we've made all our graphs and data freely available online. We hope that it will be of real use to researchers and clinicians across a wide range of applications, and we look forward to seeing the new applications they will find for the system."

Dr. Gaolin Xu, a co-author of the paper based at Shanghai Jiao Tong University, added: "During my Ph.D. in Professor Cooper's group, I developed new technologies for the detection of DNA. I always felt that there should be a better way to develop new diagnostic assay designs. When I returned to China, I continued the collaboration with my colleagues in Scotland to develop this new and exciting diagnostic system."

The team's paper, titled "Programmable Design of Isothermal Nucleic Acid Diagnostic Assays through Abstraction-based Models," is published in *Nature Communications*.

More information: Gaolian Xu et al, Programmable design of isothermal nucleic acid diagnostic assays through abstraction-based models, *Nature Communications* (2022). DOI: 10.1038/s41467-022-29101-1.
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