# An instrument-free, programmable approach for nucleic acid detection

INSPECTR is a technique for detecting nucleic acids that couples the sensitivity and specificity of nucleic acid splinted ligation with the versatile readouts of cell-free gene expression. The result is an ambient-temperature workflow that enables the detection of pathogenic viruses at low copy numbers.

**This is a summary of:**
Phillips, E. A. et al. Detection of viral RNAs at ambient temperature via reporter proteins produced through the target-splinted ligation of DNA probes. *Nat. Biomed. Eng.* https://doi.org/10.1038/s41551-023-01028-y (2023).



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## The mission

Diagnostic technologies for detecting nucleic acids that combine the sensitivity and specificity of laboratory tests (such as quantitative PCR) with the affordability and instrument-free format of lateral-flow-based antigen tests are sorely needed to democratize and decentralize access to health care[1]. Current diagnostic processes often require sending samples to a centralized laboratory equipped with costly instrumentation and infrastructure. Moreover, the turnaround time for processing these samples can be days to weeks, delaying appropriate health decisions and postponing care. The ideal diagnostic test will provide an unambiguous visual result, can be executed by an untrained user at the point of need, and can run without an external supply of power or a heat source.

## The solution

We developed a diagnostic technique based on the detection of nucleic acids that we dubbed internal splint-pairing expression-cassette translation reaction (INSPECTR). INSPECTR uses two steps; in the first, which is based on splint-ligation[2], a single-stranded DNA probe forms a ternary complex with the complementary nucleic acid target (or 'splint'). DNA polymerases then synthesize the complementary strand of the single-stranded DNA probe to form a double-stranded DNA expression cassette. For an instrument-free readout, we use cell-free gene expression[3] to enable the synthesis of proteins from the expression cassette using purified transcription and translation components outside of the cell. INSPECTR can directly detect the genome of RNA viruses with sensitivity and specificity, transducing the ligation event into chosen proteins that are readily detectable and measurable. In the present study, we used INSPECTR to produce the common reporter enzymes β-galactosidase, which enabled a colour-change readout; nanoluciferase, which luminesces; and a wide array of custom-built, dual-epitope peptides that we could capture using lateral flow to generate a visible test line. We then built libraries of INSPECTR probes that were tailored to individual pathogen nucleic acid sequences and their respective reporter readouts to test for the presence of respiratory pathogens in clinical, anterior nasal swab samples.

The modularity of the cell-free gene expression reaction allowed us to generate unique peptide reporters that were each barcoded to a separate test line on a lateral flow test, allowing the user to detect multiple target sequences from several respiratory pathogens at the same time, in one pot. We also enhanced the sensitivity of the assay by specifically amplifying only ligated probes (Fig. 1), which enabled us to develop an INSPECTR assay to detect severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in clinical samples. INSPECTR assays can therefore provide the performance of a molecular diagnostic device with the form factor and readability of an antigen test.

## Future directions

By integrating splint-ligation with cell-free gene expression, we have developed a method to transduce the presence of a nucleic acid target sequence into a chosen protein output. The flexibility of using different reporter proteins allows for the final implementation of the technology to be adapted to the specific testing needs; for instance, a quantifiable luminescent output provided by the expression of a luciferase that is proportional to viral load could be used to indicate disease progression and severity, while the multiplexed lateral flow readout could enable syndromic testing to rule in or rule out possible pathogens. This modularity makes INSPECTR a highly flexible strategy for decentralized diagnostics.

The programmability of INSPECTR enables the assay designer to select from a range of features including a clear visual readout, quantification, high sensitivity and multiplexing. However, we note that the combination of some of these capabilities, such as the highly sensitive detection of multiple RNA targets simultaneously, has not been described in this study. Furthermore, the current assay's multi-step workflow and lengthy time-to-result limit its current implementation as an at-home product. Improving the user workflow of INSPECTR by streamlining concurrent and consecutive reaction steps will improve its suitability for use by untrained users.

Beyond viral RNA targets, detection of DNA targets is an obvious next step that we have only begun to demonstrate in our extended data. By leveraging ligation site specificity[4], the INSPECTR assay could be used to detect specific single nucleotide polymorphisms, such as those associated with cancer risks or antimicrobial resistance. As a fully instrument-free nucleic acid testing method, INSPECTR can improve the accessibility of sensitive molecular tests to consumers across the health, agricultural and biodefence industries.

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## EXPERT OPINION

"Phillips et al. present a multiplexed probe ligation-based assay for the detection of pathogen-associated nucleic acids, using detection of SARS-CoV-2 genomic RNA as the timely use case. The authors appropriately explored different readout modalities, and their relative strengths and weaknesses. This technology may one day be used to encode programmable therapeutics that are translated on demand in response to the detection of a pathogen." **H. Benjamin Larman, Johns Hopkins University, Baltimore, MD, USA.**

## FIGURE

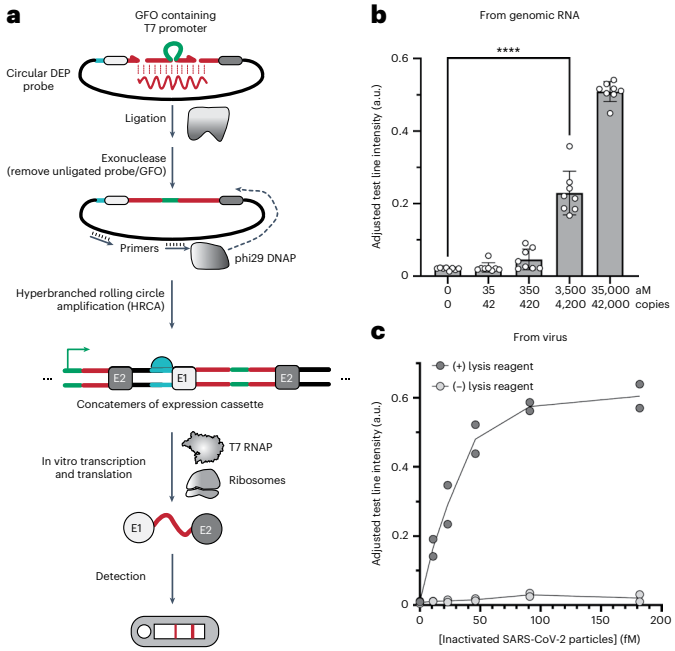


**Fig. 1 | INSPECTR enables sensitive detection of a nucleic acid target. a**, Schematic of the INSPECTR process with a lateral flow readout. Target RNA (red curved line) splints a gap-filling oligo (GFO) to the 5′ and 3′ ends of a dual epitope peptide (DEP)-encoding probe at complementary hybridization regions (solid red) to enable the enzymatic ligation of a circular expression cassette precursor. Polymerization and amplification of the precursor by DNA polymerase (phi29 DNAP) forms an expression cassette. The T7 promoter (green) is recognized by the T7 RNA polymerase (RNAP) to initiate transcription. When transcribed, the ribosome binding site (blue) is recognized by the ribosome to initiate translation of a dual epitope (E1, grey; E2, dark grey) peptide that is visualized on a lateral-flow strip. **b**, Sensitivity of the assay on a structured genomic RNA (gRNA) target. The rolling circle amplification-assisted assay was run on a dilution series of gRNA extracted from SARS-CoV-2. Concentrations of SARS-CoV-2 RNA as low as 3.5 fM (approximately 4,000 cps) could be detected on a lateral-flow strip. **c**, Detection of intact viral particles. Following the development of a rapid lysis method, intact SARS-CoV-2 could be detected with a limit of detection similar to that of extracted gRNA. 

## BEHIND THE PAPER

In our team, we explore infectious disease diagnostic technologies from a product development perspective. Since our inception, we have aimed to address the unmet need for low-cost, distributable diagnostic solutions that can be deployed without sophisticated laboratory equipment. We had already been investigating how the INSPECTR technology could inform disease status when the COVID-19 pandemic began and further exposed inequalities in access to infectious disease testing, and we stepped up to meet SARS-CoV-2 testing needs. A critical improvement to the assay sensitivity was made when we determined that an ambient-temperature target pre-amplification step could be incorporated into the workflow. This finding enabled us to test clinical samples containing SARS-CoV-2 and to differentiate SARS-CoV-2 from other respiratory infections, all without using specialized lab instruments. **E.A.P.**

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## FROM THE EDITOR

"This accurate assay for the detection of multiple nucleic acids is particularly amenable to point-of-care uses, as it works at ambient temperature, does not need instrumentation and is compatible with the standard lateral-flow format." **Editorial Team, *Nature Biomedical Engineering*.**