Paperback diagnostics

By Lauren Martz, Staff Writer

A paper-based diagnostic platform invented by **Harvard University** bioengineers could provide a cheap, portable and easy-to-use system for detecting pathogens in hospitals or developing-world settings.[1] The technology uses embedded gene expression systems and can detect isolated mRNA biomarkers, but it will need to detect sequences in complex biological samples before it can be put into practice beyond the lab.

The diagnostic was created by a team led by James Collins at the **Wyss Institute for Biologically Inspired Engineering at Harvard University** who wanted to find real-world applications for synthetic gene networks. According to Collins, those systems have not gained traction yet in treatment settings because of concerns over safety and the complex apparatus involved.

"Synthetic biology has been stuck in the lab due to the skills and equipment required," he said. He added that systems that use living cells such as bacteria require biosafety measures that prevent widespread use of the technology, so he thought a paper-based system could provide an "abiotic medium that could be used to explore and experiment safely."

The urgent need for faster and better diagnostics,[2] in particular in emerging markets, gave Collins a therapeutic application in which his technology might provide a major benefit.

The system his team produced involves freeze-drying cell-free expression systems onto small paper discs that can be stored at room temperature and widely distributed. The discs can be customized to detect specific pathogens and respond with a colorimetric output when exposed to samples containing the relevant antigen.

Collins is a founding core faculty member at the Wyss Institute and a professor of biomedical engineering and co-director of the Center of Synthetic Biology at **Boston University**. He is also a **Howard Hughes Medical Institute** investigator.

> **"The ability to build genetic networks that are active on paper creates a completely new format that is inexpensive, capable of high throughput and very portable for distribution and storage."**
> ***—Timothy Lu, Massachusetts Institute of Technology***

Paper translation

Gene expression systems contain all the machinery from a cell required to transcribe and translate genes, including amino acids, ribosomes, enzymes and supplementary factors. Although these systems are widely used in solution-based reactions, the solution-phase systems are not stable for use outside the lab.

The first step for Collins' group was to test whether a gene expression system would retain its activity when deposited onto paper discs. The team reasoned that the paper, which contains a capillary-action matrix, might support low-volume, cell-free transcription and translation of genes.

The team used commercially available gene expression systems derived from either bacterial or human cells and embedded them on paper discs together with DNA encoding a fluorescent protein, then freeze-dried the discs. When water was added to rehydrate the discs, the fluorescent protein was expressed. Although the team did not directly compare the translation efficiency of solution-phase and paper-based reactions, they found that the volume of reagent solution required for the solution-phase reactions was about fivefold greater than that needed for the paper reactions.

When the gene expression systems were freeze-dried, stored at room temperature for at least a year and reactivated with water, translation of the fluorescent protein was as efficient as translation using fresh-from-frozen systems.

Next, the team tested the technology in a diagnostic application by pairing it with a new design of a synthetic RNA switch—a construct that binds a specific RNA of interest and then changes conformation to allow translation of a signal output gene. The RNA switches designed by the team triggered translation of a gene encoding a fluorescent or colorimetric protein and generated a visual output signal only in the presence of a specific biomarker.

The team's RNA switches contained two ligated RNA segments: a 'toehold' RNA segment complementary to an mRNA biomarker and a signal output gene mRNA. When the biomarker mRNA was not present, the toehold segment blocked translation of the signal gene by obstructing its ribosomal binding site. When the biomarker mRNA was present, it bound the toehold segment, causing it to release the ribosomal binding site and allow translation (*see* **Figure 1, "Paper-based diagnostic tests"**).

As proof of concept for the new platform, the group designed synthetic RNA switches to detect genes associated with resistance to four different antibiotics. Paper discs containing the RNA switches produced a colorimetric output only when the complementary resistance gene mRNA was applied to the papers. The test detected mRNA at concentrations as low as 3 nM. That range is within the physiologically relevant concentration of mRNA in a cell, according to the study authors. Signals were detected within 20–30 minutes, which is faster than most available diagnostic technologies for infectious diseases.

Because pathogenic targets and resistance develop rapidly, the researchers wanted to ensure the test could be quickly customized. They designed and prepared a diagnostic test with sensors for 24 different Ebola virus sequences within 12 hours and used 12 different mRNA

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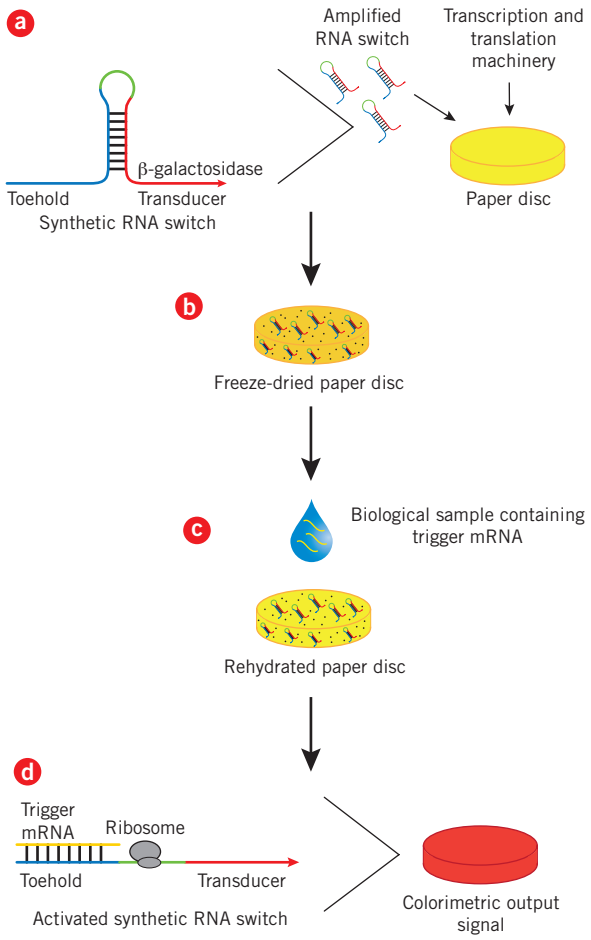

Figure 1. Paper-based diagnostic tests. [**a**] A synthetic RNA switch is designed to detect a specific oligonucleotide sequence called trigger mRNA, which acts as a marker for a particular pathogen, cell or molecule. The switch contains a toehold region that specifically binds the trigger mRNA (blue line). The toehold region is ligated to a transducer mRNA (red line), which encodes a protein to provide signal output such as β-galactosidase or GFP.

In the absence of trigger mRNA, the toehold region of the sensor restrains the conformation of the ribosomal binding site (green line) of the transducer mRNA segment and prevents it from translating the signal output protein.

Amplified mRNA switches and cell-free expression systems are applied to paper discs. The expression systems include all the elements required for the reaction to translate the signal output protein and generate a signal.

[**b**] The paper discs are freeze-dried and stored. [**c**] Rehydration activates the freeze-dried paper discs and prepares them for detection of trigger mRNA in a biological sample. [**d**] Binding of trigger mRNA (yellow line) to the toehold region of the RNA switch causes a conformational change that exposes the ribosomal binding site. This enables translation of the signal output protein, which produces a colorimetric or fluorescent signal.

sequences each from the Sudan and Zaire strains of the virus to show that the assay could accurately discriminate between the two strains.

"Our system is much cheaper and requires considerably less time for development than antibody tests," which can take months to design, Collins noted.

Results were published in *Cell*. The synthetic RNA toehold switch design was published by Collins and colleagues at the Wyss Institute in a separate study in the same issue of *Cell*.[3]

Reality bites

Researchers polled by *SciBX* said that the team will need to show the technique can detect mRNA in actual biological samples and satisfy concerns about logistics in the field to turn the idea to a reality.

"The ability to build genetic networks that are active on paper creates a completely new format that is inexpensive, capable of high throughput and very portable for distribution and storage," said Timothy Lu, an associate professor of biological engineering, and electrical engineering and computer science at the **Massachusetts Institute of Technology**.

He added that the test could be used to identify markers for a broad range of diseases, including cancer. But based on the speed, cost and portability of the system, he thought that the technology would have the most benefit in infectious diseases.

However, Lu had concerns about how well the system would fare in a real-world context. "My one primary question about this platform is about its robustness," he said. "In the presence of complex human fluids, what is the diagnostic capability?"

He added, "A test may work really well in the lab under controlled conditions, but there are a lot of complications in the real world. Many field operators won't have the training to properly use diagnostic tests, which could affect readout even with this simple test design."

In addition, he noted that extreme temperatures might affect storage and questioned whether the speed of the test would remain the same when it is applied to more complex diagnostic samples.

Alimuddin Zumla, a professor of infectious diseases and international health at **University College London**, was less upbeat about the test's potential. "They need to do an awful lot of laboratory testing and optimization to get this to work in a situation even remotely resembling actual clinical samples," he told *SciBX*. "Being able to detect a laboratory-purified mRNA transcript, which is what they have done, is a world away from being able to detect mRNA from a mixed cell extract—never mind a mixed clinical sample."

Collins told *SciBX* that his team is working to address these questions. The team is currently working with colleagues at Wyss to test whether the papers can detect the presence of an mRNA marker in more complex biological samples. He added that the paper-based tests may not be as sensitive and specific as available PCR tests, but the papers could be used as an initial indicator in triage-type settings.

However, Collins sees potential for the test beyond RNA-based diagnostics. "Our toehold riboregulator sensors are designed to detect nucleic acids," he said. "There are other riboswitches that can detect proteins and small molecules. Such switches are also compatible with our paper-based platform."

Lu agreed that—if successful—the test could go beyond diagnostics, and he suggested that the test could be adapted to incorporate sensors that monitor drug toxicity, concentration or therapeutic response.

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The Wyss Institute has filed patent applications covering the technology, and the IP is available for licensing.

Martz, L. *SciBX* 7(45); doi:10.1038/scibx.2014.1310
Published online Nov. 20, 2014

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