Trends in Biochemical Sciences | Technology of the Month

Single species RNA purification using DNA nanoswitches

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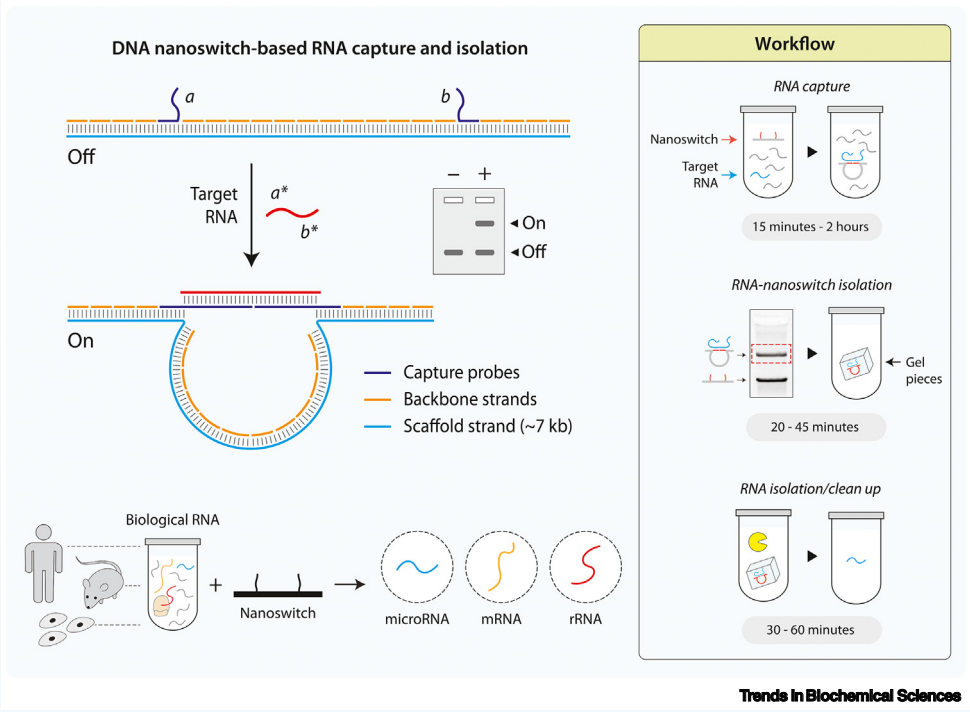
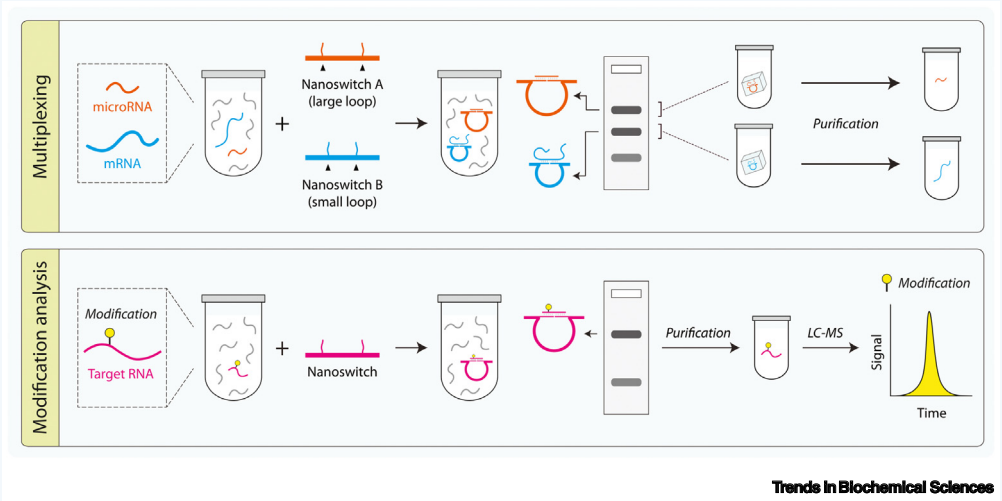


RNA purification is a critical need in biomedical research. The development of tools for analyzing structure, function, and chemical modifications of RNA have outpaced those for RNA purification. The DNA nanoswitch method is a new 'catch and release' approach for targeted purification of specific RNA sequences from complex mixtures. Capture of a target RNA reconfigures the DNA nanoswitch from the linear 'off' state to the looped 'on' state. The RNA bound to the nanoswitch can be quantified and isolated to obtain pure RNA of a single sequence. The method is useful in purifying specific microRNAs, ribosomal RNAs, and mRNAs from cellular total RNA extracts.



The nanoswitches can be programmed to capture and isolate multiple unique RNA sequences individually from a single sample. Purified RNAs are suitable for downstream liquid chromatography/mass spectrometry (LC/MS) analysis of RNA modifications, as demonstrated by identifying 2′-*O*-methyluridine (Um), 2′-*O*-methylguanosine (Gm), and pseudouridine (ψ) in 5.8S rRNA isolated from total RNA.

ADVANTAGES:

Sequence-selective purification: specific RNA sequences can be purified from biological samples in ~2–4 hours.

High yield and purity: up to 75% recovery and 99.98% purity, competitive with current beads-based purification methods.

Visual feedback of RNA capture: visual predetection of the target RNA during the purification process aids in troubleshooting and yield estimation.

Limited sample requirement: highly sensitive RNA capture of DNA nanoswitches allow purification of rare RNA molecules in limited amount of sample.

Low cost and customizable: nanoswitches can be easily customized for any RNA sequence and purifications can be performed at a low cost on the benchtop with minimal equipment requirements.

CHALLENGES:

Limited purification scale: purification scale is limited by the amount of nanoswitch and its efficient isolation, allowing femtomole–picomole scale purification.

Optimization for different RNA targets: incubation temperature and reaction time may need to be altered based on the overall length of the target and the length of the two 'capture' regions in the nanoswitch.

Manual processing: the current protocol requires processing by hand and may be difficult to automate for large-scale processing.

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Declaration of interests

No interests are declared.

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