

DNA-independent amplification of diverse RNA sequences by bacteriophage transcription polymerases

RNA replication and amplification have broad applications across biomedicine, but current methods are limited by a reliance on inefficient, multi-step protocols. Researchers at Stanford have developed a method for rapid, isothermal replication of RNA using a DNA-dependent RNA polymerase, T7 RNAP, producing large amounts of RNA from a single reaction without a DNA intermediate.

The inventors have characterized template requirements for RNA replication by T7 RNAP, and found that a diversity of RNAs with particular structures can be rapidly amplified. Under a different set of reaction conditions, a DNA 'seed' can also be used to evolve novel replicating RNA templates.

The inventors have further demonstrated the capability to conduct these reactions using a microfluidic setup, providing a means to more uniformly amplify a diverse pool of RNAs while mitigating amplification of contaminating templates.

Applications

- RNA synthesis to produce:
 - RNA/RNAi therapeutics
 - vaccines
 - agriculture/genetically modified plant products
- RNA amplification for use in:
 - diagnostics
 - high-throughput sequencing

- in vitro selection and directed evolution of RNA with desired properties

Advantages

- Direct, fast and cheap amplification of RNA starting from low input amounts
- Diversity of RNA sequences can be amplified
- No DNA intermediates required
- Could Incorporate modified nucleotides
- Single-step reaction
- Isothermal
- Can be conducted in microfluidic format

Publications

- N.Jain, L.R. Blauch, M.R. Szymanski, R. Das, S.K.Y. Tang, Y.W. Yin, A.Z. Fire [Transcription polymerase-catalyzed emergence of novel RNA replicons](#) *Science* 26 Mar 2020.

Patents

- Published Application: [WO2021007233](#)
- Published Application: [20220259645](#)
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Innovators

- Nimit Jain
- Andrew Fire
- Karen Artiles
- Julia Garcia
- Lucas Blauch
- Sindy Tang
- Yuhui Whitney Yin
- Michal Szymanski

Licensing Contact

David Mallin

Licensing Manager, Physical Sciences

[Email](#)