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Enzymatic Preparation of Small, Single-Stranded DNA Circles

High-yield enzymatic method produces exonuclease-resistant ssDNA circles as small as 16 nt for therapeutics.

Researchers at Purdue have developed a new technique for synthesizing small (≥16 nucleotides), single stranded DNA (ssDNA) circles that are resistant to exonucleases. The ssDNA circles are produced at high concentrations (100 μM) and with a high yield (97%) using already commonly utilized T4 DNA ligase.

Current methods for producing ssDNA circles cannot cyclize ssDNA that are shorter than 20 nucleotides (nt) and, because linearization is favored over cyclization precursor concentrations of more than 1 μM, large scale production is impractical. These limitations to produce small ssDNA circles also in-turn, limit the scope for development of new therapeutics or diagnostic tools to DNA sequences that are ≥20 nt. Applications for ssDNA include suppressing protein expression and pathogenic miRNA with anti-sense oligos, design of aptamers to bind to target molecules, and using ssDNA circles as a template for rolling circle amplification. Additionally, cyclization of the ssDNA removes the free ends that are otherwise exposed in its linear form, making it resistant to exonuclease degradation and increasing its lifetime in the body.

Technology Validation: The resistance to exonucleases of the circular ssDNA produced by the researchers was confirmed by immersion in 10% fetal bovine serum for 14 hours, with no obvious degradation observed. This was compared to the same length of linear ssDNA as a control, which showed degradation after 1 hour and complete dissolution by 14 hours.

Advantages:

- Synthesize DNA circles ≥16 nt.

- High throughput.

- Exonuclease resistant

Technology ID

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Category

Pharmaceuticals/Drug Discovery & Development
Materials Science & Nanotechnology/Nanomaterials & Nanostructures
Biotechnology & Life Sciences/Analytical & Diagnostic Instrumentation

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View online

- Familiar protocol and materials

- Broadly applicable

Applications:

- Biomedical therapeutics

- Biosensing

- Nanotechnology

Related Publications:

Near-Quantitative Preparation of Short Single-Stranded DNA Circles
https://doi.org/10.1002/anie.202218443

TRL: Biotechnology

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