Background and Motivation

Oligodeoxynucleotides (ODNs) are short single-stranded synthetic DNA that can be synthesized by automated synthesizer.

The ODN synthesis for each cycle contains four key steps: Detritylation, Coupling, Capping, and Oxidation.

The failure sequences are generated in each cycle in the coupling step due to incomplete coupling.

After cleavage and deprotection, full-length ODN and failure sequences are mixed together. Purification is needed to retrieve full-length ODN.

Common methods that can purify ODN are high-performance liquid chromatography (HPLC), polyacrylamide gel electrophoresis (PAGE), etc. However, they require expensive instruments and materials.

To solve these problems, we propose two simple non-chromatographic methods for ODN purification.

Methods

Catching Failure Sequences by Polymerization

Capping: In each synthetic cycle, only failure sequences are capped with methacrylamide phosphoramidite 1.

Polymerization: The failure sequences are incorporated into a water-insoluble gel by radical acrylamide polymerization while the full-length ODN is not.

Extraction: The full-length ODN is retrieved from water extraction.

Catching Full-length Sequences by Polymerization

Capping: Only full-length ODN is coupled with a methacrylamide phosphoramidite 2 via a cleavable linker.

Polymerization: The full-length ODN is polymerized into a water-insoluble gel while the failure sequences are not.

Washing: The failure sequences are removed by water extraction.

Releasing: The full-length ODN is obtained by cleaving from the gel.

Results and Discussion

Catching Failure Sequences by Polymerization

- Pure full-length ODN was confirmed by MALDI-TOF analysis (calculated mass for [M-H] - 6057, found: 6057).

Catching Full-length Sequences by Polymerization

- Pure full-length ODN was obtained from water extraction. Yield for purification process was estimated to be 83%.

Conclusion

- ODNs purification methods have been developed using polymerization.
- Both methods are simple, convenient, inexpensive, and highly efficient to achieve pure ODN in high yield.

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References