Purification of Synthetic Oligodeoxynucleotides via Catching by Polymerization

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Background and Motivation

- ◆ Oligodeoxynucleotide (ODN) is 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 pure 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 for high-throughput purification.
- ◆ 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 by **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 Sequence by Polymerization

- ◆ **Capping**: In the last synthetic cycle, 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 insoluble 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).

1. *N,N*-Dimethyl acrylamide
 2. *N,N'*-methylene bis(acrylamide), (NH₄)₂S₂O₈, TMEDA, H₂O
- (Radical polymerization)

1. Extract with water to retrieve ODN
2. Evaporate

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

Catching Full-length Sequence by Polymerization

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

1. *N,N*-Dimethylacrylamide
 2. *N,N'*-methylenebis(acrylamide) (NH₄)₂S₂O₈, TMEDA, H₂O
- (Radical polymerization)

1. HF/pyridine
 2. Me₃SiOMe
 3. Evaporate
- (Cleave full-length ODN from gel)

- ◆ The yield of pure full-length ODN from this purification process was estimated to be 72 %.

Conclusion

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

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References

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