

Purification of Synthetic Oligodeoxynucleotides via Catching by Polymerization



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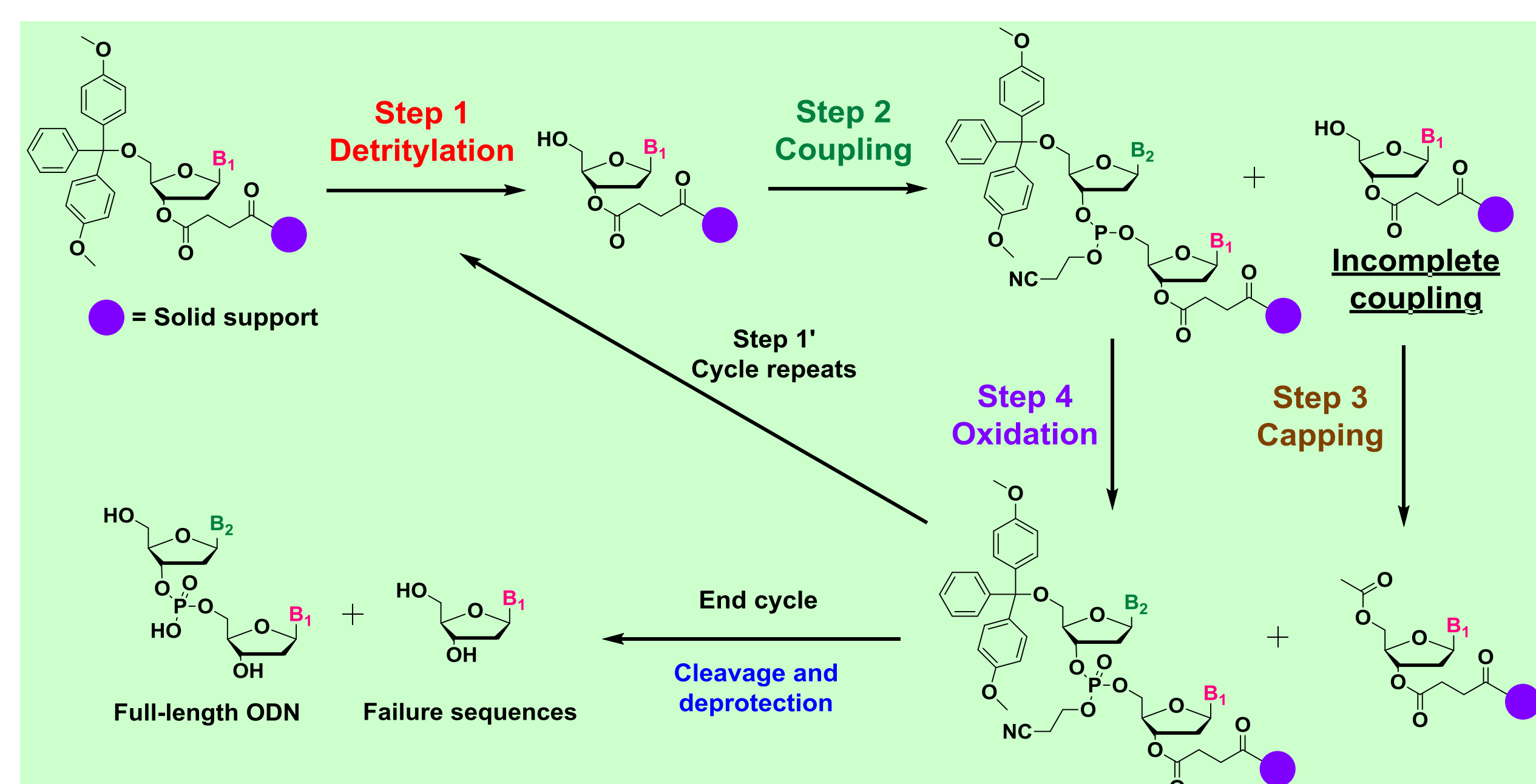
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Background and Motivation

- ◆ **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**.

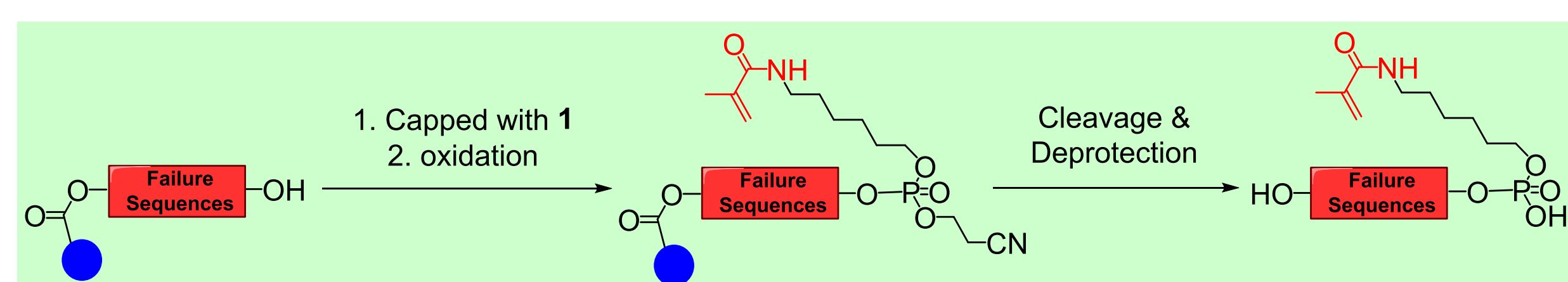
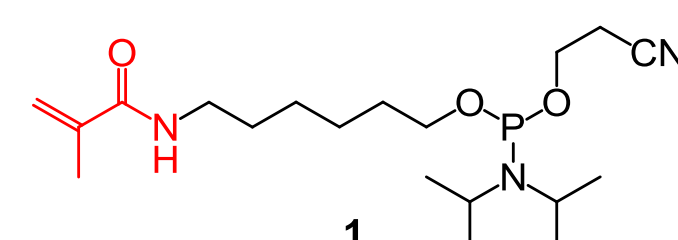


- ◆ The **failure sequences** are generated in each cycle in the **coupling step** due to incomplete coupling.
- ◆ After cleavage and deprotection, full-length ODN and failure sequences are mixed together. Purification is needed to retrieve pure full-length ODN.
- ◆ Common methods that can purify ODN are high-performance liquid chromatography (HPLC), polyacrylamide gel electrophoresis (PAGE), etc. **However, they require expensive instruments and materials.**
- ◆ To solve these problems, we propose **two simple non-chromatographic methods** for ODN purification.

Methods

Catching Failure Sequences by Polymerization

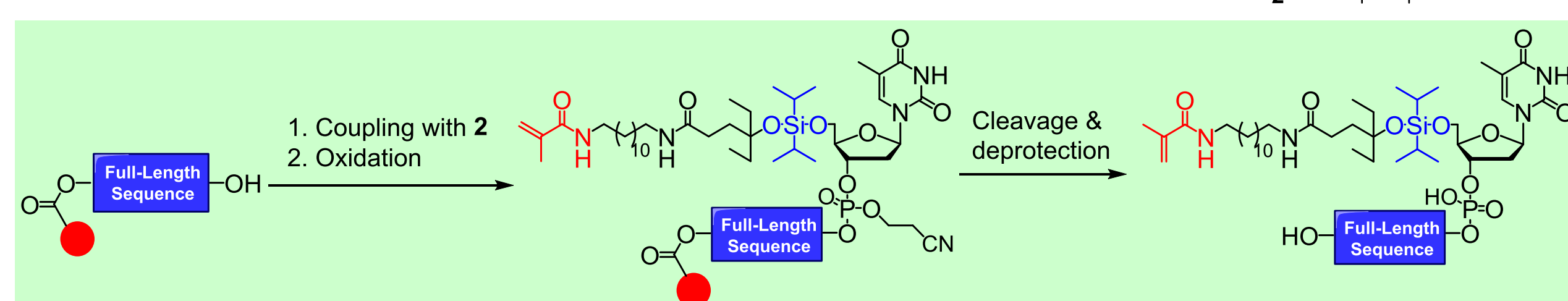
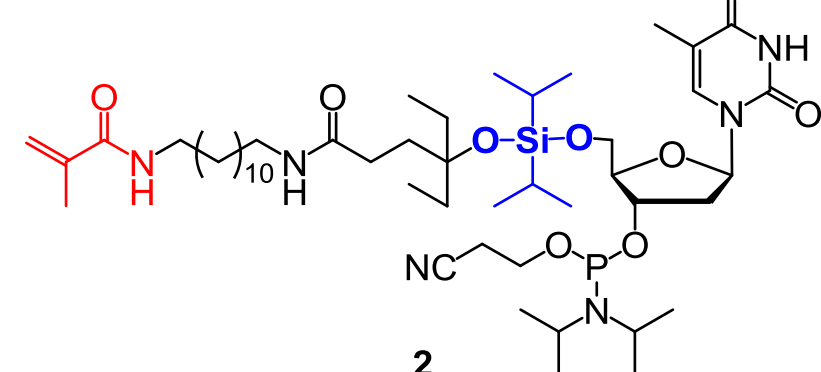
- ◆ **Capping:** In each synthetic cycle, only failure sequences are capped by **methacrylamide phosphoramidite 1**.



- ◆ **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 Sequences by Polymerization

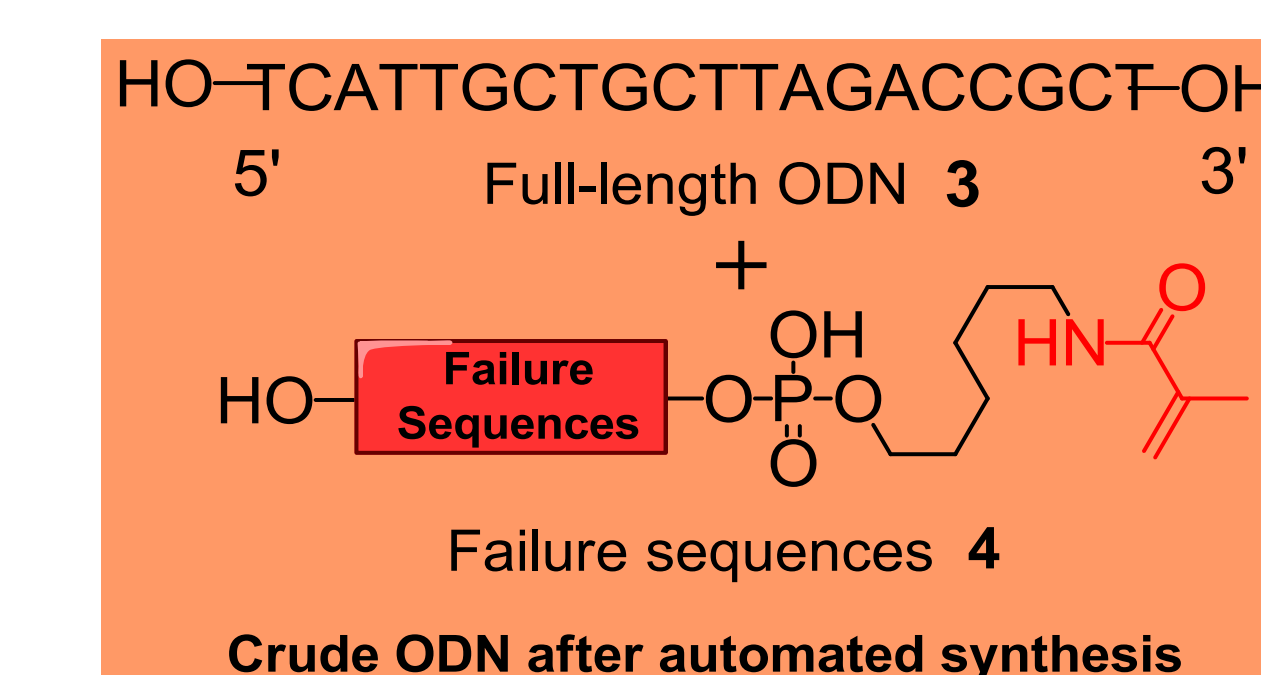
- ◆ **Capping:** Only full-length ODN is coupled with a **methacrylamide phosphoramidite 2** via a **cleavable linker**.



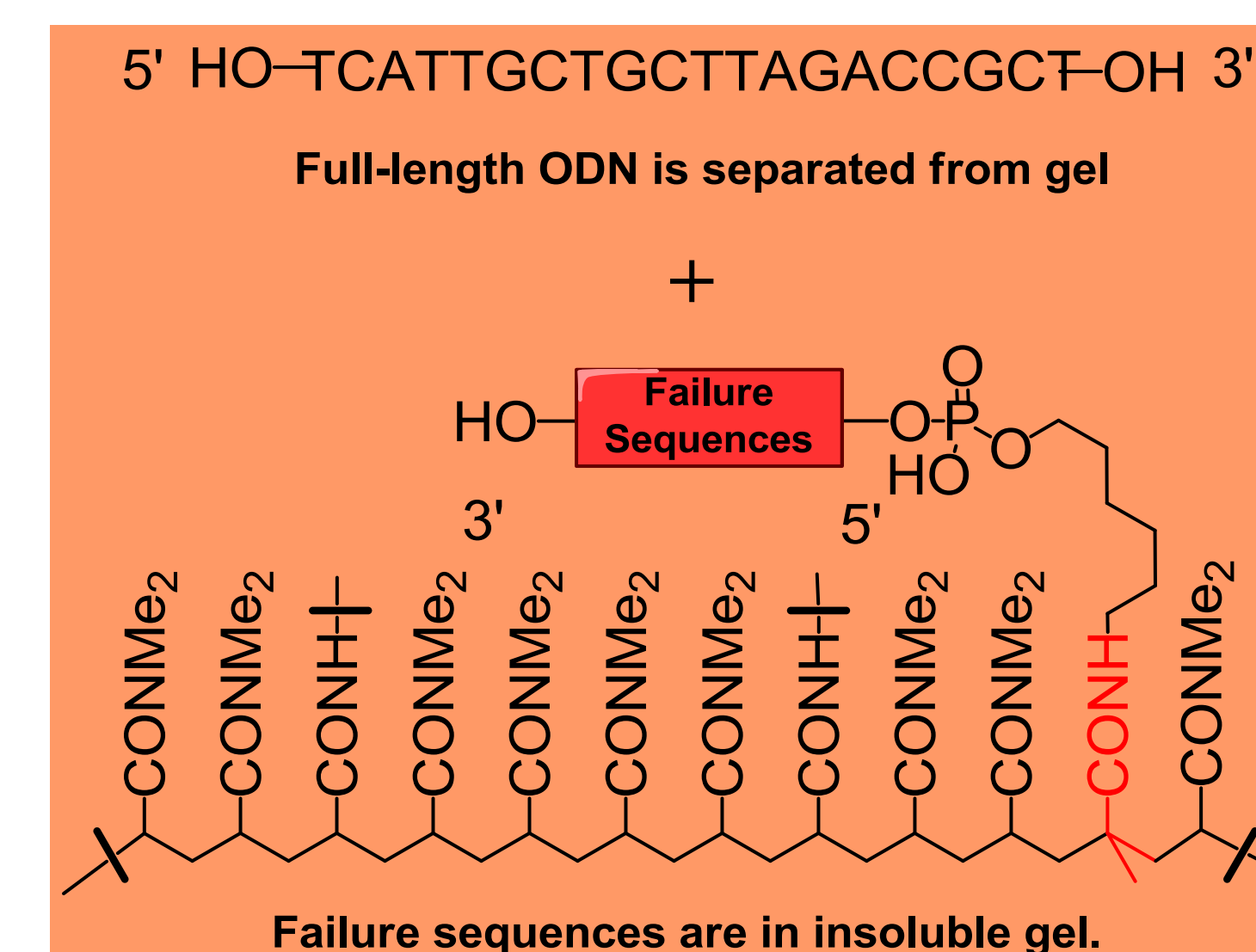
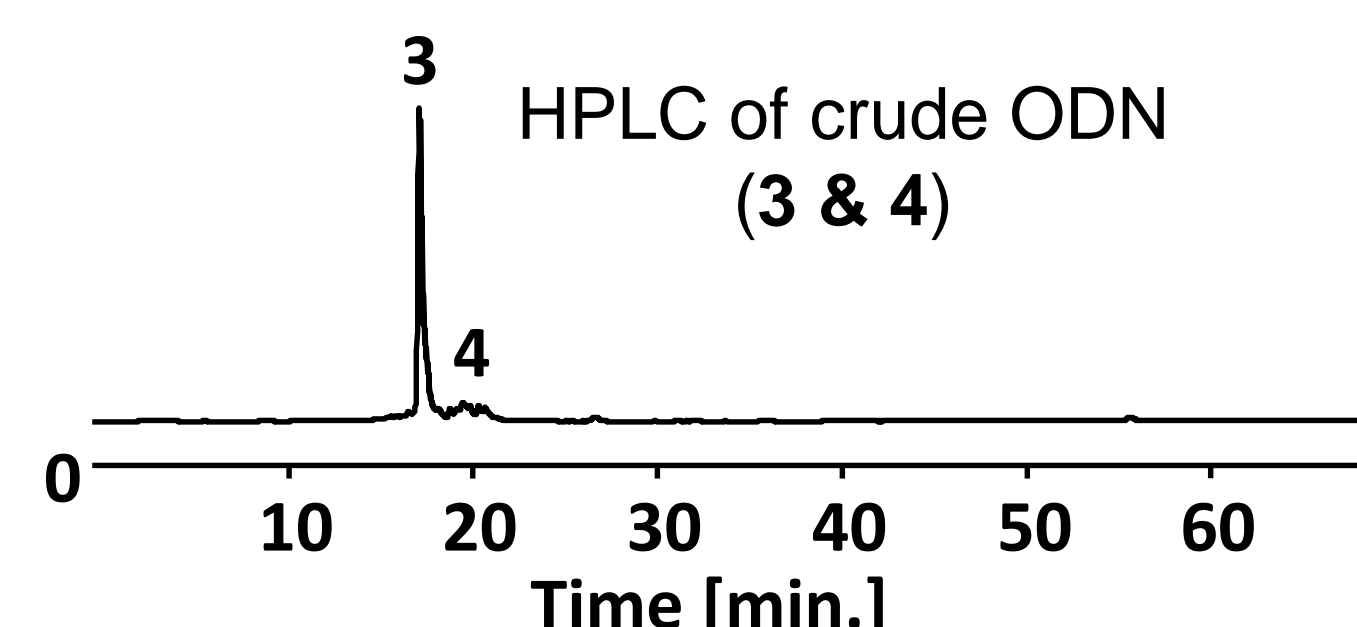
- ◆ **Polymerization:** The full-length ODN is polymerized into a water-insoluble gel while the failure sequences are not.
- ◆ **Washing:** The failure sequences are removed by water extraction.
- ◆ **Releasing:** The full-length ODN is obtained by cleaving from the gel.

Results and Discussion

Catching Failure Sequences by Polymerization



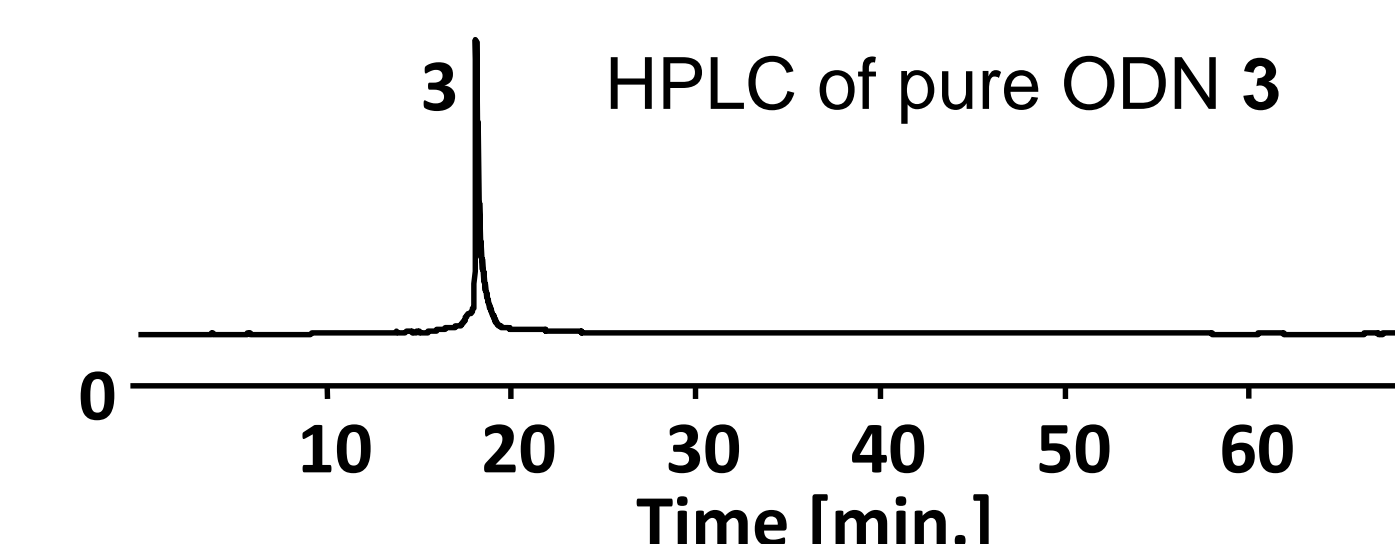
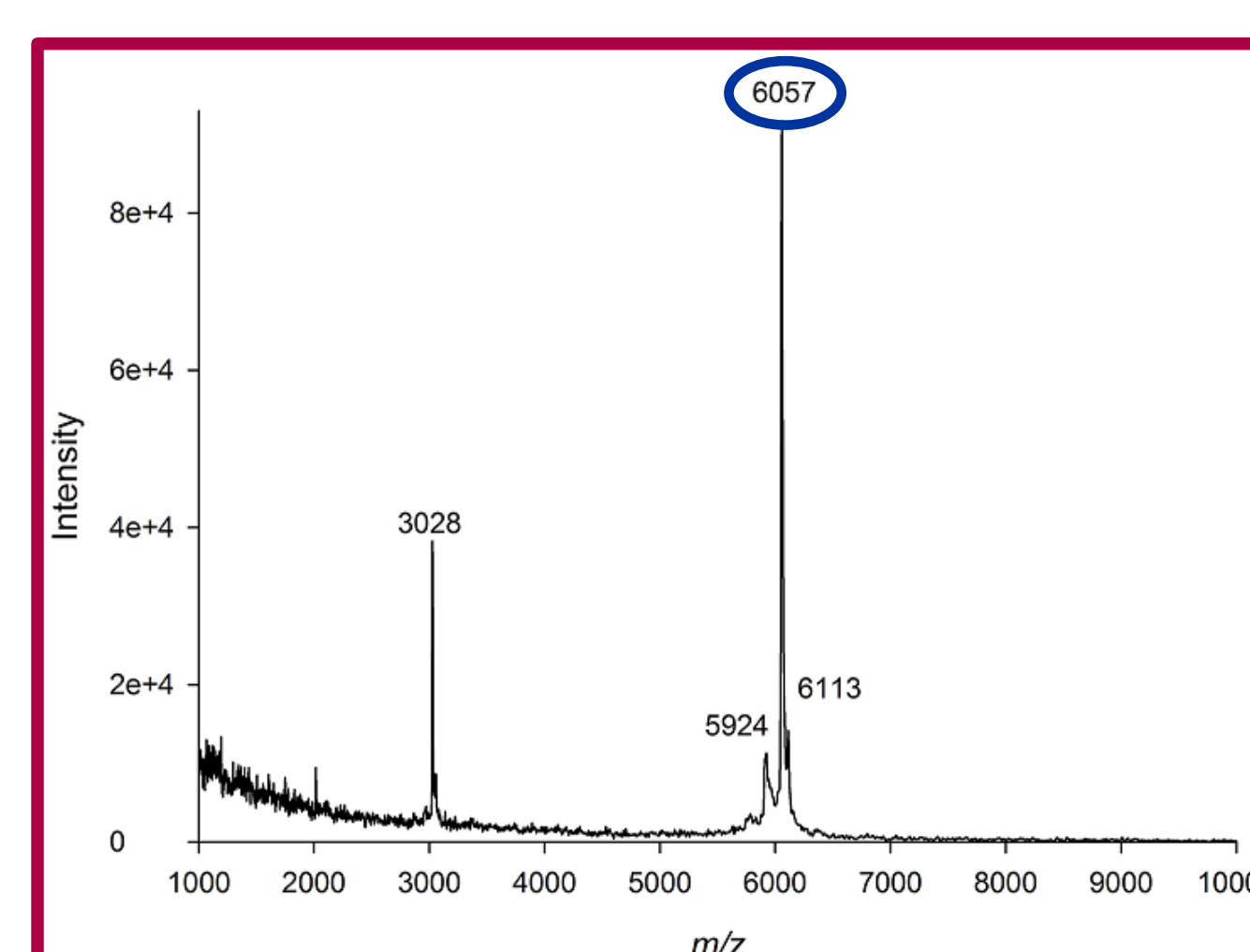
1. *N,N*-Dimethyl acrylamide
2. *N,N'*-methylene bis(acrylamide), (NH₄)₂S₂O₈, TMEDA, H₂O
(Radical polymerization)



1. Extract with water to retrieve ODN
2. Evaporate

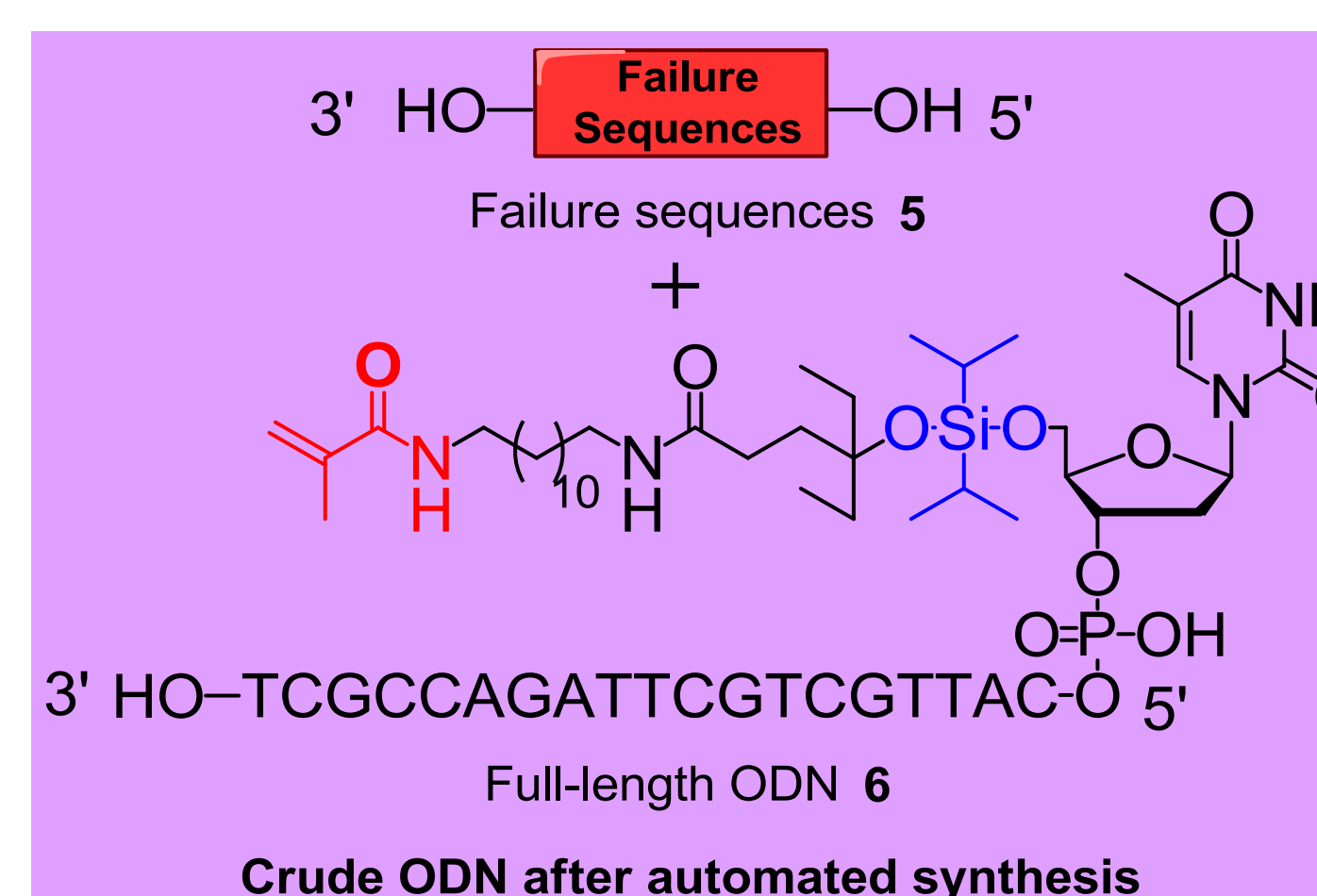


- ◆ Pure full-length ODN was confirmed by MALDI-TOF analysis (calculated mass for [M-H]⁻ 6057, found: 6057).



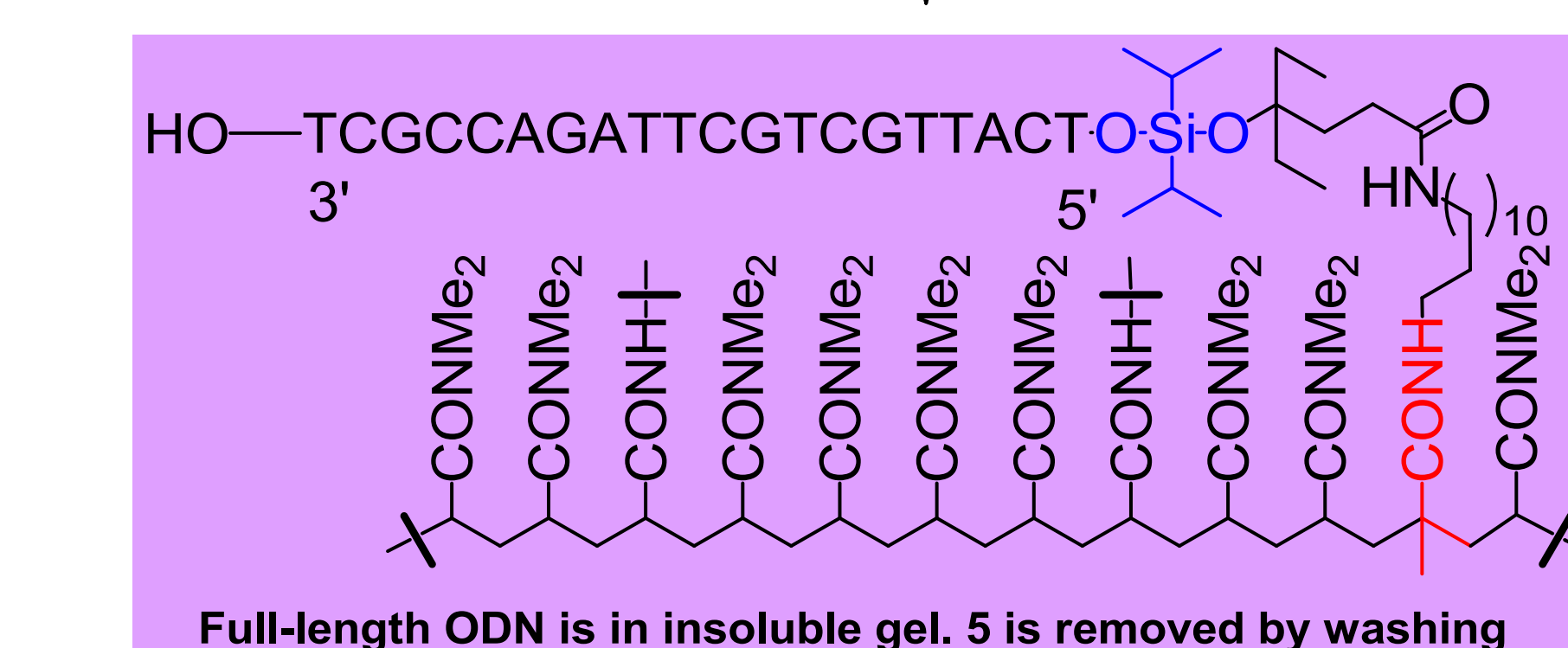
- ◆ Pure full-length ODN was obtained from water extraction. Yield for purification process was estimated to be **83 %**.

Catching Full-length Sequences by Polymerization

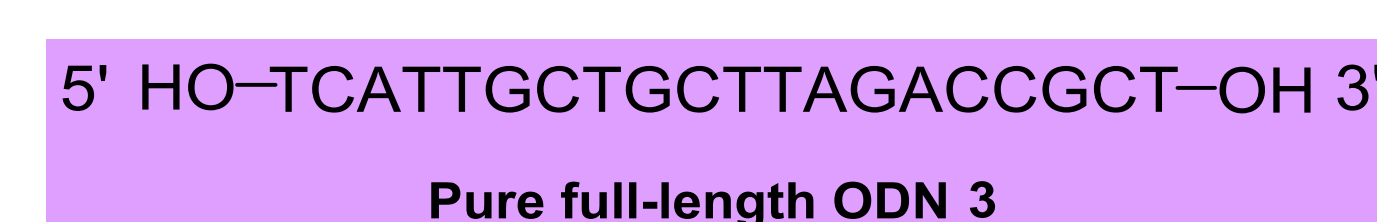


1. *N,N*-Dimethylacrylamide
2. *N,N'*-methylenebis(acrylamide) (NH₄)₂S₂O₈, TMEDA, H₂O

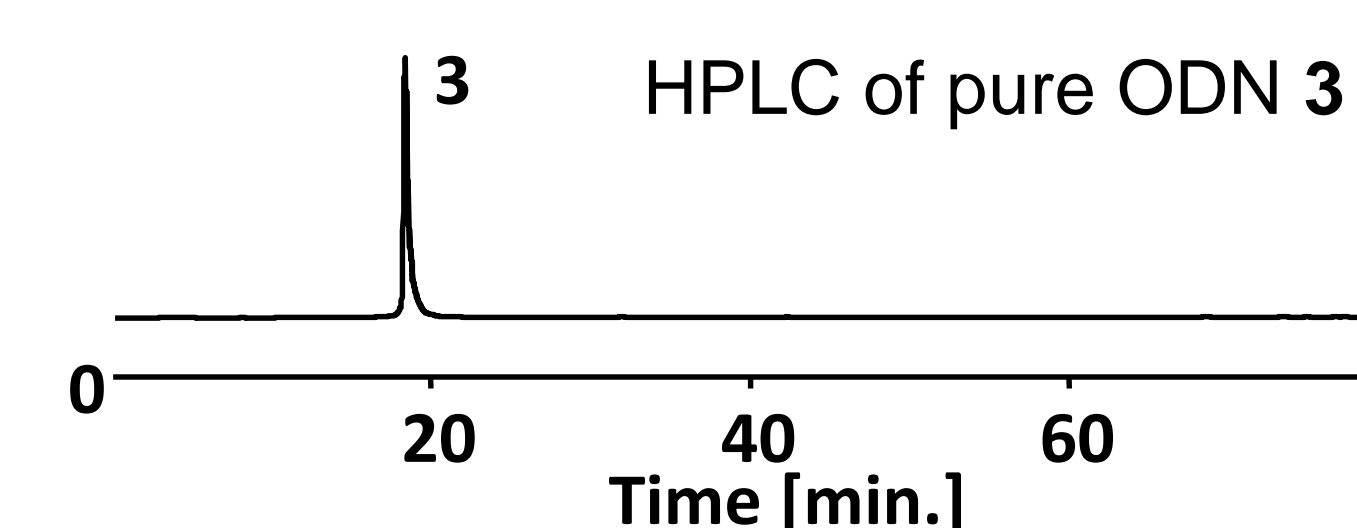
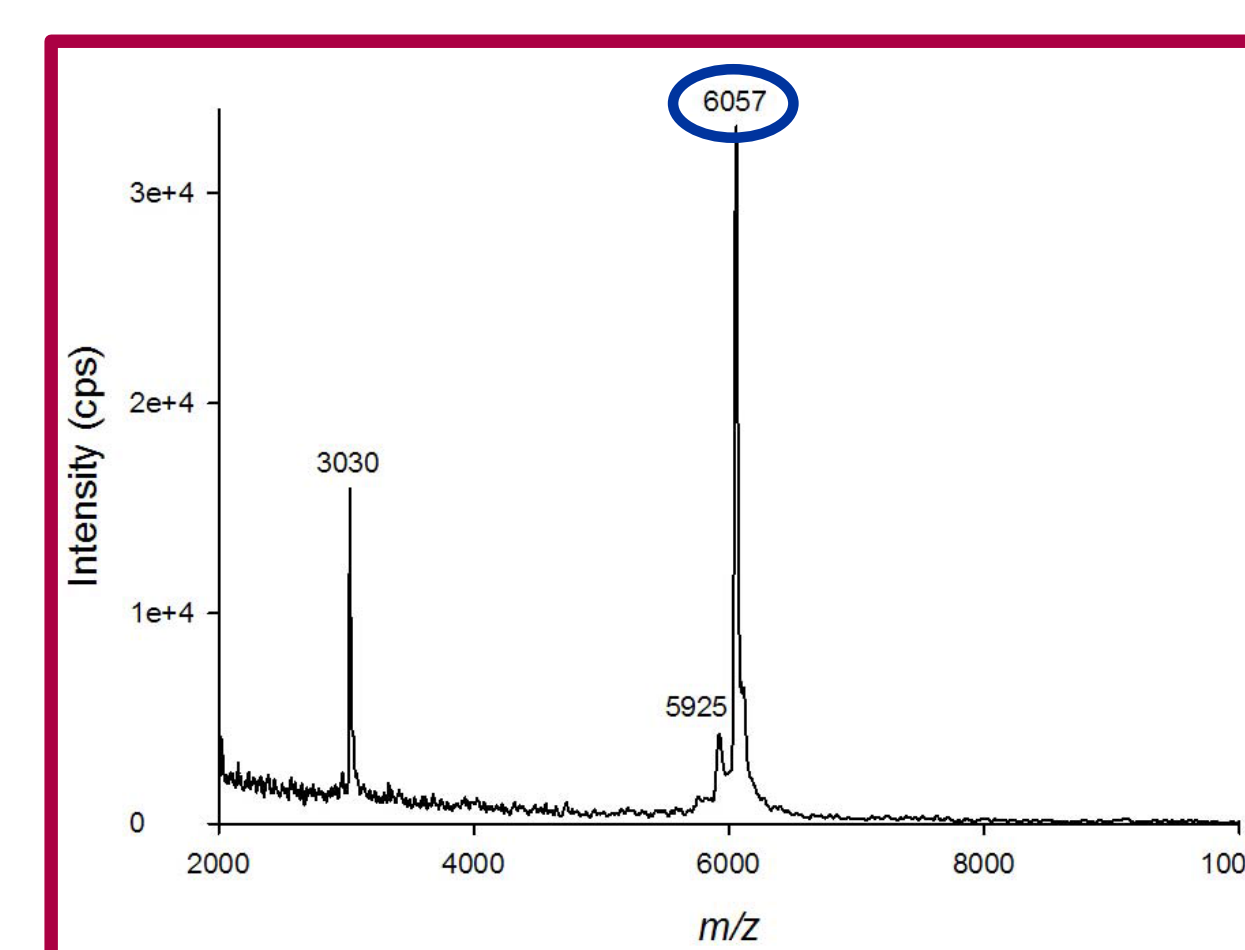
3. Wash gel with water



1. HF/pyridine
2. Me₃SiOMe



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



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

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.

Acknowledgements

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References

1. S. Fang, and S. Fueangfung. Scalable Synthetic Oligodeoxynucleotide Purification with Use of a Catching by Polymerization, Washing and Releasing Approach. *Org. Lett.*, **2010**, 12, 3720-3723.
2. S. Fang, et al. Synthetic Oligodeoxynucleotide Purification by Polymerization of Failure Sequences. *Chem. Commun.*, **2011**, 47, 1345-1347.