Solvent-free purification of D84-mer oligonucleotide using Capto™ PlasmidSelect resin

Simon Åberg, Linda Hagman, Ganesh Nawale, Per Denker
Cytiva, Björkgatan 30, 751 84 Uppsala, Sweden

Introduction

The emergence of new powerful gene therapies utilizing gene editing systems such as CRISPR-Cas9 has also sparked the need for longer oligonucleotides. The purification of oligonucleotides has traditionally been performed either by using anion exchange (AIEX) or highly solvent-dependent reversed phase chromatography (RPC).

The achievable purity of AIEX purification is highly correlated with the oligo length. The end-synthesis product length is inversely proportional to the number of coupling cycles, and the AIEX purification separates the oligo based on size by resolving secondary structure using high pH. Hence, at longer oligo lengths, the resolution for AIEX can be insufficient to separate the full-length product from the increased amount of failure sequences (Fig 2).

To overcome these challenges, we present a single-use, compatible, and solvent-free downstream process for the purification of longer oligonucleotides using first Capto™ PlasmidSelect resin to separate full-length, tritylated oligos from failure sequences. Thereafter, we used a polishing step for removal of final residual impurities to achieve a high purity (Fig 1).

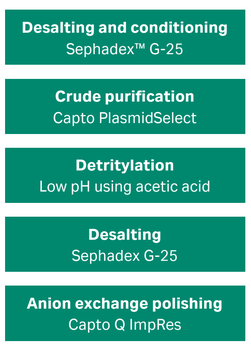

Fig 1. Process overview.

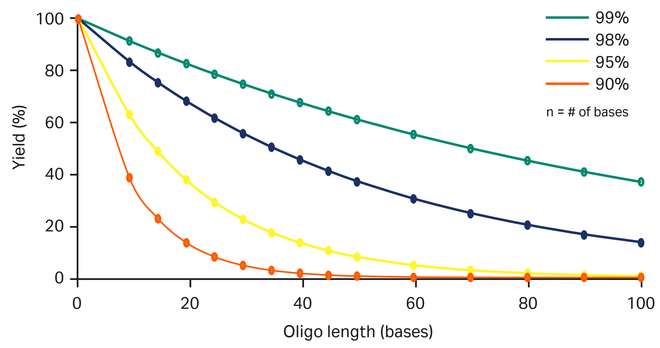

Fig 2. Full-length product yield at different coupling efficiencies.

Efficient DMTr ON purification using Capto PlasmidSelect resin

Firstly, the crude synthesized oligonucleotide was desalted and diluted to 50 mM Tris, 1.75 M ammonium sulfate, pH 7.5. Trityl on species were then separated from trityl off species using Capto PlasmidSelect resin by separating failure sequences in the flowthrough and then eluting the tritylated full-length oligo using a linear gradient toward 50 mM Tris, pH 7.5. The collected eluate was detritylated in batch-mode using pH and subsequently desalted before loaded onto Capto Q ImpRes resin for polishing of residual trityl off and shortmer species.

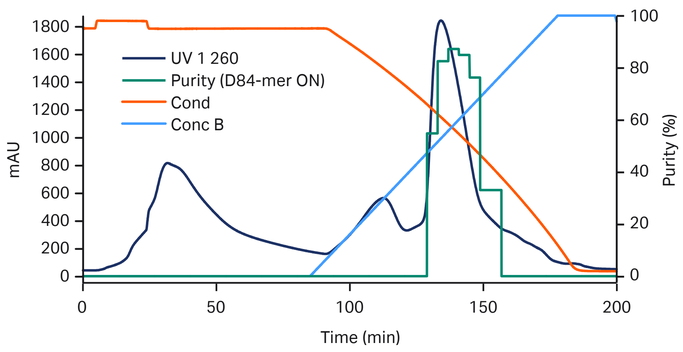

Fig 3. Separation of D84-mer trityl on full-length product and failure sequences on a 4.7 mL HiScreen™ Capto PlasmidSelect column. The sample was loaded at 10 mg oligo/mL resin at residence time of 6 min (flow velocity of 100 cm/h). Elution was performed using the same flow velocity with a linear gradient of 20 CV towards 50 mM Tris, pH 7.5 and collected in 2 mL fractions. The purity of individual collected fractions are shown in green with the % purity indicated on the right y-axis. Fractions with purity ≤ 80% were selected and pooled for detritylation.

Fast and easy detritylation and desalting

The collected eluate was detritylated in batch-mode for 45 min using a stock solution of 5 M acetic acid to a pH of < 4, yielding a final acetic acid concentration of ~ 0.1 M and pH 3.7. The detritylated sample was subsequently desalted to 10 mM NaOH, pH 12 using a HiPrep™ 26/10 desalting column.

High purity by polishing on Capto Q ImpRes resin

To obtain purity above 90%, the detritylated oligo was polished using Capto Q ImpRes anion exchange resin. Full-length trityl off species were purified in bind/elute mode using gradient elution against 1 M NaCl, 10 mM NaOH, pH 12. The eluate was collected in 0.8 mL fractions and analyzed on HPLC for purity determination. Fractions with a purity ≥ 90% were selected and pooled.

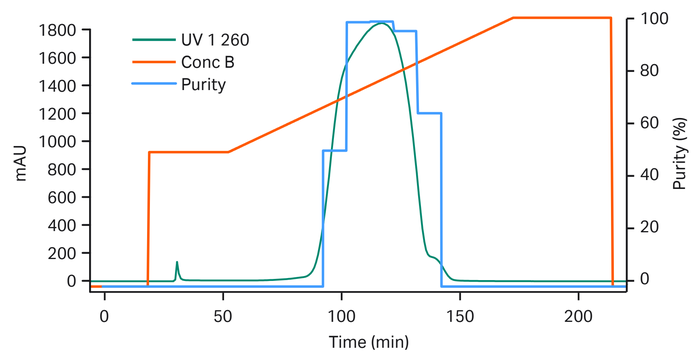

Fig 4. Polishing of detritylated oligo using 1 mL HiTrap™ Capto Q ImpRes resin. The sample was loaded at 1 min residence time (flow velocity 160 cm/h) and then eluted at 6 min residence time (flow velocity 25 cm/h) using a linear gradient of 50%B to 100%B for 20 CV against 1 M NaCl, 10 mM NaOH, pH 12 and collected in 0.8 mL fractions. The purity of individual collected fractions are shown in green with the % purity indicated on the right y-axis. Fractions with purity > 90% were selected and pooled for desalting.

Purity analysis and process recovery

In-process controls were collected for all process steps and analysed on anion exchange ultra HPLC. The crude sample from synthesis had a purity of 36% tritylated full-length oligo (Fig 5A). After the first purification step, the purity for chosen fractions was 81% (Fig 5B). The detritylation step showed a high yield with 90% detritylated full-length oligo after 45 min incubation (Fig 5C). Following the polishing step, a purity of 98% detritylated full-length oligo was obtained (Fig 5D).

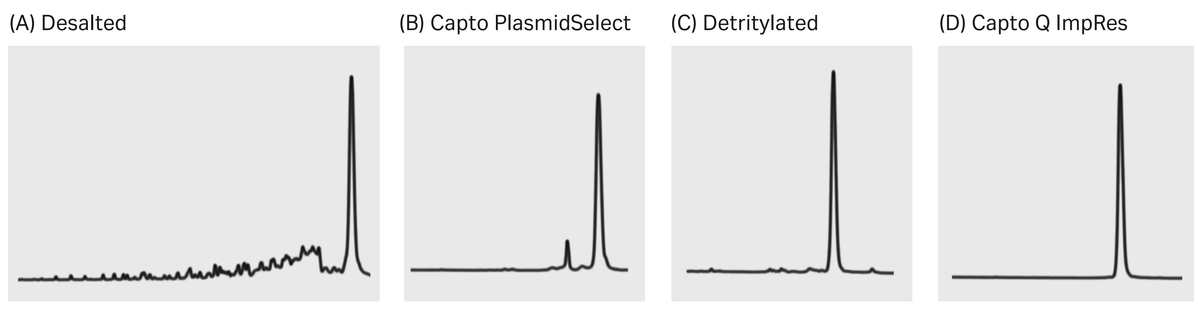

Fig 5. HPLC purity analysis of in-process control samples. (A) desalted crude synthesis pool. (B) Eluate from Capto PlasmidSelect resin. (C) Eluate after detritylation at pH 3.7 using acetic acid for 45 min. (D) Eluate after final polishing step.

Process overview

The Capto PlasmidSelect resin purification yields a recovery of 69% followed by a detritylation step with 94% recovery, showing almost complete detritylation (Table 1). The final polishing step successfully enriched the detritylated full-length oligonucleotide and had a recovery of 81% employing a pooling strategy that gave 98% purity of the 84-mer.

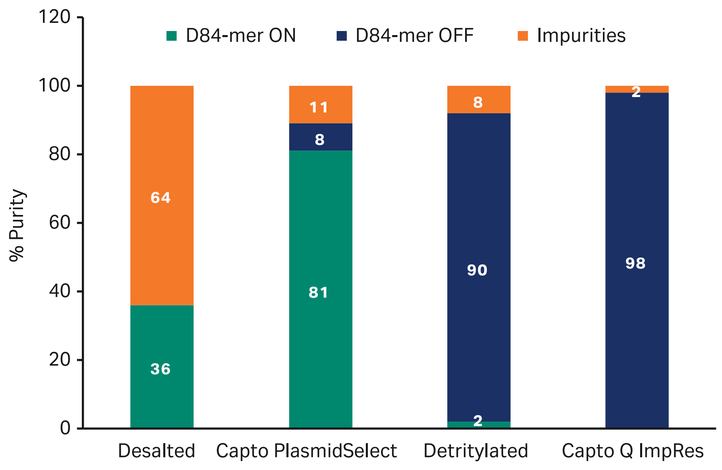

Fig 6. Purity for each process step fraction with quantified value outlined for each sample. Impurities include peaks not identified as tritylated or nontritylated full-length product such as failure sequences.

Table 1. Process summary of step recovery, total recovery, step purity, and pH for each process step

Process step	Step recovery	Total recovery	Purity	Step pH
Desalting	N/A	N/A	36%	7.5
Capto PlasmidSelect	69%	69%	81%	7.5
Detritylation and desalting	94%	65%	90%	< 4
Capto Q ImpRes	81%	53%	98%	12

Conclusions

- Purification of longer oligonucleotides using Capto PlasmidSelect resin produces ≥ 80% pure tritylated oligonucleotide.
- Solvent-free, downstream process utilizing only aqueous buffer systems can be used to purify oligonucleotides.
- High-purity oligo can be obtained using a second polishing step with Capto Q ImpRes resin reaching purities ≥ 95% detritylated full-length oligonucleotide.
- Enables efficient and easy scale-up using ÄKTA pilot™ 600, ÄKTA ready™, or ÄKTA process™ systems.

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