

Oligonucleotide development and manufacturing workflow

Solid-phase synthesis

Objective: High-fidelity synthesis of single-stranded oligonucleotides

Considerations

- Optimization of coupling conditions
- Scale up from microgram to kilogram
- Regulatory requirements
- Reagent and solvent quality, supply, and disposal

Strategies

- Choose solid support to maximize coupling efficiency and recovery
- Use the same automation software across departments for easier transfer

Removal of solid support and collection of crude oligo in aqueous ammonia solution. Optional: keep hydrophobic DMTr group on full-length oligonucleotide



Oligonucleotide purification with DMTr OFF

OPTION 1

Objective: Separation of full-length oligonucleotides from modified oligonucleotides and short-mers with DMTr off

Considerations

- Removal of all impurities by high resolution of full-length oligonucleotide from short-mers
- Use of Na⁺ as counterion to the oligonucleotide as a more biological substrate

Strategies

- Anion exchange resin that gives sufficient resolution considering the flow properties
- Use Na⁺-based elution buffers

Oligonucleotide purification with DMTr ON

OPTION 2

Objective: Capture of full-length oligonucleotides with a hydrophobic DMTr group on 5'-end

Considerations

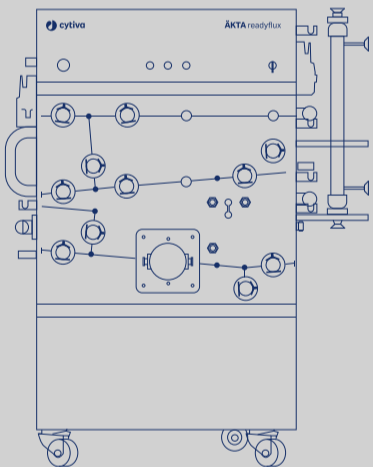
- Capture of full-length oligonucleotide and cleavage of DMTr⁺ group
- Reversed phase chromatography with flammable buffers
- Removal of all impurities

Strategies

- Enrich DMTr-on oligos utilizing RPC or HIC chromatography
- Remove DMTr group by low pH-treatment
- Add ion exchange chromatography step to remove short-mers and change counterion

* Dimethoxytrityl (DMTr) is a hydrophobic protecting group on the 5'-end of the oligonucleotide. The DMTr group is cleaved from the oligo under acidic conditions.

ÅKTA readyflux™ XL and UniFlux™ tangential flow filtration systems. ReadyToProcess™ hollow fiber cartridges



Analysis

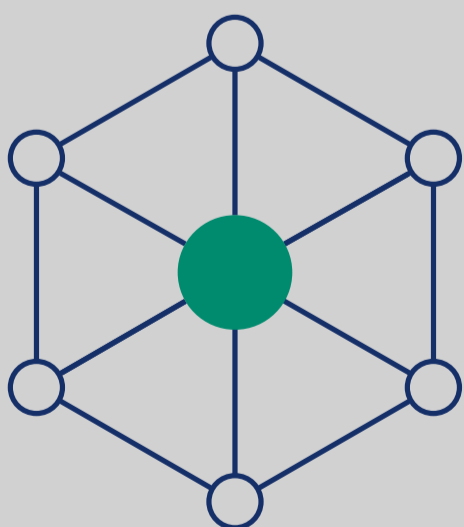
Objective: Measure purity, recovery, and identity

Considerations

- Robust and advanced analytical testing capabilities
- Quantitation of percentage full-length oligonucleotide
- Qualitative determination of oligonucleotide identity

Strategies

- Use analytical IEX-HPLC, ion-pairing RPC and MS
- Tailor analytics for final formulation (e.g., nanoparticle characterization)



Drug product

Objective: Aseptic filling of oligonucleotides

Considerations

- Standardized aseptic process that allows format changes in clinical trials/for commercial launch
- Recipe-driven process control for product stability, eliminating human intervention

Strategies

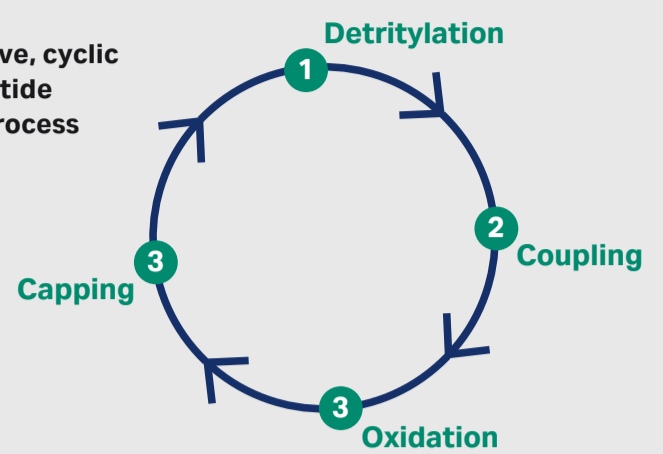
- Clinician/patient-centric delivery method in vials or pre-filled syringes, depending on indication/injection volume

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The repetitive, cyclic oligonucleotide synthesis process can be fully automated.



Cleavage and deprotection

Objective: Cleavage from solid support and optional removal of protection groups

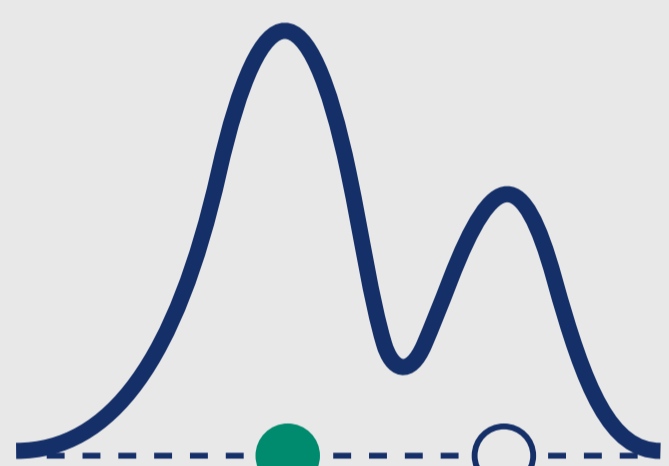
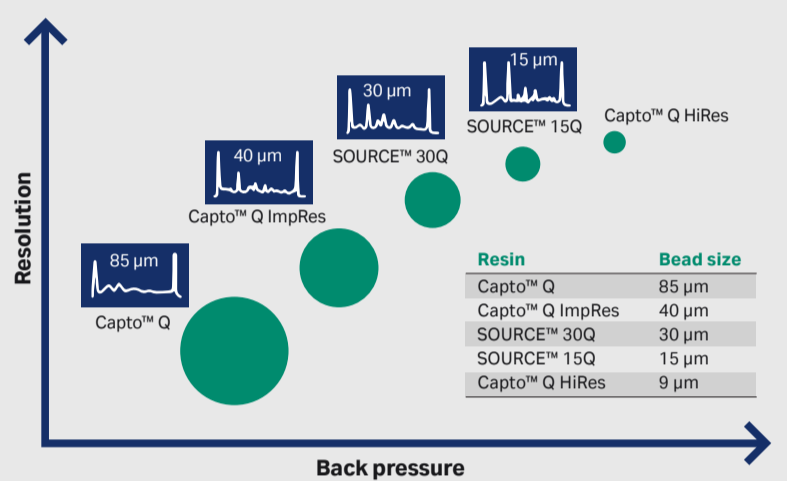
Considerations

- Cleavage and optional removal of DMTr group depend on the chemistry and overall downstream process
- Treatment in 25% ammonia at elevated temperature
- RNA requires additional cleavage step

Strategies

- Choose appropriate linker for the solid support
- Select appropriate cleavage protocols for DNA or RNA
- Decide if the DMTr 'handle' should be used to purify full-length oligos
- Develop process to treat column content with ammonia solution and elevated temperature

Cytiva ion exchange chromatography resins. Optimize resolution and throughput with appropriate bead size.



Buffer exchange

Objective: Removal of salts from the IEX purification step

Considerations

- Optimal removal of excess salts
- Minimization of yield losses
- Selection of the appropriate equipment with scale

Strategies

- Choose a size exclusion chromatography at small scale, and to separate single- and double-stranded oligos (siRNA)
- Choose tangential flow filtration for scalable buffer exchange
- Select the right filter for the oligonucleotide's elongated shape

Formulation

Objective: Creation of a stable solution, averting lyophilization

Considerations

- Evade nuclease degradation
- Avoid thermal terminal sterilization – the nanoparticle itself may degrade
- Long-term stability in solution – especially that of RNA oligonucleotides – is limited, due to sensitivity to hydrolysis

Strategies

- Drug product lyophilization (freeze-drying) step helps to remove the fill solvent
- Prefilled syringes, cartridges, and vials

Cytiva Aseptic Filling Workcells™



Learn more about oligonucleotide development and manufacturing here.