cytiva

Oligonucleotide development and manufacturing workflow

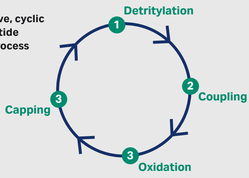
Solid-phase synthesis

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

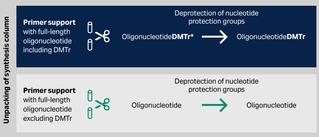
Strategies
- Choose solid support to maximize coupling efficiency and recovery
- Use the same automation software across departments for easier transfer

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



Cleavage and deprotection

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



* 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.

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

Oligonucleotide purification with DMTr OFF

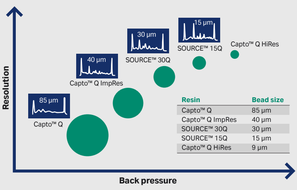
OPTION 1

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

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



Resin	Bead size
Capto™ Q	85 µm
Capto™ Q ImpRes	40 µm
SOURCE™ 30Q	30 µm
SOURCE™ 15Q	15 µm
Capto™ Q HiRes	9 µm

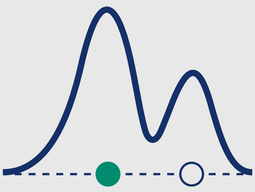
Oligonucleotide purification with DMTr ON

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

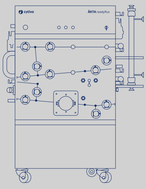
Strategies
- Enrich DMTr-on oligos utilizing RPC or HIC chromatography
- Remove DMTr group by low pH-treatment
- Add ion exchange chromatography step to remove short-mers and change counterion



* 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

Buffer exchange

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



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

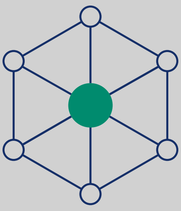
Analysis

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)

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

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

Cytiva Aseptic Filling Workcells™

cytiva.com

CY22264-21Oct21-IG

Learn more about oligonucleotide development and manufacturing **here.**

cytiva