


Oligonucleotide Process Considerations and Controls

PQRI-FDA , Rockville, MD
March 21-23, 2017

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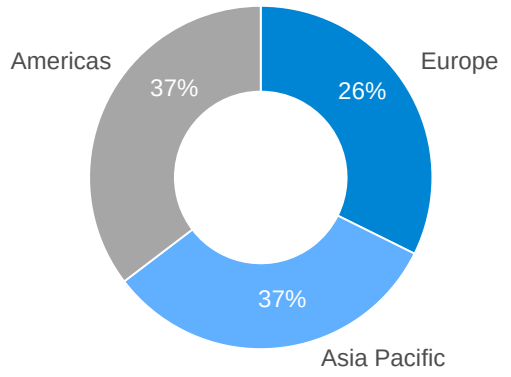
Agilent Technologies



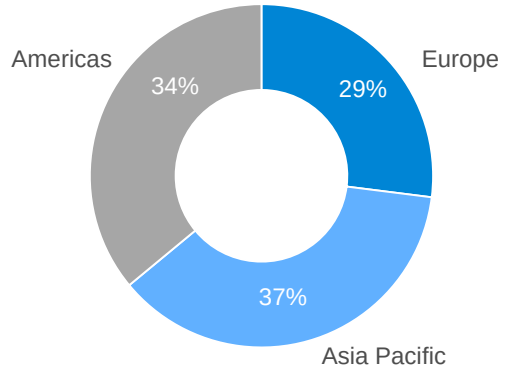

Agilent at a Glance

REVENUE IN FY16	**$4.2 billion** (more than 70% generated outside the U.S.)
EMPLOYEES	**Approximately 12,500**
PRESIDENT & CEO	**Mike McMullen**
CUSTOMER LOCATIONS	**100+ countries**
MANUFACTURING AND R&D LOCATIONS	**U.S., Europe, Asia Pacific**
STOCK TRADING	**NYSE: A**

Revenue by Geography



Headcount by Geography

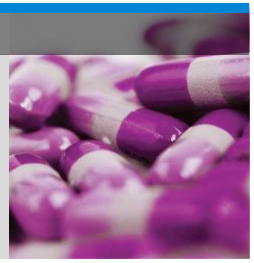


(1) Global Infrastructure Organization includes Finance, HR, Legal, IT, Workplace Services, Quality and Regulatory Assurance

Highlights of Our Solutions in Action

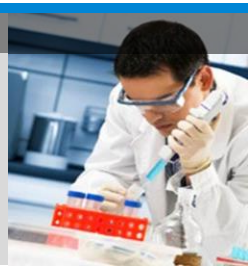
PHARMA

We help customers speed drug discovery, development and manufacturing.



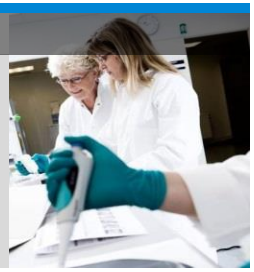
ACADEMIA & GOVERNMENT

Our analytic products support every step of multi-omics integrated biology research.



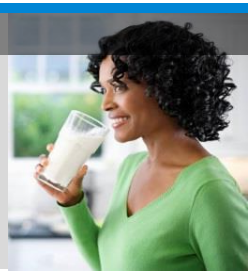
DIAGNOSTICS & CLINICAL

Our products enable pathology labs to meet accreditation demands and reach accurate diagnoses faster.



FOOD

We help safeguard the global food chain with solutions for food safety, quality and authenticity.



ENVIRONMENTAL & FORENSICS

We provide environmental labs with the tools to test water, air and soil.

Our solutions help law enforcement identify illicit drugs and analyze evidence.

CHEMICAL & ENERGY

From crude oil, natural gas and refining to specialty chemicals and alternative fuels, we help customers meet quality, safety, regulatory and environmental standards.

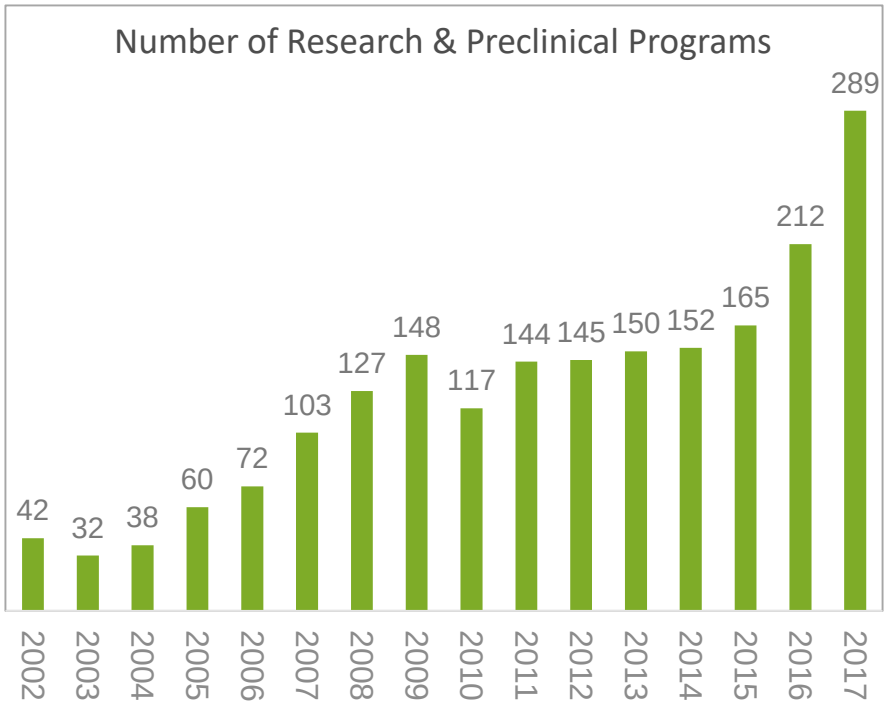
Talk Outline

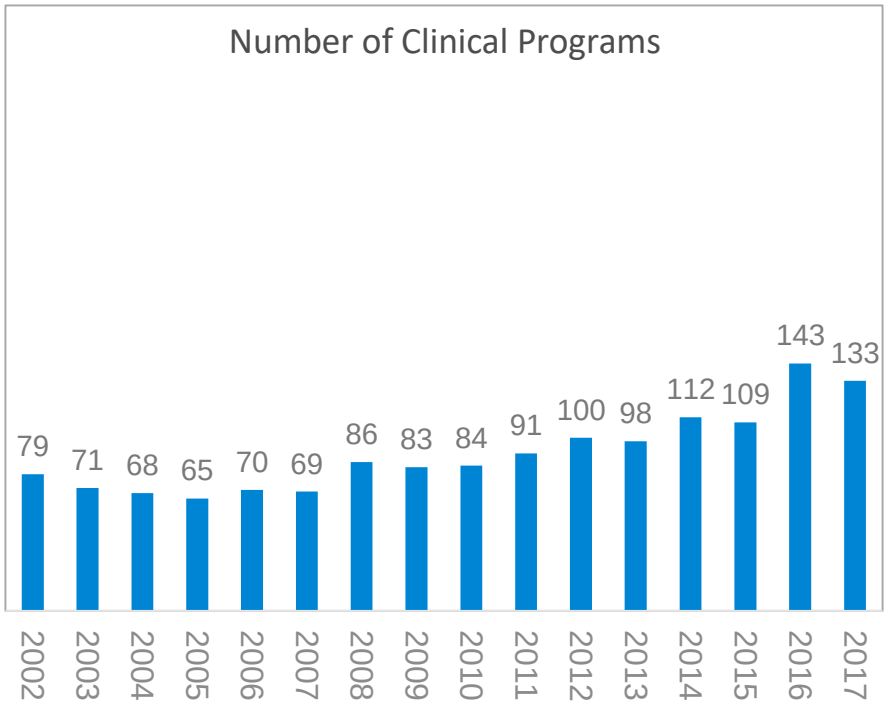
1) Oligonucleotide Overview
    a. Research and Clinical Program Trends
    b. Functional & Molecular Types of Oligo's
    c. Process Types
    d. Process Models (Origin and Control of Impurities)

2) QbD Approach
    a. Risk-based Criticality
    b. Group or Individual Unit Op approaches

3) Discussion

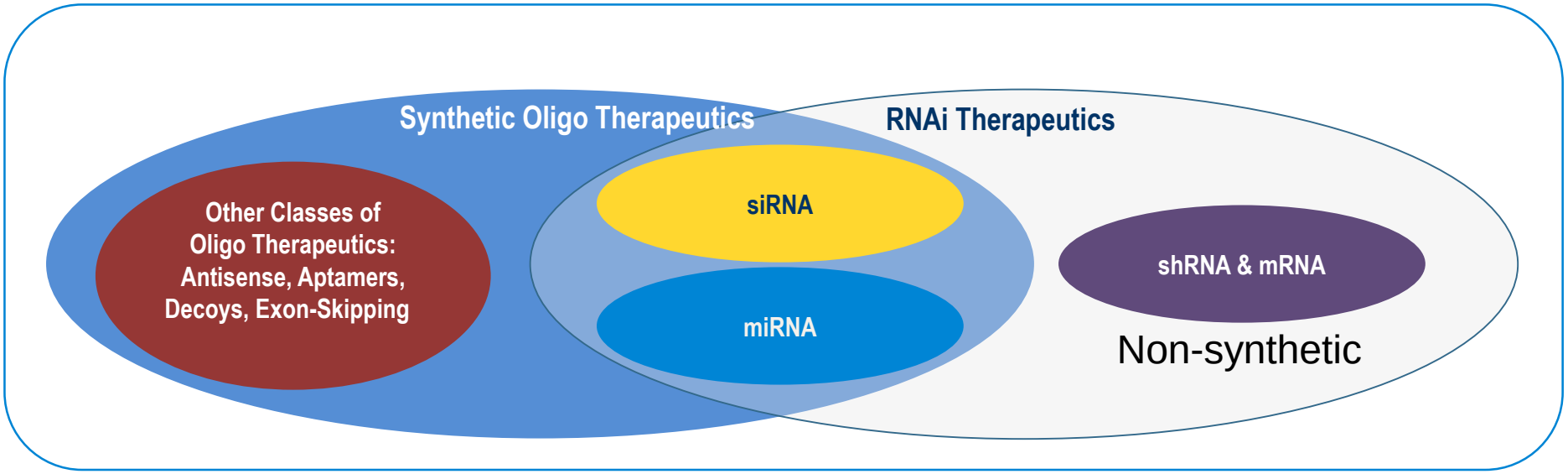
Oligo Therapeutic Research & Preclinical Programs Vs. Clinical Trials Programs

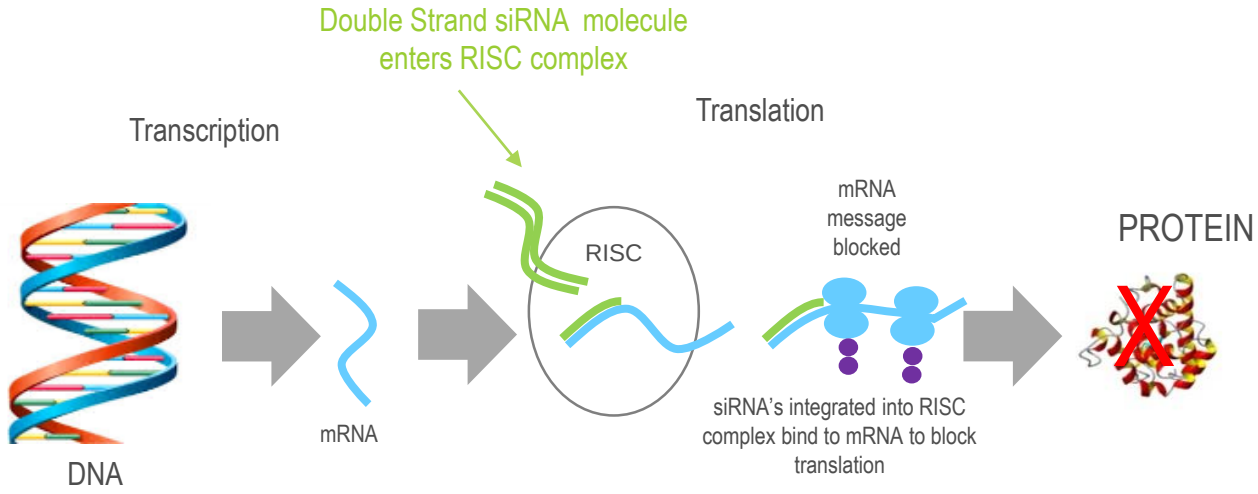




From 2016 to 2017 oligo therapeutics had largest ever increase in Research and Preclinical programs

Types of Oligonucleotide Therapeutics

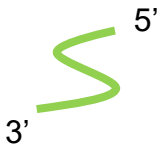




Molecular Types of Oligonucleotide Therapeutics

FLP = Full Length Product

Single Strand, miRNA



Double Strand siRNA,

Modified Single,

Modified Double

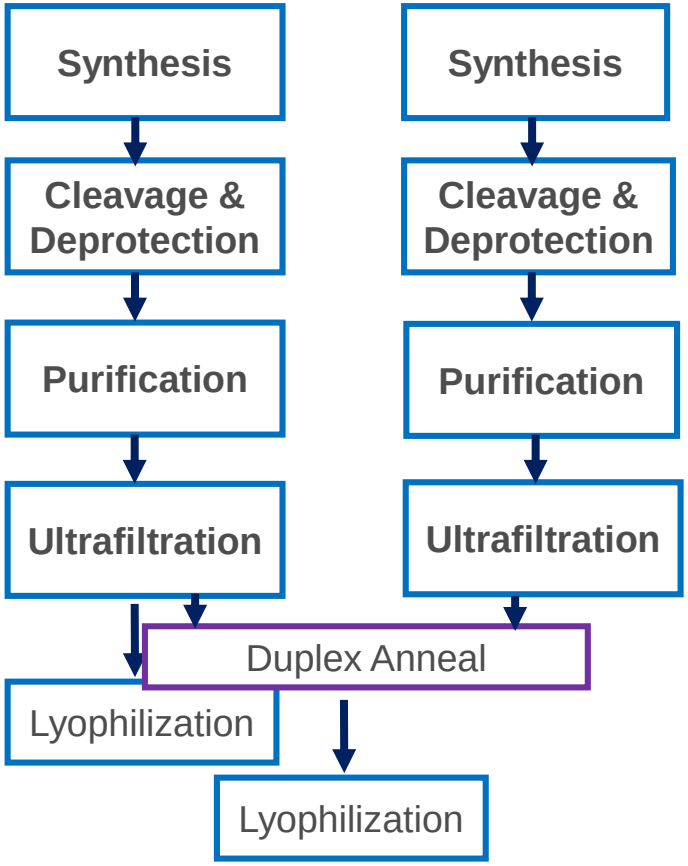
miRNA (n= 8-16)
siRNA (n= 18-27)

3' or 5', Saccharide, Cholesterol, PEG, other

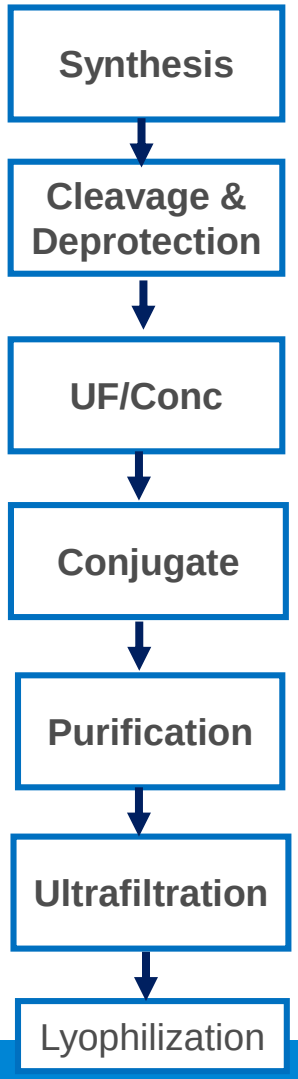
Types of Oligo MFG Processes

All Solid Phase to FLP

(natural or modified nucleotides)
(Single Strand)



Modified FLP (After SP)



Oligo MFG Processes

- Solid Phase Synthesis
- Cleavage & Deprotection
- Purification

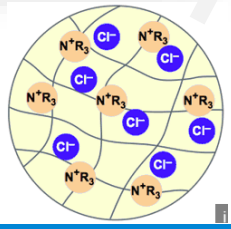
Solid phase
polymer or glass bead

Oligo MFG Processes

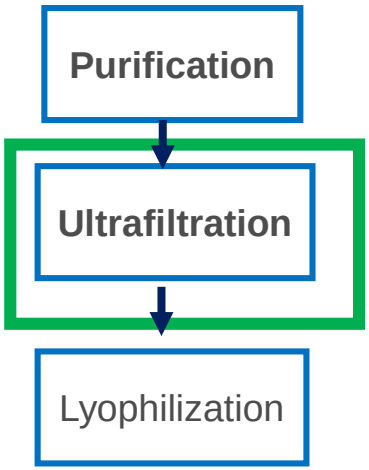
Cleavage & Deprotection

Purification

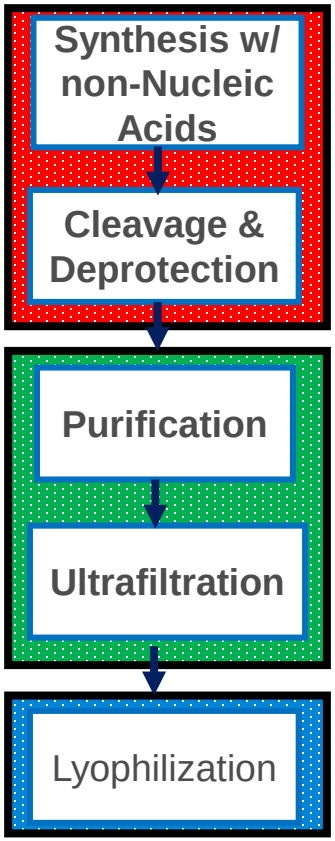
Ultrafiltration



Oligo MFG Processes



Impurities: Origin & Removal



(1) OoI

Raw Material Impurities (synthesis impact)
Synthetic Impurities (side products)

Deprotection Impurities (side products)

(2) RI

Species with reasonable Charge difference

(3) Iso

Impurity Types

Synthetic Impurities

FLP (n=20)

(n-x), Shortmer, capped

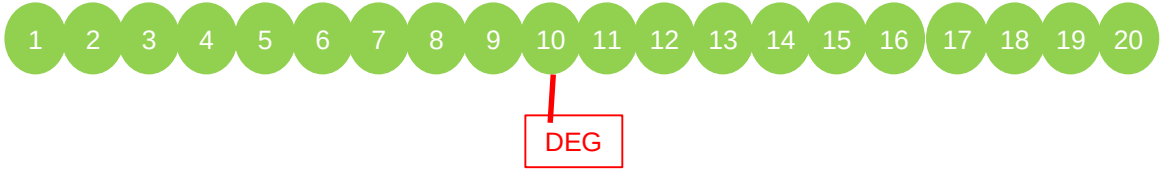
(n-x), Shortmer, missing “n”(s)

(n+x), Longmer, extra “n”(s)

Deprotection Impurities

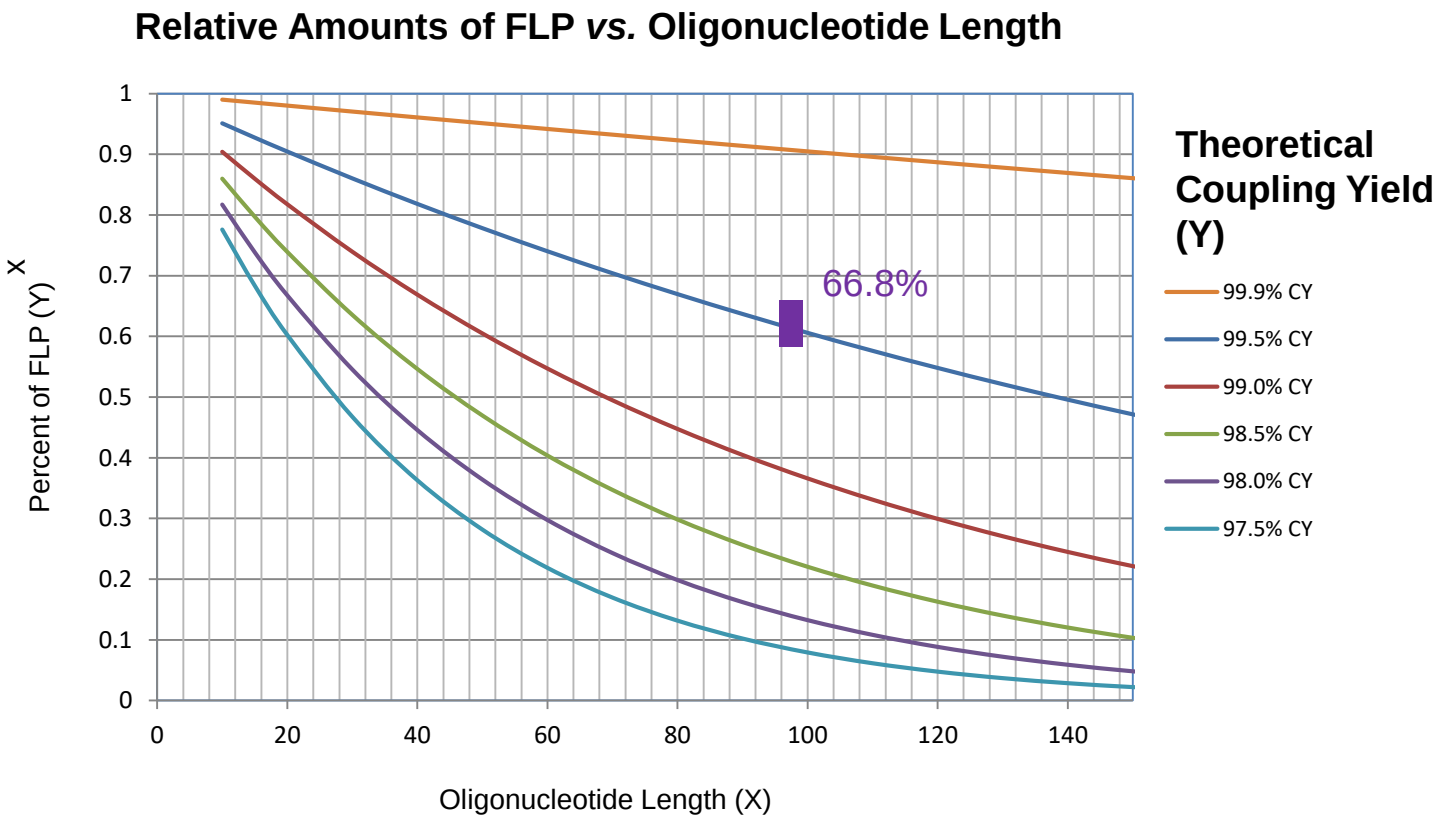
FLP(PG1), FLP(PG2), FLP(PG3)
PG (protecting group)

FLP(Deg)
Deg (degraded structure)



Coupling Yield Perspective

Example: 99% CY ~ 1% Impurities / (n)



Removal of Impurities

Difficulty of Removal

(n-x), Shortmer, missing Base(s)
(n+x), Longmer, extra Base(s)

AX

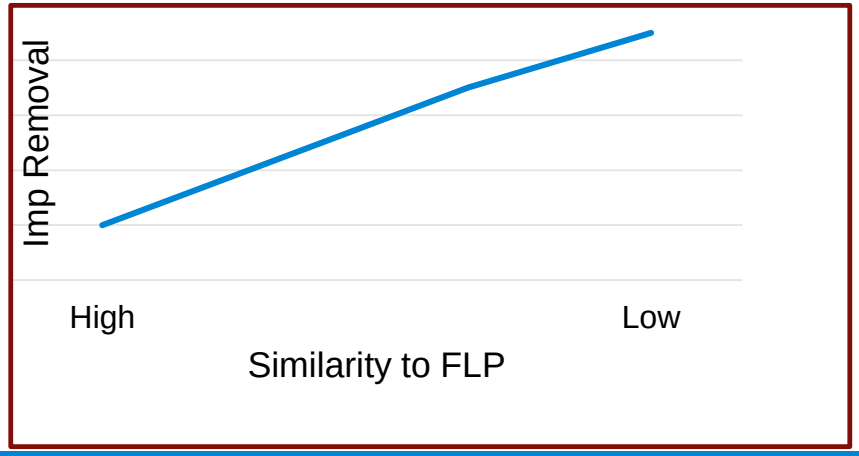
- **X = 1**
- **X = 2**
- **X = 3 or >**

FLP(PG1)$_x$, FLP(PG2)$_x$, FLP(PG3)$_x$
FLP(Deg)*

AX

- **X = 1**
- **X = 2**
- **X = 3 or >**

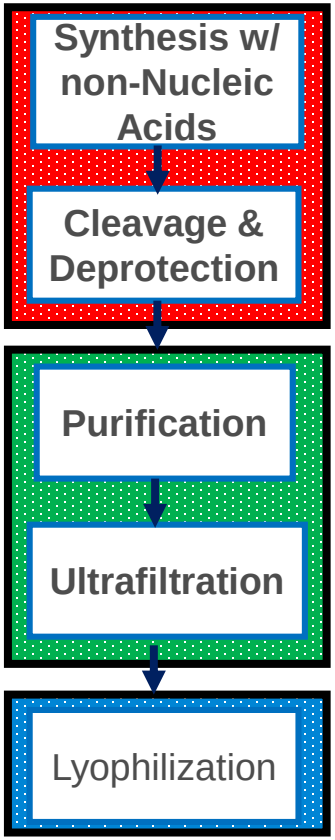
*** = unique**



Process Property Summary

- Total Impurity Level is greatly impacted by chain length, coupling yield and Deprotection yield
- Major impurities which remain in the FLP are similar molecular structures, (n-1,n+1, PG1, Deg, etc)
- Removal of impurities to low levels (<10% total) is restricted by similarity of structure and charge to that of the Full Length Product

Control Points & IP Controls



	IPT	IPAC (acceptance criteria)
Raw Material Specs		
Parameter control via Computer Time / Flow / UV		
Temperature & Time Controllers	(1) HPLC (Purity)	Yes
Parameter control via Computer Time/Flow/Conductivity/UV/pH/Temp	(2) HPLC (Purity)	Yes

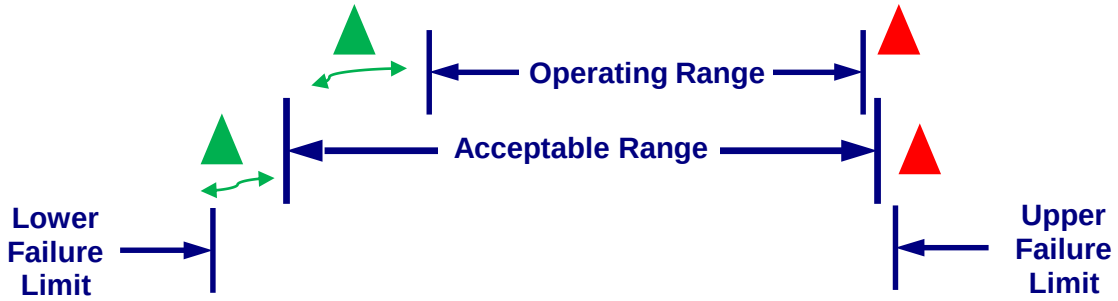
Key Considerations of In Process Assays

- Use a Representative Sample
- Prepare & Handle Sample for Accurate Analysis
  - ❖ Understand impact on analysis (e.g. degradation)
  - ❖ Some Oligo matrixes require careful handling
- Assay type fits the need
- Review the data for soundness (integrity)

❖ Consider and Implement in Development

Categorization of Process Parameters – Range Impact to Criticality

Scenario: Parameters that impact CQA



Critical Parameter

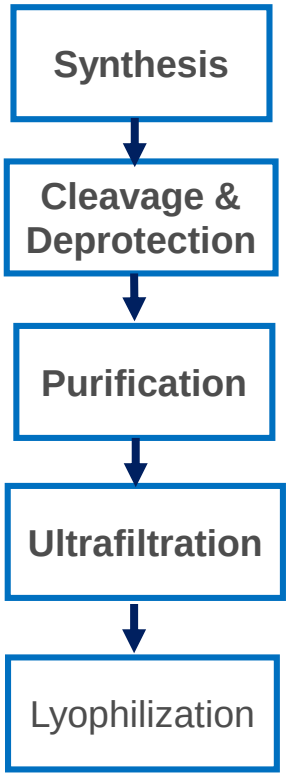
Scenario: Parameters w/minor impact to CQA

Non-critical Parameter

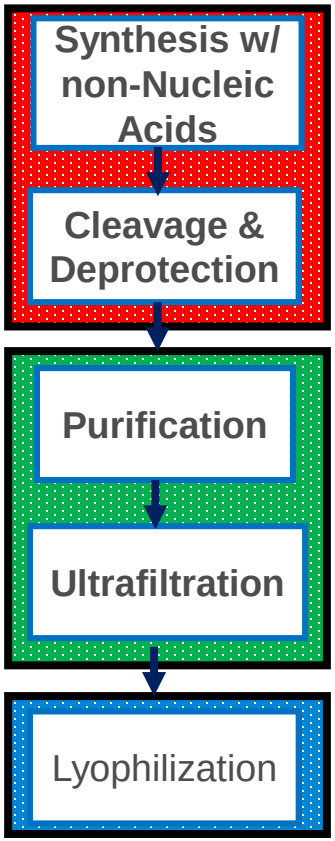
???

Design Space Options

Individual Unit Ops



Groups of Unit Ops



Discussion Items

❖ Basis for Setting Impurity Specifications

- Total Impurity Level is greatly impacted by coupling efficiency, chain length and charge similarity to FLP Major impurities with high structure similarity to FLP are difficult to remove
- Removal of impurities to low levels (<10% total) is restricted

❖ Basis for defining criticality

- Does the range Δ between PAR and NOR and PAR and failure play a role in defining criticality of a parameter

Acknowledgements
Lori Troup
Todd Kreutzian
Bob Groneberg
Jeff Beckvermit
Gary Carter

NASD Development Department

Extra Slides

Key Considerations of In Process Controls

- Use simple physical parameters (visual, computer triggered)
- Use IPC's for Physicochemical parameters wherever possible (pH, UV, Conductivity, FTIR..)
- Use in process tools (test strips, etc)