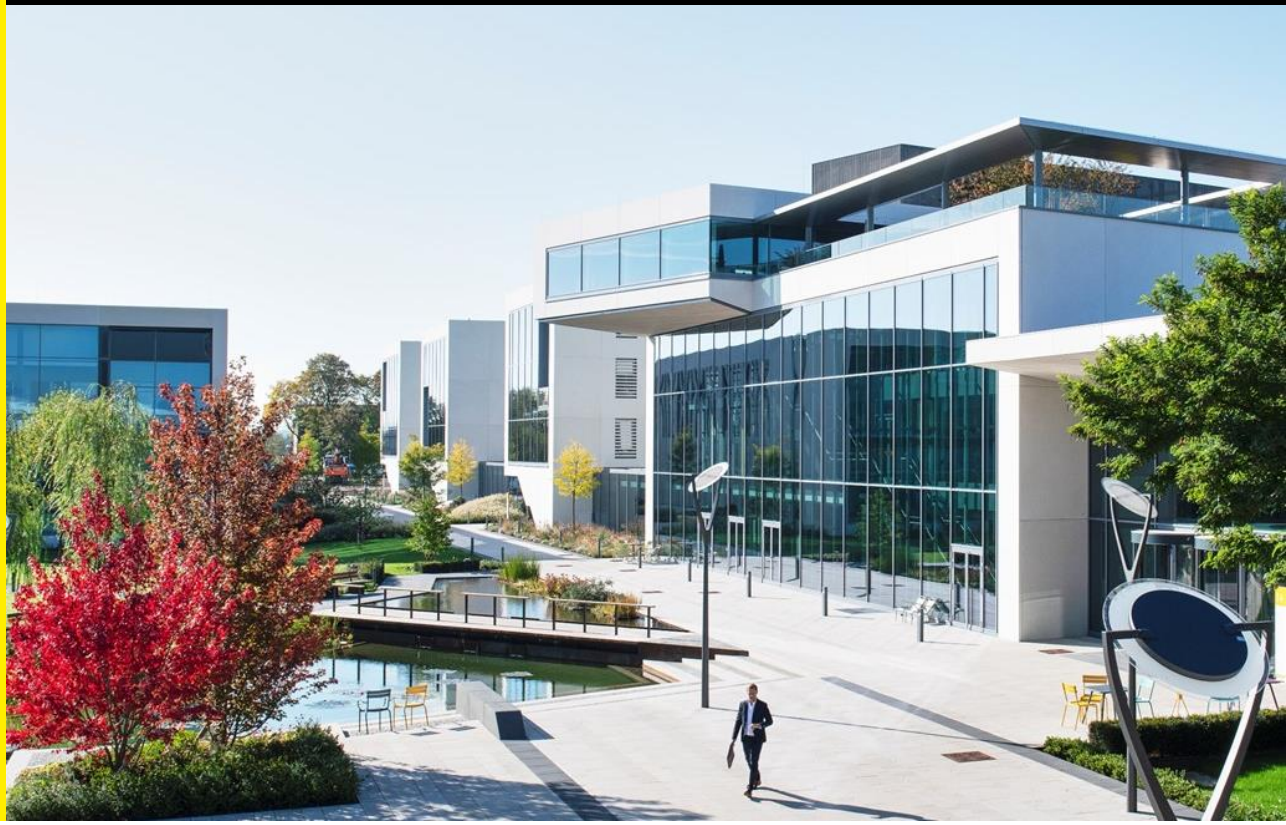
SARTORIUS

BIA separations

Simplifying Progress



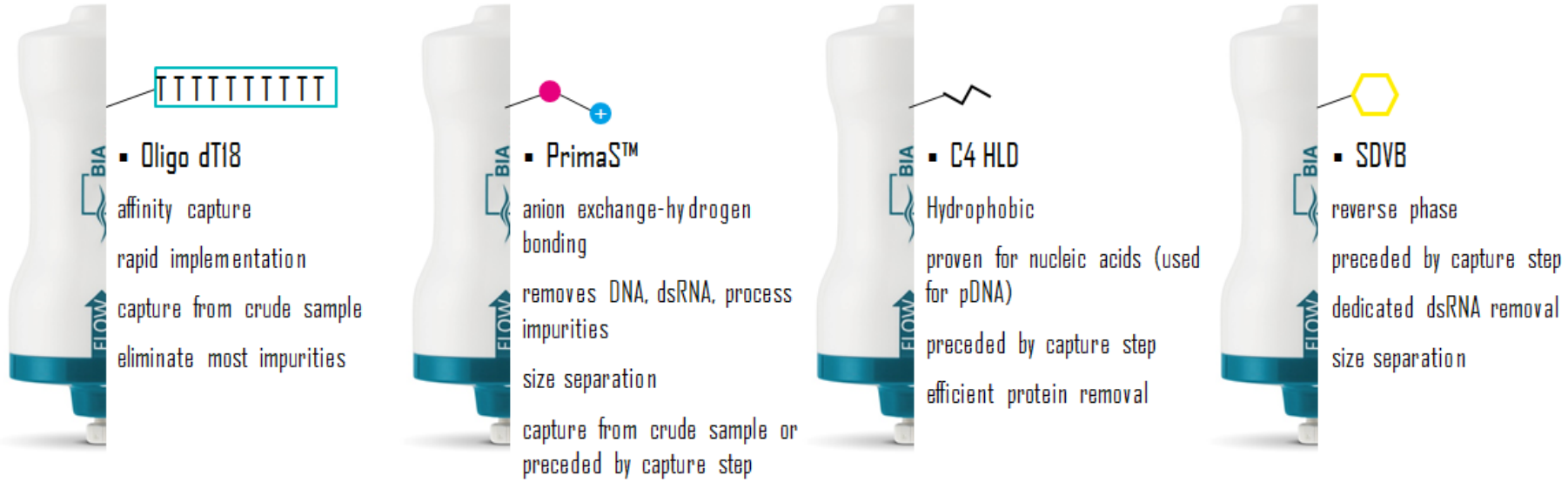
Chromatographic Tools for a High-Yielding mRNA Production Process

Nucleic Acids, 01Feb2021

Rok Sekirnik, PhD

Agenda

- Benefits of mRNA therapeutics
- mRNA production process overview
- In vitro transcription production, raw materials, products, and contaminants
- mRNA purification toolbox RNA



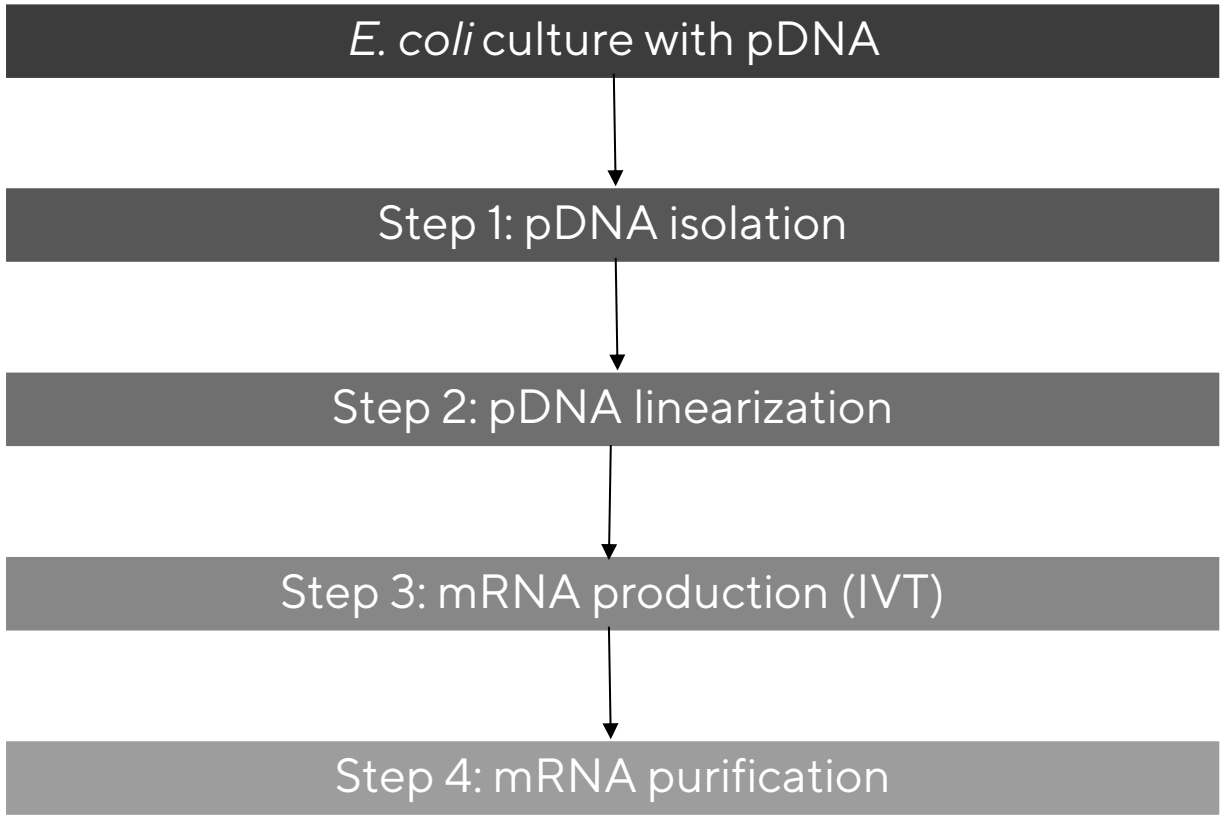
Benefits of mRNA therapeutics

Safety: it is non-infectious, non-integrating without risk for infection or insertional mutagenesis (mutation of the gene). It is degraded by normal cellular processes and its short in vivo half-life can be regulated through modifications and delivery methods.

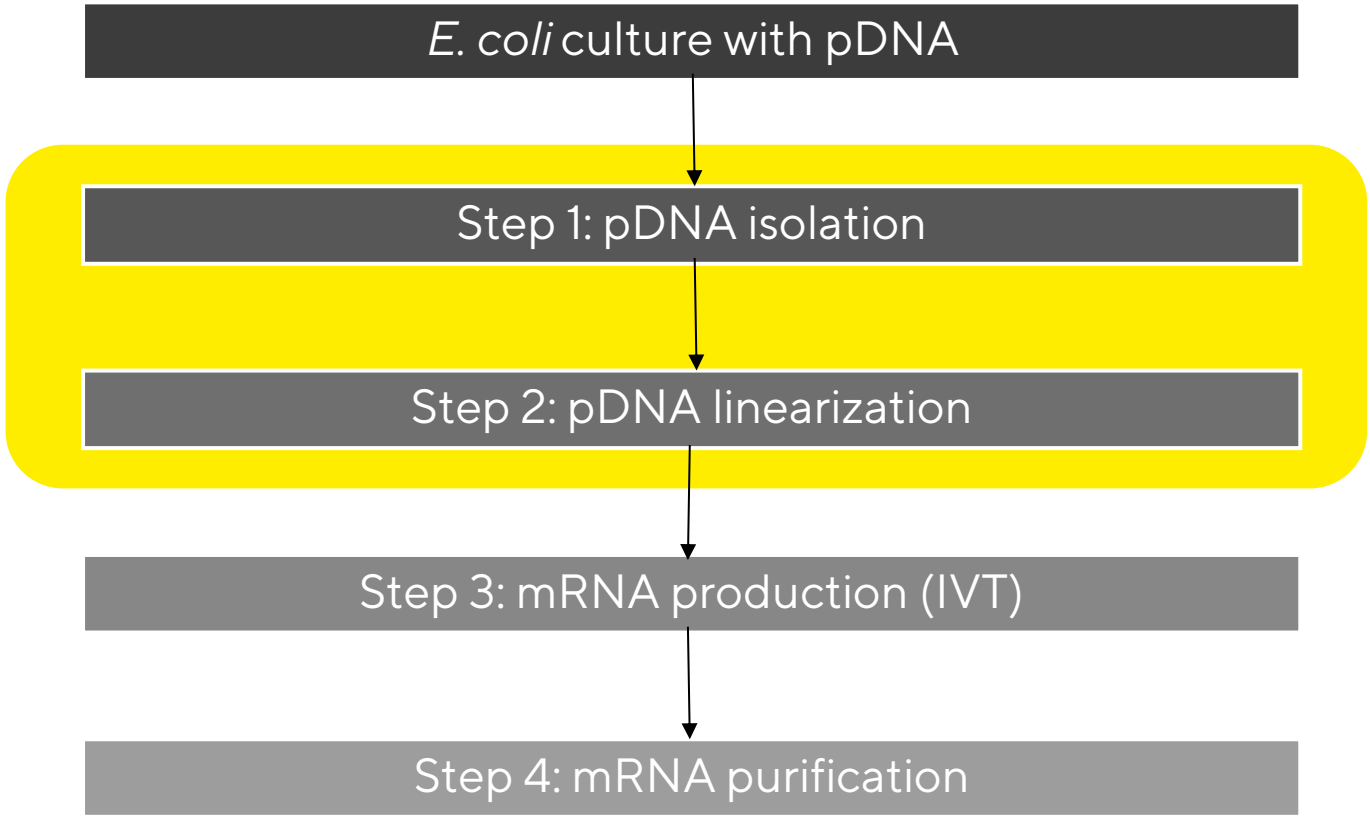
Efficacy: various modifications can improve stability and translation efficiency. It is the minimal genetic vector and anti-vector immunity can be avoided – it can be administered repeatedly.

Production: potential for rapid, inexpensive and scalable manufacturing through enzymatic reactions (no cell culture).

mRNA production process overview

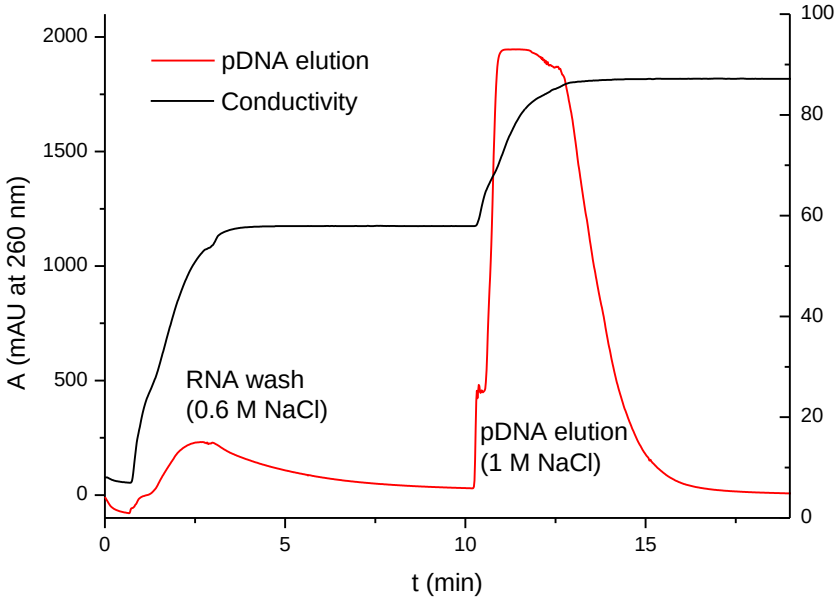


mRNA production process overview



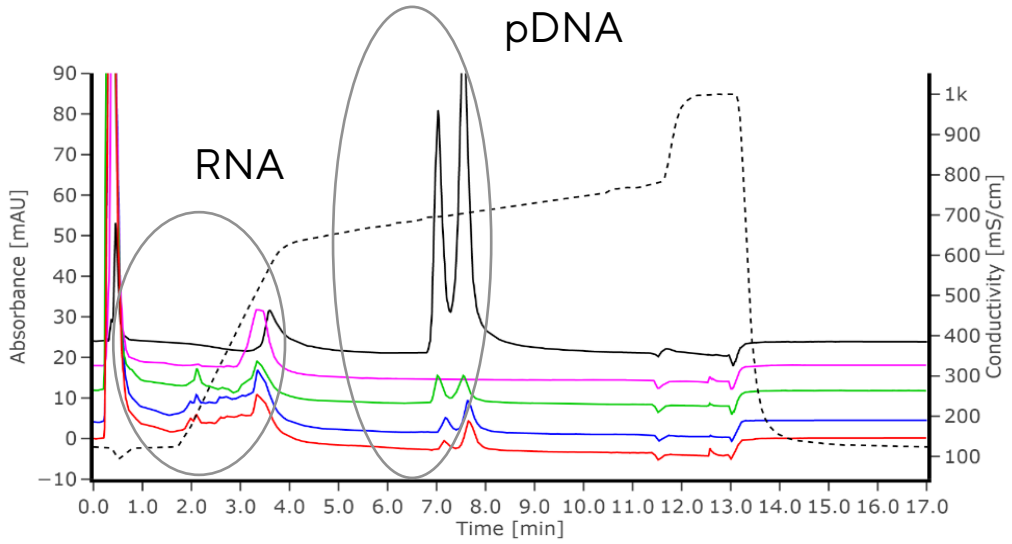
Purification of pDNA

CIMmultus DEAE column



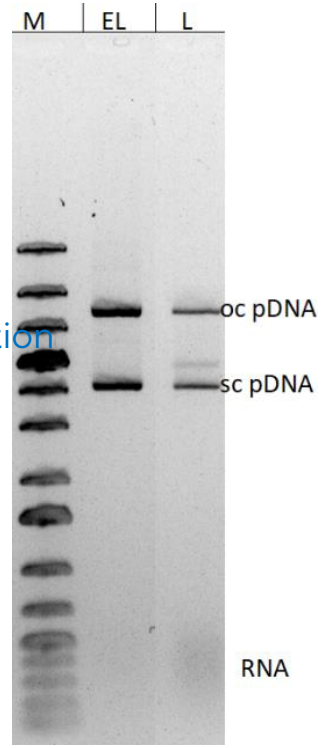
Parameters – elution fraction	
pDNA recovery in elution (%)	83
RNA removal (%)	92
oc pDNA (%)	31

HPLC CIMac™ pDNA column



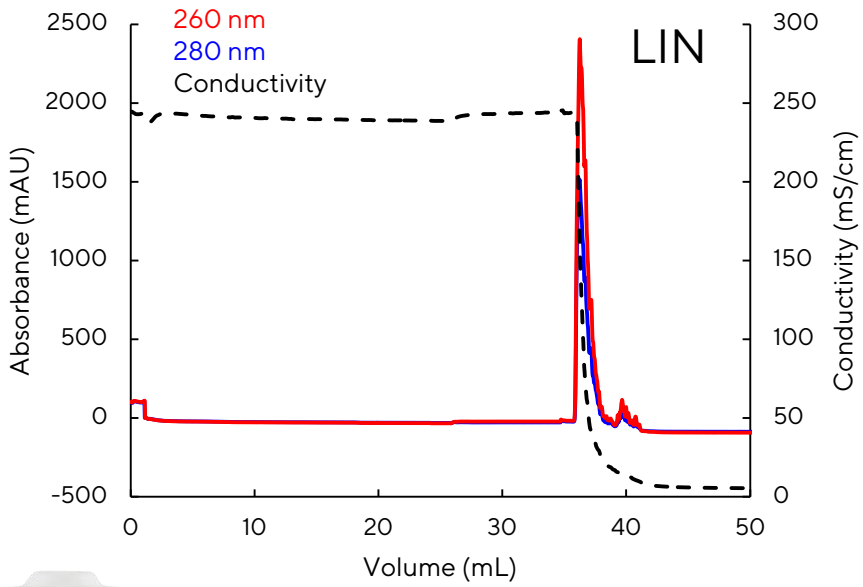
AGE

DEAE elution
RNA wash
DEAE load
Lysate after filtration
Lysate



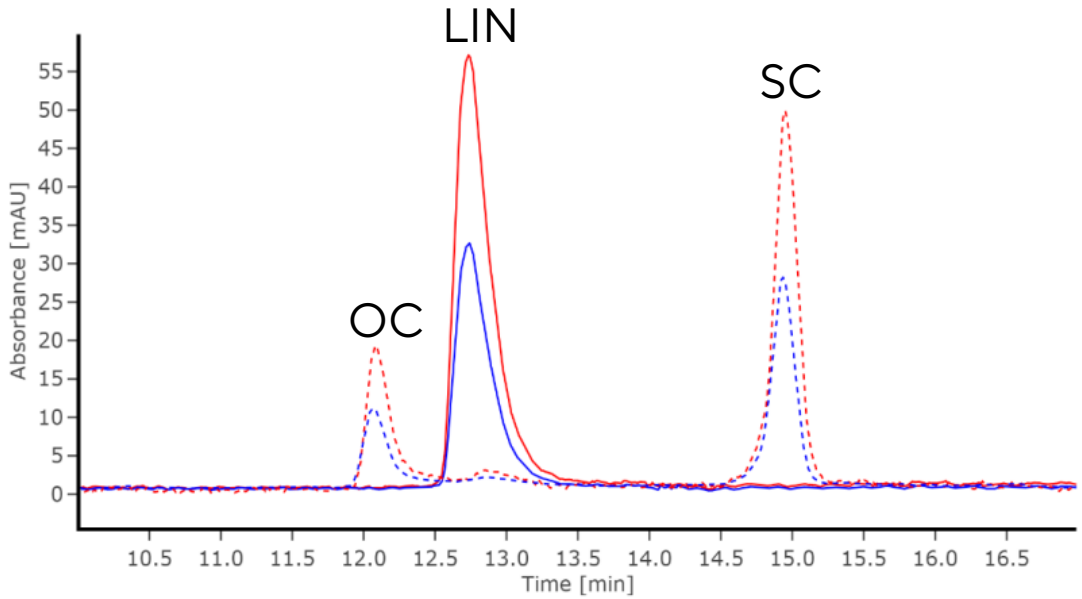
Polishing of linear pDNA - CIMmultus™ C4 HLD column

- Preparative CIMmultus™ C4 HLD

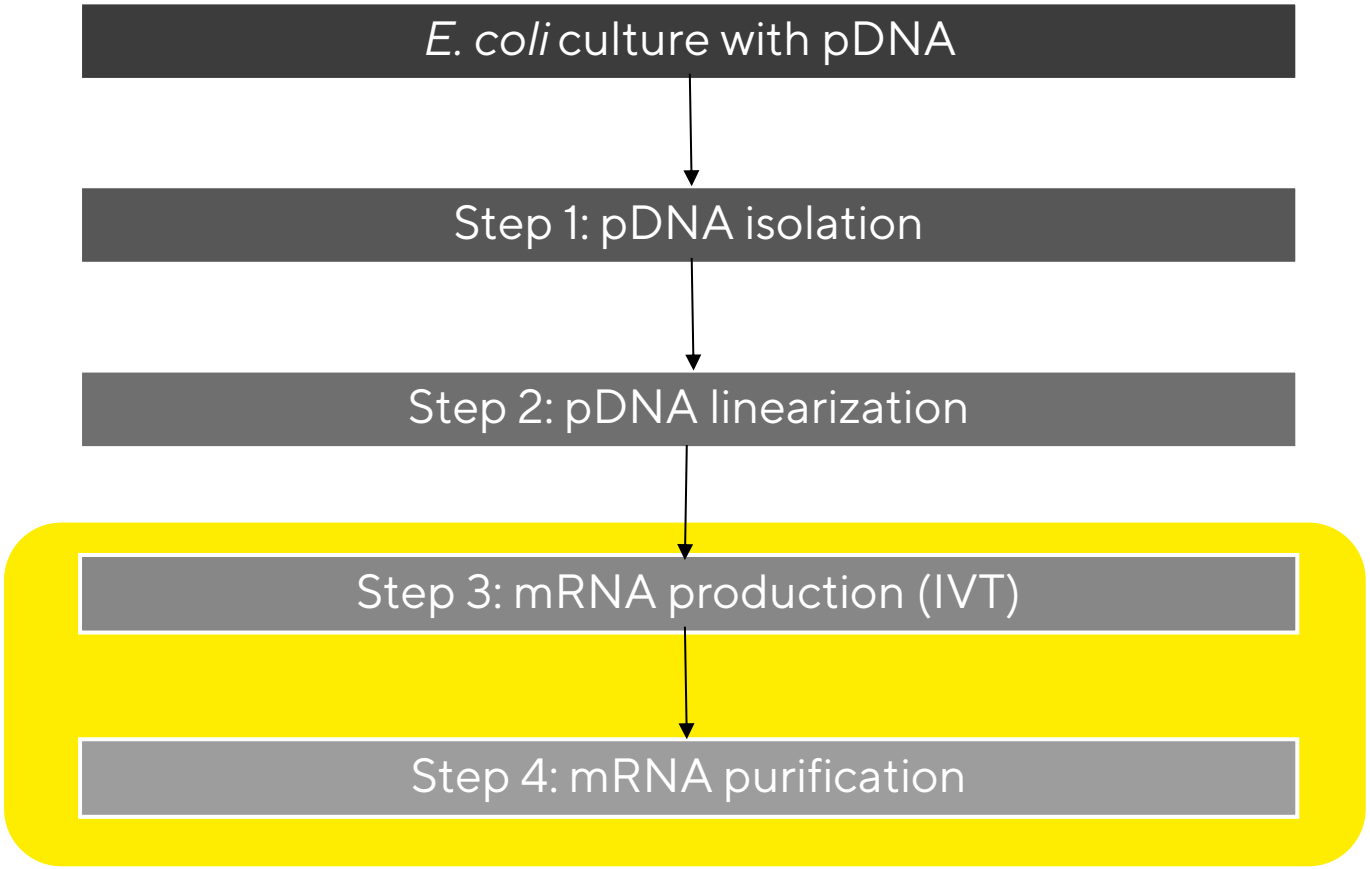


- HPLC CIMac™ pDNA

- - A260 nm before linearization
- - A280 nm before linearization
— A260 nm after linearization
— A280 nm after linearization



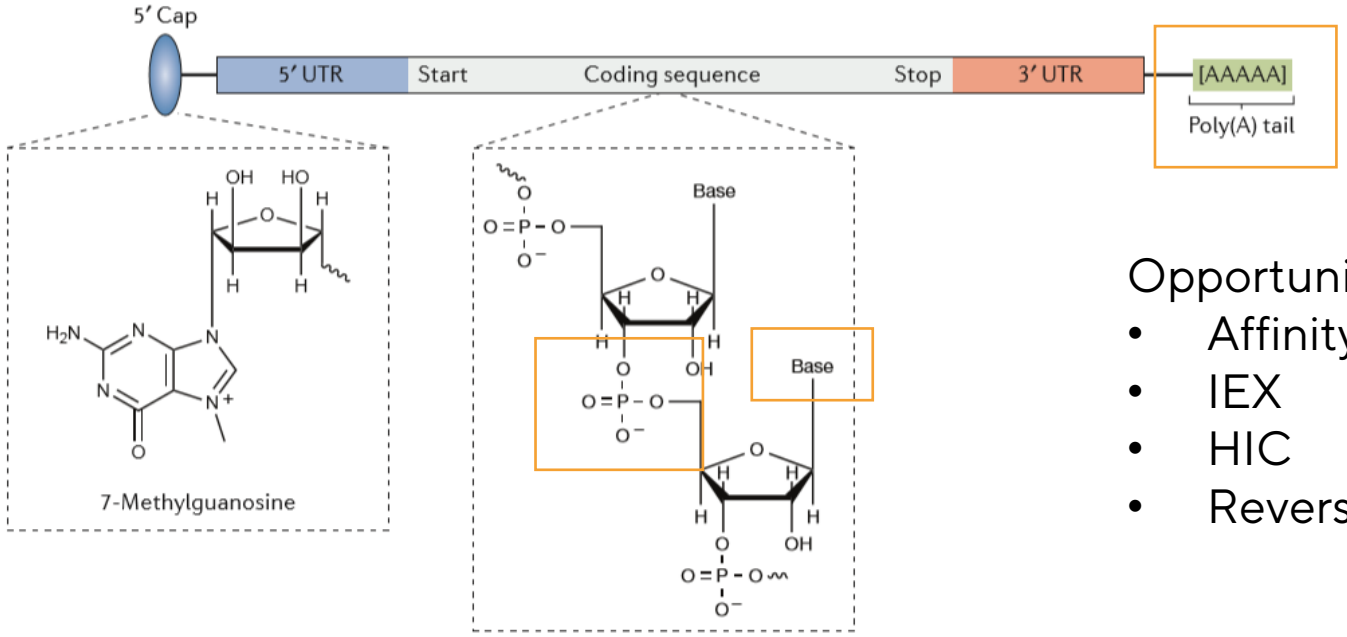
mRNA production process overview



mRNA

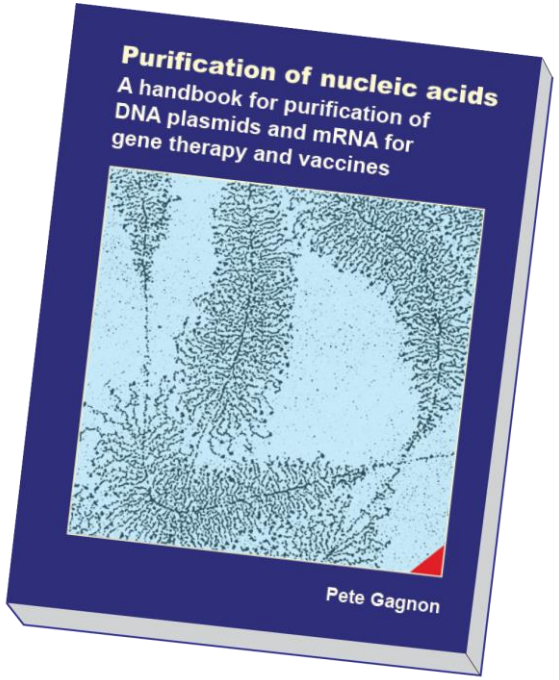
Single stranded RNA molecule, mRNA average 2000-2500 bases in nature,

however sizes range 1-15kb as therapeutics. Synthetically produced in enzymatic IVT reaction.



Opportunity for:

- Affinity
- IEX
- HIC
- Reversed phase

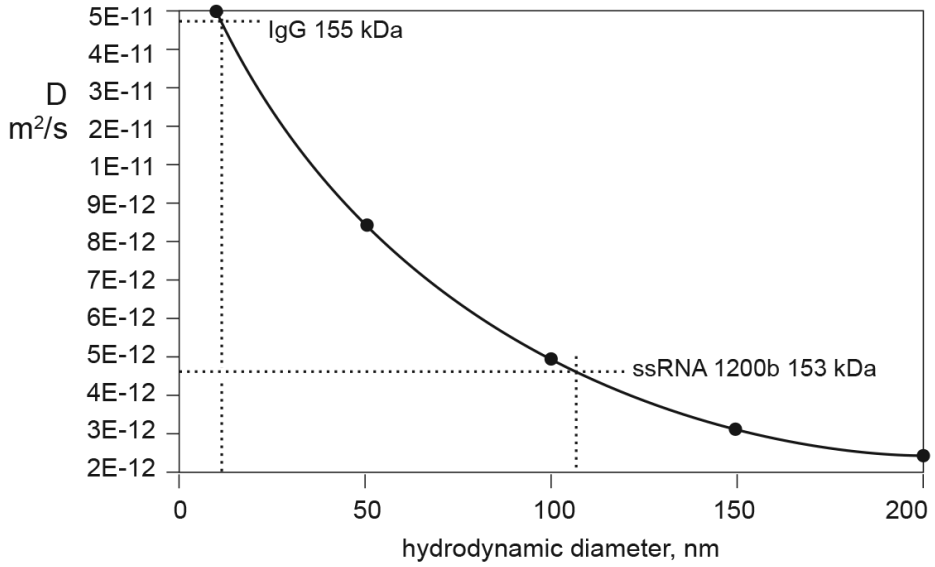


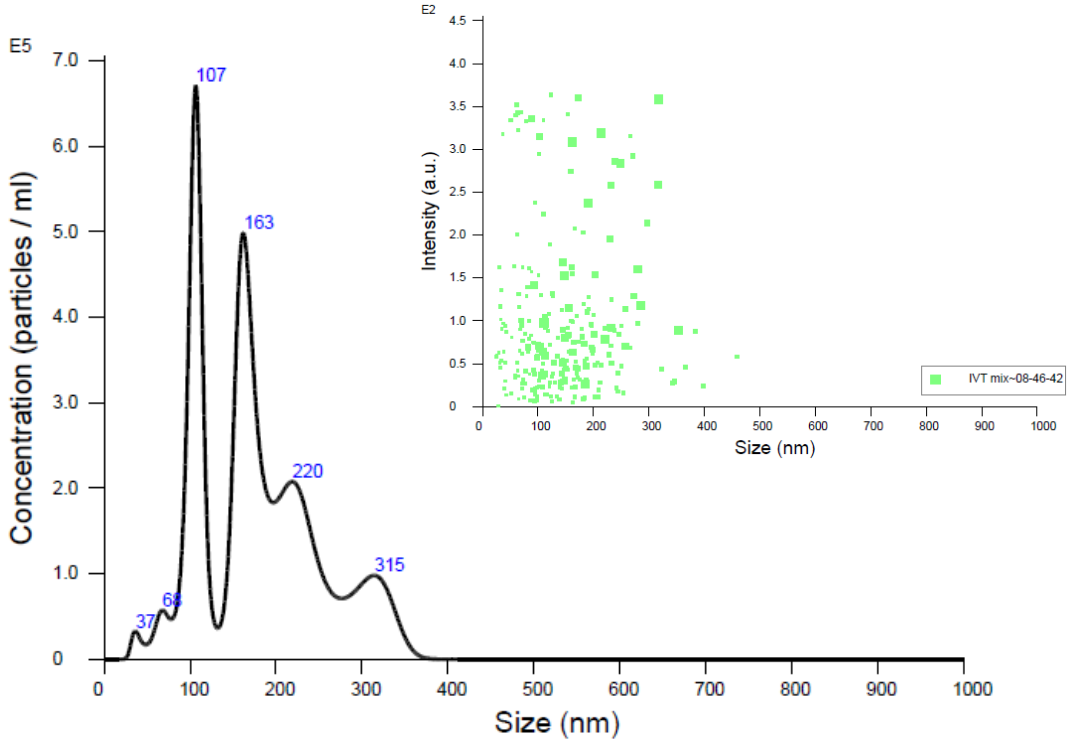
Hajj, Khalid A., and Kathryn A. Whitehead. "Tools for translation: non-viral materials for therapeutic mRNA delivery." *Nature Reviews Materials* 2.10 (2017): 17056.

Purification challenges: mRNA size = slow diffusion

Large size imposes diffusion constraints which is important in certain chromatographic media.

Large size can also limit pore accessibility and reduce binding capacity.



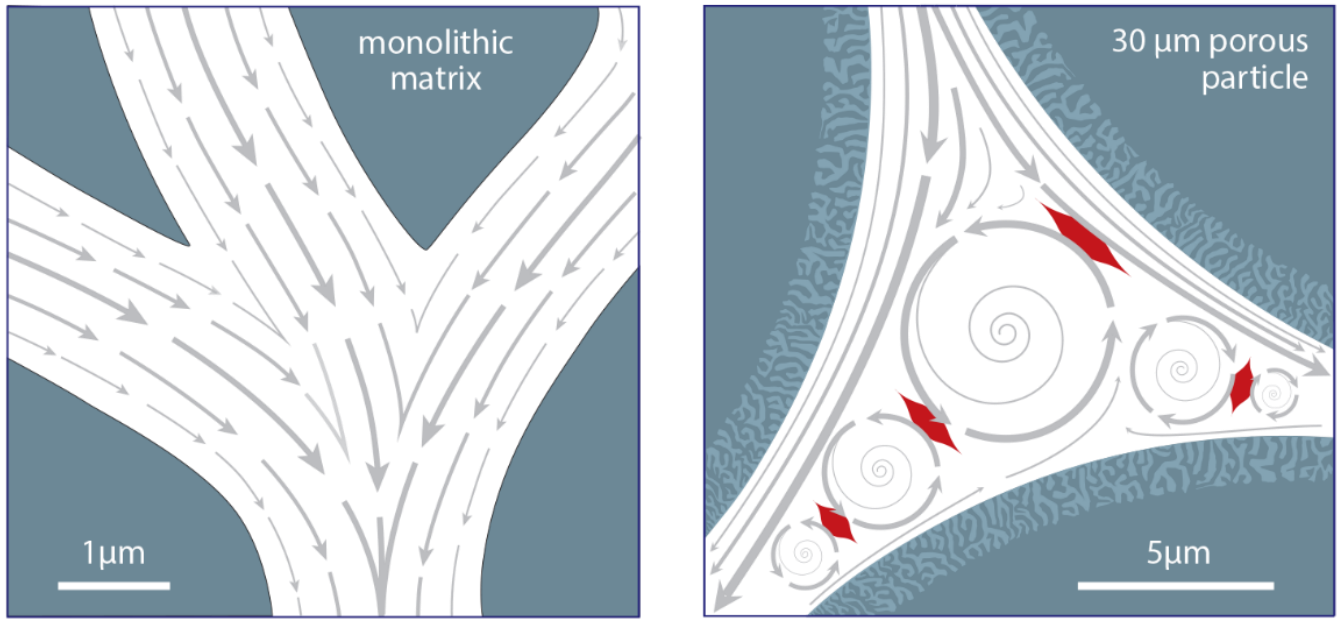


1200b ssRNA has about the same mass as IgG but it diffuses 10 times more slowly because of its more linear structure.

Purification challenges: mRNA size = shear sensitive

Laminar flow produces very low shear.

Turbulent flow produces zones of countercurrent flow that create high shear stress (red).



Large channel structure of monolith: no diffusion, no shear forces

Purification challenges: contaminants in IVT mixture

IVT mixtures contain multiple classifications of contaminants:

1. RNA variants: dsRNA, truncations, fragments, aggregates.
2. Synthesis raw materials: The DNA plasmid and enzymes.
3. The contaminants in the raw materials:
   In the plasmid: *E.coli* proteins, DNA, and RNA.
   In the enzymes: fragments, host DNA, RNA, proteins.
4. Endotoxins, if introduced during processing.

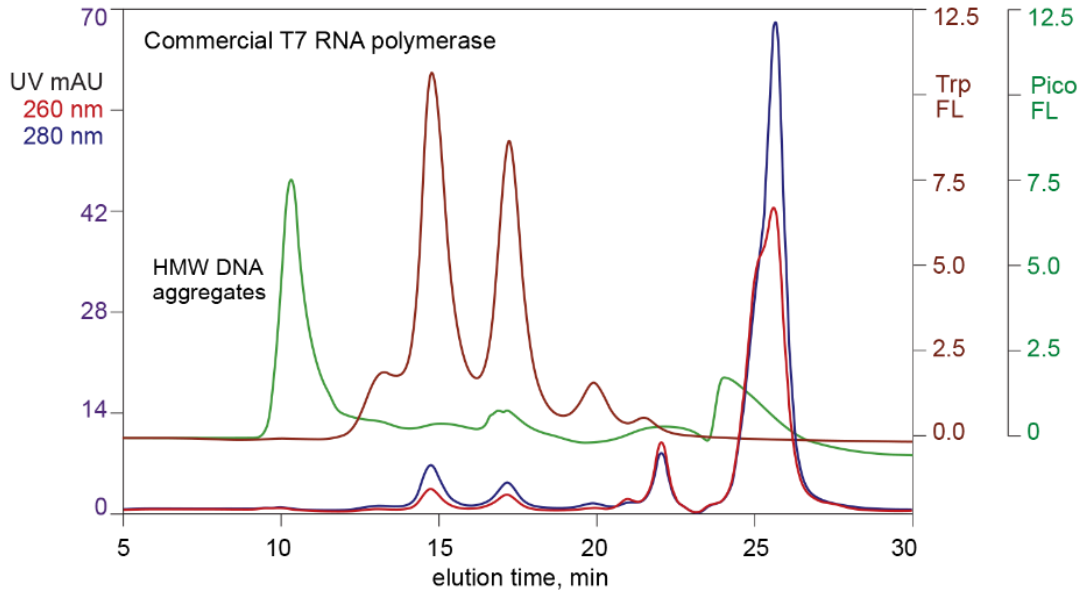
If the plasmid is digested post-synthesis, there are two more:

5. The DNAse enzyme and/or protease enzyme,
6. And the contaminants they carry, including metal ions.

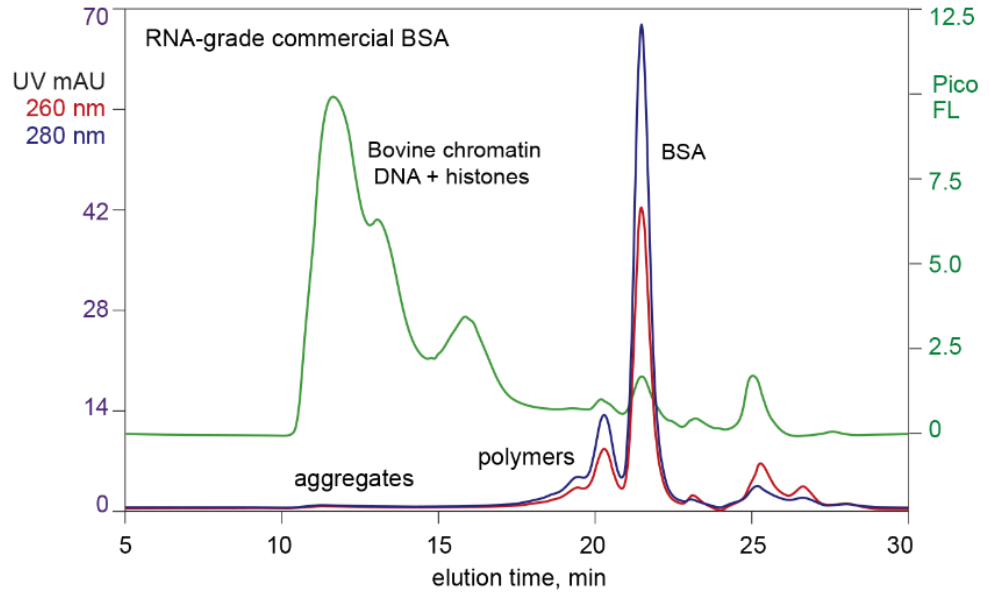
Raw material contaminants

Impurities and secondary contaminants in processing materials.

Robust raw material control is imperative.

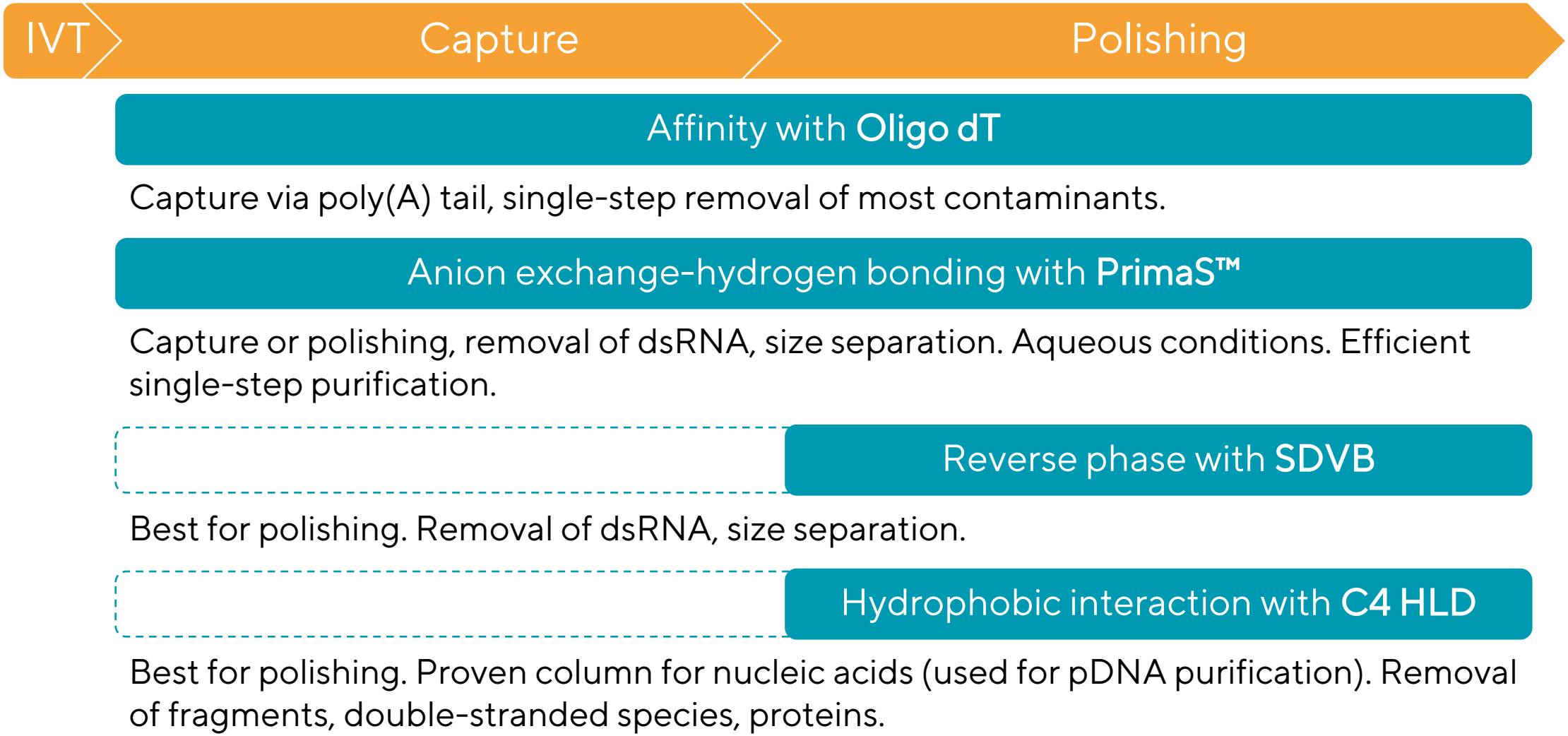


Analytical SEC. TSKgel™ G3000SWxl. 0.5 mL/min. Prestained with Picogreen.



Analytical SEC. TSKgel™ G4000SWxl. 0.5 mL/min. Prestained with Picogreen.

mRNA purification toolbox

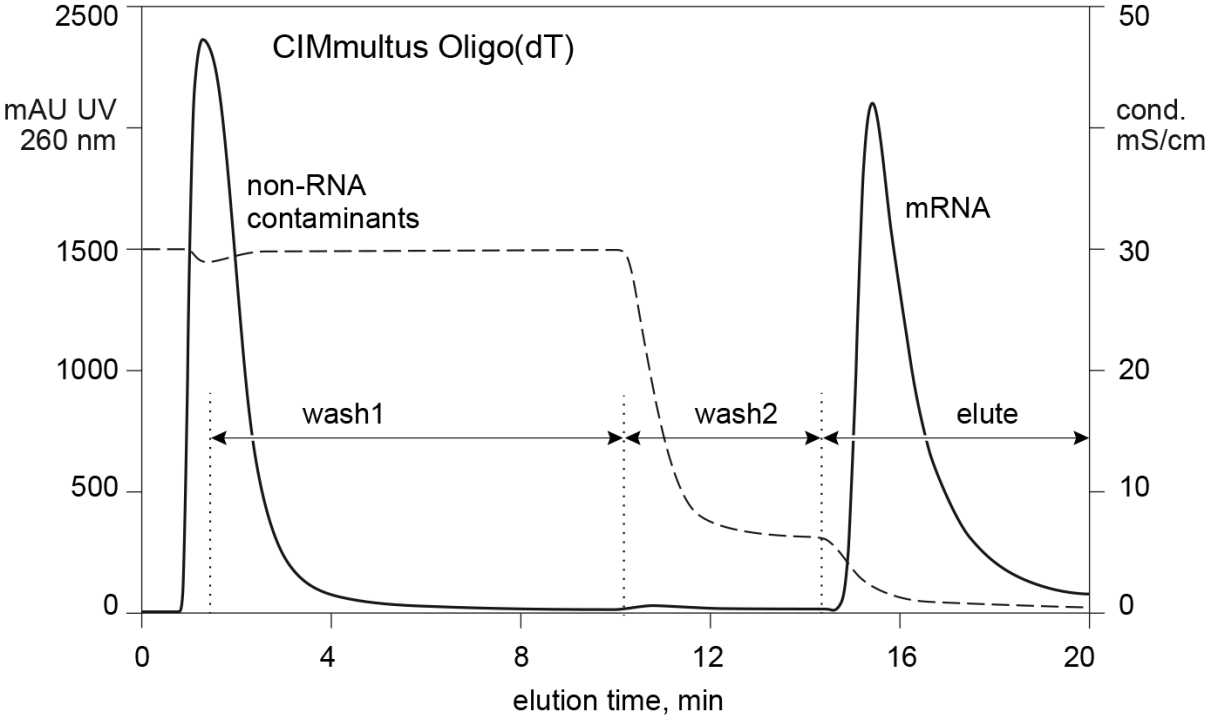


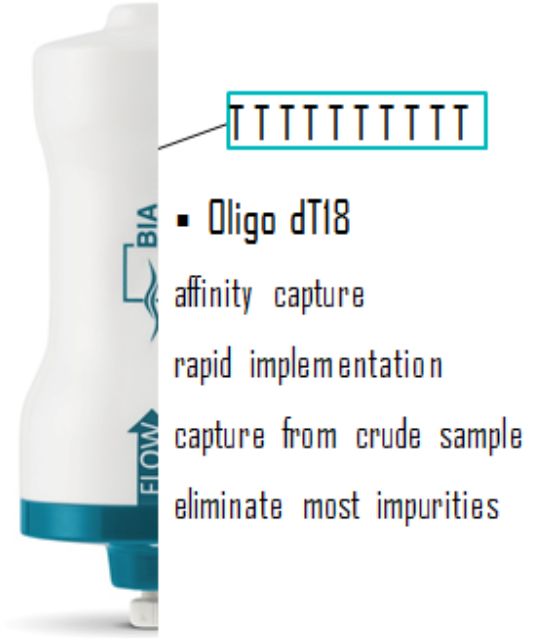
The tools: hybridisation affinity – Oligo dT

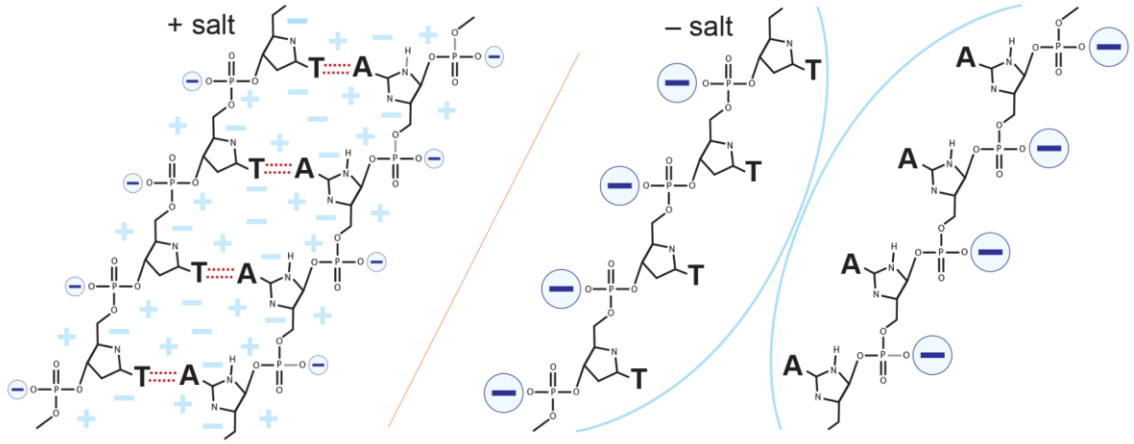
CIMmultus™ Oligo dT selectively binds the poly-A tail of mRNA.

Species lacking a poly-A tail flow through. RNA is recovered in a single step.

Binding in salt, elution in water.

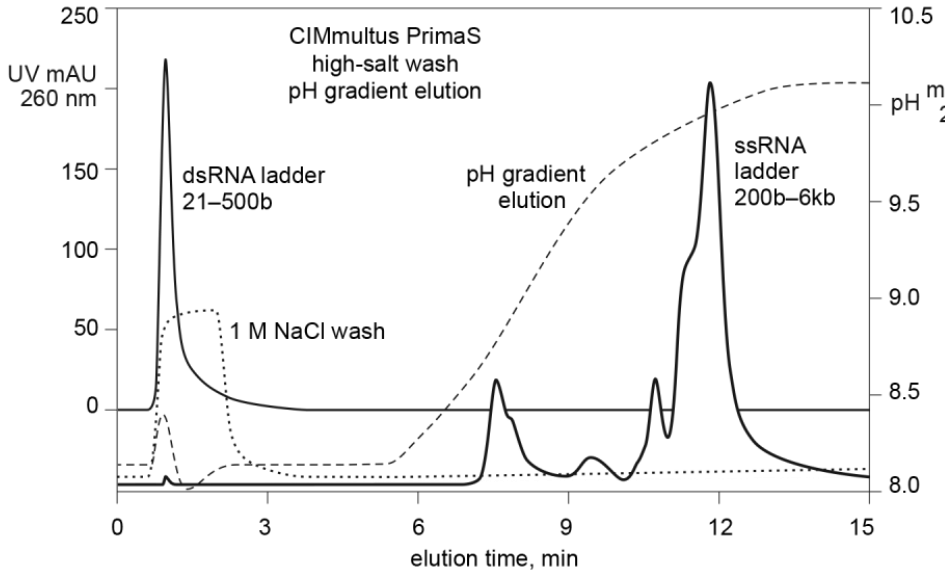


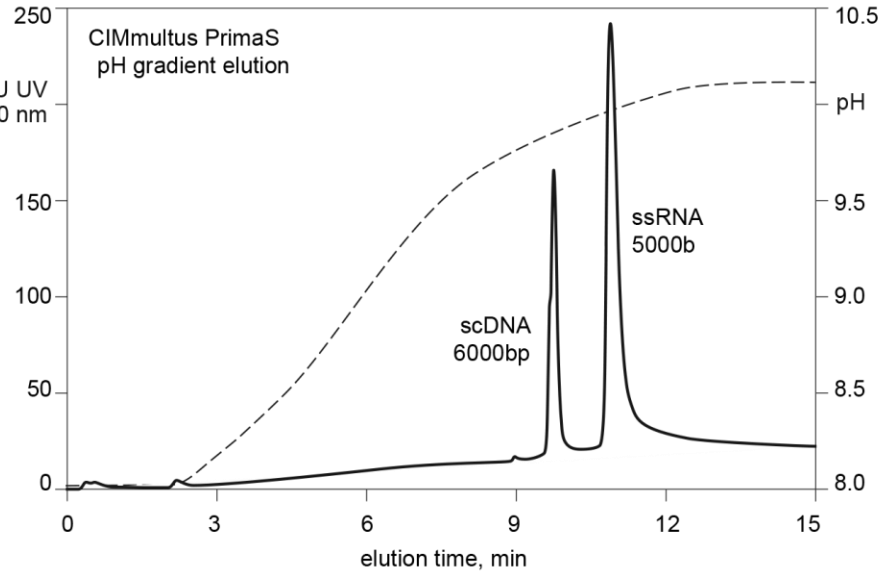




The tools: anion exchange chromatography - PrimaS

Ambient temperature, aqueous conditions for removal of DNA, ds species, and size fractionation. High salt wash followed by pH gradient elution.





- PrimaS™

anion exchange-hydrogen bonding

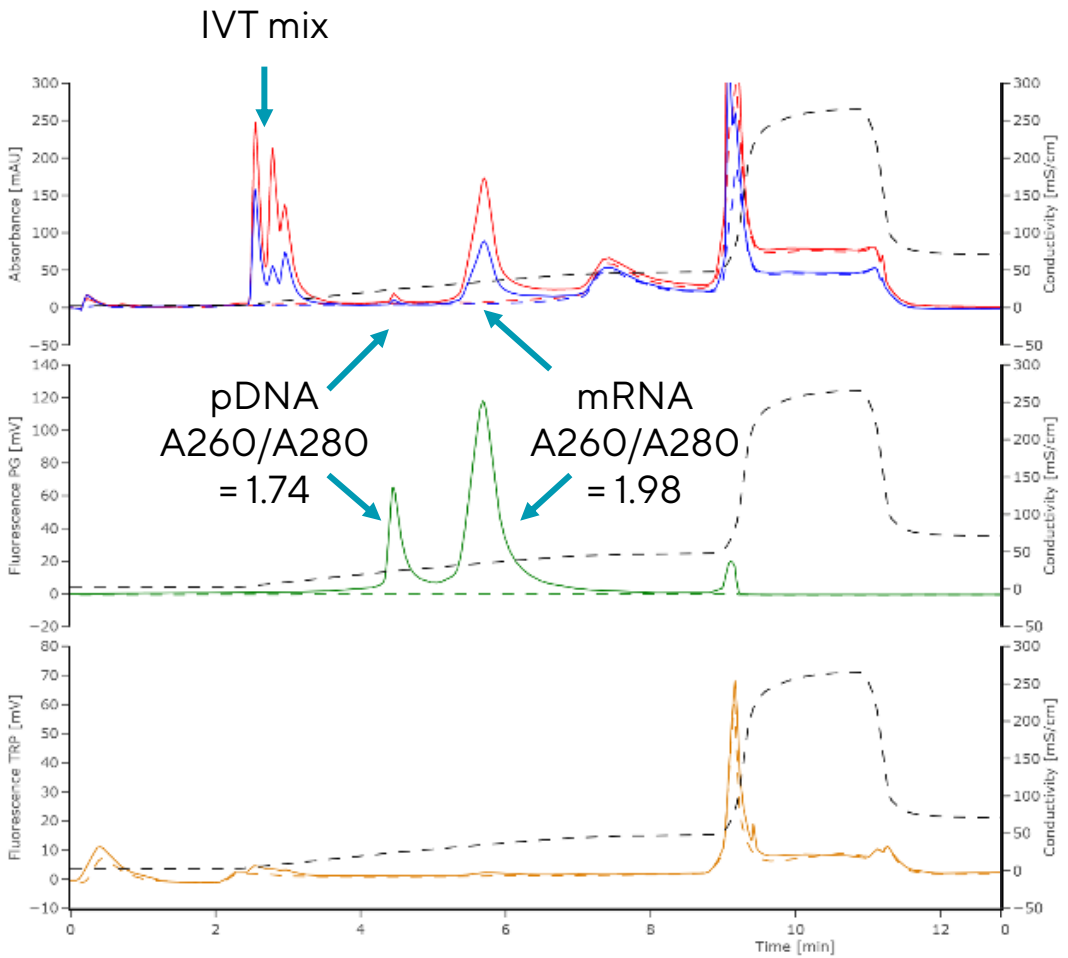
removes DNA, dsRNA, process impurities

size separation

capture from crude sample or preceded by capture step

Fractions neutralized immediately after elution show no adverse effects. Chaotropic salts like guanidine are typically more effective than NaCl. Single-stranded RNA remains bound even in saturated guanidine.

The tools: anion exchange chromatography - PrimaS



mRNA can be captured from IVT mix directly and separated from key process contaminants.

UV260

UV280

Ribogreen® fluorescence

Tryptophan fluorescence

- PrimaS™

anion exchange-hydrogen bonding

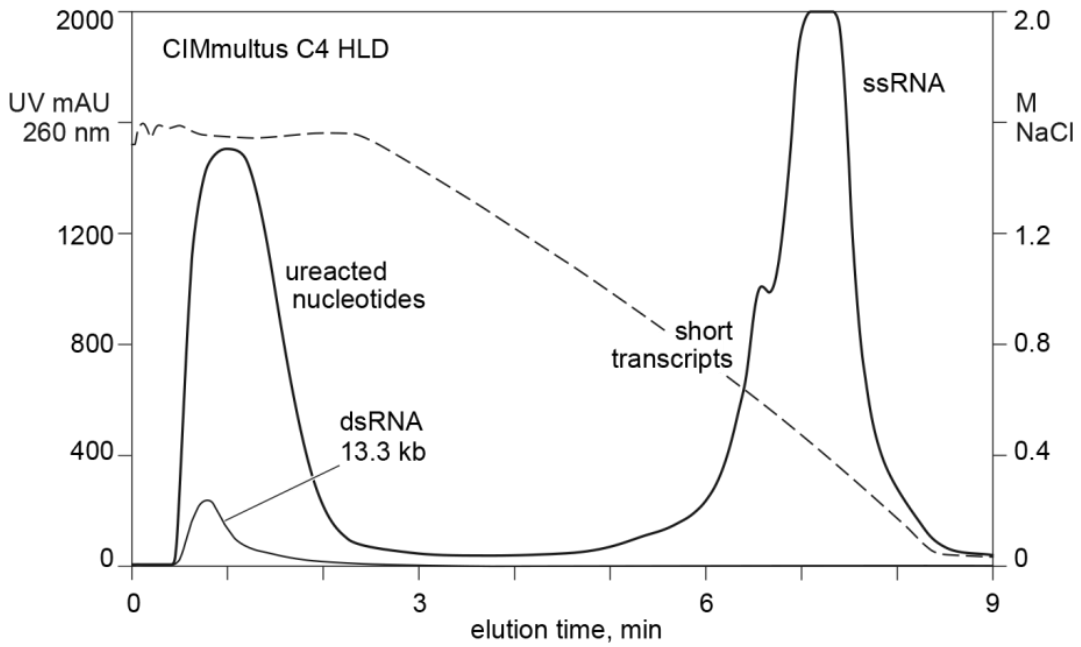
removes DNA, dsRNA, process impurities

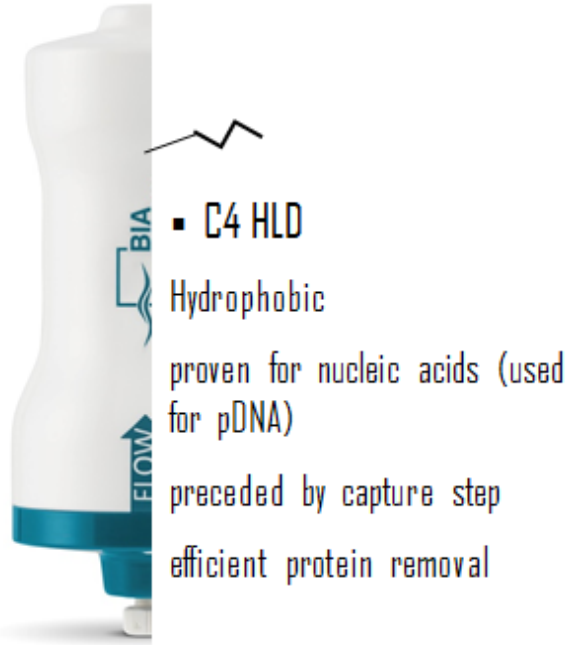
size separation

capture from crude sample or preceded by capture step

The tools: hydrophobic interaction chromatography – C4 HLD

CIMmultus C4 HLD is well-suited for protein removal. It may also separate ssRNA from truncated forms, dsRNA, and DNA. Binding is in high salt, elution to low salt.

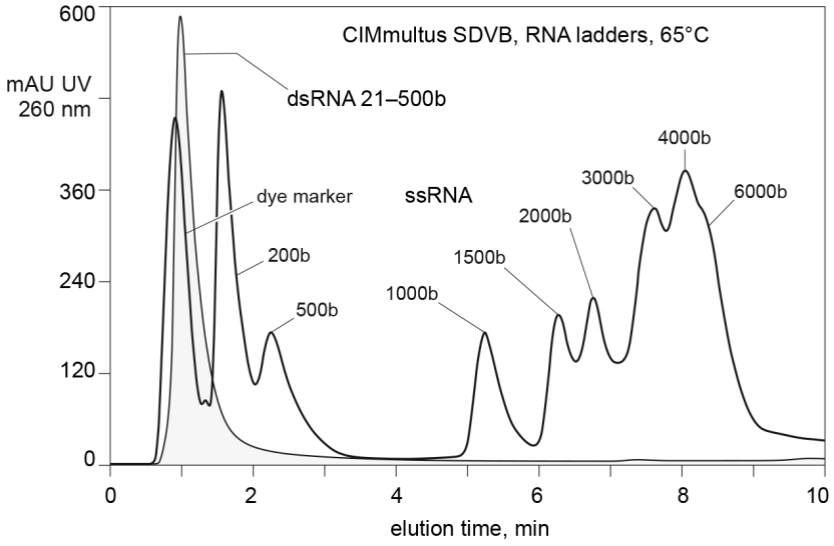


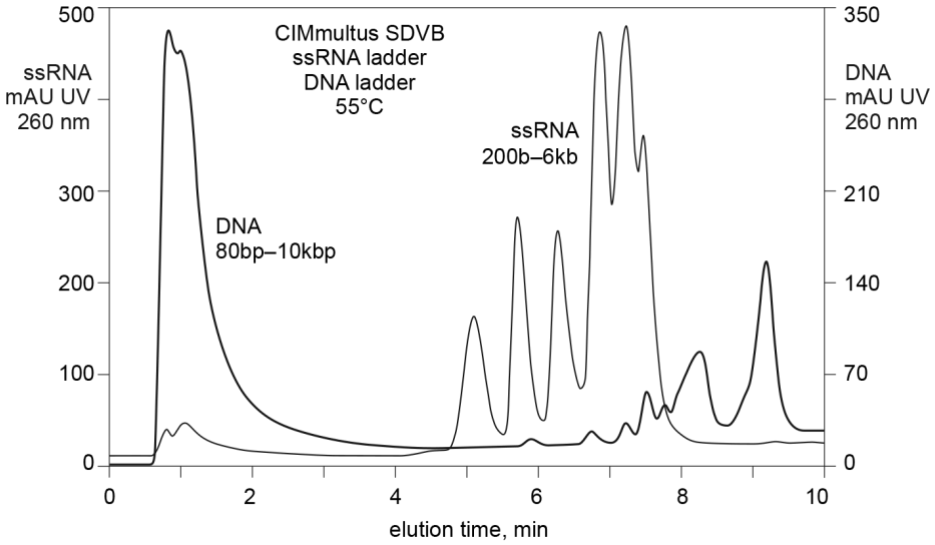


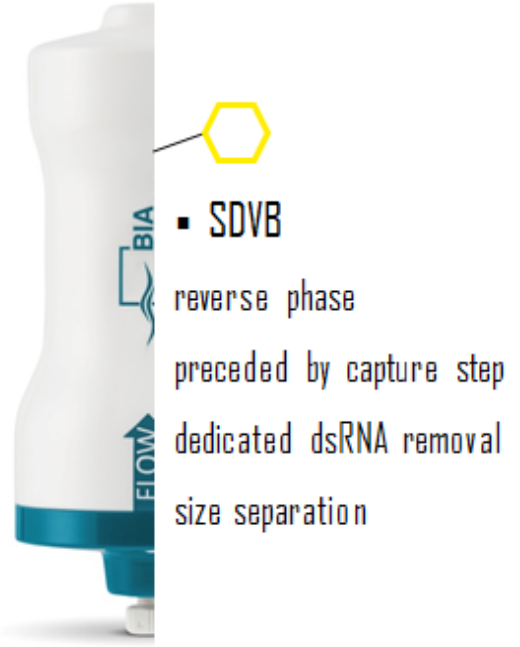
Binding can be performed with any salt that precipitates ssRNA. Each gives different selectivity but the general pattern is the same for all. DNA mostly flows through with dsRNA. Most proteins require NaOH for removal. Sample loading at industrial scale can be performed by in-line dilution.

The tools: reverse phase chromatography - SDVB

Reverse phase chromatography with SDVB is well documented for separation of ssRNA from truncated forms, dsRNA, and DNA.

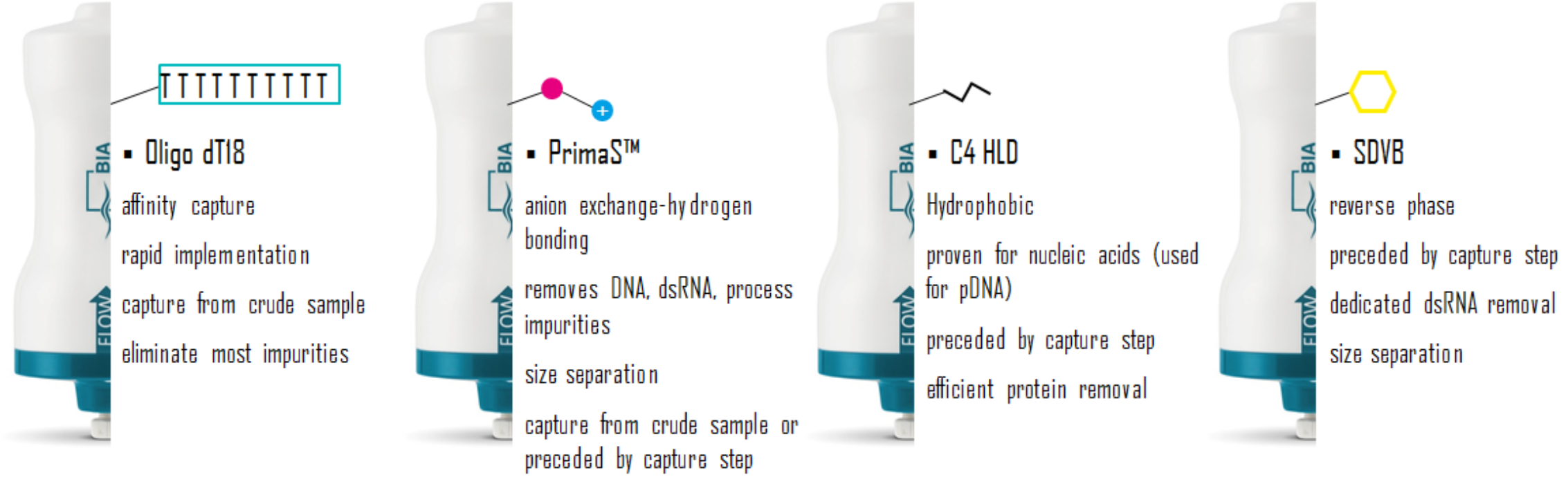






Reverse phase is generally best suited as a polishing method. Elution in an acetonitrile gradient. More information: Nwokeoji et al, J. Chrom. A 1484 (2017) 14–25 and J. Chrom. B 1104 (2019) 212–219.

A flexible toolbox for every mRNA process – universal affinity capture and dedicated second purification step



- High purification yield and recovery, fully scalable (from R&D to manufacturing)
- Chromatography columns available in CIMmultus for purification and CIMac for analytics
- SDVB is custom made, only available on request (not commercial product)

CIMmultus – process development to manufacturing

	1	4	8	40	80	400	800	4000	8000
Connector	10-32 coned UNF			Sanitary TC 1” (25 mm)					
Max pressure, bar	18	20	20	20	20	14	14	7	7
Max flow rate, mL/min	16	50	100	200	400	800	1600	4000	8000
Max flow rate, CV/min	16	12.5	12.5	5	5	2	2	1	1
Bed height, cm*	0.6	0.42	0.42	0.95	0.95	2	2	2.2	2.2

*Radial geometry, bed height and diameter not applicable same way as in axial geometry. cGMP ready columns use sanitary connector.

Analytics

The same purification options are also available in analytical format: SDVB, PrimaS, Oligo dT, C4 HLD.

Services

Development and tech transfer of purification processes and analytical methods for mRNA. This includes IVT production, development of purification process with capture and polishing steps to meet customers' requirements. Preparation of SOP and tech transfer.

Conclusions

Monoliths are ideally suited for purification of mRNA. Lack of diffusion constraints and shear forces offers a gentle and rapid purification solution.

Affinity (Oligo dT) and non-affinity (PrimaS) capture options. Affinity is easier to set up, non-affinity offers better resolution. PrimaS can be cleaned more thoroughly.

Removal of dsRNA, DNA, protein contaminants with orthogonal methods.

Monoliths are convective, flow-independent chromatography media. Use of solvents may increase viscosity of solution, but is not a limitation on operating performance.