

## Efficient, robust and scalable purification of Covid vaccines based on pDNA, mRNA, Adeno and VLP technologies

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Simplifying Progress



**SARTORIUS**

# Expert DSP Bioprocess Knowledge:

**>30 pDNA, mRNA, virus DSP cGMP processes tech transferred to CMOs, sponsors, including Corona.**

**Product impurities are one of the key reasons for treatment side effects. High purity is therefore mandatory for product safety.**

- **pDNA** including **Corona**, purity is THE key for better transfection and purer mRNA
- Minicircle DNA (shorten the pDNA)
- **ssRNA** and **dsRNA**, platform process from E.coli to **mRNA** including **Corona**
- **Adeno** virus, more than 20 years experience, including **Corona**
- **AAV** (all serotypes, > 20 tested), including **Corona**
- Influenza virus (all serotypes)
- Vaccinia/MVA
- Exosome
- Bacteriophage
- **VLPs** including Flu and **Corona**
- IVIG
- IgM and many more

# Testimonials

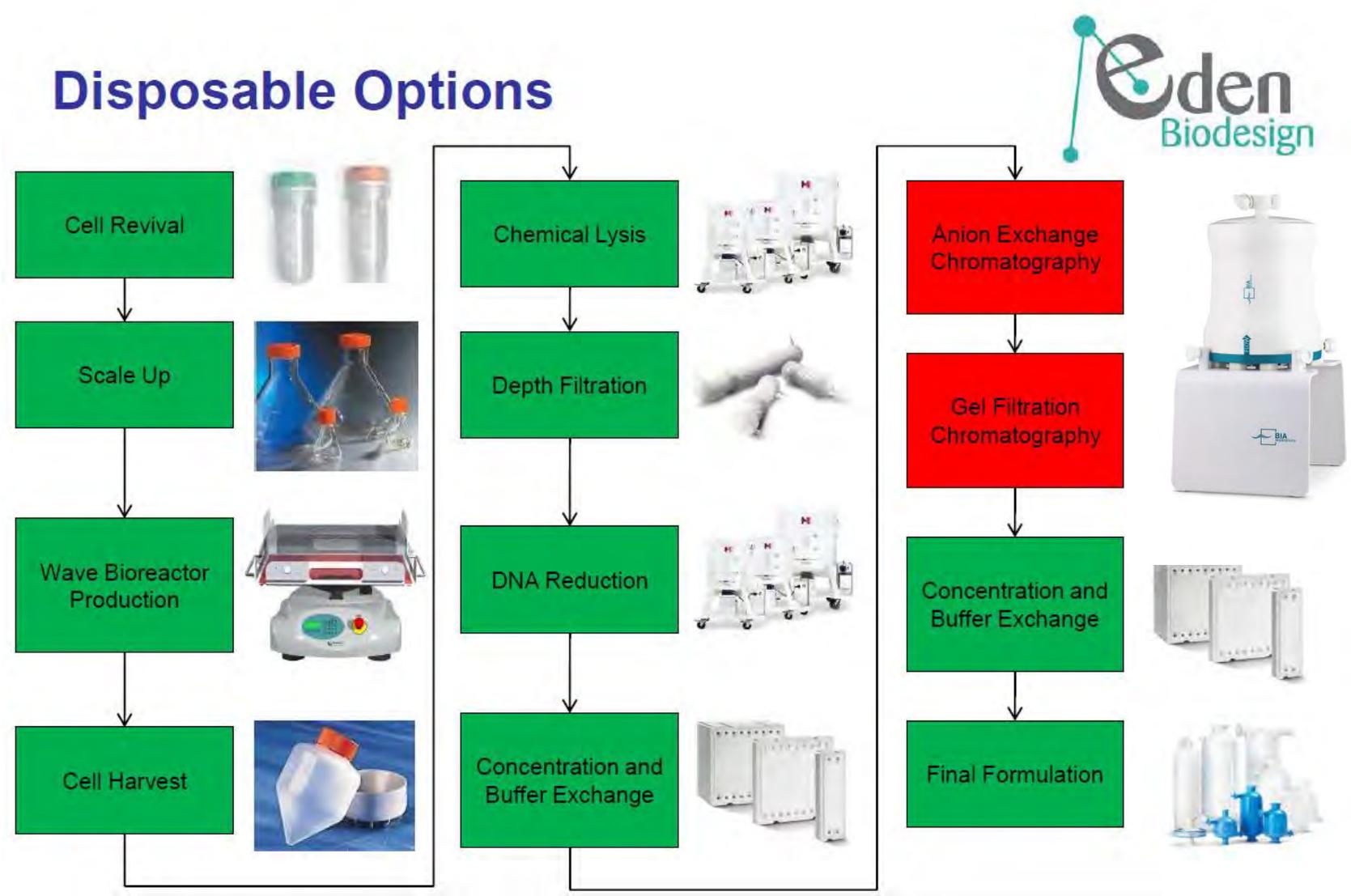
*“We are especially grateful that BIA Separations shared, and operated, with the same sense of urgency we did to help bring gene therapy to the SMA community. BIA’s experience with AAV purification and its chromatographic technology were important contributions and we look forward to our continued work together.”*

**Andy Stober**, Senior Vice President of Technical Operations for **AveXis/Novartis**

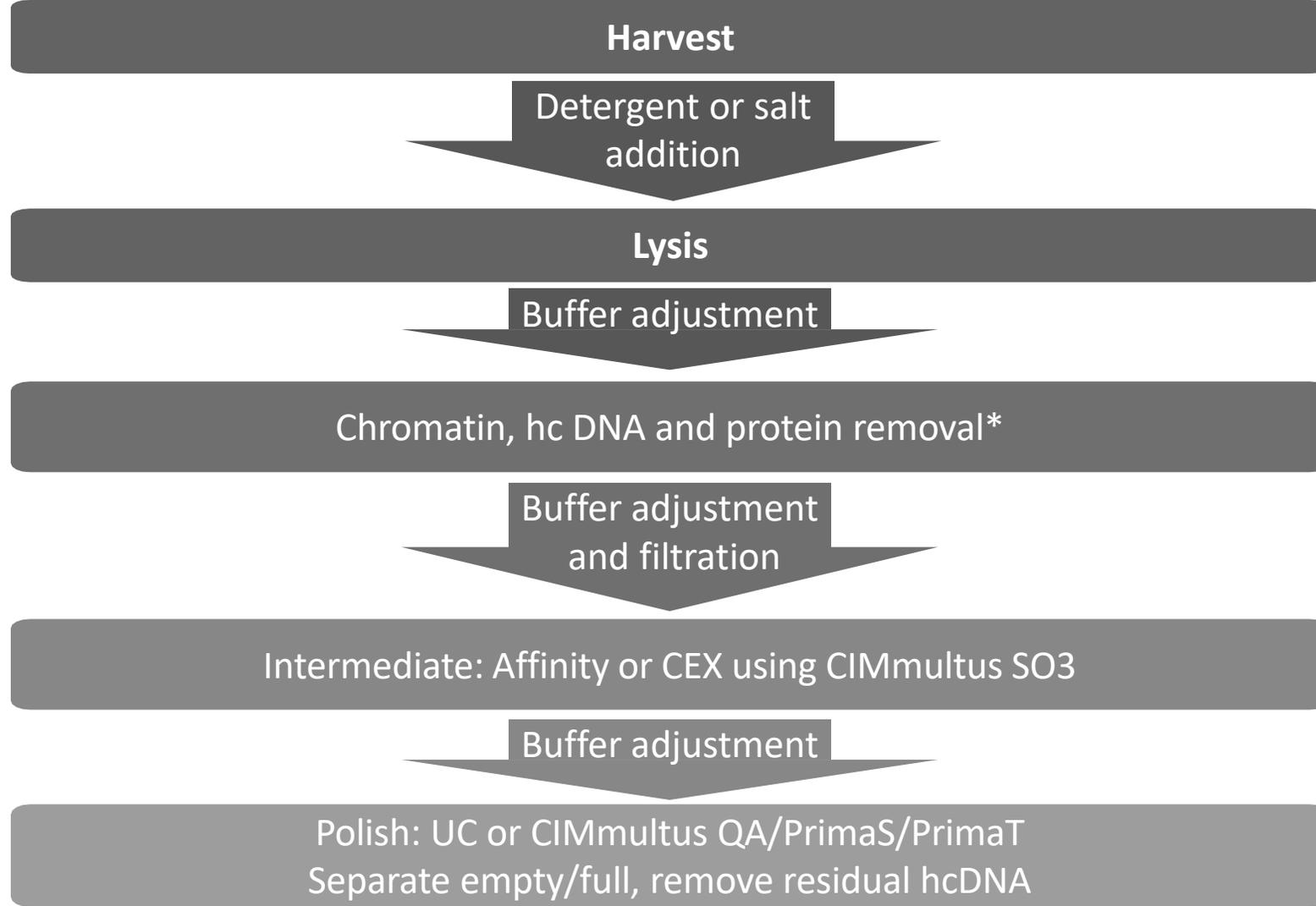
*“We use BIA Separations Monolith columns for the purification of our mRNA drug substance. The Monolith columns come in multiple sizes to meet our needs from small scale product development work to large scale cGMP manufacturing runs. We have found the Monolith columns to provide high throughput and high purity while being very robust and reliable. In addition, BIA Separations has top notch customer service that never fails to impress.”*

**Greg Kubczak**, Director of Technical Services and Manufacturing at **Arcturus Therapeutics**

# Adeno virus manufacturing platform



# AAV manufacturing platform



**\*Multiple options, alone or combined:**

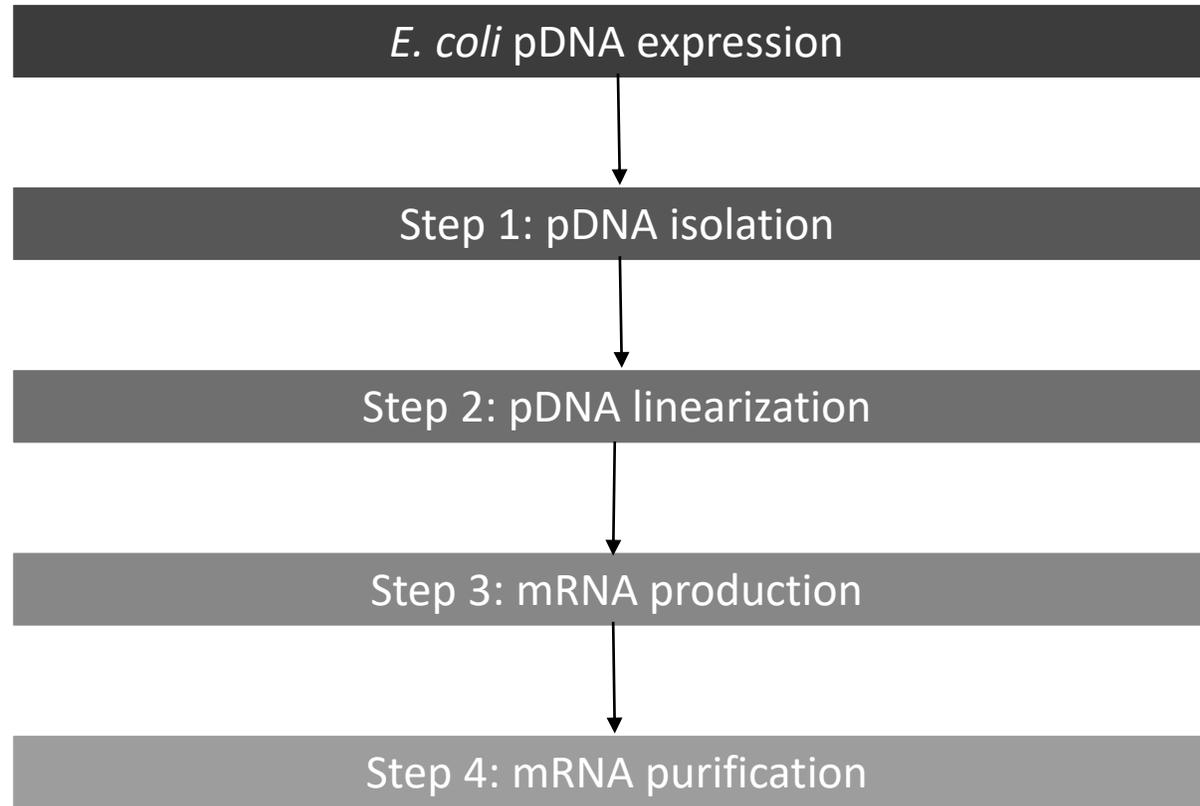
- Flocculation/DNAse treatment (Y/N)
- TFF/DNAse treatment (Y/N)
- **Solid phase extraction/DNAse treatment (Y/N)**
- DNAse treatment (Y/N) followed by acidic precipitation and direct load on the SO3 column



pDNA and mRNA manufacturing platforms

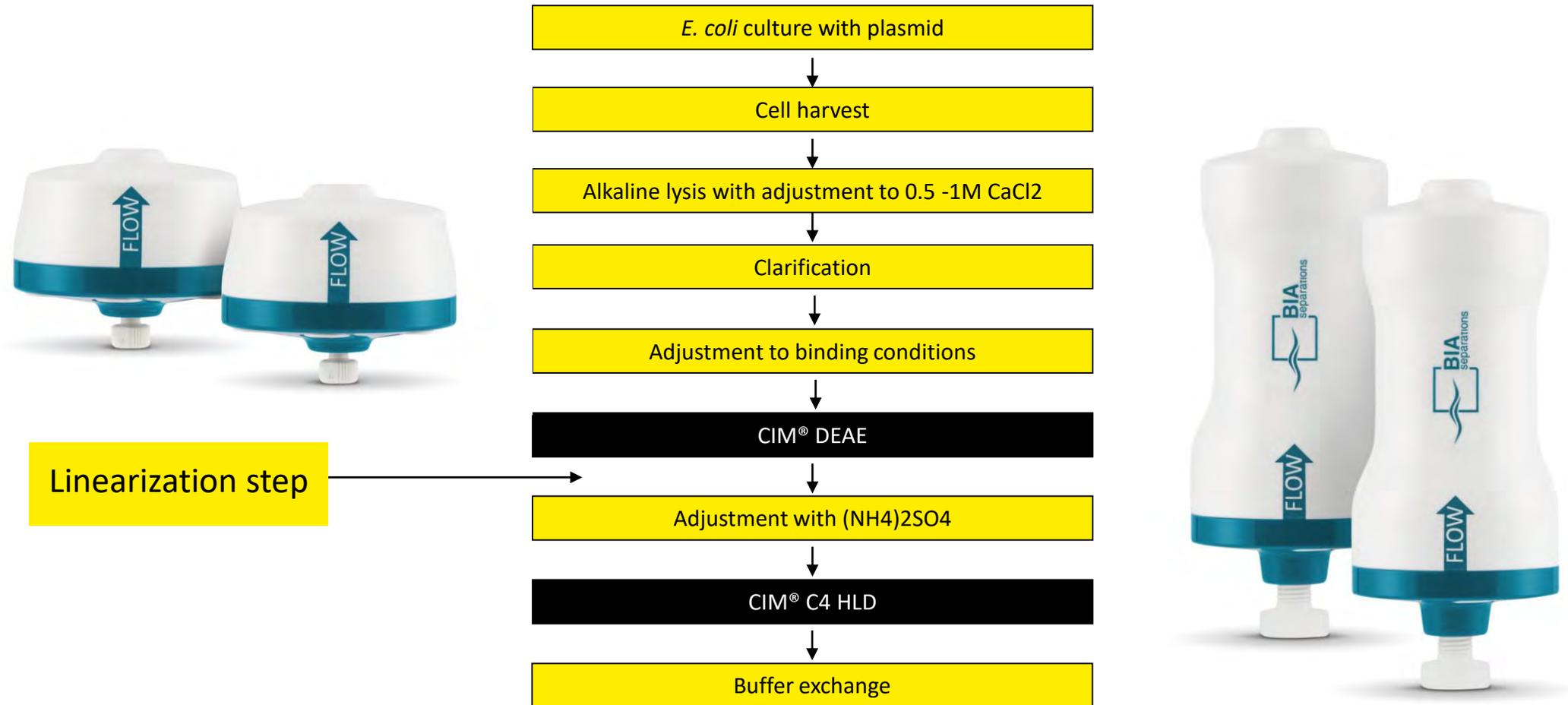
Simplifying Progress

# From *E. coli* to mRNA



Starting the process with pDNA expression allows for cheaper mRNA production, and better supply and impurity control.

# CIMmultus™ Plasmid Process Pack used for Linear pDNA Purification



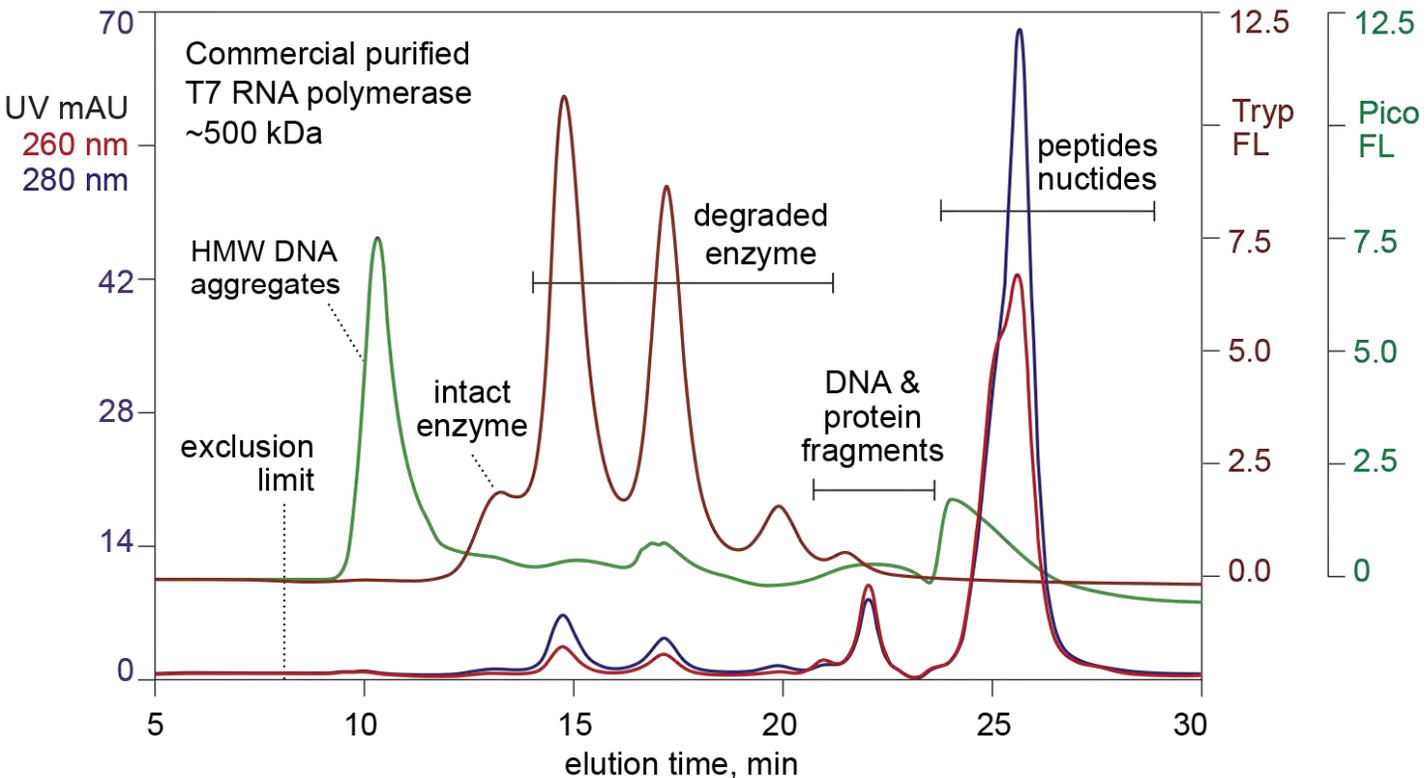
*For plasmid DNA manufacturing protocol pls visit: [www.biaseparations.com/en/featured/plasmid-dna](http://www.biaseparations.com/en/featured/plasmid-dna)*

# Step 1: pDNA Isolation Workflow

**Plasmid pIVTeGF size 3.3 kbp**, provided by Biomay GmbH, Vienna, Austria, **size of mRNA: 0.950 kbp**, enzyme for linearization: NotI, Length poly(A): 45 b

- Alkaline lysis steps:
  - E. coli biomass lysed with 0.1M NaOH and 0.5 % SDS
  - RNA precipitation with 1.0M CaCl<sub>2</sub> for impurities
- Two step filtration (CaCl<sub>2</sub> improves the filterability)
- DEAE isolation (lysate diluted with water to 35 mS/cm)

# PATfix SEC Using Multidetector Approach - Powerful Tool to Check Raw Material Purity



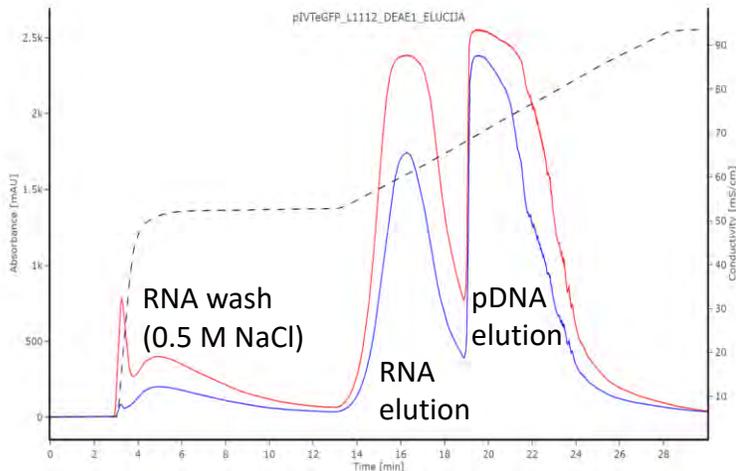
PATfix HPLC and CIMac → Process understanding

Size exclusion chromatography (SEC). TSKgel™ G3000SWxl. 0.5 mL/min.  
 Sample pre-stained with **Picogreen fluorescence** increases sensitivity of DNA/RNA related impurities.  
**Tryptophan fluorescence** increases sensitivity of protein detection over UV.

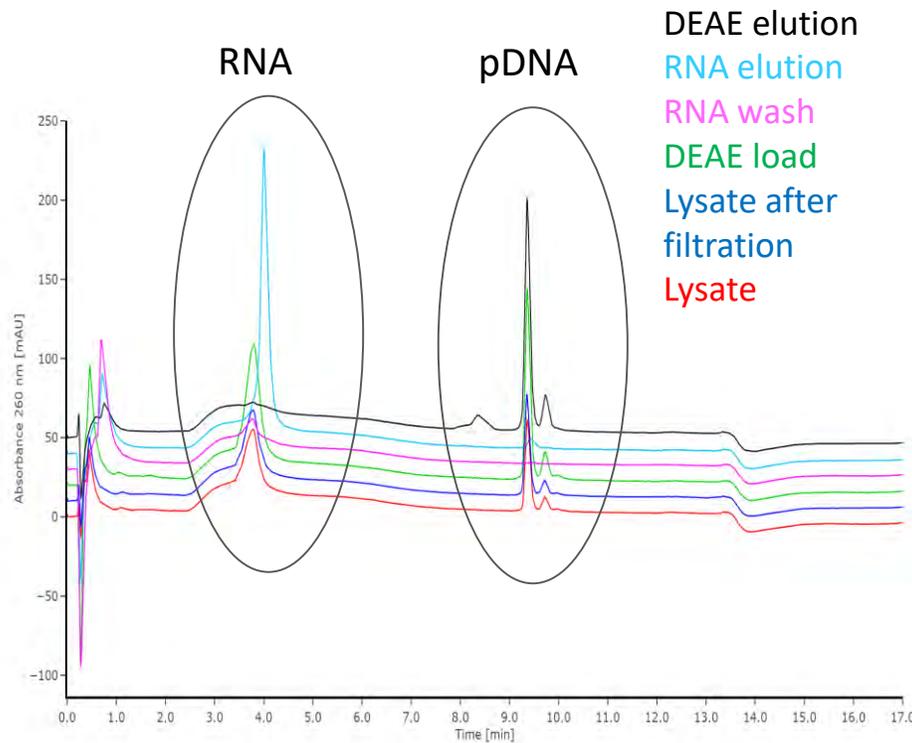
# Purification of pDNA (3.3 kbp) Using CIMmultus DEAE Column

## pDNA purification using CIMmultus DEAE column

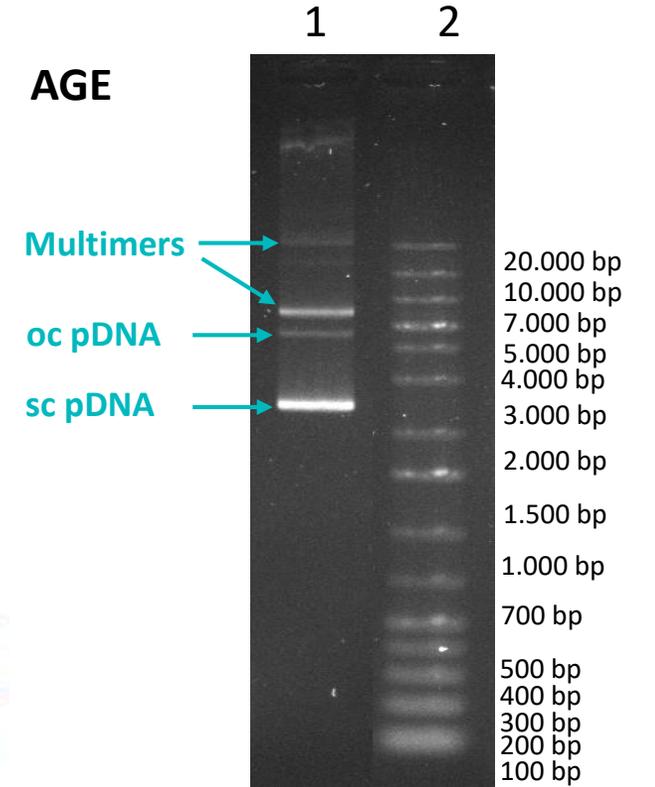
pDNA recovery in elution (%)	87
RNA removal (%)	> 99 %
oc pDNA (%)	3
Multimers (%)	25



## HPLC CIMac™ pDNA column

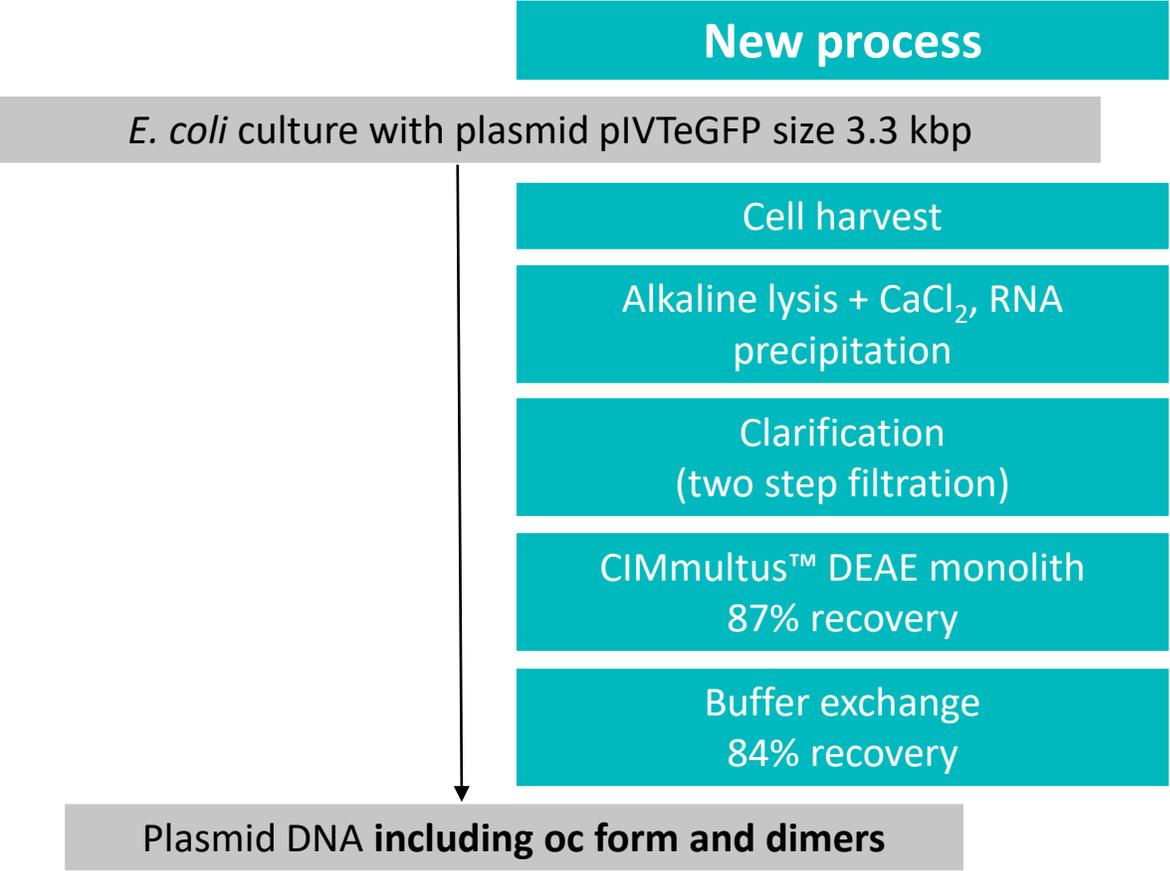


## AGE



1: GeneRuler 1 kb Plus  
2: purified plasmid

# Step 1: pDNA Isolation Summary



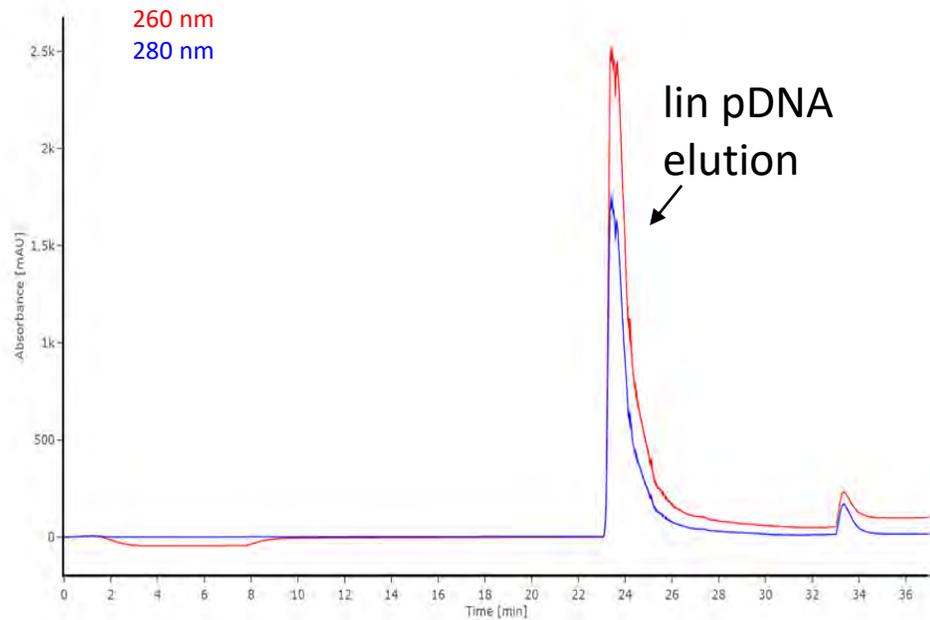
## Step 2: pDNA Linearization Workflow

- Linearization of pDNA (3.3 kbp) with restriction enzyme NotI-HF (NEB) and buffer **without BSA**, 37°C, 21h
- Purification on CIMmultus™ C4 HLD using step elution from 2.5M to 0.9M ammonium sulphate (**proteins elute with 1M NaOH only - powerful protein removal – to prevent Chromatin/Nucleosome formation**). No enzyme inactivation step needed.
- Buffer exchange: PD Midi Trap

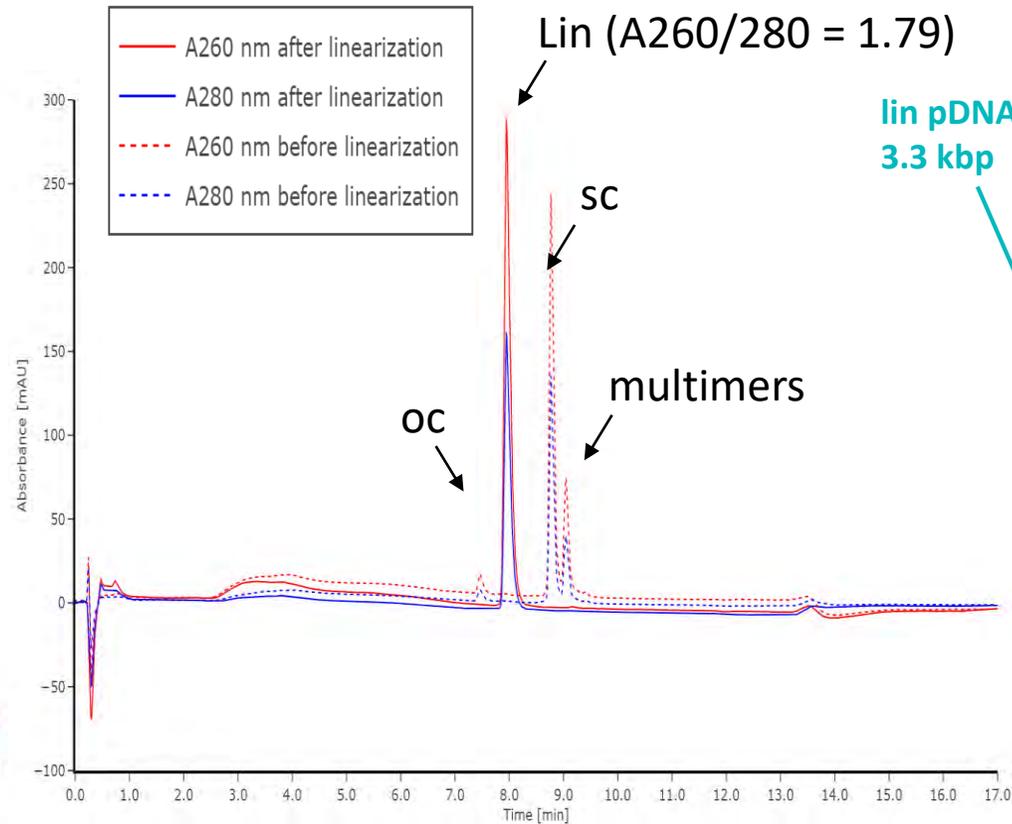
# Purification of Linear pDNA Using CIMmultus™ C4 HLD Column

## Preparative CIMmultus™ C4 HLD

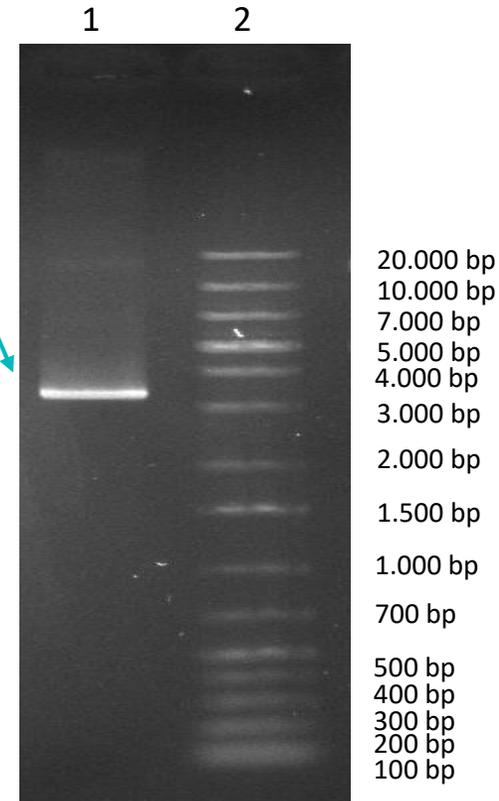
Efficient protein removal (proteins sticks to the column and elute in 1M NaOH only)



## HPLC CIMac™ pDNA analytics

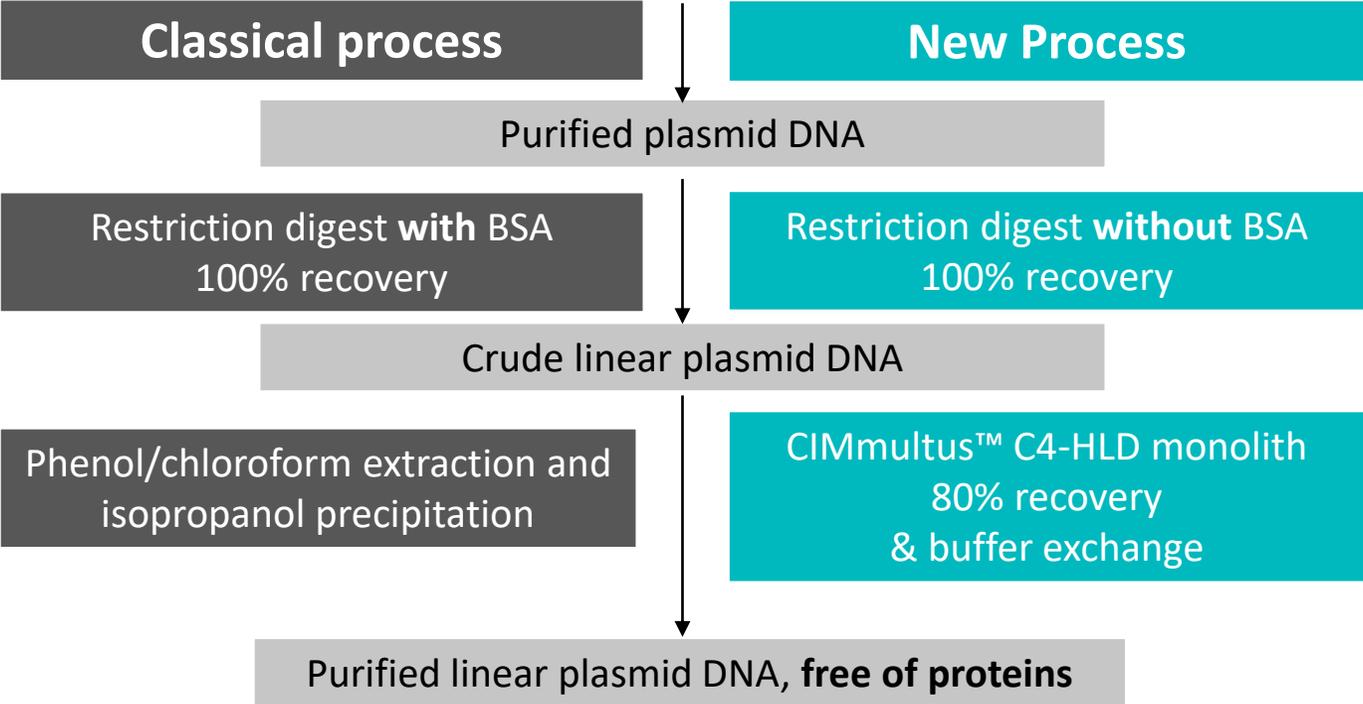


## AGE



1: linear plasmid  
2: GeneRuler 1 kb plus

# Step 2: pDNA Linearization Summary



## Step 3: mRNA Production – IVT Process Workflow

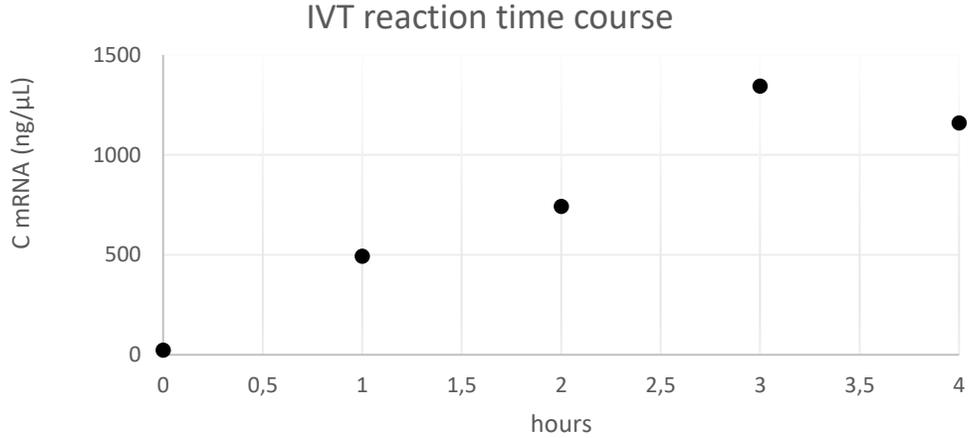
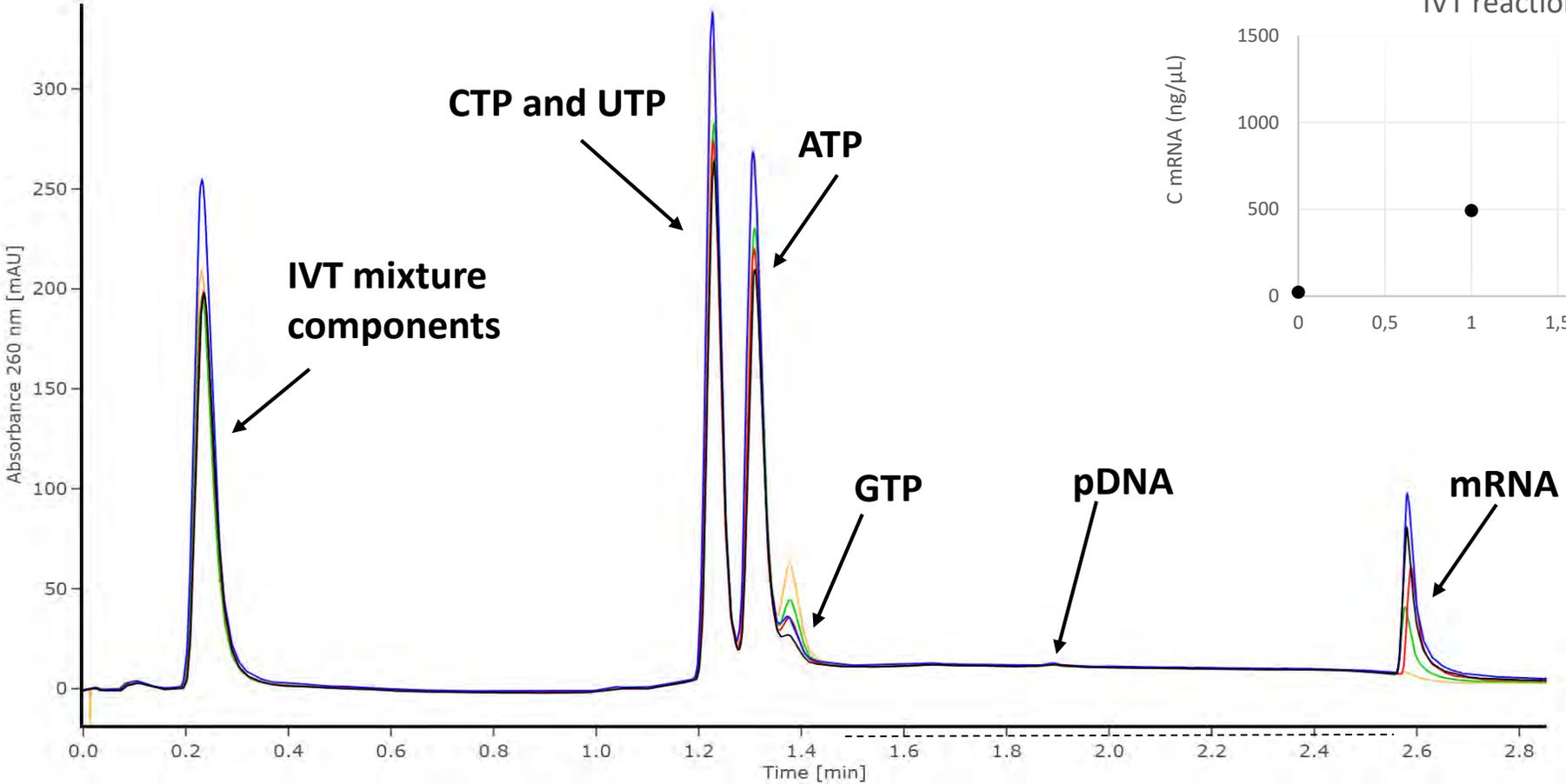
### IVT reaction (THE key step to lower manufacturing costs):

- Plasmid pIVTeGFP with encoded poly(A) tail (tail length 45 nt), T7 RNA polymerase (NEB), pyrophosphatase (NEB), RNase inhibitor (NEB), and capping reagent ARCA (NEB); incubation 40°C, 3h; inactivation with EDTA; subsequent *O*-methylation (cap1 formation), expected mRNA size: 950 nt

### LC purification:

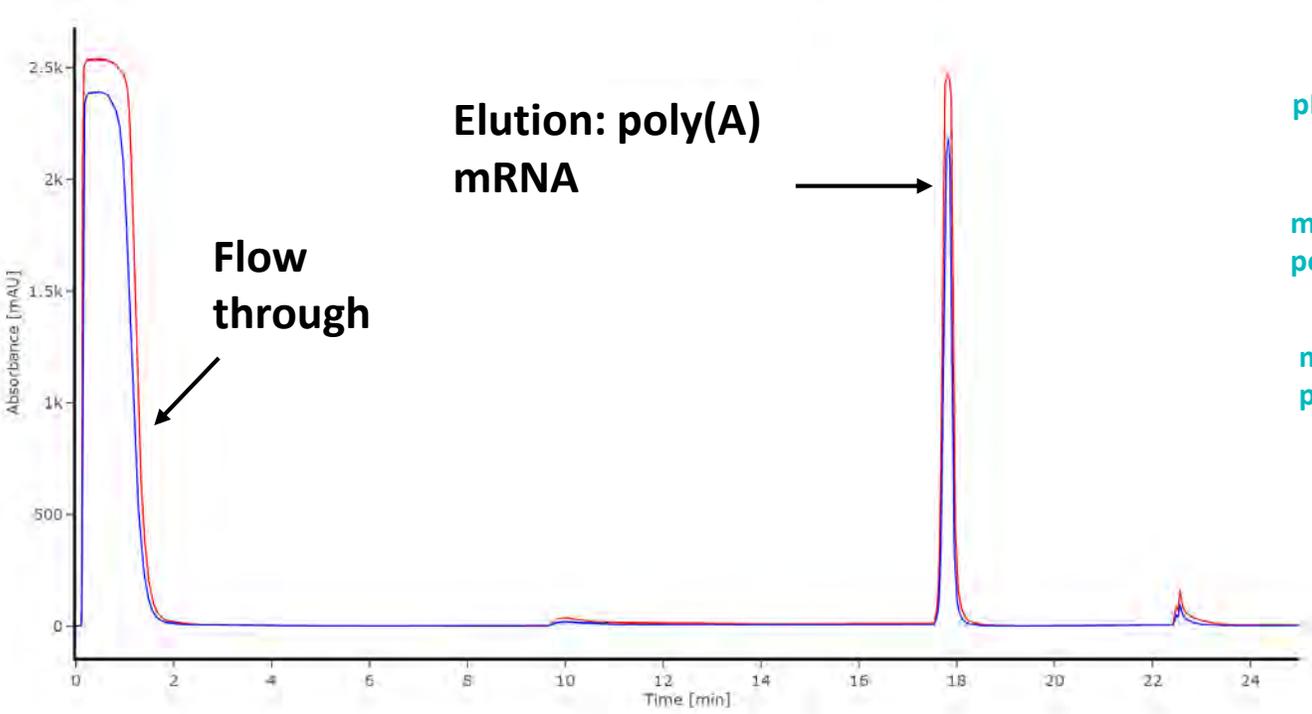
- **CIMmultus™ Oligo dT**: removal of components without poly(A) tail before capping 1 reaction and removal of product related mRNA impurities (dsRNA)

# HPLC In-process Control Using CIMac™ PrimaS Column



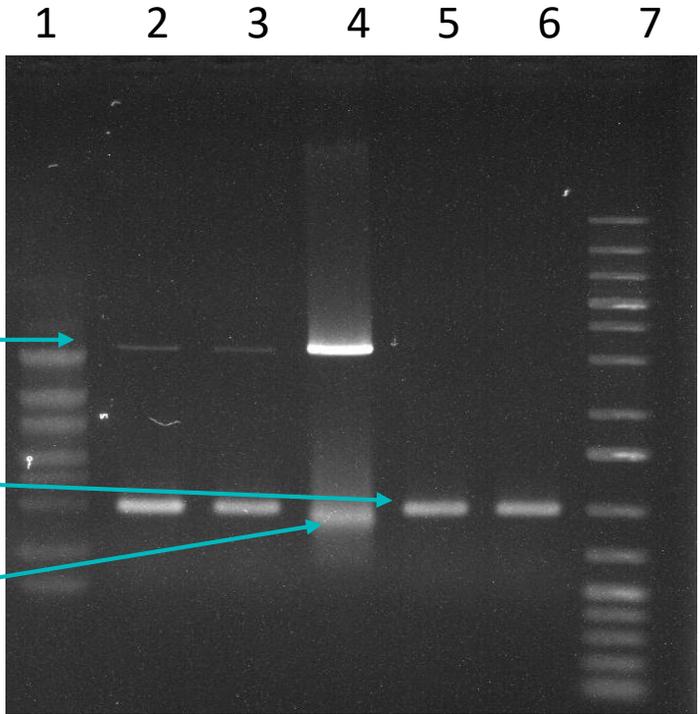
- IVTmix\_30sec A260 nm
- IVTmix\_1h A260 nm
- IVTmix\_2h A260 nm
- IVTmix\_3h A260 nm
- IVTmix\_4h A260 nm

# Purification of mRNA Cap0 Using CIMmultus™ Oligo dT



**AGE**  
(agarose gel electrophoresis)

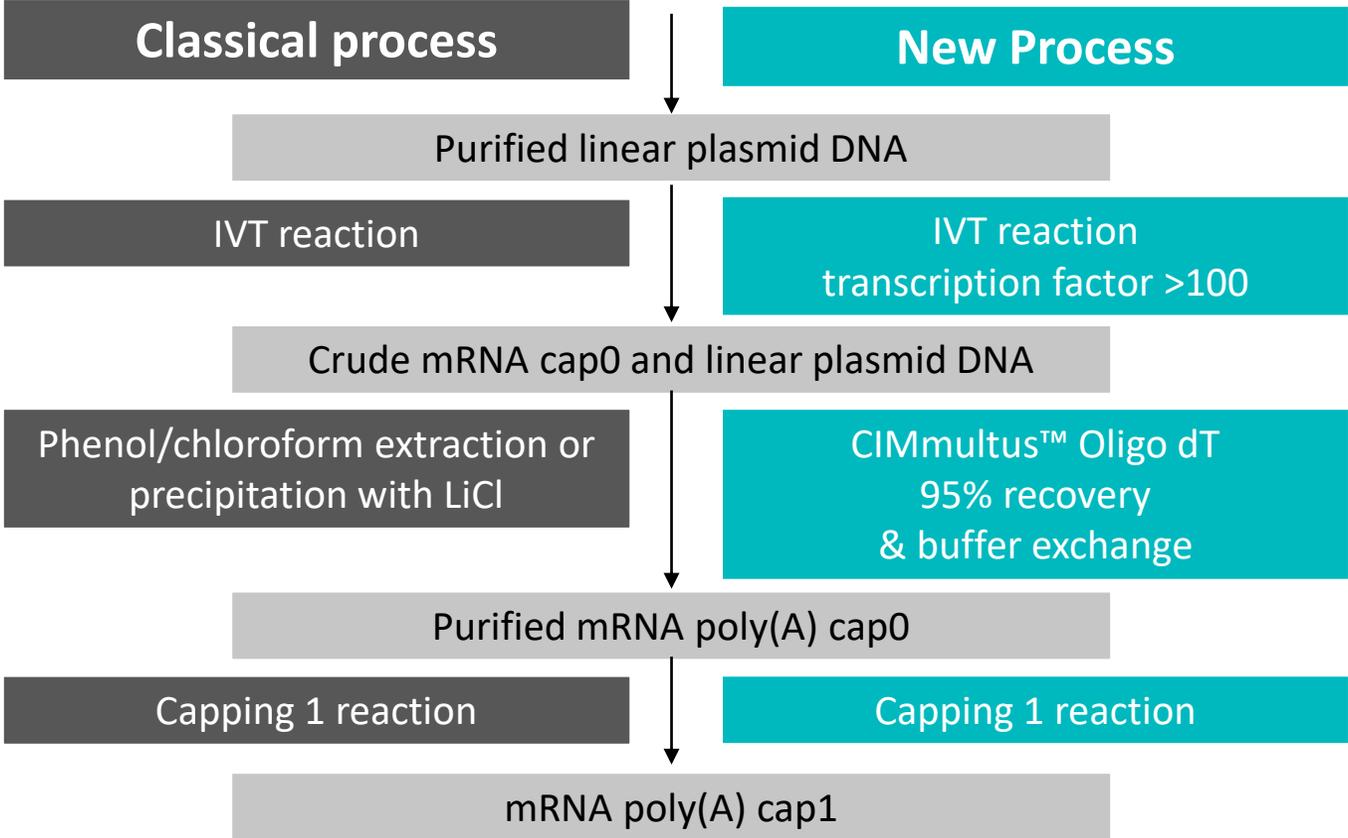
pDNA 3.3 kbp → 6000 b  
 mRNA with poly(A) tail 950 nt → 1500 b  
 mRNA without poly(A) tail → 200 b



- 1 RiboRuler HR
- 2 IVTmix
- 3 IVT mix - OligodT load
- 4 IVT mix- FT (50x concentrated)
- 5 IVTmix – OligodT Elution
- 6 IVTmix – OligodT Elution (buffer exchanged and concentrated into water)
- 7 Gene Ruler 1 kb Plus



# Step 3: mRNA Production – IVT Process Summary

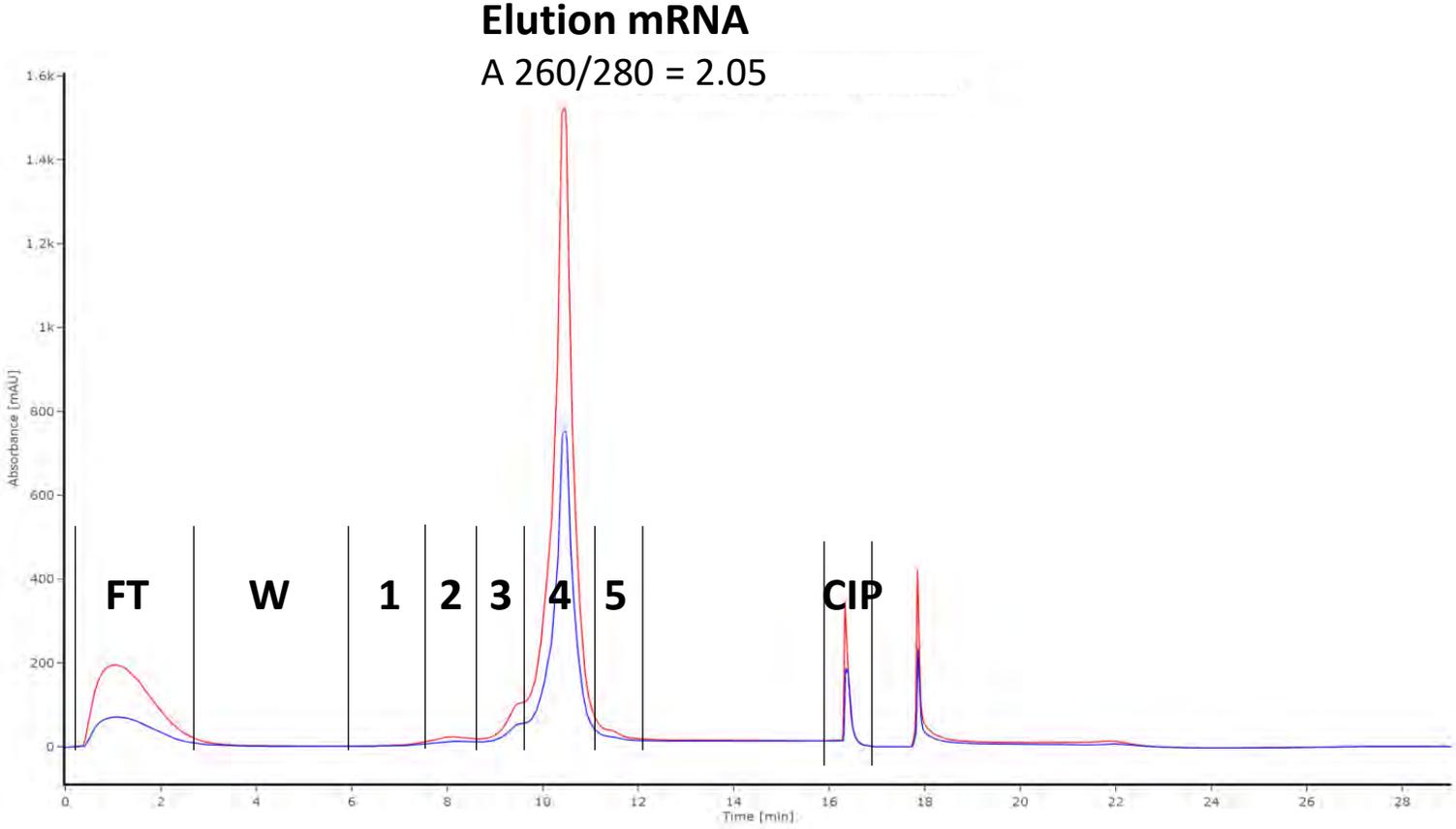


# Step 4: mRNA Cap 1 Synthesis and Purification Workflow

## LC purification:

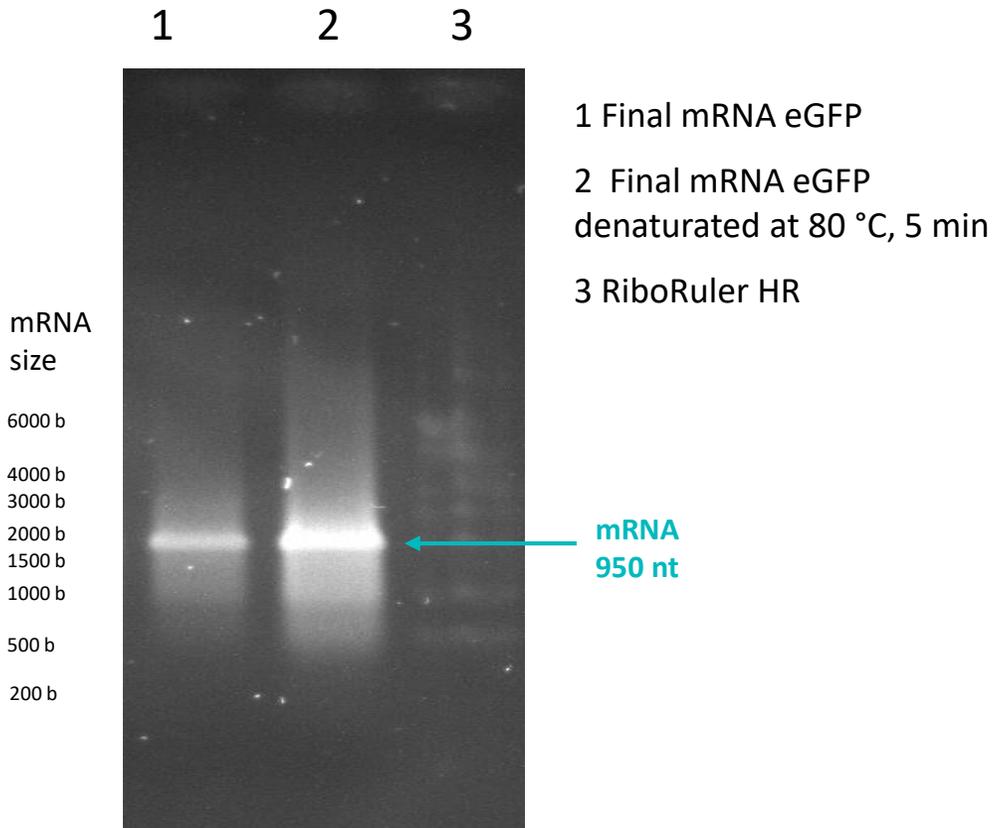
- **CIMmic™ C4 HLD:** removal of process related impurities from capping1 reaction (capping reagent, buffer components, proteins) and removal of product related mRNA impurities (dsRNA)

# Purification of Cap1 Polyadenylated mRNA Using CIMmic C4 HLD 0.2mL

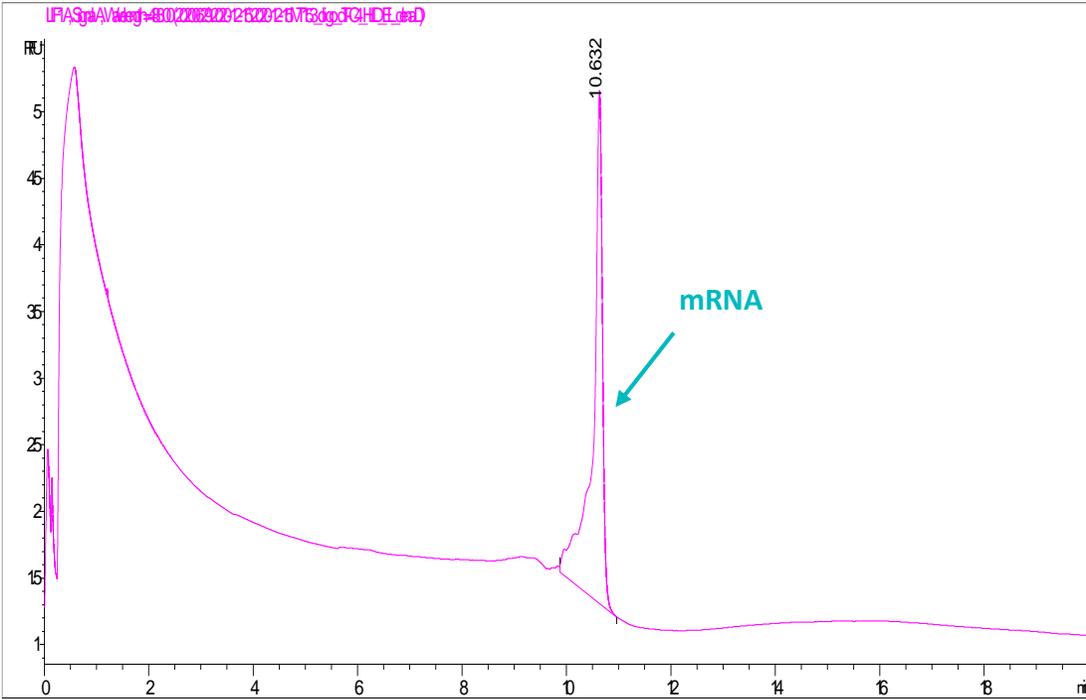


# Poly(A) mRNA Cap 1 Purity Assessment

## AGE (agarose gel electrophoresis)

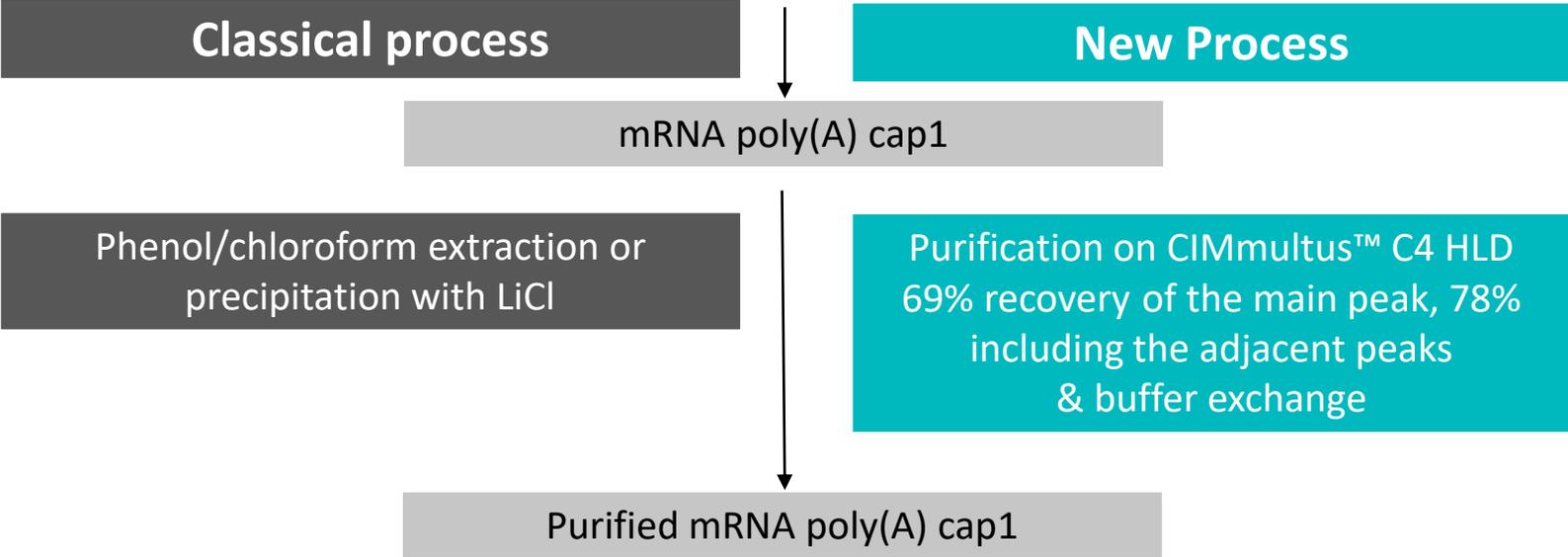


## Capillary electrophoresis



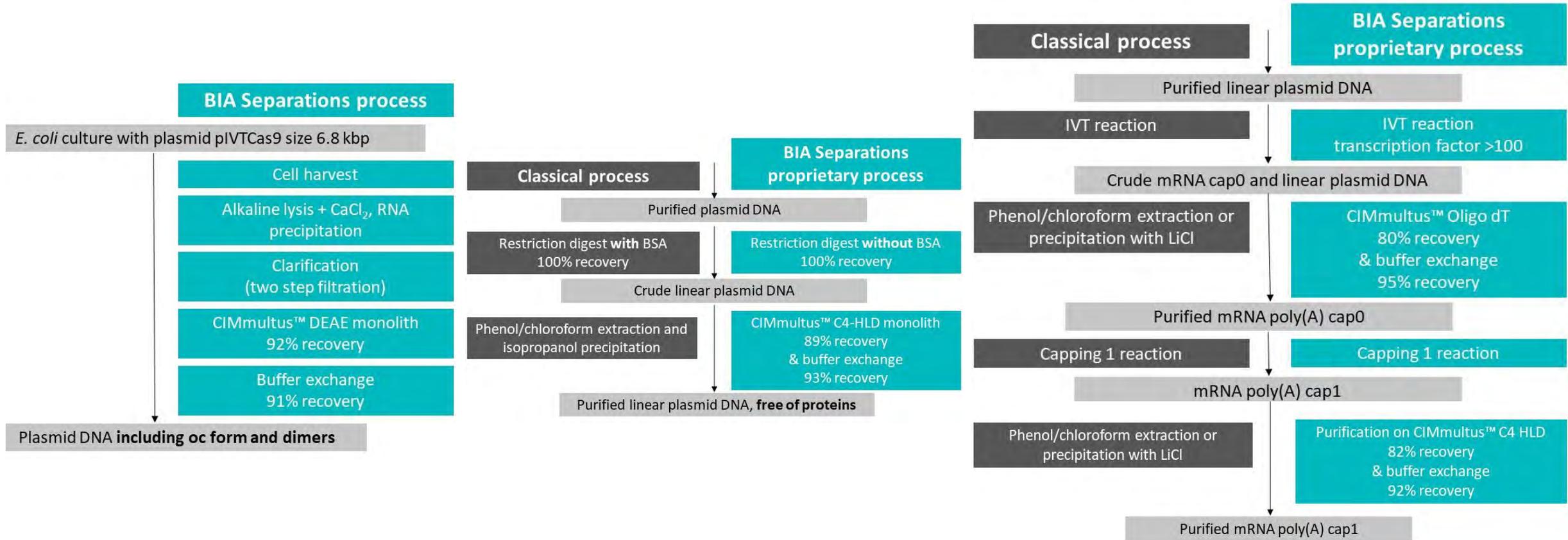
Sample denatured at 80°C, 5 min

# Step 4: mRNA Purification Summary



# Work of the process with bigger mRNA confirmed

**Plasmid pIVTCas9 size 6.8 kbp**, provided by Biomay GmbH, Vienna, Austria, **size of mRNA: 4.430 kbp**, enzyme for linearization: NdeI, Length poly(A): 45 b



# Conclusions

For the efficient and economic process of mRNA production the **pDNA production should be part of the mRNA process flow** (oc and multimers of the pDNA can be linearized, proteins should be removed before entering the IVT synthesis - not before the linearization).

**High purity of the raw materials and HPLC in-process control of the IVT process allow for high transcription number** (proteins complex with the mRNA as soon it is formed), much purer mRNA and lower consumption of expensive reagents.

**Proper management of the IVT reaction allows for high yield, lower raw material costs** and purer product with less purification steps. Most important, for cheaper manufacturing costs.

In-process control using **PATfix allows for faster process development and more robust process.**



# Thank you for your attention!

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