BIA separations
Leaders in Monolith Chromatography

Simplifying Progress

Unlock the Potential of the mRNA Vaccine Platform with Integrated Production Processes from E. coli to Highly Purified mRNA

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PDF presentation available from:
https://www.biaseparations.com/en/library/seminars-webinars?tag=nucleicacids

BIA separations

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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)
- 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

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Simplifying Progress

From *E.coli* to pure mRNA

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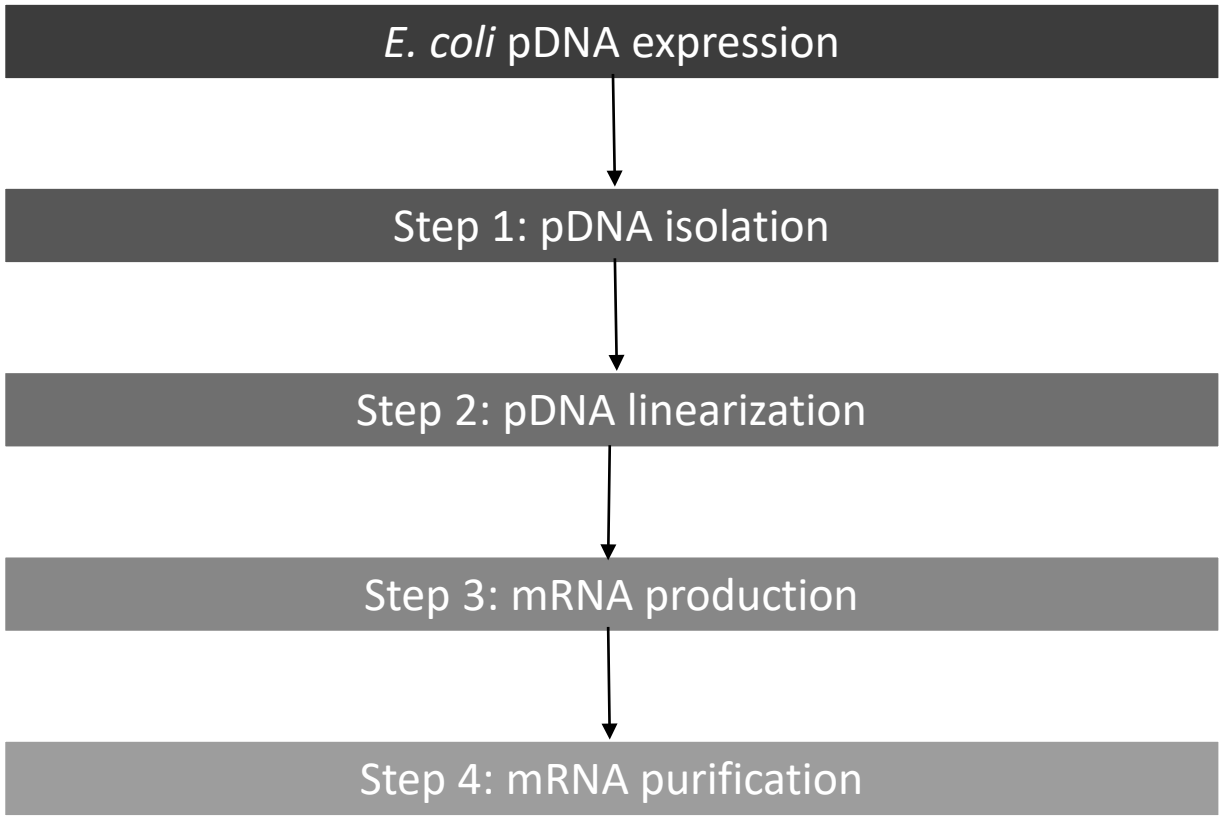
From *E. coli* to mRNA



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

Linearization step

E. coli culture with plasmid

Cell harvest

Alkaline lysis with adjustment to

0.5 -1M CaCl2

Clarification

Adjustment to binding conditions

CIM® DEAE

Adjustment with (NH4)2SO4

CIM® C4 HLD

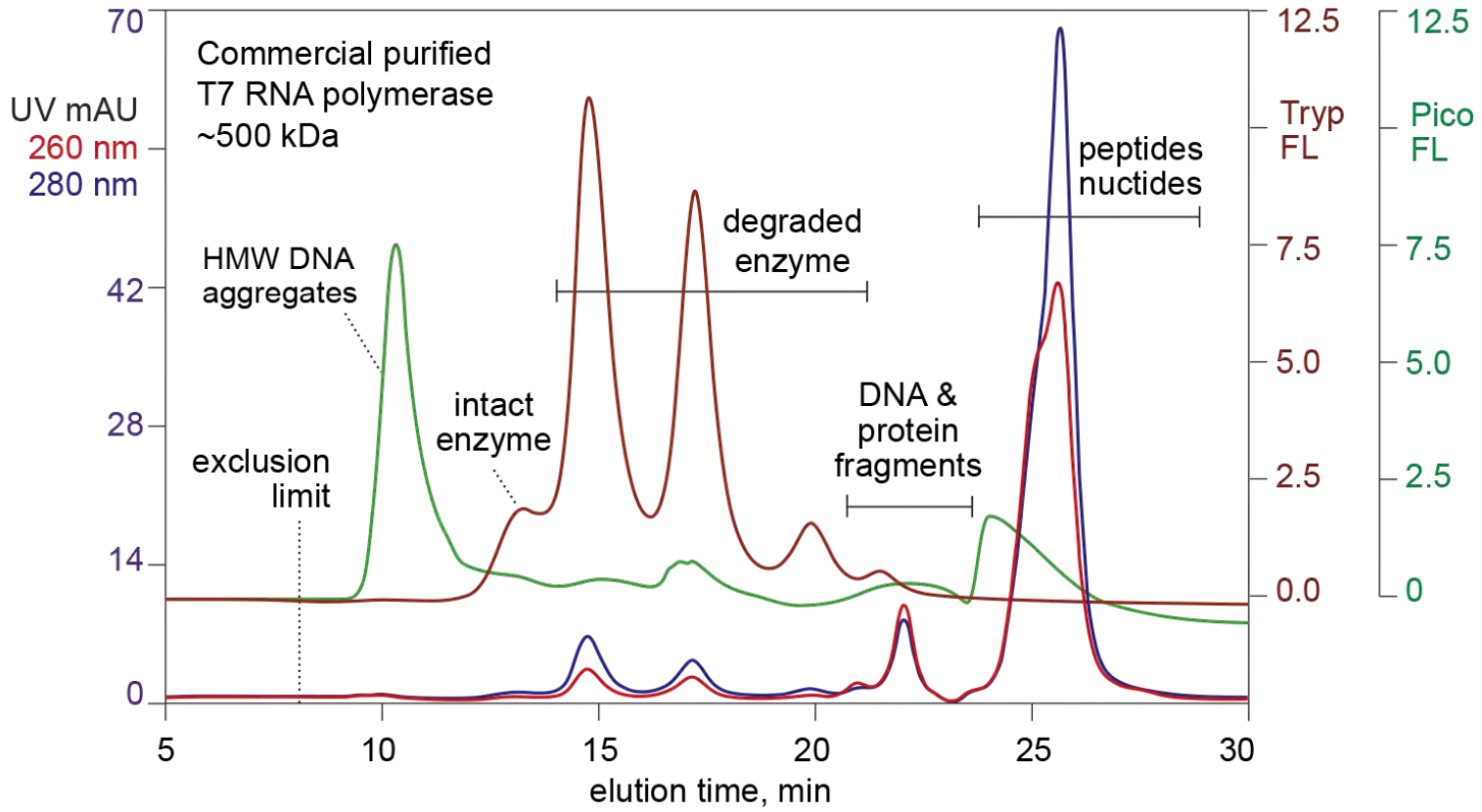
Buffer exchange

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 $CaCl_2$ for impurities
- Two step filtration ($CaCl_2$ 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

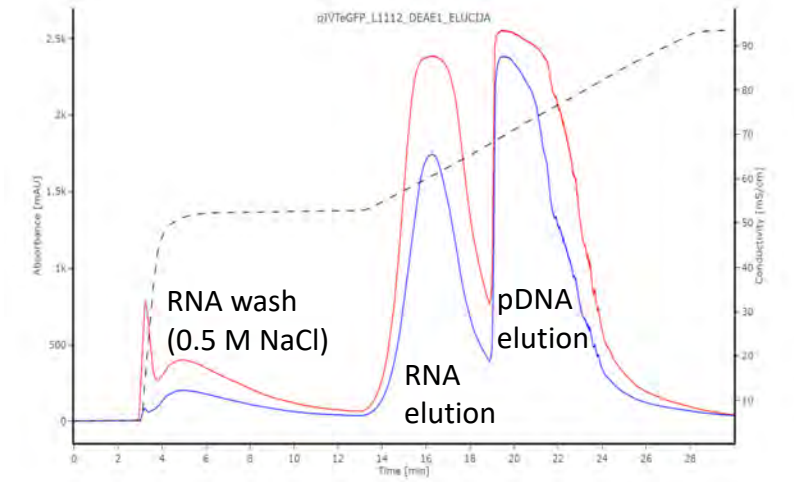


PATfix HPLC and CIMac ➔
Process understanding

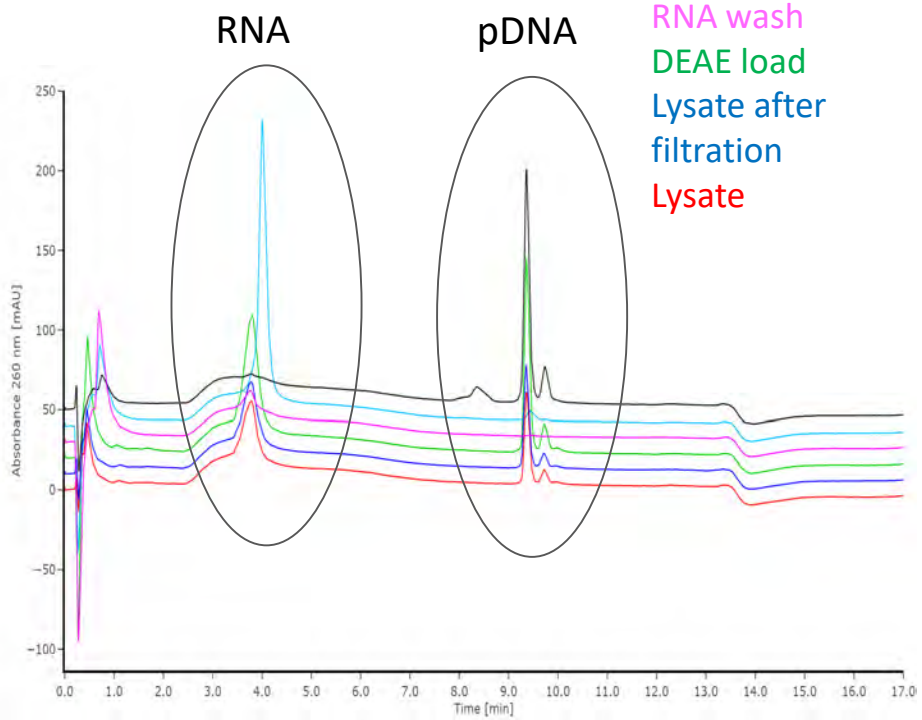
Size exclusion chromatography (SEC). TSKgel™ G3000SWxl. 0.5 mL/min. Sample pre-stained with **Picogreen fluorescence** increases sensitivity of DNA/RNA related impurities. **Tryptophan fluorescence** increases sensitivity of protein detection over UV.

Purification of pDNA (3.3 kbp) Using CIMmultus DEAE Column

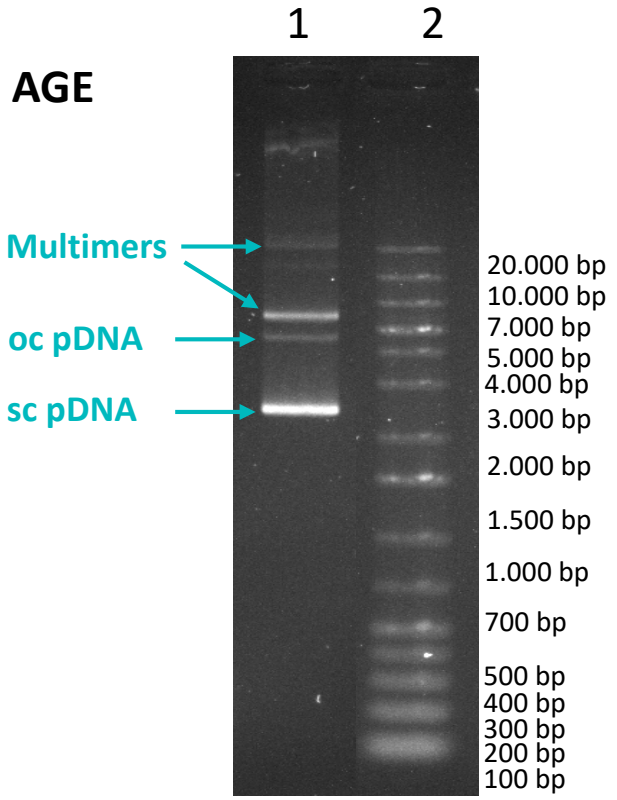
pDNA purification using CIMmultus DEAE column	
pDNA recovery in elution (%)	87
RNA removal (%)	> 99 %
oc pDNA (%)	3
Multimers (%)	25



HPLC CIMac™ pDNA column

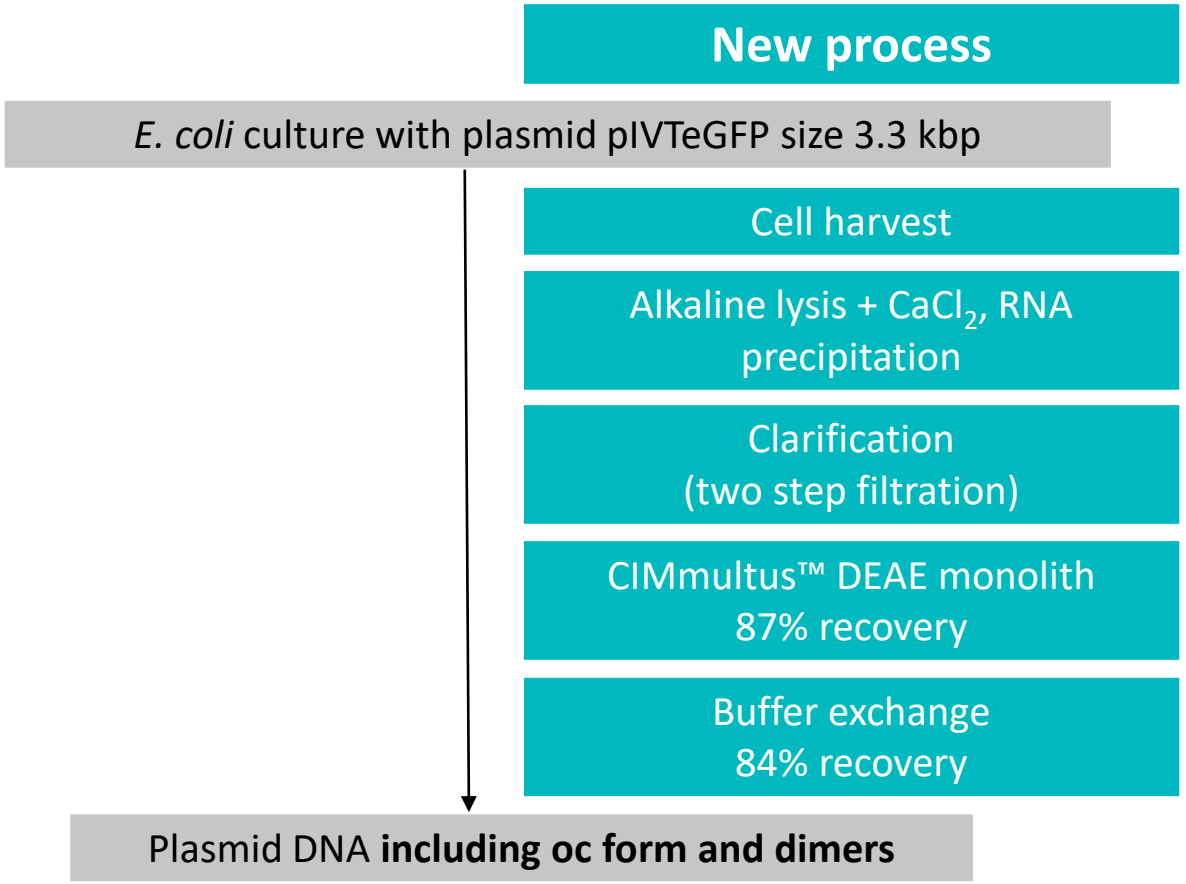


AGE



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

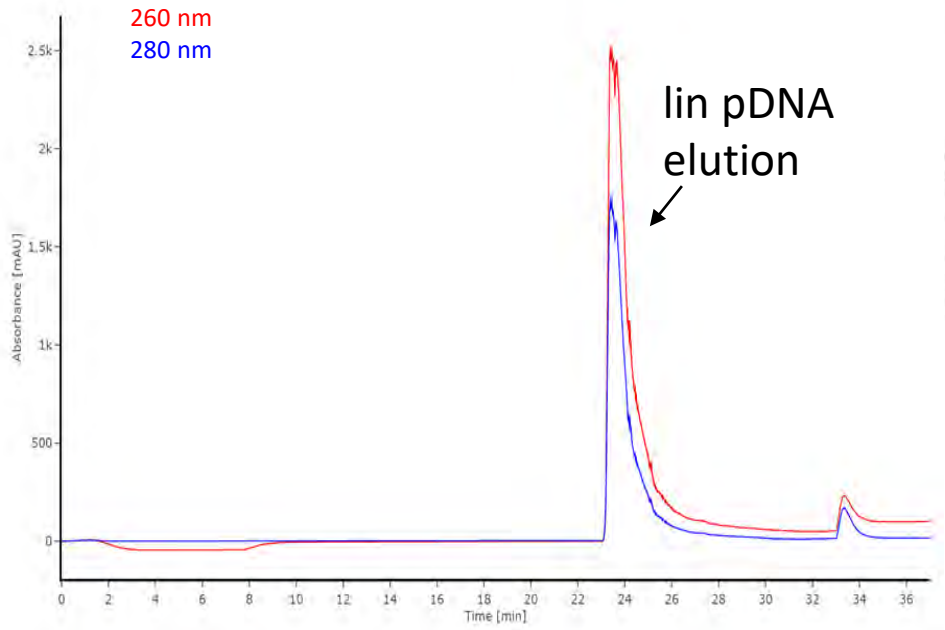
- Purification on CIMmultus™ C4 HLD using step elution from 2.5M to 0.9M ammonium sulphate **(proteins elute with 1M NaOH only - powerful protein removal – to prevent Chromatin/Nucleosome formation). No enzyme inactivation step needed.**

- Buffer exchange: PD Midi Trap

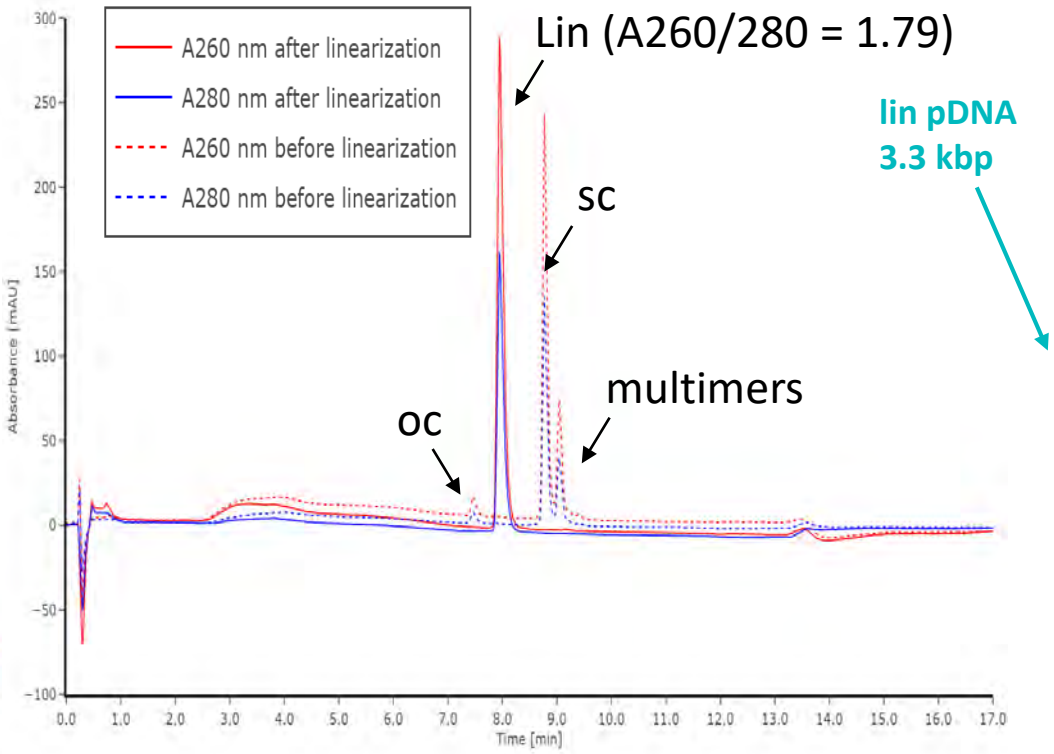
Purification of Linear pDNA Using CIMmultus™ C4 HLD Column

Preparative CIMmultus™ C4 HLD

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



HPLC CIMac™ pDNA analytics



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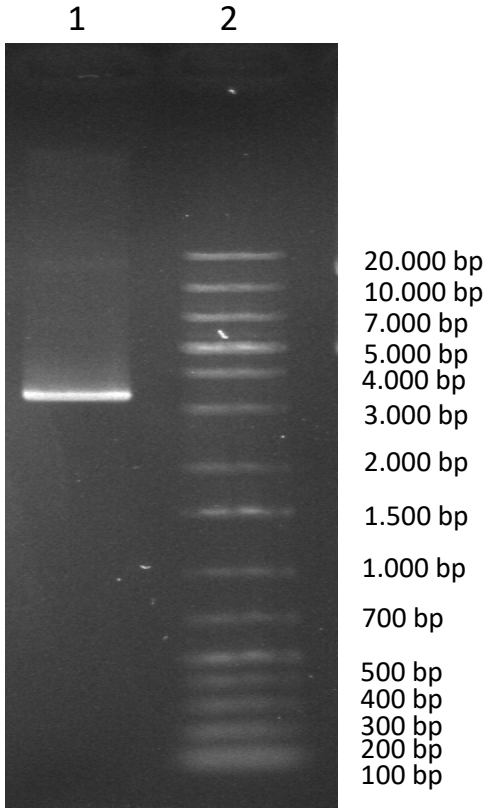
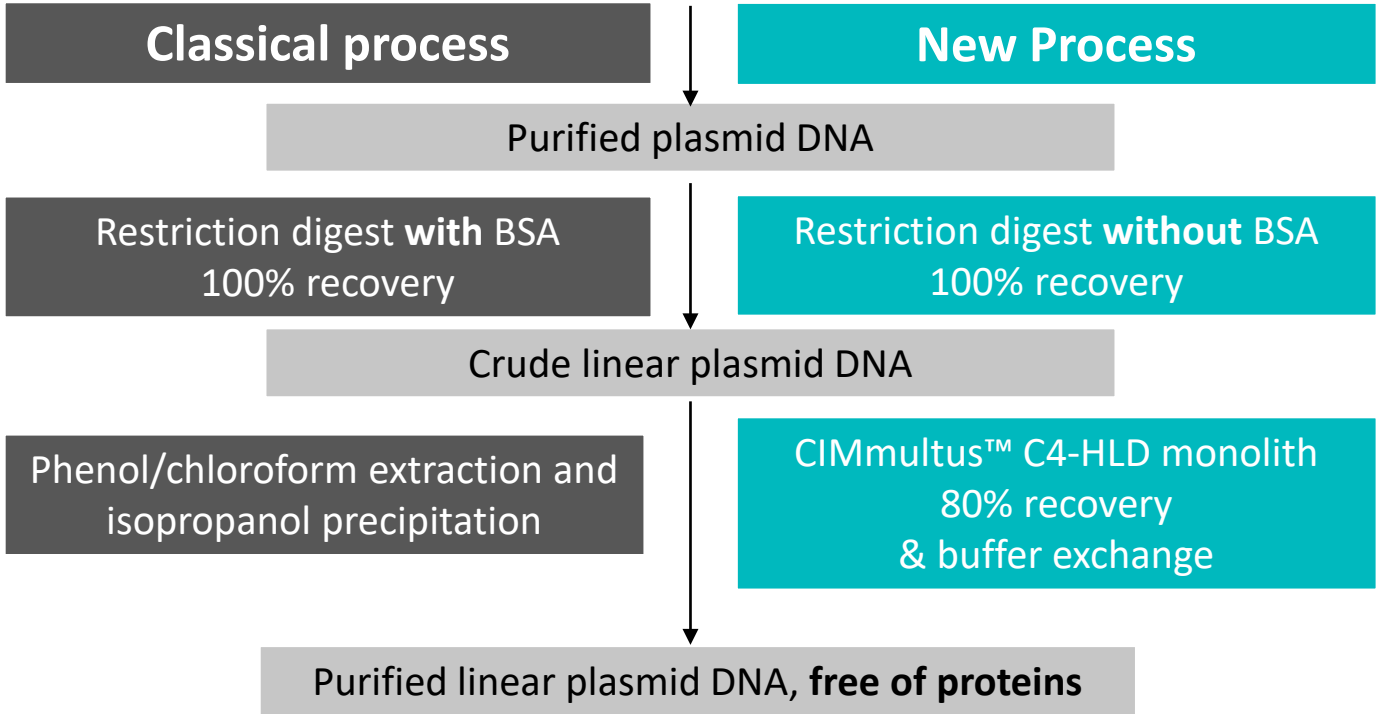


1: linear plasmid

2: GeneRuler 1 kb plus

Step 2: pDNA Linearization Summary



Step 3: mRNA Production – IVT Process Workflow

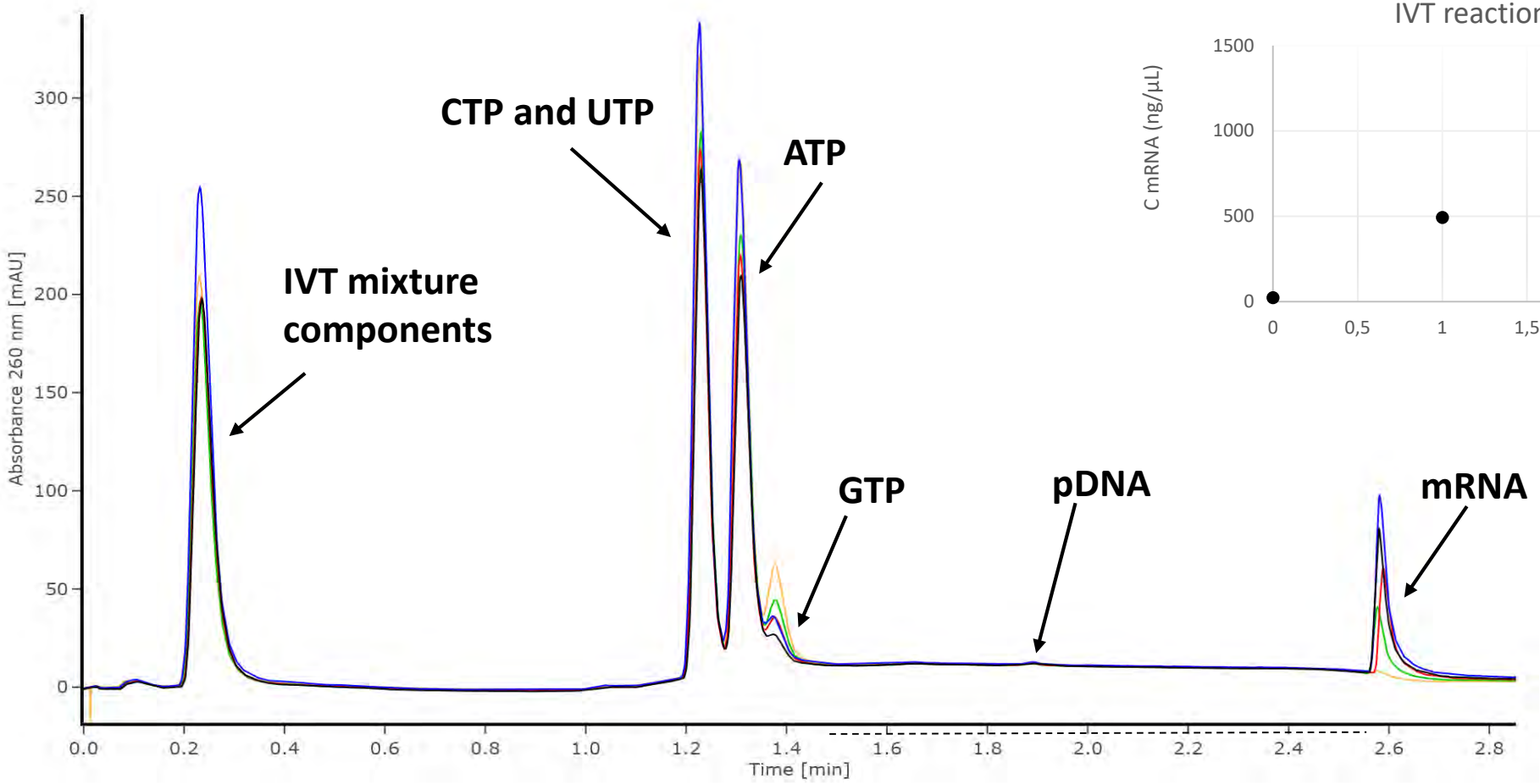
IVT reaction (THE key step to lower manufacturing costs):

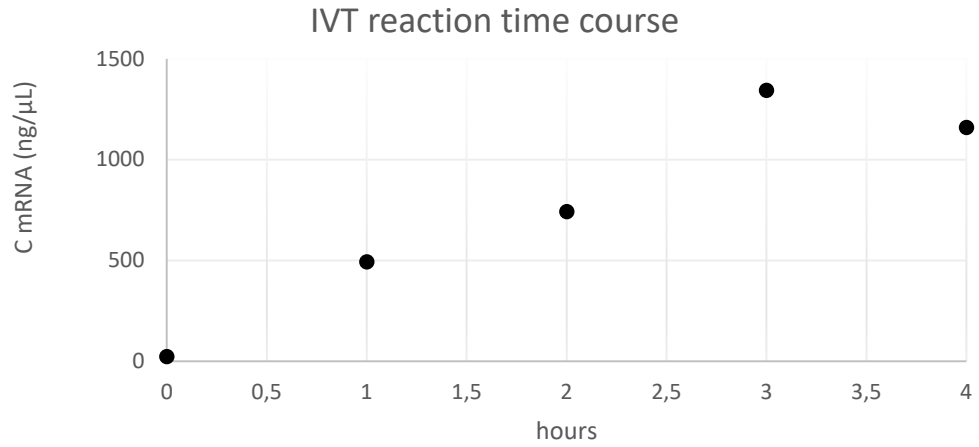
- Plasmid pIVTeGFP with encoded poly(A) tail (tail length 45 nt), T7 RNA polymerase (NEB), pyrophosphatase (NEB), RNAse inhibitor (NEB), and capping reagent ARCA (NEB); incubation 40°C, 3h; inactivation with EDTA; subsequent *O*-methylation (cap1 formation), expected mRNA size: 950 nt

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

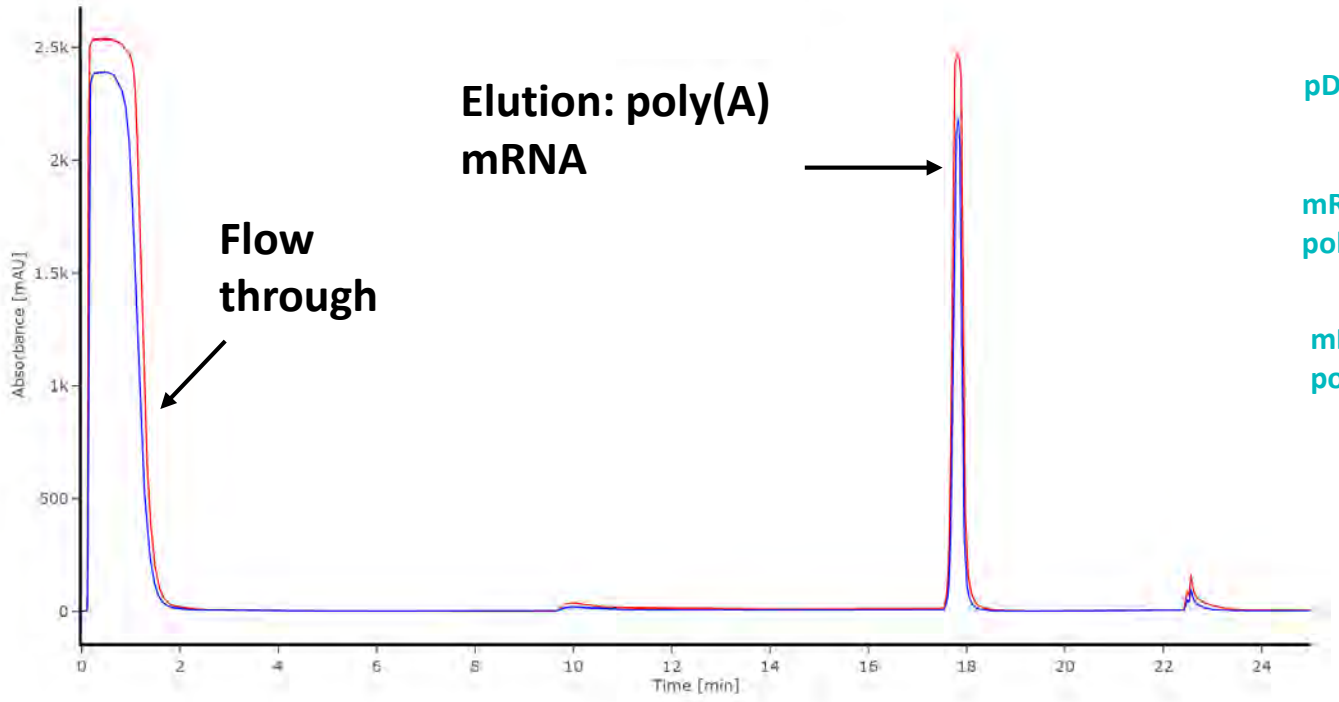
IVTmix_1h A260 nm

IVTmix_2h A260 nm

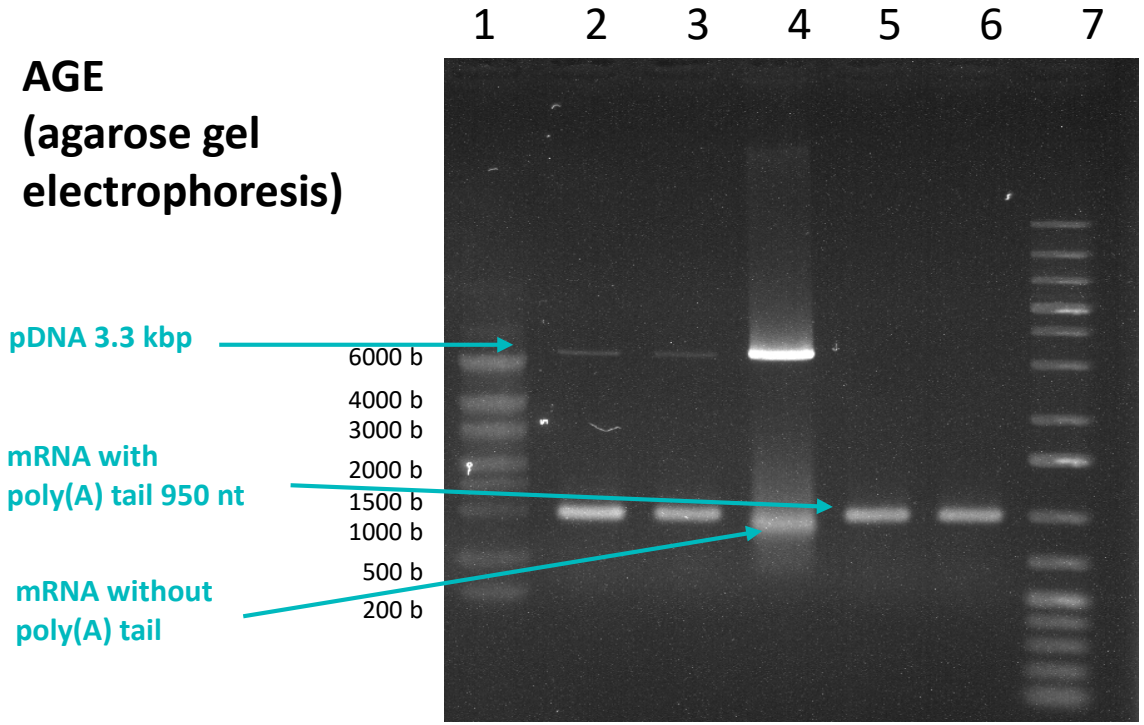
IVTmix_3h A260 nm

IVTmix_4h A260 nm

Purification of mRNA Cap0 Using CIMmultus™ Oligo dT



AGE (agarose gel electrophoresis)



1 RiboRuler HR

2 IVTmix

3 IVT mix - OligodT load

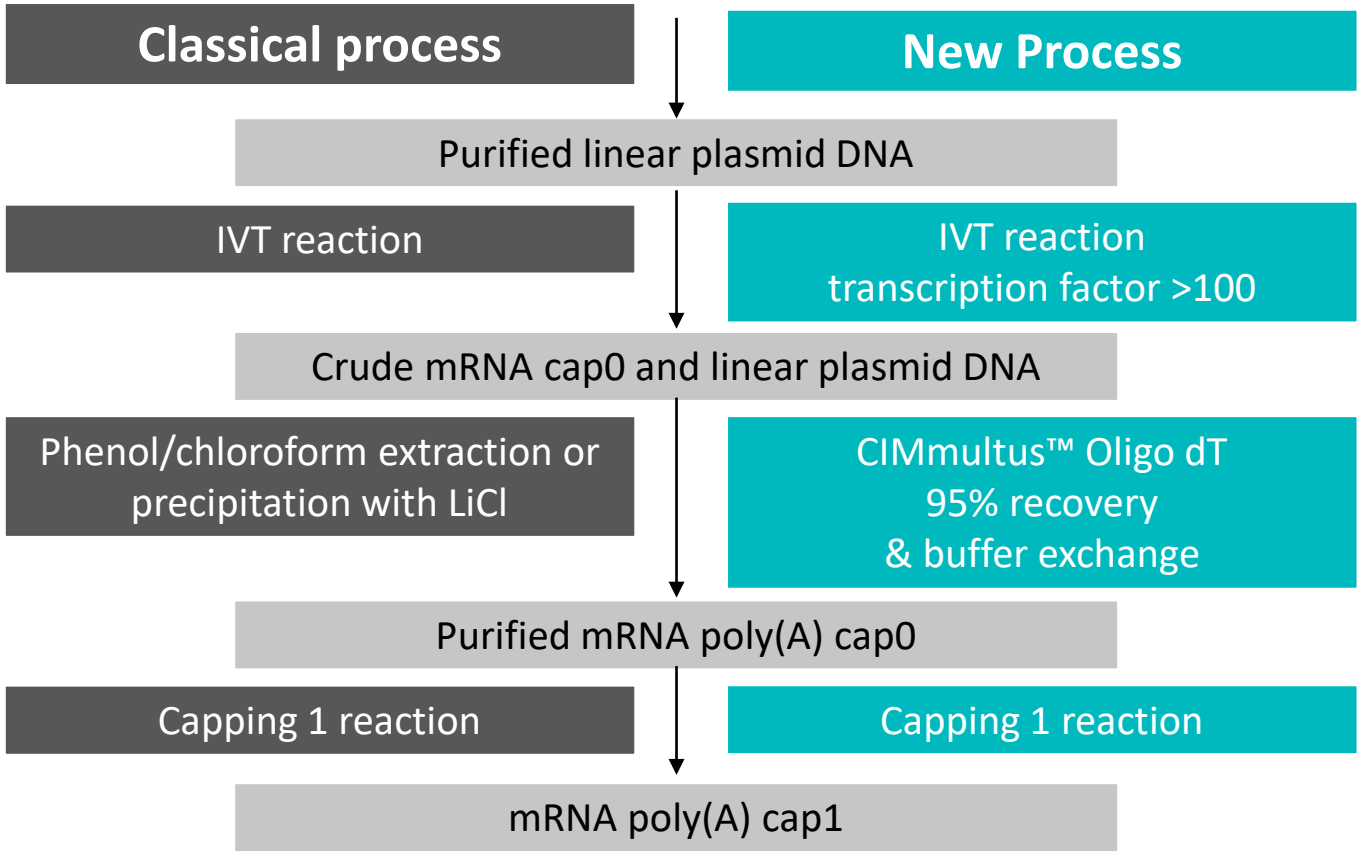
4 IVT mix- FT (50x concentrated)

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

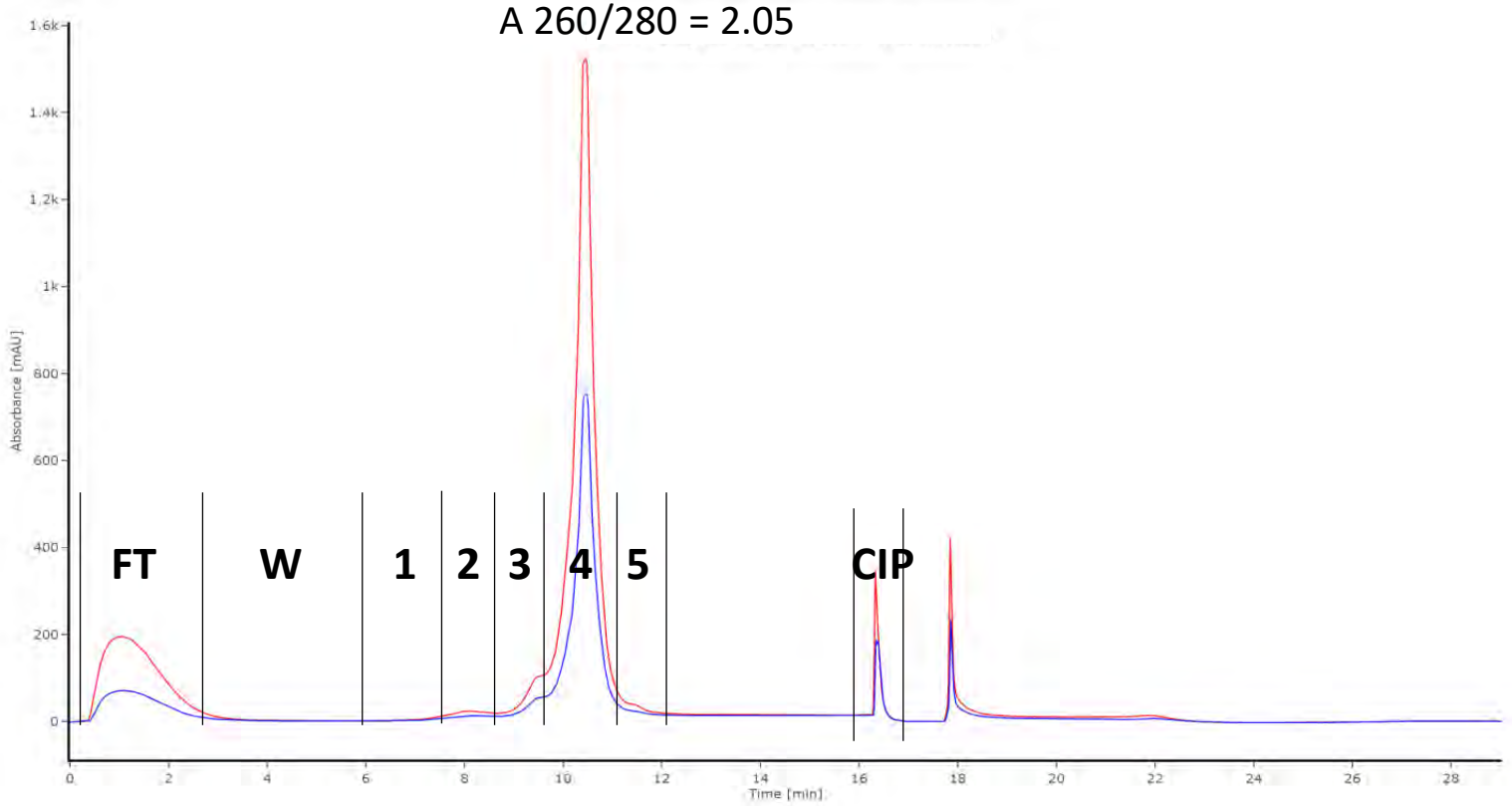
LC purification:

- **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

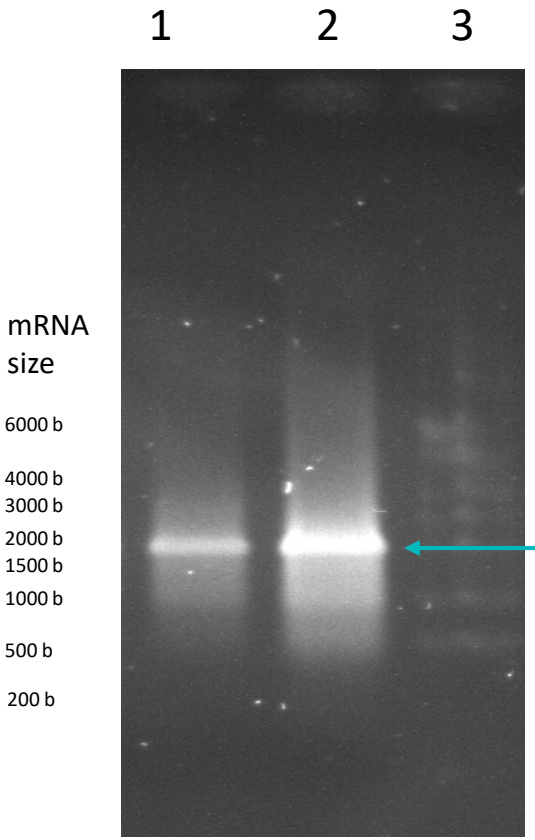
Elution mRNA

A 260/280 = 2.05



Poly(A) mRNA Cap 1 Purity Assessment

AGE (agarose gel electrophoresis)

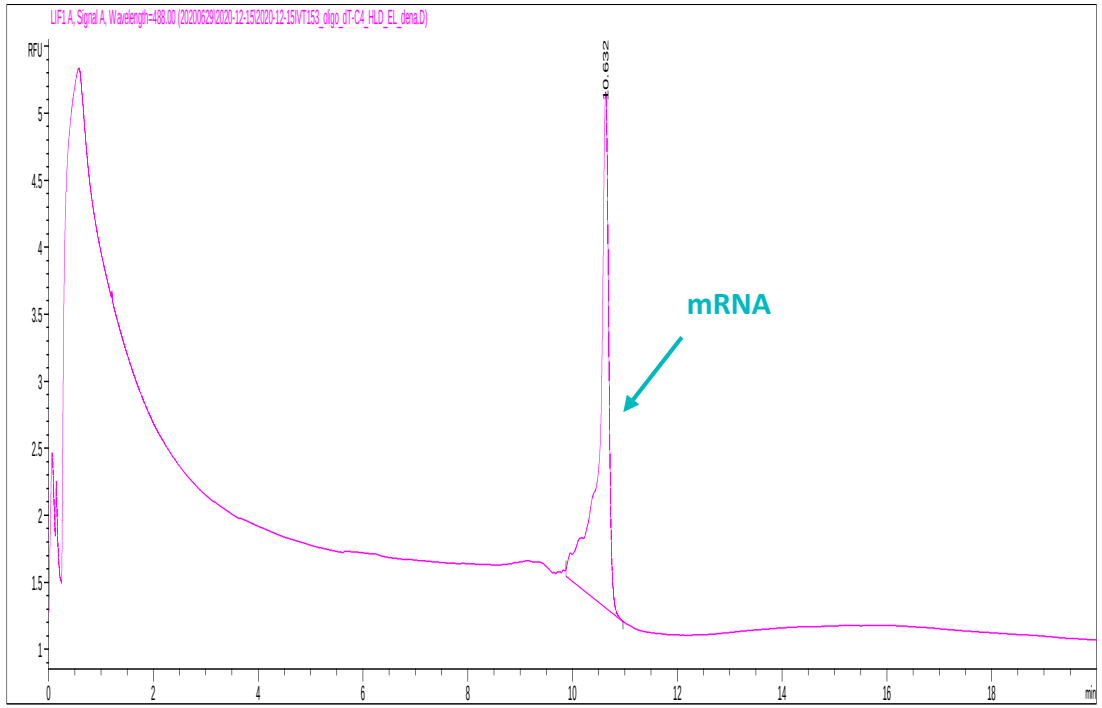


1 Final mRNA eGFP

2 Final mRNA eGFP denaturated at 80 °C, 5 min

3 RiboRuler HR

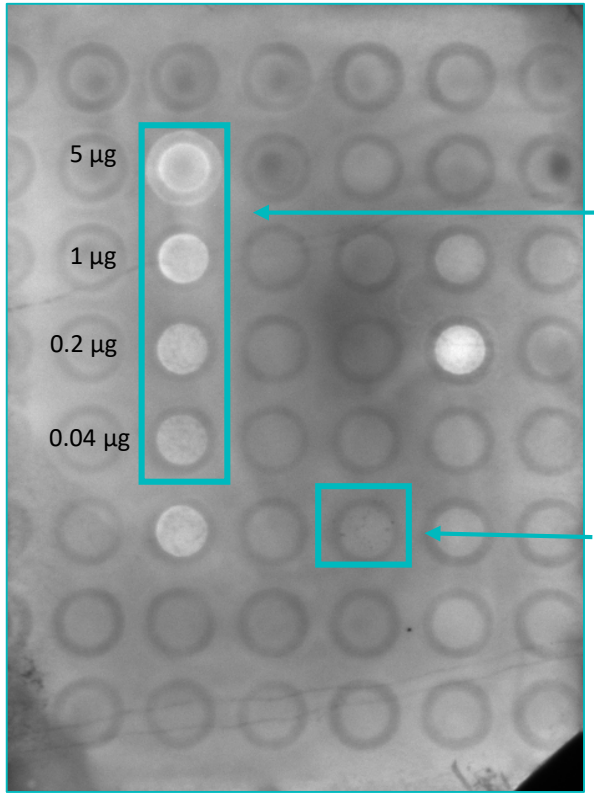
Capillary electrophoresis



Sample denaturated at 80°C, 5 min

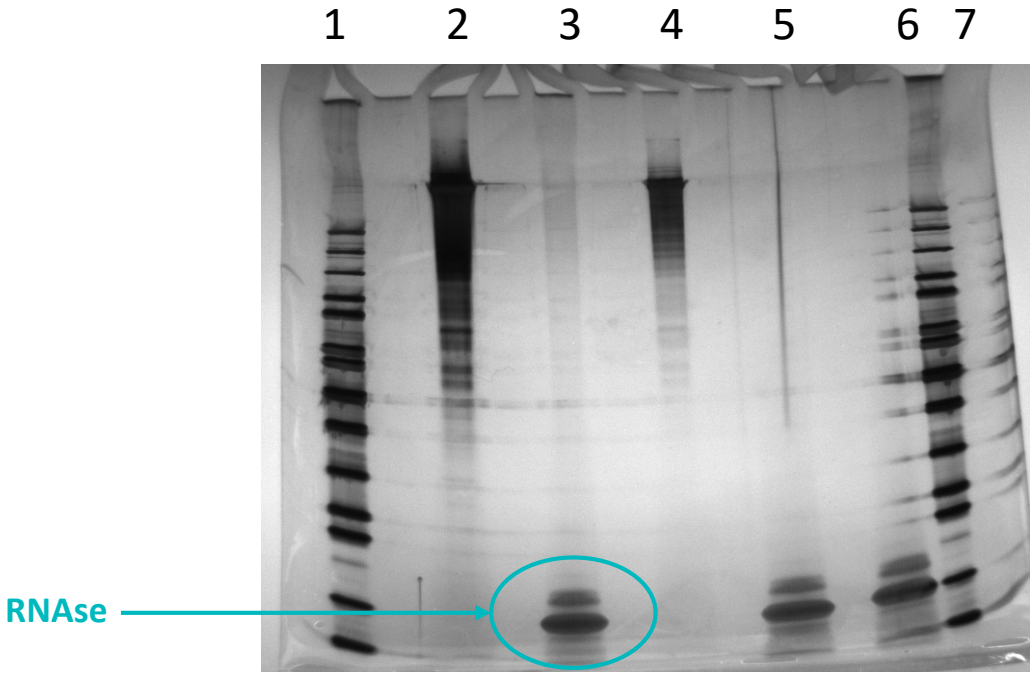
Poly(A) mRNA Cap 1 Purity Assessment

Dot blot
dsRNA detection limit 40 ng per well



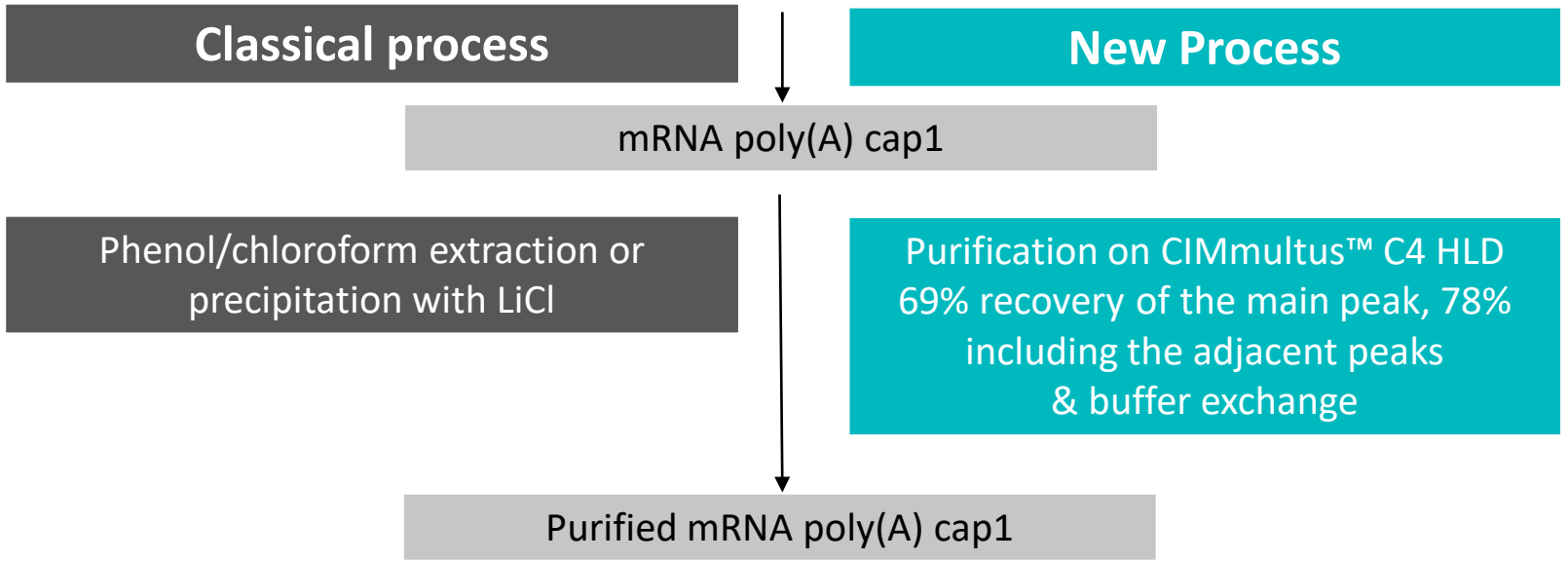
no dsRNA detected (< 1% of dsRNA in sample)

SDS-PAGE
protein detection limit: 10 ng BSA per lane



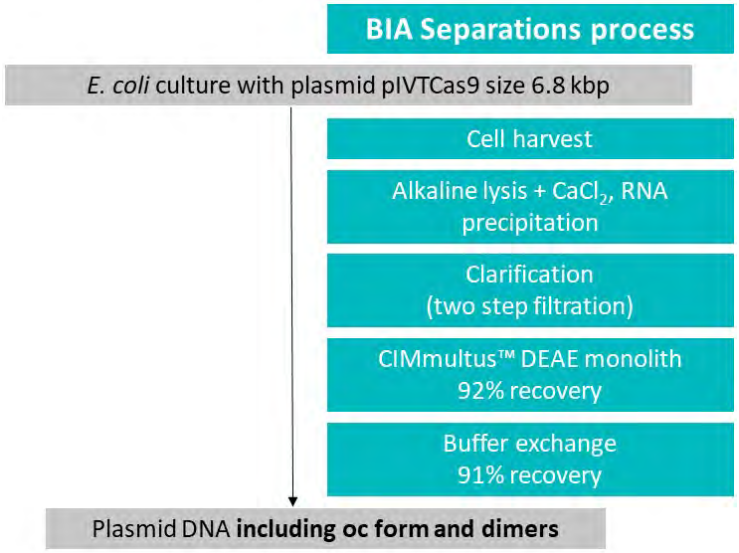
1 Protein ladder
2 Final mRNA pIVTeGFP 2.6 ug
3 Final mRNA pIVTeGFP + RnaseA
4 Oligo dT IVT153 E 0.7 ug
5 Oligo dT IVT153 E+ RnaseA
6 RnaseA
7 Protein ladder

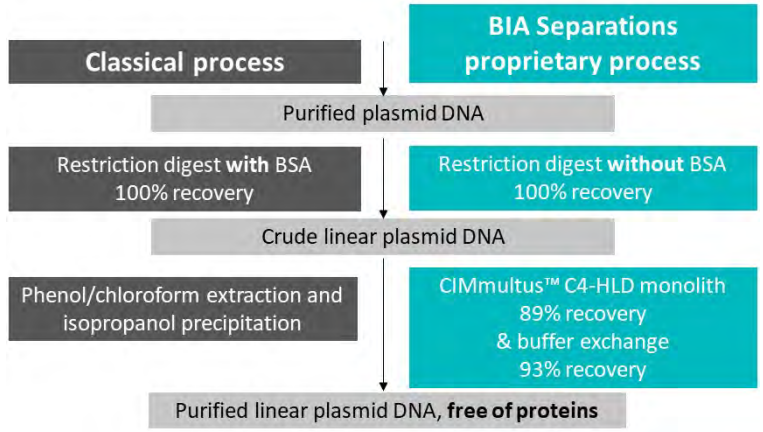
Step 4: mRNA Purification Summary

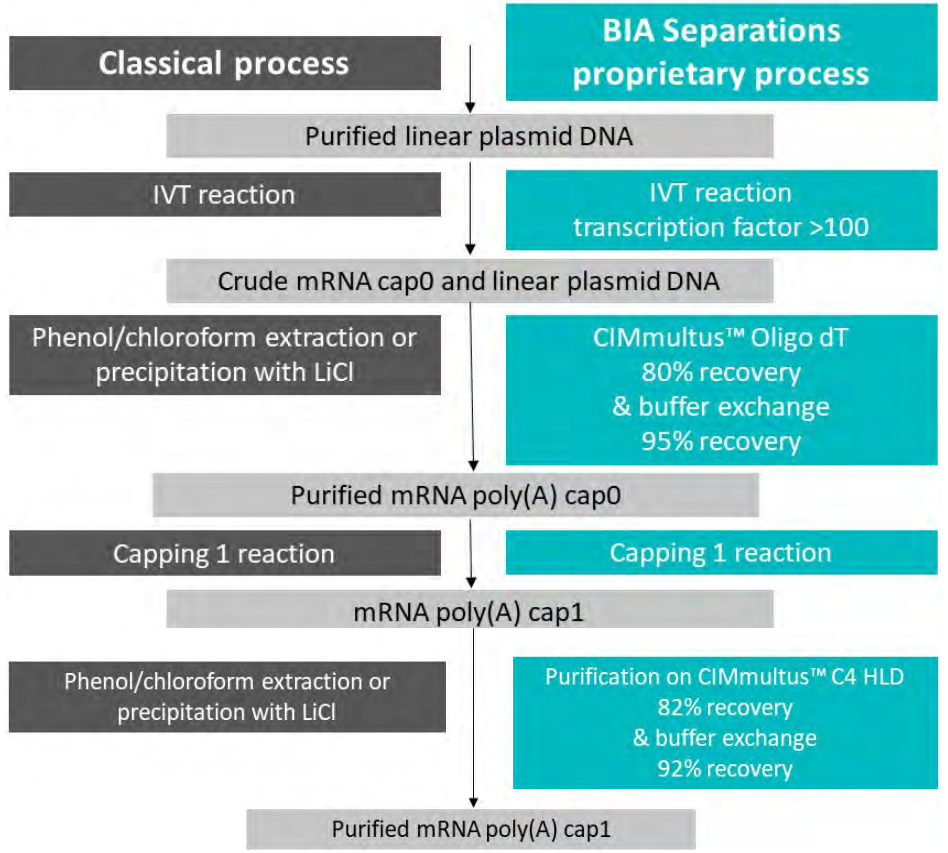


Work of the process with bigger mRNA confirmed

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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