SARTORIUS

BIA separations

Simplifying Progress

All-in-one PATfix analytical platform

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BPI, September 30, 2021

About BIA Separations - Sartorius

- The leading developer of monolith technology and the exclusive producer **of CIM® (Convective Interaction Media) monolithic chromatographic columns** for more than 20 years and with > 160 employees currently.
- A specialist in the purification of large biological molecules and viral particles for **gene therapy and the vaccine markets.**
- **Sartorius center of excellence in gene therapy** offers solutions for downstream process development and manufacturing and for analytical methods applicable to multiple large molecules, e.g. AAV, Adeno, Flu, pDNA, mRNA.
- **Supplies unique monolithic chromatographic columns** complimentary to porous particles and membranes.

SARTORIUS

Press Release

Göttingen, November 2, 2020

Sartorius closes acquisition of BIA Separations

- Combination of businesses creates a premium portfolio for advanced therapies
- Course set for successful integration

Experts in express bioproduct manufacturing process development

>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

PATfix HPLC platform

The PATfix HPLC platform

A platform for analysis and in-process control of up- and down-stream processing steps.

Four pillars of the platform:

- **Hardware: PATfix HPLC system with multiple detectors**
- **Consumables: CIMac columns + standards**
- **Software: PATfix software to allow for fast data processing**
- **Know-how: build-in user guides and methods**

Going beyond analytics, the PATfix HPLC platform gives reliable „at-line“ insights and supports rapid process development and optimization, identifying CPPs and their impact on CQAs.

During production, the PATfix platform provides in-process control and manages key performance indicators.

The 4 Pillars of the PATfix HPLC platform

PATfix System

Biocompatible (bio-inert ceramic pump heads)

Sensitive detector suite, including multi-wavelength UV and fluorescence, MALS, RI etc optional

Analytical and semi prep options (10 or 50 mL/min flow rate)

Low pressure quaternary gradients or high pressure binary gradients

CIMac Columns

Engineered for use with large biomolecules and virus particles:

- convective laminar flow
- large accessible surface area

High resolution separations

Variety of surface chemistries:

- IEX, HIC, MM, AF
- Purpose designed
- 0.1 mL column volume (CIMac pDNA 0.3 mL)
- 1 – 30 CV/min (depending on column type)

No carry-over

PATfix Software

Fast and user-friendly

Data acquisition and integration from detectors of different vendors

Data management for processing and visualization

Automated analysis of chromatographic data sets

Alarms in case of malfunction

Server-client based approach

Build-in Know-how

User guides for experimental set up and execution

Built-in validated methods and SOPs for seamless analytics

Standard included (e.g. pDNA)

CIP methods

Installation, qualification and training

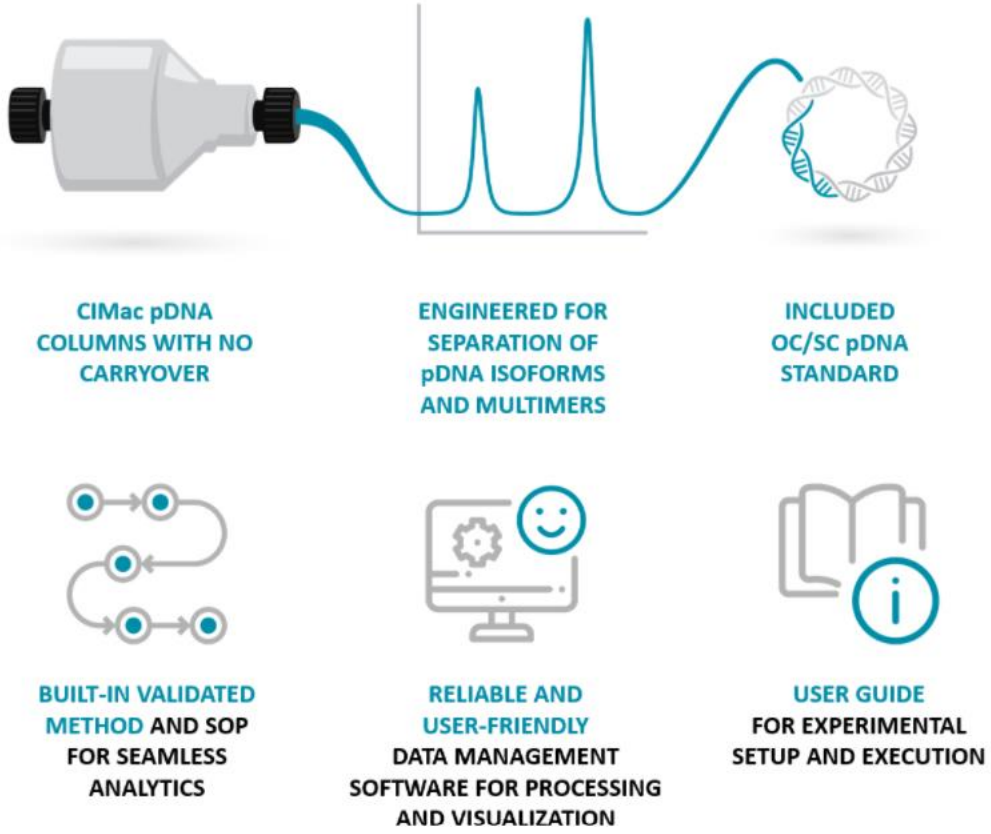
PATfix pDNA platform

PATfix pDNA Platform

Global demand for pDNA production is at an all-time high, due to increased need from Gene Therapy ramp-up. pDNA, as an enabling product, is critical in production of mRNA, AAV and other therapeutic vectors. Increasing yield and purity in the production of pDNA is a vital step in meeting such demand.

The PATfix pDNA analytical HPLC platform package is designed to give „at-line“ insight to

- support rapid process development and optimization
- provide reliable in-process control for pDNA production



PATfix™ pDNA analytics - Applications

Characterization of raw and in-process materials

At-line process monitoring & control

- Process optimization
- Batch to batch tracking

Product purity control

- Tracking of contaminant removal
- Release testing

Link between analytics and process

- Synergy due to both analytics and prep being chromo steps
- Easy buffer and method transfer

PATfix™ Analytical Platform

- Software
  - PATfix with pDNA expansion
    - A comprehensive guide for day-to-day pDNA analytics
    - Collaboration & access
    - WFH
    - Data processing
    - Information extraction
    - Data visualization

Robust → Reproducible → Fast → Sensitive → User friendly → Bio compatible

PATfix™ Analytical Platform

- **Methods**
  - Optimized for PATfix platform
  - Analysis of wide range of pDNA sizes
  - Validated (accurate & reproducible)
  - Comes with pFix standard
- **Columns**
  - Cleaning in place – no carryover
  - Range of channel sizes for big and small pDNA

Robust → Reproducible → Fast → Sensitive → User friendly → Bio compatible

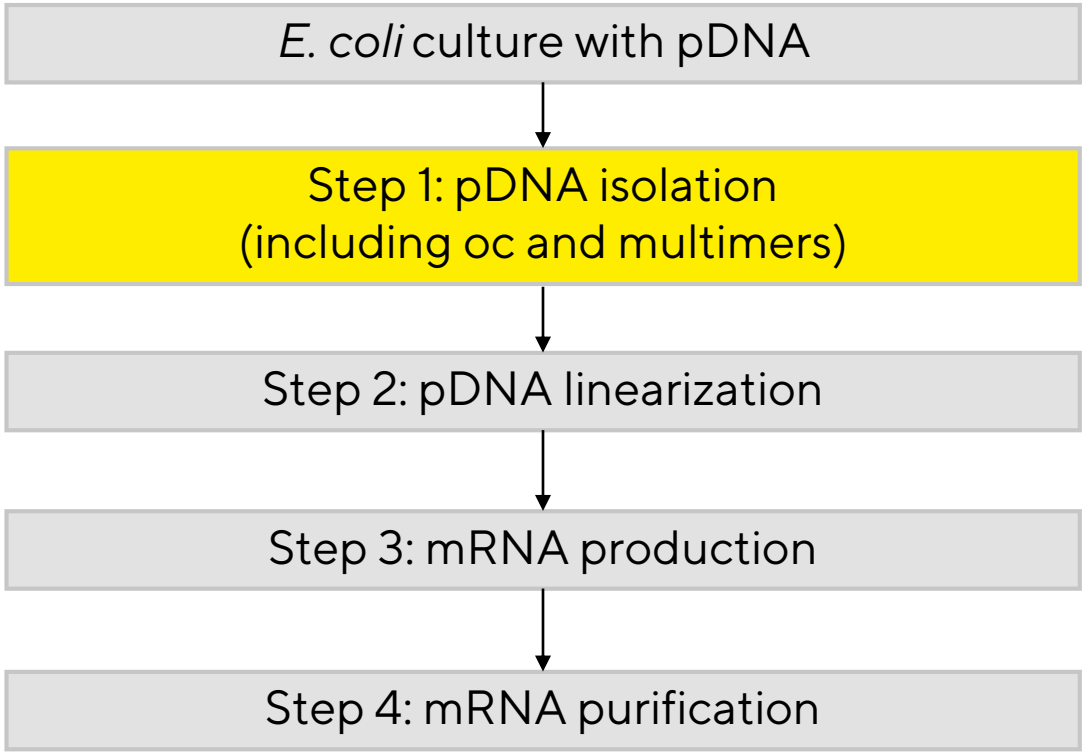
PATfix™ Analytical Platform

- Hardware
  - Pump
  - Autosampler
  - Multiwavelength detector
  - Conductivity/pH monitor
  - Column oven
  - OPTIONAL: Fluorescence detector
  - Compatible with other HPLC columns
- Process analytic services

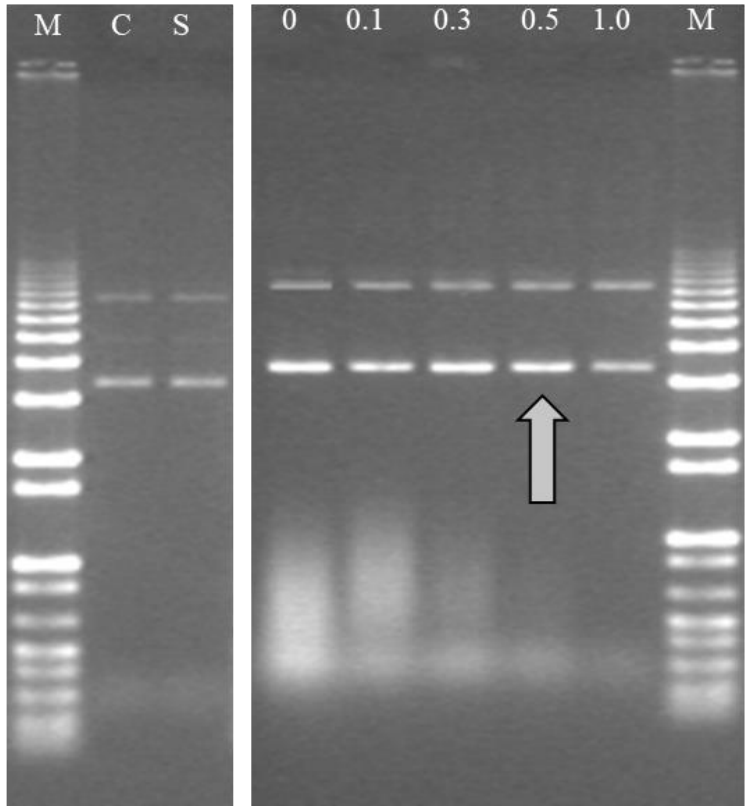
Robust → Reproducible → Fast → Sensitive → User friendly → Bio compatible

PATfix HPLC as an analytical companion to preparative chromatography

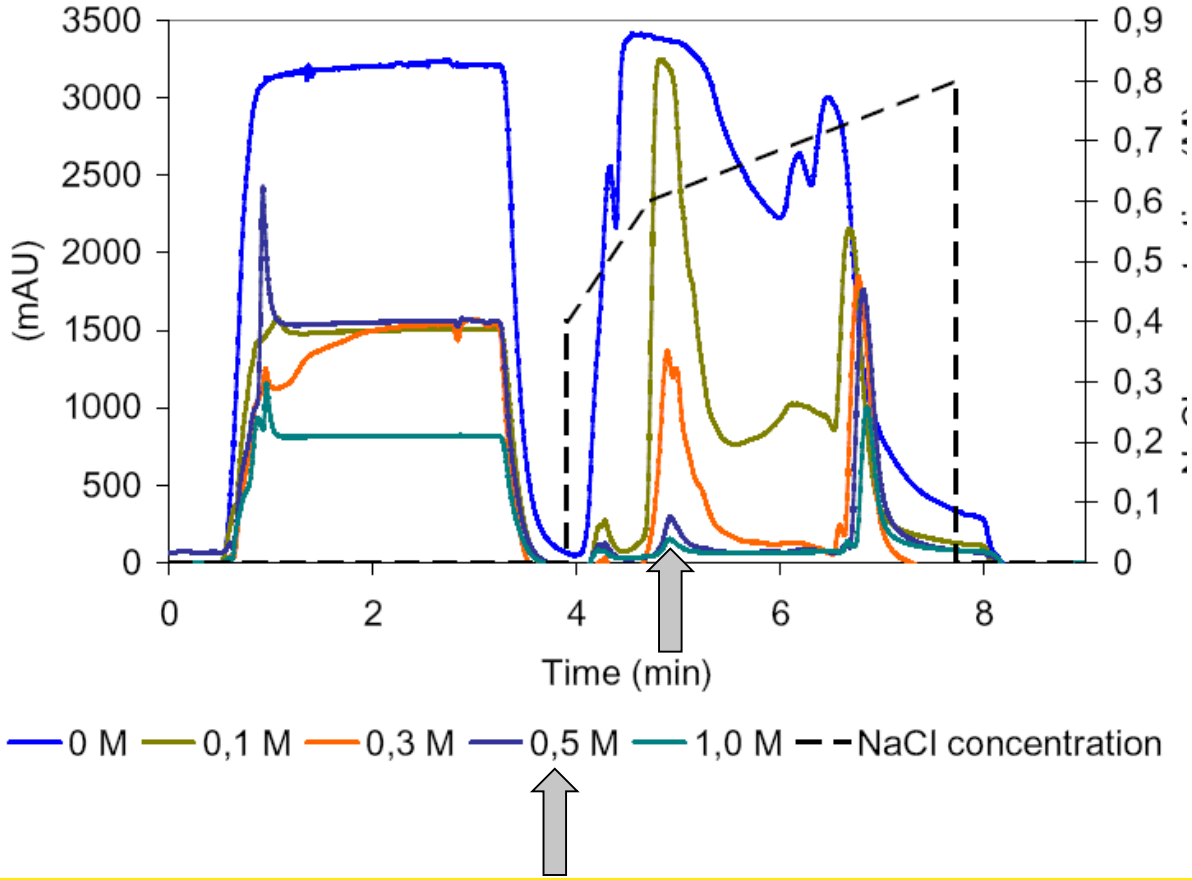
Case study: plasmid DNA analytics - from *E.coli* to pure mRNA



E.coli lysis and selective RNA precipitation - optimisation of $CaCl_2$ using PATfix pDNA platform

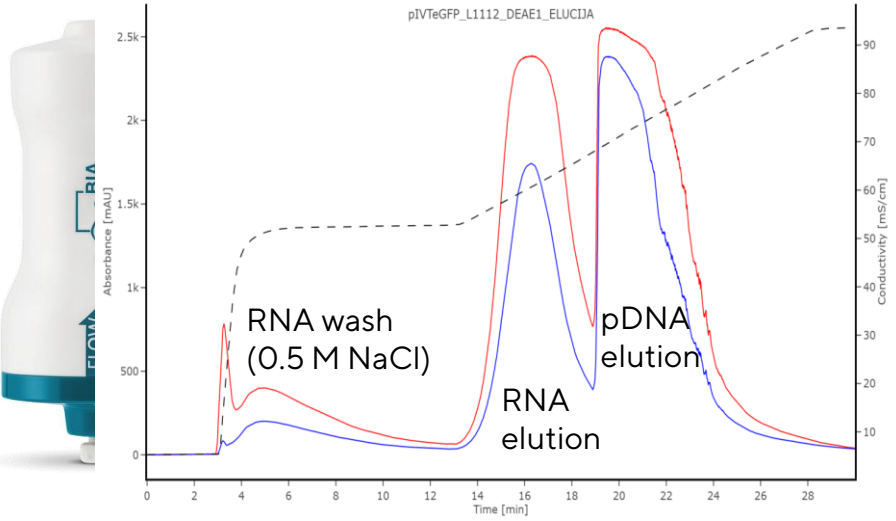


CIMac™ pDNA - analytical column



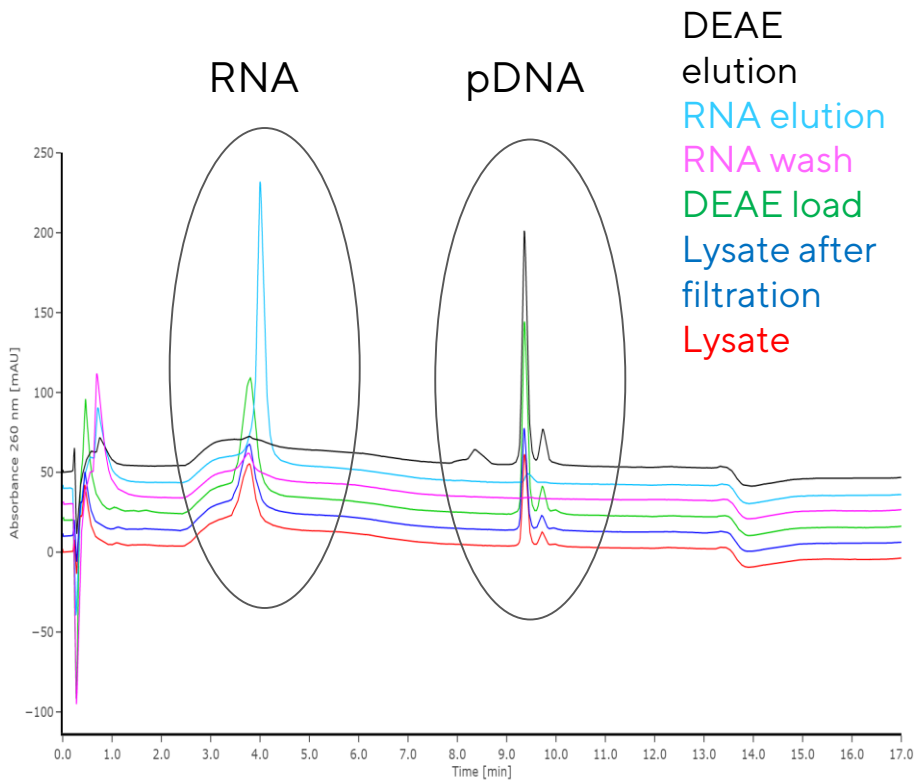
Optimisation of pDNA (3.3 kbp) purification using PATfix pDNA system

CIMmultus ™ DEAE - preparative

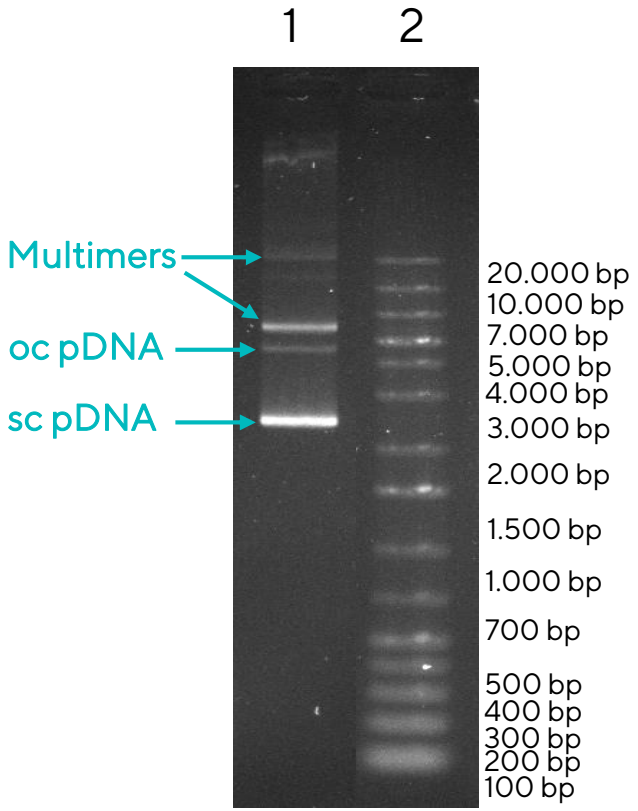


pDNA purification	
pDNA recovery in elution (%)	87
RNA removal (%)	> 99 %
oc pDNA (%)	3
Multimers (%)	25

HPLC CIMac™ pDNA - analytical column

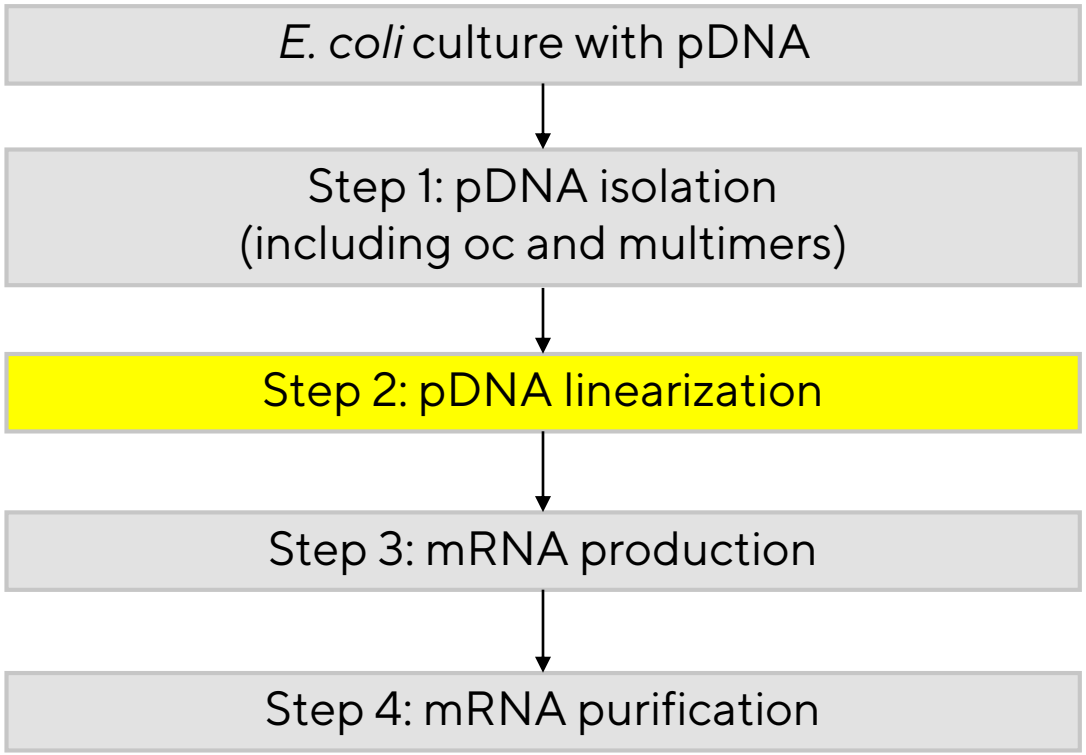


AGE



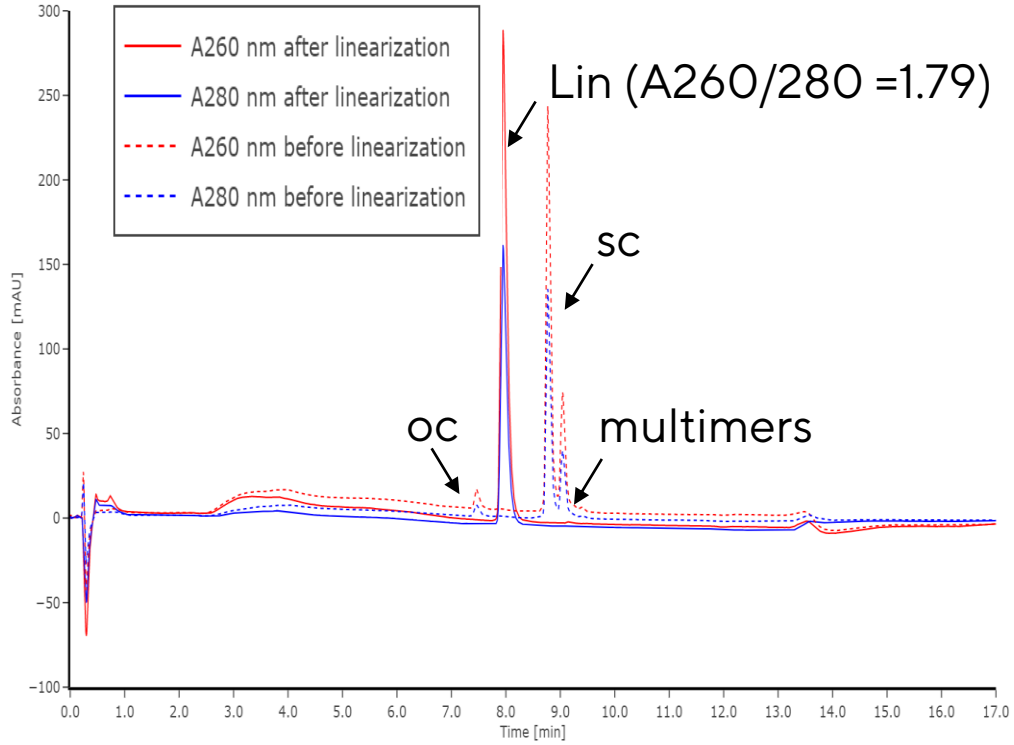
1: purified eGFP plasmid
2: GeneRuler 1 kb Plus

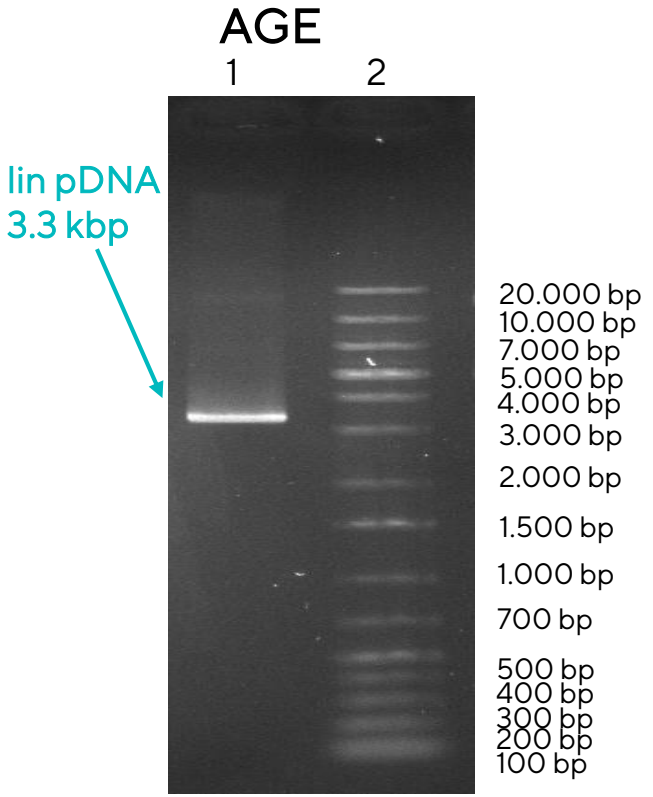
Step 2: pDNA Linearization Summary



Monitoring of pDNA linearization step using PATfix pDNA system

CIMac™ pDNA column – analytics of linearization kinetics

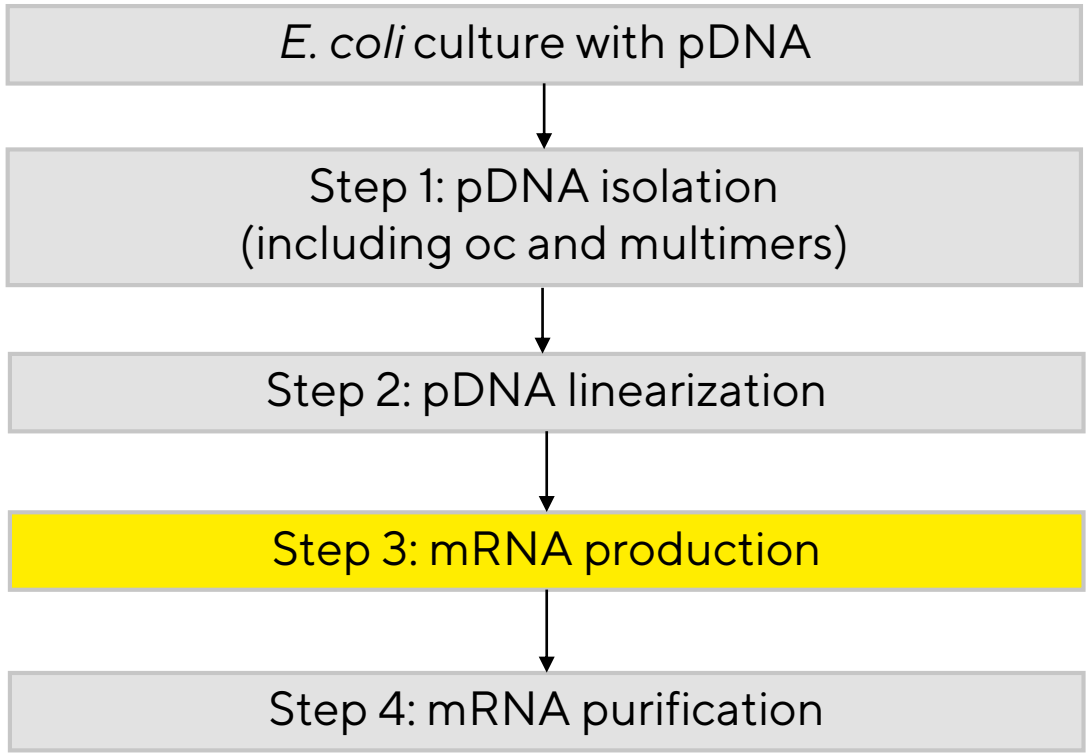




1: linear plasmid
2: GeneRuler 1 kb plus

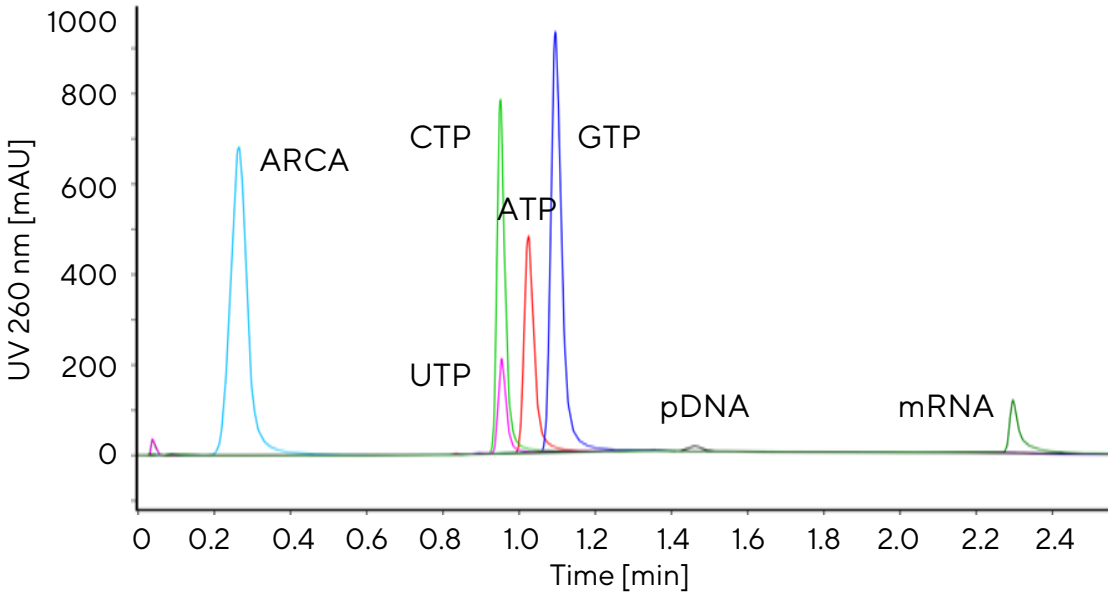
Linearization kinetics monitored by CIMac pDNA HPLC analytics. Baseline separation of linear isoform from oc/sc/multimer isoforms, 17 min run time.

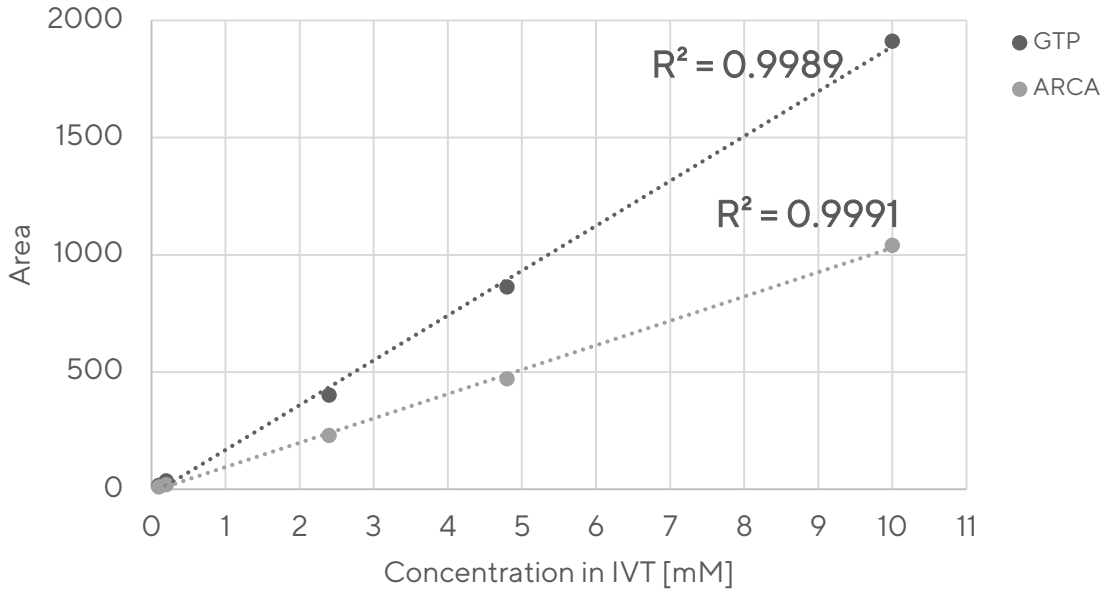
Step 3: IVT reaction kinetics



Monitoring of IVT mRNA synthesis – optimisation of pDNA amount using PATfix pDNA system with CIMac PrimaS column

- HPLC can provide valuable information on the IVT reaction and mRNA purity
- CIMac PrimaS can separate and quantify multiple individual IVT reaction components, including the pDNA in a single, rapid assay





CIMac PrimaS (P.N. 110.5118-2), Buffers: MPA (50 mM HEPES pH 7), MPB (50 mM HEPES, 200 mM sodium pyrophosphate, pH 8.5), Method: 0–1 min (100 % MPA), 1–1.8 min (gradient to 20 % MPB), 1.8–2.5 min (gradient to 80 % MPB). Full method not shown. Flow rate 2 mL/min, PATfix™ HPLC system, UV absorbance at 280 nm, injection volume 25 µL.

What is your benefit?

No need to…

- Find the right HPLC hardware
- Select the correct column
- Develop an internal standard
- Develop/optimize the analytical method
- Validate the standard + analytical method + system

Platform advantages

- Lowers initial knowledge/skill barrier
  - Ease of adoption
- Easy analytics scale-up
  - Software can pool data from multiple machines
  - New staff requires only basic analytical chromatography knowledge
- From sample to the result in 20 minutes
  - Speeds up process development

Thank you for your attention!

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