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Simplifying Progress

High Yield mRNA Production Process from *E. Coli* to Highly Pure mRNA

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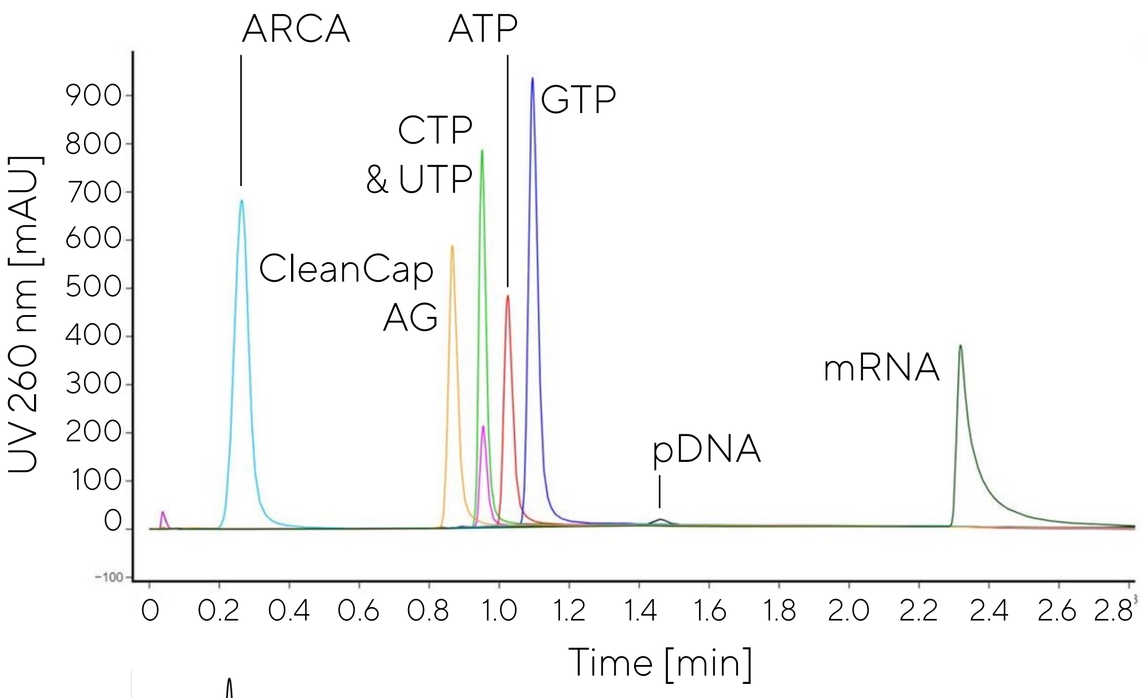
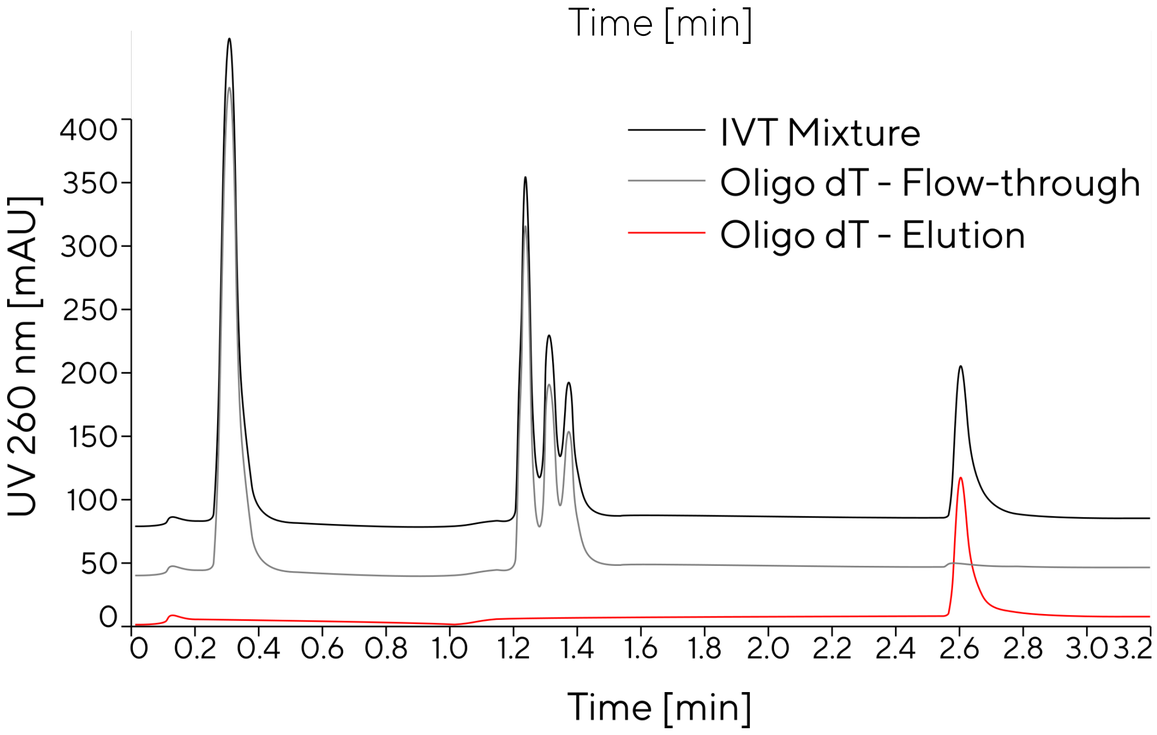
Analytical Tool for IVT Optimisation and In-Process Monitoring

Characterisation of IVT reactions, in particular for optimisation of reaction conditions, is challenging due to limited applicability of analytical assays. Common analytical methods used in laboratory and industrial settings provide information on a single IVT component at a time. For example, AGE is used to confirm RNA species, PCR for DNA or RNA, SDS-PAGE for protein contaminants, etc. Some of these methods are also time-consuming, lack automation, or require higher sample volumes.

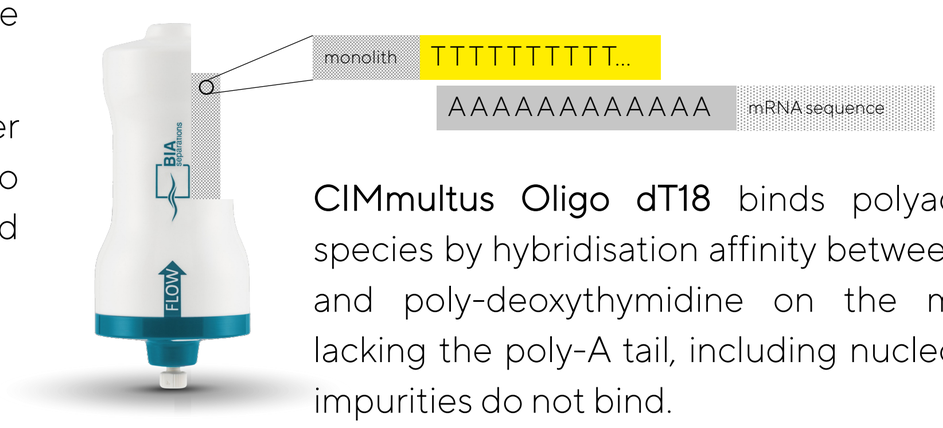
Analytical HPLC with **CIMac PrimaS** (catalog number 110.5118-2) analytical monolith provides the resolution to separate capping reagents, nucleotides, DNA template and RNA in a single, rapid assay.



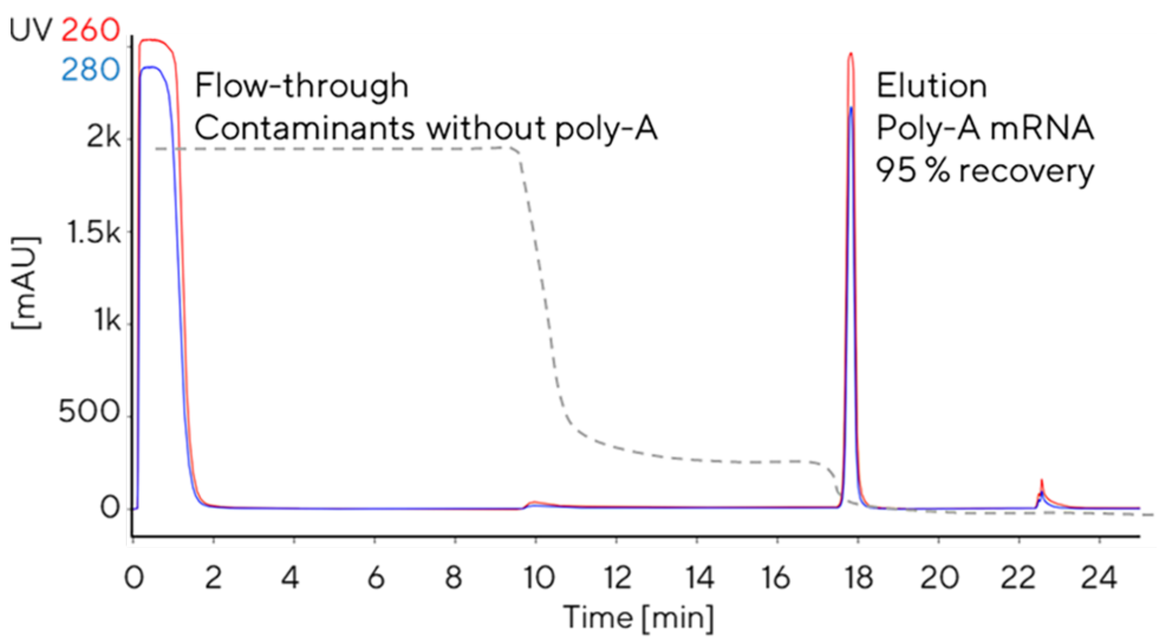


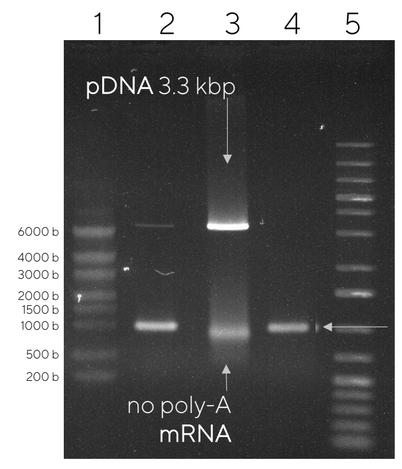
Fast Capture of mRNA from IVT Reaction Mixture

Different mRNA production protocols can employ multiple steps to produce mature mRNA with all its structural components. The 5' cap and the polyadenylated tail can be added co-transcriptionally during the IVT reaction, or as secondary enzymatic steps. Purification protocols may need to be adapted to provide intermediate purification. Polyadenylated mRNA can be purified aby Oligo dT18 affinity monolith.



CIMmultus Oligo dT18 binds polyadenylated mRNA species by hybridisation affinity between polyadenine tail and poly-deoxythymidine on the monolith. Species lacking the poly-A tail, including nucleotides, RNA, DNA impurities do not bind.





Above: Oligo dT18 purification of eGFP mRNA (950 nt mRNA with 45 nt poly-A tail). Load in 50 mM phosphate, 500 mM NaCl pH 7. Elution in 10 mM Tris.
Left: AGE, 1 – RiboRuler HR, 2 – IVT mix, 3 – Flow-through, 4 – Elution, 5 – GeneRuler 1 kb Plus

- CIMac PrimaS provides a rapid HPLC assay for **quantification** of individual IVT reaction components. mRNA, NTP, pDNA and capping reagent are resolved in ~3 min.
- Kinetics of formation of mRNA and consumption of reagents can be **measured in real-time** at different reaction conditions to find optimal IVT parameters.
- The assay provides **in-process control** during mRNA purification.

- mRNA is captured from IVT reaction mixture with **minimal sample preparation** (sample dilution only).
- CIMmultus Oligo dT produces high purity mRNA with **improved stability** compared to purification by traditional methods.
- High flow rates and high capacity for mRNA ensured by convective monolithic stationary phase result in **high purification throughput**.
- Ease of **process scalability** (from mg scale to > 100 g per run).