Simplifying Progress

Extraction of mRNA From IVT Mixtures With CIMmultus® Oligo dT Column

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Introduction

The increasing demand for messenger RNA (mRNA) as a therapeutic product requires larger production scales and more efficient extraction techniques. The fast and efficient way to purify polyadenylated mRNA using affinity chromatography on the CIMmultus® Oligo dT column is presented.

The poly-adenylated tail of mRNA interacts with covalently bound oligo dT ligands in high-salt loading conditions, where electrostatic repulsion between negatively charged backbones of both mRNA and oligo dT are reduced, and H-bonding in the T-A base pair is emphasized. High salt concentration also screens out attractive electrostatic interactions between mRNA and other components in the process sample, thus facilitating an aggregate reduction in purified products (Figure 1).

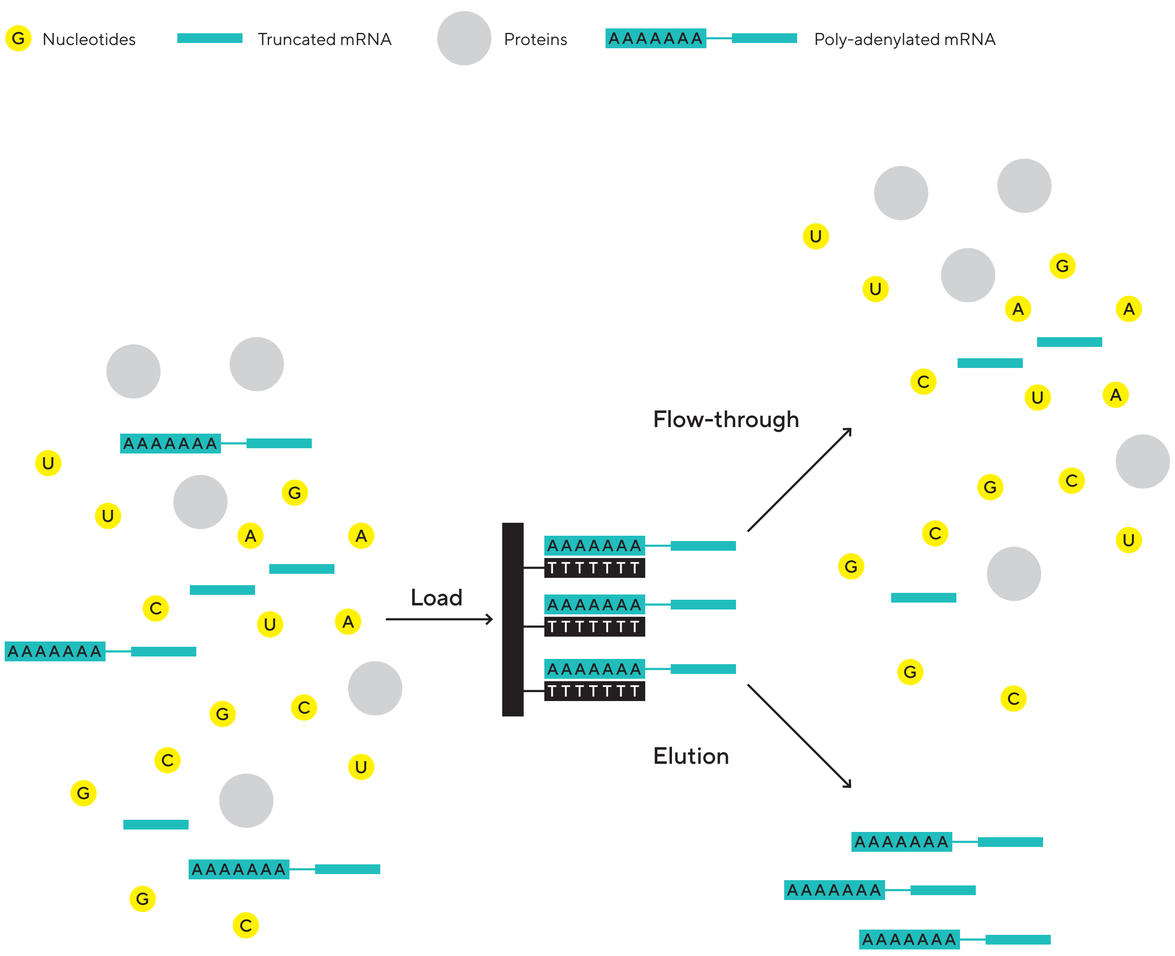

Figure 1: Schematic view of purification process using CIMmultus® Oligo dT column.

Methods

Sample: IVT reaction mixture containing approximately 180 µg of mRNA.
Chromatography done by CIMmultus® Oligo dT monolithic column.

Chromatographic conditions:
Binding buffer: 50 mM phosphate, 250 mM NaCl, 2 mM EDTA, pH 7.0
Wash buffer: 50 mM phosphate, 2 mM EDTA, pH 7.0
Elution buffer: 10 mM Tris, pH 7.0

Results - Chromatographic profile of IVT mix loaded to CIMmultus® Oligo dT column

CIMmultus® Oligo dT column was loaded with IVT reaction mixture containing approximately 180 µg of mRNA. The chromatographic profile (Figure 2) shows a large flow-through fraction predominantly containing species that do not interact with a high salt buffer column. Non-binding species are expected to be nucleotides, proteins (enzymes), and mRNA lacking poly-A tail moiety (truncated mRNA). The result is further supported by electropherograms displayed in Figure 3B, where only a small amount of material with a size between 500 and 1500 nt is observable.

Applying the intermediate wash step also elutes non-specifically bound contaminants before eluting target mRNA and lowers the conductivity of buffer, eliminating the need for extensive buffer exchange after main elution of mRNA.

By lowering the salt concentration, elution of mRNA occurs in mild conditions in low conductivity buffer at neutral pH.

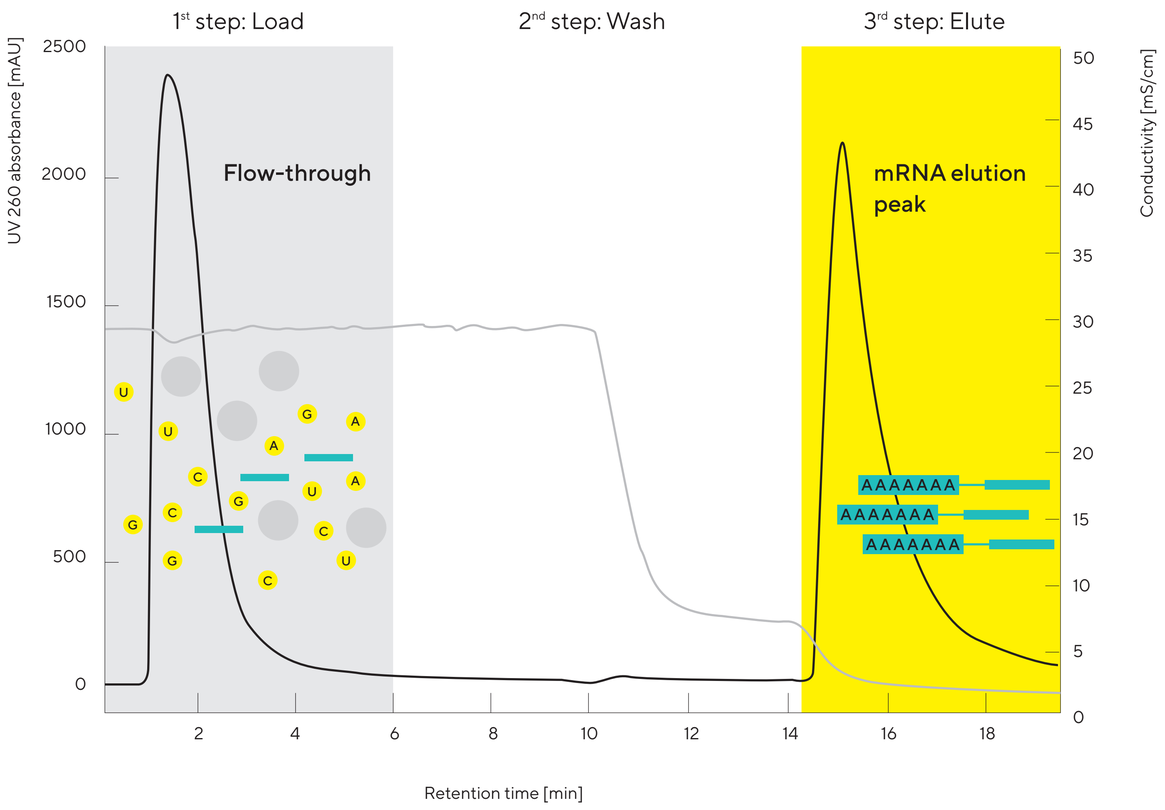

Figure 2: Chromatographic profile of IVT mix loaded to CIMmultus® Oligo dT column.

Results - Electropherograms of the loading material, flow-through, and elution fraction

Electropherograms indicate the removal of high molecular weight components (observable as shoulders at size >2000 nt on Figure 3A) and a substantial concentration of target polyadenylated mRNA (Figure 3C). The recovery of the purification process is about 80%.

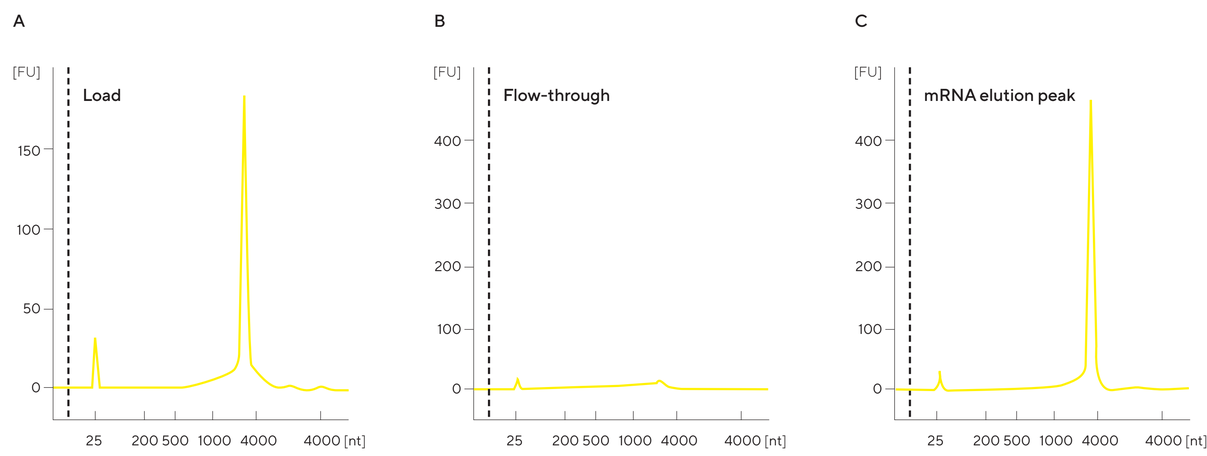

Figure 3: Electropherograms

Conclusion

- Monolith affinity chromatography can be used to isolate and purify mRNA containing a poly-A tail rapidly
- CIMmultus® Oligo dT column selectively removes impurities from the sample and is a convenient approach for the initial purification of mRNA
- With high flow rates and low shear forces, monoliths offer an efficient and mild approach to purify labile mRNA molecules