Two-step chromatographic purification of pIVTeGFP-transcribed mRNA with post-transcriptional capping

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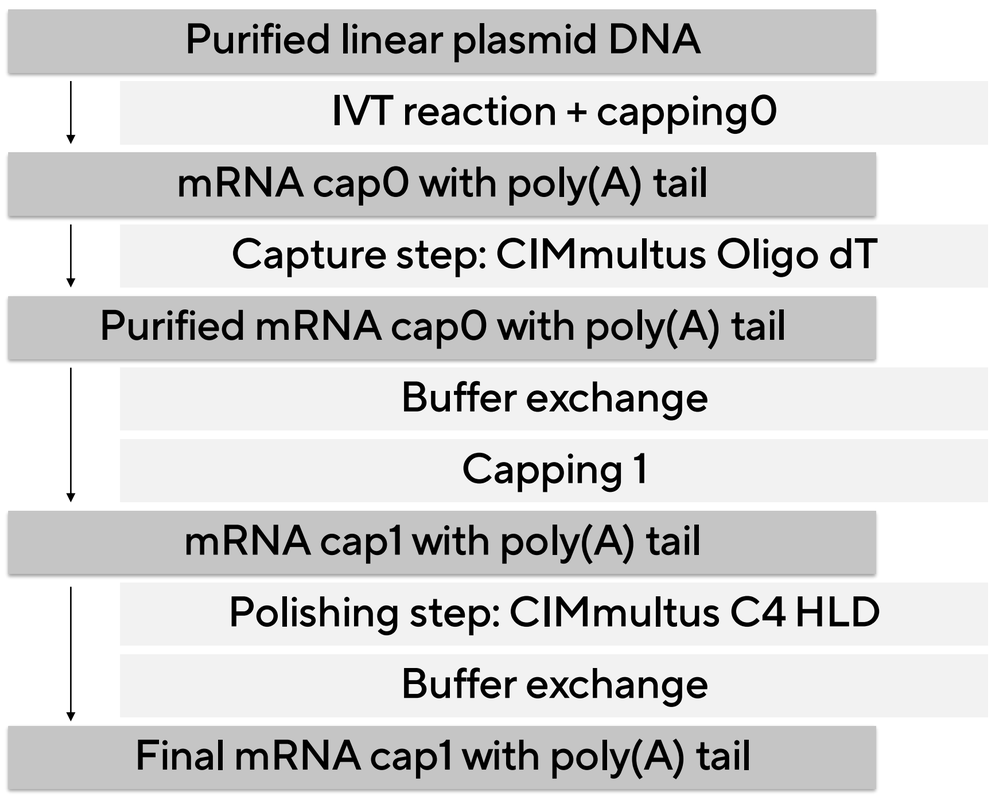
mRNA purification challenge

In mRNA production process, downstream purification of *in vitro* transcription (IVT) reaction often relies on precipitation methods which cannot provide resolution, recovery, or reproducibility to consistently produce a safe and effective product with good process economics. mRNA is a large biomolecule (mass of 1000 nt is ~ 150 kDa and >100 nm in diameter) for which porous particle chromatography lacks the ability to support high capacity and throughput to achieve good process economics. Convective flow chromatography media (e.g. monoliths) is an optimal platform for purification. A fully scalable chromatographic purification process is presented for a post-transcriptionally capped in vitro transcribed mRNA.

Process flow diagram



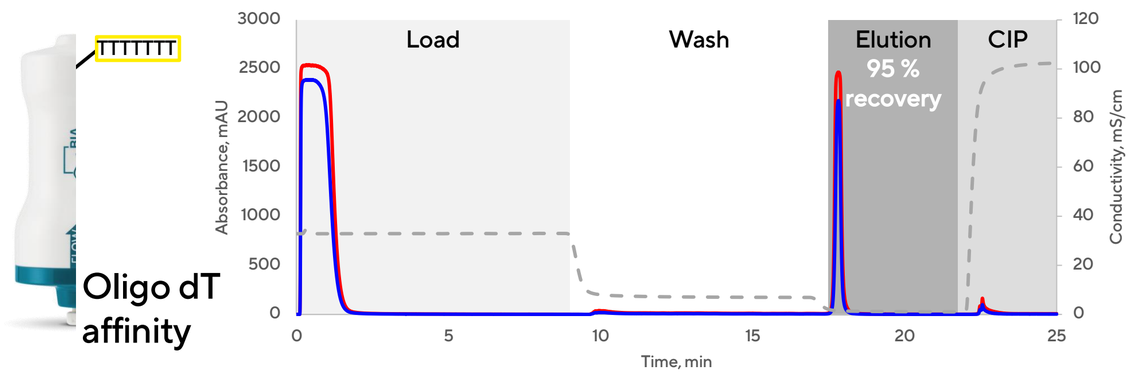
In vitro transcription reaction

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

Conclusions

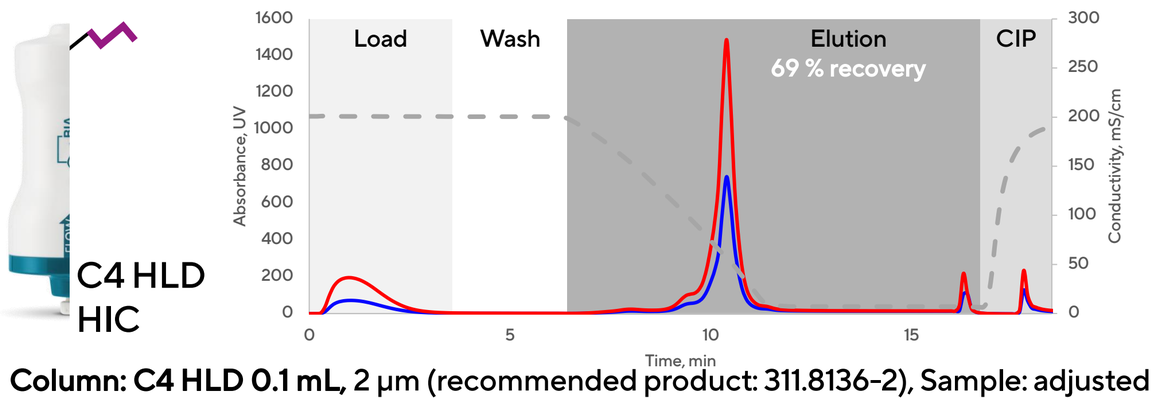
- High step recoveries for capture with Oligo dT (95%) and C4 HLD (69%)
- Purity and recovery scalable to manufacturing scale (> 16 g per cycle)
- CIMmultus™ Oligo dT, C4 HLD, PrimaS and SDVB allow for **streamlined mRNA purification** without precipitation – easier scale-up, higher yield.

Hybridisation affinity capture of mRNA from IVT reaction



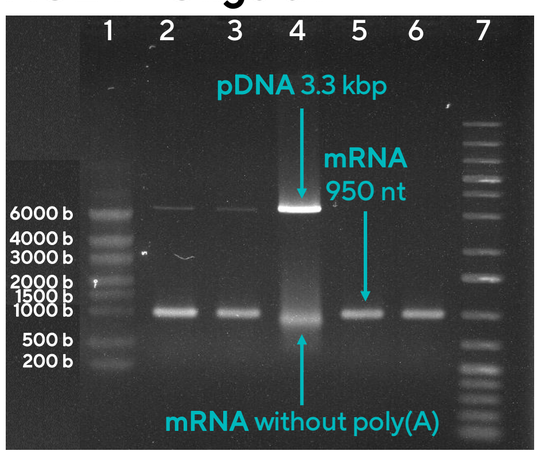
Column: Oligo dT18 0.1 mL, 2 µm (recommended product 311.1219-2), Sample: IVT reaction mixture, diluted in loading buffer, Buffers: sample dilution and load (50 mM Na-phosphate, 500 mM NaCl, pH 7.0), wash (50 mM Na-phosphate, pH 7.0), elution (10 mM Tris, pH 7.2), CIP (0.5 M NaOH). Step method, 1 mL/min, absorbance 260 nm (red), 280 nm (blue).

Hydrophobic (HIC) polishing with C4 HLD

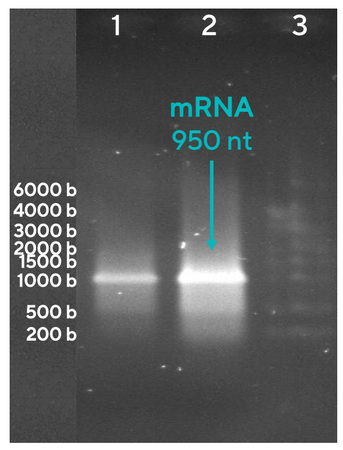


Column: C4 HLD 0.1 mL, 2 µm (recommended product: 311.8136-2), Sample: adjusted Oligo dT eluate post-capping, Buffers: mobile phase A (50 mM TRIS-HCl, 3 M NaCl, pH 7.2), mobile phase B (50 mM TRIS-HCl, pH 7.2). Method: 6min step 80% MPA, 5 min linear gradient from 80 % MPA to 0% MPA, 5 min step 0% MPB, absorbance 260 nm (red), 280 nm (blue).

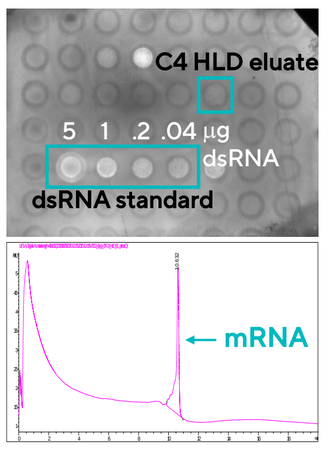
AGE 1 – Oligo dT



AGE 2 – C4 HLD



Dot-blot, CE



AGE 1: Lane 1: RiboRuler HR, Lane 2: IVTmix, Lane 3: Oligo dT load, Lane 4:Oligo dT Flow-through (50x conc), Lane 5: Oligo dT elution, Lane 6: Oligo dT elution buffer exchanged into MilliQ, Lane 7: Gene Ruler 1 kb Plus

AGE 2: Lane 1: C4 HLD eluate, Lane 2: C4 HLD eluate denatured at 80 °C, 5 min, Lane 3 RiboRuler HR.

Dot-blot: dsRNA standard (Magi2, Greentech, 1000 bp dsRNA) serial dilutions alongside C4 HLD eluate. dsRNA in sample below limit of detected (< 1% of dsRNA in sample), detection limit 40 ng .

Capillary electrophoresis (CE): C4 HLD eluate, buffer exchanged and denatured at 80 °C, 5 min. eCAP DNA capillary (Sciex), eff. l. 28 cm, Sample inj.: 2 kV, 10 s, electrophoresis: 7.8 kV, 20 min