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Increasing Dynamic Binding Capacity of Oligo dT Using CIM 96 Well Oligo dT Plates

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Affinity Purification of mRNA

Affinity-based chromatographic isolation of mRNA is robust and simple, lending itself as a useful industrial platform mRNA constructs typically contain a 3' polyA tail to increase stability in vivo, thereby affording the possibility of affinity purification using oligo deoxythymidinic acid (Oligo dT) probes covalently coupled to a solid support Poly adenylated mRNA forms a stable hybrid with Oligo dT under high-salt conditions which is destabilized when the salt is removed, allowing mRNA to be released (Figure 1).

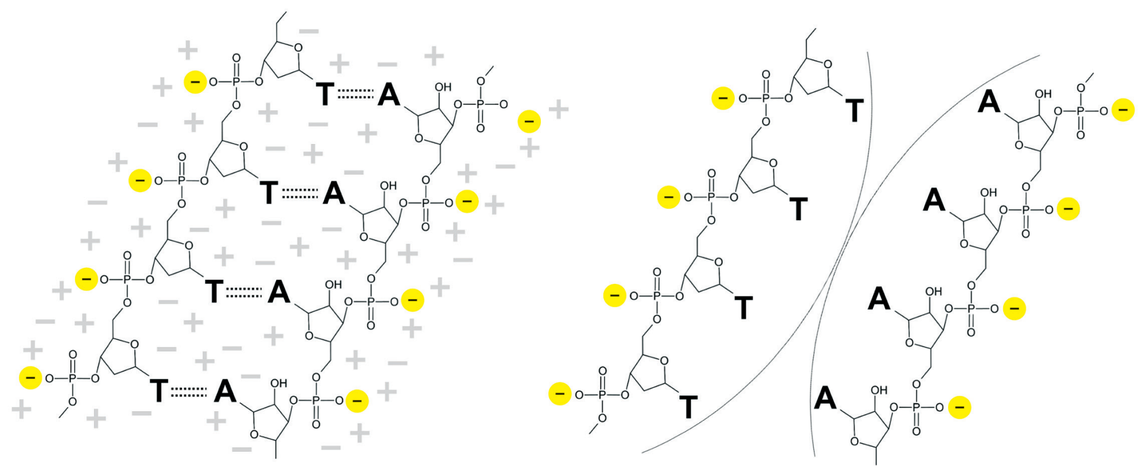

Figure 1: *Binding Interactions Between mRNA Molecules and Immobilized Oligo dT.*

Note: Left: High-salt conditions (binding), Right: Low-salt conditions (elution).

Typical dynamic binding capacity (DBC) of CIMmultus® Oligo dT for mRNA is 2-4 mg/mL ever higher IVT productivity will require higher binding capacities. Screening experiments to elucidate factors affecting CIMmultus® Oligo dT binding capacity for mRNA were performed in CIM® 96 well Oligo dT format (Figure 2). A simplified model identified NaCl, guanidine hydrochloride (Gu-HCl) and $MgCl_2$ concentration as the key factors contributing to DBC. Buffer chemistry, buffer pH, salt type and mRNA concentration had little or no effect on DBC.

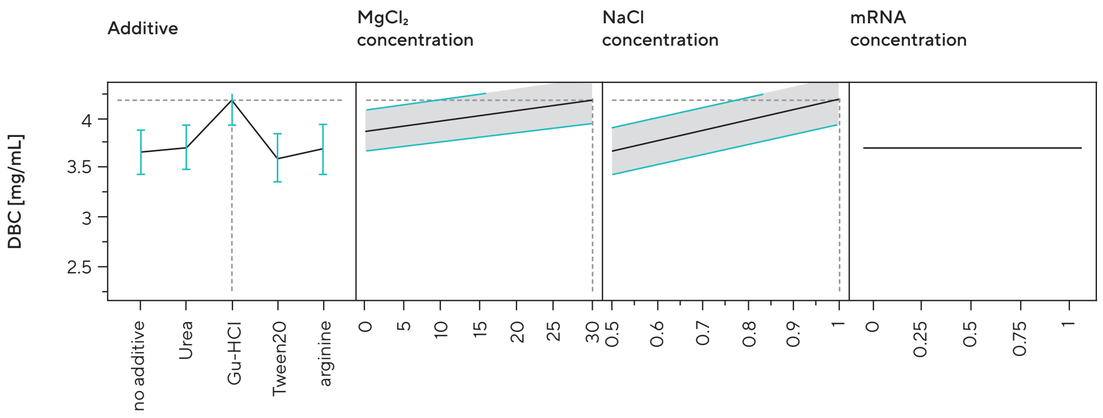

Figure 2: *Prediction Graphs of Factors With Significant Contribution to Oligo dT DBC.*

Note: Experimental results were fitted by a multiple regression model and refined with a backward selection approach with a p-value threshold of <0.05

Conclusions

- CIM 96 well Oligo dT plates were used for optimization of DBC
- The main contributing factors to DBC were identified as NaCl, Gu-HCl, $MgCl_2$.
  DBC of >6 mg/mL can be achieved with Gu-HCl
- Due to higher chaotropicity of Gu-HCl compared to NaCl, stronger binding is achieved by reducing the hydration shell around, and thus minimizing repulsive interactions between, mRNA and Oligo dT

Guanidinium, Mg^{2+} and NaCl Effects on DBC

Combinatorial effect of Gu-HCl/NaCl and $MgCl_2$ /NaCl was evaluated in 96-well format. Gu-HCl demonstrated higher impact than NaCl; DBC of 6 mg/mL was reached (Figure 3 a). Above 1 M Gu-HCl /1 M NaCl, precipitation of mRNA was observed. Effect of Mg^{2+} was also positive, though less pronounced, reaching DBC of 4.5 mg/mL (Figure 3 b). Gu-HCl alone was titrated as a loading salt, resulting in dose-response between 0.1-1 M Gu-HCl with DBC 6.4 mg/mL for 1 kb mRNA (Figure 3 c) and 3.8 mg/mL for 4 kb mRNA (Figure 3 d).

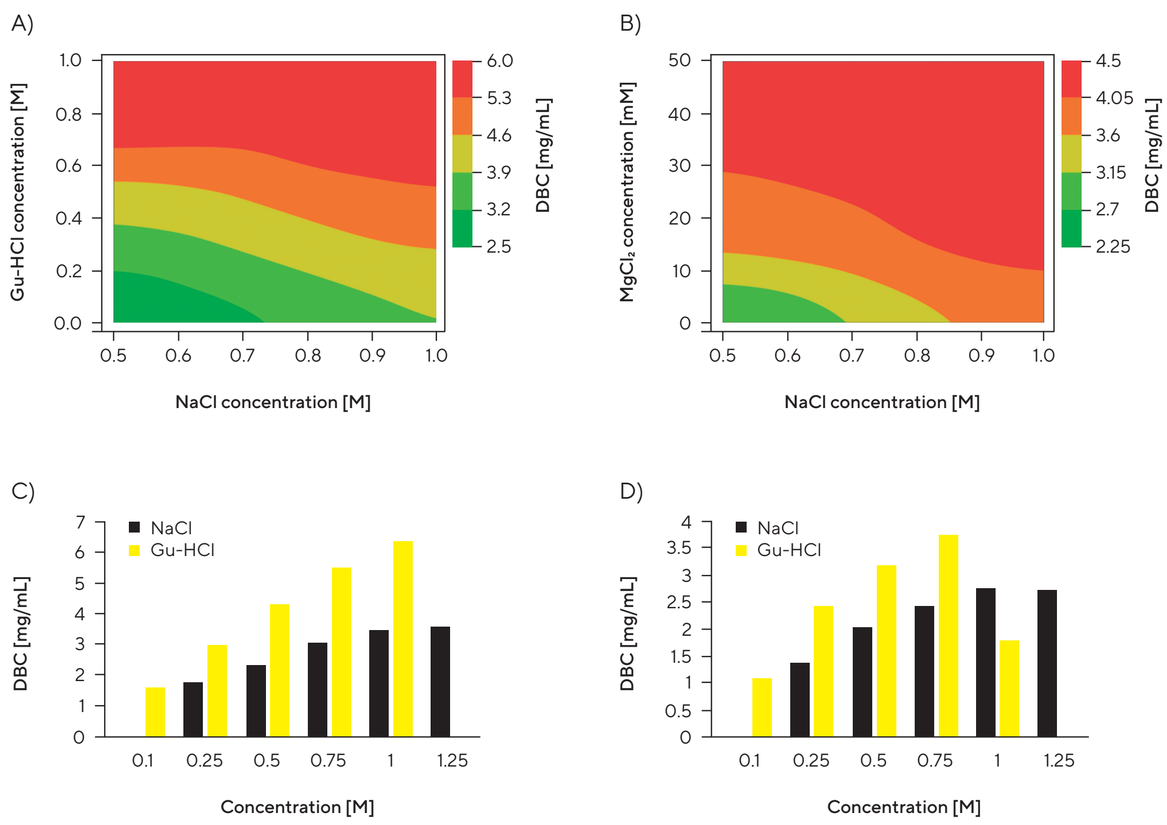

Figure 3: *Contour Plots of Oligo dT Dynamic Binding Capacity When mRNA Is Loaded In a) Gu-HCl/NaCl, b) $MgCl_2$/NaCl Combination. Titration of Gu-HCl as Loading Salt for Binding Of c) eGFP (1000 nt) or d) mRNA Encoding a Proprietary Sequence (4000 nt) to Oligo dT 96 Well Plates.*

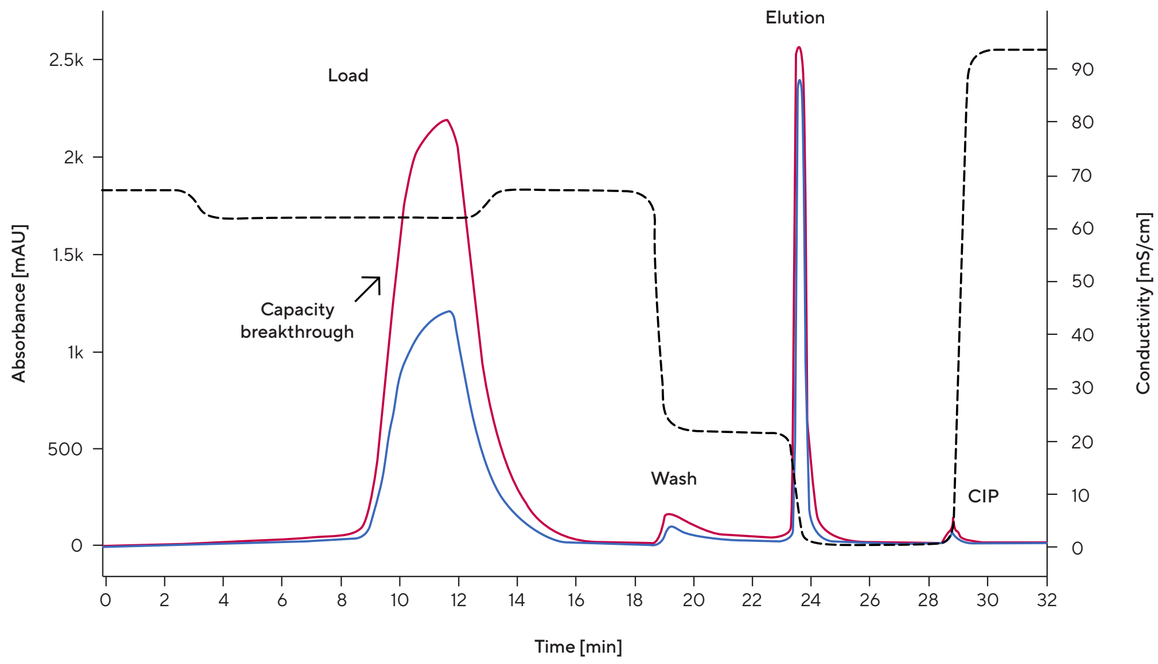

Figure 4: *Chromatogram of mRNA DBC Determination on CIM® Oligo dT (0.1 mL) Column in Presence of 0.75 M Gu-HCl. Red: UV 260 nm; Blue: UV 280 nm*

Effect of Gu-HCl was then transfered to a chromatographic separation mode using CIM® Oligo dT column (0.1 mL) confirming DBC of 5.5 mg/mL (Figure 4). A scale up to CIMmultus® Oligo dT (1 mL) column confirmed DBC of 5.5 mg/mL at 0.75 M Gu-HCl.