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Application Note

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Screening Conditions on a CIM® Oligo dT 96-Well Plate To Optimize Dynamic Binding Capacity

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Abstract

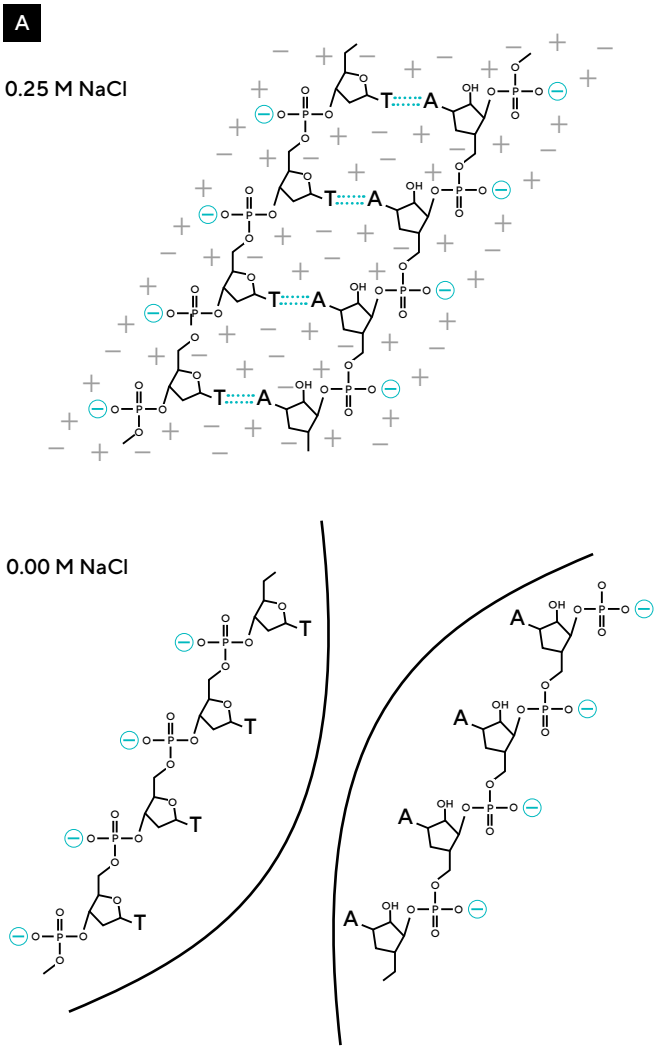
Affinity chromatography is an important method for purifying therapeutic mRNA. To keep up with the increasing efficiency of in vitro transcription reactions (IVT), affinity binding conditions must be optimized to deliver high yields and purities. In this application note, we show that multi-parallel approaches, such as screening in 96-well plate format, can significantly cut the development time by enabling the examination of multiple conditions simultaneously.

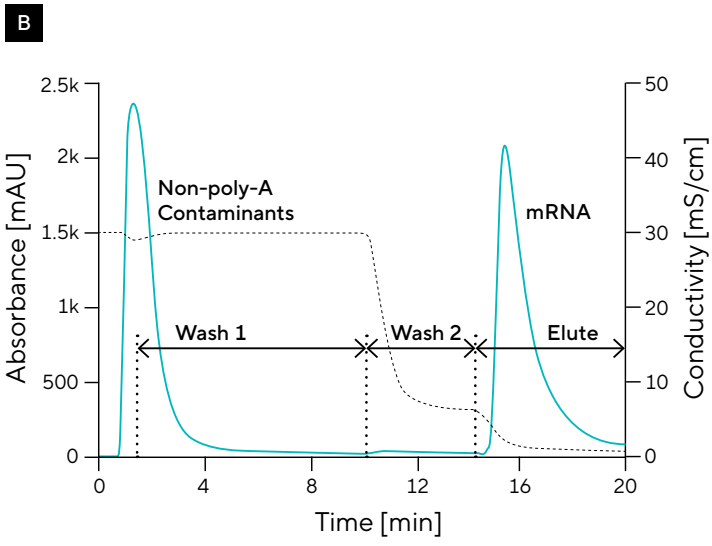
Introduction

Affinity-based chromatographic isolation of mRNA is robust and simple, lending itself as a valuable industrial platform. mRNA constructs contain a 3' polyadenylated (polyA) tail to increase stability in vivo, enabling affinity purification using oligo-deoxythymidinic acid (oligo dT) probes covalently coupled to a solid support. Under high-salt conditions, poly-adenylated mRNA forms a stable hybrid with oligo dT, which is destabilized when the salt is removed, releasing the mRNA (Figure 1).

Due to the increasing productivity of IVT reactions, finding conditions that increase the binding capacity of Oligo dT has been a strong focus of product and process development. Here, we show that multi-parallel approaches, such as screening in 96-well plate format, can significantly cut the development time by enabling the examination of multiple conditions simultaneously. 96-well plates can then be scaled up to preparative scale using tools such as the CIMmultus® product line operated by chromatographic skids.

Figure 1: *(A) Oligo-dT– mRNA Binding Interactions;*
(B) Hybridization-Affinity Chromatography of an In Vitro Transcription (IVT) Mixture on a CIMmultus® Oligo dT Column





Methods and Results

Screening Factors for Increased Dynamic Binding Capacity of Oligo dT Using 96-Well Plates

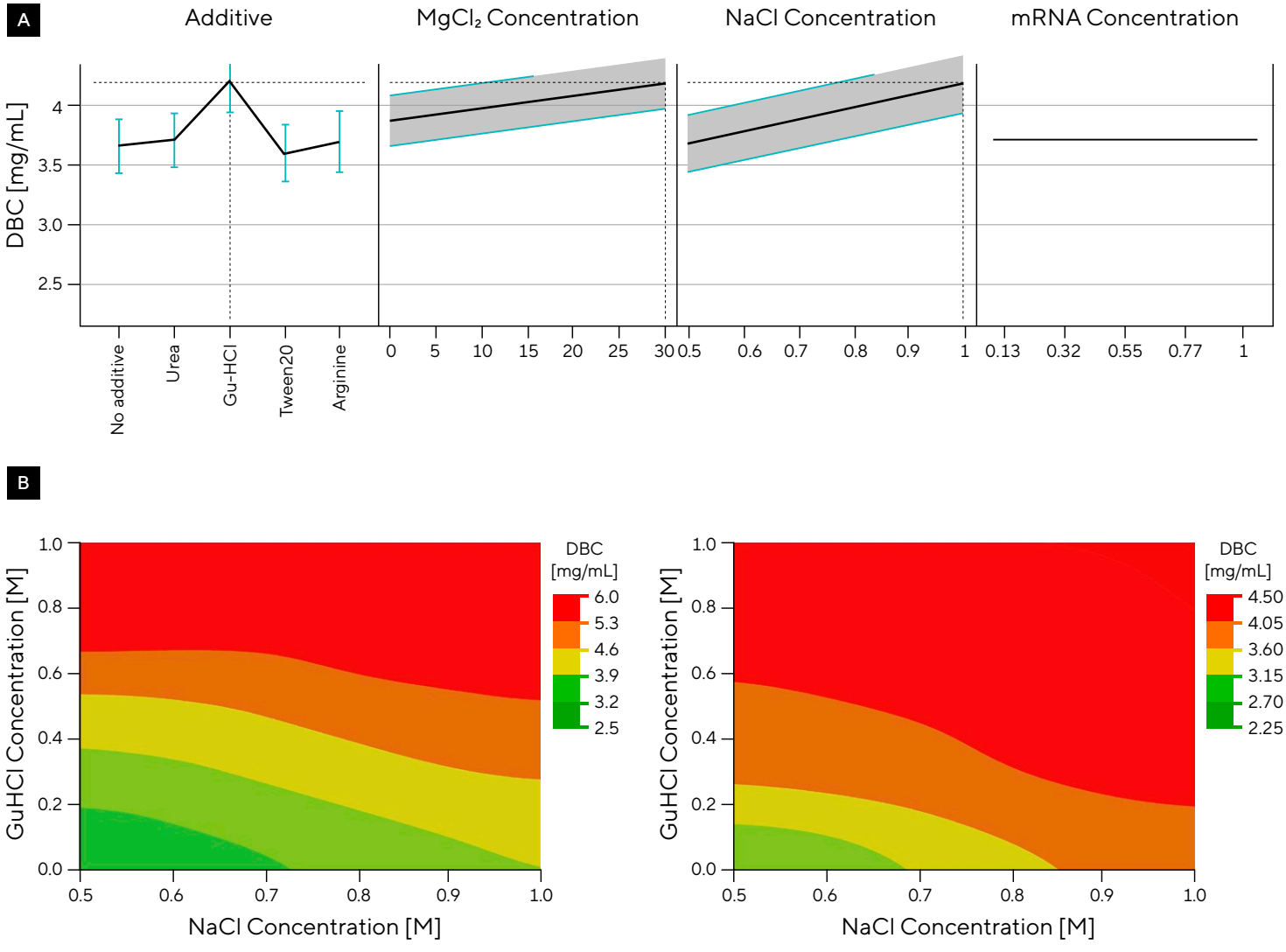
An mRNA construct coding for eGFP (950 nt) was used as a model system. Screening experiments were performed in 96-well format to identify factors that positively affect Oligo dT dynamic binding capacity (DBC). A selection of factors listed in Table 1 were tested in custom 2-level factor screening. Reliably identifying the main factors and some 2nd order interactions required us to perform 84 experiments with a pre-defined combination of listed factors. The CIM® 96-well Oligo dT plate was a suitable format for multiparallel testing of conditions.

Experimental results were fitted by a multiple regression model and refined with a backward selection approach with a p-value threshold of <0.05. A simplified model identified NaCl, guanidine hydrochloride (Gu-HCl), and $MgCl_2$ concentration as the key factors contributing to DBC (Figure 2A). Buffer chemistry, buffer pH, salt type, and mRNA concentration had little or no effect on Oligo dT DBC and were excluded from further evaluation. The effect of NaCl on the binding capacity of Oligo dT for mRNA is already described in the literature; the effect of Gu-HCl and $MgCl_2$ on DBC was greater than NaCl (Figure 2).

Table 1: *Factors Evaluated For Their Contribution to the Binding Capacity of Oligo dT Monolith Chromatography Support*

Factor	Variable
Buffer type	Tris, Phosphate
Buffer pH	7.2, 7.6, 8.0
Salt type	NaCl, KCl, CH_3COONa
Salt concentration	0.5 M, 0.75 M, 1 M
Additive	No additive, Urea, Gu-HCl, arginine, Tween-20
$MgCl_2$ concentration	0 mM, 15 mM, 30 mM
mRNA concentration	0.25 mg/mL, 0.5 mg/mL

Figure 2: *(A) Prediction Graphs of Factors With a Significant Contribution to Oligo dT DBC. (B) Contour Plots of Oligo-dT DBC When mRNA is Loaded in i) Gu-HCl/NaCl, ii) $MgCl_2$ | NaCl Combination.*



Guanidinium and Mg^{2+} Effects in Combination with NaCl on DBC

The combinatorial effects of Gu-HCl/NaCl and $MgCl_2$ | NaCl were then evaluated in 96-well format. In agreement with initial screening, using loading buffer containing Gu-HCl in combination with NaCl (in 50 mM Tris, pH 7.5) positively impacted DBC, with the effect of Gu-HCl being significantly more pronounced than the effect of NaCl (a DBC of 6 mg/mL was reached with multiple combinations of Gu-HCl | NaCl; Figure 2B). Above 1 M Gu-HCl | 1 M NaCl, precipitation of mRNA was observed. The effect of Mg^{2+} was also positive, though less pronounced (4.5 mg/mL DBC was reached for $MgCl_2$ / NaCl concentrations of 30 mM | 1 M, respectively; Figure 2B).

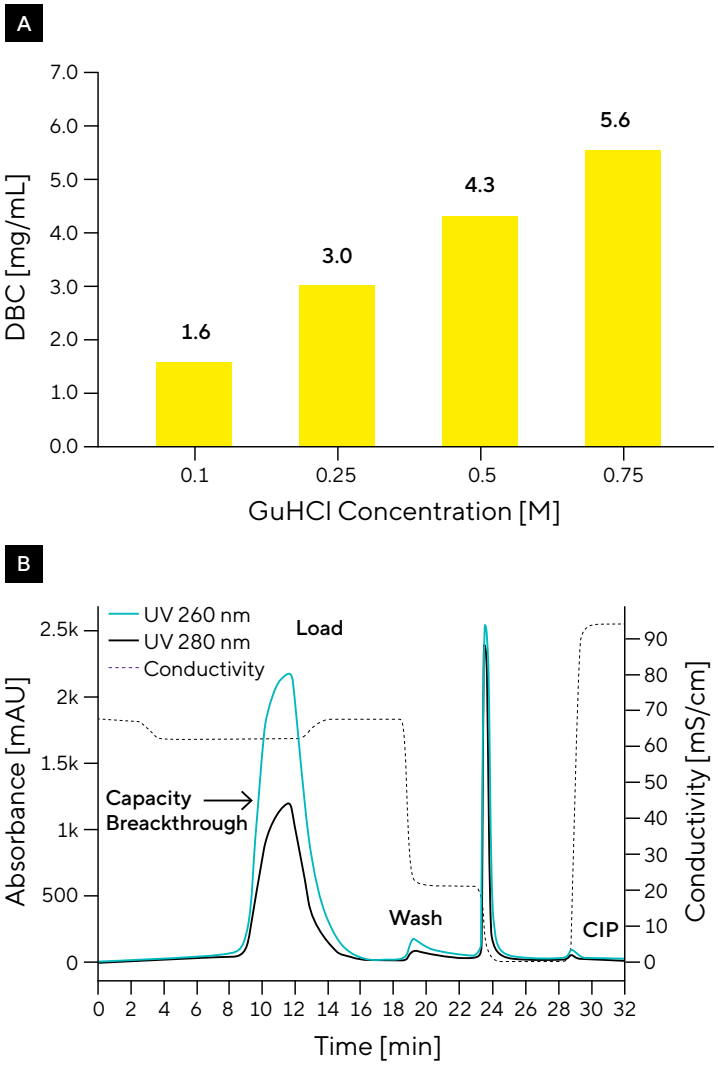
The contour plots of Gu-HCl | NaCl indicated a significantly more pronounced effect on DBC of Gu-HCl than NaCl. Since both act as chaotropic agents, reducing the hydration layer that mediates repulsive interactions between mRNA | Oligo dT negatively charged backbones, Gu-HCl alone was titrated as a loading salt. A dose-response was observed between 0.1 – 1 M Gu-HCl with 6.4 mg/mL DBC achieved when 1 M Gu-HCl was used as loading salt (Figure 3A).

The effect of Gu-HCl was also tested on a larger mRNA construct (4000 nt). A significant, though smaller, increase in DBC was observed when comparing 1 M NaCl and 0.75 M Gu-HCl as loading salts (2.9 mg/mL vs. 3.8 mg/mL, respectively). It should be noted that above 1 M concentration of Gu-HCl, precipitation of mRNA was observed when mRNA was present at 1 mg/mL; diluting mRNA to 0.25 mg/mL in loading buffer prevented precipitation.

From 96-Well Plate to Chromatography Column

We then investigated the effect of Gu-HCl on DBC in chromatographic separation mode using a 100 µL CIM® Oligo dT column (for recommended products, see Ordering Information). mRNA (800 µg) was diluted in binding buffer containing 0.75 M Gu-HCl to a final concentration of 0.16 µg/µL and loaded on the column at 5 CV/min. 550 µg was recovered in the elution fraction, corresponding to a DBC of 5.5 mg/mL (Figure 3B). The elution fraction demonstrated a DBC of 5.5 mg/mL, resulting in a recovery of 550 µg (Figure 3B).

Figure 3: *(A) Titration of Gu-HCl as a Loading Salt for Binding eGFP mRNA to Oligo-dT Column (96-Well Format); (B) Chromatogram of mRNA DBC determination on 100 µL CIM® Oligo dT Column in the Presence of 0.75 M Gu-HCl.*



Conclusion

CIM® Oligo dT18 0.05 mL Monolithic 96-well Plates were used for multi-parallel screening of binding conditions to the CIM® Oligo dT monolithic column. 96-well plates can significantly cut the development time by screening multiple conditions at once. They can be seamlessly scaled up to preparative scale (for example, the CIMmultus® product line).

NaCl, Gu-HCl, and $MgCl_2$ concentrations in the loading buffer were identified as the main contributing factors to DBC. The positive effect of NaCl on the binding of mRNA to Oligo dT is well known, whereas the effects of Gu-HCl have not been described before. Gu-HCl and Mg^{2+} can be used as additives to the NaCl-containing binding buffer. Gu-HCl can also be used instead of NaCl; it is presumed that due to its higher chaotropicity, Gu-HCl achieves stronger mRNA binding to the Oligo dT solid support. This effect is mediated by reducing the hydration shell around — and thus minimizing the repulsive interactions between — the mRNA and Oligo dT solid support.

However, it should be noted that there is a threshold of Gu-HCl concentration in combination with mRNA load concentration, beyond which precipitation may occur. A significantly higher DBC can be achieved if NaCl is replaced with Gu-HCl, with potential DBC values of over 6 mg/mL.

Ordering Information

Scaling to a different volume or format (for example, analytical or screening) is simple with CIM®. Contact your local support to find the appropriate products.

Products for Analytics and High-Throughput Screening

Catalog Number	Product Description
BIA-122.1219-2	CIM® Oligo dT18 0.05 mL Monolithic 96-well Plate (C12 Linker) (2 µm channels)
110.1219-2	CIMac™ Oligo dT18 0.1 mL Analytical Column (C12 Linker) (2 µm)

Products for Purification

Catalog Number	Product Description
311.1219-2	CIMmultus® Oligo dT18 – 1 mL (C12 Linker) (2 µm)
414.1219-2	CIMmultus® Oligo dT18 – 4 mL (C12 Linker) (2 µm)
411.1219-2	CIMmultus® Oligo dT18 – 8 mL (C12 Linker) (2 µm)
614.1219-2	CIMmultus® Oligo dT18 – 40 mL (C12 Linker) (2 µm)
611.1219-2	CIMmultus® Oligo dT18 – 80 mL (C12 Linker) (2 µm)
814.1219-2	CIMmultus® Oligo dT18 – 400 mL (C12 Linker) (2 µm)
811.1219-2	CIMmultus® Oligo dT18 – 800 mL (C12 Linker) (2 µm)
1014.1219-2	CIMmultus® Oligo dT18 – 4,000 mL (C12 Linker) (2 µm)
1011.1219-2	CIMmultus® Oligo dT18 – 8,000 mL (C12 Linker) (2 µm)

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