Optimizing downstream purification of high-quality plasmid DNA with POROS Chromatography Resins

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The demand for plasmid DNA (pDNA) has increased in recent years, but due to their physical properties there are some inherent challenges to the purification of these molecules. A typical downstream process for plasmids normally has multiple steps after fermentation, and anion exchange followed by hydrophobic interaction chromatography are commonly utilized. Thermo Fisher Scientific has developed a variety of resins well-suited for these steps, designed to simplify workflows and increase purity and yield. A series of experiments were conducted in order to evaluate POROS™ AEX resins for pDNA capture, with the goals of optimizing process conditions to maximize purity and recovery, determining the dynamic binding capacity (DBC) of POROS AEX resins for pDNA, and confirming optimal operating parameters. Some highlights of these studies, performed in collaboration with the Fraunhofer Institute for Molecular Biology, Germany, are presented here. POROS™ D50, HQ50 and XQ were selected and evaluated for plasmid capture applications.

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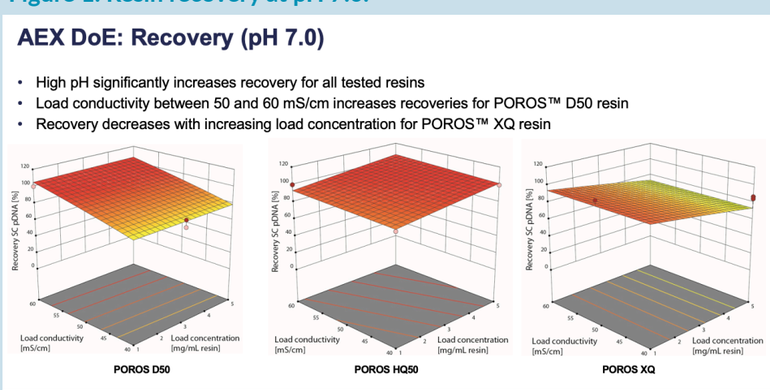
PH AND PURITY

Overall recovery at pH 7.0 was fairly high **(Figure 1)**. Notably, for the POROS™ HQ50 resin, the different parameters had little effect; in this case the load conductivity and load concentration. In contrast, for POROS™ D50, we found that with an increasing load conductivity the relative recovery of products increased. For POROS™ XQ, the recovery decreased with an increasing load concentration, i.e., with a higher quantity of plasmid loaded per volume of resin. Using a pH of 6, this initial behavior was amplified. POROS HQ50 again showed relatively stable behavior throughout the design space.

Purity for all three resins was in a good range – between 60 and 75% of total nucleic acid was supercoiled pDNA, and conditions were identified that gave close to 100% recovery for all resins.

Figure 1. Resin recovery at pH 7.0.



DYNAMIC BINDING CAPACITY

The DBC of the different resins is an important question to address, as this will ultimately dictate the process economics. The D50 resin provided the highest dynamic binding capacity **(Figure 2)**, and was therefore the best suited resin to verify our results using a scaled-up version of the experiment.

POROS D50 SCALED UP VERIFICATION

Using a scaled-up experimental procedure we verified that the binding capacity was more than 10 mg/mL **(Figure 3)**. In the gel at the bottom of Figure 3, it can be observed that in addition to the plasmid in the different salt elution steps there is a fraction of product that is eluting only once the cleaning procedure is applied (seen on the right side of the gel, in the lane labeled with CIP). Therefore, it is likely that optimizing the current elution conditions can increase the recovery.

Figure 2. POROS™ D50 dynamic binding capacity.

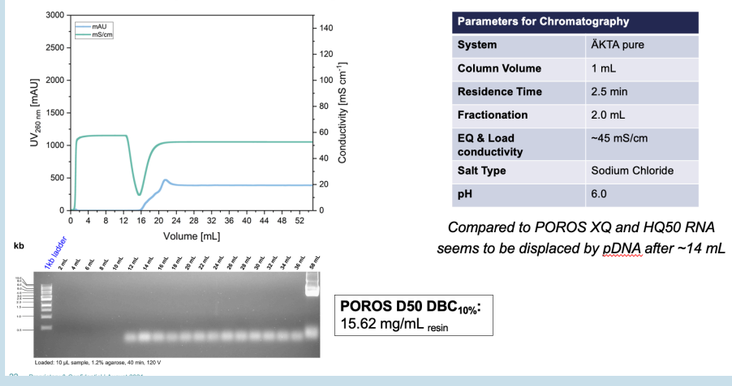
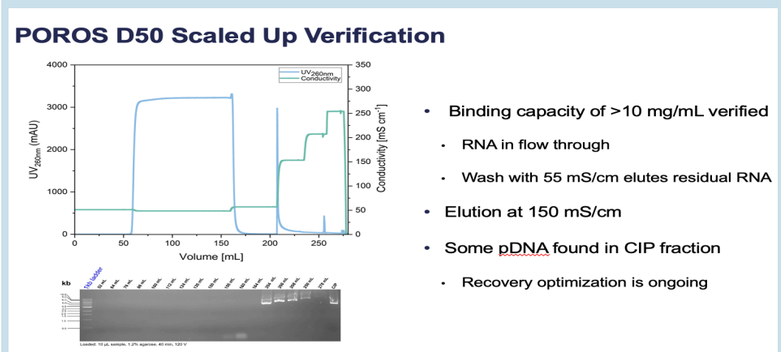


Figure 3. POROS™ D50 scaled up verification.



INSIGHTS & FUTURE DIRECTIONS

High binding capacity was obtained for all three resins, with POROS D50 demonstrating the best binding capacity. Residence time was 2.5 min, and increasing this may increase the binding capacity observed. Initial scale-up verification confirmed the high capacity, purity, and recovery for POROS D50, and work is ongoing to optimize the D50 capture step.

To explore the full study design & results, along with an author Q&A, watch the webinar or read the article

Read the full article here

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