PrimeMax™ siRNA CPG: Optimising surface area, loading, and pore architecture for enhanced synthesis yield and consistency

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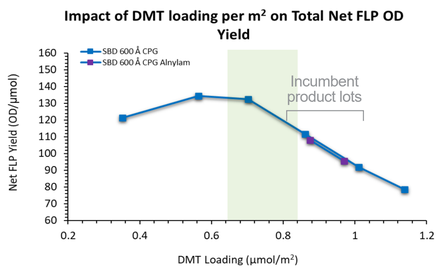
Introduction

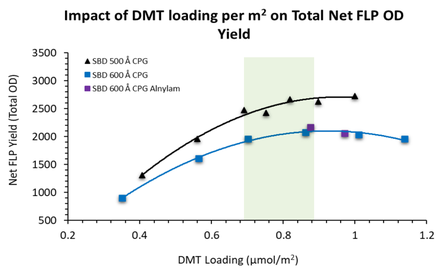
Controlled Pore Glass (CPG) remains a widely used support for solid-phase oligonucleotide synthesis of therapeutic oligonucleotides; however, performance variability linked loading density can negatively impact yields, and use of CPGs with wider pores than necessary can limit synthesis scales. The effects of CPG surface area and loading on synthesis yield and purity have been extensively studied, and the influence of pore size on oligonucleotide synthesis has been comprehensively characterised, as reported in our previous work. In this study, we present PrimeMax siRNA CPG, an engineered solid support designed to deliver consistent synthesis performance and predictable yield outcomes at maximum scales for siRNA manufacturing. PrimeMax incorporates three key advancements: (i) precise control of loading as a function of surface area rather than mass, enabling optimal utilisation of reactive sites without the detrimental effects of overloading; (ii) reduced pore diameter and CPG density, yielding an average ~50% increase in accessible surface area per column volume; and (iii) transition to trimethylsilyl (TMS) end-capped LCAA chemistry, which enhances stability and minimises potential side-reactions during synthesis. Together, these design features result in >15% improvement in efficiency (g/mmol), >20% increase in synthesis scale (mmol), and an overall productivity gain exceeding 40% in gram yield compared with conventional CPGs used for siRNA strand synthesis; without changing impurity profile. This work demonstrates how rational engineering of pore architecture and surface chemistry in CPG can directly translate into measurable gains in oligonucleotide manufacturing. By coupling enhanced efficiency with improved scalability, PrimeMax siRNA CPG establishes a new benchmark for consistency and improved productivity in therapeutic siRNA synthesis.

Background

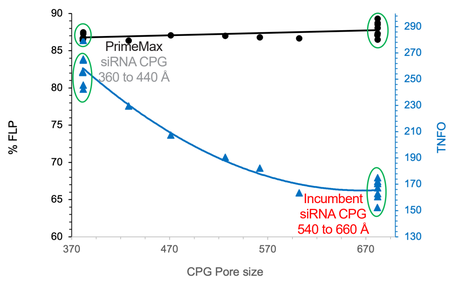
In previous work, we introduced Surface Area Normalised Loading (SANL, µmol/m²) and showed that above a critical threshold, crude yield and purity is reduced due to elevated early coupling failures,[1] and that current products are typically overloaded, sacrificing efficiency.



Optimal loading for maximum productivity is in the range of 0.85 mmol/m². New SANL specifications (green box) balance Efficiency and Productivity.



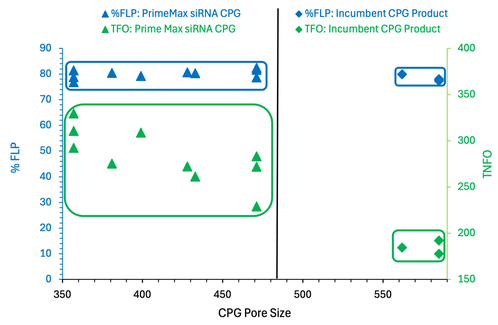
Reducing CPG pore diameter to ~400 Å preserves product purity while enabling higher loading capacity, displayed in a productivity gain of >40% in a 400 Å CPG vs Incumbent Product.



Comparison: Prime Max CPG vs incumbent product on purity for enhanced productivity

CND crude comparison of PrimeMax siRNA CPG vs incumbent product

CPG type	CPG pore size	DMT SANL (µmol/m²)	DMT loading (µmol/g)	SYN mmol scale	%FLP	Crude yield (OD/µmol)	Net FLP yield (OD/µmol) efficiency	Total net FLP OD (x10³) productivity
Incumbent CPG Product	562	0.80	83	1.73	79.8	134	107	185
Incumbent CPG Product	585	0.87	83	1.73	77.3	144	111	192
Incumbent CPG Product	585	0.91	80	1.66	78.1	137	107	178
PrimeMax siRNA CPG	357	0.63	113	2.18	81.5	165	134	292
PrimeMax siRNA CPG	357	0.70	125	2.41	78.7	164	129	311
PrimeMax siRNA CPG	357	0.79	141	2.71	76.8	158	121	329
PrimeMax siRNA CPG	471	0.64	87	1.67	82.4	166	137	229
PrimeMax siRNA CPG	471	0.73	100	1.93	81.7	173	141	272
PrimeMax siRNA CPG	471	0.79	108	2.08	78.7	173	136	283
PrimeMax siRNA CPG	381	0.69	102	2.05	80.4	167	134	275
PrimeMax siRNA CPG	428	0.68	104	2.01	80.6	168	135	272
PrimeMax siRNA CPG	433	0.74	108	2.00	80.4	163	131	261
PrimeMax siRNA CPG	399	0.72	113	2.35	79.2	166	131	309
Low Pore Size Neg Cntrl	308	0.68	128	2.66	75.5	170	128	341



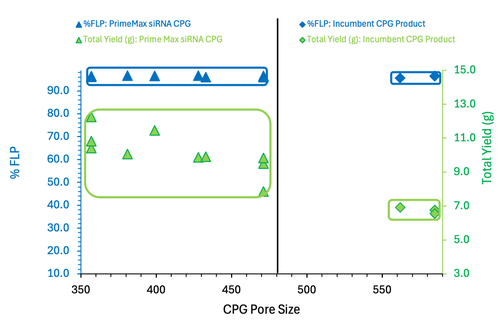
PrimeMax siRNA CPG demonstrated an average of >50% increase in Total Net FLP OD (TNFO) vs incumbent CPG and maintains product quality as evidenced by comparable crude %FLP purity and impurity profile in the (**Overlay 1**).

Negative Control CPG (308 Å) → %FLP decreases significantly, increase in late impurity observed.

Purified oligo comparison of PrimeMax siRNA CPG vs incumbent product

CPG type	CPG pore size	DMT SANL (µmol/m²)	CND crude % FLP	Mock-pool % FLP	Pre-FLP imp	Post-FLP imp	Real-pool OD (x10³)	Est yield (g/ mmol) efficiency	Total est. yield (g) productivity
Incumbent CPG Product	562	0.80	79.8	95.6	3.4	1.0	169	4.0	6.9
Incumbent CPG Product	585	0.87	77.3	96.3	2.7	0.9	166	3.9	6.8
Incumbent CPG Product	585	0.91	78.1	96.4	2.6	0.9	159	3.9	6.5
PrimeMax siRNA CPG	357	0.63	81.5	96.3	2.7	1.0	256	4.8	10.4
PrimeMax siRNA CPG	357	0.70	78.7	96.4	2.6	1.0	267	4.5	10.8
PrimeMax siRNA CPG	357	0.79	76.8	96.1	3.1	0.9	301	4.5	12.2
PrimeMax siRNA CPG	471	0.64	82.4	95.9	3.1	1.0	191	4.7	7.9
PrimeMax siRNA CPG	471	0.73	81.7	96.5	2.3	1.3	233	4.9	9.5
PrimeMax siRNA CPG	471	0.79	78.7	96.3	2.5	1.2	241	4.7	9.8
PrimeMax siRNA CPG	381	0.69	80.4	96.6	2.3	1.0	246	4.9	10.0
PrimeMax siRNA CPG	428	0.68	80.6	96.6	2.3	1.1	241	4.9	9.9
PrimeMax siRNA CPG	433	0.74	80.4	96.0	2.6	1.4	242	5.0	9.9
PrimeMax siRNA CPG	399	0.72	79.2	96.4	2.1	1.4	280	4.9	11.5
Low Pore Size Neg Cntrl	308	0.68	75.5	96.1	2.8	1.1	240	4.1	10.8

Yields reported table above are through purification. Yields through UF/DF and Lyophilisation are quite consistent and with >93% recovery, independent of solid support.

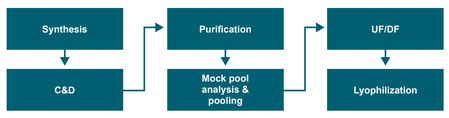


Productivity gains realised at the crude level were maintained through purification for oligos synthesised on PrimeMax siRNA CPG, with an average of >50% total yield improvement.

PrimeMax siRNA CPG maintains product quality as evidenced by comparable %FLP purity and impurity profile in final purified oligonucleotides (**Overlay 2**).

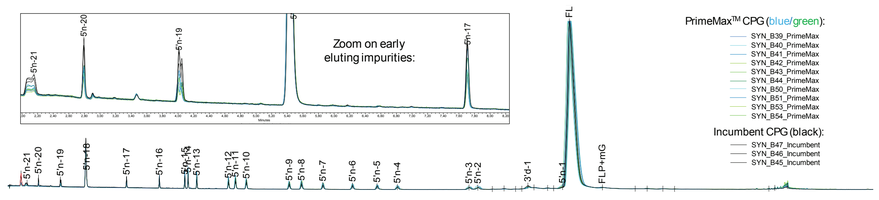
Experimental overview

- **Model sequence:** 23-mer siRNA antisense strand with 2'-OMe modified ribonucleotides.
- **Testing Workflow**



- **Synthesis setup**
  Instrument: Cytiva ÄKTA OP-100; **Column:** Cytiva FineLINE™ 35 (FL-35), packed to an 8.0 cm bed height; **Solid Support:** CPG with defined pore diameters ranging from 308 Å to 585 Å.
- **Analytical setup**
  Instrumentation: Waters Acquity UPLC I-Class, Xevo G2-XS QTof. **Column:** Waters Acquity Premier, Oligonucleotide BEH C18, 130 Å, 1.7 µm, 2.1×150 mm. **Buffers** (in H_2O); **A:** 1% HFIP, 0.2% TEA, 1% MeOH; **B:** 1% HFIP, 0.2% TEA, 40% MeOH, 5% MeCN.

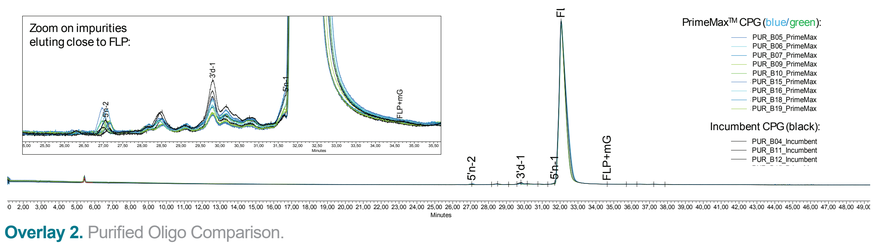
Analytical data and IPRP overlays



Overlay 1. CND crude Comparison.

Overlay 1 summary: No new impurities were observed in analysis of the crude oligos synthesised on PrimeMax siRNA CPG vs oligos synthesised on Incumbent CPG and overall impurity profile was highly consistent.

As observed in previous work, there is a notable increase in early coupling failures in oligos synthesised on Incumbent Product lots. We also observe higher levels of 3' deletions in incumbent product lot oligos.



Overlay 2. Purified Oligo Comparison.

Overlay 2 summary: Overall purity and impurity profile in the final purified oligos is equivalent for oligos synthesised on PrimeMax siRNA CPG and Incumbent CPG.

A slightly higher level of 3' deletions can be observed in oligos synthesised on incumbent CPGs. A subtle increase of 5' n^{-1} and 5' n^{-2} can be observed in the PrimeMax 357 Å CPG but average levels of these impurities is similar between purified oligos synthesised on PrimeMax and Incumbent CPGs.

Conclusion

- PrimeMax siRNA CPG demonstrates enhanced synthesis efficiency through control of surface loading and increased scale through optimised pore diameter.
- Taken together this results in a productivity gain of > 40% in PrimeMax 400 Å CPG vs Incumbent Product.
- Analytical Overlays confirms the consistency in purity and impurity profile of the final synthesised product.

References

1. Adhikari, S. P.; Ashizawa, K; Xiao S.; Sandoval E,; Schulz, M.; Dick, D.: Enhancing Yield through the Optimisation of Loading and Surface Area of CPG Solid Support. (*TIDES Boston, 2023*)
2. Adhikari, S. P.; Schoenherr, J.; Hayes, S.; Schacht, A.; Roland, A.; Meyer, R.; Renteria, A.; Choi, L.; Sandoval, E.; Hannah, D.; McKeen C.; Dick, D.: Assessing the Impact of CPG Pore Size and Loading on Yield and Purity in the Synthesis of a Model 23-mer Oligonucleotide. (*TIDES San Diego, 2025*)

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