Scalability and sustainability in mind for next generation Primer Support™ solid support

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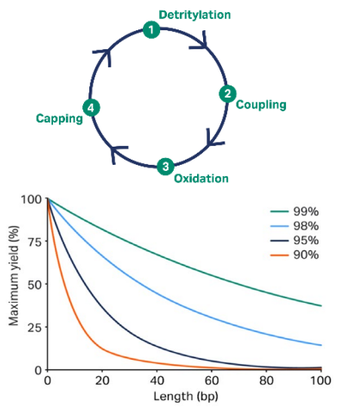
Introduction

The oligonucleotide therapeutics market is growing, and the pipeline is diverse. As this pipeline increases, scalability and productivity becomes critical to bring the life-changing oligonucleotide therapies to a global patient population. High-loaded, swellable solid supports are commonly used in synthesis of therapeutic oligonucleotides. The loading level of the swellable solid support is generally higher than alternative solid supports. The swelling between different steps of the synthesis cycle is however associated with (i) risk for high-pressure, (ii) limits on the amount of solid support that can be packed in a synthesis column, (iii) limits on having a high column-height-to-diameter ratio, and (iv) suboptimal flow distribution contributing to excessive solvent consumption. Considering these factors, Cytiva has explored different support prototypes that are optimized for synthesis of DNA and RNA oligonucleotides. These supports were observed to allow for improved synthesis scale in a given column volume without compromising on crude purity or synthesis yield compared to previous generations of our solid supports.

Solid phase synthesis



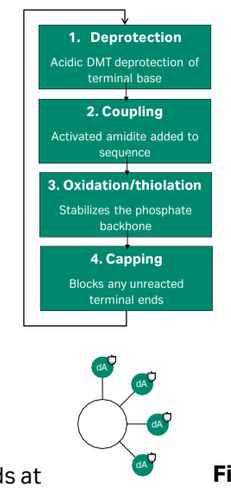


Fig 1. Synthesis cycle and theoretical yields at different coupling efficiencies.

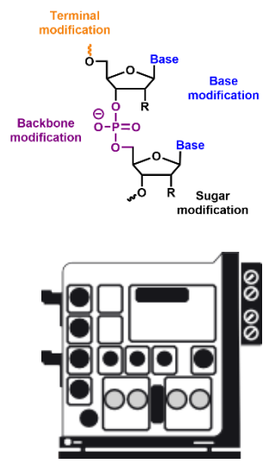


Fig 2. Chemical modifications sites and solid phase synthesizer ÄKTA oligosynt™.

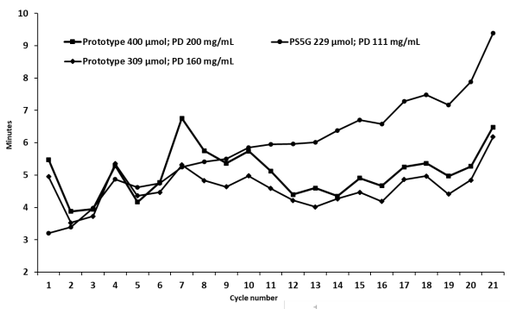
Solid support properties

Table 1. Characteristics of novel solid support

Particle size	30 to 80 µm
Packing density*	up to 220 mg/mL
Swelling in acetonitrile	similar
Swelling in toluene	
Degree of substitution†	~ 225 µmol/g
Recommended bed height‡	80 mm

* Recommendation is for a 21 mer; ~200 - 250 µmol/gm loaded support. The recommended packing density (PD) varies with the sequence length.
† Prototype dependent, possible to load up to ~400 µmol/g. Currently evaluated with 20 × 80 mm or 10 × 70 mm column dimensions.

Detritylation profiles



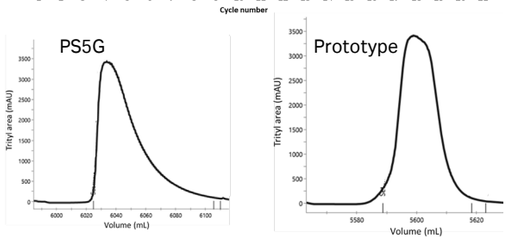
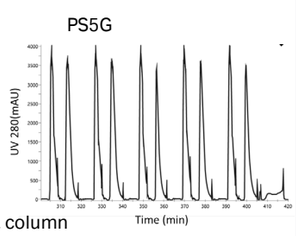


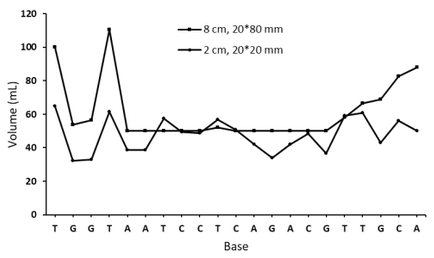
Fig 3. Packing density impact on detritylation time. Detritylation profile difference. Synthesis performed in 6.3 mL column.

Table 2. Detrit reagent consumptions

Scale, µmol	Detrit vol, mL	Support type	Reduction in solvent, %
230	122	Primer Support™ 5G (PS5G)	
239	75	Prototype	61
432	86	Prototype	70
309	74	Prototype	61

Data for 21 mer DNA sequence at base 20 in 6.3 mL column





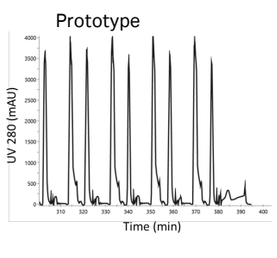


Fig 4. Bed heights' impact on detritylation consumptions (left). 190 mg/mL packing density, 225 µmol loaded dT support. Flow properties for the prototype bead make the detritylation peaks narrow (right). Synthesis performed in 6.3 mL column.

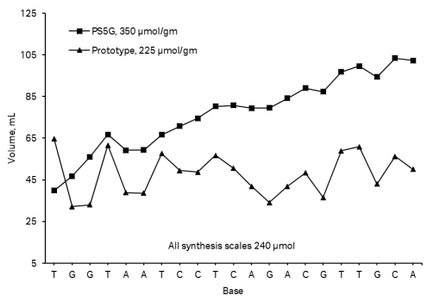


Fig 5. Different polymers comparison for detritylation consumptions.

- Quicker detritylation kinetics reduce (~ 60%) the consumptions of detrit solution (3% DCA in toluene) on prototype compared to the available solid supports.

Pressure flow properties

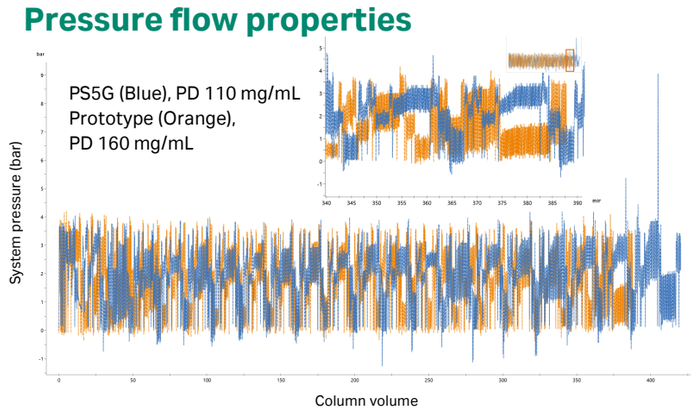
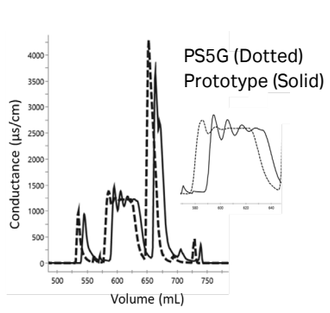


Fig 6. Pressure profile for synthesis of 21 mer DNA on prototype support in similar column dimensions; 20 × 20 mm. PS5G with 350 µmol loaded dT support, scale 240 µmol and prototype with 311 µmol loaded dT support, scale 311 µmol.



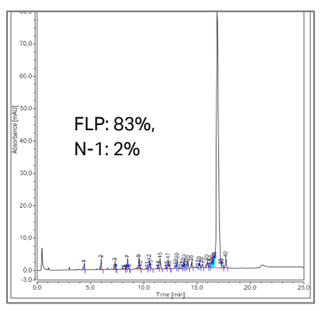


Fig 7. Reagent distribution within recirculation step of 21 mer DNA in similar column dimensions and representative HPLC* purity.

- Lower pressure for entire synthesis with extensively packed density bed and homogenous distribution over the column for each recirculation step.

*High-Performance Liquid Chromatography

Longer and sensitive oligonucleotide

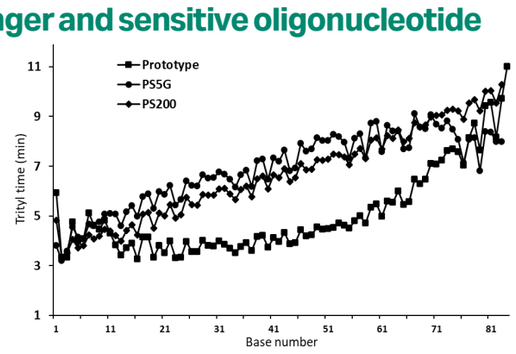


Fig 8. Comparison of detritylation time required for synthesis of 84 mer DNA. 204 µmol dT loaded prototype with PD of 79 mg/mL. 350 µmol dT loaded PS5G with PD of 50 mg/mL. PS200-Primer Support™ 200. 40 µmol dT loaded PS200 with PD of 200 mg/mL.

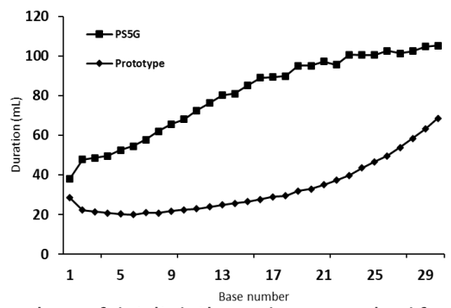


Fig 9. Comparison of detritylation volume required for synthesis of fully purine rich sequence. Prototype with PD of 190 mg/mL and PS5G with PD of 79 mg/mL.

- Detritylation reaction time and volume required is lowered on prototype solid support, which can prevent depurinations and impurity formation.

Amidite consumptions

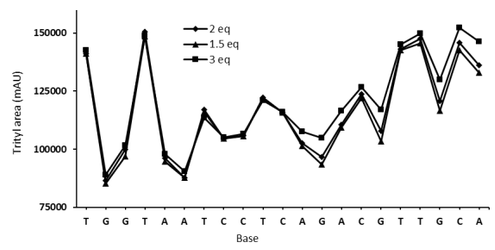


Fig 10. Detritylation profile for different amidite equivalence.

- Similar yields and purity of full-length product (FLP) .

Table 3. Yield and purity with varying amidite equivalence

Amidite eq.	Packing density, mg/mL	OD/µmol, AU260	FLP, %	Avg. coupling efficiency
1.5	208	136	83	99.1
2.0	190	145	86	99.3
3.0	190	147	87	99.3

21 mer DNA synthesis, UnyLinker loaded support.

Customer evaluations

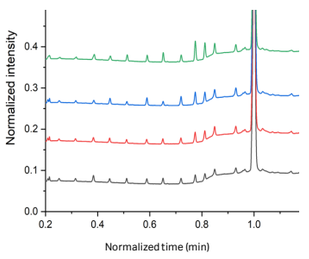


Fig 11. IPRP HPLC chromatogram of 23 mer modified RNA.*

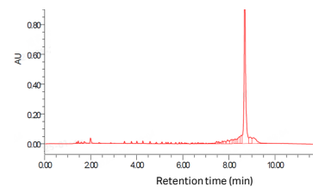


Fig 12. IPRP HPLC chromatogram of 81 mer modified DNA.*

Table 4. Yield and purity of RNA, 23 couplings with 2 eq. of amidite used for synthesis

Packing density, mg/mL	FLP, %	Avg. coupling efficiency, %
110	83	99.2
150	82	99.2
192	83	99.2
205	81	99.1

Table 5. Yield and purity of DNA, 80 couplings with 5 eq. of amidite used for synthesis

Packing density, mg/mL	FLP, %	Avg. coupling efficiency, %
70	60	99.4

*Syntheses with 10 × 70 mm column dimensions.

Yields and quality

Table 6. Yield and purity of different oligonucleotides synthesized using prototype solid support

	Bases, length	OD/µmol	OD/CV	FLP, %	N-1, %	Avg. coupling efficiency
PS5G	D13	88	3352	> 85	< 3	99.4
	D21[a]	130	4952	> 78	< 4	99.0
	D70[b]	337	6722	36	m	98.6
Prototype	D13*[c]	90	3540	90	2	99.2
	D21[c]	132	5600	78	2	98.8
	D21*[c]	127	6200	78	1	98.8
	D21†[c]	143	6200	82	1	99.1
	R23*[d]	158	5517	86	1	99.4
	D84[e]	333	5900	56	m	99.3
	R84[e]	258	4100	45	m	99.1

Packing density of [a]111 mg/mL CV, [b]62 mg/mL CV, [c]200 mg/mL CV, [d]Inclisiran AS, [e]75 mg/mL CV *UnyLinker loaded, † thiolated, D-DNA, R-RNA, m-multiple shortmers. Numbers in bases column denote length of oligonucleotide.

- Improved output (OD/CV) of synthesized oligonucleotide with similar or improved purity was observed with prototype solid supports.

Conclusions

- Higher synthesis scale in given column volume, up to 60%
- Similar swelling across synthesis cycle lower risk for high column pressure
- Reduced solvent consumption
- Quicker detritylation reduce risk for depurination
- Overall process economy and sustainability benefits

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