Primer Support 5G

OLIGONUCLEOTIDE PRODUCTION

Primer Support™ 5G is the fifth generation solid support for oligonucleotide synthesis from Cytiva. This high-loaded solid support is optimized for synthesis of DNA and RNA oligonucleotides with capability up to 70 bases. The base particle is composed of cross-linked polystyrene and delivers higher crude purity and synthesis yield compared to previous generations of our solid supports. These benefits are most evident for synthesis of RNA oligonucleotides. This support is available derivatized with DNA nucleosides, UnyLinker™, or RNA nucleosides. Primer Support 5G has been proven in scalable processes from research to industrial scale[1].

Key benefits:

- High loading capacity: A nucleoside loading of 350 µmol/g for DNA and 300 µmol/g for RNA can be used without compromising yield or purity. Suitable for synthesis of DNA and RNA oligonucleotides up to 70 bases. See the ***Synthesis of long oligonucleotides*** section.
- Cost efficient synthesis: With higher nucleoside loading, Primer Support 5G allows for major cost savings compared to our previous generations of solid supports
- Convenience and flexibility: UnyLinker can be used for any DNA starting base with any modifications that are compatible with the cleavage conditions (e.g., DNA or 2'OMe), hence enabling stocking of one support

Product characteristics

Primer Support 5G is derivatized with DNA nucleosides or UnyLinker at 350 µmol/g and RNA nucleosides at 300 µmol/g. The base matrix is made of 35–75 µm cross-linked polystyrene particles. The main product characteristics are summarized in Table 1.

Scalability

Primer Support 5G provides excellent scalability with proven consistency in purity and yield up to 1000 mmol synthesis scale[1].

[1] Data generated by Ionis Pharmaceuticals

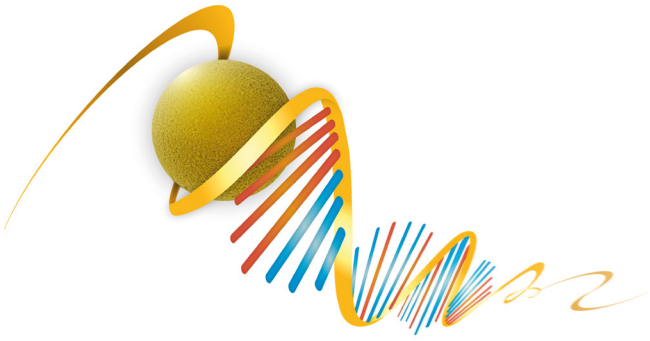


Fig 1. Primer Support 5G is a high-loaded solid support designed for oligonucleotide synthesis using phosphoramidite chemistry.

Table 1. Primer Support 5G characteristics

Matrix	porous and polydispersed divinylbenzene cross-linked polystyrene particles
Particle size	38–75 µm (400–200 mesh)
Recommended column packing volume[2]	111 mg/mL (9 mL/g)
Swelling in acetonitrile	3.5 mL/g
Swelling in toluene	7.5 mL/g
Storage	4°C to 30°C
Degree of nucleoside substitution	up to 400 µmol/g
Max recommended bed height	10 cm

[2] This recommendation is for a 20-mer. The recommended packing density varies with the sequence length; see ***Packing density of Primer Support 5G*** section for details.

Synthesis of DNA oligonucleotides

Loading optimization

Primer Support 5G allows high nucleoside loading with maintained performance in terms of yield, purity, N-1, and N+. Synthesis runs were performed on ÄKTA™ oligopilot™ plus 100 using Primer Support 5G with nucleoside loadings of 350 and 400 µmol/g. A 20-mer phosphodiester DNA oligonucleotide (5' ATA CCG ATT AAG CGA AGT TT 3') was synthesized and the synthesis parameters used are listed in Table 2 and summarized in Figure 2. The analytical data are based on ion exchange (IEX)-HPLC analysis.

Table 2. DNA oligonucleotide synthesis parameters

Amidite concentration	0.1–0.2 M in acetonitrile (ACN)
Amidite excess	1.5–2.0 equiv.
Coupling	3.0 min recirculation[1]
Activator	0.3 M BTT
Oxidation	2.0 equiv. 50 mM I_2Pyr/H_2O 9:1 (v/v)[2]
Ox CT	4.0 min[3]
Capping	0.5 Column volumes (CV), 0.5 min CT
Solid support	111 mg/mL CV
Column	6.3 mL
Oligonucleotide length	20

[1] Recommendation: 2.0–3.0 min
[2] Recommendation: 2.5 equiv.
[3] Recommendation: 1.0 min (up to 4.0 min has not shown a negative effect)

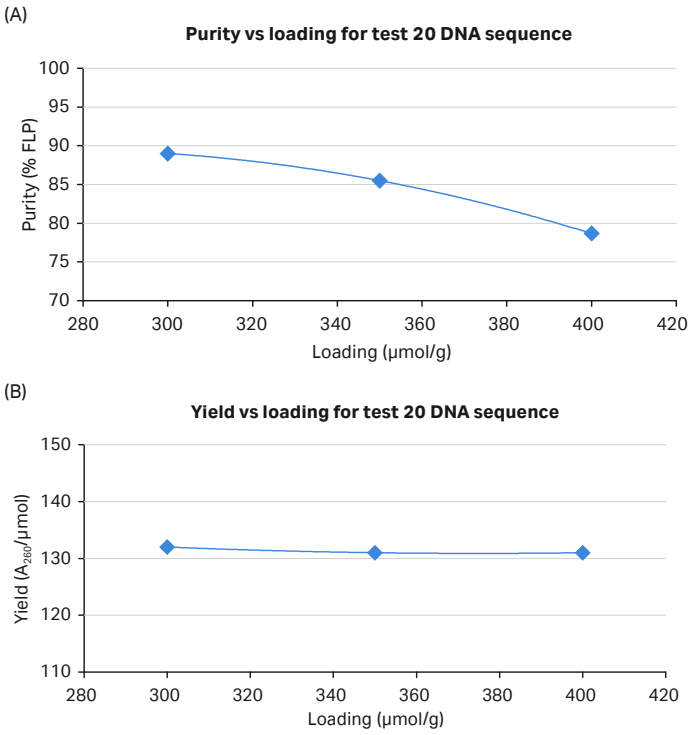


Fig 2. Synthesis of a 20-mer DNA oligonucleotide gives high yield and purity at high nucleoside loadings. Above 350 µmol/g a tendency of lowered purity was repeatedly observed. Plots showing (A) Purity vs loading and (B) Yield vs loading.

The results show that the synthesis performance of Primer Support 5G is maintained for nucleoside loadings up to 350 µmol/g with a consistent yield of 125 to 135 A_{260}/µmol and purity of 80% to 85%. The N-1 levels using Primer Support 5G, loaded at 350 µmol/g, were consistently below 2%. Based on the generated data, the preferred nucleoside loading is 350 µmol/g for synthesis of DNA oligonucleotides up to 25 bases.

Coupling optimization

Synthesis runs were carried out, using the preferred nucleoside loading of 350 µmol/g, to investigate the effect of different amounts of nucleotide monomer excess in the coupling reaction. Apart from the amidite excess, the same synthesis parameters were used as in the previous experiment (see Table 2). The results are shown in Figure 3.

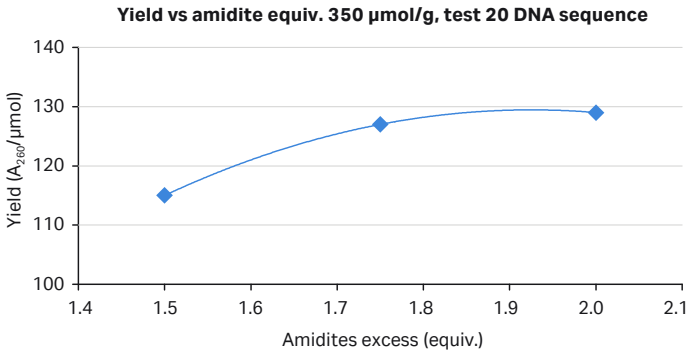


Fig 3. Synthesis of a 20-mer DNA oligonucleotide using differing amounts of monomer excess in the coupling reaction. Yield is improved up to 1.75 equiv. of amidite, without affecting purity, N-1, or N+. The plot shows yield vs amidite equiv.

The data demonstrate that between 1.5 and 2.0 equiv. of amidite, yield is improved up to 1.75 equiv. Full length purity was constantly ~85% within this range of amidite excess. Based on the data, preferred amidite excess is 1.75 equiv. for synthesis of DNA oligonucleotides. Figure 4 shows an IEX-HPLC chromatogram after synthesis of the 20-mer DNA oligonucleotide with a loading of 350 µmol/g and an excess of 1.75 equiv. of amidite during the coupling step.

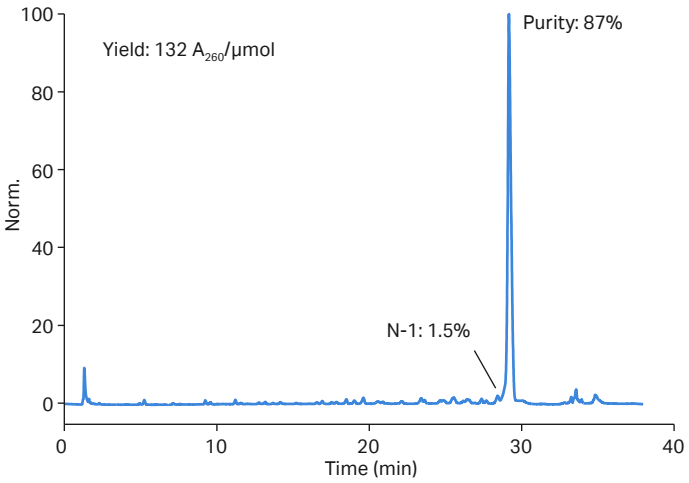


Fig 4. Chromatogram from IEX-HPLC analysis after synthesis of the 20-mer DNA oligonucleotide using Primer Support 5G with a loading of 350 µmol/g and an excess of 1.75 equiv. of amidite during the coupling step. Synthesis was performed in a 6.3 mL column.

Primer Support 5G UnyLinker

Primer Support 5G UnyLinker is a flexible alternative to standard nucleoside-derivatized solid-phase supports, because it can be used as a starting position for any first base in the sequence. Primer Support 5G UnyLinker is compatible with DNA, 2'OMe bases, and other amidites with modifications that can survive at least nine hours in ammonium hydroxide at 55°C, conditions required to cleave finished oligonucleotides from the support. Because of this requirement, UnyLinker is not suitable for use with tert-butyldimethylsilyl (TBDMS) RNA amidites.

Coupling conditions

Synthesis runs were performed on ÄKTA oligopilot plus 10 running UNICORN™ 5.31 software. Primer Support 5G was used with the preferred UnyLinker loading of 350 µmol/g. Four batches were evaluated. A standard UNICORN method was modified to extend detritylation by 2–3 min in the first cycle, double the coupling time in the first cycle, and double the capping time in the first cycle. Cleavage from the support was performed using ammonium hydroxide for 14–16 h at 55°C according to the amidite supplier's instructions. A 21-mer phosphodiester DNA oligonucleotide was synthesized using the parameters summarized in Table 3. The results are shown in Figure 5.

Table 3. DNA oligonucleotide synthesis parameters

Amidite concentration	0.1 M in acetonitrile (ACN)
Amidite excess	2.0 equiv.
Coupling	3.0 min recirculation
Activator	0.3 M BTT
Oxidation	4.0 equiv. 50 mM I_2 Pyr/$H_2$0 9:1 (v/v)
Ox CT	1.0 min
Capping	0.5 CV, 0.5 min CT
Solid support	111 mg/mL CV
Column	1.2 mL
Oligonucleotide length	21

The data in Figure 5 show the reproducibility of synthesis using the UnyLinker support.

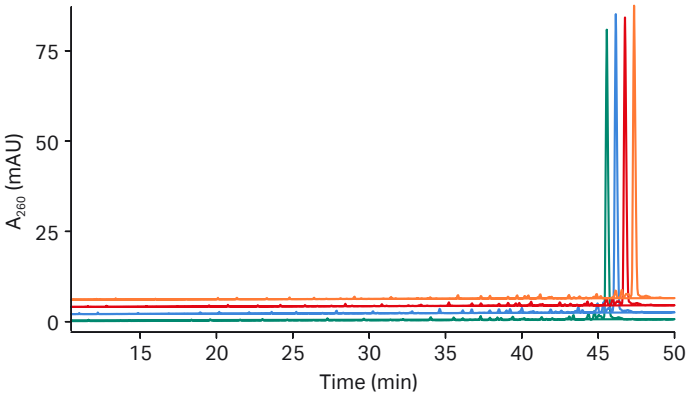


Fig 5. Chromatogram from IEX-HPLC analysis after synthesis of the 21-mer DNA oligonucleotide using Primer Support 5G with a UnyLinker loading of 350 µmol/g. Four batches of UnyLinker support were evaluated using the same synthesis parameters. Synthesis was performed on a 1.2 mL column.

Use of UNICORN software with UnyLinker

When using the UnyLinker support with Cytiva's oligonucleotide synthesizers, we recommend one or more of the following modifications to the standard UNICORN methods*:

1. Extend Detritylation by 2–3 minutes in the first cycle
2. Double Coupling in the first cycle
3. Double Capping in the first cycle

* Method templates for ÄKTA oligopilot plus 10 and 100 are available on cytiva.com/oligo under the documentation for the systems. Alternatively, search for product number 18114042 or 18113679.

Figure 6 shows an example of a modified UNICORN method.

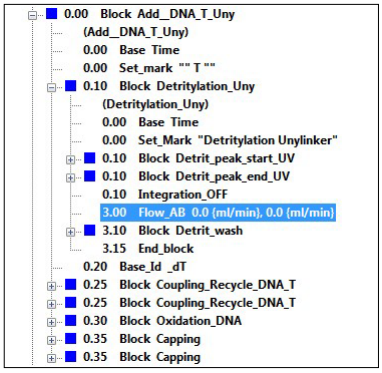


Fig 6. Standard UNICORN method with a coupling block and a capping block inserted. The Detritylation flow is stopped at 3.00 min instead of 0.5 min, so an extra 2:30 min Detritylation is added.

When using ÄKTA oligopilot plus 100 with Primer Support 5G UnyLinker, a strategy upgrade is required to handle the high loaded support. The strategy can be downloaded from our Life Sciences Web site by searching for code number 29135879. When using ÄKTA oligopilot plus 10, a UNICORN strategy upgrade is not necessary.

Synthesis of RNA oligonucleotides

Loading optimization

To maximize the nucleoside loading for synthesis of RNA oligonucleotides while maintaining performance in terms of yield and purity, a set of synthesis runs were done using Primer Support 5G with nucleoside loadings of 200, 300, and 350 µmol/g. The synthesis parameters are listed in Table 4. The sequence was a 21-mer phosphodiester RNA oligonucleotide (5′ acg uug cag acu ccu aau gg T 3′). The results are summarized in Figure 7. The analytical data are based on IEX-HPLC analysis.

Table 4. RNA oligonucleotide synthesis parameters

Amidite concentration	0.1–0.2 M in ACN
Amidite excess	1.5–3.5 equiv.
Coupling	12.0 min recirculation[1]
Activator	0.3 M BTT
Oxidation	4.0 equiv., 50 mM I_2 Pyr/H_2O 9:1 (v/v)[2]
Ox CT	1.0 min[3]
Capping	0.5 CV, 0.5 min CT
Solid support	111 mg/mL column volume
Column	6.3 mL
Oligonucleotide length	21
Deprotection	20 mL Methylamine in ethanol (33% wt)/25% NH_4OH (aq) 1:1, 55°C, 40 min Evaporate Add 5 mL DMSO Add 5 mL TEA 3HF (TREAT), 60°C to 65°C, 3 to 3.5 h

[1] Recommendation: 10.0–12.0 min

[2] Recommendation: 2.5–4.0 equiv.

[3] Recommendation: 1.0–4.0 min

As shown in Figure 7, the performance of Primer Support 5G is maintained for nucleoside loadings up to 300 µmol/g. The full length purity is consistently above 80% when the loading is up to 300 µmol/g. The yield is ~140 µmol/g even above this loading. The N-1 levels using Primer Support 5G, loaded at 300 µmol/g, were consistently below 3%. Based on the generated data, the preferred nucleoside loading is 300 µmol/g for synthesis of RNA oligonucleotides up to 25 bases.

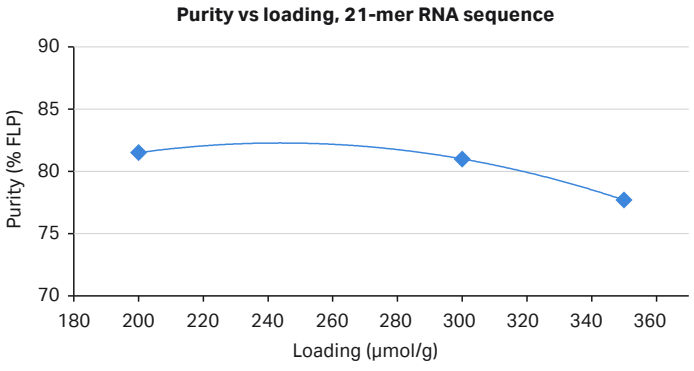


Fig 7. Synthesis of a 21-mer RNA oligonucleotide gives high yield and purity at high nucleoside loadings up to 300 µmol/g. The plot shows purity vs. loading.

Coupling optimization

Using the preferred nucleoside loading of 300 µmol/g, synthesis to investigate the effect of using different amounts of monomer excess in the coupling reaction were carried out. Apart from the amidite excess, the same synthesis parameters were used as in Table 4. The results of these evaluations are summarized in Figure 8.

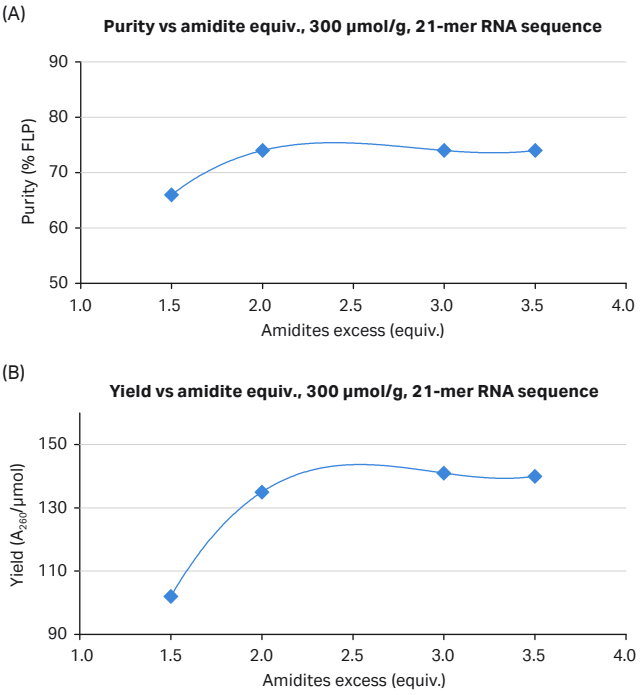


Fig 8. Synthesis of a 21-mer RNA oligonucleotide using differing amounts of monomer excess in the coupling reaction. Plots showing (A) Purity vs amidite equiv. and (B) Yield vs amidite equiv. for replicate experiments.

The generated data indicate that between 1.5 and 3.5 equiv. of amidite, yield and purity is improved up to 2.0 equiv. Based on the data, the preferred amidite excess is 2.0 equiv. for synthesis of RNA oligonucleotides (Fig 9).

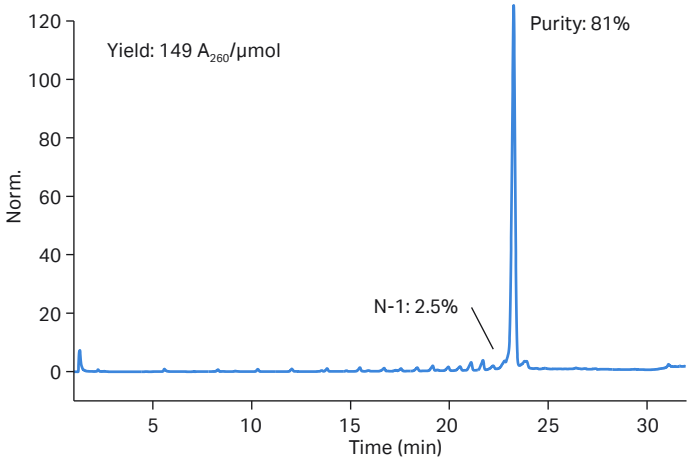


Fig 9. Chromatogram from IEX-HPLC analysis after synthesis of the 21-mer RNA oligonucleotide using Primer Support 5G with a loading of 300 µmol/g and an excess of 2.0 equiv. of amidite during the coupling step. Synthesis was performed in a 6.3 mL column.

Packing density of Primer Support 5G

Optimization of packing densities

To evaluate the effect of packing density using a nucleoside-derivatized support, we synthesized a model DNA oligonucleotide 13-mer. Five packing densities between 22 and 150 mg/mL CV were used. Oligonucleotides were synthesized on ÄKTA oligopilot plus 10 running UNICORN 5.31 software. The synthesis parameters are summarized in Table 5, and IEX-HPLC data are shown in Table 6 and Fig 10.

Table 5. DNA oligonucleotide synthesis parameters

Amidite concentration	0.1 M in acetonitrile (ACN)
Amidite excess	4.0 equiv.
Coupling	2.5 min recirculation
Activator	0.3 M BTT
Oxidation	4.0 equiv. 50 mM I_2 Pyr/$H_2$0 9:1 (v/v)
Ox CT	1.0 min
Capping	2.0 CV, 0.5 min CT
Solid support	22–150 mg/mL CV
Column	1.2 mL
Oligonucleotide length	13

As shown by the data in Table 6 and Figure 10, Primer Support 5G makes it possible to vary the packing density, and therefore the synthesis scale, in the same column from 135 to 50 mg/mL CV with similar yield per µmol and similar purity. For synthesis of a 13-mer we recommend a minimum packing density of 50 mg/mL, but we also tested two packing densities below 50 mg/mL. In this experiment 35 mg/mL gave just slightly lower yield and purity compared with 50 mg/mL (Table 6). Two runs with higher packing density than the recommendation are included. They show that there are high pressure alarms towards the end of the synthesis, but the results are still acceptable. Thus, it is possible to use slightly higher packing density if the synthesis parameters are modified.

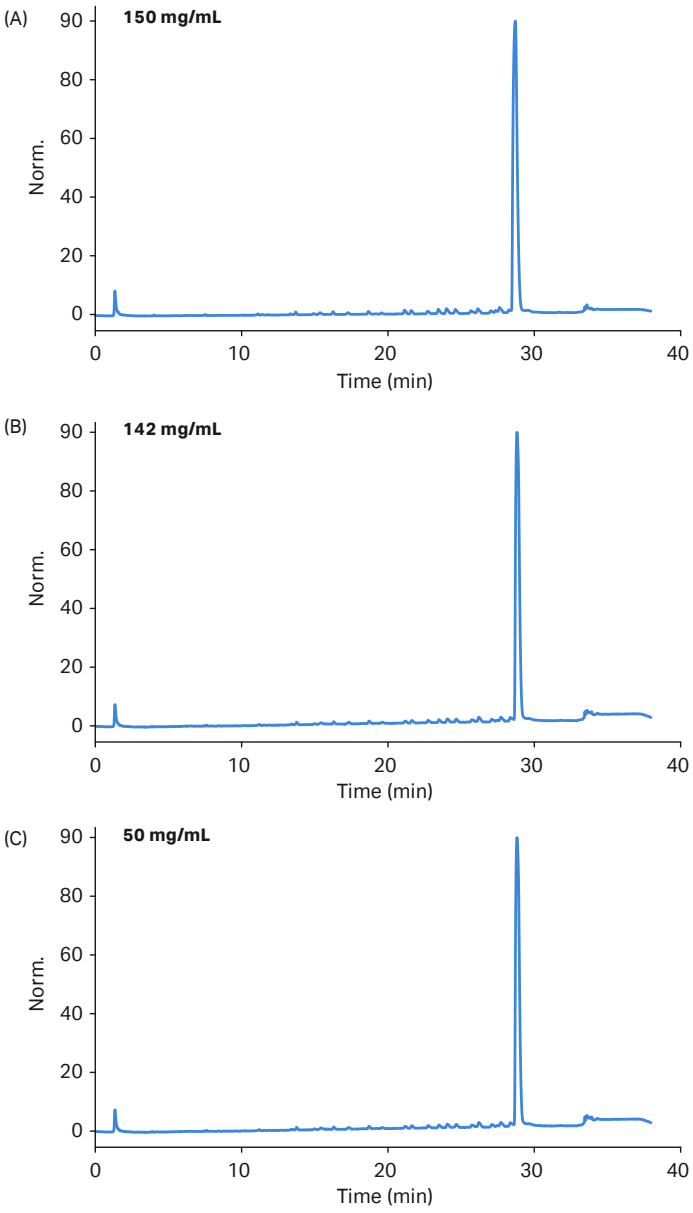


Fig 10. Chromatogram from IEX-HPLC analysis after synthesis of the 13-mer DNA oligonucleotide using Primer Support 5G with a loading of 300 µmol/g and a packing density of (A) 150, (B) 142, or (C) 50 mg/mL CV. Synthesis was performed in a 1.2 mL column.

Table 6. DNA oligonucleotide test-13 synthesis using different packing densities

Solid support (mg/mL CV)	Scale (µmol)	Yield (AU_{260}/µmol)	Total AU_{260}	Purity (% FLP)	Purity (% N-1)	Ave. coupling efficiency (%)
150*	63.5	88	5600	91	1.2	99.2
142†	60	92	5490	92	1.3	99.3
135	57	88	4950	94	1.3	99.4
50	21	89	1890	90	1.7	99.1
35	15	84	1246	87	2.1	98.8
22	10	73	675	91	1.5	99.2

* high pressure alarm in last 5 cycles in oxidation plus last 3 cycles in detritylation
† high pressure alarm in last 4 cycles, oxidation only

Synthesis of long oligonucleotides

Primer Support 5G T was used at 350 µmol/g to synthesize DNA oligonucleotides of five lengths: 30, 40, 50, 60, and 70 bases. The packing densities (Table 7) were adjusted based on recommendations for oligonucleotide length. See the ***Packing density for oligonucleotides > 30 bases*** section for more information. For synthesis of these long oligonucleotides a large excess of amidite (3.0-5.0 equiv.) and oxidation (4.0 equiv 50 mM I_2 Pyr/H_2O 9:1 [v/v]) was used to minimize the risk of coupling failure. However, an amidite excess of 2.0 equiv., oxidation of 2.5 equiv. 50 mM I_2 Pyr/H_2O 9:1 (v/v), and an Ox CT of 2.0 min should be sufficient for most syntheses.

The following analytical conditions were used for IEX-HPLC analysis of all synthesized oligonucleotides:

Buffer A:	10 mM Tris, 10 mM $NaClO_4$
Buffer A:	10 mM Tris, 300 mM $NaClO_4$
Gradient:	1%–70% in 40 min
Injection:	2 µL
Wavelength:	254 nm
Column:	Dionex NucleoPac PA100
Column temp:	35°C

The results* are summarized in Table 7 and Figure 11.

Table 7. Results of long DNA oligonucleotide syntheses

Length (bases)	Packing density (mg/mL CV)	Yield (AU_{260}/µmol)	FLP (%)	Ave. coupling efficiency (%)
30	95	198	73	98.9
40	83	255	61	98.7
50	72	303	57	98.8
60	67	362	44	98.6
70	62	337	36	98.6

FLP = full-length product

The results demonstrate that Primer Support 5G can be used successfully to synthesize oligonucleotides of at least 70 bases when the packing density is adjusted for oligonucleotide length.

* Note that results from analysis of oligonucleotides > 50 bases long are largely dependent on the injected volume, gradient, column temperature, and integration parameters used to calculate the baseline. Varying any of these parameters is likely to affect the results.

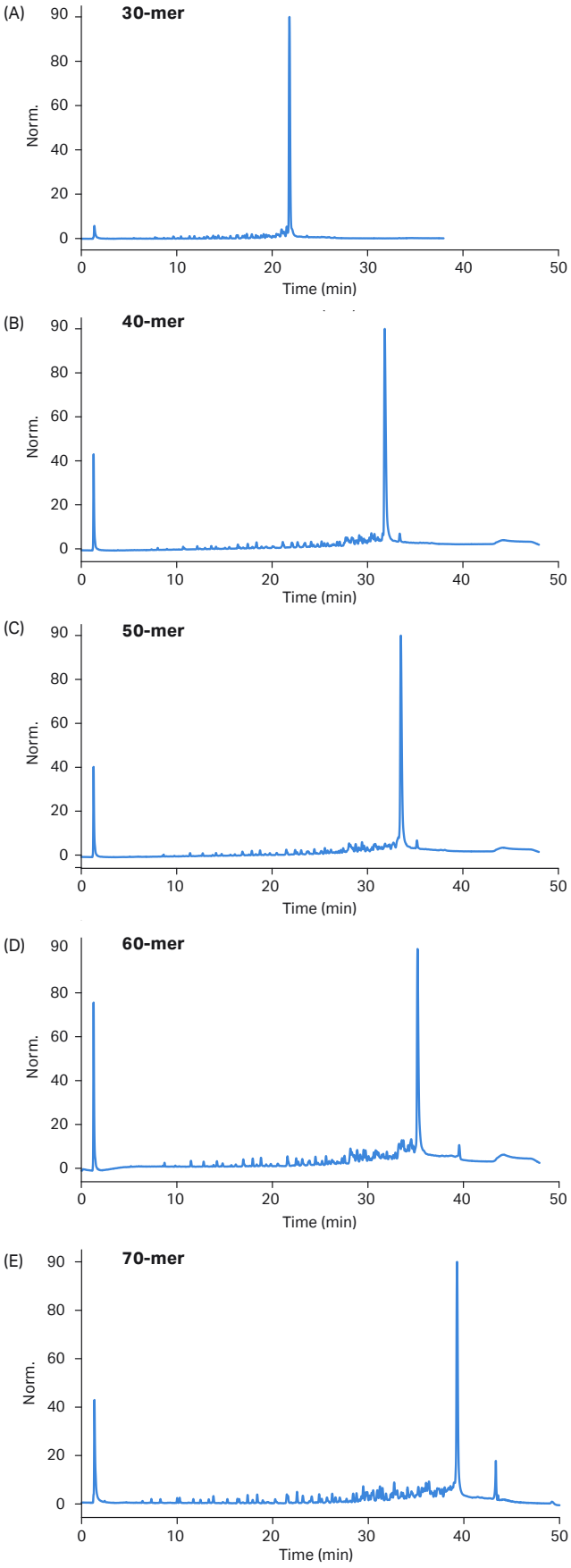
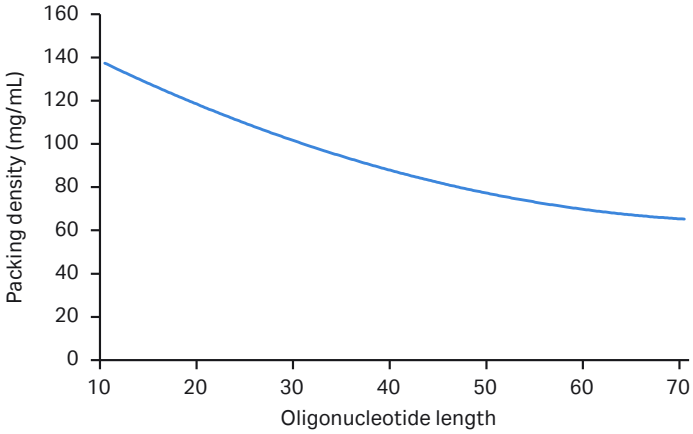


Fig 11. Chromatogram from IEX-HPLC analysis after synthesis of 30-, 40-, 50-, 60-, and 70-mer DNA oligonucleotides using Primer Support 5G and the packing densities listed in Table 7. Synthesis was performed in a 1.2 mL column.

Packing density for oligonucleotides > 30 bases

A modified packing density is an option that enables synthesis of long sequences using the same stocked solid support. Decreasing packing density as the sequence length increases helps to maintain pressure towards the end of the synthesis within acceptable levels. The recommendations in Figure 12 apply to both nucleoside- and UnyLinker-derivatized supports. Note that when synthesizing DNA or RNA oligonucleotides > 30 bases long, it is necessary to switch from 260 nm to a more sensitive UV1 wavelength to ensure efficient integration and UV watch steps. Typically, 500 nm is used.



Oligonucleotide length	13	20	30	40	50	60	70
Packing density (mg/mL CV)*	135	111	95	83	72	67	62

* Note: To synthesize RNA oligonucleotides > 25 bases, reduce the packing density by approximately 10%.

Fig 12. Packing density recommendations for Primer Support 5G based on oligonucleotide length.

Comparison of Primer Support 5G and Primer Support 40s for oligonucleotides > 30 bases

Synthesis runs were performed on ÄKTA oligopilot plus 10 using either Primer Support 5G T with nucleoside loading of 350 µmol/g or Primer Support 40s with nucleoside loading of 41 µmol/g. A 50-mer was synthesized using either support. Analysis was performed using IEX-UHPLC.

IEX-HPLC analysis results are provided in Figure 13. UHPLC was used instead of HPLC in order to improve the resolution between full-length product and shorter products.

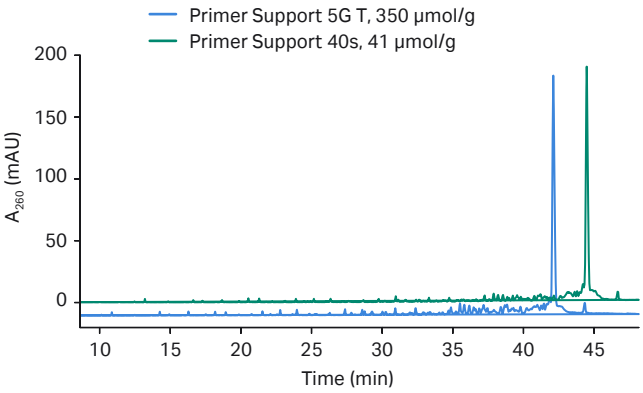


Fig 13. Chromatogram from IEX-UHPLC analysis after synthesis of a 50-mer DNA oligonucleotide using Primer Support 5G T, 350 µmol/g or Primer Support 40s, 41 µmol/g, Synthesis was performed in a 1.2 mL column.

Synthesis of 50-mer DNA oligonucleotides on both supports generated similar IEX-UHPLC profiles. The percentage of full-length product was 52% for Primer Support 5G and 54% for Primer Support 40s.

Total solution for production of therapeutic oligonucleotides

For over 25 years, Cytiva has been providing high quality solid supports for the process development and production of oligonucleotides. In addition to providing reliable solid supports, Cytiva has proven expertise in designing customized process-scale instrumentation for the synthesis of oligonucleotides from 10 mmol upwards. Our OligoProcess™ systems are custom-designed through careful planning with each production facility. Our well-established global distribution network allows material for your production facilities to be obtained world wide.

ÄKTA oligopilot plus 10 and 100, and OligoPilot 400 instruments have a proven reputation in process development and in validated manufacturing processes. Our experienced personnel have comprehensive knowledge and expertise of oligonucleotide production, regulatory aspects, and installation qualification/operational qualification (IQ/OQ).

Regulatory support

Cytiva has decades of experience working with biopharmaceutical manufacturers in validating their production processes. This expertise is also available for our oligonucleotide synthesis instruments for process development and production. In addition, we provide essential information for the registration of materials used for oligonucleotide production in the form of confidential Regulatory Support Files. For more detailed information, contact Fast Trak Validation Services or contact your local Cytiva representative.

Custom Primer Support

Cytiva offers Custom Primer Support to meet your exact needs by coupling with linkers, labels, modified bases, alternative protecting groups, or almost any molecule of your choice. Primer Support 5G can be loaded at 20 to 400 µmol/g and delivered in bulk or in prepacked, disposable Oligosynt™ columns. For more information, please contact your local sales representative or go to cytiva.com/oligo.

Ordering information

Products	Quantity	Product code
DNA350		
Primer Support 5G dA 350	1 mmol	28996425
Primer Support 5G dA 350	10 mmol	28996430
Primer Support 5G dC 350	1 mmol	28996426
Primer Support 5G dC 350	10 mmol	28996431
Primer Support 5G dG 350	1 mmol	28996427
Primer Support 5G dG 350	10 mmol	28996432
Primer Support 5G T 350	1 mmol	28996428
Primer Support 5G T 350	10 mmol	28996433
Primer Support 5G UnyLinker 350	1 mmol	29025962
Primer Support 5G UnyLinker 350	10 mmol	29025963

Products	Quantity	Product code
RNA 300		
Primer Support 5G ribo A 300	1 mmol	28996440
Primer Support 5G ribo A 300	10 mmol	28996444
Primer Support 5G ribo C 300	1 mmol	28996441
Primer Support 5G ribo C 300	10 mmol	28996445
Primer Support 5G ribo G 300	1 mmol	28996442
Primer Support 5G ribo G 300	10 mmol	28996446
Primer Support 5G ribo U 300	1 mmol	28996443
Primer Support 5G ribo U 300	10 mmol	28996447
Primer Support 5G T 300	1 mmol	29014619
Primer Support 5G T 300	10 mmol	29014620

Related products	
ÄKTA oligopilot plus 10	18114042
ÄKTA oligopilot plus 100	18113679
OligoPilot 400	28989965
OligoProcess	on request

Related literature	
ÄKTA oligopilot plus, Data file	18114466
Oligosynthesis product guide	18117275
Optimization and scaleup of siRNA synthesis	28405796

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