1-µmol synthesis of DNA using ÄKTA oligopilot plus 10

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The pilot scale synthesis instruments from GE Healthcare, ÄKTA™ oligopilot™ 10 and ÄKTA oligopilot 100 have been upgraded in regards to hardware, software, and synthesis methods. As a result of the upgrade, ÄKTA oligopilot plus 10 has become a highly efficient system for 1-µmol scale synthesis of DNA oligonucleotides.

Synthesis

DNA oligonucleotides of various lengths were synthesised using an ÄKTA oligopilot plus 10 systemt. The methods for 1 µmol scale synthesis of phosphodiester DNA oligonucleotides have been optimized and, relative to the original version of the ÄKTA oligopilot 10 system, significant improvements have been made in terms of reagent consumption, quality of the oligonucleotides synthesized, and cycle time. The reagents used for synthesis and deprotection are listed in Table 1 below.

Synthesis of oligonucleotides in the 1-µmol scale was carried out in disposable cassettes. Empty cassettes and appropriate filters are available from GE Healthcare (code number 18-1035-19). Best results have been obtained with cassettes packed with Custom Primer Support™ 40s, 25 mg/cassette. Prior to start of synthesis, the cassettes

Table 1. Reagents used for synthesis, cleavage and deprotection

Reagent	Composition/quality	Supplier	Code number
Solid support, 1g	Custom Primer Support dA 40s,	GE Healthcare	17-5214-37
Solid support, 1g	Custom Primer Support dC 40s,	GE Healthcare	17-5214-38
Solid support, 1g	Custom Primer Support dG 40s,	GE Healthcare	17-5214-39
Solid support, 1g	Custom Primer Support T 40s,	GE Healthcare	17-5214-40
Amidite dA^{bz}, 1g	0.1M, Standard phosphoroamidite, >98%	Pierce Milwaukee	27-1730-04
Amidite dC^{bz}, 1g	0.1M, Standard phosphoroamidite, >98%	Pierce Milwaukee	27-1732-04
Amidite dG^{ibu}, 1g	0.1M, Standard phosphoroamidite, >98%	Pierce Milwaukee	27-1734-04
Amidite T, 1g	0.1M, Standard phosphoroamidite, >98%	Pierce Milwaukee	27-1736-04
Acetonitrile, 2.5L	DNA synthesis grade	EMD Chemicals	AXO152/2505
Detritylation, 1L	3% DCA in toluene	EMD Chemicals	BIO832/1005
Activator, 1L	BTT (benzylthiotetrazole) 0.3 M, in ACN	EMD Chemicals	BIO166/1005
Capping A, 0.5L	20% NMI in ACN	EMD Chemicals	BIO224/0505
Capping B, 2 x 0.2L	20% Ac_2O, 30% 2,6-lutidine in ACN	EMD Chemicals	BIO347/0200 BIO349/0200
Oxidation, 1L	50 mM I_2 in pyridine/water 9:1	EMD Chemicals	BIO424/1005
Deprotection , 0.5L	20% diethylamine in ACN	EMD Chemicals	NC0017-0505
Cleavage & deprotection 2?	Concentrated ammonium hydroxide	EMD Chemicals	AX1196-1

were placed in column holders (code number 18-1142-91) connected to ÄKTA oligopilot plus 10. The reagent consumption for the optimized synthesis cycle for 1-µmol synthesis is shown in Table 2.

Table 2. Reagent consumption for 1-µmol synthesis on ÄKTA oligopilot plus 10

Reagent	Amount
Acetonitrile	11 ml
Detritylation	3 ml
Amidite	6.0 eq, 5 mg
Activator	0.3 ml
Oxidation	0.2 ml
Capping A	0.4 ml
Capping B	0.4 ml

The time for completion of one synthesis cycle is 4.5 min. This means that a 20 mer can be synthesized in less than 1.5 h.

Cleavage and base deprotection

After synthesis, the solid support (still in the cassette) was transferred to a microcentrifuge tube with the flange up. The tube was placed in a small table centrifuge and spun for about 1 min at medium speed (2000 rpm) to remove the acetonitrile inside the cassette. The cassette was then transferred to a screw-cap microcentrifuge tube, 1 ml concentrated ammonium hydroxide was added, and the ammonium hydroxide allowed to enter the cassette centrifuging for about 1 min at medium speed. The tube containing the cassette was then heated in an oven at 55°C to 60°C for 16 h (overnight) and then allowed to cool to room temperature. The cassette was then transferred to a new microcentrifuge tube and the cleavage solution still inside the cassette was collected at the bottom of the tube after centrifuging for about 1 min at medium speed. The cassette was removed from the tube, and the cleavage solution was combined with the solution in the original tube.

Yield determination

After cleavage and deprotection, the synthesis yields were determined by measuring the absorbance at 260 nm of an aliquot of the crude mixture diluted in water. In order to make the yields comparable for different synthesis scales, they are expressed as A_{260} units/µmol.

HPLC purity analysis

After cleavage and deprotection, the purity of the crude reaction mixtures were analyzed by ion exchange (IEX) HPLC using the conditions shown in Table 3.

Table 3. Conditions used for IEX HPLC analysis

HPLC system	Agilent 1100
Column	DNA Pac™ PA100
Injection volume	2µl
Sample concentration	20 to 30 A_{260} units/ml
Buffer A	1 mM Tris, 10 mM NaClO4,
Buffer B	1 mM Tris, 300 mM NaClO4
Flow rate	1000 µl/min
Gradient	1% to 55% B in 30 min for < 40-mers 1% to 70% B in 40 min for > 40-mers
Column temperature	50˚C

Results

With the procedures described above, very high synthesis efficiency is obtained. Average coupling efficiency is in the 99% to 99.5% range. Examples of HPLC analyses of crude material after synthesis and deprotection are shown opposite for a 20-mer (Fig 1) and an 80-mer (Fig 2).

20-mer phosphodiester

Yield: 155 OD/µmol
Purity: 86%
N-1: 2.8%

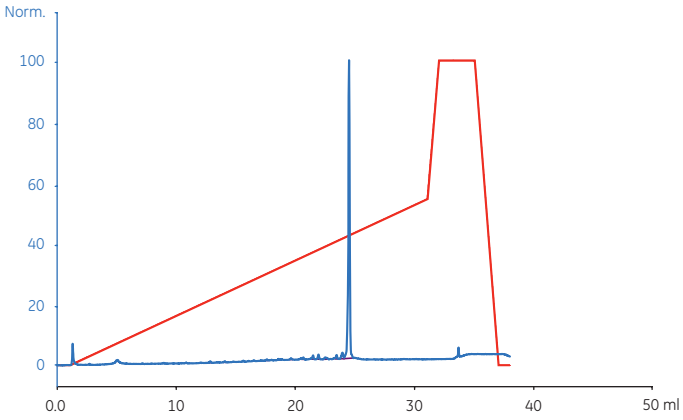

Fig 1. HPLC analysis of 20-mer phosphodiester.

80-mer phosphodiester

Yield: 618 OD/µmol
Purity: 55%
N-1: 3.5%

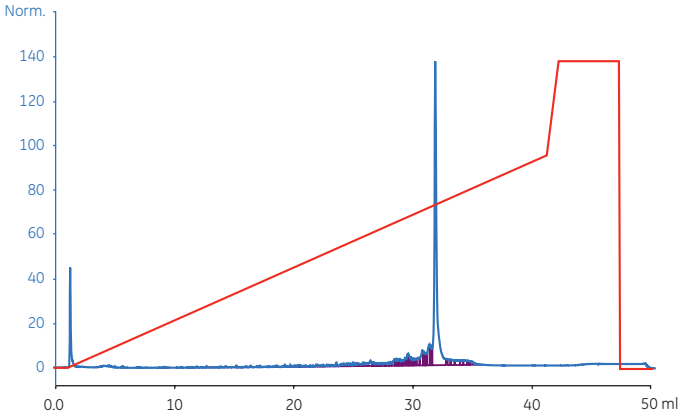

Fig 2. HPLC analysis of 80-mer phosphodiester.

Conclusions

The use of optimized synthesis methods for 1-µmol scale synthesis of DNA oligonucleotides in ÄKTA oligopilot plus 10 in combination with the use of Custom Primer Support 40s gives oligonucleotides of very high yield and purity.

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