1-µmol synthesis of RNA using ÄKTA oligopilot plus 10

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 RNA oligonucleotides

Synthesis

RNA oligonucleotides of various lengths were synthesised using an ÄKTA oligopilot plus 10 system. The methods for 1-µmol scale synthesis of phosphodiester RNA oligonucleotides have been optimized and, relative to the original version of 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 were placed in

Table 1. Reagents used for synthesis, cleavage and deprotection

Reagent	Composition/quality	Supplier	Code number
Solid support, 1g	Custom Primer Support ribo A 40s,	GE Healthcare	17-5225-17
Solid support, 1g	Custom Primer Support ribo C 40s,	GE Healthcare	17-5225-18
Solid support, 1g	Custom Primer Support ribo G 40s,	GE Healthcare	17-5225-19
Solid support, 1g	Custom Primer Support ribo U 40s,	GE Healthcare	17-5225-20
Amidite rA^{bz}, 1g	TheraPure® phosphoroamidite, 2'TBDMS	Pierce Milwaukee	27-1903-02
Amidite rC^{ac}, 1g	TheraPure® phosphoroamidite, 2'TBDMS	Pierce Milwaukee	27-1805-02
Amidite rG^{iso}, 1g	TheraPure® phosphoroamidite, 2'TBDMS	Pierce Milwaukee	27-1906-02
Amidite rU, 1g	TheraPure® phosphoroamidite, 2'TBDMS	Pierce Milwaukee	27-1904-02
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, 2x0.2LL	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	33% Methylamine in ethanol/conc ammonium hydroxide 1:1		
Desilylation	Triethylamine 3HF Dimethylsufoxiide		
Precipitation	n-butanol		

column holders (code number18-1142-91) 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	10 eq, 10 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 14 min. This means that a 20-mer can be synthesized in less than 4.5 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. A mixture of 33% methylamine in ethanol and 1 ml of ammonium hydroxide was added and the solution allowed to enter the cassette by centrifuging for about 1 min at medium speed. The tube containing the cassette was then heated in an oven at 60°C to 65°C for 2 h 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 centrifugation for about 1 min at medium speed. The cassette was then removed from the tube and the cleavage solution was combined with the solution in the original tube. The cleavage solution was evaporated to dryness in a speedvac.

Desilylation

The crude RNA oligonucleotide, still carrying the 2'-TBDMS groups was dissolved in 0.1 ml of DMSO. 1 ml of Triethylamine3HF (TREAT) was added, the tube was capped, and the mixture was shaken vigorously to ensure complete dissolution. The bottle was heated in an oven at 60°C to 65°C for 3 to 3.5 h. The tube was removed from the oven and cooled to room temperature.

Precipitation

The solution containing the completely desilylated oligonucleotide was cooled on dry ice. 2 ml of ice-cold n-butanol (-20°C), was carefully added in 0.5 ml portions to precipitate the oligonucleotide. The precipitate was filtered and washed with1 ml ice-cold n-butanol and the precipitate was then dissolved in 1M TEAA (triethylammonium acetate).

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 $NaClO_4$
Buffer B	1 mM Tris, 300 mM $NaClO_4$
Flow rate	1000 µl/min
Gradient	1% to 55% B in 30 min
Column temperature	50˚C

Results

With the procedures described above, very high synthesis efficiency is obtained. Average coupling efficiency is in the 98.5% to 99% range. Examples of HPLC analyses of crude material after synthesis and deprotection are shown below for a 21-mer (Fig 1).

21-mer phosphodiester

Yield: 122 OD/µmol
Purity: 71%
N-1: 3%

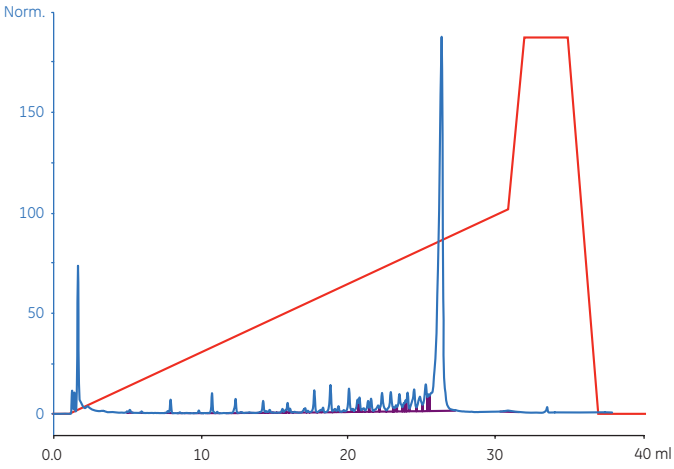

Fig 1. HPLC analysis of 21-mer phosphodiester.

Conclusions

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

Related literature

Application Note: Optimization and scale-up of siRNA synthesis, Code No 28-4057-96.

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28-4094-58 AA 04/2006