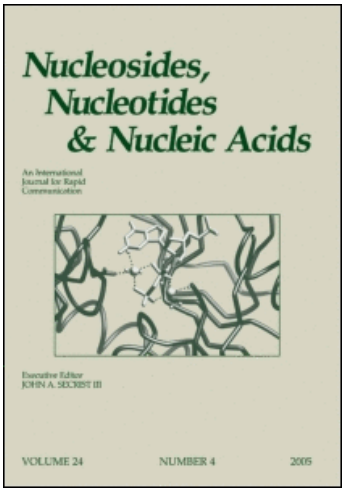

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Easily Available Column Device for Manual Oligonucleotide Synthesis on Solid Support by P(III) Methodology

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EASILY AVAILABLE COLUMN DEVICE FOR MANUAL OLIGONUCLEOTIDE SYNTHESIS ON SOLID SUPPORT BY P(III) METHODOLOGY

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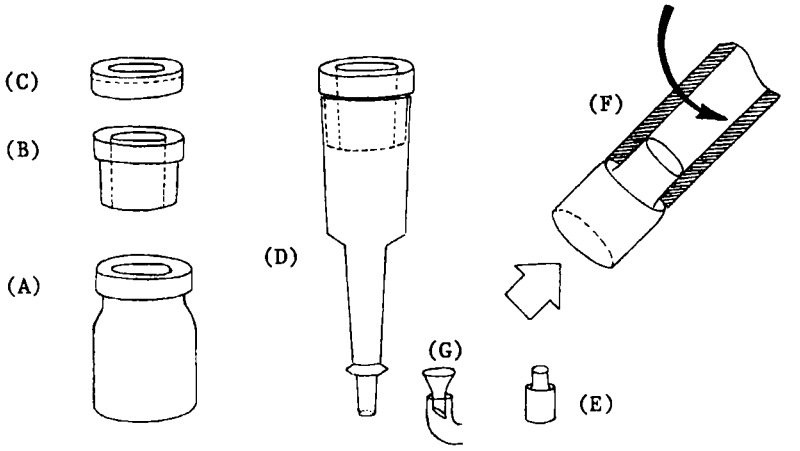
Abstract: New variant of the manual method of oligonucleotide synthesis by "in situ" P(III) methodology will be presented.

Manual techniques based on the application of syringes[1,2], filtration funnels[3,4] and other devices[5] have been used despite of remarkable progress in automation of oligonucleotide synthesis. Here we would like to present the manual method using commercially available polyethylene chromatography column (Bio-Rad) equipped with the frit which porosity allows for appropriate flow of solvents and reagents without use of pressure or suction.

Chemistry: the following protocol based on application of derivatized CPG support and "in situ" made deoxynucleoside-3'(2-cyanoethyl)N,N-diisopropylphosphoramidites[4,6] has been used for the synthesis of oligodeoxynucleotides up to 18 units long with coupling efficiency 93-96% as checked by detritylation. Scale 1.5 μmole.

1. detritylation: flow-3% DCA/methylene chloride, 150sec.(dA 100sec)
2. wash: flow-CH_2Cl_2 1min, 3% 2,6-lutidine/CH_2Cl_2 15sec, CH_2Cl_2 30sec,
3. drying: capped column, flow CH_3CN (10ml), argon passage (3min),
4. coupling: capped column and bottom closed, 0.2M "in situ" made amidites (15 eqv.) followed by 0.5M tetrazole in acetonitrile(60 eqv.); rotating 7 min.
5. wash: flow- CH_3CN 30sec,
6. capping: 0.1ml Ac_2O followed by 6% w/v DMAP in 2,6-lutidine/THF 2:8 v/v, 2min.
7. wash: flow- CH_3CN (not predried) 30sec,
8. oxidation: stop-flow: 0.1M iodine in THF/2,6-lutidine/H_2O 8:4:0.2,1min
9. wash: flow- CH_3CN 1min, CH_2Cl_2 30 sec.



Technique: Amidites were prepared in sealed bottles (A) and injected to the column containing support through "press-in" teflon cap (B) equipped with pressed-on membrane coated aluminium seal (C). Bottom of the column (D) was closed with rubber sleeve stoper (E). During coupling the column was rotated (F) (inlet of rotary evaporator could be used). Other reagents were delivered in flow-stop flow mode to the open column with outlet made from silicon rubber tube (G). Device allow easy scale-up.

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