DNA molecule synthesis

Part 1: Iterative Synthesis of ACTCTGAT

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Step 1.1: Coupling Reaction (First Nucleotide Addition: A)

    Option 1.1.1: TdT-mediated incorporation of 3'-ONH2-dATP in phosphate
buffer.
        - Component A: Immobilized initiator oligonucleotide on beads (at 1
µM final concentration)
        - Component B: ZaTdT-R335L-K337G (at 2 mg/mL final concentration)
        - Component C: 3'-ONH2-dATP (at 0.50 mM final concentration,
purified as in Part 2)
        - Component D: CoCl2 (at 0.50 mM final concentration)
        - Component E: phosphate buffer (pH 6.8) (at 50 mM final
concentration)
        - Component F: NaCl (at 100 mM final concentration)
        (Operational details: Combine all components, ensuring they are
prepared with HPLC-grade water and free from aldehyde/ketone contamination.
Incubate the reaction mixture at 30 °C for 5 min with gentle tilting and
rotation to keep beads suspended.)

Step 1.2: Post-Coupling Wash

    Option 1.2.1: Wash beads with 1X Binding & Washing (B&W) Buffer.
        - Component A: Beads from Step 1.1
        - Component B: 1X B&W Buffer
            - Recipe: 5 mM Tris-HCl (pH 7.5), 0.5 mM EDTA, 1 M NaCl, 0.1%
Tween-20
        (Operational details: Use a magnetic stand to separate the beads
for 1 minute. Remove the supernatant. Add 1X B&W Buffer, resuspend the
beads, separate, and discard the supernatant. Repeat wash cycles 2-3
times.)

Step 1.3: Deblocking Reaction

    Option 1.3.1: Cleavage of the 3'-ONH2 protecting group using sodium
nitrite in acetate buffer.
        - Component A: Washed beads from Step 1.2
        - Component B: Cleavage Reagent
            - Recipe: 700 mM NaNO2 in 1 M aqueous sodium acetate buffer, pH
5.5
        (Operational details: Resuspend the beads in the Cleavage Reagent
and incubate at room temperature for 5 minutes with gentle rotation. This
step may be repeated to ensure complete cleavage.)

Step 1.4: Post-Deblocking Wash

    Option 1.4.1: Wash beads with 1X Binding & Washing (B&W) Buffer to
remove deblocking agent.
        - Component A: Beads from Step 1.3
        - Component B: 1X B&W Buffer
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            - Recipe: 5 mM Tris-HCl (pH 7.5), 0.5 mM EDTA, 1 M NaCl, 0.1%
Tween-20
        (Operational details: Use a magnetic stand to separate the beads
for 1 minute. Remove the deblocking buffer. Add 1X B&W Buffer, resuspend
the beads, separate, and discard the supernatant. Repeat wash cycles 2-3
times.)

Step 1.5: Subsequent Synthesis Cycles for ACTCTGAT

    Option 1.5.1: Repeat steps 1.1 through 1.4 for the remaining 7
nucleotides.
        (Operational details: For each cycle, perform the Coupling (Step
1.1), Post-Coupling Wash (Step 1.2), Deblocking (Step 1.3), and Post-
Deblocking Wash (Step 1.4). The specific 3'-ONH2-dNTP used in the coupling
step is changed for each cycle according to the target sequence ACTCTGAT.)
        - Cycle 2 (C): Use 3'-ONH2-dCTP.
        - Cycle 3 (T): Use 3'-ONH2-dTTP.
        - Cycle 4 (C): Use 3'-ONH2-dCTP.
        - Cycle 5 (T): Use 3'-ONH2-dTTP.
        - Cycle 6 (G): Use 3'-ONH2-dGTP.
        - Cycle 7 (A): Use 3'-ONH2-dATP.
        - Cycle 8 (T): Use 3'-ONH2-dTTP.
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