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Supporting Information

Copying of RNA Sequences without Pre-Activation

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Contents

1 Materials and Methods

1.1 Reagents and Instruments

Reagents

Reagents, buffers and solvents were obtained from Aldrich/Fluka/Sigma, Acros Organics, GenScript, or VWR. Oligonucleotides were purchased from Biomers in HPLC-purified form. Epoxy-functionalized magnetic beads (Dynabeads M-270 Epoxy) were from Invitrogen. Dowex 50WX8 ion exchange resin was obtained from Sigma in its H^+ form. A sample of the resin (10 g) was washed with acetone (100 mL), methanol (100 mL), dichloromethane (100 mL), and water, until the filtrate was pH neutral, and then converted to the NH_4^+ form by stirring with aqueous ammonia (25%, 50 mL) for 1 h and washing with water until the filtrate was pH neutral. Immobilization of the 5'-mercaptohexyl-modified capture strands was performed as previously described.[S1]

Condensation Buffer

The optimized reaction medium, referred to as "general condensation buffer" that is mentioned in the text and in General Protocols 1-3 was freshly prepared by adding an aqueous solution of HEPES buffer (0.5 M), $MgCl_2$ (0.08 M), 1-ethylimidazole (0.15 M) and the stated amount of nucleotides, adjusted with NaOH to pH 7.5 (65 µL) to EDC hydrochloride (10 mg, 52 µmol), to give a final EDC concentration of 0.8 M. After vortexing, the solution was immediately used for primer extension or oligomerization assays.

MALDI-TOF mass spectrometry

Unless otherwise noted, MALDI-TOF mass spectra were acquired on a Bruker Reflex IV or a Bruker Microflex spectrometer in linear negative mode. A matrix mixture consisting of 0.2 M 2,4,6-trihydroxyacetophenone as matrix and 0.03 M diammonium citrate as comatrix in ethanol:water (2:1 *v*/*v*) was used. Then, 0.5 µL of the sample solution was mixed with 0.5 µL of the matrix solution on a stainless steel target, reduced to dryness *in vacuo* and transferred into the instrument.

HPLC

Ion-exchange HPLC was performed using a Nucleogel SAX 1000-8 column (Macherey Nagel), with a gradient of NH_4Cl (0 to 0.1 M in 5 min, then 0.1 to 0.5 M in 30 min) in buffer (Tris, 5 mM, in acetonitrile:water, 20:80, *v*/*v*, pH 7.5) with a flow rate of 1 mL/min and detection at $\lambda = 260$ nm.

1.2 General Protocols

General Protocol 1. *Primer Extension in Solution*

The following protocol was used for primer extension assays in solution, as shown in Figure 1. All reactions leading to the results in Table 1 were performed thus, at different pH and salt concentrations, and temperature, as indicated. To *N*-ethyl -*N*'-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, 10 mg, 52 µmol) was added 65 µL of a solution containing HEPES (0.2 M, 13 µmol), NaCl (0.4 M, 26 µmol), $MgCl_2$ (0.16 M, 10.4 µmol), 1-ethylimidazole (0.1 M, 6.5 µmol), and the nucleoside 5'-monophosphate (NMP, 0.02-0.1 M, 1.3-6.5 µmol) at pH 7.5. Of the resulting solution, a sample (10 µL) was transferred to a lyophilized mixture of template (600 pmol) and primer (500 pmol) and mixed. The resulting solution was incubated at 0 °C. Analytical samples (0.5 µL) were drawn at stated intervals, diluted with aqueous ammonium acetate buffer (10 µL, 1 M NH_4OAc, pH 7.0) and treated with Dowex 50WX8 cation exchange resin (1 mg, NH_4^+ form) for 10 min. The mixture was centrifuged, and 0.5 µL of the supernatant was analyzed by MALDI-TOF mass spectrometry.

General Protocol 2. *Primer Extension on Beads*

To a suspension (5 µL) containing magnetic beads (5 mg/mL, 25 µg, loaded with capture oligonucleotide; see Figure S1, below) in buffer containing HEPES (0.5 M, 2.5 µmol) and $MgCl_2$ (0.08 M, 0.4 µmol), pH 7.5, were added solutions of template (0.6 µL, 100 µM, 60 pmol) and primer (0.5 µL, 100 µM, 50 pmol), and the mixture allowed to hybridize at 0 °C for 15 min. To start the reaction, the supernatant was aspirated and a freshly prepared solution of condensation buffer (5 µL), containing nucleoside 5'-monophosphate (NMP, 0.02-0.1 M, 0.1-0.5 µmol), at pH 7.5 was added. Where needed, the reaction volume was transferred to a polypropylene vessel containing the lyophilized downstream-binding oligonucleotide (5 nmol), followed by vortexing for 5 s and incubation at 0 °C. After 5 d, the supernatant was replaced with a freshly prepared solution of the same composition. For monitoring the progress of primer extension, samples (0.5 µL) of the freshly vortexed suspension were drawn at time points indicated, the supernatant was removed, and the magnetic beads were washed with ammonium acetate buffer (2 *x* 1 µL, 1 M NH_4OAc, pH 7.0). The beads were then suspended in deionized water (2 µL) and heated to 80 ° C for 5 min. The resulting supernatant containing the oligonucleotides was added to a vial containing a few beads of Dowex 50WX8 cation exchange resin (1 mg, NH_4^+ form). After 10 min, 0.5 µL of the supernatant was analyzed by MALDI-TOF mass spectrometry.

General Protocol 3. *Untemplated Oligomerization*

Freshly prepared condensation buffer, as described above, containing either AMP (0.15 M, 9.75 µmol), UMP (0.15 M, 9.75 µmol), CMP (0.15 M, 9.75 µmol), or GMP (0.02 M, 1.3 µmol) at pH 7.5 was prepared by adding the solution to EDC hydrochloride, mixing and allowed the mixture to react at 0 °C. Samples (2 µL) were drawn at stated intervals, diluted with buffer (200 µL; Tris, 5 mM, in acetonitrile:water, 20:80, v/v; pH 7.5), and analyzed via anion-exchange HPLC. Samples (0.5 µL) of individual fractions were analyzed by MALDI-TOF mass spectrometry.

2 Supplementary Data

2.1 Data for Oligonucleotides

Structure of capture oligodeoxynucleotide used for immobilizing templates on magnetic bead particles (compare Figure 3 of the manuscript).

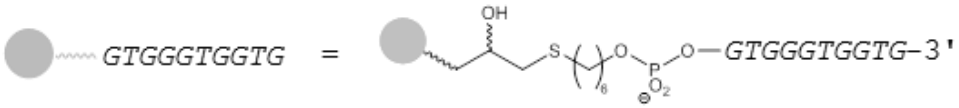

Figure S1. Sequence of the capture oligodeoxynucleotide and structure of the linker to the bead surface. The immobilization method was described previously.[S1]

Mass spectrometric data

Calculated masses are average masses, and masses found are the maxima of the unresolved isotope pattern. MALDI-TOF mass spectra with external calibration have a mass accuracy of approx. 0.1%, so that a mass of 2000 Da is determined with a mass accuracy of approx. ± 2 Da.

Primer extension in solution

Masses of primers **2** and **5** and their extended derivatives, as shown in spectra in Figure 2 of the main manuscript. The assays followed General Protocol 1.

Primer **2**: $[M-H]^-$ calculated for $C_{75}H_{94}N_{27}O_{56}P_7$ 2485.4, found 2485

Primer **2** + G: $[M-H]^-$ calculated for $C_{85}H_{106}N_{32}O_{63}P_8$ 2830.4, found 2830.7

Primer **2** + GC: $[M-H]^-$ calculated for $C_{94}H_{118}N_{35}O_{70}P_9$ 3136.4, found 3135.4

Primer **5**: $[M-H]^-$ calculated for $C_{79}H_{96}N_{37}O_{51}P_7$ 2595.4, found 2595.6

Primer **5** + U: $[M-H]^-$ calculated for $C_{88}H_{107}N_{39}O_{59}P_8$ 2901.4, found 2901.5

Primer extension on immobilized templates

Masses of primer **7** and extended versions of it from assays on beads, as shown in spectra in Figure 3 of the main manuscript. The assays followed General Protocol 2.

Primer **7**: $[M-H]^-$ calculated for $C_{76}H_{94}N_{29}O_{56}P_7$ 2525.4, found 2523.9

Primer **7** + A: $[M-H]^-$ calculated for $C_{86}H_{106}N_{34}O_{62}P_8$ 2852.4, found 2852.5

Primer **7** + C: $[M-H]^-$ calculated for $C_{85}H_{106}N_{32}O_{63}P_8$ 2830.4, found 2830.5

Primer **7** + G: $[M-H]^-$ calculated for $C_{86}H_{106}N_{34}O_{63}P_8$ 2871.4, found 2872.2

Primer **7** + U: $[M-H]^-$ calculated for $C_{85}H_{105}N_{31}O_{64}P_8$ 2830.4, found 2829.5

Untemplated oligomerization in solution

Masses of oligoadenylates formed in solution after 30 d at 0 °C. The assay followed General protocol 3. Spectra are shown in Figure 4 of the main manuscript.

A_2, $[M-H]^-$ calculated for $C_{20}H_{26}N_{10}O_{13}P_2$ 675.1, found 674.3

A_3, [M-H]- calculated for $C_{30}H_{38}N_{15}O_{19}P_3$ 1003.2, found 1003.5

A_4, [M-H]- calculated for $C_{40}H_{50}N_{20}O_{25}P_4$ 1333.2, found 1333.8.

A_5, [M-H]- calculated for $C_{50}H_{62}N_{25}O_{31}P_5$ 1662.3, found 1663.1

A_6, [M-H]- calculated for $C_{60}H_{74}N_{30}O_{37}P_6$ 1991.3, found 1992.9

A_7, [M-H]- calculated for $C_{70}H_{86}N_{35}O_{43}P_7$ 2320.4, found 2323.0

A_8, [M-H]- calculated for $C_{80}H_{98}N_{40}O_{49}P_8$ 2649.4, found 2653.2

A_9, [M-H]- calculated for $C_{90}H_{110}N_{45}O_{55}P_9$ 2978.5, found 2982.9.

3 References for Supporting Information

[S1] A. Kaiser, S. Spies, T. Lommel, C. Richert, *Angew. Chem. Int. Ed.* **2012**, *51*, 8299–8303.