

Overview of Methods for Large-Scale RNA Synthesis

Marcin Ryczek, Martyna Pluta, Leszek Błaszczyk * and Agnieszka Kiliszek *

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznań, Poland;
mryczek@ibch.poznan.pl (M.R.); mpluta@ibch.poznan.pl (M.P.)

* Correspondence: blaszcz@ibch.poznan.pl (L.B.); kiliszek@ibch.poznan.pl (A.K.)

Materials and Methods

1. DNA /RNA synthesis and purification

Synthesis of oligonucleotides were carried out on Applied Biosystem 392 DNA/RNA synthesizer using phosphoramidite method. Phosphoramidites were purchased from Chemgenes, LGC Link and Glen Research. All oligomers were cleaved from the CPG support by AMA protocol – ammonium hydroxide/methylamine in water (40%) (1:1 v/v) for 4 h. DMT-ON oligomers were further deprotected and/or purified with Glen-Pak DNA or Glen-Pak RNA purification cartridges (Glen Research) according to manufacturer protocol. DMT-OFF RNA oligomers were deprotected with 1 M tetra-n-butylammonium fluoride in tetrahydrofuran at 37°C for 20 h. Oligomers were desalted on illustra NAP-25 columns (GE healthcare) or Bio-Scale Mini Bio-Gel P-6 Desalting Cartridge (Bio-Rad). The purity of oligomers was estimated using HPLC or 10-15% denaturing polyacrylamide gels.

2. In vitro transcription

An in vitro transcription reaction contained 1 µg of linearised plasmid or chemically synthesized DNA, 15 µg of in-house produced T7 RNA polymerase, 4 mM mixture of NTPs and transcription buffer (40 mM Tris-HCl pH 8.0, 1 mM spermidine, 0.01% Triton X-100 and 5 mM DTT). Magnesium ions concentration was adjusted for each RNA construct and type of the ribozyme (typical concentration range: 20-32 mM). In case of the 2'-methoxy modified DNA template, prior to in vitro transcription reaction, 500 ng of each strand was annealed at 95°C for 5 min and incubated for 10 min at room temperature. Transcription reaction was carried out for 4h or overnight at 37°C. Next, template DNA was digested using 10 U of DNase I following incubation at 37°C for 20 min. Reaction products were purified using EurX Universal RNA/miRNA Purification Kit (EurX) and analyzed on HPLC or 10-15% denaturing polyacrylamide gels.

3. HPLC analysis

Approximately 5 µg of RNA sample were injected on Thermo Fisher Scientific DNAPac PA 100 4 × 250 mm column. Separation was carried out at 1 ml/min flow rate in room temperature in gradient of 0-60% of 0.5 M sodium perchlorate buffer B in 25 mM Tris pH 8.0 for 45 minutes.