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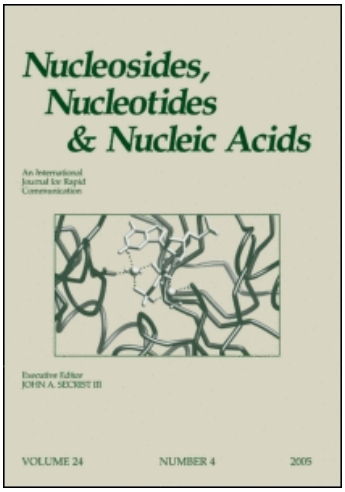

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MESSENGER RNA ISOLATION USING NOVEL PNA ANALOGUES

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MESSENGER RNA ISOLATION USING NOVEL PNA ANALOGUES

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ABSTRACT

Homo-Thy hetero-oligomer probes composed of *trans*-4-hydroxy-*L*-proline based PNA-like (HypNA) monomers and phosphono PNA (pPNA) monomers demonstrated strong binding to complementary poly A^+ RNA strands. We used a mixture of chimeric oligomers containing both "linear" and "clamping" PNA-analogues to develop an mRNA isolation procedure and demonstrate the improved recovery of RNA molecules with secondary structure at the 3′end as well as RNAs with short poly A tails.

Current techniques for the isolation of mRNA require the hybridization of oligo dT to the 3′ poly A tail present on all mRNAs (1). Given the high amount of stable secondary structure in RNA, resulting from RNA/RNA interactions, it is difficult for oligonucleotides to penetrate these structures and hybridize to the target RNA sequence. Peptide nucleic acids (PNAs) (2–5) are a relatively new class of DNA mimics that may be able to overcome some of the problems associated with oligo-dT based purification of mRNA. These DNA mimics bind to complementary DNA or RNA stretches with affinities significantly higher than those of the corresponding deoxyribooligonucleotides (6,7). Furthermore PNAs are resistant to nucleases allowing for a DNase digest of captured material (8). Finally some PNAs can "invade" into double stranded nucleic acids, resulting in

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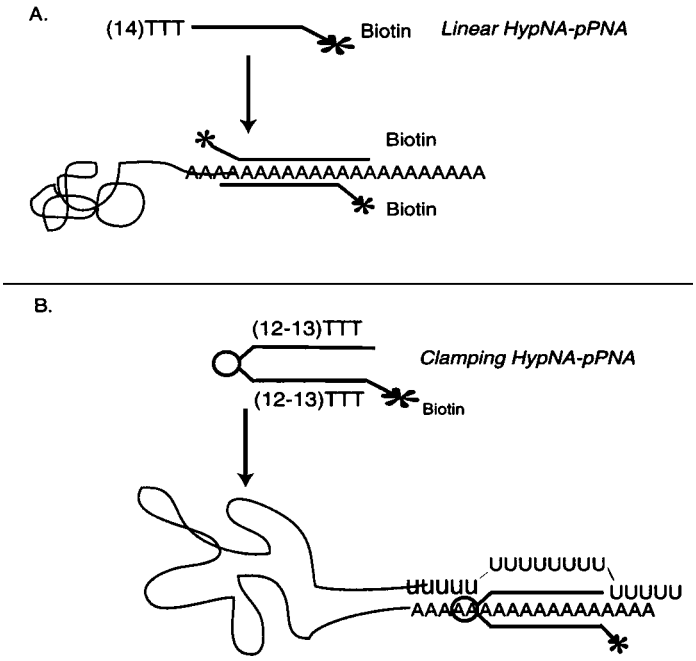

Figure 1. Schematic drawing of linear (A) and clamping (B) HypNA-pPNA poly-Thy hetero oligomers hybridizing.

the capture of mRNAs with high degree of secondary structure (9). However, their poor water solubility and tendency to self-aggregate limit the biological applications of PNAs. Recently, novel classes of PNA-related oligonucleotide mimics containing *trans*-4-hydroxy-*L*-proline based (HypNA) monomers and phosphono PNA (pPNA) monomers have been designed (10–11). The introduction of negative charges into the PNA backbone leads to excellent solubility characteristics of pPNAs and HypNAs while preserving the high affinity of these mimics to DNA and RNA targets.

In this study, an mRNA isolation procedure using poly-Thy PNA-analogues has been developed. We used a mixture of chimeric oligomers containing both "linear" and "clamping" molecules (Fig. 1) (12). The "linear" chimeras contain a chain (12–15 residues) of pPNA monomers or alternating HypNA and pPNA units in a 1:3 ratio. The "clamping" PNA-analogue consists of two pPNA or HypNA-pPNA chains connected via a flexible linker. We tested these poly-Thy oligomers for their ability to bind to RNA transcripts with extensive secondary structure and short poly-A tails (Table 1) and demonstrated that mRNA purified using negatively charged PNA analogues is free of genomic DNA contamination. We subsequently developed a line of mRNA isolation Kits, mVADER, using PNA-analogues and currently offer these Kits to the research community.

As it is shown in Figure 2, $pPNA_2$/DNA triplex is more thermodynamically stable than $pPNA_2$/RNA triplex. This ensures that even in the absence of a DNase

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Table 1. The Comparison of the Elution Efficiency of Poly A $^{+}$RNA Transcripts from Oligo-dT and from Clamping Oligo-Thy pPNA*

	Oligo-dT (% recovery)	Clamping mimic (% recovery)
ss RNA	7.6	52.4
stem/loop RNA	2.0	23.6
ds RNA	8.6	39.7

*Approximately 11 pmoles of each transcript were incubated with 100 pmoles of either biotinylated oligo dT or biotinylated clamping oligo-Thy pPNA. After extensive washing, the captured RNA was eluted off the probes with water. Recovered RNA transcripts were quantified by spectrophotometric analysis and agarose gel analysis.

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digest, by using water at ambient temperature the researcher can be confident that no genomic DNA will be co-purified with the mRNA.

Finally we have used pPNA as well as HypNA-pPNA hetero oligomers (linear and clamping) in a mRNA isolation procedure from both cells and tissue. Essentially 10–15 molar excess of biotinylated PNA probe is allowed to hybridize to the target poly-A^{+} messages in a cell lysate for up to 2 hrs at room temperature. Addition of streptavidin coated magnetic beads captures the PNA/RNA complexes. Following

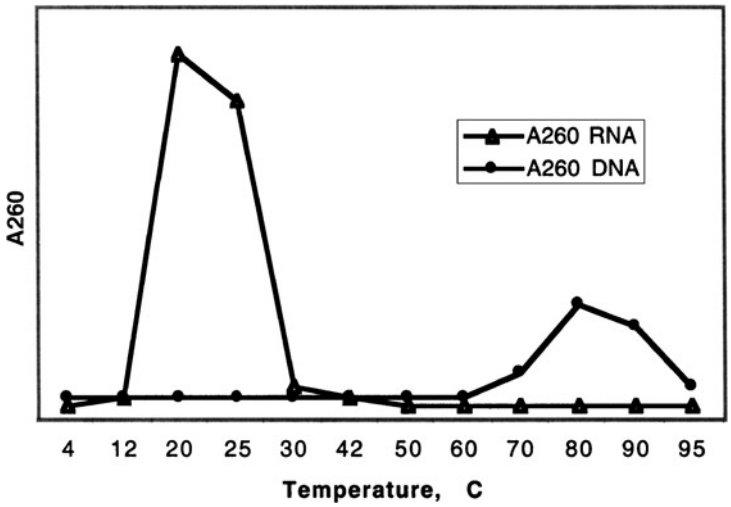

Figure 2. In two separate experiments, poly-rA_{30} and dA_{30} were allowed to anneal to a linear pPNA for 1 h at room temperature. Streptavidin coated magnetic beads were subsequently added to capture the oligo/pPNA complexes. After extensive washing, the captured RNA or DNA was eluted off the mimic oligomer probe with water at 4°C, 22°C, 50°C, 70°C and 90°C, successively. PNA_2 /RNA triplexes dissociate at room temperature, while PNA_2/DNA triplexes dissociate at higher temperatures.

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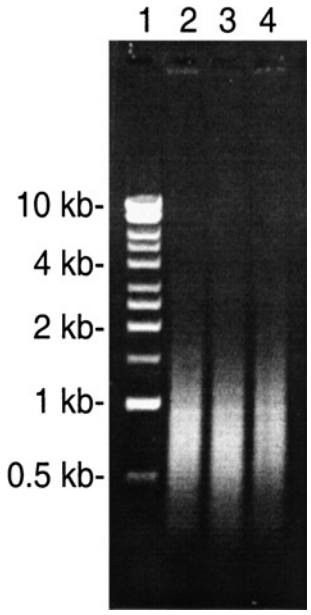

Figure 3. Analysis of mRNA isolated from total RNA in 3 separate enrichment experiments using Total mVADER. Lane 1 - dsDNA ladder, lanes 2, 3 and 4 - 1 μg of HypNA-pPNA selected mRNA.

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washes of the beads with a low salt buffer and an optional DNase digest, the mRNA is eluted off the PNA analogue using water at ambient conditions. We have developed this technique for the isolation of mRNA using PNA analogues from various amounts of cells or quantities of tissue, including a 96-well version and a version for the isolation of mRNA from total RNA (Fig. 3).

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