

LETTER TO THE EDITOR

## The position of site-directed cleavage of RNA using RNase H and 2'-O-methyl oligonucleotides is dependent on the enzyme source

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It has been shown that RNA can be site specifically cleaved by RNase H using 2'-O-methyl RNA/DNA chimeras to direct the cleavage site (Inoue et al., 1987; Hayase et al., 1990; Lapham & Crothers, 1996; Xu et al., 1996). In these studies, the chimeric splint positions four base paired deoxyribonucleotides to the 5' side of the position of desired cleavage, as shown in Figure 1A. In each of these studies, the cleavage position has been determined to be that shown in the figure with a reaction efficiency of greater than 90%.

Recently, a paper from the Steitz laboratory (Yu et al., 1997) reported the application of chimeric-directed RNA cleavage as a diagnostic tool in determining positions that have been methylated at the 2' hydroxyl position. It was reported that a chimera containing four deoxyribonucleotides resulted in efficient cleavage of a target RNA at the site 5' to the ribonucleotide that base pairs to the 5'-most deoxyribonucleotide, as shown in Figure 1B. This cleavage site is different from that described previously (see above). For another target RNA, a chimera containing three deoxyribonucleotides was found to direct specific RNase H cleavage more efficiently than a four deoxyribonucleotide-containing chimera. Again, the cleavage site was 5' to the ribonucleotide that base pairs to the 5'-most deoxyribonucleotide (Fig. 1B).

We have determined that the apparent discrepancy between these findings can be traced to the source of the RNase H enzyme. In the earlier studies, the RNase H (*Escherichia coli*, cloned) was purchased from either Pharmacia, Sigma, or Takarashuzo. The later work from

the Steitz lab utilized RNase H from Boehringer Mannheim (*E. coli*, cloned). An experiment comparing the Pharmacia versus the Boehringer Mannheim enzymes on the same substrate and chimera was performed (data not shown), with the Pharmacia enzyme cleaving as shown in Figure 1A and the Boehringer Mannheim enzyme cleaving as in Figure 1B.

It is unclear why enzymes from different suppliers derived from the same source show different activities. We note that the Boehringer Mannheim RNase H storage buffer does not contain EDTA and is relatively low in salt compared with that from Sigma and Pharmacia. Other differences in the purification protocols may also be contributing to the observed differences. Therefore, when precise cleavage using chimeric oligonucleotides is required (i.e., for ligations or site-specific labeling) we recommend caution in the construction of the oligonucleotides and choice of supplier of enzyme.

### REFERENCES

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**A** Pharmacia (cat. # 27-0894), Sigma (cat. # R-6501) or Takarashuzo RNase H

RNA: 5' — NNNNNNNNNNNNNNNNNN — 3'  
 2'-O-methyl RNA/DNA chimera: 3' — NNNNNNNNNNNNNNNN — 5'

**B** Boehringer Mannheim (cat. # 786 349) RNase H

RNA: 5' — NNNNNNNNNNNNNNNNNN — 3'  
 2'-O-methyl RNA/DNA chimera: 3' — NNNNNNNNNNNNNNNN — 5'



RNA: 5' — NNNNNNNNNNNNNNNNNN — 3'  
 2'-O-methyl RNA/DNA chimera: 3' — NNNNNNNNNNNNNNNN — 5'



**FIGURE 1.** Underlined letters represent 2'-O-methyl RNA, bold letters represent the deoxyribonucleotides, and the observed cleavage positions are shown as arrows.