A rapid method for determining the orientation of poly (A) containing cDNAs

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We have designed a 17 base oligonucleotide that can be used for rapid screening of cDNA libraries to determine the orientation of the inserts and the presence of poly (A) tails. Such information is needed for the preparation of single stranded DNA and antisense RNA probes, but is usually obtained by DNA sequencing or other laborious analysis.

We have been screening cDNA libraries using labelled cDNA to identify clones coding for abundant mRNAs. These libraries were constructed using both the S1 nuclease and the RNase H methods. However, only 20% of the positive clones had poly (A) tails. The remainder appear to have been damaged during cloning. To facilitate our screening project, we designed an oligonucleotide (see below) complementary to and spanning three consecutive segments of the recombinant DNA molecules: the poly (A) tail, the Eco R1 linker used in constructing the cDNA library, and 4 bases of the vector that are unique in sequence to one side of the Eco R1 cloning site. Since the vector sequence is different on each side of the cloning site, the oligonucleotide will hybridize to clones in only one orientation.

The oligonucleotide was end labeled with [³²P] and hybridized for 3 hours in 6X SSC/10X Denhardt's solution with plaque lifts on nitrocellulose from a lambda library. The filters were washed with 6X SSC for 5 minutes at 5° intervals between 25 and 55°. Between 35 and 45°, three categories of plaques were visible: a strong, an intermediate, and a weak background signal. At higher temperatures only the strong and intermediate signals persisted. From each category, several clones were picked for DNA sequence analysis. All of the strongly labeled plaques were clones containing the poly (A) tails in the downstream orientation and had perfect complementarity to the oligonucleotide. Clones in the intermediate category contained 1 to 3 mismatches which resulted from: (1) upstream orientation, (2) damaged Eco R1 linker, or (3) any other cloning artifact resulting in as few as one mismatch. Plaques displaying weak background signals contained clones with 5 or more mismatches with the oligonucleotide and lacked poly (A) tails. Use of this oligonucleotide allows the rapid determination of poly (A) containing clones with a specific orientation, and provides information needed for synthesis of antisense probes. It also eliminates from consideration damaged clones lacking Eco R1 linkers or poly (A) tails. This work was supported by (Public Health Service) Grant HD 17722-06 to M.W. from the National Institute of Health.

	vector	Eco R1 linker	Poly (A) tail
Oligonucleotide	5' - CAGG	AATTCGG	TTTTTT- 3'