PerkinElmer®
For the Better

Methods for preparing labeled hybridization probes

The following chart summarizes the common methods of DNA and RNA probe generation and their relative advantages. These methods are most commonly used with our ^{32}P and ^{35}S nucleotides, but can also be utilized with ^{3}H nucleotides.

Method	Enzyme used	Specific activity (dpm/µg)	Features	Optimal nucleotides
Nick translation	DNA Pol I	10^8-10^9	-High specific activity -Trouble-free -Inexpensive *Hairpin loops of DNA can be formed	dCTP, dATP, dTTP
Random Priming	Klenow	10^8-10^9	-Highest specific activity *Need primer	dCTP, dATP,dTTP
Primer Extension	Klenow	10^7-10^8	-Partially single-stranded probe	dCTP, dATP, dTTP
3' End Labeling	Terminal transferase	10^7	-Highly specific -Oligo probes	3'-deoxy-ATP (cordycepin)
5' End Labeling	T4 Polynucleotide Kinase (T4 PNK)	10^7	-Highly specific -Oligo probes	gamma phosphate labeled-ATP
Fill-in	Klenow	10^7	-Highly specific -For 5' or 3' overhangs after restriction enzyme digest	dCTP, dATP, dTTP
T4 DNA labeling	T4 DNA Polymerase	10^7	End labeling for blunt ends. Can be made strand-specific by restriction enzyme digestion	dCTP, dATP, dTTP
RNA Probes	SP6 RNA polymerase	10^7-10^8	DNA/RNA has high binding coefficient	UTP, CTP, GTP
RNA Probes	T7 RNA polymerase	10^7-10^8	DNA/RNA has high binding coefficient	UTP, CTP, GTP
cDNA synthesis	Reverse transcriptase	10^7	High specificity for probing recombinants	dCTP, dATP, dTTP