Oligonucleotide labeling
(Random Hexamer Primed and/or Directed primed)

Procedure:

(1) DNA (GI fragment)	50-100ng
(2) ddH_2O	to 50µL
(3) Primers	3.0µL*
(4) Boil	10min
(5) Chill on ice	
(6) Solution A	2µL
(7) HEPES	2µL
(8) dA	1µL
(9) dG	1µL
(10) dT	1µL
(11) BSA	2µL
(12) ^{32}P-dCTP	5µL
(13) Klenow	1µL
(14) 37^{o}C	~1h
(15) STOP	100µL

(15) Run G50 column (gravity or spin)

Stock solutions and stuff:

(1) Primers (random hexamers) 1.8 mg/ml in $T_{10}E_{.1}$ (90 A_{260} Units/ml)

(2) Sol'n A

(a)	Solution O	56µL
(b)	2-ME	1µL

(3) Sol'n O

		10ml	[Final]
(a)	Tris base	1.5g	1.25M
(b)	MgCl	0.25g	0.125M
(c)	pH	to 8.0	
(d)	ddH_2O	to 10ml	
(e)	s/f		

(4) HEPES

		10ml	[Final]
(a)	HEPES	4.8g	2M
(b)	pH (NaOH)	to 6.6	
(c)	ddH_2O	to 10ml	
(d)	s/f		

(5) dNTP 10mM stocks (make up according to Maniatis)

(6) BSA 10mg/ml (I use the stuff sent w/restriction enzymes)

(7) ^{32}P-dCTP (3000 Ci/mMole; NEN 013H)

(8) Klenow ~5U/µL

(9) Stop

		10ml	[Final]
(a)	5M NaCl	40µL	20mM
(b)	1M Tris (7.6)	200µL	20mM
(c)	0.5M EDTA	40µL	2mM
(d)	10% SDS	250µL	0.25%
(e)	Orange G	3mg	0.03%
(f)	Blue Dextran	100mg	1.0%
(g)	ddH_2O (sterile)	to 10ml	

Notes:

All stock solutions are kept in the -20°C.
Use either low melt agarose and β-agarase or a commercial kit to gel isolate template DNAs.
*For directed priming reactions I use 3µL of a 125ng/3uL stock.
Make Solution A fresh every 3 months or so.

Ref:

Feinberg and Vogelstein (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem. 132: 6-13.

Hodgson and Fisk (1987) Hybridization probe size control: optimized oligo labeling. Nuc. Acids Res. 15:6295