Random Primer Labeling of DNA

1. **Template DNA:** Linearize DNA by restriction digestion. Remove the enzyme by phenol:chloroform:isoamyl-alcohol extration and ethanol precipitate the DNA or isolate on low-melt agarose gel by electrophoresis and purify. Use 50 - 75 ng of DNA as template (DNA template can be in molten agarose).

2. **Denaturing of template DNA:** The DNA can be in low melt agarose add 30 μL of sterile water to the gel slice before melting (usual use ~30 μL of the melted DNA containin agarose band). Heat in an eppendorph tube at 95 - 100 C for 2 -8 minutes, rapidly chill or ice. (Do not chill too long if using low-melt agarose, sometimes you can add it directly to th reaction). Quickly assemble the labeling reaction in another tube during the DNA denaturation process.

3. **Assembling the Reaction:** Assemble the reaction on ice in the order listed.

5 X Labeling buffer	10 μL
Mixture of unlabeled dNTP (1.5 μM)	2 μL
Denatured DNA template	50 ng (or 30μL)
Acetylated BSA (10 mg/mg)	2 μL (optional)
α-^{32}P dATP (50μCi, 3000Ci/mmol	2 μL
Klenow Enzyme (5 Units)	1 μL
Sterile water to final	50 μL

Mix reaction together gently and incubate at room temperature overnight.

4. **Termination of Reaction:** Heat reaction to 95 -100 C for 5 minutes and then chill in an ice bath. I usually add 250 μL of stop buffer (containing EDTA) so the agarose won't solidify after chilling and use directly in hybridization or store at -20 C until use.

5. **Determination of Incorporation:** TCA preciipitation (optional)
1) Add dilluted labeling sample (1 μL each) on 2 glass filters. Let the filters air dry.
2) In a glass beaker wash 1 filter with 10 mL of 10% TCA for 10 min. Wash twice with 5% TCA (10 min each). Wash once with 1-2 mL of acetone and let filter air dry.
3) Add scintillation cocktail and count in a scintillation counter.

Solutions:

5X Labeling Buffer: (from Promega U1151)

250 mM	Tris-HCl, pH 8.0
25 mM	$MgCl_2$
10 mM	DTT
1 mM	HEPES, pH 6.6
26	A_{260} U/mL random hexadeoxyribonucleotides

dNTP Mixture
Mix 1 μL of 3 dNTPs (1.5mM stock solutions) to yield 3 μL solution at 500 μm for each dNTP.