

## How to make hot probes for your Southern

### Step 1. Prepare reaction cocktail on ice

2.5 $\mu$ L	0.5 mM 3dNTP mix (no dATP)	(NEB #N0446S)
2.5 $\mu$ L	10X Klenow Buffer	supplied with Klenow order
5.0 $\mu$ L	3000 Ci/mmol $\alpha$ - <sup>32</sup> P dATP	your radioactive supplier
1.0 $\mu$ L	Klenow (3 to 8 units)	(NEB #M0210S)
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11 $\mu$ L /rxn		

**Step 2.** Combine your probe (30 to 100 ng) with random 9mers (1 to 5  $\mu$ g, from NEB #S1254S) in total volume of 14  $\mu$ L. USE A SCEW-CAP EPPENDORF or an eppendorf clamp to prevent an explosion of radioactive material while boiling in the Denaturing step!!!.

2-3 $\mu$ L	DNA probe	
5 $\mu$ L	1 $\mu$ g/ $\mu$ L 9mer	(NEB #S1254S)
6-7 $\mu$ L	water	
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14 $\mu$ L		

**Denature** by boiling (make sure cap is secure) for 2 to 3 minutes (using boil beads is easiest), spin down, then place on ice.

Step 3. Add 11  $\mu$ L of the reaction mix from Step 1 to the denatured DNA (final volume 25  $\mu$ L) and incubate 37°C for 30 minutes.

### Step 4. Stop reaction by adding:

1 $\mu$ L	0.5 M EDTA	
3 $\mu$ L	10 mg/ml tRNA	(Ambion #7119)
100 $\mu$ L	TE buffer	

**Step 5.** Remove unincorporated labelled nucleotides with ProbeQuant G-50 Microcolumns. (Amersham # 27-5335-01 for a box of 50 columns).