

³²P labeling of large DNA Fragments.

1. Purify DNA fragment from gel and phenol/CHCl₃ extract a couple of times to remove residual agarose

2. Aliquot 200-500ng of DNA into screw cap tube and add ddH₂O to a final volume of 40ul

DNA	- 10ul (will vary)	} Δ 100°C 5-10 mins - quick spin and place on ice immediately.
ddH ₂ O	- 30ul	
Primers (hexa/nanomers)	- 20ul	
5x dCTP Buffer	- 20ul	} add to tube on ice * use Stratagene Primer II Kit
3000cpm/ml ³² P-dCTP	- 20ul	
Klenow	- 2ul	
	<u>102ul</u>	

- incubate tube at 37°C for 15-30 mins
- add stop 2ul and mix gently
- centrifuge to get labeled DNA to the bottom (will condense in cap)
- NucTrap to clean up
- Cant probe 1ul spotted on filter etc.
- Boil 5-10 mins before use.