Radioactive Probe Preparation

Order Prime-a-Gene Labeling System from Promega (Cat # U1100)
Order from CORD Perkin Elmer Life Science alpha-32P-dCTP, 3000 CI/mmol, 10mCI/ml (Cat # BLU-513H)

1] PCR the gene of interest and clean up with Qiagen PCR purification kit
2] Spec to determine concentration (usually 10-50ng/ul)
3] Place 25ng of clean PCR rxn in 0.5ml tube and bring up to 5ul with nuclease free H2O
4] Denature for 2 min at 95C in PCR Machine
5] Rapidly chill in ice water bath for at least 2 min
6] Spin down to bring everything to bottom
7] Make dNTP mixture
    -from the kit, mix an equal amount of dATP, dGTP, dTTP
8] Assemble reaction in the order shown in a 0.5ml tube
    -25ul Nuclease free H2O
    -10ul 5X Labeling buffer
    -2ul of dNTP mixture (dATP, dGTP, dTTP)
    -5ul of denatured template
    -2ul Nuclease free BSA
    -5ul alpha-32P-dCTP
    -1ul Klenow Polymerase
9] Mix gently and centrifuge briefly to bring down contents
10] Incubate reaction at room temp for 60min
11] Terminate reaction by heating to 95C for 2min and rapidly chilling in ice water bath
12] Add 2.083ul of 0.5M EDTA
13] Prepare G-50 spin column by spinning in 1.5ml tube at 2Xg for 1 min
14] Blot the extra drop of water off of the end of the column after spinning and place in a new 1.5ml tube
15] Load the 50ul probe sample above directly to the middle of the slanted G50 column
16] Spin at 2Xg for 1 min
17] Take one microliter of sample and check with Geiger counter