5/30/07
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OLIGOLABELLING

Feinberg, A.P., Vogelstein, B. (1984) Anal. Biochem. **137:** 266-27
Feinberg, A.P., Vogelstein, B. (1983) Anal. Biochem. **132:** 6-13

Combine in O-ring sealed microfuge tube: 50 – 100 ngs linearized DNA
1.5 µl Random primers 0.2U/µl (Pharmacia)
H_2O to 5µl

Cap tightly; 100ºC for 3'; plunge into ice.
Microfuge 1"

Add the following to tube:
6µl 5 x buffer
2.5µl 5 mg/ml BSA
5 µl 250 µM dGAT TPs
5 µl α ^{32}P-dCTP (400-800 Ci/mmol or up to 3000 Ci/mmol)
6 µl H_2O
3 – 4 units Klenow ($0.30/unit)

Incubate @ RTº > 1 h. (Klenow has essentially no activity after a couple hours of incubation.)

Remove 0.5 µl RXN to 100 µl TE/0.5 mg/ml carrier DNA to estimate incorporation. See protocol.

SPOT 10 µl:	~ 170,000
FILTER 10 µl:	~ 120,000

Can get 8 x 10^8 cpm/µg

		Stock	1 ml
5 x buffer =	1 M Hepes, 6.6	2 M	500 µl
	250 mM Tris-Cl, 8	1 M	250 µl
	25 mM $MgCl_2$	1 M	25 µl
	50 mM 2-ME	14.4 M	3.5 µl
	H_2O		221.5

I often use the probe without removing unincorporated nucleotides since I often get 70-80% incorporation.

If incorporation drops, make new buffer.

Boil the probe 10 minutes before using in O-ring tube.

Quench on ice.

Pipet it into hybridization solution.

NOTE: High specific activity nucleotides (3000 Ci/mmole) and probes are more susceptible to radiochemical decomposition. Therefore, use this material only when you need the extra sensitivity.