Lab-protocol for:

Labelling of cDNA target prepared according to Hertzberg Amp Modified protocol

Labelling of the amplified targets.

X	100ng of cDNA + internal standard	1	95°C for 60s
10 µL	5x PCR buffer	2	95°C for 30s
10 µL	5x label dNTP mix	3	50°C for 30s
0.5 µL	MaraAP1 (100µM)	4	72°C for 10 min.
3 µL	CyX (Cy3 or Cy5, 1 mM)	5	GOTO 2, 9 times
Y µL	H_2O	6	4°C, 10 h.
0.6 µL	Ampli-Taq (perkin Elmer)	7	END
Total	50 µL		

1. Remove unincorporated nucleotides using Microcon 30 or using AutoSeq G-50 (Amersham).
2. Hybridize or store at -20°C.

Additional information:

Primer:

MaraAP1: 5 ´- CCA TCC TAA TAC GAC TCA CTA TAG GGC-3'

Buffers:

5x PCR Buffer:

335 mM Tris-HCl (pH 8.8),
20 mM $MgCl_2$,
80 mM $(NH_4)_2SO_4$.
0.17 mg/mL BSA.

5x Label dNTP mix: UTP

400 μM dATP, dCTP and dGTP,
100 μM dTTP in H_2O.