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Use of labeled primers in polymerase chain reaction (LP-PCR) for a rapid detection of the product

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The polymerase chain reaction (PCR (1)) can amplify DNA of known sequences up to 10^{15} fold. Analytically, the amplified DNA's are usually electrophoresed in gels and detected either by staining or by hybridization after blotting. We propose the use of labeled primers in the chain reaction to detect the product rapidly and quantitatively. **Methods:** Primers, 100 pmol each, were labeled with ^{32}P-ATP (2 ul, 7000 Ci/mmol, ICN) and polynucleotide kinase (5 u, Boehringer) in 10 ul of 50 mM Tris-HCl, pH 8.3, 10 mM $MgCl_2$ and 5 mM DTT at 37 degree for 30 min. The PCR mixture (GeneAmp kit, Perkin Elmer Cetus) containing 2.5 ul of the kination mixture and 0.25 ug of a sample DNA in a total volume of 25 ul was made in a 0.5 ml microfuge tube as recommended by the manufacturer. The solution was incubated in a Thermal Cycler (Perkin Elmer Cetus; 94, 55 and 72 degrees for 0.5, 0.5 and 1 min respectively, per cycle). A portion of the product was mixed with 1/4 vol. of a dye solution containing 50 % glycerol and 1 % SDS and applied onto a 5 % polyacrylamide gel in 0.5 x TBE (20 x 40 x 0.03 cm, 1 cm/lane). After electrophoresis for 1 h at 40 W with cooling by the aid of a fan, the gel was dried and contacted to a Kodak XAR film.

The procedure (including exposure to the film) ends within 6 h and involves neither restriction enzyme digestion nor hybridization. Relative band intensities among samples in LP-PCR for the first 25 cycles are essentially identical to those in the Southern blotting (Fig. A vs. B). LP-PCR is suitable for the examination of known sequences in many samples, e.g., clinical surveillance of genetic abnormalities within particular sequences, and may, in some cases, replace the Southern blotting technique.

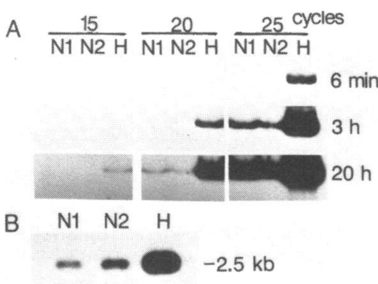

Figure legend A. The 20 b primers (sequences of human c-myc intron 2, bracketing an Alu-repeat 390 b apart) were synthesized with a 380A DNA Synthesizer (ABI). DNA from leukocytes of normal adults (N1 and N2) and from HL60 (H) which has about 30 fold amplified c-myc (2) were examined. Cycles of PCR and the exposure times are indicated. B. Southern blotting of the same DNA samples digested with Pvu II. c-myc exon 3 fragment was used as a probe.

References 1. Saiki, R. et al., Science, 239:487-491 (1988)
2. Dalla Favera, R. et al., Nature, 299:61-63 (1982)

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