A universal procedure for primer labelling of amplicons

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ABSTRACT

Detection and visualisation of nucleic acids is integral to genome analyses. Exponential amplification procedures have provided the means for the manipulation of nucleic acid sequences, which were otherwise inaccessible. We describe the development and application of a universal method for the labelling of any PCR product using a single end-labelled primer. Amplification was performed in a single reaction with the resulting amplicon labelled to a high specific activity. The method was adapted to a wide range of PCRs and significantly reduced the expense of such analyses.

At present, there are several methods for the amplification of DNA and RNA. Constant to the myriad of protocols and applications of nucleic acid amplification technologies, however, is the labelling of amplified DNA sequences. Labelling of DNA fragments, for successive visualisation, is achieved using a variety of stains or via the incorporation of fluorescent, luminescent, radioactive or other visualisation, is achieved using a variety of stains or via the incorporation of fluorescent, luminescent, radioactive or other visualisation, is achieved using a variety of stains or via the incorporation of fluorescent, luminescent, radioactive or other visualisation, is achieved using a variety of stains or via the incorporation of fluorescent, luminescent, radioactive or other visualisation, is achieved using a variety of stains or via the incorporation of fluorescent, luminescent, radioactive or other visualisation, is achieved using a variety of stains or via the incorporation of fluorescent, luminescent, radioactive or other visualisation, is achieved using a variety of stains or via the incorporation of fluorescent, luminescent, radioactive or other visualisation, is achieved using a variety of stains or via the incorporation of fluorescent, luminescent, radioactive or other visualisation, is achieved using a variety of stains or via the incorporation of fluorescent, luminescent, radioactive or other visualisation, is achieved using a variety of stains or via the incorporation of fluorescent, luminescent, radioactive or other visualisation, is achieved using a variety of stains or via the incorporation of fluorescent, luminescent, radioactive or other visualisation, is achieved using a variety of stains or via the incorporation of fluorescent, luminescent, radioactive or other visualisation, is achieved using a variety of stains or via the incorporation of fluorescent, luminescent, radioactive or other visualisation, is achieved using a variety of stains or via the incorporation of fluorescent, luminescent, radioactive or other visualisation, is achieved using a variety of stains or via the incorporation of fluorescent, luminescent, radioactive or other visualisation, is achieved using a variety of stains or via the incorporation of fluorescent, luminescent, radioactive or other visualisation, is achieved using a variety of stains or via the incorporation of fluorescent, luminescent, radioactive or other visualisation, is achieved using a variety of stains or via the incorporation of fluorescent, luminescent, radioactive or other visualisation, is achieved using a variety of stains or via the incorporation of fluorescent, luminescent, radioactive or other visualisation, is achieved using a variety of stains or via the incorporation of fluorescent, luminescent, radioactive or other visualisation, is achieved using a variety of stains or via the incorporation of fluorescent, luminescent, radioactive or other visualisation, is achieved using a variety of stains or via the incorporation of fluorescent, luminescent, radioactive or other

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To the increased primer. Thermal cycling parameters were not altered in response to the increased Tm of the chimeric template-specific primer and non-specific amplification products were absent from the described applications.

Therefore, instead of obtaining a new fluorescently labelled set of primers for each new loci of interest, two unlabelled primers were synthesised, specific to the genome sequence, with one primer extended to contain the universal priming adapter sequence. Universal primers, as described here, may be any arbitrary sequence which is compatible with the PCR conditions employed and not being present within the template genome’s DNA sequence. Labelling of the universal primer may also be accomplished using any of the currently available methods, including fluorescent, chemiluminescent, digoxigenin and radioactive nucleotide analogues. This protocol, unlike recently described advances in PCR and not being present within the template genome’s DNA sequence.

Figure 2. Effect of altering the ratio of template specific-labelling primer chimera to a universal labelling primer. (A) The quantity of amplified phycocyanin gene (30 cycles) from the cyanobacterium *Microcystis* NIES111 (4) using 10 pmol forward primer (PCβ) with 1 pmol reverse chimera (PCαURP) and 10 pmol labelling primer (URP) (lane 2), 1 pmol PCαURP and no URP (lane 3), 0.1 pmol PCαURP and no URP (lane 4), and 0.1 pmol PCαURP and 10 pmol URP (lane 5). URP is the universal reverse sequencing primer labelled with TAMRA (ABI, Foster City, CA). Lane 1 is the molecular weight marker 6X174/HaeIII (Boehringer Mannheim, Germany) and the 2% agarose gel in TAE was stained with ethidium bromide. (B) Incorporation of [α-32P]-dATP end-labelled URP into the microsatellite locus D18S363 (GDB number 195027) from a heterozygous individual. Twenty pmol of forward primer (D18S363F) combined with 20 pmol (lane 1), 2 pmol (lane 2) and 0.2 pmol (lane 3) of the reverse chimeric primer (D18S363R-URP) and 20 pmol of end-labelled URP.

AMPLICONS

Figure 3. Automated electrophoresis and detection of universal primer labelled PCR fragments. (A), (B) and (C) Amplicons of the (CA)n dinucleotide repeat locus D18S363 (GDB number 195027). (D), (E) and (F) Amplicons from the same three individuals at the (CA)n dinucleotide repeat locus D7S435 (GDB number X54666). Primer ratio used was 1:10 (forward chimeric primer:reverse primer) with incorporation of the universal forward primer end-labelled with TAMRA (ABI). (G) Selective partial digest of the phycocyanin amplicon (4) from *Microcystis wesenbergii* NIES111 using the restriction enzyme HaeIII. The amplicon was labelled by the described method with a chimeric:universal primer ratio of 1:10. The universal primer was labelled with TAMRA (ABI). Fragment sizes, in bp, are indicated for each peak.

Acrylamide gel electrophoresis and detection of the universal primer labelled PCR product was performed using an automated DNA fragment analyser (Pharmacia Biotech, Uppsala, Sweden) equipped with an external detector (automated imaging system: Molecular Dynamics, Sunnyvale, CA) using the scanner software in a semi-automated mode. Fragment sizes were determined by using size markers (Amersham, Buckleton, UK).

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REFERENCES