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Enhanced detection of tritium-labelled PCR products.

Due to the low energy β particle emission of Tritium(^3H), this isotope is not used for DNA labelling in Molecular Biology as the labelled products are impossible to detect. Currently ^{32}P is the isotope of choice for DNA labelling due to its high energy emission and thus short exposure times required for DNA detection. However, this high energy of ^{32}P also makes it hazardous, requiring special shielding for use and causing high exposure to others during gel and sample handling. Furthermore, the short half-life of this radionucleotide (^{32}P) often results in much of the product being wasted and labelled samples being reactive for short time spans, resulting in multiple labelling reactions having to be continuously carried out.

Enhanced Autoradiography was used to detect Tritium(^3H) labelled PCR amplimers from the Hras1 VNTR, a 28 bp repeat which is present in 30 to 100 copies. Following PCR amplification with deoxy(5- ^3H)Cytidine5'-triphosphate included in the reaction components, the products were electrophoresed through a 1% agarose midi gel and Southern blotted on to a nitrocellulose membrane (Amersham). The EA-Wax was then melted onto the membrane, and then exposed to film at -700C .

The products were clearly detected and indeed an additional band (labelled x), which had not been detected on the ethidium bromide stained gel, was apparent.

Advantages of Enhanced Autoradiography in this experiment:

1. This experiment indicated that DNA can be easily labelled with Tritium(^3H) and the signal now detected.
2. This experiment could also be extended to probe labelling for Southern Blotting and to products run on polyacrylamide gels.

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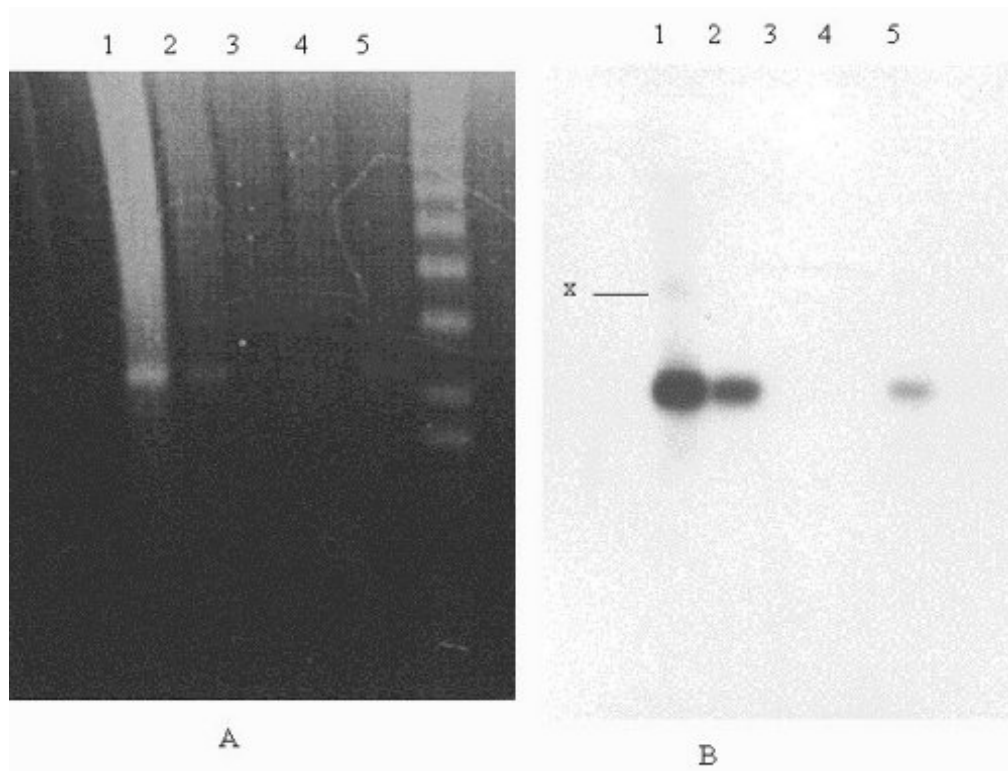


Figure 1. **Detection of PCR products by:** A. Ethidium Bromide staining and B. Enhanced Autoradiography. X indicates a band which was not detected on the Ethidium Bromide gel.