

Thesis Title Analysis of HLA Class II Gene Polymorphisms using
 Nonradioactive Probes

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ABSTRACT

We have established a nonradiative method of HLA class II genotyping and applied this to analysis of the central Thai population. We have investigated the use of non-isotopic methods for the detection of sequence specific oligonucleotide probes (SSOs) hybridized to PCR amplified products. Using the novel digoxigenin derivative DIG-11-ddUTP, labelled SSOs were hybridized to PCR amplified products which had been immobilized on Gene Screen membrane and UV - cross - linked.

Detection of hybridization of DIG-11-ddUTP - oligonucleotides can be accomplished using alkaline phosphatase coupled anti-DIG-FAB fragments and

chemiluminescent substrated Lumigen - PPD. The results can be stored on X-ray film and it also allows for multiple exposures over a 24 hours period.

The advantages of this technique, are that membranes can easily be stripped and re-probed at least 10 times, labelled probes can be stored at -20 °C and we have been able to re-use the hybridization solution containing the probe for up to 4 hybridization without much loss of signal.

DNA from 46 control panel cells was used to evaluate nonradioactive method of hybridization, and a further 106 unrelated individuals from a panel of 179 central Thais were used for analysis of DQA1 allele frequencies in this population using the primers and SSOs, the protocols of 11th International Histocompatibility Workshop (IHWs). The detection system was found to be as sensitive and reliable as ³²P-labelling of SSOs

The use of chemiluminescent system to detect hybridization of SSOs to PCR amplified DNA is a reliable , sensitive , specific , and safe method.