Improvement of IGCR technique using FRET

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ABSTRACT
The fluorescent detection system has been introduced into the study on denaturation/reassociation process of DNA fragments in gel, for improving In-Gel Competitive Reassociation technique, one of genome subtraction methods. The annealing behaviour of the mixture of 3'-Fluorescein-labelled and 5'-Cy5-labelled DNA fragments was analysed by Fluorescence Resonance Energy Transfer (FRET) technique from donor Fluorescein to acceptor Cy5. We showed that two fluorescent dyes labelled at 3' and 5' ends of DNA fragments caused FRET in both the solution and the gel. The characterisation of fluorescence-labelled fragments in gel and the changes of their fluorescence intensity will be reported.

INTRODUCTION
Genomic DNA subtraction methods (1) including In-Gel Competitive Reassociation (IGCR) technique (2,3) are not easy to handle and are relatively complicated and require good technical skills. This is because of the complexity of the human genome, which makes these methods difficult from the point of view of reproducibility and efficiency. And also, all gene-hunting methods require a lot of time, labour and money. IGCR technique, however, has significant advantages that enable the cloning of DNA fragments that differ between, usually closely related, target and control genomes without the need for specific probes. By improving the IGCR method, we want to overcome the problems associated with this technique making its utility comparable to that of the positional cloning. In addition, the impressive advances in resolution by the new version of IGCR have allowed for more precise and quantitative studies of genetic abnormalities.

We investigated a monitor system for fluorescence resonance energy transfer (FRET) that would be used for the IGCR technique, and the basic strategy for the FRET on a hybridisation was shown in Figure 1. Using this monitor, the fluorescent characterisation of labelled DNA fragments was analysed in the solution and the gel.

MATERIALS AND METHODS
We tested many probes and their combination for FRET study to improve IGCR technique and finally selected Fluorescein (Ex=494 nm, Em=517 nm) and Cy5 (Ex=647 nm, Em=665 nm). DNA fragments labelled by Fluorescein at 3' end or Cy5 at 5' end (20, 30 & 60 bp) were ordered and purchased from Amersham Pharmacia Biotech, Inc.

Figure 1. The strategy for FRET on IGCR technique.
Figure 2. FRET upon hybridization in solution for DNA oligomer (20 bases).

In Solution

The mixture of 5'-Fluorescein-labelled double helix DNA fragment (20 bp) and 3'-Cy5-labelled double helix (20 bp) which has the same sequences as the Fluorescein-labelled DNA was investigated. Figure 3 shows the time dependent changes in the emission of Fluorescein and Cy5 excited at 494 nm with the exposure of the gel containing the DNA mixture from the same denaturation buffer as that of IGCR (3) to the same reassociation buffer as that of IGCR (3). The increase of Cy5 fluorescence in the reassociation buffer indicates that the new heteroduplex of Fluorescein-labelled strand and Cy5-labelled strand was formed, resulting FRET between the two labels has occurred in the polyacrylamide gel. The characterization of other fragments in different sizes are in progress.

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REFERENCES