



Dr. F. PELLESTOR

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Dr. KRISHNAJA A.P.
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Dear Dr. Krishnaja:

Sorry for my delayed answer, I was off the last month.

Concerning the PRINS technique, I send you a copy of a further paper involving a detailed protocol for PRINS reaction on sperm and recent reprints.

We performed PRINS reactions on either thermocycler Hybaid Omnigene or Techne PCH-3 equipped with a plate block for 4 slides (Hybaid Limited, 111-113 Waldegrave road, Teddinton, Middlesex, TW11 8LL, U.K., Phone (44) 181 614 1000, Fax (44) 181 977 0170). In USA, you can also find a similar apparatus called "MISHA", with a block for 20 slides (Shandon Inc, 171 Industry Drive, Pittsburgh, PA 15275, Phone : 1-800-547-7429 or 1-412-788-1133).

You need imperatively to use a PCR machine with a flat plate block (type Techne PHC-3 or Hybaid Omnigene). Indeed, the total control of temperature (annealing and elongation) is essential to get an efficient PRINS reaction. You cannot obtain labelling on slides with a traditional PCR machine, because the temperature at the upper surface of the slides will never be the specific temperature you wish. The optimal conditions can slightly vary when using different equipment. Thus, the Hybaid machine integrates a simulated slide control process. This is important because the temperature at the upper surface of the slide may be 2 - 3°C lower than on the block.



On the other hand, you need to use highly purified primers. In case of the primer 21A, the nucleotide at the 3' end is specific to the alpha-satellite sequence of chromosome 21. A partial synthesis of sequences degenerated in 3' end will lead to the partial loss of the primer specificity.

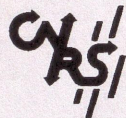
Up to now, the PRINS labeling technique works only for detection of repeat DNA sequences, such as alpha-satellite, satellite II or III and Alu sequences. To date, there are only few results concerning detection of unique sequences by PRINS. In particular, Troyer from Kansas State University, has successfully adapted PRINS for detection of unique sequences on porcine chromosomes (Cytogenet Cell Genet 1994; 67: 199-204 ; Mammalian Genome 1994; 5: 112-114).

I hope these suggestions will help you in your further PRINS experiments.

Good luck for your further experiments. Don't hesitate to contact me again for more information on PRINS.

Best wishes,

Dr. Franck Pellestor



CENTRE NATIONAL DE LA RECHERCHE SCIENTIFIQUE

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UPR 1142

Montpellier, 14. 08. 2000

Dear Dr Knishnaja,

Please find enclosed:

- a detailed PRINS protocol on sperm and lymphocytes. It is an unpublished manuscript.
- a copy of a recent paper with the sequences of all our primers.

Good luck for your PRINS experiments.

With my best wishes.

Franck PELLESTOR

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