G - 79

DETECTION OF *Mycobacterium tuberculosis* DNA IN CLINICAL SAMPLES PURIFIED BY SEPHAGLAS

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Tuberculosis is one of the most important infection disease in the world, particularly in developing countries. The tradicional methods to identify the illness are still of little sensitivity (direct detection of the organism in specimens) or very slowly growing (the culture of mycobacteria can take as long as 6 weeks). The detection of *M. tuberculosis* species in biological material by polymerase chain reaction (PCR) has been reported with success. However, despite the sucess of PCR to detect *M. tuberculosis DNA* directly in clinical specimens, the implementation of methods in routine laboratories for public health has not been easily incorporated. Problems like contamination, inhibition, laborius methods and expensive cust are not still totaly resolved. As the sensitivity of PCR is quite depend of a efficient preparation of specimen before amplification we have worked to find a method to purify the DNA from samples. We describe a method for preparation of diferents samples from patient including blood and sera for detection of *M. tuberculosis* by PCR analysis with sensitivity to detect after amplification about 3 mycobacteria in agarose gel. One set of 111 specimens were analysed (46 sputum, 3 urine, 2 feces, 14 spinal fluid, 16 blood and 30 sera). The PCR was done after two diferents extraction protocols. In both the bacteria was broken by heat and amplification was performed before and after of purification by sephaglas. The results were compared with traditional methods. Our results showed that it is possible to amplify *M. tuberculosis* of the diferent samples with exception of feces with both protocols. However, depending of the sample the amplification was only possible after purification by sephaglas. In some samples the sensitivity increased after the sephaglas step. Although the results open the possibility to analyse sera for the diagnosis of tuberculosis by PCR, several tests have to be done to standardize the method.

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G - 80

Sex determination in water-buffalo (*Bubalus bubalis*) using bovine (*Bos taurus*) probes in multiplex PCR system.

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Sex determination of preimplantation embryos has a major impact on animal breeding. Control of sex ratio in livestock would give rise to a more flexible breeding and production strategies. Numerous methods have been investigated to accomplish this task such as karyotyping, H-Y antigen and Y-specific probes. However, all of these methods have drawbacks of being insufficiently accurate or extremely time consuming. The PCR technology has been employed successfully for accurate and precise bovine sex determination. We have started to use multiplex PCR system also for sex determination in buffaloes (*Bubalus bubalis*). For this, buffalo DNA was purified from blood samples of both sexes harvested in EDTA vacuum tubes, using the classic phenol-chloroform protocol. The DNAs extracted were submitted to two PCR systems, the first one (multiplex PCR) using bovine 1715 satellite DNA specific oligonucleotides (5' TGGAAGCAAAGAACCCCGCT 3' and 5' TCGTGAGAAACCGCACACTG 3') and male specific BRY4a primers (5' CTCAGCAAAGCACACCAGAC 3' and 5' GAACTTTCAAGCAGCTGAGGC 3'), and the second system using male specific BRY.1 oligonucleotides (5' GGATCCGAGACACAGAACAGG 3' and 5' GCTAATCCATCCATCCTATAG 3'). The PCR were performed for 40 cycles (94°C, 1 min.; 55°C, 1 min.; 72°C, 1 min.). The amplified products, bovine satellite 216bp fragment and 301bp fragments yielded from both male specific primers sets, were detected on 2,5% agarose-gel by electrophoresis and ethidium-bromide staining. Two bands, 216bp and 301bp, were observed in male samples and only one 216bp band was seen for the female samples in the first system. In the second system, the fragment of 301bp was observed only in male samples. These data suggest that sexing method using PCR has a potential use for sex determination in buffalo embryos.

G - 81

ULTRASENSITIVE HUMAN THYROTROPIN (hTSH) IMMUNORADIOMETRIC ASSAY (IRMA) SET UP BY THE IDENTIFICATION AND MINIMIZATION OF NONSPECIFIC BINDINGS

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An IRMA of hTSH, based on magnetic solid phase separation, was studied to identify the origin and chemical nature of its nonspecific bindings (B_0), in order to be able to reduce this phenomenon and its negative effects and possibly improve the sensitivity rank of this type of assay.

These nonspecific effects were identified as a product of the interaction between an altered form of radioiodinated anti-hTSH monoclonal antibody (^{125}I-mAB) and the uncoupled magnetizable cellulose particle (matrix). Apparently this form of ^{125}I-mAB is a type of aggregate that can be partly resolved from the main peak on Sephadex G-200 and further minimized via a single preincubation with the same matrix. Solid phase saturation with milk proteins, tracer storage at 4^{0}C and serum addition during incubation was also found to be particularly effective in preventing its formation.

These findings were used in order to reproducibly decrease nonspecific bindings to values < 0.1% (or < 70 cpm), thus increasing the signal-to-noise ratio (B_{60}/B_0) up to values of 300-500. This way hTSH radioassays were obtained with functional sensitivities of about 0.05 mIU/L and analytical sensitivities of the order of 0.02 mIU/L, which are excellent for magnetic IRMAs. A more optimistic sensitivity calculation, based on Rodbard's definition, provided values down to 0.008 mIU/L. Such sensitivities, moreover, were obtained in a very reproducible way and all over the useful tracer life.

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G - 82-

DETECTION OF POTATO VIRUS Y STRAIN M BY DOT - BLOT HYBRIDIZATION USING PROBE PRODUCED BY POLYMERASE CHAIN REACTION (PCR)

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The PVY strain M (PVY^M) was isolated from *Capsicum annuum* plants (pepper) cultivar (cv.) Margareth in the Rio de Janeiro State. This cultivar is resistant to the PVY strains N, AT, F, FT and W. The purpose of this study is to produce specific probe to identify this strain. The replicative form of viral RNA (ds RNA) was isolated from leaf tissue of pepper plants infected by PVY^M. By PCR a fragment to 900 bp was obtained from ds RNA and radioactive probe were produced. The PVY^M was mechanically inoculated to pepper cv. Apolo, Carl Yolowonder, Hércules, Ikeda, Magda, Margareth, Myr 10 and PI 201232, and infection was monitored by Dot - Blot test. Only cv. Myr 10 was resistant to the viral strain. This probe can be utilized in the monitoring program for this viral strain.

G - 83

BIODEGRADATION OF ORGANOCHLORINATED COMPOUNDS.

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Every year hundreds of tons of organochlorinated compounds are spilled in the environment. Due to their properties such as recalcitrancy and toxicity, these compounds can be hazardous to flora, fauna and human beings.

The purpose of our research is to investigate the mechanisms involved in biodegradation of organochlorinated compounds, especially 4,5,6- Trichloroguaiacol (TCG). In the course of our work we have identified 9 types of aerobic bacteria isolated from pulp cellulose production effluent. These microorganisms exhibited the hability to grow in a concentration of TCG $3x10^4$ folds higher than that found in the effluent. Furthermore, some of these microorganisms were able to decrease the concentration of TCG when growing in rich medium.

Molecular analysis revealed the presence of plasmid DNA in bacteria from two of the isolated bacteria. We are now investigating the role of such plasmids in the process of biodegradation of TCG.

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G - 84

EVALUATION OF A CLASS OF RECOMBINATION DEFICIENT YEAST CELLS (*pso3-1*) AS HOSTS FOR ARTIFICIAL CHROMOSOME (YAC) LIBRARIES

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Yeast has proved to be an excellent host in which to clone large fragments of exogenous DNA as yeast artificial chromosomes (YACs). YACs contain all the DNA sequences necessary for yeast chromosome function and are mainly useful to construct large-scale physical maps of DNA, to walk along large segments of mammalian chromosomes, to examine structural and functional aspects of large genes. Experiences with libraries of human YAC clones has indicated that the most common artifact is the presence of clones containing two or more unrelated pieces of DNA (i.e. segments arising from different sites in the original genome).If undetected, these chimeric YAC clones will generate wrong results by above-mentioned methods. Therefore, currently available YAC libraries demand constant attention to the possibility that any particular YAC insert is chimeric. We investigated if the pso mutation (*pso3*), that when homozygous in diploids lowers the rate of gene conversion and mitotic recombination, has an effect on the rate of inter-YAC recombination. Several genetic crosses were made between the *pso3-1* mutant and YAC-containing strains and it was possible to obtain diploid homozygous YAC-containing *pso3-1* mutants. YAC in *pso3* mutant strains appear to be just as stable as those in w.t. strains. When we forced the loss of a YAC-contained gene in the *pso3-1* background, this did not occur via inter-YAC recombination, but rather by gene conversion, i.e. recombinational processes between YAC and yeast genomic DNA.

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