ISOLATION OF SPECIFIC CELLS FROM FORENSIC SAMPLES FOR HUMAN DNA TYPING

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DNA typing using Short Tandem Repeats (STRs) holds the greatest promise in forensic applications. A multiplex PCR can be coupled with the direct detection of amplified products using capillary electrophoresis.

Although PCR based technology is very sensitive, some difficulties are encountered when examining samples from dirty matrices. These problems can be attributed to the presence of PCR inhibitors in these samples. Some biological samples contain high amounts of PCR inhibitors such as bile salts (feces) and myoglobin (skeletal muscle).

We report here a study that describes the use of immunomagnetic cell-separation to isolate human cells from all kinds of matrices and mixtures with non-human biological materials. Since this method selectively purifies human cells, the problems with PCR inhibitors can be avoided. A slight modification of this technique allows to purify or remove specific cell types from mixtures with other cell types e.g., the specific isolation of epithelial cells of the suspect from a knife covered with blood from the victim, or sperm cells from vaginal epithelial cells.

The selection of human cells is based upon the use of immunomagnetic particles (Dynabeads®). These particles can be coated with a monoclonal antibody against a specific target. DNA from the cells isolated using the immunomagnetic particles was extracted using the Chelex® method.

We used this methodology successfully on a variety of samples such as blood, bloodstains, saliva and saliva stains (wiped off from pop cans using a sterile cotton swab). For these experiments a monoclonal anti-human HLA class I antigen mouse antibody (AbHLAI) was used. The paramagnetic beads were coated with a monoclonal antibody specific for Fe on all mouse IgG.

The samples (pure blood or saliva, or bloodstains or saliva on a sterile cotton swab) were, after an initial incubation with PBS/BSA buffer, incubated with the coated beads. After incubation the samples were placed in a magnetic separation device and the fluid was removed leaving the coated Dynabeads® with the cells bound to the AbHLAI. After some washing steps, Chelex® was added for DNA extraction. DNA was quantitated using Quantiblot® Human DNA quantitation kit. All samples gave good interpretable profiles after multiplex PCR and analysis on the ABI 310.

The binding of cells by the AbHLAI was confirmed by microscopic investigation under a light microscope. The average ratio of binding is 4 to 10 magnetic beads/cell. The amount of coated beads added allows to limit the maximum amount of cells isolated. The risk of adding too much DNA to the PCR reaction, which can disturb the PCR reaction, can so be eliminated.

In conclusion, the use of immunomagnetic particles can be very useful in isolation of cells from samples with a low amount of DNA and to avoid the presence of PCR inhibitors. Using specific immunoglobulins this methodology creates the possibility to avoid mixed profiles resulting from the mixture of different cell types (e.g. blood and epithelial skin cells). A great promise is the specific isolation of sperm cells from a vaginal rinsing in a rape case. Often there is a low amount of sperm and/or a lack of total separation of the sperm cells from the vaginal epithelial cells during DNA extraction.