Comparison of Chemiluminescent and Radioactive Methods of DNA Typing

L.R. Liss and G.R. Hudson

Promega Corporation, Madison, Wisconsin, U.S.A. 53711-5399

INTRODUCTION

DNA typing has the potential to completely replace the immunological and biochemical genetic tests that have been used over the past decade in parentage testing and forensic analysis. The method is easily performed, but there are several incubation periods which result in the procedure taking a number of days to complete. Nevertheless, DNA typing is attractive because: 1) it is a single technique, 2) with the exception that identical twins cannot be distinguished, conclusive results are always obtainable since the same blot of genomic DNA can be sequentially stripped and tested with different probes, 3) once a blot has been made, the reprobings are extremely quick and easy to perform, 4) it is cost-competitive with the other group of tests, 5) it is more sensitive than the other methods in that far less sample is required for the analysis.

RESULTS

The genomic DNAs used in this study were isolated via an organic extraction method. HaeIII restriction endonuclease (Promega Corporation) was used to at five units per microgram to completely digest human genomic DNA in five hour reactions.

Radioisotopic Detection

Following the electrophoresis of the digested genomic DNA in a 1X TEA, 1% agarose gel, a Southern blot was made. The transfer of the DNA profile resulting from electrophoresis was accomplished by a six hour alkaline transfer (GenePrint™ Technical Manual). It was found that overnight hybridization with ^{32}P-labeled YNH24 probe (D2S44) invariably led to an enhanced signal from the VNTR loci when compared to the results from a three hour hybridization. Nevertheless, it is important to note that the three hour hybridization always yielded a satisfactory result when five micrograms of genomic DNA per lane were being probed.

Advances in Forensic Haemogenetics 3
Edited by H. F. Polesky and W. R. Mayr

Nonradioisotopic Detection

Probe sequences labeled with digoxigenin-dUTP (Boehringer Mannheim) were hybridized to blots of genomic DNA. Detection was effected by the subsequent binding of anti-digoxigenin polyclonal antibody conjugated with alkaline phosphatase (Boehringer Mannheim), and the action of this enzyme on the substrate 5-bromo-4-chloro-3-indoyl phosphate (BCIP, Promega Corporation) in the presence of the enhancer nitro blue tetrazolium (NBT, Promega Corporation). The sensitivity of the method is such that it is necessary to have five micrograms of genomic DNA per lane to obtain a satisfactory result. Alkaline transfer methods were curiously incompatible with the nonradioisotopic procedure and a six hour neutral transfer method was utilized instead (Maniatis et al, 1982).

Chemiluminescent Detection

The use of a chemiluminescent alternative to the BCIP/NBT substrate/enhancer combination was recently described (Beck et al, 1989). The new substrate is a phosphorylated dioxetane which upon dephosphorylation by alkaline phosphatase forms an unstable intermediate. The intermediate decays emitting light over time in a pH and temperature dependent manner. It was unnecessary to alter the nonradioisotopic procedure until the last step where the phosphorylated dioxetane chemiluminescent substrate (PDCS) was used instead of the BCIP/NBT combination. Thus blots which had been previously probed and treated with the immunoglobulin-alkaline phosphatase conjugate were then immersed in a solution of PDCS for five minutes, sandwiched between sheets of thin, transparent plastic and placed in direct contact with X-ray film. The chemiluminescent method is faster and more sensitive than the colorimetric procedure.

DISCUSSION

The DNA typing procedure is evolving rapidly becoming faster, easier, cheaper, and more sensitive. Technological improvements will radically push the procedure toward these objectives.

Some general conclusions from this work are: 1) it was necessary to use a neutral transfer method in conjunction with the nonradioactive procedure. However, the overnight transfer was shortened to six hours with no apparent loss of signal. 2) depurination is unnecessary for the quantitative transfer of DNA with the size range of alleles detected by the probes used in this study. 3) the greatest source of variation is the membranes themselves. Membranes from the same lot, and more rarely different parts of the same membrane, showed a five-fold range of binding efficiency. 4) in the chemiluminescent method, steady state emission of light is not achieved for

three hours. During this "ramp-up" period an increasing amount of light is emitted. By the third hour a constant amount of light is emitted which persists for several days. A one hour exposure of a fully ramped blot clearly reveals the DNA type of a microgram or less of human gemomic DNA. 5) the sensitivity of the radioactive procedure is such that YNH24 alleles could be read from one microgram of human genomic DNA after overnight autoradiography. The nonradioisotopic method which used BCIP/NBT was found to be at least five-fold less sensitive (24 hour detection). The chemiluminescent method yielded an image in one hour that was comparable to that obtained from overnight autoradiography. 6) the chemiluminescent method described here could not be used to distinguish alleles in samples that contained much less than one microgram of genomic DNA. For although light is emitted for several days, the background light precludes any greater sensitivity with the system that was studied. Nevertheless, the steady state emission of light afor a long time allows the investigator to take multiple exposures as with autoradiography in order to secure the highest quality image.

SUMMARY

1) radiolabeled YNH24 probe can detect VNTR alleles in as little as 40 nanograms of genomic DNA following a Southern transfer to nylon, probing and prolonged (weeks) autoradiography.
2) the chemiluminescent procedure described here routinely detects YNH24-specific VNTR alleles in one microgram of genomic DNA after a single hour of imaging. The result is comparable in quality and sensitivity to that obtained from the analysis of the same amount of genomic DNA by overnight autoradiography.
3)compared to the radioactive and chemiluminescent methods, colorimetric detection with BCIP/NBT is less reproducible, less sensitive, and slower since it requires a 24 hour enzymatic reaction.
4) the chemiluminescent detection of alleles is quickly and easily done. The method is currently suitable for applications where as little as one microgram of genomic DNA is available to be run in a single lane of the analytical gel. Most significantly, the hazards of working with radioactivity and of its disposal are avoided.

REFERENCES

Beck, S., et al, (1989) Nucleic Acids Research 17(13):5115-5123

GenePrint™ DNA Typing: Technical Manual. Promega Corporation

Maniatis, T., et al, (1982) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor