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Detection of Highly Polymorphic Human CA Repeat Markers Using the Sequi-Gen® II PAGE Apparatus

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Introduction

Genetic linkage analysis has been used to identify the location of many major genes, for example, cystic fibrosis, myotonic dystrophy, and adenomatous polyposis gene (APC). In the past few years genetic linkage of inherited disease genes in families has been greatly facilitated by the availability of highly polymorphic microsatellites composed of di-, tri-, and tetra-nucleotide repeats as first described by Weber and May [(*Am. J. Hum. Genet.*, 44, 388–396 (1989)].

These tandem repeats are distributed randomly and occur at high frequency throughout the genome. These repeats also show a high level of individual variation in the number of repeat units. Heterozygosities in excess of 75% are common. However, the great advantage of these highly informative markers is that they are detectable by PCR* (polymerase chain reaction).

We have been interested in determining the chromosomal location of two inherited diseases, breast cancer and malignant melanoma. A number of families have been genotyped using PCR and microsatellite markers and these have been used in two point and multipoint linkage analysis. It is therefore possible to localize genes that may be important in the inherited predisposition of the disease, for example the BRCA1 gene on 17q [Easton, D. F. *et al. Am. J. Hum. Genet.*, in press].

Genes involved in the initiation and progression of tumorigenesis can also be identified by loss of heterozygosity (LOH) or allele loss studies. For example, the deleted colon cancer (DCC) gene was localized solely as a result of such studies [Fearon, E. R. et al., *Science*, **247**, 49-56 (1990)]. Detection of LOH is achieved by comparing tumor and normal DNA from the same individual using highly polymorphic markers (*e.g.*, microsatellite repeats). The DNA marker used has to be heterozygous in the normal DNA. Loss of one of the alleles (*i.e.*, hemizygosity) in the tumor DNA indicates loss of genetic material from one of the chromosome homologues. This loss of genetic material in the tumor is indicative of deletion of a tumor suppressor gene. The identification of microsatellite DNA markers that are highly polymorphic have therefore enabled thorough analysis and localization of genomic regions in which tumor suppressor genes may lie. These multi-allelic repeats can also be used to detect loss of heterozygosity in fresh as well as in archival material such as formalin fixed tissues embedded in wax or fresh tissue on microscopic slides.

Methods

Since the specific alleles amplified will differ by only one or two nucleotides, it is important to have a technique that will allow good separation of such closely spaced fragments. We have used the long, thin gel format of the Sequi-Gen II system to achieve such separation using either a non-denaturing or denaturing gel system.

PCR products (90-250 bp) are generated under standard conditions. For a denaturing gel (0.4 mm, 6% acrylamide, 5.5 M urea, 1x TBE), 3 µl of PCR product is added to 4 µl of deionized formamide and 1.5 µl of loading dye. The samples are preheated to 75 °C for 2 minutes before loading onto the gel. The samples are run for 2-3 hours at a constant power of 50 watts using a 21 x 50 cm Sequi-Gen II electrophoresis cell (1x TBE; running buffer).

Visualization of the PCR products is then usually performed by transferring the DNA from the gel (still on the glass plate) onto a Hybond N* nylon membrane, pre-wet with 10x TBE. 3M Whatman paper is layered on top of the nylon membrane and the DNA is transferred for 2 hours. After transfer, the membrane is soaked in 0.4 M NaOH for 20 minutes and washed in 5x SSC for an additional 5 minutes. Hybridization using one of the PCR products that has been radioactively end-labeled using terminal transferase occurs using an Amisine hybridization buffer (PEG and SDS) at 42 °C overnight. The membrane is washed once with 2x SSC, 0.1% SDS and exposed to X-ray film for 2-4 hours or overnight.

Alternatively, the PCR products are visualized by electrophoresis in non-denaturing polyacrylamide gels (0.4 mm, 6% acrylamide and 1x TBE) using a 21 x 50 cm Sequi-Gen II cell and staining with ethidium bromide.

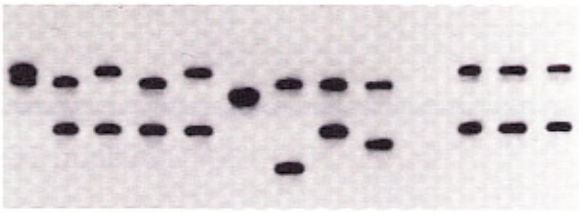

Fig. 1.

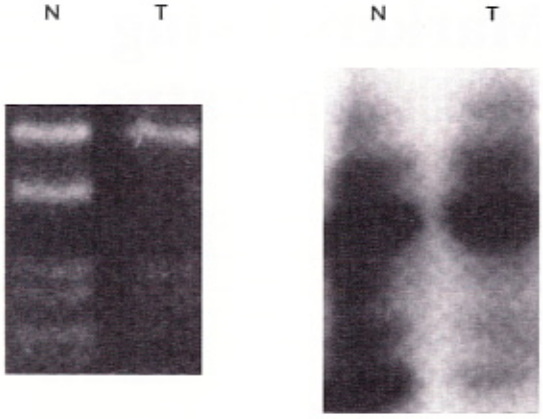

Fig. 2.

Results

Figure 1 shows PCR amplification of human polymorphic CA repeat sequence. PCR was used to amplify a specific chromosomal region of DNA from family members with malignant melanoma. The PCR samples were detected using one of the primers that had been end-labeled. Using this type of separation and highly informative markers, it is extremely easy to identify the individual alleles within the family.

Figure 2 shows LOH studies performed on DNA from a breast cancer patient as visualized by either ethidium bromide or radioactive labeling. This patient is constitutionally heterozygous for the marker used (lane N). In the tumor from this patient however (lane T) a substantial signal reduction of one of the two alleles can be observed. In this case the wild type allele is lost while the disease allele is retained indicating deletion of a tumor suppressor gene.

References

1. Screening of Multiple DNA Samples for Detection of Sequence Changes, Mashiyama, S., Sekiya, T., and Hayashi, K., *Technique*, **2**, 304 (1990).
2. PCR-SSCP: A Simple and Sensitive Method for Detection of Mutations in the Genomic DNA, Hayashi, K., *PCR Methods and Applications*, **1**, 34-38 (1991).

New Product Update

The Sequi-Gen II has been replaced with the new Sequi-Gen GT. All of the features that make the Sequi-Gen ideal for microsatellite and SSCP analysis have been kept in the new model. In addition, the new models offer a more convenient horizontal casting method and a size developed specifically for microsatellite and SSCP analysis (38 cm wide x 30 cm long). Please request bulletin 2006 for more information.

Ordering Information

Catalog Number	Product Description
Sequi-Gen GT Sequencing Systems	
165-3860	**Sequi-Gen GT System,** 21 x 40 cm**
165-3861	**Sequi-Gen GT System,** 21 x 50 cm**
165-3862	**Sequi-Gen GT System,** 38 x 30 cm**
165-3863	**Sequi-Gen GT System,** 38 x 50 cm**
165-3802	**Sequi-Gen GT/PowerPac 3000 System,** 21 x 40 cm, 100/120 V†
165-3805	**Sequi-Gen GT/PowerPac 3000 System,** 21 x 40 cm, 220/240 V†
165-3803	**Sequi-Gen GT/PowerPac 3000 System,** 21 x 50 cm, 100/120 V†
165-3806	**Sequi-Gen GT/PowerPac 3000 System,** 21 x 50 cm, 220/240 V†
165-3810	**Sequi-Gen GT/PowerPac 3000 System,** 38 x 30 cm, 100/120 V†
165-3811	**Sequi-Gen GT/PowerPac 3000 System,** 38 x 30 cm, 220/240 V†
165-3804	**Sequi-Gen GT/PowerPac 3000 System,** 38 x 50 cm, 100/120 V†
165-3807	**Sequi-Gen GT/PowerPac 3000 System,** 38 x 50 cm, 220/240 V†

* PCR is covered by U.S. patent number 4,683,202 issued to Cetus Corp.

** Sequi-Gen GT systems include GT IPC assembly (IPC and bonded inner glass plate, outer glass plate, and clamp set), GT universal base, GT safety covers with cables, stabilizer bar, precision caster assembly (precision caster base, gasket, tubing, luer tapers, tubing, and syringe), 0.40 mm vinyl sharkstooth comb and spacers, gel temperature indicator, leveling bubble, drain port connector, and instruction manual.

† Sequi-Gen GT/PowerPac 3000 systems include the appropriate Sequi-Gen GT system, PowerPac 3000 Power Supply, PowerPac temperature probe, and PowerPac instruction manual.

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