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Microsatellite Analysis of Colorectal Tumor DNA using the Sequi-Gen® GT System

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Microsatellites are short repetitive sequences spread over the entire genome. Due to high levels of allelic diversity, microsatellites are analyzed in paternity and forensic tests as well as in molecular oncology. The genome of healthy persons normally contains two copies of each genomic segment (one paternal and one maternal allele). This situation is called heterozygous. Many tumors, especially colorectal cancers, show losses of genomic segments when matched specimens of normal and tumor tissue are compared. Defects leading to deletions in one of the two alleles are termed loss of heterozygosity[1] (LOH). In a number of studies, specific LOHs were described for different tumors indicating that most cancers have characteristic LOH patterns.

In addition to LOH, another process involved in tumorigenesis, microsatellite instability[2,3,4] (MIN), has been described. The characteristic feature of MIN, expansions or contractions in the short repeated sequence motifs, can easily be detected by polyacrylamide gel electrophoresis. This paper describes a method to analyze microsatellite instability in colorectal tumor DNA using the Sequi-Gen GT system combined with silver staining.

Tumor DNA Isolation from Formalin Fixed PET

- Prepare 5 µm sections from routine paraffin embedded tissue (PET) material on microscopic slides (in parallel, DNA from a matched normal tissue or DNA from peripheral blood monocytic cells must be analyzed).
- Incubate the slides for 1 hour at 65 °C.
- Incubate the slides 2 x 15 minutes in xylene, 2 x 15 minutes in 100% ethanol, 2 x 15 minutes in 96% ethanol, and 2 x 15 minutes in 70% ethanol.
- Put the slide into a water filled cuvette, stain with hematoxilin/eosine, and use this section for microdissection.
- Add 50 µl digestion buffer (50 mM Tris-HCl, pH 7.5; 5% Tween 20; 1 mM EDTA; 300 µg proteinase K) per cm^2 microdissected tissue and incubate the samples at 50 °C until tissue lysis is observed.
- Inactivate proteinase K by incubating the samples at 94 °C for 15 minutes.

Table 1. Master-Mix for 1 Reaction (rc)

	µl	Final Concentration	Stock Solution
H_2O	34.3		
DMSO	2.5	5 %	100 %
10x Taq Reaction buffer incl. $MgCl_2$	5	1x	10x
dNTPs	4	0.2 mM	2.5 mM
Upstream primer†	1	0.3 µM	15 µM
Downstream primer†	1	0.3 µM	15 µM
Taq-DNA-Polymerase	0.2	0.02 U/µl	5 U/µl
Total	**48**		

† BAT26, BAT40, D2S123, D17S250, D5S346 (loci to be analyzed for MIN study in colorectal cancer)

PCR* Amplification

- Add 48 µl PCR master-mix to 2 µl DNA template (corresponding to about 50 ng DNA) according to the scheme shown in Table 1.
- Amplify microsatellites by PCR (usually 30 cycles: 1 minute 94 °C/ 1 minute 50–60 °C depending on primers/1 minute 72 °C, final elongation:7 minutes 72 °C).

Gel Electrophoresis

Separate amplified fragments by denaturing polyacrylamide gel electrophoresis.

- Prepare the 6.7% polyacrylamide (19:1 crosslinked) 7M urea solution by combining 21 g urea; 8.4 ml 40 % acrylamide (19:1 acrylamide:bis-acrylamide) solution; 5 ml 10x TBE, and dd H2O to 50 ml. Immediately prior to casting the gel add 400 µl 10% ammonium persulfate and 50 µl TEMED.
- Assemble the Sequi-Gen GT apparatus (both the 21 x 40 and the 38 x 30 units work well) using 0.4 mm spacers and cast the gel according to the instruction manual.
- Allow gel to polymerize for 1 hour at room temperature.
- Pre-run for 15 minutes at 2,300 V until a gel temperature of 55 °C is reached.
- Meanwhile, pipet 3 µl PCR-product to 3 µl loading buffer (formamide containing 0.1% xylene cyanol, 0.1% bromophenol blue, 10 mM EDTA) and denature samples 5 minutes at 94 °C.
- Load denatured samples onto pre-run gel.
- Let the gel run at 50 °C (2,000 V, 400 mA) until the xylene cyanol has migrated 25 cm.

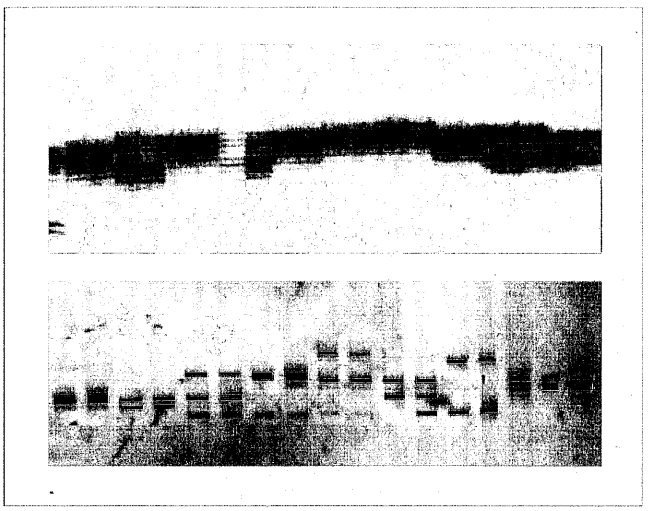
Allele Detection by Silver Staining

After the run, disassemble the Sequi-Gen GT unit, and silver stain the gel with Silver Stain Plus or a similar product. Take care to prevent the gel from sliding off the glass plate and into the staining tank. We have made a lucite frame which surrounds the gel, holding it in place as staining solutions are poured on and off.

After staining, carefully place a piece of filter paper onto the gel. Separate the filter paper with the attached gel from the glass plate, cover the gel with plastic film, and dry the gel in a gel dryer.The results of MIN assessments of two microsatellite loci are shown in Figure 1. The dried gels can be stored for years, and reamplification of excised bands even years later is possible.

Summary

Microsatellite detection by silver staining after separation on the Sequi-Gen GT nucleic acid electrophoresis cell is a convenient, non-radioactive method. It is facilitated by the lucite frame, which keeps the polyacrylamide gel attached to the glass plate. This method allows microsatellite investigation in any standard laboratory, and eliminates the problems associated with radioactive waste disposal. Furthermore, this method provides the most accurate way to excise bands directly from even long-term stored gels and allows easy reamplification of eluted bands.



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