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Loss of Heterozygosity Detection with GeneMarker®

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Introduction

Loss of Heterozygosity (LOH) occurs when a somatic cell contains only one copy of an allele due to non-disjunction during mitosis, segregation during recombination, or deletion of a chromosome segment. LOH becomes critical when the remaining allele contains a point mutation that renders the gene inactive. This is a common occurrence in cancers where a tumor suppressor gene is affected. Tumor suppressor genes code for proteins that regulate the cell's life cycle. Thus, they are critical in preventing tumor formation. The human retinoblastoma (pRb) was the first tumor suppressor protein found to be dysfunctional in a number of types of cancer **(1)**. Another protein, tumor protein 53 (TP53), is central to many of the cell's anti-cancer mechanisms. It plays a role in apoptosis, genetic stability, and inhibition of angiogenesis **(2)**. It has also been found that TP53 is involved in the DNA repair support function of pRb, thus linking these two cancer regulating pathways **(3)**.

Recently, a vaccine that prevents women from contracting human papillomavirus (HPV) has been developed for use in the United States **(4)**. The significance of this vaccine is that it has the potential to prevent cervical cancer. Cells infected with HPV produce oncogenic proteins that can bind with and inactivate the pRb protein. Since one of the major functions of the pRb protein is to prevent cells from dividing it is a key factor in regulating the cell cycle. Without this specific protein, a cell will continue to divide and become cancerous **(5)**. Examples of other major cancers caused by loss of heterozygosity include breast, ovarian, and colorectal cancer. **(6, 7, 8)**

GeneMarker fragment analysis software has been developed to aid researchers and clinicians in the detection of LOH within cancer cells. Using a unique allele calling algorithm, GeneMarker uses the germ line reference to compare and detect LOH in patient samples.

Procedure

1. Create a tab-delimited text file (in GeneMarker's **Filename Group Tool**) that pairs reference trace filenames with patient trace filenames
2. Upload LOH data
3. Use the **Run Wizard** *Animal Fragment Analysis* parameter settings to call alleles
4. Create a panel with the **Panel Editor** tool and **Run Wizard** again to compare sized data to panel
5. Select **LOH Analysis** from the **Applications** menu to open the *LOH Analysis Settings* box
6. Upload paired filename text file to the *Group File* field
7. Select ratio analysis type (peak height or peak area) and set the LOH ratio parameters
    a. Default: *LOH < 0.70 > 1.30*
8. Click *OK* and the LOH main analysis window appears
9. Click **Print** icon in the main toolbar to print the *LOH Clinical Report*

Results

The concept for determining LOH is the comparison of a suspect tissue sample to a normal tissue sample with all expected alleles. In GeneMarker, a patient trace is compared to a reference trace. Samples with loss of heterozygosity are immediately apparent in two displays – the electropherogram and the ratio plot **(Fig. 1)**. In the electropherogram, the reference trace is in light red behind the patient trace. When a peak is absent in the patient trace, only the reference trace peak remains. In the ratio plot, the absence of a patient trace peak is indicated by a red dot that falls outside the user-defined bounds of the LOH ratio (indicated by dashed lines). Blue dots within the LOH ratio bounds indicate a patient trace with the expected number of peaks.

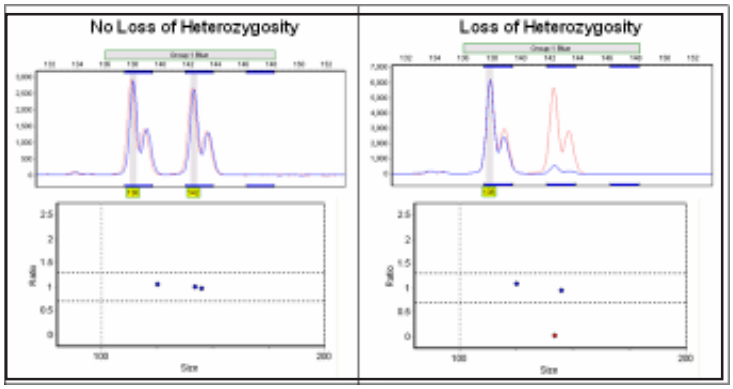

Figure 1. Example of LOH trace and ratio plots.

In addition to the electropherogram and ratio plot, a clinician can use the LOH score parameter in the report table to qualify a given marker. LOH score is calculated using the peak intensity or area ratio between normal and tumor samples. For complementary peaks in both reference and sample trace the score will equal 1.0. In the case of complete loss of heterozygosity at a given position, the LOH score will be 0.0.

Discussion

Loss of heterozygosity detection is a key component in distinguishing cancerous from non-cancerous tissue. The ability to do this type of analysis quickly and accurately is an extreme advantage to clinicians determined to fight the disease. In addition to GeneMarker's LOH analysis tool, an easy-to-read report can be printed for clinical review. The LOH Clinical Report includes patient/reference traces, ratio plots and a table of peak statistics.

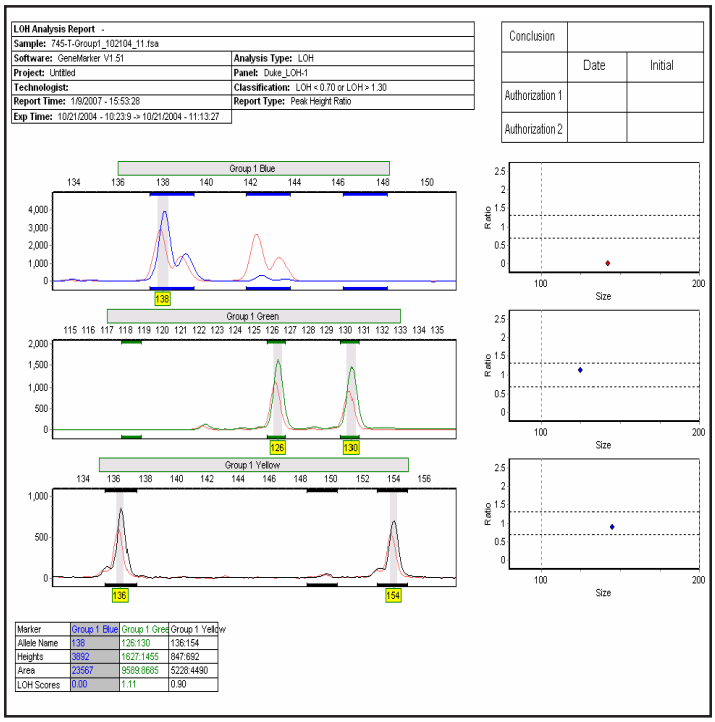

Figure 2. LOH Clinical Report

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