

Assaying for Loss of Heterozygosity in Tumor Samples Using Relative Fluorescent Quantitation on Capillary Electrophoresis Systems



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ABSTRACT

Several capillary electrophoresis based fragment analysis applications require comparison of peak heights across samples as a relative quantitation method. Screening for Loss of Heterozygosity (LOH), Microsatellite Instability (MSI) and detection of chromosomal deletions and duplications are typical examples of such applications. Optimized chemistries, a robust and reliable electrophoresis platform as well as accurate analysis software are all essential to the success of these assays. In this study we use a LOH assay to demonstrate relative quantitation using capillary electrophoresis. Microsatellite markers were run on the Applied Biosystems 3130x/ Genetic Analyzer and peak heights were compared across paired samples from tumor and healthy tissues. Using GeneMapper® v3.7 Software samples were identified as LOH candidates based on a threshold enabling rapid identification for downstream review. Our results highlight the 3130 Series Systems in conjunction with GeneMapper® Software as an optimal solution for relative fluorescent quantitation assays.

INTRODUCTION

LOH Assay

In the two-hit model used to describe Tumor suppressor gene (TSG) inactivation, the first mutation or "hit" results in a heterozygous state for the TSG with one wild-type allele and one mutant allele. If a second hit (deletion) follows, the result is the loss of the wild-type allele also known as Loss of Heterozygosity (LOH). LOH has been shown to occur in a number of different chromosomal regions and screening for LOH has demonstrated reliability in the early detection in cancers such as bladder cancer as well as for LOH/MSI classification of colon tumors. In this poster data from LOH assays performed on bladder cancer samples using paired DNA samples from urine pellet and blood will be presented. Two sets of multiplex reactions that each amplify eight microsatellite markers were run on the 3130x/ Genetic Analyzer and the data analyzed in GeneMapper software v3.7. We will highlight the use of the results in early detection and follow-up of bladder cancer.

MATERIALS AND METHODS

Two sets of 8 microsatellite markers were amplified using the conditions described below. For more information on the markers please contact Professor Oudet.

PCR conditions for amplification of 8 microsatellite markers:

- **DNA = *10 to 20ng
- Taq Gold = 5 units
- Betaine = 1M
- Labeled primer#1 = 0.08µM (for each marker)
- Unlabeled primer #2 = 0.08µM (for each marker)

*Based on PicoGreen ds DNA Quantification Kit

**Each sample was run in duplicate. These conditions allow high reproducibility of Allele ratio and the Allelic Imbalance.

Electrophoresis on the 3130x/ Genetic Analyzer

- PCR product (Diluted 1:8) = 2 µl
- GeneScan™ 500LIZ® size std = 0.1 µl
- HiDi™ Formamide = 7.9 µl

Samples were denatured at 95°C for 5 minutes, chilled to 4°C and run on a 36cm capillary array with POP-7™ polymer. The standard run module FragmentAnalysis36_POP7 and dye set G5 were used.

RESULTS

Multiplex reaction of 8 microsatellite markers

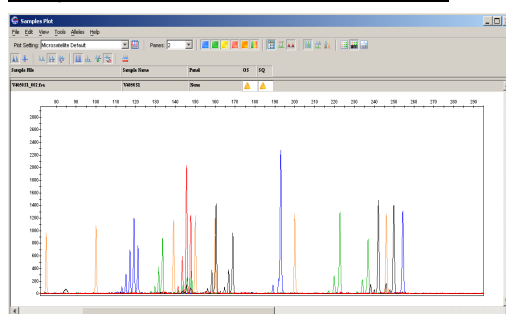


Figure 1. Five-dye electropherogram of 8 microsatellite markers run on the 3130x/ Genetic Analyzer. The markers were amplified using one of four dyes FAM™, VIC®, NED™ and PET™ (shown by the blue, green, yellow and red peaks). The GS500LIZ size standard is shown by the orange peaks.

Professor Oudet's lab has developed an advanced and automated system for QC of amplification based on parameters such as checking of duplicates between each other, homology of amplification of urine and leucocytes, peak height and resolution. Please contact his laboratory for more information.

Data Analysis with GeneMapper software

The GeneMapper software or higher provides advanced data analysis management features that enable precise fragment sizing and accurate allele scoring in an automated fashion.

Allele calling in GeneMapper software v3.7

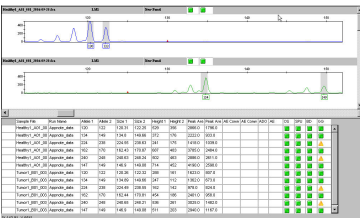


Figure 2. Electropherogram of a microsatellite marker from a normal (top panel) and tumor sample (bottom panel).

The Report Manager Feature

Analysis software that can accurately and automatically score samples for LOH is critical to the successful completion of the assay. The new report manager feature in GeneMapper software enables users to perform customized multi-step calculations and generate a final report that flags candidate LOH samples.

Report Manager feature for performing relative peak height comparisons

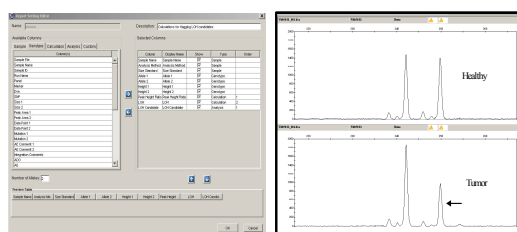


Figure 3. The report manager (left panel) in GeneMapper software can be used to compare peak height ratios of healthy and tumor samples (right panel)

RESULTS

Multi-step calculations using the Report Manager

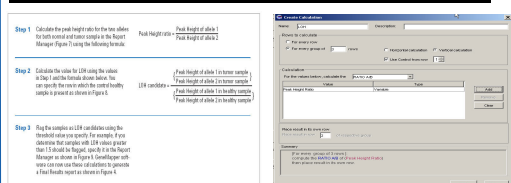


Figure 4. Calculations shown on the left panel were set up in the report manager (right panel) to identify and flag LOH candidate samples. The calculation show here is an example of one type of calculation that can be used. Users can set up the report manager for whatever calculation they want to specify.

Final report with LOH candidates flagged for review

Sample Name	Marker	Allele 1	Allele 2	Size 1	Size 2	Height 1	Height 2	peak H ratio	comparison	LOH cand.
1	JMI1	1	120	122	120.31	122.25	529	356	1.486	
2	JMI1	1	120	122	120.26	122.32	288	191	1.508	1.2038
3	JMI1	2	134	149	134.89	149.86	372	178	2.118	1.0434
4	JMI1	2	134	149	134.89	149.86	247	112	2.2054	1.0434
5	JMI1	3	224	238	224.55	238.83	241	175	1.3771	
6	JMI1	3	224	238	224.49	238.95	162	142	1.1426	0.0304
7	JMI1	4	192	170	192.42	170.87	887	463	1.9224	
8	JMI1	4	192	170	192.44	170.81	454	186	2.4489	1.7161 candidate
9	JMI1	5	240	248	240.63	248.24	502	463	1.0842	
10	JMI1	5	240	248	240.65	248.21	536	261	2.0536	1.8941 candidate
11	JMI1	6	147	149	148.9	149.06	174	452	1.5786	
12	JMI1	6	147	149	148.9	149.06	511	303	2.6732	1.8935 candidate

Figure 5. With the report manager samples with cut-off can be quickly identified. The cut-off was statistically determined on a marker base. The presence of 2 or more markers that showed allelic imbalance was indicative of the presence of cellular clones. This report can be printed or exported for downstream analysis and further review.

It should be noted that Professor Oudet's lab uses an advanced calculation method for calculating allelic imbalance. Refer to Schneider et al., cancer research 60, 4617-4622, August 15, 2000 for more information.

CONCLUSIONS

The Applied Biosystems 3130 series Genetic Analyzers in conjunction with GeneMapper software v3.7 provides an optimal solution for performing relative fluorescent quantitation assays such as LOH. Users have a complete system starting with electrophoresis, data collection, fragment size calling to final data analysis with the generation of a results report where LOH candidates are flagged.

Highlights of the systems are:

- Robust reliable instrument platform. Compared to a gel based system the Capillary Electrophoresis system offers features such as ease of use, automation and simple maintenance procedures.
- Streamlined workflow from assay to analysis that comes from integration of the instrument platform with GeneMapper software
- Reduced assay times. In a span of 4.5 hours an assay can be performed on 12 patient DNAs (Please contact Professor Oudet for more information)
- Short run times resulting in faster turnaround times and therefore increased throughput
- Rapid data analysis facilitated by software features such as the report manager, Quality Values and remote auto-analysis via network

TRADEMARKS/LICENSES

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