

Microsatellite-instability testing using the QIAxcel[®] system

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The QIAxcel system was successfully used for microsatellite-instability testing in biomedical research using 3 mononucleotide markers with normal and tumor tissue from colon.

Introduction

Microsatellites are repeated DNA sequences that are commonly found in normal cells. In cells with mutations in DNA repair genes, some microsatellite sequences accumulate errors and become longer or shorter. The presence of abnormally long or short microsatellites is referred to as microsatellite instability (MSI). Detection of microsatellite instability in tumor cells suggests mutation in a DNA mismatch repair gene. For example, MSI is detected in more than 90% of colon cancers arising in patients with hereditary non-polyposis colorectal cancer (HNPCC) and in only 10–15% of sporadic colorectal carcinomas (1). Testing samples for MSI can help determine whether genetic testing for HNPCC is appropriate.

Materials and methods

DNA was extracted from formalin-fixed, paraffin-embedded sections of normal and tumor tissue from colon using the QIAamp[®] DNA FFPE Tissue Kit. MSI analysis was performed using a panel of 3 mononucleotide markers: BAT-25, BAT-26, and NR27 (2, 3); primer sequences are provided in Table 1. Amplification reactions (20 µl) were prepared with 10–100 ng extracted DNA, 1 × PCR Buffer, 1.5 mM MgCl₂, 0.25 mM dNTP, 0.5 µM of each primer, and 0.5 units Platinum[®] Taq DNA Polymerase (Invitrogen). Reactions were subjected to PCR amplification: initial incubation at 95°C for 5 minutes, followed by 40 cycles of 95°C for 30 seconds, 54°C for 45 seconds, and 72°C for 60 seconds, and a final incubation at 72°C for 10 minutes.

Table 1. Oligonucleotide primers used for microsatellite analysis

Marker	Gene	Primer sequences	Product size (bp)
BAT-25	KIT	5'-CTCGCCTCCAAGAATGTAAGT-3' 5'-CTATGGCTCTAAAATGCTCTGTTC-3'	114
BAT-26	MSH2	5'-TGACTACTTTTGACTTCAGCC-3' 5'-AACCATTCAACATTTTAAACCC-3'	122
NR-27	BIRC3	5'-AACCATGCTTGCAAACCACT-3' 5'-CGATAATACTAGCAATGACC-3'	89

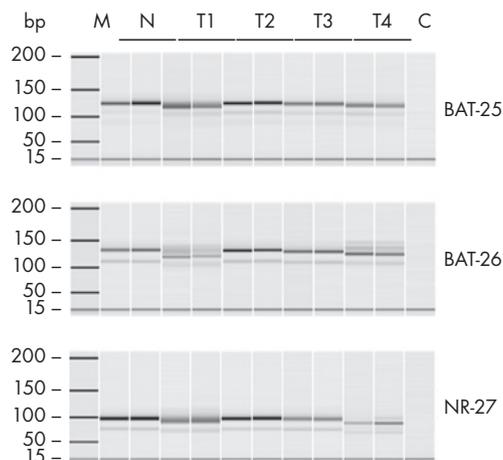


Figure 1. Rapid determination of microsatellite instability using the QIAxcel System. Duplicate samples were prepared from normal colon tissue (N) and colorectal tumors (T1–T4). PCR products were analyzed using the QIAxcel DNA Screening Kit. MSI-H tumors (T1 and T4) exhibited allelic shifts in all 3 microsatellite markers. MSS tumors (T2 and T3) did not exhibit allelic shift. M: 50 bp ladder, C: Negative control.

PCR products were analyzed on the QIAxcel system using the QIAxcel DNA Screening Kit and QX Alignment Marker 15 bp/400 bp. Separation was performed with a customized protocol at 2 KV voltage using 40 second (samples) and 30 second (alignment marker) injection time and 700 second separation time. Samples were classified as “MSI high” (MSI-H) when more than one of the analyzed markers was unstable, “MSI low” (MSI-L) when only one microsatellite locus was unstable, and “microsatellite stable” (MSS) when changes in microsatellites could not be detected.

Results and discussion

The QIAxcel system was evaluated for microsatellite-instability analysis using 3 mononucleotide markers (BAT25, BAT26, and NR27). A typical gel image is presented in Figure 1. The QIAxcel capillary electrophoresis system provided a rapid and effective screening method for assessing MSI in tissue samples for biomedical research. The QIAxcel reduced the labor required after PCR amplification by decreasing the time to result for 11 samples to 30 minutes, compared to 4 hours using standard polyacrylamide gel electrophoresis.

Conclusions

- The QIAxcel system was successfully used for MSI testing in biomedical research.
- Using the QIAxcel system for MSI testing is faster and more cost-effective than using standard polyacrylamide gel electrophoresis.

References

1. Aaltonen, L.A., et al. (1998) Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. *New Engl. J. Med.* **338**, 1481.
2. Boland, C.R., et al. (1998) A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res.* **58**, 5248.
3. Nardon E., et al. (2010) A multicenter study to validate the reproducibility of MSI testing with a panel of 5 quasimonomorphic mononucleotide repeats. *Diagnostic Mol. Pathol.* **19**, 236.

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