



Elchrom Scientific

Detection of APC Gene Mutations on Precast Spreadex[®] Gels

Application Note

Spreadex[®] Gels
SEA 2000[®] Apparatus

INTRODUCTION

Familial adenomatous polyposis (FAP) is an autosomal inherited disease, affecting 1 person in 2000. Patients with FAP develop hundreds to thousands of polyps in the colon and rectum during their second and third decade, and the polyps often progress to cancer. Because FAP patients have a high risk of colorectal cancer, it is important to test members of affected families. The presymptomatic diagnosis relies on detection of inherited germ line mutations in the adenomatous polyposis coli (APC) gene (1).

In the APC gene, the majority of deletions and insertions were detected at positions containing repetitive sequences within the coding region. The most frequent germ line mutations include deletion of the 5 bp sequence AAAAG at codon 1307-1311, and mutations in the AAAACAAAA sequence at codon 1060-1063. Other mutations occur in the region near or within the repetitive sequence at codons 1156 and 1564.

Mutations in the APC gene were analyzed by electrophoresis on Elchrom precast Spreadex gels that were run in the SEA 2000 apparatus.

Advantages of the method used here include:

- Fast analysis, only 85 min is sufficient to complete the electrophoresis
- Full reproducibility, due to precast gels and precise temperature control in the SEA 2000 apparatus
- High throughput, as up to 50 samples can be run on one gel
- Precise size estimation (within 1 bp), for checking the length of amplified DNA
- Simple procedure, with Mini gels and SYBR Gold staining

RESULTS

Figure 1 shows the results obtained by analysis of 5 samples on a Spreadex EL 300 S-2x13 gel. Thanks to high resolving power of the Spreadex gel, amplified DNA fragments in all lanes are fully resolved, including the 86 and 91 bp pair (lane 2).

It is important to analyze several regions of the APC gene. Apparently there exists a correlation between position of the mutation and phenotypic manifestation of the disease. FAP patients of profuse type develop over 5000 polyps, while those of the sparse type develop fewer than 5000 polyps. The patients affected by a mutation in the APC gene between codons 1250 and 1464 tend to be of the profuse phenotype (2).

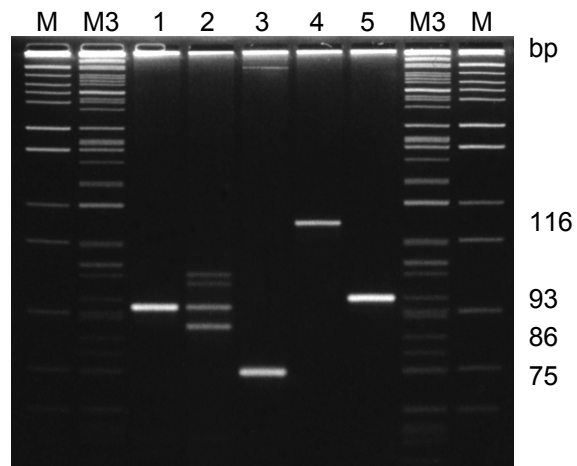


Figure 1. Analysis of APC gene mutations on a Spreadex EL 300 Wide Mini S-2x13 gel. Lanes: 1 - codon 1309 negative control (91 bp); 2 - codon 1309 positive control (91 bp normal allele and 86 bp mutated allele); 3 - codon 1061 negative control (75 bp); 4 - codon 1546 negative control (116 bp); 5 - codon 1156 negative control (93 bp); M-Elchrom M1 Marker; M3-Elchrom M3 marker. As expected, the heterozygous sample in lane 2 shows two heteroduplex bands (above the 91 bp band). Note the perfect match of the 93 bp band from the sample in lane 5 and the 93 bp band from the M3 marker.

Experimental Procedures

Equipment

Electrophoresis was performed in Elchrom's SEA 2000 submarine electrophoresis apparatus. Temperature of the running buffer was kept constant at 55°C by connecting the SEA 2000 to a circulating water bath equipped with an external temperature probe. The probe is inserted through the lid of the SEA 2000 into the running buffer. The bath automatically makes adjustments in the temperature of the circulating water in order to keep the buffer temperature at a desired value during electrophoresis.

Sample preparation

PCR samples were generated according to standard protocols. A 1.5 µl portion of each sample was mixed with 5 µl of diluted (1:3) loading buffer. The loading buffer, 1 ml (5X), is provided with every box of Elchrom precast gels. The samples (6 µl) were loaded to a Spreadex EL 300 gel.

Running Conditions

The running buffer in the SEA 2000 apparatus was heated to 55°C before one of the Spreadex EL 300 Wide Mini S-2x13 gels was placed in the apparatus. Heating of the buffer takes about 30 min. The Spreadex gel shown in Figure 1 was run at 10 V/cm (120 V) for 85 min at 55°C.

Detection

The Spreadex gel was stained with SYBR Gold in Elchrom's Easy Stain Tray, destained and then photographed.

References

1. Ando, H., Miyoshi, Y., Nagase, H., Baba, S., and Nakamura, Y. (1993) Detection of 12 germ-line mutations in the adenomatous polyposis coli gene by polymerase chain reaction. *Gastroenterology*, 104, 989-993.
2. Miyaki, M., Tanaka, K., Kikuchi-Yanoshita, R., Muraoka, M. and Konishi, M. (1995) Familial polyposis: recent advances. *Critical Reviews in Oncology/Hematology* 19, 1-31.

Acknowledgement

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ORDERING INFORMATION

Product	P/N
Basic Submarine Electrophoresis System	2031E
SEA 2000 Electrophoresis Apparatus	2001E
Power Supply 200 V/2000 mA	2029E
Spreadex EL 300 Mini Gels (10 gels)	3413
Spreadex EL 300 Wide Mini S-2x13 (12 gels)	3426
Spreadex EL 300 Wide Mini S-2x25 (12gels)	3442
Spreadex EL 300 Wide Mini S-4x13 (24 gels)	3452
Spreadex EL 300 Wide Mini S-4x25 (24 gels)	3492
40X TAE Running Buffer (20 tubes of 50 ml)	3031
Destaining Solution (DST) 100 X (50 ml)	3037

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©Elchrom Scientific AG, Gewerbestrasse 8, 6330 Cham, Switzerland, Phone +41 41 747 2550, Fax +41 41 743 2536 e-mail: service@elchrom.com; www.elchrom.com

