Elchrom Scientific
Excellence in Electrophoresis

Application Note

SSCP Analysis of CYP2D6 Mutations

GMA™ Gels

SEA 2000® Electrophoresis System

Introduction

The single strand conformational polymorphism (SSCP) method relies on different migration rates of single stranded DNA fragments in a non-denaturing gel (1, 2). Two single stranded DNA fragments whose sequence differs in just one base often migrate at sufficiently different rates to allow separation of the two bands. The success of an SSCP analysis critically depends on maintenance of a low constant temperature during electrophoresis (1-3).

The CYP2D6, along with other cytochrome P450 enzymes, appears to play a role in metabolism of many therapeutic agents, as well as in metabolism of various environmental compounds including some carcinogens. Genetic polymorphism of the CYP2D6 gene is well characterized, allowing the use of genotyping for examining the correlation between specific alleles and certain diseases (4, 5).

Elchrom's GMA™ (Gene Mutation Analysis) gels have been optimized for detecting DNA mutations by SSCP. The gels are further optimized for electrophoresis in the submarine mode using Elchrom's SEA 2000 apparatus.

Advantages of the method used here include:

- Full reproducibility, thanks to precast GMA gels and maintenance of the buffer temperature within 0.1°C in the SEA 2000 apparatus
- High throughput, as up to 50 samples can be run on one gel
- Simple procedure with SYBR Gold instead of silver staining or the use of radiocative labels

Results

Figure 1 shows the results obtained by SSCP analysis of 6 human DNA samples. The samples were obtained by PCR amplification using standard procedures. Purity of the amplified fragments was checked by electrophoresis on a Spreadex® EL 600 gel (not shown). It is important to use only single-band PCR products for SSCP, because the presence of unspecific amplification products may cause misinterpretation of the SSCP results. As can be seen from Figure 1, the mutant samples are easily distinguished from the wild type samples.

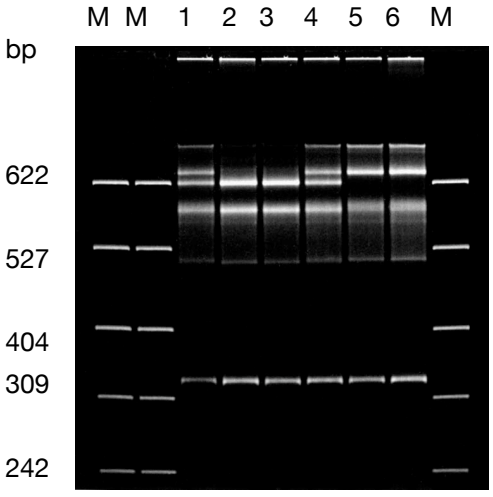

Figure 1. SSCP analysis of CYP2D6 samples on a precast GMA™ Wide Mini S-2x13 gel. After PCR, the samples were treated with formamide as described in Experimental Procedures. Electrophoresis was carried out at 6 V/cm for 15 h at 9.0°C. Lanes: M-M1 Marker (Elchrom); 1, 4 - *4/wt; 2, 3 -wt/wt; 5, 6 - *4/*4.

Experimental Procedures

Equipment

Electrophoresis was performed in Elchrom's SEA 2000® submarine electrophoresis apparatus. Temperature of the running buffer was kept constant at 9.0°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 prepration

PCR samples were generated according to standard protocols. Purity of the amplified DNA fragments was checked on a Spreadex® EL 600 gel. Sample denaturing solution was prepared by mixing 990 µl of formamide with 10 µl of 1 M NaOH just prior to use. A few grains of Bromphenolblue were added and dissolved by mixing. Portions of the denaturing solution (7 µl) were pipetted into 0.6 ml microcentrifuge tubes, followed by addition of 3 µl of the PCR samples. The tubes were placed in a thermal cycler and incubated for 5 min at 95°C. Hot tubes were quickly transferred to an ice bath using the forceps provided with the SEA 2000®. Within five minutes the samples (6 µl) were loaded to a GMA gel.

Running Conditions

The running buffer in the SEA 2000® apparatus was cooled to 9°C before the GMA™ gel was placed in the apparatus. Cooling of the buffer takes about 30 min, during which time the SSCP samples are prepared. The GMA™ Wide Mini S-2x13 gel shown in Figure 1 was run at 6 V/ cm (72 V) for 15 h at 9.0°C.

Detection

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

References

1. Orita, M., Suzuki, Y., Sekiya, T., and Hayashi, K. (1989) Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics, 5, 874-879.
2. Hayashi, K. (1991) PCR-SSCP: a simple and sensitive method for detections of mutations in the genomic DNA. PCR Methods Appl. 1, 34-38.
3. Hongyo, T., Buzard, G.S., Calvert, R.J., Weghorst, C.M. (1993) "Cold SSCP": a simple, rapid and non-radioactive method for optimized single-strand conformation polymorphism analyses. Nucleic Acids Res., 21, 3637-3642.
4. Broly, F., Marez, D., Sabbagh, N Legrand, M., Millecamps, S., Lo-Guidice, J.M., Boone, P., and Meyer, U.A. (1995) An efficient strategy for detection of known and new mutations of the CYP2D6 gene using single strand conformation polymorphism analysis. Pharmacogenetics, 5, 373-384.
5. Marez, D., Sabbagh, N., Legrand, M., Lo-Guidice, J.M., Boone, P., and Broly, F. (1995) A novel CYP2D6 allele with an abolished splice recognition site associated with the poor metabolizer phenotype. Pharmacogenetics, 5, 305-311. Basic

Acknowledgement

The CYP2D6 samples were obtained by courtesy of M. Stefanovic and A.M. Ivanisevic, Clinical Institute of Chemistry, University Hospital "Sestre Milosrdnice", Zagreb, Croatia.

Order Information

Product	P/N
ORIGINS by Elchrom Scientific, 230 V	2100E
ORIGINS by Elchrom Scientific, 115 V	2100U
SEA 2000® Apparatus, 230V	2001E
SEA 2000® Apparatus, 115V	2001U
GMA™ Mini Gels (10 gels)	3501
GMA™ Wide Mini S-2x13 Gels (12 gels)	3526
GMA™ Wide Mini S-2x25 Gels (12 gels)	3548
40 x TAE Running buffer (20 tubes a 50 ml)	3031
Destaining Solution (DST) 100 x (50 ml)	3037
Easy Stain Tray	2344
Power Supply, 200 V/2000 mA, incl. timer	2029E
PeelIT™	2355

Contact

Elchrom Scientific AG, Gwerbestrasse 8,
6330 Cham, Swtzerland,
Phone +41 41 747 2550, Fax +41 41 743 25 36
www.elchrom.com, e-mail: service@elchrom.com