

## Multiplex MS-PCR for the Simultaneous Detection of Three Genes Associated with Thrombosis

Application Note  
Spreadex® Gels  
ORIGINS by Elchrom™

### Introduction

Mutations in several genes that code for coagulation proteins highly correlate with venous thromboembolism (VTE). Homozygous carriers of a factor V aberration have a 100-fold higher risk of developing VTE. Mutations in factor V and prothrombin can contribute to the development of thrombosis and are detected in ~50% of all patients (1).

In addition to these established factors enhancing thrombotic risk, further targets and other genetic variations are under investigation. Mutations in the thermolabile methylenetetrahydrofolate reductase (MTHFR) e.g. can be correlated to mild hyperhomocysteinemia.

Multiplex MS-PCR allows to screen multiple point mutations and to determine the genetic disposition for thrombosis in patients. It relies, however, to the ability to accurately determine the presence or absence of diagnostic bands for each gene under investigation.

Endler et al. (2001) used multiplex MS-PCR to simultaneously detect mutations in the FV, FII and the MTHFR genes. They found that among 70 known patients with recurrent venous thrombosis, 44% of the patients were heterozygous for the factor V mutation FV:R506Q(G1691A). Of the control group, however, only 9% were heterozygous for this mutation.

When using Elchrom high resolution Spreadex gels run in Origins electrophoresis system to separate the DNA fragments from single-tube PCR mixes, the following advantages were achieved:

- Multiplex PCR analysis is done on a mini gel with 4cm running distance.
- Reliable identification of bands due to accurate size separation.
- Full reproducibility due to precast gels and controlled running conditions.
- Unequivocal interpretation of genetic background.
- High throughput, as up to 100 samples can be run simultaneously.
- Fast analysis allows getting the results the same day.

### Results

Figure 1. shows the results obtained by multiplex mutagenically separated PCR analysis of the FV, FII and the MTHFR genes. Single tube PCR reactions were electrophoresed to determine the presence or absence of the FV:R506Q(G1691A) mutation, the FII:G20210A variant, as well as the MTHFR:A223V (C677T) mutation. Patients which are heterozygous for all three variants can be reliably and unambiguously identified due to the presence of 6 clearly visible and distinct bands.

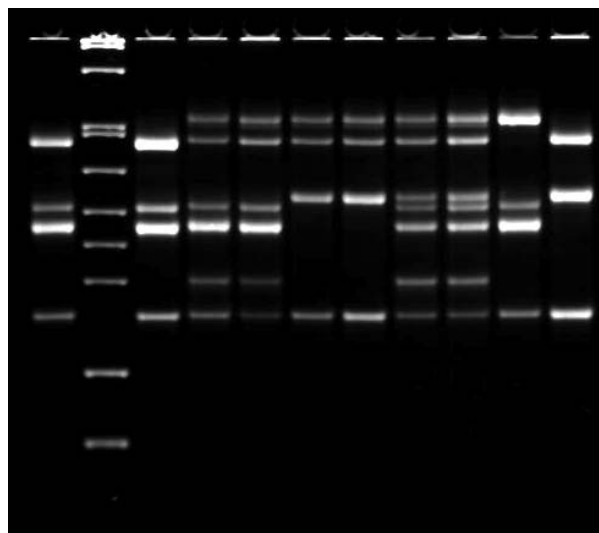


Figure 1

Gel electrophoresis of multiplex MS-PCR on a Spreadex EL 400 Wide Mini S-4x25 Gel at 120V, 55 °C for 2.5h. The bands are from top to bottom: FV Mutant 506 Gln, FV WT 506 Arg, MTHFR WT 677C, MTHFR Mutant 677T, FII Mutant 20210A, FII WT 20210G.

## Experimental Procedure

### Sample Preparation

DNA samples were amplified by PCR in 50 µl volume using AmpliTaq Gold (Perkin Elmer Cetus) in a single PCR tube according to Endler et al. (2001). The primers used for the amplification of FV, FII and MTHFR genes were described by Ulvik et al. (1998).

### Electrophoresis

Samples of the PCR reactions were electrophoresed on Spreadex EL 400 Wide Mini S-2x13 gels. DNA fragments in the size range from 180 to 246 bp were resolved on 4 cm running distance. The samples were run at 120 V for 2.5 h in Elchrom submarine electrophoresis system.

### Detection

Elchrom Scientific Spreadex gels were stained with SYBR Green for 20 min and destained in double distilled water for 40 min prior to photography at 306 nm.

### References

1. Clinical studies and thrombin generation in patients homozygous or heterozygous for the G20210A mutation in the prothrombin gene. (1998) Kyrle P.A., Mannhalter C., Beguin S., Stumpflen A., Hirschl M., Weltermann A., Stain M., Brenner B., Speiser W., Pabinger I., Lechner K., Eichinger S. *Arterioscler. Thromb. Vasc. Biol.* 18(8):1287-1291.
2. Multiplexed mutagenically separated PCR: simultaneous single-tube detection of the factor V R506Q (G1691A), the prothrombin G20210A, and the methylenetetrahydrofolate reductase A223V (C677T) variants. (2001) Endler G., Kyrle P.A., Eichinger S., Exner M., Mannhalter C. *Clinical Chemistry* 47(2): 333-335
3. Simultaneous determination of methylenetetrahydrofolate reductase C677T and factor V G1691A genotypes by mutagenically separated PCR and multiple-injection capillary electrophoresis (1998). Ulvik A., Ren J., Refsum H., Ueland PM. *Clinical Chemistry* 44(2):264-269.

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## Ordering Information

Product	P/N
Spreadex EL 400 Mini, (10 gels)	3414
Spreadex EL 400 Wide Mini S-2x13 (12 gels)	3427
Spreadex EL 400 Wide Mini S-2x25 (12 gels)	3443
Spreadex EL 400 Wide Mini S-4x25 (24 gels)	3493
Spreadex EL 400 Wide Mini S-4x13 (24 gels)	3453
Basic Submarine Electrophoresis System	2031
Origins by Elchrom™	2100 E
Power Supply, 200 V/2000 mA, including timer	2029 E
40 x TAE Running buffer (20 tubes of 50 ml)	3031
Easy-Stain Gel Tray	2344

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