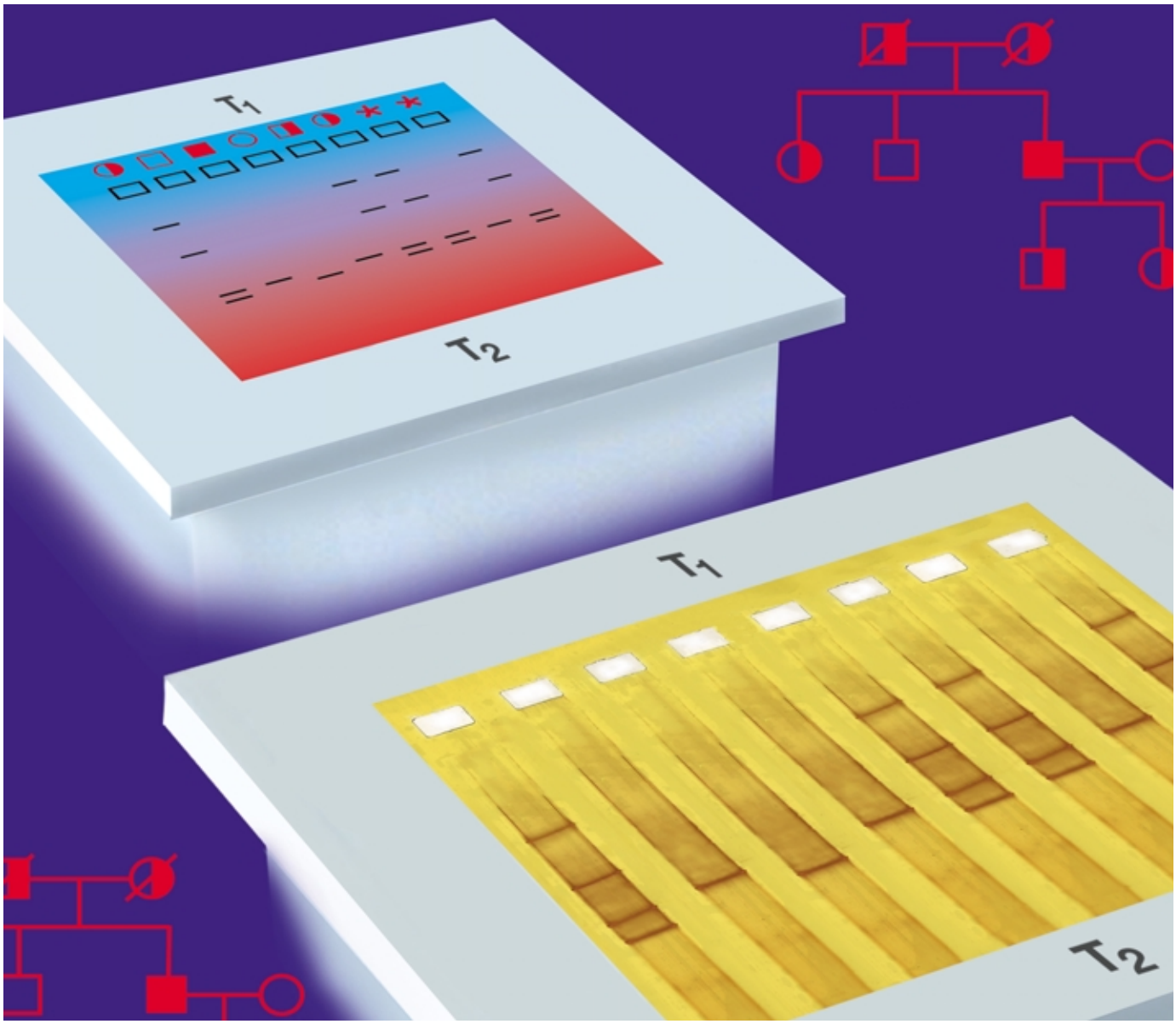
TGGE Temperature Gradient Gel Electrophoresis

Mutation analysis without ambiguity



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TGGE System – Mutation analysis

Typical applications for TGGE *(currently used methods)*

- Cancer mutation screening *(SSCP or sequencing)*
- Polymorphism screening *(RFLP)*
- HLA typing *(RFLP or sequence specific PCR)*
- Clonality analysis of lymphomas *(DGGE)*
- Bacterial genotyping *(DGGE)*
- Population studies *(Heteroduplex analysis)*

DNA applications unique to TGGE

- Differentiation of amplicon and competitor for quantitative PCR
- Fidelity assay for thermostable polymerases

RNA applications unique to TGGE

- Secondary structure analysis of RNA viroids

Protein applications unique to TGGE

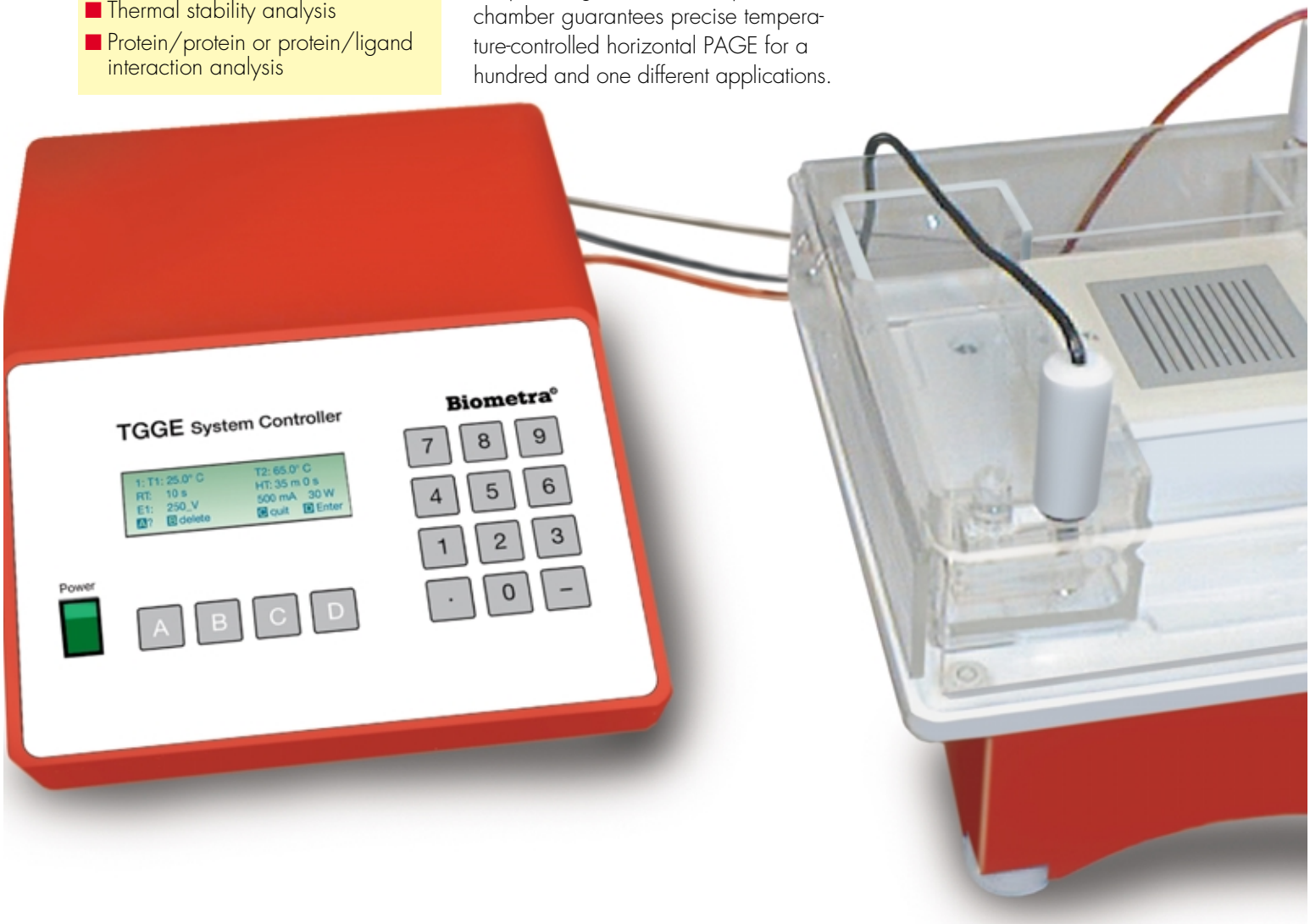
- Thermal stability analysis
- Protein/protein or protein/ligand interaction analysis

One instrument for all applications

The new, Peltier element-based **T**emperature **G**radient **G**el **E**lectrophoresis (TGGE) System from Biometra is a universal detection system designed especially for rapid mutation analysis. By applying a temperature gradient during the electrophoretic separation of DNA or RNA, fragments of identical length but different sequence can be separated. Any method for mutation screening such as SSCP, heteroduplex analysis, DGGE (chemical gradient) or TTGE can be performed with the TGGE System without the need for extra equipment. In addition, many other applications can be performed such as bacterial genotyping, tumor clonality assay and conformational analysis of proteins or viral RNA. The unique design of the electrophoresis chamber guarantees precise temperature-controlled horizontal PAGE for a hundred and one different applications.

Mutation analysis without chemical gradient

DNA fragments with point mutations will exhibit different melting behavior and therefore different conformation compared to the wild type DNA. By varying the denaturing conditions with a temperature gradient, TGGE achieves unparalleled separation and therefore sensitivity[1]. The TGGE System is superior to other gradient systems because of the linearity and reproducibility of its Peltier-controlled temperature gradient. Chemical gradients such as those used in DGGE are not as reproducible, are difficult to establish and often do not completely resolve heteroduplices[2].



Beyond all expectations

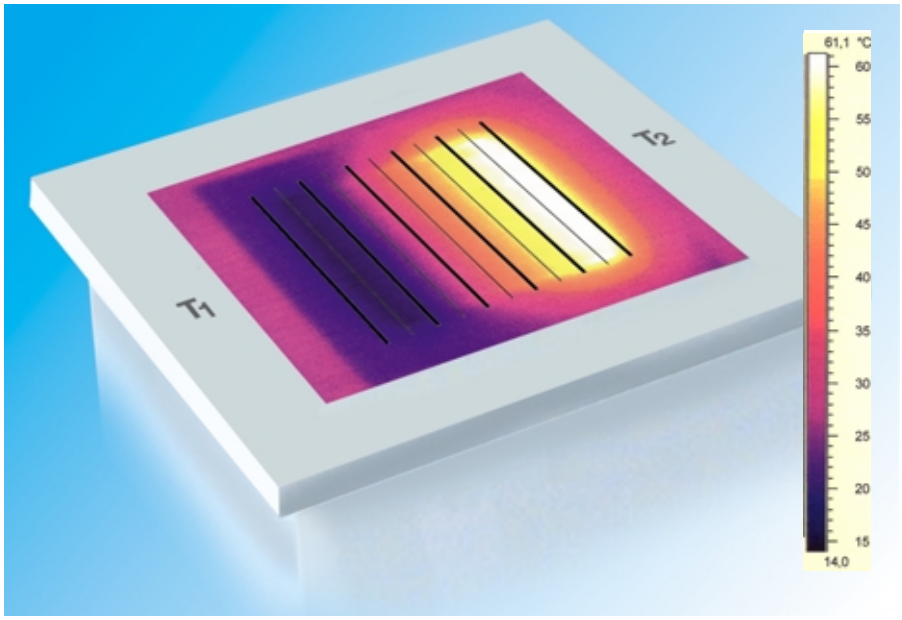


Figure 1: The thermal image during electrophoresis superimposed on the size of the gradient block reveals a constant linear temperature increase between the first and last marked line of the gradient block.

Temperature gradient ensures highest accuracy

The TGGE System uses precisely regulated Peltier elements to establish a temperature gradient across the gel during electrophoresis. The broad temperature range of 4°C – 80°C allows the separation of DNA fragments even with high GC content. The microprocessor-driven control of the Peltier elements together with the design of the gradient block ensures the absolute linearity of the temperature gradient. Thermal measurements show a steady and linear temperature increase from the first marked line on the gradient block to the last line (figure 1). The temperature accuracy of the gradient block allows the resolution of fragments with melting temperatures that differ by as little as 0.5°C.

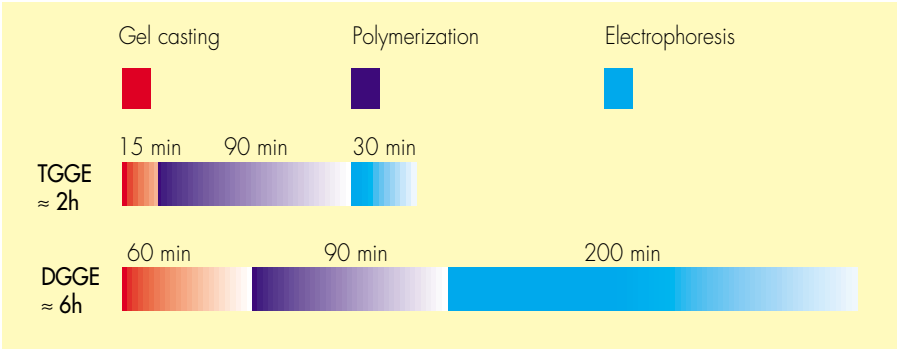


Figure 2: Comparison of typical experimental time using the TGGE System and chemical gradients (DGGE).

Short run lengths – fast results

The gel dimension of the TGGE System has been downsized in order to achieve short run times combined with the highest possible resolution. The short hands-on time of the TGGE System in comparison to other screening methods like DGGE (figure 2) allows to run 3 times more gels. The maximum run distance of 5 cm for parallel TGGE allows the separation of approximately 30 different fragments.

Therefore even complex patterns which are common for bacterial genotyping can be resolved. Due to the small size of the electrophoresis unit only small amounts of material are necessary. With silver staining, the most sensitive staining method, lowest amounts (0.03 ng/mm^2) of DNA and RNA can be visualized.

1. Scholz, R.B. et al. (1993): Rapid screening for Tp53 mutations by temperature gradient gel electrophoresi Human Mol. Gen. 2 (12): 2155-2158
2. Cremonesi, L. et al. (1997): Double-gradient DGGE for optimized detection of DNA point mutations. BioT
3. Fan, E. et al. (1993): Limitations in the use of SSCP analysis. Mutat. Res. 288: 85-92
4. Sheffield, V.C. et al. (1993): The sensitivity of single-strand conformation polymorphism analysis for the de
5. Finke, R. (1996): Theoretical basis and application of molecular diagnostics. Exp. Clin. Endocrinol. Diabe

For highest reproducibility

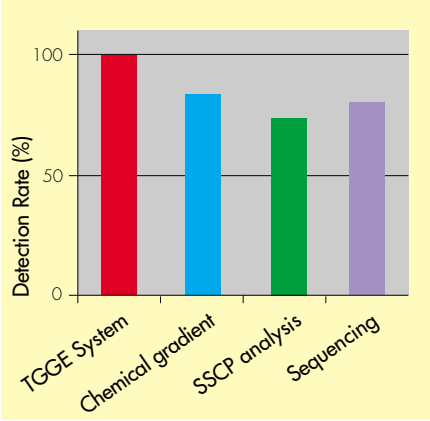


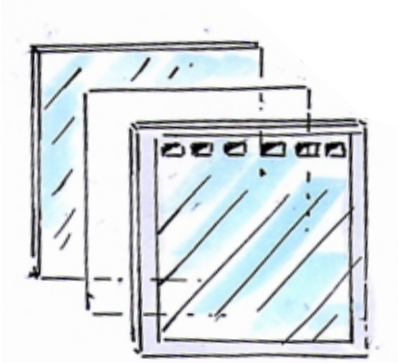
Figure 3: TGGE outperforms other mutation detection systems, such as DGGE (chemical gradient), SSCP analysis and sequencing, giving almost 100 % detection rates.

100% detection rate for mutation analysis

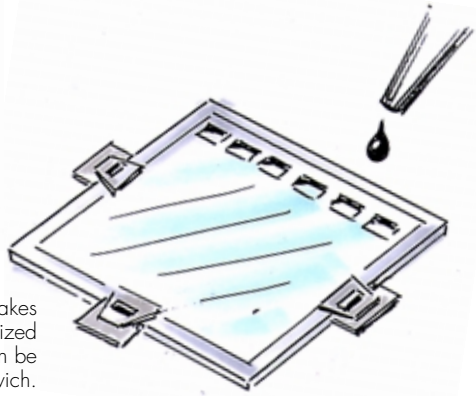
Whilst TGGE is superior to DGGE, SSCP analysis only shows published detection rates of 80% or less (figure 3). This is because factors such as temperature and ionic buffer strength exhibit a large influence over the resolution[3,4].

Sequencing is now used in most cases for verifying mutations or polymorphisms. However, this method is not able to differentiate unambiguously between homozygotes and heterozygotes. The high costs and long run times prohibit the use of sequencing as the standard method for mutation screening[5]. In contrast the TGGE method offers virtually 100% detection rate at a reasonable price and at less than a quarter of the time.

Easy handling improves reproduction of results

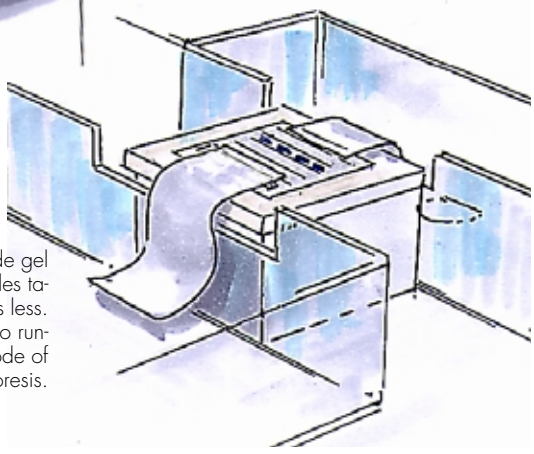


For gel pouring a simple sandwich of two glass plates with a gel support film is clamped together. No need for spacers and combs.

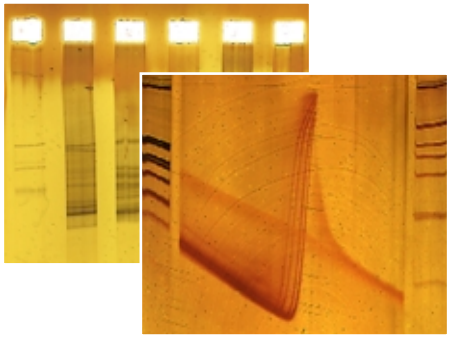


Pouring the polyacrylamide solution takes only 1 minute. After 1.5 h the polymerized gel fixed to the support film can be retrieved from the sandwich.

The gel is placed on top of the metal gradient plate. A few drops of a thermal coupling solution are spread on the metal plate to optimize the thermal contact to the gel.



Horizontal polyacrylamide gel electrophoresis of up to 18 samples takes only 30 min – 1 h, sometimes less. Pre-cut electrode wicks soaked into running buffer allow a semi-dry mode of electrophoresis.



The polyacrylamide gel is stained by a rapid silver staining protocol. Depending on the technique applied the resulting gels look like conventional PAGE gels (parallel TGGE: back) or shows sigmoidal curves (perpendicular TGGE: front).

omparison with SSCP analysis.

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04, Suppl. 4: 92-97

Application: Prion research

The TGGE System: A fast method for differentiation between wild type and mutant DNA of the human prion protein gene

M. Wulfert, Universität Düsseldorf, Institut für Physikalische Biologie, 40225 Düsseldorf

Abstract. Certain forms of spongiform encephalopathy in animals and humans are caused by an abnormal form of the so called prion protein[1]. For studying a mutation at codon 102 of the human prion protein gene (Pro>Leu)[2] it was necessary to distinguish between PCR fragments which have been amplified from wild type and from mutant DNA. With the TGGE System the optimal separation conditions could be defined in less than 3 h (electrophoresis run time: 18 min). Mutant and wild type DNA could clearly be distinguished due to a T_M difference of about 1°C. The short run time of the system allowed immediate analysis of samples which dramatically decreased experimental time.

Method. Prior to TGGE analysis a profile of the gene was calculated using the software POLAND which is available in the internet (http://www.biophys.uni-duesseldorf.de/service/polandform.html). The two primers, 1⊕ and 5⊖ include nucleotide positions 270-390 of the gene, which contains the codon 102. DNA template of wild type and mutant type was amplified in 100 µl reaction volume. A sample of 20 µl was mixed with 20 µl loading buffer (0.2 x Na-TAE, 8 M urea, dyes) and applied to a 8% polyacrylamide TGGE gel (acrylamide/bisacrylamide 30:1, 8M urea, 0.2 x Na-TAE). After a 3 min pre-run at 300 V, 12 mA at constant 20°C, the gel slot was rinsed with running buffer (0.2 x Na-TAE). The gel was covered by a film to prevent desiccation. The temperature gradient was established and electrophoresis started again at 300 V, 30 mA. After a run time of 15 min the gel was silver stained.

Results. The graph calculated by POLAND (figure 1) shows the denaturing behavior of the gene at different temperatures. For optimal separation of mutant and wild type DNA the mutation site should reside in a sequence range which melts earlier than the surrounding sequence.

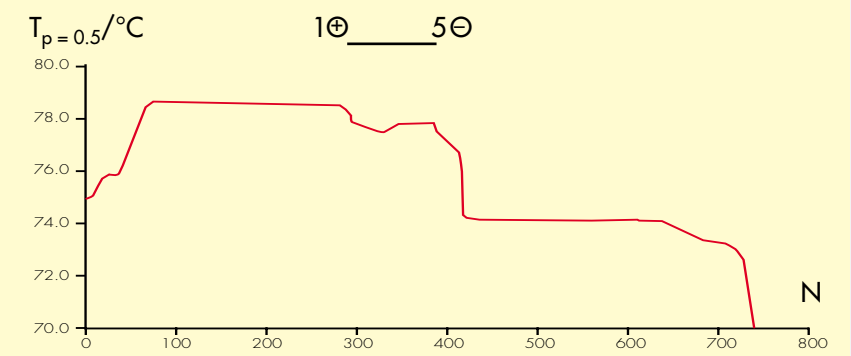

Figure 1: The temperature of 50% base pairing probability of each base stack ($T_{p = 0.5}$) vs. nucleotide position (N) of the human prion protein ORF. Positions of primer 1⊕ and 5⊖ and amplified region inbetween are indicated.

Therefore we used primers which include nucleotide positions 270 – 380. By using 8 M urea during PAGE it was possible to use 20°C lower temperature than calculated. After a perpendicular TGGE with a broad temperature range of 30°C – 70°C the resulting gel showed four sigmoidal bands in the middle area (figure 2).
Two of the bands represent the homoduplices of the wild type and the mutant whereas the other two bands represent the two possible heteroduplices (figure 3). In a narrow temperature range of 50°C – 53°C the four DNA fragments separate from each other and can easily be distinguished.

Discussion. The TGGE System is a powerful and rapid tool for distinguishing DNA fragments of the same length but different sequences from each other. It was possible to separate a complex PCR mixture in less than 3 h including a 1 h silver staining procedure. The broad temperature range of the perpendicular TGGE defines the best conditions for separation in one experimental run. Subsequently, it is possible to run multiple samples in parallel TGGE at a narrower temperature range.

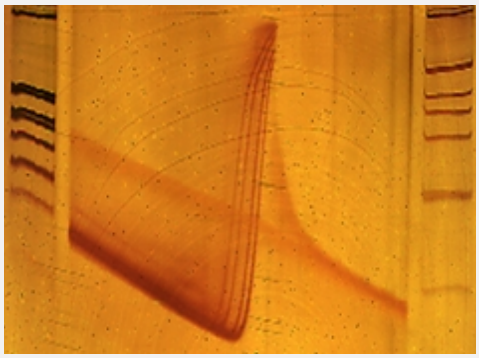

Figure 2: Separation of partial sequences of wild type and mutant human prion protein gene by perpendicular TGGE.

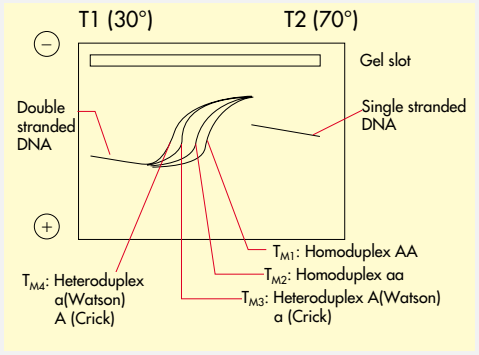

Figure 3: Schematic drawing of a perpendicular TGGE run of a PCR amplified mixture of wild type and mutant DNA.

References.

1. Prusiner, S.B. et al. (1997) Ann. Rev. Genet. 31: 139 – 175
2. Wiese, U. et al. (1995) Electrophoresis 16: 1851 – 1860

Technical Specifications/ Ordering Information

Technical Specifications	
TGGE System	Electrophoresis unit with Peltier element-powered gradient block and 2 removable electrophoresis buffer chambers; Controller with integrated power supply and connector cable; Starter Kit with glass plates, Polybond film, electrode wicks and cover film
Gradient Block	
Temperature range of gradient block	5 – 80°C
Maximum linear temperature range (above 20°C)	45 K, e.g. 30°C – 75°C
Temperature accuracy	±0.3°C
Temperature uniformity	±0.3°C/2 mm
Gradient block size	6 cm x 6 cm
Glass plate size	9 cm x 9 cm
Gel size	7.4 cm x 8.2 cm
Run distance	5 cm (perpendicular TGGE), 5.5 cm (parallel TGGE)
Size of electrophoresis unit (l x w x h)	22.5 cm x 22.5 cm x 23 cm
Weight	4.2 kg
TGGE System Controller	
Functions	Microprocessor control of Peltier elements, power supply and control of electrophoresis
Program stores	100
Program mode	const. V, const. A, const. W, V/h integration, time controlled
Display, language	4 line LCD, English and German
Mains voltage	230 V or 115 V
Voltage	max. 400 V
Current	0 – 100 mA
Wattage	30 VA
Interfaces	1 parallel port (Centronics)
	1 serial port (RS232)
Size (l x w x d)	31 cm x 22 cm x 11.5 cm
Weight	3.8 kg

Ordering Information	Order No.
TGGE System, 230 V; electrophoresis unit with Peltier element-powered gradient block and 2 removable electrophoresis buffer chambers, Controller with integrated power supply, Starter Kit and manual	024 - 000
TGGE System, 115 V, dito	024 - 090
TGGE Starter Kit with 3 Bonding glass plates, 3 types of glass plates with slots, pre-cut electrode wicks, pre-cut Polybond film and cover film	024 - 003

Accessories	Order No.
TGGE electrode wicks, pre-cut 7 x 7 cm, 100/pkg	024 - 015
TGGE Bonding glass plate, 9 x 9 cm, w/o spacer	024 - 021
TGGE glass plate, 9 x 9 cm, 8 slots	024 - 022
TGGE glass plate, 9 x 9 cm, f. perpendicular TGGE	024 - 023
TGGE glass plate, 9 x 9 cm, f. diagonal TGGE	024 - 024
TGGE glass plate, 9 x 9 cm, 12 slots	024 - 025
TGGE glass palte, 9 x 9 cm, 18 slots	024 - 026
TGGE Polybond film, pre-cut 8.8 x 8.8 cm, 25/pkg	024 - 030
TGGE Cover glass plate and 10 hydrophobic cover films	024 - 031
Gel casting clips, 3/pkg.	010 - 007

PCR (polymerase chain reaction) is covered by patents issued to Hoffmann-La Roche.
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Biometra GmbH · Postfach 1544 · D-37005 Göttingen
phone +49-(0)551/50 68 6-0 · fax +49-(0)50 68 6-66
e-mail: info@biometra.co.uk · Web site: www.biometra.de

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