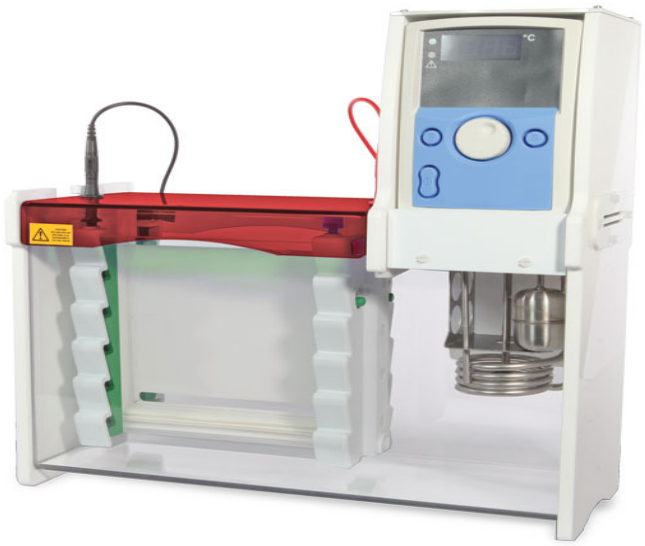
Instruction Manual
The Denaturing Gradient
Gel Electrophoresis System

Catalogue Numbers
VS20WAVE-DGGE



Version: VS20WAVE-DGGE/CDGE/HA awp1.5.2015

Contents

Section 1

General Information
1.1)Safety Instructions

- WHEN USED CORRECTLY, THESE UNITS POSE NO HEALTH RISK. HOWEVER, THESE UNITS CAN DELIVER DANGEROUS LEVELS OF ELECTRICITY AND ARE TO BE OPERATED ONLY BY QUALIFIED PERSONNEL FOLLOWING THE GUIDELINES LAID OUT IN THIS INSTRUCTION MANUAL.
- ANYONE INTENDING TO USE THIS EQUIPMENT SHOULD READ THE COMPLETE MANUAL THOROUGHLY.
- THE UNIT MUST NEVER BE USED WITHOUT THE SAFETY LID CORRECTLY IN POSITION.
- THE UNIT SHOULD NOT BE USED IF THERE IS ANY SIGN OF DAMAGE TO THE EXTERNAL TANK OR LID.
- ACRYLAMIDE IS A POWERFUL NEUROTOXIN IN SOLUTION FORM. POLYMERIZED GELS CAN CONTAIN SOME UNPOLYMERIZED SOLUTION AND PROTECTIVE GLOVES AND CLOTHING MUST BE WORN.
- THESE UNITS COMPLY WITH THE STATUTORY CE SAFETY DIRECTIVES: 73/23/EEC: LOW VOLTAGE DIRECTIVE: IEC 1010-1:1990 plus AMENDMENT 1:1992
  EN 61010-1:1993/BS EN 61010-1:1993

1.2) Introduction to the VS20WAVE-DGGE

DGGE, known as denaturing gradient gel electrophoresis, is an important molecular biology technique used to study mutations and DNA polymorphisms. DGGE gels separate similarly sized PCR products that differ according to their melting temperature (Tm), usually as a result of single base pair mutations. The VS20WAVE-DGGE is designed primarily for parallel denaturing gradient gel electrophoresis, where the electrophoresis and the denaturing gradient run in the same direction. DGGE gels are formed by mixing two solutions containing acrylamide (structural material) and varying amounts of denaturants (urea and formamide) to form a denaturing gradient, whereby double stranded DNA fragments of different sequence will be denatured during electrophoresis. The VS20WAVE-DGGE temperature control unit combines buffer recirculation with a heat sensor to facilitate precise temperature control to within ±0.02°C, allowing the gel temperature to be set to the melting temperature of the amplified DNA polymorphism or mutation of interest. In addition to parallel DGGE, the VS20WAVE-DGGE may be used to perform constant denaturing gel electrophoresis (CDGE) and heteroduplex analysis (HA).

VS20WAVE-DGGE - Applications			
	DGGE	**CDGE**	**HA**
Description	i. Determines the denaturing conditions required to identify unknown mutations ii. Works on the principle that increasing denaturant concentrations melt DNA in a domain-specific manner, and the mutation or polymorphism of interest is in the DNA domain with the lowest T_m iii. Requires parallel DGGE – a technique where DNA samples are resolved at uniform temperature in gels containing a formamide and urea denaturant gradient parallel to the direction of electrophoresis iv. Results in partial melting of DNA to produce a branched molecule identified by its reduced mobility within the gel.	i. Rapid screening method for multiple samples containing an identified mutation ii. Requires DGGE beforehand to establish optimal denaturing conditions to identify a specific mutation iii. No denaturant gradient required as multiple samples are screened on a constant denaturant gel iv. Increases throughput and alleviates bottlenecks	i. Used when it is difficult to detect a homoduplex mutation by DGGE ii. Requires denaturation and re-annealing of wild-type and mutant DNA mixed together, usually within a PCR reaction iii. Resultant heteroduplexes are less stable and melt at a lower denaturant concentration than wild-type and mutant homoduplex molecules, allowing them to be identified by reduced mobility within the gel iv. Requires parallel DGGE, or may be performed overnight in a TBE gel made from special high-resolution acrylamide
VS20WAVE-DGGE Application Benefits	i. GM100 gradient mixer and optional MU-D01 peristaltic pump simplify casting of denaturing gradient gels ii. New VS20WAVE electrophoresis insert and cam caster for leak free casting iii. Temperature control unit provides consistent run temperatures between 45-70°C iv. High resolution 20x20cm format	i. Uses constant denaturant gels cast with new VS20WAVE electrophoresis insert and cam caster for leak free casting ii. Temperature control unit provides constant run temperature during electrophoresis iii. Maximum 96-sample throughput (48 samples per gel)	i. New VS20WAVE electrophoresis insert and cam caster for leak free casting ii. Gradient mixer simplifies DGGE option iii. Optional temperature control for reproducibility iv. High resolution 20x20cm format

1.3) Product Information

The VS20WAVE-DGGE unit is supplied as a complete package, and includes: a Gel Tank with Safety Lid; a Gel Casting Inner Module; Glass Plates with Bonded Spacers and Combs of matching spacer thickness; plus a Gradient Mixer, Temperature Control Unit and Power Cables. The table below describes the main individual components, while further details are shown overleaf in section 1.5) Specifications.

Gel Tank and Safety Lid	Durable, robust gel tank and safety lid fully compliant with latest CE safety directives. Asymmetric lid design and colour-coded thumb locators prevent polarity reversal.
Inner Module	Dual purpose PAGE insert eliminates time-consuming transfer of glass plates between separate casting and running modules. Vertical screw-clamp technology reduces the number of screws required for set up, significantly reducing assembly time.
Glass Plates & Combs	Robust 4-mm-thick glass plates have bonded spacers for convenience. Bonded spacers and combs are colour-coded according to thickness and easy identification. The widest selection of combs allows separation of up to 96 samples.
Gradient Mixer	100mL gradient mixer supplied as standard to ensure efficient gradient formation by mixing and delivering high- and low-density denaturant solutions. A flat-base design and support handle allow the GM100 to be secured to a retort stand, enabling it to be easily mounted on a magnetic stirring plate (e.g. CSL-STIR) while the mixing chamber may accommodate a magnetic stirrer to form a linear gradient. The optional MU-D01 peristaltic pump is also recommended for delivery of linear and reproducible gels.
DGGE Temperature Controller	The redesigned VS20WAVE-DGGETC temperature control unit combines buffer recirculation with a heat sensor and 1.4kW heating element to facilitate precise temperature control to within ±0.2°C, allowing the gel temperature to be set to the melting temperature (Tm) of the amplified polymorphism or mutation of interest.
Power Cables	Protective retractable connectors are compatible with the 4mm power outputs of most power supplies. Optional adaptors available.

1.4)Packing List

The packing list should be referred to as soon as the units are received to ensure that all components have been included. The unit should be checked for damage when received. Please contact your supplier if there are any problems or missing items.

Each unit consists of the following:

Quantity	Description	Cat. Number
	Complete VS20WAVE-DGGE unit consists of the following:	VS20WAVE-DGGE
1	• Inner running module	VS20WAVEDIRM
1	• Main Tank	VS20WAVE-TANK
1	• Temperature Controller	VS20WAVE-DGGETC
1	• Gradient Mixer	GM100
1	• Lid	VS20WAVELID
Pk/2	Notched Glass Plates	VS20NG
Pk/2	Plain Glass Plates with bonded 1mm spacers	VS20PGS1
2	1mm Thick, 24 Sample Comb	VS20-24-1
1	Casting Base	VS20WAVECAST
1	Silicone Casting Mat	VS20DCASTM
1	Power Cables.	CSL-CAB

1.5) Specifications

Technical Specification - VS20WAVE-DGGE	
WAVE electrophoresis insert and tank	
Max. Number of Gels	2 per run
Plate Dimensions (WxH)	20x20cm
Active Gel Dimensions (WxH)	16x17.5cm
Spacer Thicknesses	0.75, 1, 1.5 and 2mm
Max. Sample Capacity	96 samples; 48 per gel
Standard Combs	2x 1mm 24-sample
Available Combs	1, 5, 10, 18MC, 24, 30, 36MC, 48; as per VS20WAVE unit
Max. Buffer Volume	8.5L
Unit Dimensions	40.5x17x44cm (WxDxH)
Weight	8Kg
Recommended Power Supply	CS-500V
Temperature Control Unit	
Temperature Control	PID
Operating Temperature Range	Ambient-100°C
Working Temperature Range (DGGE)	45-70°C
Buffer Recirculation Mechanism	Stirring
Temperature Uniformity/Stability at 37°C	±0.05/0.02°C
Setting/Display Resolution	0.1°C
Stored Temperature Values	4
Safety	Fluid-level float switch; isolated; IEC 1010 / CE
Heater Power at 230V/110VAC	1.4/1.3kW
Electrical Power at 230V/100VAC	1.5/1.4kW (50-60Hz)
Gradient Mixer	
Total Volume	100ml
Volume of Reservoir and Mixing Chambers	50ml
Internal Diameter of Outlet Port	2mm

This apparatus is rated POLLUTION DEGREE 2 in accordance with IEC 664. POLLUTION DEGREE 2, states that: “Normally only non-conductive pollution occurs.
Occasionally, however, a temporary conductivity caused by condensation must be expected”.

1.6) Care and Maintenance

- ### Cleaning Large Format Vertical Units

  Units are best cleaned using warm water and a mild detergent. **Water at temperatures above 60^0 C may damage the unit and its components.**
  The inner module should be thoroughly rinsed with warm or distilled water to prevent build up of salts, but care should be taken not to damage the enclosed electrode while vigorous cleaning is not necessary or advised. Air-dry before use.

- ### The unit should only be cleaned with the following:

  Warm water with a mild concentration of soap or other mild detergent. Compatible detergents include dishwashing liquid, Hexane and Aliphatic hydrocarbons. The unit should not be left in detergent for more than 30 minutes. Always rinse thoroughly in distilled water afterwards and allow to drain.

- ### DO NOT expose the unit to the following, otherwise damage WILL result:

  Acetone, Phenol, Chloroform, Carbon Tetrachloride, Methanol, Ethanol, Isopropyl Alcohol & Alkalis.

- ### RNase Decontamination

  This may be performed as follows:
  Clean the units with a mild detergent as described above.
  Wash with 3% hydrogen peroxide (H_2O_2) for 10 minutes.
  Rinse with 0.1% DEPC- (diethyl pyrocarbonate) treated distilled water,
  Caution: DEPC is a potential carcinogen. Always take the necessary precautions when using it. RNaseZAP™ (Ambion) can also be used. Please consult the instructions for use with acrylic gel tanks.

Section 2
Initial Set Up

2.1) Initial set up of the VS20WAVE-DGGE Tank

Note: Before setting up the Gel Tank please ensure that it has been properly cleaned and dried.

1. Note the position of the lid on the unit. This shows the correct polarity and the correct orientation of the cables. Black is negative and red positive.
2. Remove the lid from the unit.

Note: Detach the lid from the tank and VS20WAVEDIRM before fitting the cables to the lid. Failure to do this could twist the gold plugs on the VS20WAVEDIRM and distort and break the platinum wire electrodes, rendering the warranty invalid.

3. Screw the cables into the tapped holes as fully as possible so that there is no gap between the lid and the leading edge of the cable fitting.
4. Refit the lid.

***CAUTION* - VS20WAVE-DGGETC**
Do NOT attempt to use the temperature controller in the tank unless it has been filled with buffer. Failure to do this will damage the unit and present a potential FIRE risk.

2.2) Cleaning the Glass Plates

- Clean a set of glass plates for each gel first with distilled water and then with 70 % ethanol.
- One set of glass plates constitutes one notched glass plate and one plain glass plate with bonded spacers.
- When making a triple glass plate sandwich, place one notched glass plate with bonded spacers on a level bench surface with the spacers uppermost. Then overlay a second notched plate (without bonded spacers), followed by a plain glass plate with bonded spacers, to form a triple glass plate sandwich. Gently insert the triple glass plate sandwich, with the plain glass plate outermost, into the VS20WAVEDIRM as described in 2.3.3. Casting Stand Assembly.
- Repeat for the other side of the VS20WAVEDIRM.
- **REMEMBER to use the YELLOW clamps for triple glass plate sandwiches**

Note: All glass plates, gel casting modules, casting base and accessories must be completely dry before set-up. Wet components are more likely to misalign and cause leaks.

2.3) Gel Cassette Assembly

- Assemble the glass plates so that the bottom of the glass plates and the spacers are perfectly aligned; the standard glass plates supplied with the WAVEDSYS, WAVEDSYS-CU and WAVETETRAD systems are supplied with bonded spacers and do not require manual alignment. However, if using free spacers, which are not included as standard, these need to be perfectly aligned. This is best performed using a small spacer or comb to push the spacers apart.

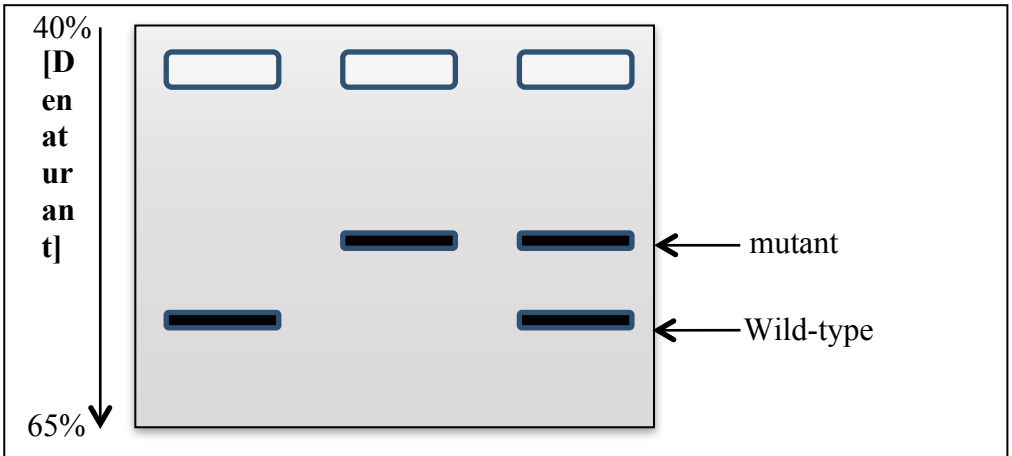
NOTE: The glass plates with bonded spacers have an arrow in the top of the spacers which are slightly longer than the glass plate to indicate the top.

Section 3
Denaturing Gel Electrophoresis

3.1 Introduction to Denaturing Gradient Gel Electrophoresis – DGGE and CDGE

Denaturing Gradient Gel Electrophoresis (DGGE) is an electrophoretic method to identify single base pair changes within a DNA segment. The separation techniques upon which DGGE is based were first described by Fischer and Lerman in 1983[1]. In a denaturing gradient acrylamide gel, double-stranded DNA, which is subjected to an increasing denaturant concentration, will melt in discrete segments called "melting domains". The melting temperature (Tm) of these domains is sequence-specific. When the Tm of the lowest melting domain is reached, the DNA becomes partially melted, creating a branched molecule whose mobility is reduced within a polyacrylamide gel. Since the Tm of a particular melting domain is sequence-specific, the presence of a mutation alters the melting profile of that DNA when compared to the wild-type sequence. Consequently, DNA containing mutations will encounter mobility shifts at different positions in the gel compared to the wild-type. If the fragment completely denatures then DNA migration once again becomes a function of its size.

In DGGE, the denaturing environment is created by a combination of uniform temperature, typically between 50 and 65 °C, and a linear denaturant gradient formed with urea and formamide that is either parallel or perpendicular to the direction of electrophoresis. A solution of 100% chemical denaturant consists of 7 M urea and 40% formamide. Perpendicular DGGE gels are often difficult to set up and use a much broader denaturant gradient range, such as 0-100% or 20-70%; whereas parallel DGGE gels use a narrower denaturant range (e.g. 40-65%) to achieve better fragment separation[2]. Consequently, the VS20WAVE-DGGE has been optimised only to perform parallel DGGE. A schematic diagram showing a parallel denaturing gradient gels with homoduplex wild-type and mutant fragments is shown below.



A schematic diagram of parallel denaturing gradient gel electrophoresis, where the denaturing gradient is parallel to the direction of electrophoresis. Typically, wild-type and mutant alleles of a gene exon are separated in an acrylamide gel with a parallel gradient of 40-65% denaturant concentration. Standard running conditions for an 8% acrylamide (1x TAE) gel are at 150V for up to 5 hours at 60°C. From left to right: lane 1 wild-type fragment; lane 2: mutant fragment; lane 3: wild-type and mutant fragments.

[1] Fischer, S. and Lerman, L., *Proc. Natl. Acad. Sci.*, **80**, 1579–1583 (1983).

[2] Myers, R., Maniatis, T., and Lerman, L., *Methods Enzymol.*, **155**, 501–527 (1987).

In performing denaturing gel electrophoresis both the mutant and wild-type DNA fragments are run within the same gel, which allows mutations to be detected by differential migration of mutant and wild-type DNA. The mutant and wild-type fragments are usually amplified beforehand using the polymerase chain reaction (PCR) to create sufficient amounts of DNA to load on the gel. Optimal resolution is attained when the molecules do not completely denature and the region to be screened for mutation is in the lowest melting domain. Addition of a 30–40 base pair GC clamp to one of the PCR primers ensures that the region screened is in the lower melting domain and that the DNA remains partially double-stranded for optimal gel resolution[3]. Psoralen-derivative PCR primers serve as an alternative to GC-clamps[4]; but because they covalently link the two DNA strands at one end, they should not be used when isolating a DNA fragment that will be ultimately sequenced from the gel. Although DNA fragments as large as 1 kb in length may be run on a denaturing gel, only the lower melting domains will be available for mutation analysis. For complete analysis of fragments over 1 kb in length, more than one PCR reaction should be performed[5].

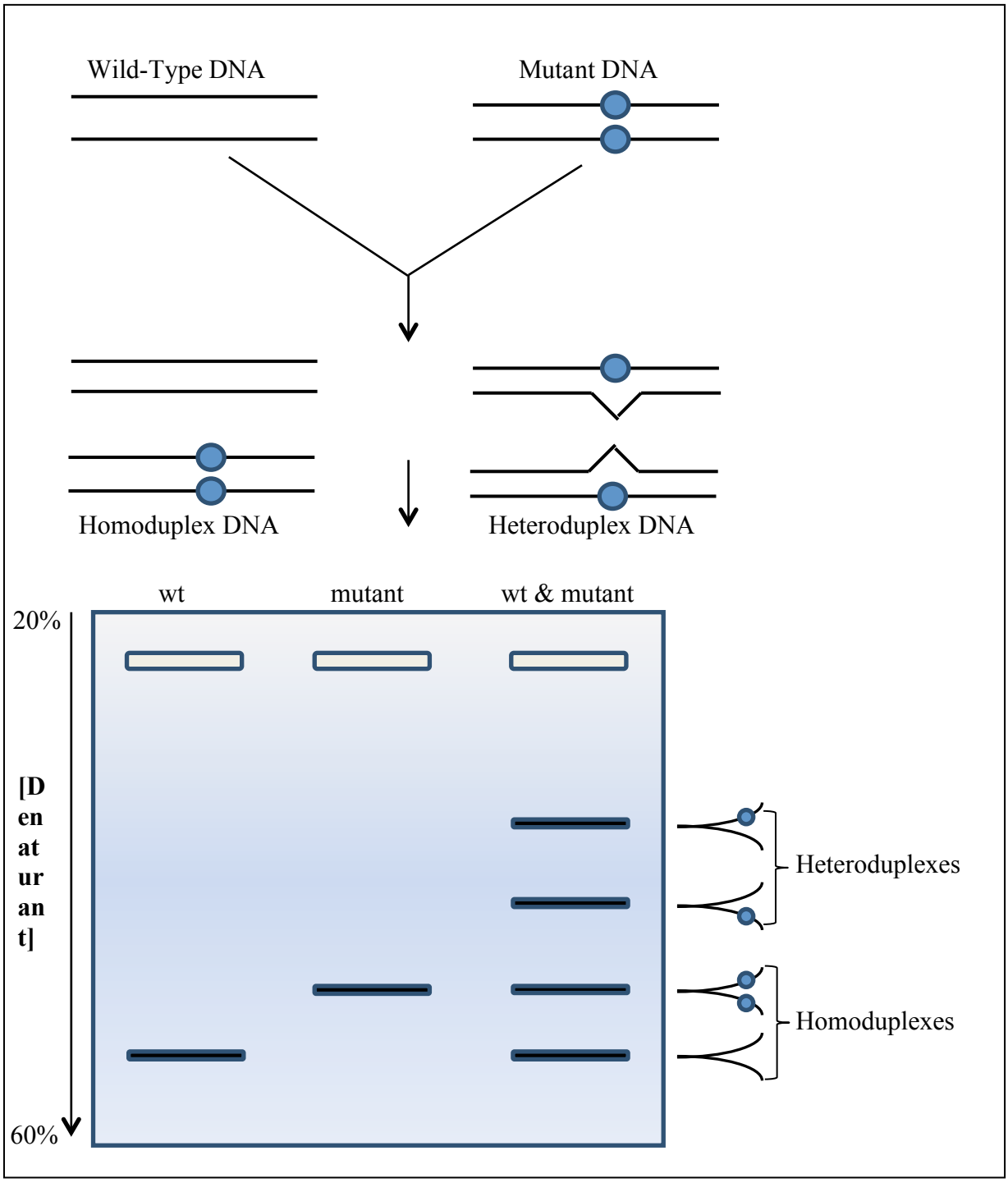
The thermodynamics of the transition from double-stranded to single-stranded DNA during the melting process have been described by a computer program developed by Lerman in 1984. POLAND analysis software, readily available through the University of Dusseldorf website (G Steger; http://www.biophys.uni-duesseldorf.de/html/local/POLAND/poland.html), predicts the melting behaviour of the DNA product of interest, which yields insights into the positions for optimal primer placement and GC clamping.

The method of creating heteroduplex molecules aids detection of homoduplex mutations. It is typically performed when it is not originally possible to resolve a homoduplex mutation. Analysis of heteroduplex molecules can therefore increase the sensitivity of DGGE. Heteroduplexes may be formed by adding the wild-type and mutant template DNA molecules in the same PCR reaction or by adding separate PCR products together, before denaturing them and allowing them to re-anneal. This results in a heteroduplex that has a mismatch between the double strands which causes a distortion in its usual conformation. This induces a destabilizing effect which makes the DNA strands to denature at a lower concentration of denaturant. The heteroduplex bands always migrate more slowly than the corresponding homoduplex bands under specific conditions (please see the figure overleaf).

[3] Myers, R., Fischer, S., Lerman, L., and Maniatis, T., *Nucl. Acids Res.*, **13**, 3131–3145 (1985).

[4] Costes, B., Girodon, E., Ghanem, N., Chassignol, M., Thuong, N., Dupret, D., and Goossens, M., *Hum. Mol. Genet.*, **2**, 393–397 (1993).

[5] Smith-Sorensen, B., Hovig, E., Andersson, B., and Borresen, A., *Mutat. Res.*, **269**, 41–53 (1992).



Schematic illustration of wild-type (wt) and mutant DNA fragments first denatured and the re-annealed to generate 4 fragments – i.e. 2 heteroduplexes and 2 homoduplexes, as shown here run out on a parallel denaturing gel. Denaturation and re-annealing alters the melting behaviour of the heteroduplexes relative to the homoduplexes, to the extent that the heteroduplexes melt at a much lower denaturant concentration than the homoduplexes and are resolved at different positions within the gel, thus enabling their visualization and detection.

3.1.1) Parallel Denaturing Gradient Gel Reagent Preparation

The concentration of denaturant to use within a parallel DGGE gel depends on the sample being analysed within the VS20WAVE-DGGE system. Unlike perpendicular DGGE, a relatively narrow denaturant gradient range is applied, usually 25 to 30%, although the extremities of a parallel denaturing gradient gel may be as low as 10% and as high as 70%. The concentration of acrylamide may also vary, depending on the size of the fragment analyzed. Both 0% and 70% denaturant should be made as stock solutions, based on a 100% denaturant solution containing a mixture of 7 M urea and 40% deionized formamide.

Stock Solutions

40% Acrylamide/Bis (37.5:1), 100ml

However, for different percentage crosslinking, use the equation below to determine the amount of Bis to add. The example stock solution is for an acrylamide/bis ratio of 37.5:1.

Polyacrylamide gels are described by reference to two characteristics:
1) Total monomer concentration (%T)
2) Crosslinking monomer concentration (%C)

$$\%T = \frac{\text{gm acrylamide + g bis-acrylamide}}{\text{total volume}} \times 100\%$$

$$\%C = \frac{\text{gm bis-acrylamide}}{\text{gm acrylamide + g bis-acrylamide}} \times 100\%$$

WARNING: Acrylamide is a neurotoxin, and especially dangerous when used in powdered form! Always wear a mask to prevent inhalation, or better still use commercial acrylamide solutions.

50x TAE Buffer, 1L. This may be purchased using code TAE50X1L or made up as follows:

Reagent	Amount	Final Concentration
Tris base	242g	2M
Acetic Acid, glacial	57.1ml	1M
0.5M EDTA, pH 8.0	100ml	50mM
Distilled water	To 1000mL final volume.	

Mix and, preferably, autoclave for 20-30 minutes. Store at RTP.

Please consult the table below for the recommended % acrylamide/ bis required for a particular DNA size range.

Gel Percentage	Basepair Separation (bp)
6%	300-1000bp
8%	200-400bp
10%	100-300bp

0% Denaturing Solution

	6% Gel	8% Gel	10% Gel	12% Gel
40% Acrylamide / Bis	15ml	20ml	25ml	30ml
50x TAE buffer	2ml	2ml	2ml	2ml
Distilled water	83ml	78ml	73ml	68ml
Total volume	100ml	100ml	100ml	100ml

Degas for 10-15 minutes. Filter through a 0.45µm filter, and store away from sunlight in a refrigerator for 1 month at 4°C.

To make 100ml 70% Denaturing Solution

	6% Gel	8% Gel	10% Gel	12% Gel
40% Acrylamide / Bis	15ml	20ml	25ml	30ml
50x TAE buffer	2ml	2ml	2ml	2ml
Formamide (deionized)	28ml	28ml	28ml	28ml
Urea	29.4g	29.4g	29.4g	29.4g
Distilled water	To 100ml			
Total volume	100ml	100ml	100ml	100ml

Degas for 10-15 minutes. Filter through a 0.45µm filter, and store away from sunlight in a refrigerator for 1 month at 4°C. It may be necessary re-dissolve the denaturant after cold storage. If so, place the bottle within a 37°C water bath, preferably stirring the contents for faster dissolution (e.g. SWB-10L).

For 100ml denaturing solutions of maximum concentration less than 70%, the formamide and urea content may be adjusted as follows:

Denaturing Solution	10%	20%	30%	40%	50%	60%
Formamide	4ml	8ml	12ml	16ml	20ml	24ml
Urea	4.2g	8.4g	12.6g	16.8g	21g	25.2g

1ml, Ammonium Persulphate (APS) 10%.

Dissolve 0.1g APS in 1ml distilled water. Store at -20°C for 1 week.

Gradient Dye Solution

Reagent	Amount	Final Concentration
Bromophenol blue	0.05g	0.5%
Xylene cyanol	0.05g	0.5%
1x TAE buffer	10ml	1x

Store at room temperature.

2x Gel Loading Dye

Reagent	Amount	Final Concentration
2% Bromophenol blue	0.25 ml	0.05%
2% Xylene cyanol	0.25 ml	0.05%
100% Glycerol	7.0 ml	70%
dH2O	2.5 ml	
Total volume	10.0 ml	

Store at room temperature.

3.1.2) Gel Volumes for Parallel Denaturing Gradient Gels

The table below provides the required gradient volumes to make one 16x17.5cm (width x height) parallel denaturant gradient gel in the VS20WAVE-DGGE inner running module, using the GM100 gradient mixer. The volume per chamber represents the volumes required for the high percentage mixing (A) and low percentage reservoir (B) chambers in the gradient mixer. To make gradient gels, using a gradient mixer, it is advisable to make one gel at a time, before washing out the apparatus and setting it up again to prepare a second gradient gel.

The volume per chamber requires a surplus volume of denaturant mixture, because the excess volume in each chamber is necessary to ensure that the correct volume of gel solution enters and fills the gel sandwich. An excess gel volume also accounts for the volume required to fill the silicon tube.

Spacer thickness	Volume per 16 x 17.5cm gel	Volume per chamber
0.75mm	28ml	14ml
1mm	34ml	17ml
1.5mm	48ml	24ml

3.1.3) Sample Preparation

1. Before performing DGGE, the PCR reaction should be optimised to ensure that the samples are free of any unwanted PCR products which might interfere with gel analysis. PCR products can be evaluated for purity by agarose gel electrophoresis before being loaded onto a denaturing acrylamide

gel. Cleaver Scientific recommends the MSCHOICE and MSMIDI96 units for checking PCR products.

2. For a parallel denaturing gel, load 180-300ng of amplified DNA per well (usually 5-10% of a 100µl PCR volume from a 100ng DNA template). A wild-type control should be run on every gel.
3. Add an equal volume of 2x loading dye to the sample.
4. Heteroduplexes, if required, can be generated in two ways. The first method is to amplify both the wild-type and mutant DNA samples in the same tube during PCR, whereas in the second method both the wild-type and mutant samples may be amplified in separate tubes and heteroduplexes then formed by mixing an equal amount of wild-type and mutant within one tube, which is heated at 95°C for 5 minutes and stabilised at 65°C for a further hour, before allowing to cool slowly to room temperature.

3.1.4) Temperature Controller

The VS20WAVE-DGGETC temperature controller, shown below, maintains the desired temperature of the VS20WAVE-DGGE during electrophoresis. VS20WAVE-DGGETC temperature control unit combines buffer recirculation with a heat sensor and 1.4kW heating element to facilitate precise temperature control to within ±0.2°C. This allows the gel temperature to be set to the melting temperature (Tm) of the amplified polymorphism or mutation of interest. However, in the interests of safety and successful operation a few moments should be spent becoming acquainted with the features of the VS20WAVE-DGGETC before using it.

CAUTION! SAFETY CUT OUT AND ALARM LIGHT

The VS20WAVE-DGGETC is fitted with a float switch (9) that monitors the level of liquid during operation. If the liquid level within the VS20WAVE-DGGE tank were to fall below the level of the float switch during operation, the safety cut out would be activated causing the alarm light to appear. If this happens, disconnect the VS20WAVE-DGGE tank from the power supply, before removing the lid and adding sufficient buffer to re-immerse the float switch and resume electrophoresis.

NEVER OPERATE the temperature controller unless the buffer level at least covers the float switch.

Over-Temperature Dial (6)

The over-temperature dial is factory preset for safety. Since a maximum temperature of 65°C is generally accepted for denaturing gel electrophoresis within the scientific literature, it should not be necessary to set the over-temperature dial in excess of 70°C. The over-temperature dial may be readjusted by gently turning the dial to the desired setting using a flat-bladed screw driver.

N.B. Heat damage to the unit from excessive temperatures will invalidate the warranty.

Adjusting the buffer temperature (7, S [Select] button; 8, Dial).

Setting the buffer temperature is simple:

1. **Press S (7) –** the display will flash
2. **Rotate the Dial (8) to the desired buffer temperature** – e.g. 65°C
3. **Press S to store the temperature value** – the temperature is now set at 65°C, and the heater light will come on to show that heating is underway.
4. **Allow the buffer to reach temperature** (i.e. 65°C in this example).

3.1.5) Pre-heating the Running Buffer

1. Fill the VS20WAVE-DGGE electrophoresis tank with 7 L of 1x TAE running buffer.

N.B. Linear and double-stranded DNA migrates faster in TAE buffer, although buffering capacity becomes depleted particularly during extended runs. Consequently, it is advisable to avoid reusing TAE buffer, as the migration rate and band resolution of DNA molecules may be adversely affected in subsequent DGGE experiments.

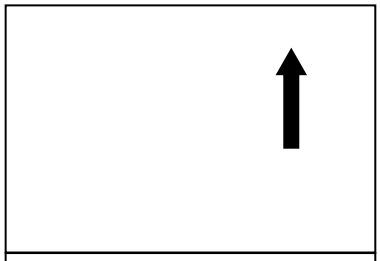
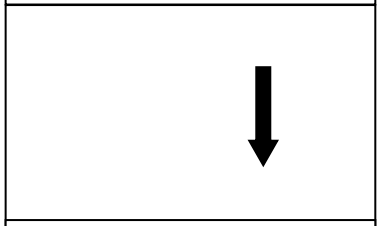
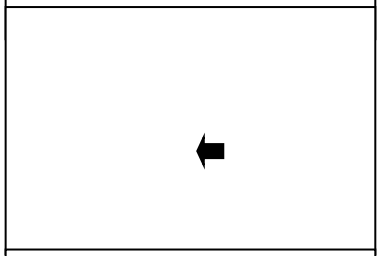
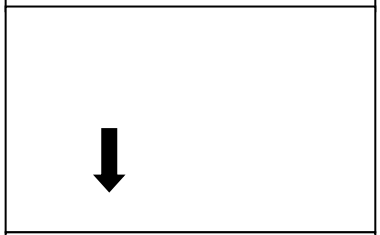
2. With the VS20WAVE-DGGETC temperature control unit in situ within the electrophoresis tank, connect the power cord to the temperature control unit and turn the power, pump, and heater on. The lid should be replaced on the VS20WAVE tank during preheating, to prevent evaporation and accidental scalding.

3. Set the temperature controller to the desired temperature as described in the section Temperature Controller. Set the desired temperature.

4. Preheat the buffer to the set temperature, which may take 1 to 1.5 hours. Heating the buffer in a microwave helps reduce the preheating time, but do take care not to overheat as it will take longer for the buffer to cool than it will to heat up.

3.1.6) Assembling the PAGE insert for Parallel Gradient Gel Casting

For parallel gel formats, a 16 x 17.5cm (w x h) gel sandwich size is recommended. To ensure proper alignment, clean and dry all plates and spacers before assembly. Use caution when assembling the glass plate sandwiches. Wear gloves and eye protection at all times, as acrylamide is neurotoxin.

1. Loosen the vertical screw-pins in the PAGE insert to release the locking mechanism, allowing the gel clamps to sit in the resting slots.	
2. Insert a gel cassette into each side of the inner buffer chamber in the PAGE insert, and begin tightening the vertical screw-pins.	
3. Continue to tighten the screw-pins until the gel clamps glide out of the resting slots and fix firmly against the glass plates pushing them upright.	
4. Check the bottom of the glass plates to ensure that they are flush together, and place the PAGE insert on the casting base; make sure that the cams are facing downwards.	
5. Insert cams and turn until tightened, usually after 90 degrees, drawing the PAGE insert onto the casting to form a leak-proof seal.	
6. The PAGE insert is now ready for gel casting.	

3.1.7) Gradient Mixer Installation and Casting Parallel Gradient Gels

1) Secure the gradient mixer to a retort stand by using the red handle supplied and attach a 25cm piece of silicon tubing to the outlet port of the mixing chamber. Very carefully pass the tubing over to the PAGE insert and by using some masking tape fix the other end of it, connected to a syringe needle, between the glass plate cassettes. The needle should be positioned at the top of the gel in the middle of the glass plates. If using the optional but highly recommended MU-D01 peristaltic pump carefully thread the silicon tubing through the pump head before affixing the syringe needle to the glass plates, as described above. For polyacrylamide slab gels a flow rate of 5ml per minute (10 rpm) is sufficient.
2) After setting up the gradient mixer as stated above, place it on a magnetic stirrer by readjusting the height of the gradient mixer on the retort stand. Place a small magnetic stirring bar in the mixing chamber. Cleaver Scientific recommends the CSL-STIR and MU-S16 silicon tube (I.D. 1/8"). Please see the schematic diagram overleaf. However, if using gravity to fill the gel cassettes adjust the height of the gradient mixer so that it is above the PAGE insert.
3) Close the stopcock valves for the reservoir and mixing chambers.
4) Prepare the high and low denaturant concentration solutions in separate 50mL Falcon tubes, labelled **'HIGH'** and **'LOW'** respectively, as described in **Parallel Gradient Gel Preparation**.
   Optional: Add 100□l of Gradient Dye Solution per 5mL of high density solution to serve as a visual indicator of gradient gel formation.

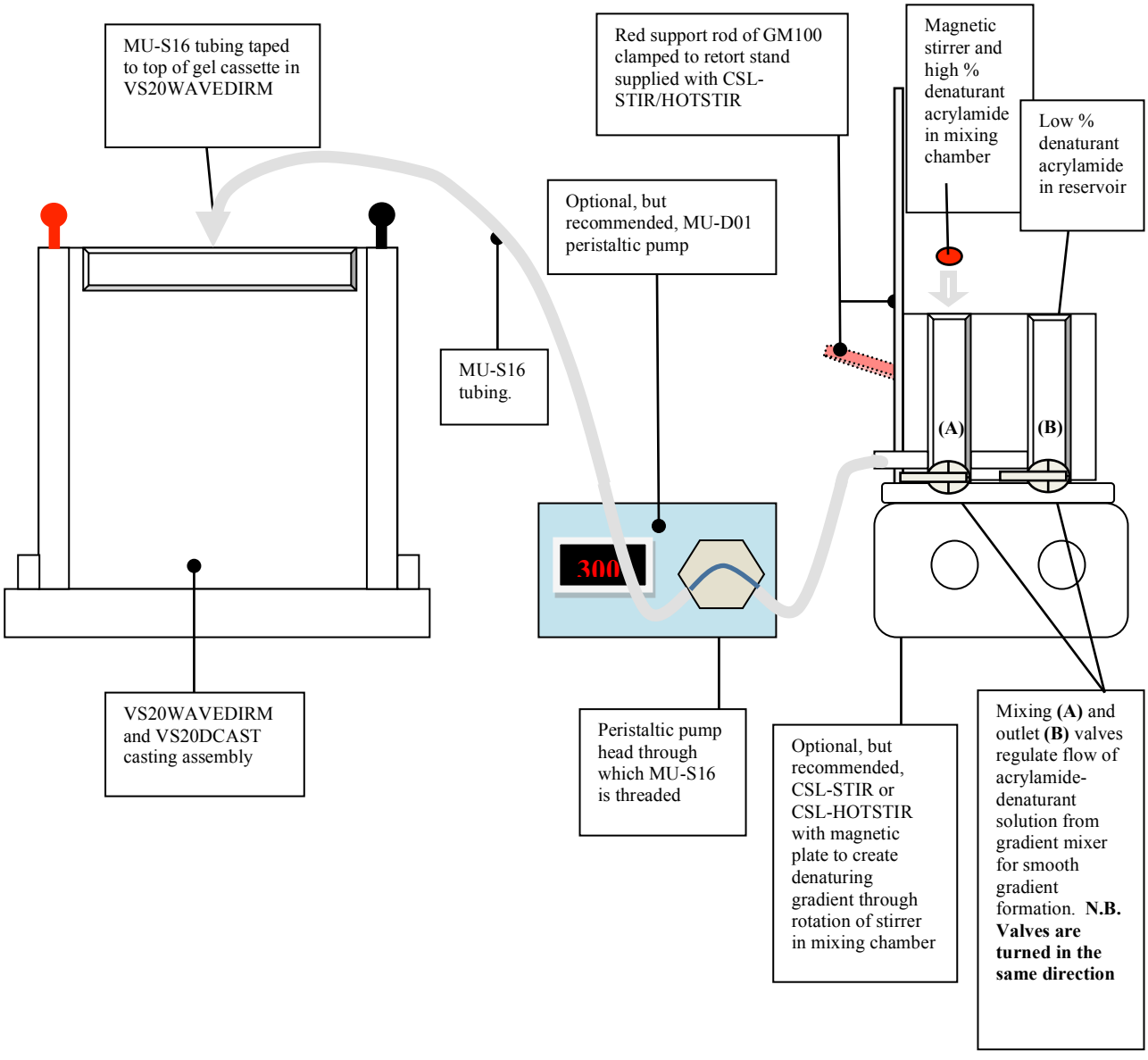
   ***DO NOT ADD POLYMERISING AGENTS AT THIS POINT UNTIL FIRST REVIEWING STEPS 5-12 BELOW*.**

5) **BEFORE PROCEEDING WITH THE FOLLOWING STEPS it is important to become familiar with the workings of the gradient mixer as described below, because once the polymerising agents are added to the acrylamide there is a maximum timeframe of 10 minutes to cast the gradient gel before polymerisation. A couple of trial runs with distilled water should be performed if necessary.**
6) Add a final concentration of 0.09% (v/v) each of ammonium persulphate and TEMED solutions to the high and low percentage denaturant solutions. The 0.09% (v/v) concentrations allow no more than 10 minutes to finish casting the gel before polymerisation. Replace the cap and mix by inverting each tube gently several times.
7) Add the low percentage denaturant solution to the reservoir chamber and fill the stopcock valve with this solution by opening the valve, allowing the tube in it to fill before, again, closing the valve. **PROCEED QUICKLY AND DO NOT ALLOW ACRYLAMIDE TO ENTER THE MIXING CHAMBER.**
   Now transfer the high percentage denaturant solution to the mixing chamber.
8) Start the magnetic stirrer.
9) Start the peristaltic pump and simultaneously open the outlet port of the mixing chamber of the gradient mixer. Open the connecting valve between the mixing and reservoir chambers.
10) Run the acrylamide denaturing gel mix slowly down from the upper centre of the gel cassette. Avoid any bubble formation, as bubbles inhibit polymerisation.

11) Allow all of the solution in the mixing chamber to empty before turning off the peristaltic pump.

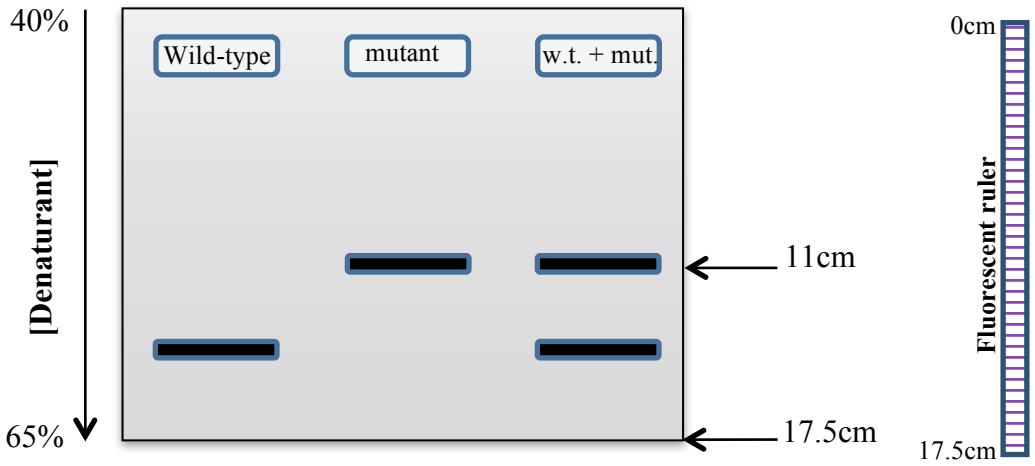
12) Remove the tube from the glass cassette assembly. Insert the comb and allow the acrylamide solution to polymerise properly for 1 hour. Detach the gradient mixer from the retort stand and wash thoroughly in warm soapy water. Rinse with distilled water and drain before preparing a second gradient gel if desired.

Schematic Diagram of the VS20WAVE-DGGE Gradient Gel Casting Set Up



3.2) Introduction to Constant Denaturing Gel Electrophoresis (CDGE)

Constant Denaturing Gel Electrophoresis (CDGE) is a modification of DGGE. In CDGE, the denaturant concentration that gives optimal resolution from a parallel or perpendicular DGGE gel is held constant[6]. The optimal concentration of denaturant to use for a CDGE is determined from the maximum split between wild-type and mutant DNA, as seen in corresponding the perpendicular or parallel denaturing gel. To calculate the concentration of denaturant required for a CDGE gel, first place a fluorescent ruler along the axis of the denaturant gradient when taking a photograph. Then, determine the distance along the gradient where the maximum split is seen between wild-type and mutant bands. If, for example, the distance is 11 cm, divide this distance by the length of the gel and then multiply by the denaturant range (e.g. 25%; or 40-65%). For example, (11 ÷ 17.5) x 25% = 16 %, when rounded up to 1 significant figure. Add this number to the starting denaturant concentration to determine the optimum concentration to use for CDGE (40% + 16% = 56%). This same calculation is also applied to samples run on perpendicular DGGE gels.



A schematic diagram showing how parallel DGGE might be applied to determine the optimum denaturant concentration used in a CDGE gel. The distance along the gradient where the maximum split is seen between samples is 11 cm. The denaturant concentration of the gradient at this distance is 56%. Therefore, the CDGE gel should use a denaturant concentration of 56%. From left to right: lane 1 wild-type fragment; lane 2: mutant fragment; lane 3: wild-type and mutant fragments (w.t+mut.).

Once DGGE has been applied to determine the optimum denaturant concentration to identify a specific mutation, a CDGE gel can be then used to screen samples for the presence of the mutation. With no gradient required, rapid, high throughput screening is possible. As in DGGE, heteroduplex analysis can be used to help in resolving wild-type and mutated fragments when it is not possible to detect a mutation by running homoduplex fragments.

[6] Hovig, E., Smith-Sorensen, B., Uitterlinden, A., and Borresen, A., *Pharmacogenetics*, **2**, 317–328 (1992).

3.2.1) Constant Denaturing Gel Reagent Preparation

The concentration of denaturant is determined from a parallel DGGE gel when using the VS20WAVE-DGGE system. The concentration of acrylamide may vary, depending on the size of the fragment that is being analysed. Both 0% and 100% denaturant should be made as stock solutions. A 100% denaturant is a mixture of 7 M urea and 40% deionized formamide.

Stock Solutions

40% Acrylamide/Bis (37.5:1), 100ml

However, for different percentage crosslinking, use the equation below to determine the amount of Bis to add. The example stock solution is for an acrylamide/bis ratio of 37.5:1.

Polyacrylamide gels are described by reference to two characteristics:
1) Total monomer concentration (%T)
2) Crosslinking monomer concentration (%C)

$$\%T = \frac{\text{gm acrylamide + g bis-acrylamide}}{\text{total volume}} \times 100\%$$

$$\%C = \frac{\text{gm bis-acrylamide}}{\text{gm acrylamide + g bis-acrylamide}} \times 100\%$$

50x TAE Buffer, 1L. This may be purchased using code TAE50X1L or made up as follows:

Reagent	Amount	Final Concentration
Tris base	242g	2M
Acetic Acid, glacial	57.1ml	1M
0.5M EDTA, pH 8.0	100ml	50mM
Distilled water	To 1000mL final volume.	

Mix and, preferably, autoclave for 20-30 minutes. Store at RTP.

0% Denaturing Solution

	6% Gel	8% Gel	10% Gel	12% Gel
40% Acrylamide / Bis	15ml	20ml	25ml	30ml
50x TAE buffer	2ml	2ml	2ml	2ml
Distilled water	83ml	78ml	73ml	68ml
Total volume	100ml	100ml	100ml	100ml

Degas for 10-15 minutes. Filter through a 0.45µm filter, and store away from sunlight in a refrigerator for 1 month at 4°C.

To make 100ml 100% Denaturing Solution

	6% Gel	8% Gel	10% Gel	12% Gel
40% Acrylamide / Bis	15ml	20ml	25ml	30ml
50x TAE buffer	2ml	2ml	2ml	2ml
Formamide (deionized)	40ml	40ml	40ml	40ml
Urea	42g	42g	42g	42g
Distilled water	To 100ml			
Total volume	100ml	100ml	100ml	100ml

Degas for 10-15 minutes. Filter through a 0.45µm filter, and store away from sunlight in a refrigerator for 1 month at 4°C. It may be necessary re-dissolve the denaturant after cold storage. If so, place the bottle within a 37°C water bath, preferably stirring the contents for faster dissolution (e.g. SWB-10L).

To cast constant denaturing gradient gels, use the formula below to determine the volume of 0% and 100% denaturing solutions needed to achieve the desired denaturant concentration.

1. (% desired denaturant) (total gel volume needed) = ml of 100% denaturant solution

2. (total gel volume needed) - (ml of 100% denaturant) = ml of 0% denaturant solution

Example: To cast a 56% constant denaturing gel, use 32 ml total volume for a 16 x 17.5 cm gel with a 1.0 mm bonded spacer.

1. (0.56)(32 ml) = 17.9 ml of 100% denaturing solution needed

2. (32 ml) - (17.9 ml) = 14.1 ml of 0% denaturing solution needed

Please consult the table below for the recommended % acrylamide/ bis required for a particular DNA size range.

Gel Percentage	Basepair Separation (bp)
6%	300-1000bp
8%	200-400bp
10%	100-300bp

1ml, Ammonium Persulphate (APS) 10%.

Dissolve 0.1g APS in 1ml distilled water. Store at -20°C for 1 week.

Gradient Dye Solution

Reagent	Amount	Final Concentration
Bromophenol blue	0.05g	0.5%
Xylene cyanol	0.05g	0.5%
1x TAE buffer	10ml	1x

Store at room temperature.

2x Gel Loading Dye

Reagent	Amount	Final Concentration
2% Bromophenol blue	0.25 ml	0.05%
2% Xylene cyanol	0.25 ml	0.05%
100% Glycerol	7.0 ml	70%
dH2O	2.5 ml	
Total volume	10.0 ml	

Store at room temperature.

3.2.2) Gel Volumes

The table below shows the required volume per gel based on gel size and spacer thickness/

Spacer thickness	Volume per 16 x 17.5cm gel
0.75mm	25ml
1mm	32ml
1.5mm	46ml

3.2.3) Sample Preparation

1. Before performing DGGE, the PCR reaction should be optimised to ensure that the samples are free of any unwanted PCR products which might interfere with gel analysis. PCR products can be evaluated for purity by agarose gel electrophoresis before being loaded onto a denaturing acrylamide gel. Cleaver Scientific recommends the MSCHOICE and MSMIDI96 units for checking PCR products (please visit www.cleaverscientific.com).
2. For a parallel denaturing gel, load 180-300ng of amplified DNA per well (usually 5-10% of a 100µl PCR volume from a 100ng DNA template). A wild-type control should be run on every gel.
3. Add an equal volume of 2x loading dye to the sample.
4. Heteroduplexes, if required, can be generated in two ways. The first method is to amplify both the wild-type and mutant DNA samples in the same tube during PCR, whereas in the second method both the wild-type and mutant samples may be amplified in separate tubes and heteroduplexes then formed by mixing an equal amount of wild-type and mutant within one tube, which is heated at 95°C for 5 minutes and stabilised at 65°C for a further hour by allowing to cool slowly to room temperature.

3.2.4) Temperature Controller

The VS20WAVE-DGGETC temperature controller, shown below, maintains the desired temperature of the VS20WAVE-DGGE during constant denaturing gel electrophoresis. VS20WAVE-DGGETC temperature control unit combines buffer recirculation with a heat sensor and 1.4kW heating element to facilitate precise temperature control to within ±0.2°C. This allows the gel temperature to be set to the melting temperature (Tm) of the amplified polymorphism or mutation of interest. However, in the interests of safety and successful operation a few moments should be spent becoming acquainted with the features of the VS20WAVE-DGGETC before using it.

CAUTION! SAFETY CUT OUT AND ALARM LIGHT

The VS20WAVE-DGGETC is fitted with a float switch (9) that monitors the level of liquid during operation. If the liquid level within the VS20WAVE-DGGE tank were to fall below the level of the float switch during operation, the safety cut out would be activated causing the alarm light to appear. If this happens, disconnect the VS20WAVE-DGGE tank from the power supply, before removing the lid and adding sufficient buffer to re-immerse the float switch and resume electrophoresis.

NEVER OPERATE the temperature controller unless the buffer level at least covers the float switch.

Over-Temperature Dial (6)

The over-temperature dial is factory preset for safety. Since a maximum temperature of 65°C is generally accepted for denaturing gel electrophoresis techniques within the scientific literature, it should not be necessary to set the over-temperature dial in excess of 70°C. The over-temperature dial may be readjusted by gently turning the dial to the desired setting using a flat-bladed screw driver.

N.B. Heat damage to the unit from excessive temperatures will invalidate the warranty.

Adjusting the buffer temperature (7, S [Select] button; 8, Dial).

Setting the buffer temperature is simple:

1. **Press S (7) –** the display will flash
2. **Rotate the Dial (8) to the desired buffer temperature** – e.g. 65°C

3. **Press S to store the temperature value** – the temperature is now set at 65°C, and the heater light will come on to show that heating is underway.
4. **Allow the buffer to reach temperature** (i.e. 65°C in this example).

3.2.5) Pre-heating the Running Buffer

1. Fill the VS20WAVE-DGGE electrophoresis tank with 7 L of 1x TAE running buffer.
N.B. Linear and double-stranded DNA migrates faster in TAE buffer, although buffering capacity becomes depleted particularly during extended runs. Consequently, it is advisable to avoid reusing TAE buffer, as the migration rate and band resolution of DNA molecules may be adversely affected in subsequent CDGE experiments.
2. With the VS20WAVE-DGGETC temperature control unit in situ within the electrophoresis tank, connect the power cord to the temperature control unit and turn the power, pump, and heater on. The lid should be replaced on the VS20WAVE tank during preheating, to prevent evaporation and accidental scalding.
3. Set the temperature controller to the desired temperature as described in the section Temperature Controller. Set the desired temperature.
4. Preheat the buffer to the set temperature, which may take 1 to 1.5 hours. Heating the buffer in a microwave helps reduce the preheating time, but do take care not to overheat as it will take longer for the buffer to cool than it will to heat up.

3.2.6) Assembling the PAGE insert for CDGE Gel Casting

For parallel gel formats, a 16 x 17.5cm (w x h) gel sandwich size is recommended. To ensure proper alignment, clean and dry all plates and spacers before assembly. Use caution when assembling the glass plate sandwiches. Wear gloves and eye protection at all times, as acrylamide is neurotoxin.

1. Loosen the vertical screw-pins in the PAGE insert to release the locking mechanism, allowing the gel clamps to sit in the resting slots.	
2. Insert a gel cassette into each side of the inner buffer chamber in the PAGE insert, and begin tightening the vertical screw-pins.	
3. Continue to tighten the screw-pins until the gel clamps glide out of the resting slots and fix firmly against the glass plates pushing them upright.	
4. Check the bottom of the glass plates to ensure that they are flush together, and place the PAGE insert on the casting base; make sure that the cams are facing downwards.	
5. Insert cams and turn until tightened, usually after 90 degrees, drawing the PAGE insert onto the casting to form a leak-proof seal.	
6. The PAGE insert is now ready for gel casting.	

3.2.7) Casting CDGE Gels

With the PAGE insert set up for casting, start making the appropriate denaturant gel solution following the directions below.

1. Add the required amounts of low density and high density solutions required for the desired denaturant percentage (see CDGE calculation) to a 50ml Falcon tube. Replace the cap and mix gently by inverting the tube.
2. Remove the cap and add a final concentration of 0.09% (v/v) each of ammonium persulphate and TEMED solutions to the denaturant solution. The 0.09% (v/v) concentrations allow no more than 10 minutes to finish casting the gel before polymerisation. Replace the cap again and mix by inverting each tube gently several times.
3. Insert a comb in the gel sandwich and tilt it so the teeth are at a slight angle (e.g. 20-30 degrees from the top left hand corner of the gel). This will prevent air from being trapped under the comb teeth when pouring the gel solution.
4. Pour or pipette the gel solution into the sandwich until the gel solution covers the wells of the comb. Straighten the comb to the desired well depth, by pushing it downwards at the angle between the glass plates. Add more gel solution if needed.
5. Allow the gel to polymerize for about 60 minutes. After polymerization, remove the comb by pulling it straight up, both slowly and gently.
6. Proceed to the section on Electrophoresis.

Section 4

4.1) Introduction to Heteroduplex Analysis (HA)

Heteroduplex Analysis (HA) is based on conformational differences in double-stranded DNA caused by the formation of heteroduplex molecules.[7] Heteroduplex molecules have a mismatch in the double-strand, which induces a distortion in their usual conformation that makes them migrate more slowly within a polyacrylamide gel than their corresponding homoduplex molecules. Consequently, it is this difference in mobility that enables heteroduplexes to be detected within a gel; to the extent that heteroduplexes with as little as one mismatch show a difference in mobility in a gel compared to their homoduplex counterparts. Heteroduplexes may be generated in the following ways: during PCR of a heterozygous individual, or by adding mutant and wild-type DNA in the same PCR reaction, or by denaturation and renaturation of mutant and wild-type DNA in a single tube. Both mutant and wild-type samples are run on the same gel and the mobility of the fragments is compared.

The sensitivity of heteroduplex analysis is 80–90% in small DNA fragments (< 300 bp).[8] The sensitivity of mutation detection can be improved when used in conjunction with SSCP (single-strand conformation polymorphism).[9] A polyacrylamide analogue has been developed (MDE™ or DEM™) which enhances the ability to detect mutations in

[7] Myers, R., Larin, Z., and Maniatis, T., *Science*, **230**, 1242–1246 (1985).

[8] White, M., Carvalho, M., Derse, D., O'Brien, S., and Dean, M., *Genomics*, **12**, 301–306 (1992).

[9] Glavac, M., Glavac, D., and Dean, M., *Hum. Mol. Genet*., **3**, 801–807 (1994).

heteroduplex samples when compared to conventional polyacrylamide gels.[10] The addition of urea to the gel can create a mildly denaturing environment that may increase the separation of heteroduplexes, making mutation detection easier.[5]

A variation of heteroduplex analysis is Conformation Sensitive Gel Electrophoresis (CSGE). This technique exploits the observation that a mildly denaturing environment will enhance the ability of single base pair mismatches to induce conformational changes.[11] These changes also increase the differential migration of heteroduplex and homoduplex molecules. Samples are electrophoresed in 6–10% polyacrylamide gels (99:1), with tris-taurine buffer and 10% ethylene glycol with 15% formamide as denaturants. Bis (acryloyl) piperazine (BAP) or piperazine diacrylamide (PDA) can also be used instead of bis as a cross-linker.[12] BAP or PDA cross-linker helps to improve the gel strength and increase the pore size in the gel. PCR fragment sizes for CSGE are often between 300–800 bp in length for optimum mutation detection.

4.1.1) Reagent Preparation - Heteroduplex Analysis (HA)

The concentration of acrylamide may vary, depending on the size of the fragment that is being analysed. Accordingly, a 40% stock solution containing acrylamide and bis-acrylamide (bis) or a 2x DEM solution should be made or purchased.

Stock Solutions

40% Acrylamide/Bis (37.5:1), 100ml

However, for different percentage crosslinking, use the equation below to determine the amount of Bis to add. The example stock solution is for an acrylamide/bis ratio of 37.5:1.

Polyacrylamide gels are described by reference to two characteristics:
1) Total monomer concentration (%T)
2) Crosslinking monomer concentration (%C)

$$\%T = \frac{\text{gm acrylamide + g bis-acrylamide}}{\text{total volume}} \times 100\%$$

$$\%C = \frac{\text{gm bis-acrylamide}}{\text{gm acrylamide + g bis-acrylamide}} \times 100\%$$

1

10x TBE Buffer, 1L. This may be purchased using code TBE10X1L or made up as follows:

Reagent	Amount	Final Concentration
Tris base	108g	0.89M
Boric Acid	55g	0.89M
0.5M EDTA, pH 8.0	40ml	50mM
Distilled water	To 1000ml final volume.	

Mix. Autoclave for 20-30 minutes. Store at RTP.

[10] Keen, J., Lester, D., Inglehearn, C., Curtis, A., and Bhattacharyya, S., *Trends Genet.*, **7**, 5 (1991).

[11] Ganguly, A., Rock, M., and Prockop, D., *Proc. Natl Acad. Sci.*, **90**, 10325–10329 (1993).

[12] Williams, C., Rock, M., Considine, E., McCarron, S., Gow, P., Ladda, R., McLain, D., Michels, V., Murphy, W., Prockop, D., and Ganguly, A., *Hum. Mol. Genet.*, **4**, 309–312 (1995).

40% Acrylamide/Bis Solutions (1x TBE)

	6% Gel	8% Gel	10% Gel	12% Gel
40% Acrylamide / Bis	6ml	8ml	10ml	12ml
10x TBE buffer (see note)	4ml	4ml	4ml	4ml
Urea (optional)	See note	See note	See note	See note
TEMED	40 µl	40 µl	40 µl	40 µl
10% APS	400 µl	400 µl	400 µl	400 µl
Total volume	40ml	40ml	40ml	40ml

Add distilled water to 40ml and mix. Cast the gel immediately after the addition of the TEMED and APS catalysts.

Note: For 0.5X TBE, add 2ml; for 15% urea, add 6g.

To make 2 x DEM Solution (0.6x TBE)

Reagent	1x DEM Gel	0.8x DEM Gel
2x DEM	20ml	16ml
10x TBE buffer (see note)	2.4ml	2.4ml
Urea (optional)	See note	See note
TEMED	40 µl	40 µl
10% APS	400 µl	400 µl
Total volume	40ml	40ml

Add distilled water to 40ml and mix. Cast the gel immediately after the addition of the TEMED and APS catalysts.

Note: For 0.5X TBE, add 2ml; for 1X TBE, add 4ml. For 15% urea, add 6g.

1ml, Ammonium Persulphate (APS) 10%.

Dissolve 0.1g APS in 1ml distilled water. Store at -20°C for 1 week.

2x Gel Loading Dye

Reagent	Amount	Final Concentration
2% Bromophenol blue	0.25 ml	0.05%
2% Xylene cyanol	0.25 ml	0.05%
100% Glycerol	7.0 ml	70%
dH2O	2.5 ml	
Total volume	10.0 ml	

Store at room temperature.

1x TBE Running Buffer

Reagent	2 L Buffer	7 L Buffer
10x TBE buffer	200ml	700ml
Distilled water	1800ml	6300ml

0.6x TBE Running Buffer

Reagent	2 L Buffer	7 L Buffer
10x TBE buffer	120ml	420ml
Distilled water	1880ml	6580ml

CSGE Analysis

For a different percentage crosslinking, use the equation below to determine the amount of crosslinker to add. The example stock solution is for an acrylamide/PDA ratio of 99:1.

40% Acrylamide/PDA (99:1)

Reagent	Amount
Acrylamide	198g
PDA or BAP	2g
Distilled water	To 500ml final volume.

Filter through a 0.45µm filter. Store at 4°C.

DANGER! Acrylamide is a neurotoxin and can be easily inhaled in its powdered form. Always wear a mask when dispensing it.

10x TTE Buffer

Reagent	Amount	Final Concentration
Tris base	107.8g	0.89M
Taurine	18.8g	0.15M
EDTA	1.9g	5mM
Distilled water	To 1000ml final volume.	

Mix. Autoclave for 20-30 minutes. Store at RTP.

10% Acrylamide/PDA Gel

Reagent	Amount	Final Concentration
40% Acrylamide / PDA (see note)	10ml	10%
10x TTE	2ml	0.5x
Formamide	6ml	15%
Ethylene Glycol	4ml	10%
Distilled water	17.6ml	
TEMED	40 µl	
10% APS	400 µl	
Total volume	40ml	40ml

Cast the gel immediately after the addition of the TEMED and APS catalysts.

Note:
For a 6% acrylamide gel use 6ml of 40% stock
For a 8% acrylamide gel use 8ml of 40% stock
For a 12% acrylamide gel use 12ml of 40% stock

1x TTE Buffer VS20WAVE-DGGE Outer Tank

Reagent	8 L Buffer
10x TTE Buffer	800ml
Distilled water	7200ml

However, fill to 7L initially.

0.25x TTE Buffer VS20WAVEDIRM PAGE insert

Reagent	800ml Buffer
10x TTE Buffer	20ml
Distilled water	680ml

4.1.2) Gel Volumes

The table below shows the required volume per gel based on gel size and spacer thickness/

Spacer thickness	Volume per 16 x 17.5cm gel
0.75mm	25ml
1mm	32ml

4.1.3) Sample Preparation

1. Heteroduplexes can be generated in two ways. The first method is to amplify both the wild-type and mutant DNA samples in the same tube during PCR, whereas in the second method both the wild-type and mutant samples may be amplified in separate tubes and heteroduplexes then formed by mixing an equal amount of wild-type and mutant within one tube, which is heated at 95°C for 5 minutes and stabilised at 65°C for a further hour, before allowing to cool slowly to room temperature.
2. Before performing HA, the PCR reaction should be optimised to ensure that the samples are free of any unwanted PCR products which might interfere with gel analysis. PCR products can be evaluated for purity by agarose gel electrophoresis before being loaded onto a denaturing acrylamide gel. Cleaver Scientific recommends the MSCHOICE and MSMIDI96 units for checking PCR products (please visit www.cleaverscientific.com).
3. Approximately 180-500ng of heteroduplex DNA per well (usually 5-15% of the total PCR volume from a 100ng DNA template). A wild-type control should be run on every gel.
4. Add an equal volume of 2x loading dye to the sample.

4.1.4) Temperature Controller

The VS20WAVE-DGGETC temperature controller maintains the desired temperature of the VS20WAVE-DGGE. Its temperature and resultant buffer temperature may be set as described previously. Because the temperature controller is not required for heteroduplex analysis, runs are usually performed at room temperature.

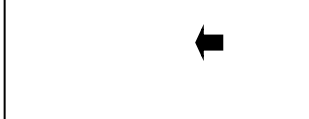
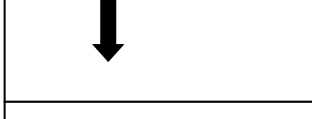
4.1.5) Adding the Running Buffer

1. Remove the lid from the VS20WAVE-DGGE tank.
2. Add 7 L of running buffer to the electrophoresis tank. Add a further 640ml to VS20WAVEDIRM insert. For CSGE, note that the buffer concentration within the VS20WAVEDIRM insert is different from the buffer concentration in the outer tank.

Note: To improve heat dissipation during electrophoresis, the VS20WAVE-DGGE tank should filled with buffer to the 'max fill' mark.

4.1.6) Assembling the PAGE insert for HA Gel Casting

For parallel gel formats, a 16 x 17.5cm (w x h) gel sandwich size is recommended. To ensure proper alignment, clean and dry all plates and spacers before assembly. Use caution when assembling the glass plate sandwiches. Wear gloves and eye protection at all times, as acrylamide is neurotoxin.

1. Loosen the vertical screw-pins in the PAGE insert to release the locking mechanism, allowing the gel clamps to sit in the resting slots.	
2. Insert a gel cassette into each side of the inner buffer chamber in the PAGE insert, and begin tightening the vertical screw-pins.	
3. Continue to tighten the screw-pins until the gel clamps glide out of the resting slots and fix firmly against the glass plates pushing them upright.	
4. Check the bottom of the glass plates to ensure that they are flush together, and place the PAGE insert on the casting base; make sure that the cams are facing downwards.	
5. Insert cams and turn until tightened, usually after 90 degrees, drawing the PAGE insert onto the casting to form a leak-proof seal.	
6. The PAGE insert is now ready for gel casting.	

4.1.7) Casting HA Gels

With the PAGE insert set up for casting, start making the appropriate denaturant gel solution following the directions below.

1. Add the required amount of DEM solution (see HA Reagent Preparation) to a 50ml Falcon tube. Replace the cap and mix gently by inverting the tube.
2. Remove the cap and add a final concentration of 0.09% (v/v) each of ammonium persulphate and TEMED solutions to the denaturant solution. The 0.09% (v/v) concentrations allow no more than 10 minutes to finish casting the gel before polymerisation. Replace the cap again and mix by inverting each tube gently several times.
3. Insert a comb in the gel sandwich and tilt it so the teeth are at a slight angle (e.g. 20-30 degrees from the top left hand corner of the gel). This will prevent air from being trapped under the comb teeth when pouring the gel solution.
4. Pour or pipette the gel solution into the sandwich until the gel solution covers the wells of the comb. Straighten the comb to the desired well depth, by pushing it downwards at the angle between the glass plates. Add more gel solution if needed.
5. Allow the gel to polymerise for about 60 minutes. After polymerisation, remove the comb by pulling it straight up, both slowly and gently.
6. Proceed to the section on Electrophoresis.

Section 5
Electrophoresis

5.1) Finalising Set Up of the WAVEDGGE Tank for Electrophoresis

DGGE and CDGE Gels

1. The electrophoresis tank should contain 7 L of the appropriate running buffer.
2. When the running buffer has reached the desired temperature, switch off the VS20WAVE-DGGETC and disconnect the power cord. Remove the lid.
3. Gently remove the comb from each gel, taking care not to damage the wells. Detach the PAGE insert from the casting base by unlocking and turning the cam pins.
4. Transfer the PAGE insert to the VS20WAVE-DGGE tank, noting that the insert will only fit in one orientation.
5. Reconnect the power cord and turn back on the VS20WAVE-DGGETC. Wash the wells with running buffer to flush out any unpolymerised acrylamide or leached denaturants from the wells. If necessary, add more buffer to the “max fill” line of the electrophoresis tank. Return the lid to PAGE insert within the tank.
6. Allow the system to reach the set initial temperature before loading samples. This may take 10–15 minutes.

Heteroduplex Gels

1. The electrophoresis tank should contain at least 2 L of the appropriate running buffer.
2. Gently remove the comb from each gel, taking care not to damage the wells. Detach the PAGE insert from the casting base by unlocking and turning the cam pins. Transfer the PAGE insert to the VS20WAVE-DGGE tank, noting that the insert will only fit in one orientation.
3. Wash the wells with running buffer to flush out any unpolymerised acrylamide. If necessary, add more buffer to the "max fill" line of the electrophoresis tank. Return the lid to PAGE insert within the tank.

5.2) Sample Loading

1. Remove the lid and, having first ensured that the wells are free of any impurities, load the samples using a pipette and a sequencing loading tip. Be careful not to pierce the wells during sample delivery.

5.3) Running the Gel

1. Attach the red and black power cables, which should be already screwed into the lid, to the corresponding ports within a suitable DC power supply. Cleaver Scientific recommends the CS-500V programmable power supply or the nanoPAC-500.

   ### DGGE and CDGE Gels

   For DGGE and CDGE run the gel at 130 volts. Apply power to the VS20WAVE-DGGE and begin electrophoresis. As a precaution, always set the voltage, current, and power limits when possible.

N.B. The voltage should not exceed 180 V; otherwise electrophoretic heating may affect results.
Optional: If the power supply has a built-in timer, set the power supply timer to the desired run time, noting that the run time should be determined empirically for each fragment being analysed. As a reference during electrophoresis, two marker dyes in the 2x gel loading dye can be used to determine when to stop a DGGE and CDGE run. The dyes are bromophenol blue (dark blue) and Xylene cyanol (light blue).

Heteroduplex and CSGE Gels

1. For heteroduplex analysis, run the gel at 100 volts for 16–20 hours. The run time should be determined empirically for each fragment being analysed. CSGE gels are typically run between 1–2 mA. Check that the power supply in use does not shut off when the current is below 2 mA.
2. Begin electrophoresis. As a precaution, always set voltage, current, and power limit when possible.

N.B. The VS20WAVE-DGGETC temperature control unit is **SWITCHED OFF** for heteroduplex and CSGE runs.

5.4) Removing the Gel

1. Following electrophoresis, turn off the power supply and temperature control unit. Disconnect the power cord and electrical leads, and allow the heater to cool for approximately 1 minute in the buffer.
2. Gently remove the PAGE insert from the VS20WAVE tank, taking care to avoid contact with the heating element of the temperature control, which may be **HOT!**
3. Transfer the PAGE insert to a clean bench surface covered with paper towels. Twist the red and black screws on the PAGE insert to unlock the sliding clamps. Push the sliding clamps upwards to release the pressure on gel cassettes. Remove the gel cassettes and gently pry open the glass plates in the centre, using a spacer of the corresponding thickness or a gel-release tool.
4. If running two gels, cut the corner of one gel so it may be easily distinguished from the other and therefore identified.
5. Empty the buffer from the tank and rinse the heating element of temperature control unit with tap water followed by distilled water.

5.5) Staining and Photographing the Gel

DGGE, CDGE and Heteroduplex Gels

1. Carefully tease the gel, using either a spacer or a spatula, from the glass plate to which has attached, into a suitable dish or tub containing 250 ml of running buffer and 25 □l of 10 mg/ml ethidium bromide (50 □g/ml).
2. Stain for 5–15 minutes.
3. After staining, carefully transfer the gel into a dish containing 250 ml of 1x running buffer.
4. Destain for 5–20 minutes.
5. Place the gel on a UV transilluminator of a suitable gel documentation system and photograph (the Cleaver Scientific microDOC [CSL-MDOCUVTS312] and omniDOC [CSL-OMNIDOC], both with 312nm transilluminators, are ideal for this purpose – please contact info@cleaverscientific.com). 1D image analysis with band pattern matching within individual gels may be performed using Phoretix 1D, while Phoretix 1D Pro may be used for band pattern matching between different gels.

Section 6
Troubleshooting – Common Questions

6.1) Equipment

Problem	Cause	Solution
Temperature Controller		
Unit fails to power	On off switch on controller off Fuse is blown Lid isn't fitted Fuse in power cord Float might not be up enough because there is not enough liquid or it may be stuck.	Turn switch on at back of controller Insert spare fuse from power lead inlet Fit lid properly and make sure unit is full of buffer Put new fuse in plug Make sure float is adjusted properly and free moving and up enough by filling with buffer
No display with power on	Burned out fuse or inner circuitry	Do not attempt to open housing as warranty will be immediately invalidated. Contact your distributor or manufacturer
Display shows "Cut"	Over-temperature cut-out has operated	Check the set temperature is correct and that the over-temperature cut-out temperature is set at least 5°C above the set temperature.
Temperature does not rise when expected	Set temp is lower than liquid temp Set temperature is too close to ambient	Check that the bath set temperature is correct Increase the set temperature or fit accessory cooling e.g. cool pack
Temp continues to rise when not expected	Set temp is higher than liquid temp	Check that the DGGE controller set temperature is correct
Alarm light on	Over-temperature cut-out has operated Low liquid level float switch has operated	Check the over-temperature cut-out is set appropriately Check that the liquid level in the WAVE-DGGE tank is adequate
Display shows "Flot"	Liquid level has dropped below minimum level	Check that the liquid level in the WAVE-DGGE tank is adequate
Display shows "–Al-"	High temperature warning alarm has tripped	Check that the set temperature is correct Check that the setting for the high temperature alarm is correct Check that the liquid level in the WAVE-DGGGE tank is adequate

Casting Gels		
Leaking during gel casting	Incorrect assembly of gel sandwich	Check that the spacers and glass plates are flush before pouring acrylamide; check with a small volume of water beforehand
	Over-turned cams	Cams should not be turned more than 90 degrees in alternate directions
	PAGE insert and glass plates assembled on casting base	All perform glass plate assembly on flat surface, before transferring to casting base; never on the casting base
	Chipped glass plates	Check bottom of glass plates for chips; replace if necessary

6.2) Applications

Parallel DGGE	
Normal and mutant DNA unresolved	1. Perform time course 2. Recalculate gradient range or run a time course gel
Air bubbles in gel	• Clean plates
Fuzzy DNA bands	• Clean wells before use; check for matching comb and spacer thickness; increase polymerisation time
Bands do not migrate far enough into gel	1. Increase run time 2. Decrease acrylamide concentration 3. Decrease denaturant concentration
DNA leaks between wells	1. Acrylamide not polymerised; add more APS and TEMED (0.1% final conc.) 2. Degas acrylamide before casting 3. Allow to polymerise over 60' 4. Do not overload sample wells; reduce sample volume
Skewed or distorted bands	1. Impurities in acrylamide; filter or check shelf life date 2. Carefully load DNA samples; do not puncture wells
CDGE	
Normal and mutant DNA unresolved	Repeat parallel DGGE to recalculate constant denaturant concentration
Air bubbles in gel	• Clean plates

Fuzzy DNA bands	• Clean wells before use; check for matching comb and spacer thickness; increase polymerisation time
Bands do not migrate far enough into gel	1. Increase run time 2. Check and if necessary decrease acrylamide concentration 3. Re-check denaturant concentration and decrease if necessary
DNA leaks between wells	1. Acrylamide not polymerised; add more APS and TEMED (0.1% final conc.) 2. Degas acrylamide before casting 3. Allow to polymerise over 60' 4. Do not overload sample wells; reduce sample volume
Skewed or distorted bands	1. Impurities in acrylamide; filter or check shelf life date 2. Carefully load DNA samples; do not puncture wells

6.2) Applications (Continued)

Heteroduplex Analysis	
Normal and mutant DNA unresolved	1. Optimise DEM concentration 2. Add 15% urea to gel 3. Adjust voltage or run time so that samples travel at least 15cm from well
Air bubbles in gel	• Clean plates
Fuzzy DNA bands	• Clean wells before use; check for matching comb and spacer thickness; increase polymerisation time to at least 60'
Bands do not migrate far enough into gel	1. Increase run time 2. Check and if necessary decrease acrylamide concentration 3. Re-check denaturant concentration and decrease if necessary
DNA leaks between wells	1. Acrylamide not polymerised; add more APS and TEMED (0.1% final conc.) 2. Degas acrylamide before casting gel 3. Allow to polymerise over 60' 4. Do not overload sample wells; reduce sample

	volume
Skewed or distorted bands	1. Impurities in acrylamide; filter or check shelf life date 2. Carefully load DNA samples; do not puncture wells

Section 7
Ordering Information

VS20WAVE-DGGE	Complete Denaturing Gradient Gel Electrophoresis System, 20x20cm; includes: temperature control unit, cam casting base, glass plates with 1mm bonded spacers, 2x 24-sample combs and gradient mixer – 240 VAC version
VS20WAVE-DGGE$	VS20WAVE-DGGE – 110VAC version
VS20WAVE-DGGETC	VS20WAVE-DGGE Temperature Control Unit – 240VAC version
VS20WAVE-DGGETC$	VS20WAVE-DGGETC – 110VAC version
GM100	Gradient Mixer, 100ml
CSL-STIR	CSL Magnetic Stirrer, 19x19cm
MU-D01	Single Peristaltic Pump
MU-S16	Silicon tube I.D. 1/8", 25 ft, for MU-D01
CS-500V	omniPAC Power Supply, 500V, 800mA, 300W
Phoretix 1D	1D image analysis with band pattern matching
Phoretix 1D Pro	1D image analysis with band pattern matching between different gels
VS20WAVE-DGGEKIT	VS20WAVE Package Deal; includes: VS20WAVE-DGGE, CSL-STIR, MU-D01, MU-S16, CS-500V – 240 VAC version
VS20WAVE-DGGEKIT$	VS20WAVE-DGGEKIT – 110 VAC version

For further details of products compatible with the VS20WAVE-DGGE, please contact your local authorised dealer or visit www.biocomdirect.com

Warranty

This unit has a warranty against manufacturing and material faults of twelve months from the date of receipt by the customer.

If any defects occur during this warranty period, the Cleaver Scientific Ltd. (CSL) will repair or replace the defective parts free of charge.

This warranty does not cover defects occurring by accident, misuse or defects caused by improper operation.

Units where repair or modification has been performed by anyone other than CSL or an appointed distributor or representative are no longer under warranty from the time the unit was modified.

Units fitted with accessories or repaired parts not supplied by CSL or its associated distributors have an invalidated warranty.

CSL cannot repair or replace, free of charge, units where inappropriate solutions or chemicals have been used.

For a list of these please see the Care and Maintenance subsection.

If a problem does occur then please contact your supplier or Biocom on:-

Biocom Ltd
6 Lomond Crescent
Bridge of Weir
PA11 3HJ
United Kingdom

tel/fax: +44 1505 615976
e-mail: support@biocomdirect.com
web: www.biocomdirect.com