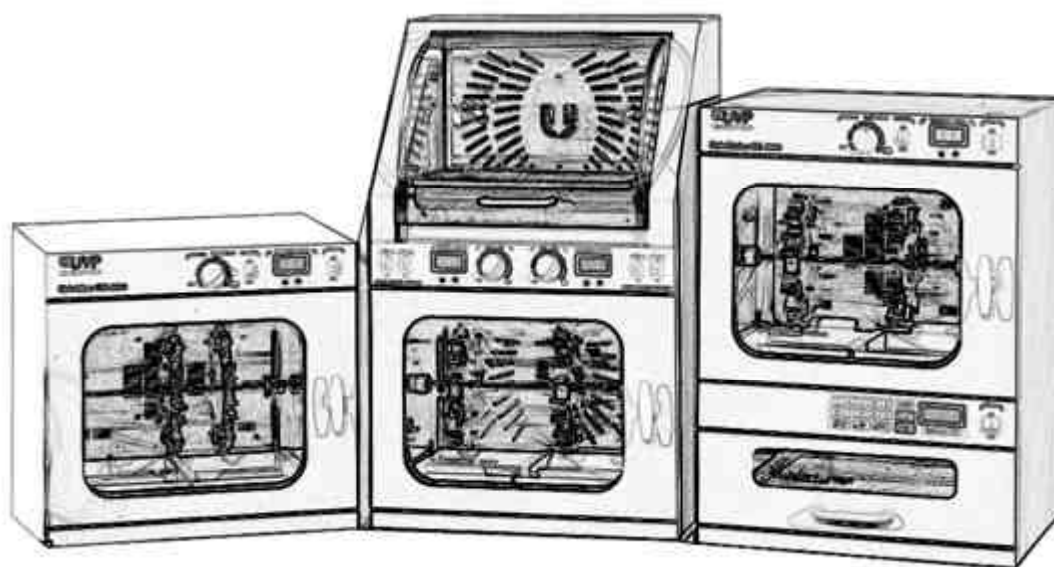


Hybridizer, HybriLinker and Multidizer Hybridization Ovens

Operating Instructions



HB-1000 Hybridizer

HM-4000 Multidizer

HL-2000 HybriLinker

IMPORTANT: Please read these instructions before operating your UVP Hybridizer, Multidizer or HybriLinker systems to familiarize yourself with operation procedures.



UVP, LLC 2066 W 11th Street, Upland, CA
Tel: (800) 452-6788 / (909) 946-3197
Fax: (909) 946-3597

Ultra-Violet Products Ltd., Unit 1
Trinity Hall Farm Estate, Nuffield Road
Cambridge CB4 1TG UK
Tel: +44(0)1223-420022 / Fax: +44(0)1223-420561

Web site: www.uvp.com

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INTRODUCTION

The HB-1000 Hybridizer is a cost-effective, high-performance, 10-bottle hybridization oven with state-of-the-art microprocessor temperature and variable speed controls. The HL-2000 HybriLinker System is designed with a combination hybridizer and crosslinker components. This equipment is intended to be used in the laboratory environment. The HM-4000 Multidizer System is designed as a multifunctional unit with a variety of motion options. The lower chamber operates in the same manner as the HB-1000. The HM-4000 has an additional upper chamber for use of a reciprocating shaker tray, an orbital rocker tray or an acrylic carousel. Refer to Accessories for ordering information.

The HB-1000 hybridizer can be set to operate anywhere from 10 to 18 RPM, enabling consistent saturation of samples, whether it be for washing or hybridizing. Having the ability to remove the rotary carousel wheel and replace it with an interchangeable rocker plate enables all hybridization protocols in one reliable benchtop unit. An easily removable protective tray under the carousel allows easy clean-up of spilled media.

A touch sensitive key pad and microprocessor provides accurate temperature control from ambient +10°C to 99.9°C. The keypad is located just below a large LED readout. Once the desired hybridization temperature is entered, the readout displays the current temperature inside the chamber. The chamber environment is calibrated at 68.0°C; at this temperature, the accuracy is $\pm 0.5^{\circ}\text{C}$ (HB-1000 and HL-2000). An internal circulating fan assists uniform distribution of heating throughout the chamber.

Two different hybridizer bottle sizes are available for use within the hybridizer using the standard carousel provided with the unit. The rotary carousel wheel can hold up to twenty (20) 35 x 150 mm bottles or ten (10) 35 x 300 mm bottles. In addition, combinations of both size bottles may be used at the same time. Optional carousel kit for oversized bottles is available.

WARNING!

There may be build up of pressure within the hybridization bottles when they are taken from ambient to hybridization temperature. To help in relieving some of this pressure, preheat the solutions and bottles. Also, be sure bottles are opened at the same temperature at which the hybridization was done. Do not allow the bottles to cool before opening.

To assure hybridization bottles remain leakproof and pressure proof, prevent temperatures above 70°C. If temperatures above this are used without first relieving the pressure within the bottle, there is a risk that bottles will leak and/or break due to the internal pressure build up.

If the bottles are accidentally taken above 70°C without relieving the pressure, DO NOT open the hybridizer door. Turn off the hybridizer and allow it to cool before opening the door and checking the contents of the oven. Should the bottles be used above 70°C, relieve the pressure by unscrewing and re-tightening the cap.

If using Radioactive material see DECONTAMINATION section of this manual.

CAUTION!

The HL-2000 HybriLinker System and HM-4000 Multidizer System are designed to be carried or lifted by two (2) people.

HYBRIDIZATION OVEN SPECIFICATIONS

HB-1000 Hybridizer Hybridization Oven

| Part Numbers | Volts/Hz |
|-----------------------|---|
| 95-0030-01 | 115V/60Hz |
| 95-0030-02 | 230V/50Hz |
| 95-0030-03 | 100V/50Hz |
| Specifications | |
| Net Weight: | 43 lbs |
| Temperature: | Ambient +10°C to 99.9°C |
| Heating Element: | 1200 watts |
| Temperature Display: | LED |
| Rotation Speed: | Off, 10 to 18 RPM |
| Bottle Capacity: | Twenty (20) 35 x 150 mm, Ten (10) 35 x 300 mm |
| Dimensions: | Exterior 17.5W x 16H x 15D in. (445 x 406 x 381 mm) Interior 14W x 10.75H x 9.25D in. (356 x 273 x 234 mm) |

HL-2000 HybriLinker System

The HybriLinker System combines a hybridization oven and a crosslinker in one space-saving unit. Each section has separate power supplies and for independent operation.

| Part Numbers | Volts/Hz |
|---|--|
| 95-0031-01 | 115V/60Hz |
| 95-0031-02 | 230V/50Hz |
| 95-0031-03 | 100V/50Hz |
| Specifications | |
| Unit Net Weight: | 60 lbs |
| Dimensions: | Exterior 17.5Wx24.6Hx15D in. (445 x 625 x 381 mm) |
| Hybridization Oven | |
| Temperature: | Ambient +10°C to 99.9°C |
| Temperature Display: | LED |
| Heating Element: | 1200 watts |
| Rotation Speed: | Off, 10 to 18 RPM |
| Bottle Capacity: | Twenty (20) 35 x 150 mm, Ten (10) 35 x 300 mm |
| Dimensions: | Interior 14W x 10.75H x 9.25D in. (356 x 273 x 234 mm) |
| Crosslinker | |
| Microprocessor controls | 8 watt Shortwave UV (254nm) tubes |
| Large LED displays time or energy | Tactile membrane keypad |
| Preset or user-selected UV time exposure settings | Internal interlocking safety system |
| Preset or user-selected UV energy settings | |
| Maximum UV energy setting | 999,900 microjoules per cm ² |
| Dimensions: | Interior 11.375W x 3.75H x 11.5D in. (288 x 95 x 292 mm) |

HM-4000 Multidizer Hybridization Oven

| Part Numbers | Volts/Hz |
|--------------|-----------|
| 95-0340-01 | 115V/60Hz |
| 95-0340-02 | 230V/50Hz |
| 95-0340-03 | 100V/50Hz |

Specifications

| | |
|----------------------|---|
| Net Weight: | 75 lbs |
| Temperature Display: | LED |
| Dimensions: | Exterior 17.5W x 16H x 15D in. (445 x 406 x 381 mm) |

Upper Chamber

| | |
|--------------------|---|
| Temperature: | Ambient +10°C to 80°C |
| Heating Element: | 500 Watts |
| Shaker Tray Speed: | 54 - 106 cycles/min. |
| Orbital Shaker: | 10 - 27 cycles/sec; adjustable angle ± 0 to 7 degrees (0 to 14 degrees included angle) |
| Carousel Rotation: | 12 - 20 rpm |

Lower Chamber

Same as the HB-1000 Hybridizer

ACCESSORIES

Bottles: Borosilicate glass bottles with polypropylene cap and teflon seal.

| | |
|----------------------------|------------|
| Bottle, Small, 35 x 150 mm | 07-0194-01 |
| Bottle, Large, 35 x 300mm | 07-0194-02 |
| Bottle, Oversize | Call UVP |

Bottle Carousels:

| | |
|---|------------|
| Carousel Kit for Four Oversized Bottles | 76-0066-02 |
| Drive Shaft Rod Assembly | 76-0064-03 |
| Carousel Kit, Metal for 10 Large or Sm. Bottles | 76-0070-01 |
| Carousel, Acrylic for HM-4000 upper chamber | 76-0089-01 |

Other Equipment:

| | |
|---|------------|
| Rocker Tray, 12.5W x 10D in. (318 x 254 mm) | 98-0067-03 |
| Removable plate is manufactured of stainless steel fabrication. For HB-1000 and HL-2000 only. | |

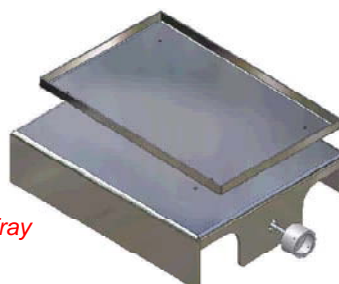
| | |
|--|------------|
| Orbital Motion Tray (for HM-4000) 10.3"W x 8.2"D | 98-0067-01 |
|--|------------|

| | |
|---|------------|
| Reciprocating Shaker Tray (for HM-4000), 10"W x 6"D | 98-0067-02 |
|---|------------|

For replacement parts, see "Replacement Parts" Section of this manual.



Orbital Motion Tray



Reciprocating Shaker Tray



SET-UP INSTRUCTIONS

Two different bottle sizes are available for use within the hybridizer. The rotary wheel carousel can hold up to twenty (20) 35 x 150 mm bottles or ten (10) 35 x 300 mm bottles. In addition, combinations of both sizes bottles may be used at the same time. Follow the instructions below to install the optional oversize bottle carousel kit.

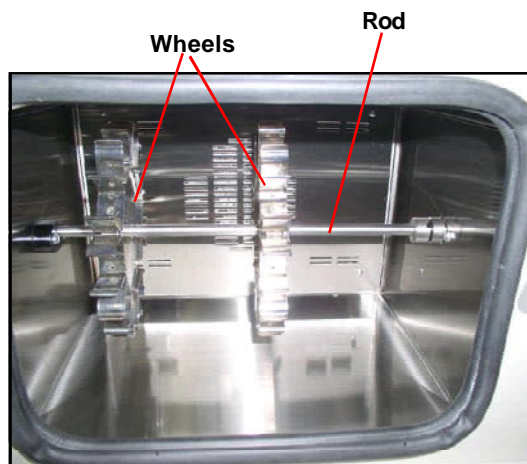
- To insert bottle holder carousel (see Pic. 1), slide the wheels (2) onto the rod and tighten the thumb screws. Insert the rod into the holder on the right wall. Slide the rod into the holder on the left wall of the chamber.
- To adjust for various bottle lengths (see Pic. 2), simply loosen the thumb screws on the inside of the bottle clip wheel and adjust to desired width.
- If skewed bottle positioning is required, loosen the thumb screw for only one bottle clip wheel (see Pic. 2). Slightly rotate the wheel until the required angle is obtained. Retighten the thumb screw.
- To insert bottles into bottle clips, gently push bottle into clips (see Pic. 3). Place one side at a time for large bottles.

NOTE: ALWAYS USE AN EVEN NUMBER OF BOTTLES AND LOAD BOTTLES OPPOSITE EACH OTHER TO BALANCE THE BOTTLE HOLDER. THIS PREVENTS EXCESSIVE WEAR OF THE BEARINGS AND DRIVE MOTOR.

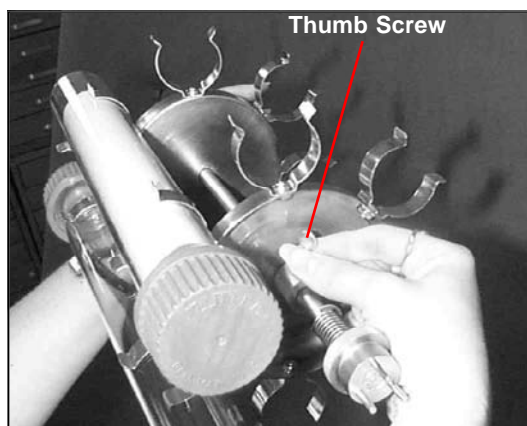
Bottle Care

Hybridization bottles are made of lead-free borosilicate glass which provides excellent long term reliability. Bottle includes a polypropylene cap and teflon seal.

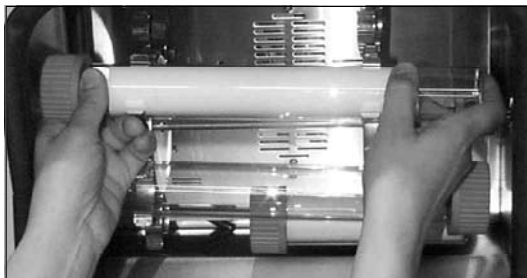
- It is important to check your bottles regularly for chips, stress fractures, and cracks. If these occur, the bottle must be discarded.
- Ensure bottles are stored either in a suitable rack or with caps replaced in between experiments. This will protect the bottle and sealing area.
- Replace "O" rings/Teflon® seals when worn or leaky; replace "O" rings or seals every six months.
- Wear protective gloves to protect your hands in the event of an accidental breakage.
- Never over tighten caps on bottles. Hand tightening is sufficient.
- If the cap is difficult to unscrew, NEVER ATTEMPT to force the bottle cap open. Allow the bottle to cool and retry. If the cap remains stuck, discard the bottle.
- The bottles should not be used at temperatures above 70°C.



Pic. 1 - Inserting Bottle Holder Carousel



Pic. 2 - Adjusting for Bottle Lengths



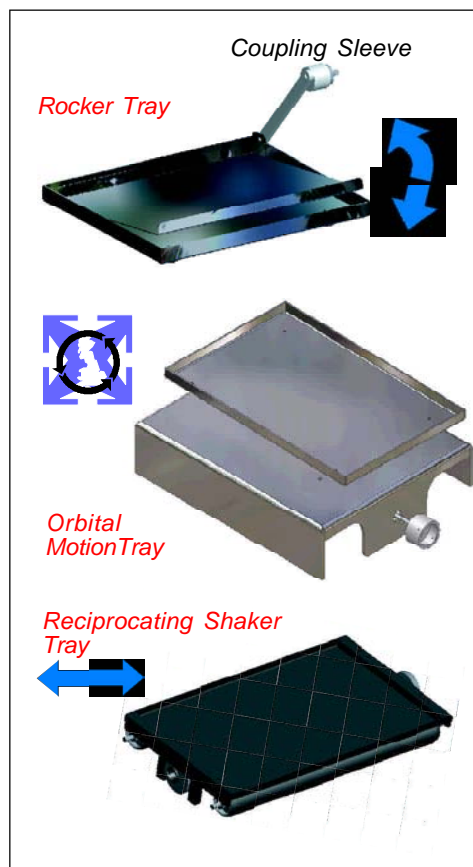
Pic. 3 - Inserting Bottles

Installing the Rollers and Trays

To insert rollers in the top chamber of the HM-4000 Multidizer, slide the rollers into the receptacle first on the left side of the chamber and then the right side.

To install the **Rocker Tray**, remove the metal carousel from the chamber. Place the tray into the chamber with the coupling arm to the right side. Place the coupling sleeve over the motor drive shaft such that the pins on the inside of the coupling sleeve engage the slots in the motor drive shaft of the chamber. Rotate the sleeve counter-clockwise 30° until locked in place. To remove the tray, reverse the process.

The **Orbital Rocker Tray** and **Reciprocating Shaker Tray** are designed for the use in the upper chamber of the HM-4000. To install, place the tray over the rollers.

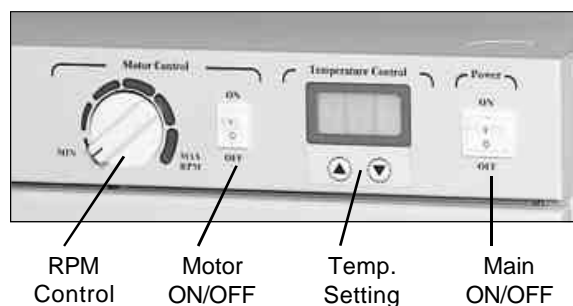


OPERATING INSTRUCTIONS

This equipment is not intended for interconnection with any other devices. Use of this equipment other than intended may create a safety hazard and/or malfunction.

Using the Hybridization Oven

- Place the unit on a level working surface and provide adequate room in front of the door to open it easily.
- Plug the female end of the power cord into the unit.
- To turn on the main power supply, depress the Power switch toward "ON" on the right side of the control panel.
- To adjust the temperature setting to the necessary degree, press either the "UP" or "DOWN" buttons below the temperature control display to raise or lower the displayed degree (displayed in degrees Celcius). This setting is adjustable from ambient +10°C to 99.9°C. See Temperature Control Instructions below for more detail.
- To turn on the motor for the bottle holder, press the motor control power switch to the "ON" position.
- To adjust the bottle carousel to the desired RPM, simply turn the motor control knob clockwise to increase the RPM and counterclockwise to decrease the RPM.



Minimum RPM = OFF/10

Maximum RPM = 18

NOTE: The inside of the hybridizer should be cleaned with a damp cloth. The bottle carousel assembly can be cleaned with soap and water.

Setting the Temperature Controls

Whenever power is applied to the controller, the software revision number is displayed for several seconds. While the software revision number is being displayed, the intensity of the display digits alternates between full and half brightness, and on some models the two outside decimal points blink on and off. After several seconds, the display reverts to showing the controlled temperature. In this mode, at most a single decimal point is illuminated, and the display intensity is steady.

Altering the Setpoint

The current setpoint value can be altered using the “UP” and “DOWN” buttons while the setpoint is being displayed. To change the setpoint from normal mode, proceed as follows:

- Momentarily press then release either the “UP” or the “DOWN” button. The LED intensity will flash to indicate that the displayed value is the current setpoint.
- Increase or decrease the setpoint value by pressing “UP” or “DOWN” buttons respectively. If either button is held down for more than several seconds, setpoint value will increase or decrease continuously.
- When the desired setpoint is reached, wait approximately five seconds without pressing either button; the display will revert to normal mode showing the actual temperature.

The new setpoint becomes effective and is stored in the non-volatile memory when the display reverts to the normal mode.

Calibrating the Temperature Reading

The hybridizer is calibrated at the UVP factory. UVP recommends temperature recalibration be performed at the UVP factory as recalibration by the user may void the warranty. Obtain a RGA (Returned Goods Authorization) number from UVP prior to returning a unit to the factory. Call UVP’s customer service department in Upland, California at (800) 452-6788 or (909) 946-3197 or Cambridge, UK at +44(0)1223-420022.

Decontamination

Bottles and Caps

- Soak items in a diluted detergent solution overnight.*
- Remove from detergent and rinse items with distilled water.*
- If items are still contaminated, gently scrub with an abrasive cloth or brush, if necessary, continue to soak items in the detergent solution for a longer period of time.*

Oven Chamber

- The oven chamber and drip pans may be decontaminated by wiping clean with a decontaminating agent, then repeat with distilled water.*

*These decontamination methods may not remove all contaminants. Refer to Federal, State, and Local Guidelines and Biological Protocols to assure decontamination.

NOTE: If any unit requires service, a returned goods authorization number (RGA) must be obtained from UVP’s customer service department prior to returning any item to UVP. If Radioactive or Biological Hazardous material has been present within the unit, Radioactive decontamination and Biological clean-up as per current Federal, State, and Local Guidelines and Biological Protocols must be performed, BEFORE returning the unit.

Using the Crosslinker

The crosslinker portion of the HL-2000 HybriLinker operates independently from the hybridizer. The crosslinker provides an exposure instrument which utilizes shortwave 254nm ultraviolet energy for many applications and protocols. The crosslinker's internal microprocessor measures and controls UV output, ensuring maximum energy efficiency. UV crosslinking takes seconds as compared to hours for oven baking. The UV crosslinking procedure optimizes the use of the ultraviolet energy while ensuring that crosslinking is consistent. This equipment is not intended for interconnection with any other devices. Use of this equipment other than intended may create a safety hazard and/or malfunction.

WARNING! The crosslinker is a powerful source of ultraviolet (UV) radiation. Even though it is not easily accessible, do not attempt to disengage or override the internal safety interlocks. Exposure to the UV radiation may result. If the UV sources remain on when the door is open, the unit is malfunctioning and use should be discontinued until the unit has been serviced. Do not expose unprotected eyes or skin to UV radiation.

1. Place the unit on a level working surface. Be sure there is adequate room in front of the door to open it easily.

2. Plug the female end of the power cord into the unit.

3. Plug the male end of the power cord into a properly grounded electrical outlet. The proper operating voltage of the Crosslinker is found on the product information label. Note: For 230V models, or those requiring special power cord connectors, ensure that the proper configuration of the male connector or the plug has been properly connected to the power cord.



Crosslinker Control Panel

4. Turn the On/Off switch to the On position. NOTE: WHEN TURNED ON THE CROSSLINKER DEFAULTS TO THE LAST USED UV EXPOSURE SETTING.
5. The last UV exposure setting will now be displayed on the LED and the last function setting will be noted by glowing red spot(s) on the display panel.
6. Place your sample requiring exposure into the chamber.
7. The Crosslinker can be operated on the following settings:

Preset ultraviolet energy exposure setting

- Push the PRESET and then ENERGY on the tactile touchpad. The red light at each position should now be lit and the preprogrammed UV exposure setting of 120,000 microjoules per cm² is displayed in the LED as 1200. Note: THE LED DISPLAYS 1200. THIS MUST BE MULTIPLIED BY 100 TO OBTAIN EXPOSURE.
- Push START. After a slight delay to energize the UV tubes, the LED will begin to countdown.
- The unit automatically stops at the end of the exposure cycle and will beep FIVE times. Exposure is now complete.

NOTE: Though the PRESET UV EXPOSURE SETTING is factory set at 120,000 microjoules per cm², it is possible to change this if necessary, as follows:

- Push and hold the PRESET on the tactile touchpad until you hear an audible signal then push ENERGY also on the touchpad.
- Set your new Preset UV exposure by pushing the numbers on the touchpad.
- Remember: THE LED DISPLAY MUST BE MULTIPLIED BY 100 TO OBTAIN THE PROPER EXPOSURE SETTING. The new setting will appear on the LED display.
- Push ENTER on the touchpad. The new setting is now installed.

Preset ultraviolet time exposure

- Push PRESET and then TIME on the tactile touchpad. The red light at each position should now be lit and the preprogrammed UV exposure time of 2 minutes should be displayed in the LED.
- Push START on the touchpad. After a slight delay to energize the UV tubes, the LED will begin to countdown.

NOTE: THE TIME EXPOSURE IS SET IN MINUTES AND TENTHS OF A MINUTE.

- The unit will automatically stop at the end of the exposure cycle and will beep five times. Exposure is now complete.

NOTE: Though the PRESET ULTRAVIOLET TIME EXPOSURE is factory set to 2 minutes, it is possible to change this if necessary, as follows:

- Push and hold the PRESET on the touchpad until you hear an audible signal, then push TIME.
- Set your new PRESET UV TIME EXPOSURE by pushing the numbers on the tactile touchpad. The new setting will appear on the LED display.
- Push ENTER and the new setting will be installed.

User set UV energy exposure

Sometimes it may be necessary for you to set your own standards for exposure. This is easily accomplished as follows:

- Push the ENERGY on the tactile touchpad then set your energy exposure requirements by pushing the numbers on the touchpad.
- The energy exposure settings should now be displayed on the LED in flashing mode.
- REMEMBER: YOUR ENERGY EXPOSURE SETTINGS DISPLAYED MUST BE MULTIPLIED BY 100. If settings are correct, push ENTER on the touchpad.
- Push START on the touchpad. After a slight delay to energize the UV tubes, The LED will begin to countdown. The unit will stop automatically at the end of the exposure cycle and will beep five times. Exposure is now complete.

User set UV time exposure

- Setting your own UV time exposure can be achieved as follows:
- Push the TIME on the tactile touchpad and then set your requirements by pushing the numbers. Your time exposure settings will be displayed on the LED in flashing mode. REMEMBER THAT YOUR TIME EXPOSURE SETTINGS ARE SET IN MINUTES AND TENTHS OF A MINUTE. If your settings are correct, push ENTER on the tactile touchpad.
- Push START. After a slight delay to energize the UV tubes, the LED will begin to countdown. The unit will stop automatically at the end of the exposure cycle and will beep five times. Exposure is now complete.

8. At the end of the exposure cycle, simply open the door and remove your sample.
9. Operational Notes:
 - a. To abort an exposure, press STOP on the tactile touchpad. The LED will display the remaining exposure.
 - b. To restart an aborted exposure, press START on the touchpad. The exposure will continue from the point at which the exposure was aborted.
 - c. To reset an aborted exposure, press the RESET. The LED and touchpad will return to the last -used setting.
 - d. The unit will not operate with the door open. Opening the door during a cycle aborts the cycle. Reclosing the door will reset the cycle to the last entered cycle used. To restart, press the START key.

Care and Cleaning

NOTE: ALWAYS UNPLUG UNIT FROM ELECTRICAL SUPPLY BEFORE CLEANING OR DRYING THE UNIT.

The units are built to provide you with trouble-free operation. To ensure correct operation:

1. Wipe ANY water from inside and outside the unit with a soft cloth or sponge.
2. Use soap and water with a soft cloth or sponge to clean the unit.
3. Do not allow chemicals to remain on unit surfaces.
4. Never clean unit with abrasive pads or cleaners.
5. Never clean unit with acetone or chloroform.
6. Clean the UV sensor regularly with a soft cloth and alcohol.

Changing the UV Wavelength of the Crosslinker

The Crosslinker is shipped with shortwave UV tubes. However, if user requirements and applications change, users have the ability to change the ultraviolet wavelength and recalibrate the UV sensor and microprocessor to the new UV wavelength. This is accomplished by purchasing FIVE tubes of the NEW UV wavelength and the proper UVP calibration sensor.

| TUBE P/N | WAVELENGTH | SENSOR P/N | DESCRIPTION |
|------------|------------------|------------|------------------|
| 34-0006-01 | 365 nm Longwave | 97-0016-02 | UVX-36 Longwave |
| 34-0042-01 | 302 nm Midrange | 97-0016-04 | UVX-31 Midrange |
| 34-0007-01 | 254 nm Shortwave | 97-0016-01 | UVX-25 Shortwave |

UV Wavelength Calibration Procedure

1. After disconnecting the unit from the electrical supply, install the five new tubes.
2. Plug the corresponding UV calibration sensor into the back of the Crosslinker chamber, just below the pull-out tray.
3. Place the UV calibration sensor in the middle of the tray of the exposure chamber.
4. Close the Crosslinker door.
5. Invoke the calibration mode of the Crosslinker by pushing/holding down STOP on the tactile touchpad and turning the power to the unit ON.
6. A sequence of tones will be heard from the Crosslinker and a 180 second count will display on the LED when the calibration procedure has started.
7. No calibration occurs during this 180 second period. This time sequence allows for the UV tube sources to warm-up and stabilize.
8. Upon completion of the 180 second period, measurements are automatically made with the UV calibration sensor and the crosslinker sensor. These measurements are compared to limits of acceptability and/or if sensor operation is within range.
9. If sensor readings are unacceptable, an error code (01, 02, 03 or 04) will flash on the LED Press any key to stop.
10. Check all connections and redo calibration. If the same error message appears, call UVP.
11. This calibrated operation will continue following any unsuccessful recalibration. Replace the new UV wavelength tubes with the previously removed old wavelength tubes.
12. Successful recalibration to the NEW UV wavelength changes the values in the microprocessor and numeric setting value is displayed on the LED. To return to original or another wavelength the proper UV calibration sensor is needed.

REPLACEMENT PARTS

Replacement parts lists are provided below for the HB-1000 Hybridizer and HL-2000 HybriLinker Systems. Repairs or replacement other than specified in the following procedures shall be done only by authorized service personnel.

Replacement Parts for HB-1000 Hybridizer

| DESCRIPTION | QTY. | PART NO. |
|---|------|-------------------------------|
| Fuses, 10 amp, 250V, 5 x 20 mm, slo-blo | 2 | 56-0002-04 (for all voltages) |
| Main switch | 1 | 53-0165-01 |
| Power Cord | 1 | 58-0085-01 |
| Motor Control Knob | 1 | 72-0030-01 |
| Adjustable Feet | 4 | 72-0058-01 |
| Door Handle | 1 | 22-0110-01 |
| Drip Tray | 2 | 10-0292-01 |
| | | |
| Power Cord, 230V UK | 1 | 58-0085-02 |
| Power Cord, 230V Euro | 1 | 58-0085-03 |

Replacement Parts for HL-2000 HybriLinker/Crosslinker

| DESCRIPTION | QTY. | PART NO. |
|---|------|-------------------------------|
| Tubes, 254nm shortwave | 5 | 34-0007-01 |
| Fuses, 10 amp, 250V, 5 x 20 mm, slo-blo | 2 | 56-0002-04 (for all voltages) |
| Main switch | 1 | 53-0165-01 |
| Micro switch, door interlock | 1 | 53-0072-01 |
| Ballasts, 100V | 6 | 42-0005-03 |
| Ballasts, 115V | 6 | 42-0005-01 |
| Ballasts, 230V | 6 | 42-0005-04 |
| Starters, 100V | 6 | 53-0001-02 |
| Starters, 115V | 6 | 53-0001-02 |
| Starters, 230V | 6 | 53-0132-01 |
| Power Cord | 1 | 58-0085-01 |
| Motor Control Knob | 1 | 72-0030-01 |
| Door Handle, hybridizer | 1 | 22-0110-01 |
| Drip Tray | 2 | 10-0292-01 |
| Adjustable Feet | 4 | 72-0004-01 |
| Power Cord, 230V UK | 1 | 58-0085-02 |
| Power Cord, 230V Euro | 1 | 58-0085-03 |

MAINTENANCE, REPAIR AND TECHNICAL ASSISTANCE

UVP offers technical support for all of its products. If you have any questions about the product's use, operation or repair, please call or fax UVP Customer Service at the following numbers:

UVP, LLC In the US toll free (800) 452-6788 or (909) 946-3197, fax: (909) 946-3597
2066 W. 11th Street, Upland, CA 91786 USA

Ultra-Violet Products Ltd. In Europe/UK call +44(0)1223-420022 or fax: +44(0)1223-420561
Unit 1, Trinity Hall Farm Estate, Nuffield Road, Cambridge CB4 1TG UK

NOTE: A **Returned Goods Authorization (RGA) number** must be obtained from UVP Customer Service prior to returning any product to UVP.

Replacement Parts for HM-4000 Multidizer

| DESCRIPTION | QTY. | PART NO. |
|---|-------------|-------------------------------|
| Hood, acrylic (for upper chamber) | 1 | 10-0317-01 |
| Fuses, 15 amp, 250V, 5 x 20 mm, slo-blo | 2 | 56-0044-04 (for all voltages) |
| Main switch, rocker | 1 | 53-0165-01 |
| Carousel, Rod | 1 | 76-0064-01 |
| Carousel, Spindle Bottle Holders | 2 | 76-0070-01 |
| Rollers | 2 | 76-0039-01 |
| Roller Insert and Cams | 1 | 76-0309-01 |
| Cable, 15A/125V | 1 | 58-0180-01 |
| Cable, 230V | 1 | 58-0180-02 |
| Knob, Motor Control | 2 | 72-0030-01 |
| Adjustable Feet | 4 | 72-0058-01 |
| Door Handle, Compression Latch | 1 | 22-0110-01 |
| Drip Tray (for upper chamber) | 1 | 10-0318-01 |
| Drip Tray (for lower chamber) | 2 | 10-0292-02 |
| Teflon O Ring Seal for 35mm bottle | 1 | 66-0036-01 |
| Teflon O Ring Seal for 70mm bottle | 1 | 66-0036-02 |

WIRING DIAGRAMS

Wiring diagrams are provided on the followings pages.

- Diagram 1** **Wiring Diagram for hybridization section of HL-2000 and HB-1000 and the lower Chamber of the HM-4000 Multidizer**
- Diagram 2** **Wiring Diagram for the crosslinker section of the HL-2000**
- Diagram 3** **Wiring Diagram for the upper chamber of the HM-4000 Multidizer**

Please note all voltages are covered in the same wiring diagrams.

Diagram 1 -Wiring Diagram for the hybridizer section of the HL-2000 and HB-1000

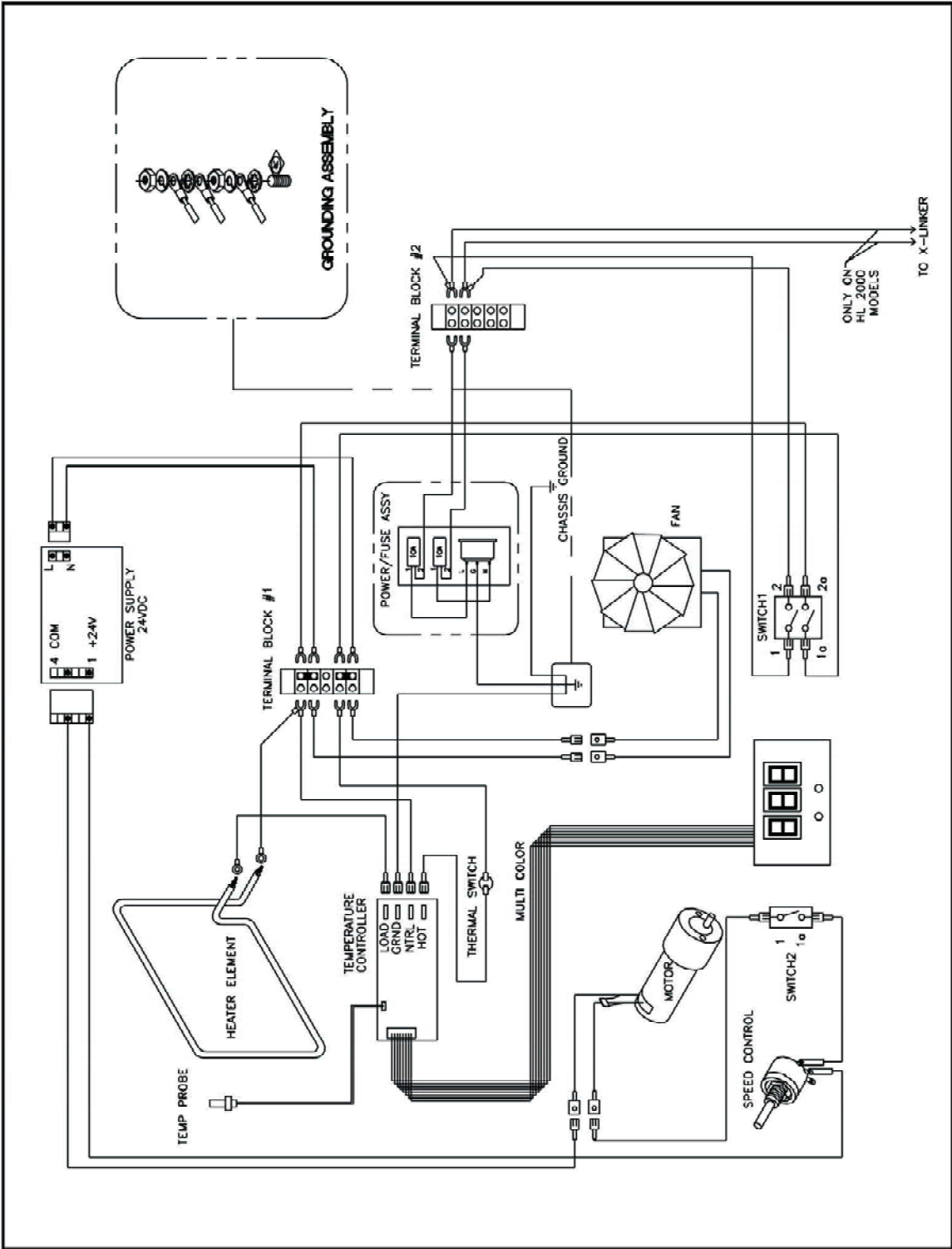
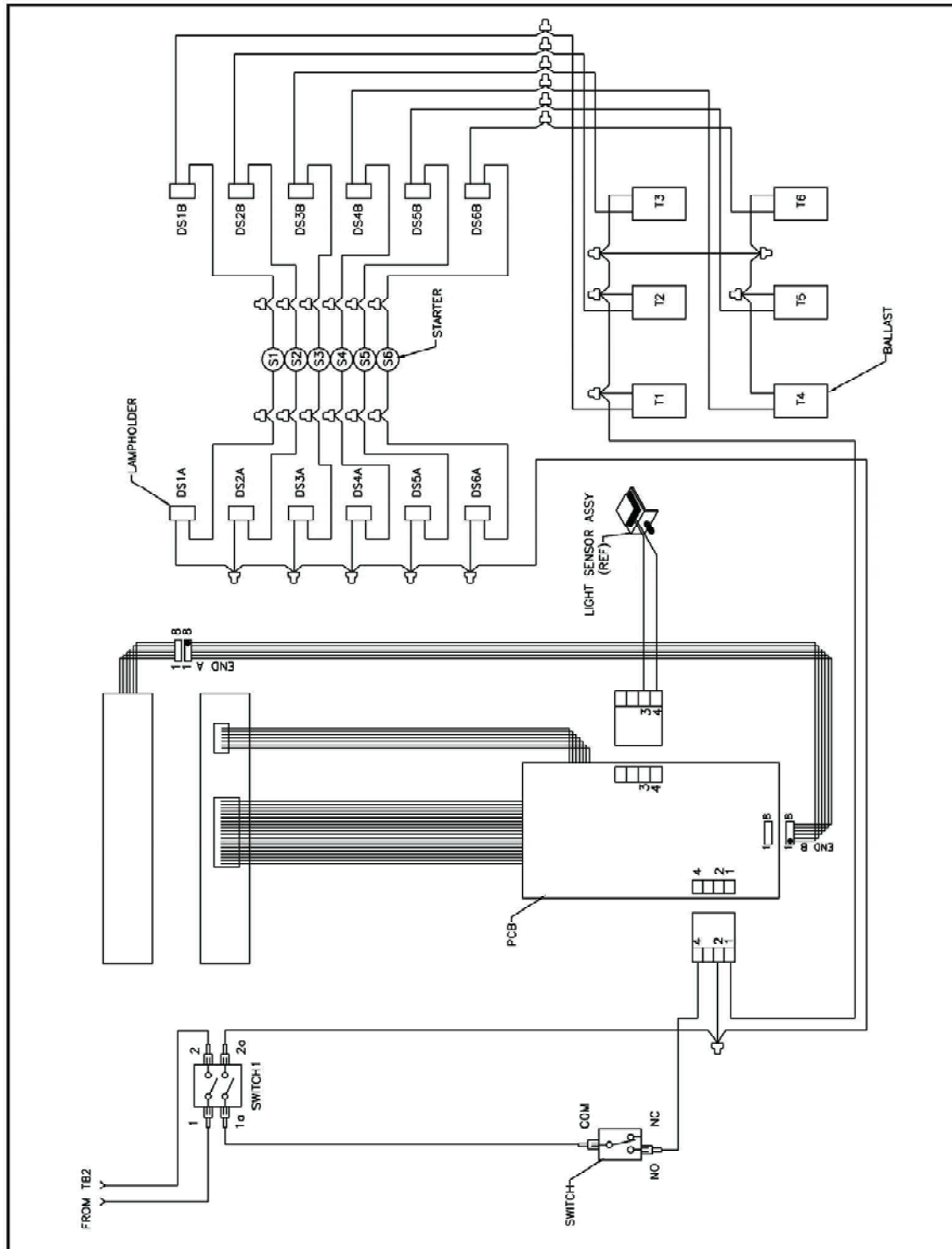


Diagram 2 -Wiring Diagram for the crosslinker section of the HL-2000



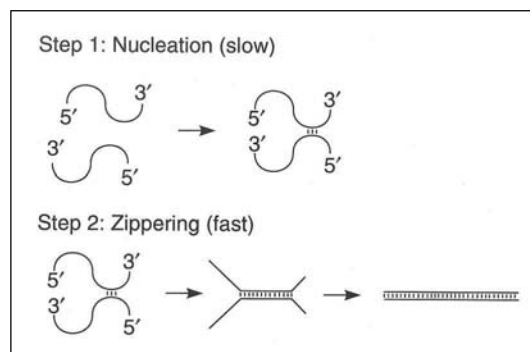
HYBRIDIZATION TECHNIQUES

There are really two main steps to a hybridization reaction: hybridizing two strands of complementary DNA and detection of the hybridized DNA. Nucleic acid hybridization is a mechanism where strands of DNA in a single stranded state have their complements bind together. The proximity of the DNA strands to each other determines the frequency of the binding events and is in fact successful binding is proportional to their concentration. The concentration of the target (nucleic acid you are looking for) is the independent variable in all hybridization reactions.

Since the target concentration is usually the unknown variable, an excess of labeled probe (what you use to find the target) will drive the reaction, thus decreasing the time for the probe to hybridize to a target. This is simply increasing the chances of a probe bumping into a target. But with an enormous amount of probe around (in solution or on the surface of a membrane) the background signal will also be enormous. The typical approach to correct for excess background (noise) on a membrane or slide hybridization: wash in a low salt buffer as this favors the disassociation of unbound probe from the membrane/slide and non-complementary DNA. In solutions a probe can be enzymatically degraded by using a single strand specific nuclease.

Mechanisms of Nucleic Hybridization

Hybridization occurs with a process called nucleation whereby the two separate nucleic acid strands come into close proximity of each other. A duplex region is formed where a minimum of three bases of one strand complements to those on the second strand. If the remainder of the strands are complementary, the two strands will anneal or zipper together very quickly. The rate-limiting step in nucleic acid hybridization is the duplex formation, which again explains why probe to target concentrations are critical.



Experimental Protocols

There are many different protocols available on the web, in journals, and in text references and we reference several at the end of this text.

1: Concentration of Species

Target: How much target molecule depends on the species you expect to find. Cellular constituents may be expressed in large or small amounts; the trick is to start with enough target (~25 µg) and determine experimentally.

Probe: plan to have more probe than expected target. To answer questions about adding too much probe; run an extinction experiment: serially increase the amount of target by a factor of two and use a fixed amount of probe. Hybridize for a short length of time and quantitate the amount of probe that has hybridized. As long as the signal increases and shows linearity there is excess probe (Fig 2 left). If the signal levels off and a loss of linearity noticed, then the probe is not in excess (Fig 2 right).

2: Length of Probe

The goal is to increase hybridization efficiency while minimizing background. In most cases probes range from 20 – 1000 bps.

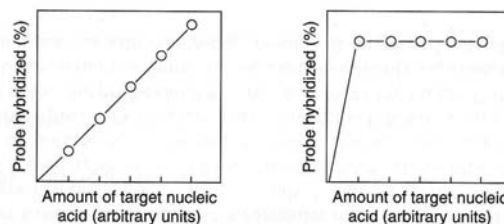


Figure 2. Analysis for probe excess in hybridization reactions.

3: Salt Concentration and Temperature

Nucleic acid requires salt (monovalent cations) to reduce the ionic effects of the phosphate backbone, and heat as a form of non-denaturing kinetic energy. Because the salt concentration and temperature effect each other, knowing the thermostability of the hybrid probe is helpful. Hybridization rate varies directly with the sodium ion concentration between 0.03 and 1.2 M. Most protocols run between 0.5 and 1.1 M Sodium.

| Situation | Response |
|---|---|
| G+C = 45-55% | follow normal protocol |
| G+C < 45% | Lower salt and temperature |
| G+C > 55% | Raise salt and temperature |
| Evidence of probe target mismatching | Lower salt and temperature |
| Target and probe is degraded on aqueous | Hybridize in a formamide-based buffer solution |
| Unacceptable high background | Use less probe Hybridize at lower salt/ higher temperature Wash with lower salt higher temperature Incubate with very low salt/change nuclease(solution) Use a smaller probe or a different probe Clean probe of contaminants prior to use |

4: Aqueous or Denaturing Hybridization Buffer

If hybridization takes place in an aqueous salt environment of 0.8 to 1.2M salt, the $T_{M/2}$ (the temperature at which the half of the duplex molecules will dissociate under a given set of conditions) can be 90°C. This is high enough to degrade DNA, RNA and some proteins. It is therefore possible to add formamide as a denaturing / temperature lowering agent because for every percent of formamide in the reaction the $T_{M/2}$ is reduced by 0.65°C. Therefore, at 80% formamide, reactions can be performed in the 40 - 55°C range. However the rate of formamide-based hybridization is at least three-fold lower than that of aqueous hybridization requiring longer incubations.

Protocol 1: Random priming method for tagging DNA with fluorescein-labeled nucleotide and others

This method uses DNA polymerase to incorporate Fluorescein-11- dUTP into double stranded DNA probes. This protocol can be used to incorporate **any** tagged nucleotides.

Equipment

- | | |
|--|---|
| <input type="checkbox"/> Micropipettes and tips | <input type="checkbox"/> Boiling water bath |
| <input type="checkbox"/> 1.5 mL Microcentrifuge tubes | <input type="checkbox"/> Microcentrifuge |
| <input type="checkbox"/> Cap lock for Microcentrifuge tube | <input type="checkbox"/> Water bath set to 37°C |

Reagents

- ☐ Deionized, sterile water
- ☐ EDTA, 0.5 M
- ☐ Klenow DNA polymerase , 4-5 units/ μ L
- ☐ Nucleotide mix (300 μ m each of dATP, dCTP, dGTP and 60 μ m dTTP)
- ☐ Random nonamer (9-mer) primers, 2.5 μ g/ μ L in water
- ☐ Reaction buffer, 10X: 50mM MgCl₂, 10mM 2-Mercaptoethanol, 500 mM Tris-HCl, pH 7.5

- ☐ Tagged nucleotide: fluoresceine-11-dUTP
- ☐ Template DNA in water (5ng/mL)

Procedure

1. Pipette 10 mL of template DNA plus 10 mL of water into a microcentrifuge tube and cap tightly. Cover cap with a cap lock or bend a paper clip in half and secure over the microcentrifuge tube.
2. Place the tube into the boiling water bath for 5 minutes.
3. Immediately place tube on ice for 5 minutes.
4. Centrifuge for 15 seconds in microcentrifuge.
5. Add the reagents listed below to a fresh tube on ice in the following order:
6. 10 mL Nucleotide mix
7. 5 mL Tagged nucleotide
8. 5 mL Reaction buffer (x10)
9. 5 mL Random primers
10. 10 mL Boiled DNA
11. 14 mL Water
12. 1 mL DNA polymerase
13. Mix gently and incubate at 37°C for 1 hour
14. Stop the reaction by adding 2 mL EDTA
15. Store probes at -20°C in the dark.

Protocol 2: Hybridization to Nylon or Nitrocellulose

Hybridization to nylon or nitrocellulose membranes containing Nucleic Acid is accomplished by adding single-stranded probe to the membranes that have been previously incubated with prehybridization solution. The prehybridization and hybridization solutions both contain buffers designed to prevent adventitious binding of the probe to the filters.

Reagents and Equipment

- ☐ Prehybridization/hybridization solution [45% formamide, 5X SSPE (0.9 M NaCl, 50mM sodium phosphate buffer, pH 7.4, 5mM EDTA), 0.1% SDS, 5X Denhardt's solution (0.1% each of Ficoll, polyvinylpyrrolidone, and bovine serum albumin), and 100 mg/mL of denatured salmon sperm DNA). Mix well and remove aggregates before use.

Notes: When preparing prehybridization/hybridization solutions, add dry reagents directly to the formamide/SSC solution. Incubate with mixing at 40-50°C for 2 hours or until dissolved. Store at -20°C. SDS will precipitate at room temperature but remain in solution at 37°C.

- | | |
|--|---|
| <input type="checkbox"/> UVP Hybridization bottle(s) and caps | <input type="checkbox"/> 15 mL plastic tube |
| <input type="checkbox"/> Boiling Water Bath | <input type="checkbox"/> Bucket of ice |
| <input type="checkbox"/> Gloves | <input type="checkbox"/> Plexiglass shield |
| <input type="checkbox"/> UVP Minidizer™, HybriCycler™, or Hybridizer™ Hybridization Oven | |

Procedure

1. Add 15 mL of prehybridization solution to each hybridization bottle containing the blot. Remove bubbles between the glass and blot. Cap the blots and close the Hybridizer.
2. Incubate the blot at 42°C for 1 hour.

3. Remove prehybridization solution and replace with 10 mL of hybridization solution.
4. Pipette 1×10^6 counts per minute of radio labeled probe or 200ng of biotinylated DNA into a 15-mL plastic tube. Seal the tube with a plastic cap and poke a hole in the top with a syringe needle to prevent pressure build-up during boiling.
5. Denature the probe by placing the samples in the boiling water bath and heating for 10 minutes. Immediately transfer the tube to ice for 5 minutes (to prevent renaturation). Add 5 mL of hybridization buffer to the probe and transfer to the hybridization bottle containing the blot: AVOID pouring the probe directly onto the blot.
6. Incubate in the UVP HybriCycler, Hybridizer, or Minidizer 6 to 8 hours at 42 to 56 degrees.

Washing the blot

- ☐ Tupperware container (sized to contain the blot)
- ☐ 0.1X SSC, 0.1% SDS (pre-warmed to 50°C)
- ☐ 2X SSC, 0.1 % SDS (room temperature)
- ☐ 2X SSC (room temperature)
- ☐ 0.15X SSC, 0.1% SDS (pre-warmed to 50°C)
- ☐ Gloves
- ☐ Filter Paper
- ☐ Cardboard
- ☐ Plastic wrap
- ☐ Tape

Non radioactive probes

1. Wash blots in 2X SSC, 0.1% SDS for 3 minutes at room temperature (repeat one)
2. Wash filter in 0.15X SSC, 0.1%SDS for 15 minutes at 50°C (repeat once)
3. Store blots in 2X SSC at room temperature

Radioactive probes

Additionally you will need:

- ☐ X-Ray film holder
- ☐ X-Ray film
- ☐ Intensifying screen

Procedure

1. Remove blot from hybridization tube and transfer to Tupperware container
2. Rinse briefly in 50°C 0.1X SSC, 0.1% SDS.
3. Remove this solution to radioactive waste and wash
4. Wash blot three more times in the same solution.
5. After the final wash, dry blot on filter paper for 10 minutes. This is a good time to quickly pass your hand held radioisotope reader (beta or gamma counter) over your blot to get a general idea as to the exposure time you will need for the x-ray film. Hot blots are 20 minutes to 2 hours. Not so hot blots can be left overnight (8 hours).
6. Tape the blot to a cardboard backing.
7. Cover with plastic wrap to prevent the blots from sticking to the x-ray film.
8. Place the cardboard containing the blots into the X-ray film folder.
9. In the darkroom, place a piece of X-ray film over the blot(s).

10. Place an intensifying screen on top of the film.
11. Close the film folder and clamp it.
12. Store at -70°C . The low temperature reduces light scattering and increases the length of exposure time. Expose the blot for 20 minutes to 24 hours.

Protocol 3: Chemiluminescence detection: HRP-tagged, Alkaline phosphatase (AP) probes or antibody conjugates

Equipment

- ☐ Clear plastic cling-wrap or Clear transparent sheet protector
- ☐ UVP EC3 or AC1 Darkroom with Cooled CCD camera
- ☐ Pipette

Reagents

Chemiluminescence

- ☐ ECL™ (or other) detection reagent 1
- ☐ ECL™ (or other if required) detection reagent 2
- ☐ Membrane following hybridization.

Procedure

1. Mix equal volumes of detection reagents 1 and 2
2. Pipette the mixture over the surface of the membrane and leave at room temperature for 1 minute.
3. Drain the sample and wrap it transfer to Darkroom
4. Close the darkroom and image using CCD camera.

References

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Current Protocols in Molecular Biology; 1987, John Wiley and Sons; ISBN 0-471-50338-X