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Stratalinker® UV Crosslinker

INSTRUCTION MANUAL

Model 1800

Catalog #400071 (120 V), #400072 (230 V) and #400672 (100 V)

Model 2400

Catalog #400075 (120 V), #400076 (230 V) and #400676 (100 V)

Revision #122003

IN #70034-06

Warning *To avoid possible injury, read this entire instruction manual before operating the Stratalinker UV crosslinker.*

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Stratalinker® UV Crosslinker 1800 and 2400

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Stratalinker® UV Crosslinker 1800 and 2400*

MATERIALS PROVIDED

Materials provided	Quantity
Stratalinker® UV crosslinker	1
254-nm UV light bulbs (model 1800: 8 watts each; model 2400: 15 watts each)	5

ADDITIONAL MATERIALS REQUIRED

Hybridization membranes
Replacement UV bulbs

Note *Replacing existing bulbs with bulbs of a different wavelength requires a recalibration of the unit to deliver accurate quantities of energy. Please contact the Stratagene Technical Services Department for more information.*

* U.S. Patent Nos. 5,814,523, 5,395,591, 5,288,647 and patents pending.

Revision #122003/IN #70034-06

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INTRODUCTION

The Stratalinker® UV crosslinker is designed to crosslink DNA or RNA to nylon, nitrocellulose, or nylon-reinforced nitrocellulose membranes. The crosslinking process takes only 25–50 seconds, in contrast to the traditional method of baking filters at 80°C for 2 hours. Additionally, crosslinking has been shown to significantly increase hybridization signals when compared to oven-baking. For optimal crosslinking performance, each Stratalinker UV crosslinker is equipped with an internal photodetector designed to compensate for the natural shift in power output of aging ultraviolet bulbs.

The Stratalinker UV crosslinker may be used for Northern, Southern, dot or slot blot analysis,[1–3] colony or plaque screening, nicking of DNA in agarose gels prior to blotting,[4] dimer formation to perform partial digests for rapid restriction mapping,[5] UV sensitivity testing for host strain verification,[6] and UV irradiation of PCR samples.[7] The Stratalinker UV crosslinker may be used in the *Autocrosslink*, *Time*, or *Energy* modes, and the apparatus is available in two convenient sizes—the Stratalinker 1800 UV crosslinker for smaller membranes and the Stratalinker 2400 UV crosslinker for larger membranes.

SAFETY CONSIDERATIONS

- Do not operate the Stratalinker UV crosslinker with the door open. Doing so will emit dangerous UV energy and seriously affect results.
- Do not look directly at the UV bulbs while the Stratalinker UV crosslinker is in use. Serious eye injury could result from overexposure to ultraviolet light.
- Do not tamper with the door seals or allow soil or residue from cleaning products to accumulate on the sealing surfaces.
- Do not disrupt the sensor in the rear of the unit when removing the UV bulbs.
- Be sure the electric cord plug fits snugly into the wall socket and is kept dry. Do not immerse the cord or plug in water or hang the cord over the edge of a counter.
- Do not use the Stratalinker UV crosslinker for any purposes other than those described in this manual.
- Do not expose the Stratalinker UV crosslinker to extreme temperatures.
- Do not attempt to service the Stratalinker UV crosslinker. Contact Stratagene's Technical Services Department for assistance.

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COMPONENTS

Refer to Figures 1 and 2 for front and interior diagrams of the Stratalinker UV crosslinker.

No.	Component	Function
1	Power switch	Powers the Stratalinker UV crosslinker when the switch is toggled
2	Door latch	Secures and releases the door
3	Energy button	Enables the user to set the energy dose from 0–9999 microjoules × 100
4	Time button	Enables the user to set the time from 0–999.9 minutes
5	Autocrosslink button	A preset optimal energy for Stratagene's membranes
6	Start button	Starts the selected operation
7	Reset button	Clears the information previously entered into the control panel
8	Number pad	Enables the user to set the time or energy level
9	Minutes LED	The green LED indicates the crosslinker is operating in the *Time* mode and the display shows the number of minutes remaining in the cycle
10	Microjoules LED	The yellow LED indicates the crosslinker is operating in the *Energy* mode and the display shows the number of microjoules × 100 being applied
11	LED Display	Displays the selected time or energy level
12	UV light bulbs	Source of UV radiation. To remove the bulbs, toggle the power switch to the off position, carefully rotate the bulb one-quarter turn and pull gently
13	Starter (except catalog #400076)	Starts the light bulbs. To remove the starter, toggle the power switch to the off position and rotate the starter counterclockwise one-quarter turn
14	UV sensor	Measures the UV light emitted by the UV bulbs. The crosslinker must be recalibrated if the UV sensor is replaced

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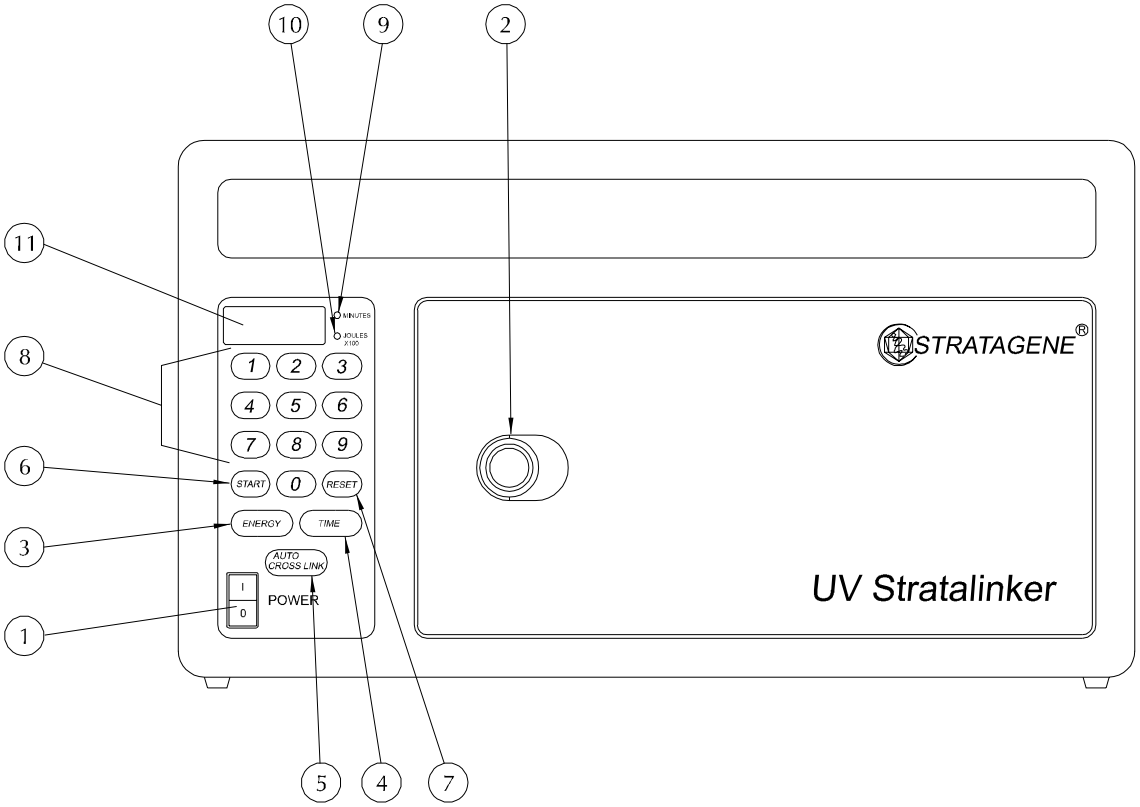


FIGURE 1 Front view of the Stratalinker® UV crosslinker.

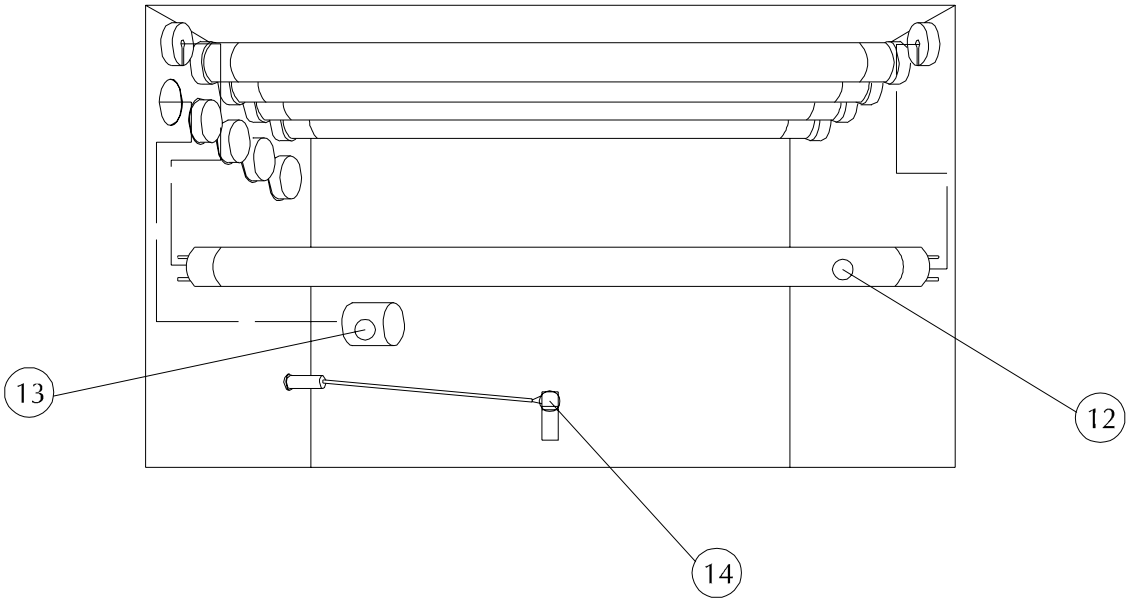


FIGURE 2 Interior view of the Stratalinker® UV crosslinker.

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EQUATIONS

Joules (J) = Watts × time (sec)
Ergs = J × 10^{-7}

OPERATING INSTRUCTIONS

Note *The following guidelines were determined using Stratagene's PosiBlot® pressure blotter with Stratagene's Duralon-UV™ nylon membranes. Other products or techniques may alter the results.*

Assembling the Stratalinker® UV Crosslinker

1. Plug the power cord securely into the rear of the Stratalinker UV crosslinker. Connect the plug to an outlet of appropriate voltage that has been grounded to prevent shock.

2. Press the *Power* button. The digital display will read 0000. If this does not appear, press *Reset*. The beeper will sound and the display will read 0000.

Selecting the Mode of Operation

The mode of operation selected depends on the specific results desired. Below is a brief explanation of each operating mode.

Energy Mode

When selecting the *Energy* mode, the beeper will sound and the yellow indicator next to the digital display will illuminate. The numbers on the display represent microjoules/cm^2 × 100. Enter the specific microjoule level desired and begin the irradiation. Stratagene recommends 120,000 microjoules for most membranes; if selecting this amount, the LED display will read 1200. If an error is made while entering the energy level, press *Reset* to clear the display and then reenter the desired value.

Time Mode

When selecting the *Time* mode, the beeper will sound and the green indicator next to the digital display will illuminate. The numbers on the display represent minutes. Enter the specific length of exposure desired in minutes from 0.1–999.9.

Autocrosslink Mode

When selecting the *Autocrosslink* mode, the beeper will sound and a preset exposure of 1200 microjoules (× 100) will be displayed. No further entries are required. The crosslinking will be complete in ~25–50 seconds.

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Irradiating Membranes

Note *The Stratalinker UV crosslinker has been optimized for use with Stratagene's nitrocellulose, Duralose-UV™ (reinforced nitrocellulose), and Duralon-UV (nylon) hybridization membranes.*

The Stratalinker UV crosslinker can be used to link nucleic acids to membranes after Southern blotting, Northern blotting, slot blotting, dot blotting, colony lifts, or plaque lifts. When crosslinking, a covalent bond forms between the amino group of the nylon and the thymine of the DNA (or uracil of RNA) at an energy of 120,000 microjoules, utilizing a 254-nm light source (a process similar to thymine dimerization). The smallest DNA size that Stratagene has successfully UV-crosslinked to filters is 17 bp.

Stratagene has found that a setting of 120,000 microjoules/cm^2 (1200 on the LED display) is optimal for attachment of RNA or DNA to nylon, nitrocellulose, or reinforced nitrocellulose membranes in any of the procedures listed above.

1. Place one or two sheets of absorbent paper lightly dampened with transfer buffer (such as 10× SSC) on the floor of the Stratalinker UV crosslinker. Place the membrane on top of the absorbent paper with the side with the attached nucleic acids **facing upwards,** enabling direct irradiation of the nucleic acids by the ultraviolet bulbs.

   Note *To obtain the best results, place the membrane into the Stratalinker UV crosslinker while the membrane is still damp (but not dripping) after the DNA or RNA transfer step.*

2. Close the door of the Stratalinker UV crosslinker.

3. Press the *Start* button to initiate the desired function. When the UV light bulbs turn on, the display will immediately begin to count down from the entered value. If the door is opened at any point during exposure, the irradiation will stop and the remaining exposure value will be displayed. (Close the door to continue irradiation.)

4. When the irradiation is complete, the beeper will sound for approximately 3–4 seconds. The *Autocrosslink* setting will take approximately 25–50 seconds.

5. Remove the membranes and close the door. The irradiation is now complete. Clean the floor of the Stratalinker UV crosslinker after each use to remove any residual salt.

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APPLICATIONS

Membrane Blotting

The Stratalinker UV crosslinker is used in place of the drying and vacuum baking step in Northern, Southern, and dot or slot blotting procedures.[1–3] For Northern blotting, Duralon UV membranes are recommended. Stratagene **does not recommend** Duralose UV membranes for Northern blotting.

DNA or RNA that has been blotted onto nitrocellulose, nylon, or hybrid membranes can be bound to that membrane using the Stratalinker UV crosslinker on the *Autocrosslink* setting.

DNA Nicking

The Stratalinker UV crosslinker can also be used to nick ethidium bromide-stained DNA in the agarose gel, in place of the depurination wash.[4]

Depurination of high-molecular-weight DNA may be more efficient than nicking. HCl changes the purines and NaOH cleaves them during depurination, whereas nicking with UV also forms thymine dimers which interfere with hybridization. Stratagene recommends depurination for large DNA (2–10 Mb). Use 80,000 μJ/cm^2 as a starting point for nicking. 150,000 μJ/cm^2 is the recommended setting for megabase nicking.

Gene Mapping

Partial digests can be generated using the Stratalinker UV crosslinker to create thymine dimers prior to digestion with restriction enzyme. The formation of dimers at or near the recognition site inhibits site cleavage.[5]

RecA Screening

The Stratalinker UV crosslinker can also be used to screen recA mutations.[6] Mutations in recA prevent repair of UV-induced damage, preventing irradiated recA– cells from growing.[8] The recA genotype of a strain may be determined in the following manner:

1. Lightly touch a colony of the strain with a sterile toothpick and streak out the cells in a single line onto a fresh NZY plate.

   Note *Do not insert the toothpick deep into the colony or pick up the entire colony. Only a small number of cells is necessary.*

2. Streak out a positive (recA+) and a negative (recA–) control parallel to the sample streak on the same plate using the technique described above.

3. Prerun the Stratalinker UV crosslinker for two minutes as follows:

   - Turn the *Power* switch to the on position.
   - Press the following *buttons* in this order: *2*, *0*, *Time*, *Start*.

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4. After the unit has prerun, place the NZY plate on the floor of the Stratalinker UV crosslinker. Cover the plate with the lid so that half of each line of cells is covered.

5. Expose the cells to 10,000–15,000 μJ (100-150 on the LED display).

6. Incubate the plates at 37°C overnight.

Expected Results

A known recA– strain will **not** grow when exposed to UV radiation.

MISCELLANEOUS USES

- Reducing primer extension reaction false positives

- Inactivating contaminating DNA in reaction buffer that may act as a template in primer extension reactions.[9] Up to 100 μl of reaction buffer in a 500-μl microcentrifuge tube may be treated by laying the tube on its side in the Stratalinker UV crosslinker (making sure the buffer does not run into the cap) and irradiating it at 200,000–300,000 μJ/cm^2 (2000-3000 on the LED display). More than one tube may be irradiated simultaneously.

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TROUBLESHOOTING

Observation	Suggestion(s)
The display does not light	There is no power to the unit. Secure the plug into the back of the unit and into the wall socket, and then depress the circuit breaker on the back panel
The display does not count down after *Start* is pushed	The *Energy* or *Time* modes have not been selected. Select the desired mode (*Autocross-link* selects *Energy* automatically)
	The door switch is not engaged. Close the door completely
	The UV sensor is not completely pushed into the connector on the interior sidewall. Ensure connections are secure
Countdown is slower than normal (e.g., *Autocrosslink* takes more than one minute)	UV bulbs are burned out. Check for burned-out bulbs and replace as necessary
	The starter is out. Replace the starter. Rotate the defective starter one-quarter turn counterclockwise and pull out
	The UV sensor is blocked and may need to be replaced. Contact the Stratagene Technical Services Department

SPECIFICATIONS

Weight

Model 1800: 9.7 kg
Model 2400: 15.5 kg
Model 2400 (220V): 18.9 kg

Dimensions (in cm)

Model 1800, internal: 33.7 W × 18.1 D × 16.8 H
Model 1800, external: 46.2 W × 22.4 D × 26.7 H
Model 2400, internal: 47.7 W× 34.3 D × 16.8 H
Model 2400, external: 58.9 W × 38.9 D × 26.7 H

Bulbs

Model 1800: 8 watts each
Model 2400: 15 watts each

Power Delivered

Model 1800: ~3000 µwatts/cm^2
Model 2400: ~4000 µwatts/cm^2

Starters

Sylvania starters (FS-5 for model 1800 and FS-2 for model 2400) may be used as replacement starters.

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MAINTENANCE

The Stratalinker 1800 and 2400 UV crosslinkers were designed to be durable and long lasting. Care must be taken to ensure proper operation and reliable performance. Failure to maintain the Stratalinker UV crosslinker in its proper condition may reduce its effectiveness.

- Operate the Stratalinker UV crosslinker on a flat, stable surface.
- Clean up any spills immediately. Do not allow chemicals to build up on either the inner or outer surfaces of the crosslinker.
- Wash the floor of the crosslinker after each use to remove any salt. All components may be cleaned with a soft cloth and nonabrasive cleanser. Do not use acetone or chloroform.
- Replace light bulbs and starters as needed.
- If you suspect that the UV sensor may need to be replaced, please contact your Stratagene sales representative or the Stratagene Technical Services Department. (If the UV sensor is replaced, the unit must also be recalibrated.)

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ENDNOTES

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