

User Bulletin

ABI PRISM 377 DNA Sequencer

June 5, 1997

SUBJECT: Modifications for Subambient Temperature Operations

Purpose of this User Bulletin

This user bulletin describes how to modify your ABI PRISM™ 377 DNA Sequencer to accommodate an external gel cooling system for run temperatures below the standard temperature. The standard run temperature is ambient plus 10°C up to 60°C.

The following topics are included:

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Purpose of an External Cooling System

Performing SSCP The ABI PRISM 377 DNA Sequencer provides gel temperature control from 10°C above the ambient temperature to a maximum of 60°C. To perform PCR Single-stranded Conformation Polymorphism (SSCP) protocols at lower temperatures (below 10°C above ambient), you must attach a cold water bath to the instrument.

Refer to *PCR SSCP Analysis: A Guide to Fluorescent PCR Single-stranded Conformation Polymorphism Analysis on the ABI PRISM™ 377 DNA Sequencer* (P/N 904413), for further information.

Hardware and Software Requirements

Overview To provide gel temperature control for operations at reduced temperatures, you must have all of the following:

- ♦ an external cold water bath connected to the ABI PRISM 377
- ♦ 30 k-ohm thermistors installed on the instrument
- ♦ the ABI PRISM 377 Data Collection 2.1 software

Hardware Requirements The water bath, polyurethane tubing, and tubing connectors needed for the instrument modification must meet the following minimum requirements.

Hardware	Minimum Requirements
Water Bath	Cooling capacity: 250 watts at 20°C
	Heating capacity: 500 watts up to 60°C
	Pump flow rate: 1 gal/min or 4 L/min at pressure head of 3 m
	Automatic Shutdown: At high temperature and low liquid-level
Polyurethane Tubing	6 ft. (1/4-in. i.d., 3/8-inch o.d.)
Tubing Connectors	1/4-in. female NPT, 1/4-in. male barbed fittings (NESLAB P/N 126000000007)

Any external water bath that meets these requirements should work properly. The NESLAB Model RTE111 meets these requirements and has been tested with the instrument.

Refer to the user manual for the water bath you select, follow proper procedures, and ensure that all product safety and regulatory requirements applicable to your location are met.

Changing Thermistors for SSCP

Most ABI PRISM 377 instruments have 100 k ohm thermistors installed. To perform SSCP at temperatures less than 22°C, you must replace the 100 k ohm thermistors with 30 k ohm thermistors.

To determine which thermistors are installed on your instrument, upgrade to the Data Collection 2.1 software, and check the Log file from a recent run. The configuration information in the Log makes reference to the type of thermistors installed.

Schedule a service call with your PE Applied Biosystems service engineer for replacement of the 100 k ohm thermistors if you wish to run SSCP at the lower temperatures.

Software Requirements

The Data Collection 2.1 software upgrade contains all required features to accommodate the external cold water bath, including new chiller modules.

Refer to the user bulletin, *ABI PRISM 377 DNA Sequencer Data Collection Software 2.1 Enhancements* (P/N 904945) for software installation instructions, descriptions of features, and instructions for using the new modules.

Requirements for Specific Run Conditions

Run Conditions	Software	Thermistor (ohms)	External Bath
>10°C above ambient to 60°C	1.1 or 2.1	100 k	not required
SSCP from 22°C to ambient +10°C	2.1	100 k	required
SSCP at <22°C	2.1	30 k	required

How the External Cold Water Bath Functions

Disengages Internal System

The ABI PRISM 377 configuration with the external cold water bath attached is similar to Figure 1 when viewed from the back. New modules in the Data Collection 2.1 software disengage the instrument's internal pump system and switch to the external cold water bath. When you turn the external valve so the arrow points down, access to the internal gel temperature control unit is closed off.

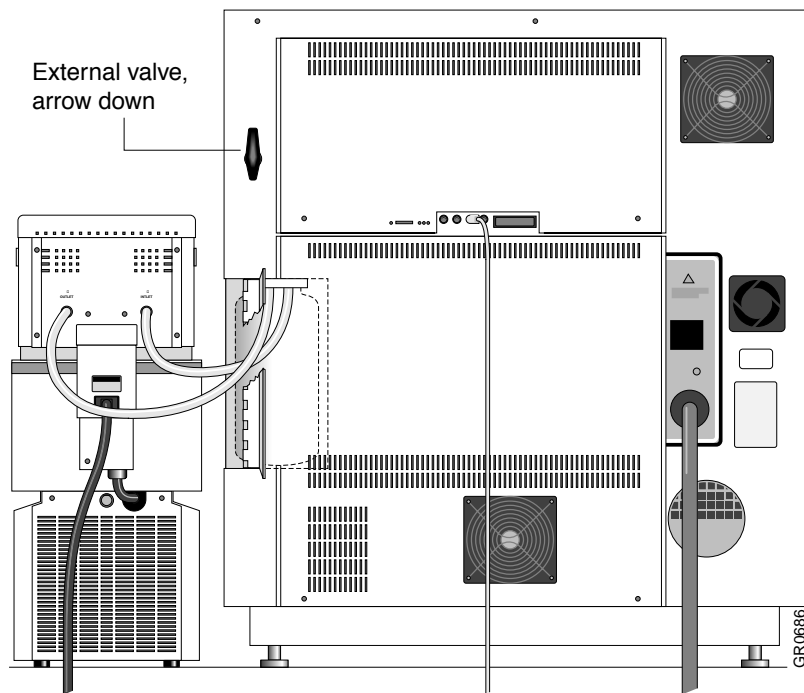


Figure 1. Rear view of the ABI PRISM 377 with external water bath attached

Water Bypasses the Gel Temperature Control Unit

With the external cold water bath attached, water passes directly into the front and back gel heat-transfer plates in the instrument, bypassing the gel temperature control unit (Figure 2).

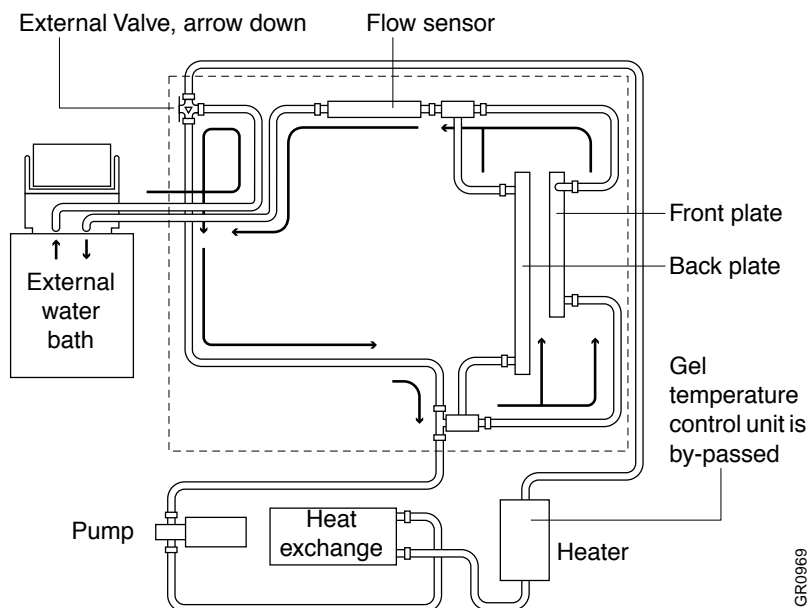


Figure 2. ABI PRISM 377 interior view with arrows showing water flow. Dotted line shows ABI PRISM 377 components used with the external water bath.

Standard Configuration Run Conditions with External Bath Attached

If the external water bath you use has heating capacity to 60°C, you can run the instrument under standard configuration conditions (ambient temperature plus 10°C to 60°C) with the external bath attached.

Frequent monitoring of the water level is required when the external bath is used during standard runs. For long periods of use, disconnect the external water bath, reinstall the water bottle and tubes, and use the standard run modules (not the chiller modules) in the Data Collection 2.1 software Modules folder.

Refer to the *Data Collection Software 2.1 Enhancements* user bulletin for the names of all the Data Collection 2.1 software modules and for setting preferences to switch between the standard run modules and the chiller modules.

Installing the External Cold Water Bath

Positioning the Machines

To position the ABI PRISM 377 and external water bath:

Step	Action
1	Place the ABI PRISM 377 on a table or counter that provides easy rear access.
2	Facing the ABI PRISM 377, position the water bath on the right side of the instrument and at the same height.
3	Plug both machines into an external power supply.

Determining Intake and Outlet

To identify the intake and outlet flow tubes on the instrument:

Step	Action
1	Turn on the ABI PRISM 377.
2	Open the right side panel where the water bottle for the internal pump system is mounted.
3	Unscrew the water bottle, and slowly pull it down to expose the ends of the tubes, but do not remove the bottle.
4	Observe the direction of water flow from the tubes into and out of the bottle to identify the intake and outlet tubes. Note Water coming into the bottle is from the instrument outlet, and water being drawn out of the bottle is pumped into the instrument intake. Some instruments have a small cutout at the bottom of the water intake tube.
5	Turn off the ABI PRISM 377.

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Disconnecting the Internal Pump

To disconnect the instrument's internal pump:

Step	Action
1	Remove the water bottle, and pull down the intake and outlet tubes to remove them.
2	Store the tubes and water bottle for later use.
3	Step to the rear of the instrument, and turn the external valve so that the arrow points down for the external position.
4	Cut two pieces of polyurethane tubing approximately three feet (one meter) long to connect the external water bath to the intake and outlet openings on the instrument.
5	Insert one end of a piece of tubing into the intake port on the instrument.
6	Repeat step 5 with the second piece of tubing in the outlet port.

Attaching the External Cold Water Bath

To attach the external cold water bath to the instrument:

Step	Action
1	Wrap the threads of the intake and outlet ports on the external water bath with Teflon tape.
2	Attach a metal tubing connector to each port.
3	Connect the free end of the intake tube to the outlet fitting on the back of the external water bath.
4	Connect the free end of the outlet tube to the intake fitting on the back of the external water bath.

Operating at Subambient Temperatures

Performing a Run at Subambient Temperatures

After your ABI PRISM 377 is modified for subambient temperature runs, perform the following steps each time you use it:

To perform a run at subambient temperatures:

Step	Action
1	Follow all instructions in the external water bath user's manual.
2	Fill the external water bath with cold, deionized distilled water, and add antifreeze as recommended by the manufacturer. ! WARNING ! CHEMICAL HAZARD. Antifreeze may cause respiratory tract, skin, and eye irritation. Wear chemical-resistant gloves and safety glasses when handling, and always use in a well-ventilated area.
3	Check that all tubing is tightly connected.
4	Turn on power to the external water bath, start the circulating pump, and add water as needed to maintain the proper level. IMPORTANT If the water level drops below the level recommended by the manufacturer, the circulation pump shuts off, and the required temperature is not maintained.
5	Set the external cold water bath temperature about 3–4°C below the desired gel plate temperature, and monitor the temperature in the Status screen under the Window menu.
6	Place a lid on the bath to minimize evaporation.
7	Add water and antifreeze during operation as needed to maintain the manufacturer's recommended water level.
8	Monitor the temperature and humidity in the lab (refer to the following section, "Avoiding Condensation").
9	Refer to the user bulletin, <i>ABI PRISM 377 DNA Sequencer Data Collection Software 2.1 Enhancements</i> (P/N 904945) for software installation procedures.

Avoiding Condensation

Severe Damage May Result

Operating the ABI PRISM 377 DNA Sequencer at subambient temperatures may cause condensation to form inside the instrument, which can cause decreased performance and severe damage. To avoid condensation, you must keep the water temperature above the dew point (temperature at which water vapor condenses into liquid) or lower the humidity in the room.

Consult the following tables to determine the dew point of water at various relative humidities and ambient temperatures in your lab.

CAUTION Condensation can cause decreased performance and severe damage to the instrument. Monitor the room temperature and humidity, consult the following condensation tables, and maintain the water bath temperature setting above the corresponding dew point.

Dew Point Table

Ambient Temp. 15°C		Ambient Temp. 20°C		Ambient Temp. 25°C		Ambient Temp. 30 °C		Ambient Temp. 35°C	
Relative Humidity (%)	Approx. Dew Point (°C)	Relative Humidity (%)	Approx. Dew Point (°C)	Relative Humidity (%)	Approx. Dew Point (°C)	Relative Humidity (%)	Approx. Dew Point (°C)	Relative Humidity (%)	Approx. Dew Point (°C)
100	15	100	20	100	25	100	30	100	35
90	14	90	18	90	23	90	29	90	33
80	12	80	16	80	21	80	26	80	31
70	10	70	14	70	19	70	24	70	29
		60	12	60	17	60	21	60	26
		50	10	50	14	50	19	50	23
				40	10	40	15	40	19
						30	10	30	15
								20	9

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