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User Bulletin

ABI PRISM® 377 DNA Sequencer With 96-Lane Upgrade

January 14, 1999 (updated 01/2001)

SUBJECT: Optimizing Resolution on the 96-Lane Upgrade

In This Bulletin

This user bulletin provides:

- Information on where to obtain your free copy of the ABI PRISM® DNA Sequencing Analysis Software Version 3.3 and GeneScan® Analysis Software Version 3.1.
- Provides recommendations to maximize resolution when using the ABI PRISM® 377 96-Lane Upgrade.

Latest Version of Software

If you currently own version 3.0, 3.1, or 3.2 of the ABI PRISM® DNA Sequencing Analysis Software, you can download a free copy of the latest version, 3.3, from our website:

www.appliedbiosystems.com/techsupport

If you currently own version 2.1 or later of the GeneScan® Analysis Software, you can download a free copy of the latest version, 3.1, from our website:

www.appliedbiosystems.com/techsupport

The “timer bug” in the older versions of the DNA Sequencing Analysis Software and GeneScan Analysis Software has been resolved in the current versions.

Applied Biosystems

Recommendations for Optimizing Resolution

Introduction

We have installed the 96-lane upgrade on more than 1,000 ABI PRISM® 377 Instruments. The recommendations that follow come from experience gained in our research centers and our users' experience with the 96-lane upgrade.

To optimize the overall quality of data received from a 96-lane upgrade:

- Reduce the amount of DNA loaded in each lane as compared to the amount loaded on the XL-Upgrade or the 36-Lane instrument.
- Minimize the concentration of salts in the sample.
- Load samples at the bottom of the well.
- Use the Sakabe/Water Run-In protocol.

Lowering the Amount of DNA

Resolution increases when the amount of DNA per well is decreased.

To lower the amount of DNA in each lane:

- Use less DNA in sequencing reactions and increase the number of cycles.
- Resuspend the sample in a larger volume of loading buffer.
- Load between 0.5–1 µL of the sample in each well.

Note You may have to optimize your resuspension and loading volumes by doing a series of titrations, and running gels to determine the best combination of signal strength and resolution.

Minimizing Salt Concentration

Excess salts in a sample lead to lowered resolution. Salts accumulate in a sample when:

- The loading buffer of the cycle sequencing reactions has a high concentration of salts.
- Salts are used to purify extension products from a cycle sequencing reaction.

To minimize the concentration of salts in a sample:

- Use Applied Biosystems 25 mM EDTA Loading Buffer.
- Clean up unincorporated dye terminators using salt-free or low-salt methods. We recommend in the following order:
  - Spin columns
  - Isopropanol precipitation protocol for BigDye terminators
  - Ethanol/Sodium Acetate precipitation protocol with 1–2 extra rinses using 70% ethanol
  - Ethanol/$MgCl_2$ precipitation protocol for Rhodamine dye terminator and dRhodamine terminator chemistries

For details on spin columns and precipitation protocols, refer to the *ABI PRISM® User Bulletin: Precipitation Methods to Remove Residual Dye Terminators from Sequencing Reactions* or the *ABI PRISM® Automated DNA Sequencing Chemistry Guide*. These documents can be obtained from the Applied Biosystems WWW site: (www.appliedbiosystems.com/techsupport).

Tips for Optimal Loading

Resolution can be improved by loading samples close to the gel surface rather than from the top of the well.

To load samples:

Step	Action
1	To expel the urea leached into the wells, rinse wells sufficiently with either: ♦ ddH_2O if using the Sakabe/Water Run-In protocol (see page 4) ♦ 1X TBE if using the standard protocol
2	Draw sample(s) into a tip or multi-channel loader with a needle diameter of 0.2–0.25 mm. **Note** We recommend the multi-channel loaders made by Kloehn or World Precision Instruments®.
3	Position the tip(s) close to the bottom of the well (gel surface). Very slowly dispense up to 1 µL of sample into each well, withdrawing the syringe tip as the sample is being expelled.

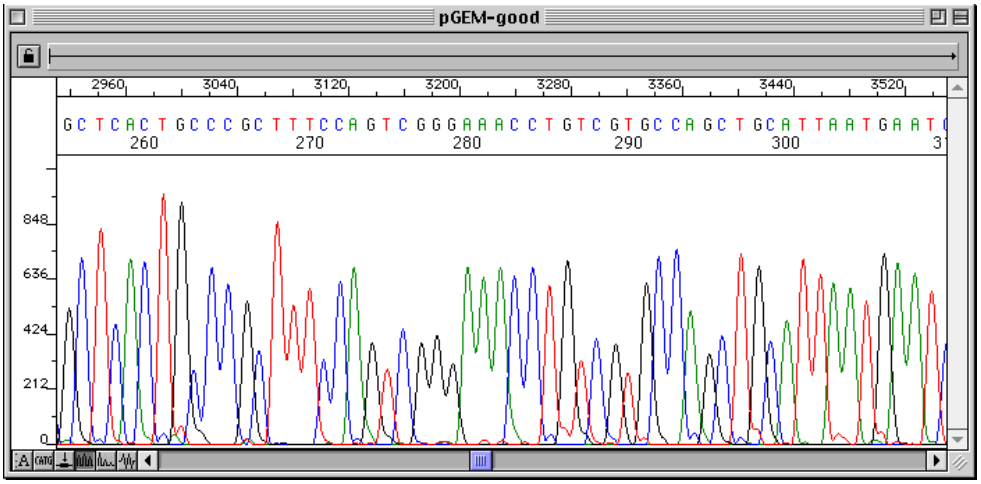
Sakabe/Water Run-In Protocol

If the resolution of your run does not improve sufficiently by following the above suggestions, we recommend using the Sakabe/Water Run-In protocol. This protocol helps minimize band broadening and improves stacking.

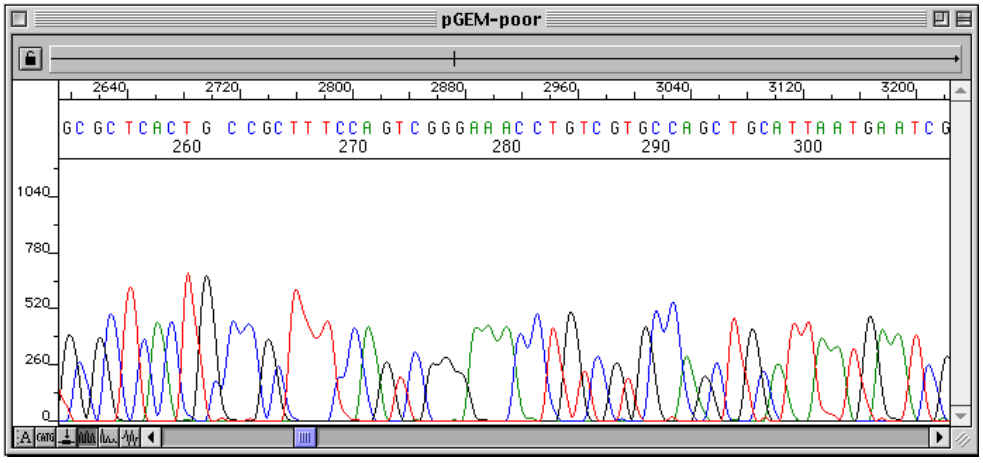
Example

Two control pGEM samples (A and B) were used to demonstrate the improvement in resolution provided by the Sakabe/Water Run-In protocol. The samples were run on two different gels. Sample A was run using the Sakabe/Water Run-In protocol and sample B was run using the standard protocol.

pGEM Sample A



pGEM Sample B



Procedure for the Sakabe/Water Run-In Protocol

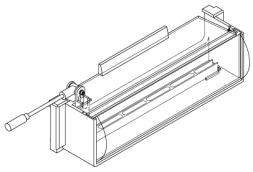
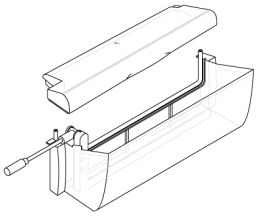
IMPORTANT The final concentration of buffer in the buffer chambers and in the gel is 1X for the Sakabe/Water Run-In protocol.

To perform the Sakabe/Water Run-In protocol:

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Prepare gel solution.</td></tr>
<tr><td>2</td><td>Pour the gel and let it polymerize for 2 hours.</td></tr>
<tr><td>3</td><td>Pull the casting comb out of the gel and clean the sample loading region of all gel fragments.

IMPORTANT All gel fragments must be removed from the gel loading region prior to inserting the sharks tooth comb.</td></tr>
<tr><td>4</td><td>Insert the sharks tooth comb with only water in the comb region.

IMPORTANT Do not add 1X TBE to ease the comb in.</td></tr>
<tr><td>5</td><td>Mount the gel in the cassette and place it on the instrument.</td></tr>
<tr><td>6</td><td>Do a plate check.</td></tr>
<tr><td>7</td><td>Add:

a. Deionized water[a] to the upper buffer chamber in the following amounts:
<table>
<tr><th>If you have a...</th><th>Volume of dH2O to Add (mL)</th></tr>
<tr><td>White upper buffer chamber (old model)
  </td><td>540</td></tr>
<tr><td>Clear upper buffer chamber (new model)
  </td><td>594</td></tr>
</table>
a. Results may vary depending on local variations in water quality. If you do not see enough of a stagger between odd and even lanes, substitute the deionized water with the same amount of 0.25–0.5X TBE solution in the upper buffer chamber. This solution must be made up to 1X TBE in step 15 of this procedure.

b. 1X TBE in the lower buffer chamber (approximately 600 mL) up to the over flow line.</td></tr>
<tr><td>8</td><td>Start the prerun and immediately pause it.

Note Once started and paused, the prerun module continues to bring the gel up to 51 °C. No electrophoresis takes place.</td></tr>
</table>

To perform the Sakabe/Water Run-In protocol: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>9</td><td>Rinse the loading region well:
a. Take a 50-cc syringe of water with a needle on the end of it.
b. Drag the needle along the glass ledge in front of the comb while dispensing the water.

IMPORTANT Be careful not to let the needle touch the comb.</td></tr>
<tr><td>10</td><td>Load the odd lanes:
a. Position the tip(s) close to the bottom of the well (gel surface).
b. Very slowly dispense up to 1 µL of sample into each well, withdrawing the syringe tip as the sample is being expelled.</td></tr>
<tr><td>11</td><td>Start a prerun and pause it after 2 minutes.</td></tr>
<tr><td>12</td><td>Repeat step 9.</td></tr>
<tr><td>13</td><td>Load the even lanes using the technique described in step 10.</td></tr>
<tr><td>14</td><td>Resume the prerun for 1.5 minutes and cancel.</td></tr>
<tr><td>15</td><td>Add the following amount of 10X TBE carefully to the upper buffer chamber to get a final volume of 1X TBE:

IMPORTANT Use the following amount only if you started with the volumes of water in step 7.

<table>
<tr><th>If you have a...</th><th>Add...</th><th>To get...</th></tr>
<tr><td>White upper buffer chamber

Note This chamber can hold a total volume of 600 mL.</td><td>60 mL of 10X TBE</td><td>600 mL of 1X TBE.</td></tr>
<tr><td>Clear upper buffer chamber

Note This chamber can hold total volume of 660 mL.</td><td>66 mL of 10X TBE</td><td>660 mL of 1X TBE.</td></tr>
</table>
</td></tr>
<tr><td>16</td><td>Mix the contents of the upper buffer chamber with a clean 50-mL syringe.</td></tr>
<tr><td>17</td><td>Start the run.</td></tr>
</table>

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