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User Bulletin

ABI PRISM® 377 DNA Sequencer

December 7, 1999 (updated 03/2001)

SUBJECT: Achieving Longer High-Accuracy Reads on the 377 Sequencer

About This Bulletin

Longer sequencing read lengths are now possible on the ABI PRISM® 377 DNA Sequencer by using a revised buffer and gel system with a water run-in protocol for focusing the sample at loading. Gels are analyzed using a new long read basecaller and the ABI PRISM® DNA Sequencing Analysis software.

In This Bulletin

This user bulletin describes the following topics:

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Instrument Platforms

This protocol is for use with all models and upgrades of the ABI PRISM 377 DNA Analyzer. The well-to-read length of the plates must be 48 cm.

Downloading Required Software

There are two software updates that you must have to analyze data using this protocol:

- ABI PRISM DNA Sequencing Analysis Software version 3.3 or 3.4

  These versions of DNA Sequencing Analysis Software can be obtained from our web site. If you currently own version 3.0 or later, of the software, you can download a free update to the latest version:
  www.appliedbiosystems.com/techsupport

- 377 Long Read Basecaller v3.3.1b2

  The basecaller should be downloaded and placed in the Sequencing Analysis folder. To install the basecaller, follow the directions located in the Read Me file.
  www.appliedbiosystems.com/techsupport

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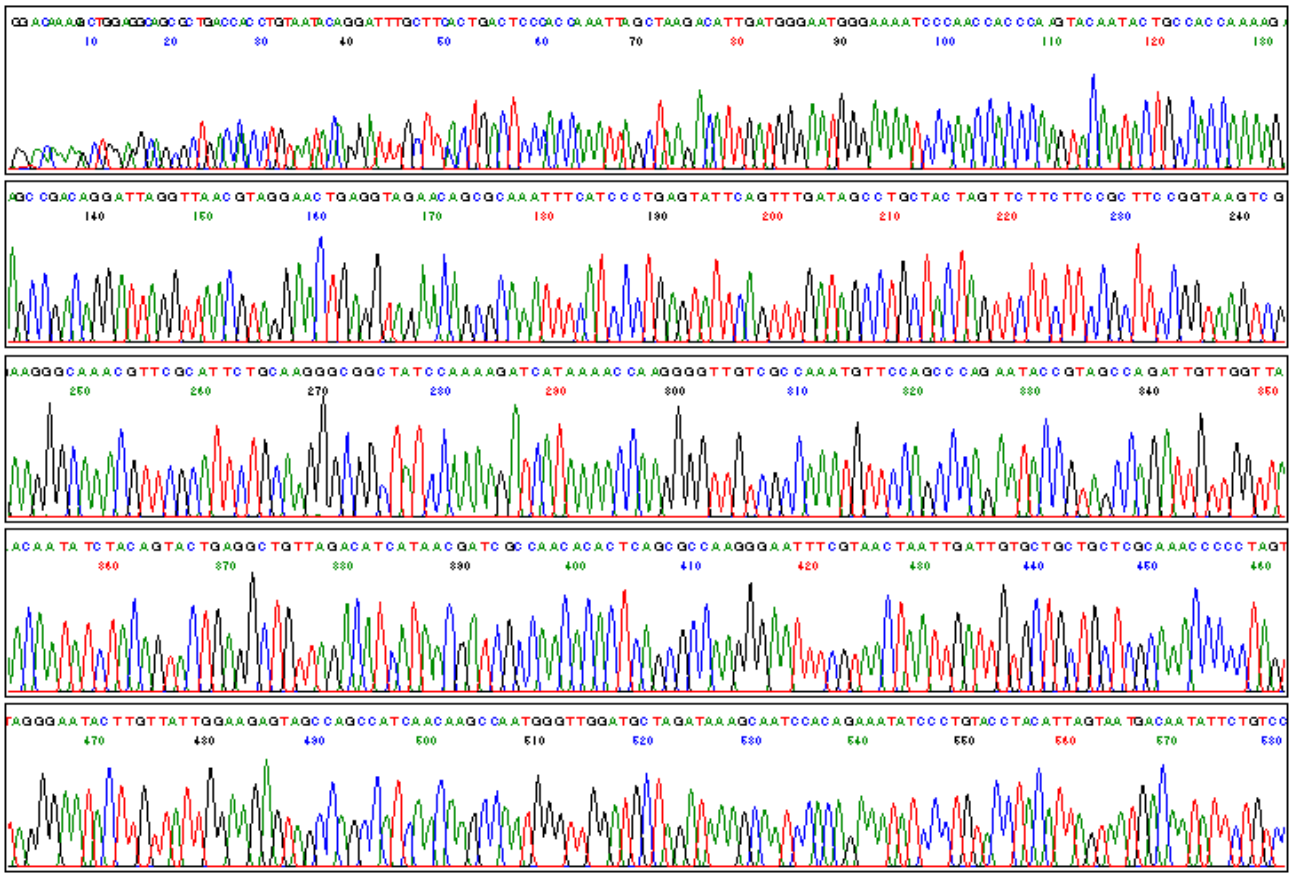
Example Results Using This Protocol

Longer Read Lengths

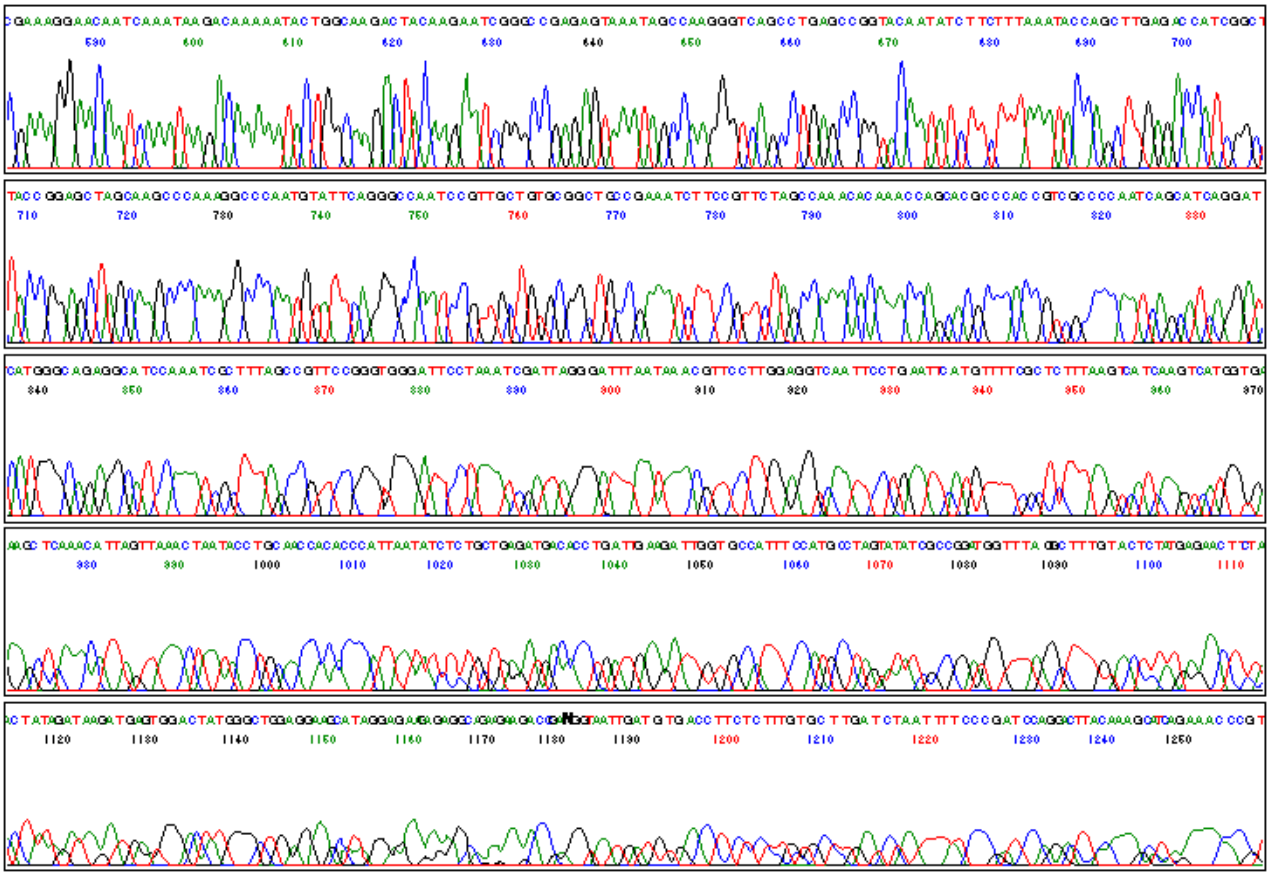
This protocol, if followed correctly, can improve data resolution and increase the number of bases called by the ABI PRISM DNA Sequencing Analysis Software.

Electropherogram Showing Longer Read Lengths

An electropherogram showing data obtained using this protocol is shown below. This electropherogram was generated using BigDye™ terminator chemistry on an ABI PRISM 377 DNA Sequencer with 96-Lane Upgrade.



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Requirements for Achieving Longer Reads

Summary of Requirements

This procedure requires the following:

- Using a water run-in loading protocol
- Using a 48-cm well-to-read length gel
- Using 2X Tris TAPS EDTA (TTE) running buffer in the upper buffer chamber
- Pouring a 4.25% PAGE-PLUS gel made with 1.2X TTE
- Using 1X TTE running buffer in the lower buffer chamber
- Modifying and saving the Seq Run 48E-1200 run module
- Loading minimal sample volumes, *e.g.*, 0.5 µL on a 96-lane gel
- Analyzing data using the LR-377 Basecaller v3.3.1b2

Chemistries to Use

Longer gel reads can be achieved using any of the chemistries, including:

- ABI PRISM® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit
- ABI PRISM® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit v2.0
- ABI PRISM® BigDye™ Primer Cycle Sequencing Ready Reaction Kit
- ABI PRISM® dRhodamine Terminator Cycle Sequencing Ready Reaction Kit

Resuspension and Loading Volumes of DNA

To optimize your resuspension and loading volumes you may have to do a series of titrations and run gels to determine the best combination of signal strength and resolution.

Refer to the appropriate chemistry protocol for recommendations about resuspension and loading volumes. Optimal results are achieved when the amount of DNA per well is decreased and signal strengths are <500 relative fluorescent units (RFUs).

IMPORTANT For 96-lane gels, we recommend that you resuspend the DNA in 2–3 µL of loading buffer and load 0.5 µL of the sample onto the gel.

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Safety

Documentation User Attention Words

Five user attention words appear in the text of all Applied Biosystems user documentation. Each word implies a particular level of observation or action as follows.

Note This word is used to call attention to information.

IMPORTANT This word calls attention to information that is necessary for correct operation of the kit or instrument.

CAUTION **This word informs the user that damage to the instrument could occur if the user does not comply with the information. It also indicates a potentially hazardous situation that could result in minor or moderate injury to the user.**

! WARNING ! This word informs the user that serious physical injury or illness to the user or other persons could occur if these required precautions are not taken.

! DANGER ! Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Chemical Hazard Warning

! WARNING ! CHEMICAL HAZARD. Some of the chemicals used with Applied Biosystems instruments are potentially hazardous and can cause injury, illness or death.

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- **Minimize contact with and inhalation of chemicals. Wear appropriate personal protective equipment when handling chemicals (*e.g.*, safety glasses, gloves, or clothing). Consult the listing in the MSDS.**
- **Do not leave chemical containers open. Use only with adequate ventilation.**
- **Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended on the MSDS.**
- **Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.**

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You can order free additional copies of MSDSs for chemicals manufactured or distributed by Applied Biosystems using the contact information below.

To order MSDSs...	Then...
Over the Internet	Use www.appliedbiosystems.com/techsupport a. Select **MSDS Search** button b. Enter keywords (or partial words), or a part number, or the MSDS's Documents on Demand index number c. Select **Search** d. Select the Adobe® Acrobat symbol to view, print, or download the document, or check the box of the desired document and delivery method (fax or e-mail)
By automated telephone service from any country	Use Documents on Demand
By telephone in the United States	Dial 1-800-327-3002, then press **1**

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<table>
<tr><th>To order MSDSs...</th><th>Then...</th></tr>
<tr><td>By telephone from Canada</td><td>
<table>
<tr><th>If you want ordering instructions in...</th><th>Then dial 1-800-668-6913 and...</th></tr>
<tr><td>English</td><td>Press 1, then 2, then 1 again</td></tr>
<tr><td>French</td><td>Press 2, then 2, then 1</td></tr>
</table>
</td></tr>
<tr><td>By telephone from any other country</td><td>See a list of our Regional Sales Offices.</td></tr>
</table>

For chemicals not manufactured or distributed by Applied Biosystems, call the chemical manufacturer.

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Preparing the Buffer and Pouring the Gel

Reagents Required for Making Buffer and Gel

The following reagents are required for making 10X TTE buffer and pouring a 4.25% PAGE-PLUS gel.

Material	Source
10X TTE or ♦ Tris ♦ TAPS acid ♦ Na_2EDTA	Teknova (P/N 0275-1L) or ♦ Bio-Rad (P/N 1610719) ♦ Sigma (P/N T5130) ♦ Sigma (P/N ED2SS)
Ammonium persulfate	Bio-Rad (P/N 1610700)
PAGE-PLUS	Amresco (P/N E562)
TEMED	Bio-Rad (P/N 1610800)
Urea	Bio-Rad (P/N 1610731)
Water, deionized	Major laboratory supplier or Merck (P/N 1.15333.2500)

Preparing and Storing the 10X TTE

The stock solution (10X) TTE is 500 mM Tris-base, 500 mM TAPS, and 10 mM Na_2EDTA.

! WARNING ! CHEMICAL HAZARD. Tris TAPS EDTA (TTE). May cause eye, skin, and respiratory tract irritation. May be harmful if swallowed. Consult the manufacturer's MSDSs for additional information. Wear splash-resistant safety goggles and appropriate chemical-resistant clothing and gloves.

To prepare 1 liter of 10X TTE:

Step	Action
1	Combine the following reagents in a 1-L beaker: **Reagent** / **Quantity** Tris — 60.55 g TAPS acid — 121.65 g Na_2EDTA — 3.72 g Deionized water — *q.s.* to 1 L
2	Mix well.
3	Optional. Check the pH of the 10X TTE. The pH should be 8.3 ± 0.3. If the pH is not in this range, then discard the buffer. **Note** Store the 10X TTE at room temperature for up to 4 weeks.

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Preparing and Pouring the Gel

To prepare a 48-cm well-to-read gel containing 4.25% PAGE-PLUS gel, 6 M urea, and 1.2X TTE, follow the instructions below.

Ingredient	Quantity
Urea	18 g
40% PAGE-PLUS	5.3 mL
10X TTE	6 mL
Deionized water	21.5 mL
10% ammonium persulfate (APS)	250 µL
TEMED	25 µL
Total	**50 mL**

To prepare and pour the gel:

Step	Action
1	Prepare the glass plates as described in the *ABI PRISM 377 DNA Sequencer User's Manual* (P/N 4307164). **Note** Glass plates and all other gel pouring equipment must be ready for use prior to adding the polymerizing reagents to the gel solution.
2	Prepare the stock solutions listed in the table above.
3	Weigh out the urea and carefully transfer it to a stoppered, graduated cylinder. **! WARNING ! CHEMICAL HAZARD. Urea can cause irritation to the skin, eyes, and respiratory tract. Avoid inhalation and contact with skin, eyes and clothing. Always work in a fume hood. Obtain a copy of the MSDS from the manufacturer. Wear appropriate protective eyewear, clothing, and gloves.**
4	Using a pipette, add 5.3 mL of 40% PAGE-PLUS and 6.0 mL of 10X TTE buffer to the cylinder. **CHEMICAL HAZARD. PAGE-PLUS. Acrylamide is harmful if in contact with the skin or if swallowed. Acrylamide may cause eye, skin, and respitory tract irritation. It may also cause an allergic reaction. Exposure may cause damage to the nervous system, kidneys, and reproductive system. Acrylamide is a possible cancer and birth defect hazard. Please refer to the manufacturer's MSDS for additional information. Wear splash-resistant safety goggles and appropriate chemical-resistant clothing and gloves.**
5	Add 21.5 mL of deionized water to bring the solution to a final volume of 50 mL.
6	Mix the contents thoroughly to dissolve the urea.
7	Filter the solution through a 0.2-µm cellulose nitrate filter.
8	Degas for 2–5 minutes, and transfer the solution to a wide-mouthed container. **Note** Degas time for all gels should be constant to ensure a reproducible polymerization rate for all gels. **IMPORTANT** If the plates are not clean and mounted in the gel cassette or other device, clean and mount them now before adding the polymerizing agents to your solution.

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Polymerizing the Gel

To polymerize the gel:

Step	Action
1	Add 250 µL of freshly made 10% APS to the gel solution, and swirl carefully to mix without introducing air bubbles. **Note** Accuracy and reproducibility are important when making the 10% APS solution. Significant variation in this reagent can produce changes in data quality. **! WARNING ! CHEMICAL HAZARD. Ammonium persulfate (APS) is harmful if swallowed, inhaled, or absorbed through the skin. It is extremely destructive to mucous membranes, eyes, and skin. Inhalation can be fatal. Always work in a fume hood. Obtain a copy of the MSDS from the manufacturer. Wear appropriate protective eyewear, clothing, and gloves.**
2	Add 25 µL of TEMED, and swirl carefully to mix without introducing air bubbles. **! WARNING ! CHEMICAL HAZARD. TEMED is extremely flammable, and can be very destructive to the skin, eyes, nose, and respiratory system. Keep TEMED in a tightly closed container. Avoid inhalation and contact with the skin, eyes, and clothing. Always work in a fume hood. Obtain a copy of the MSDS from the manufacturer. Wear appropriate protective eyewear, clothing, and gloves.**
3	Immediately pour the gel.
4	Wrap the gel in cellophane to prevent drying.
5	Allow the gel to polymerize for 2 hours before using.

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Preparing the 377 Data Collection Software

Overview

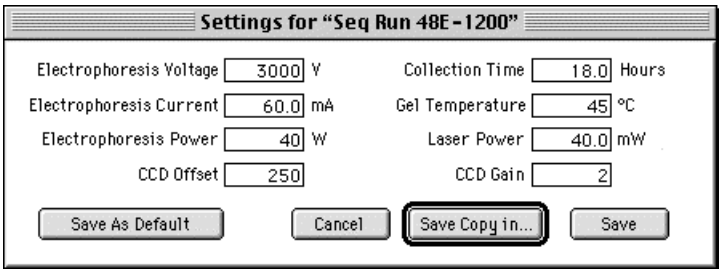
Now that you have prepared the gel, you will need to modify the Seq Run 48E-1200 run module. The run module is located in the ABI PRISM 377 Data Collection Software.

Prerun Module

Use the Seq PR 48E-1200 prerun module. It is not necessary to modify the prerun module.

Modify the Seq Run 48E-1200 Module

Modify the Seq Run 48E-1200 module in the ABI PRISM 377 Collection Software. The module file is modified such that it runs under constant power instead of constant voltage. For additional information about modifying the module, refer to the *ABI PRISM 377 DNA Sequencer User's Manual* (P/N 4307614).

Step	Action
1	Launch the ABI PRISM 377 Data Collection Software.
2	Choose **New** from the **File** menu and click on the **Sequence Run** icon.
3	Select **Seq Run 48E-1200** from the **Run Module** pop-up menu.
4	Click the page graphic to the right of the **Run Module** pop-up menu. In the dialog box that appears, change the settings to those shown below.  **Note** The **Collection Time** can range from 13 to 18 hours depending on the amount of data that you need to collect. **Note** If you have worked out alternative parameters for **CCD Offset** and **CCD Gain** on your instrument, use these values to increase signal strength.
5	Click **Save Copy in...**.
6	a. Enter a name for the new module, *i.e.,* **Seq Run 48E-1200 LR**. b. Select the **Modules** Folder in the 377 Collection Software. c. Click **Save**.

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Preparing the Instrument and the Samples for Gel Electrophoresis

Inserting the Comb and Performing the Plate Check

To insert the comb and perform the plate check:

Step	Action
1	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.
2	Insert the sharks tooth comb or a paper comb. Make sure that only water is present in the comb region. **IMPORTANT** When inserting a paper comb, the comb region of the gel must be dry. This is because the paper comb is compressed when dry, making it easy to slide the comb in. The comb then swells in the presence of liquid. The part numbers for the Single-Use Shark's Tooth combs are listed below:
3	Mount the gel in the cassette and place it on the instrument.
4	Perform a plate check.

Comb Size	Part Number
36 lane (0.18 mm)	4309451
48 lane (0.18 mm)	4309453
64 lane (0.18 mm)	4309455
96 lane (0.33 mm)	4309457

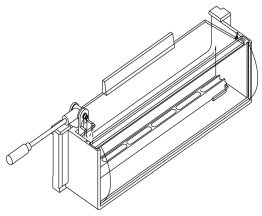
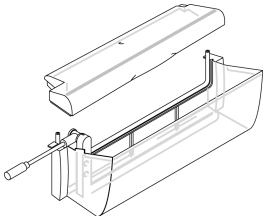
Prepare the Buffer Chambers

To prepare the buffer chambers:

Step	Action
1	Rinse the upper and lower buffer chambers with deionized water. **Note** The purpose of this step is to remove any residual buffer that may be present in the chamber. If you are switching from TBE to TTE gels or TTE to TBE gels, you will need to do this rinse.

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To prepare the buffer chambers: *(continued)*

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>2</td><td>Add deionized water to the upper buffer chamber in the following amounts:
<table>
<tr><th>If you have a...</th><th>Add...</th></tr>
<tr><td>White upper buffer chamber (old model)
</td><td>480 mL of deionized water.</td></tr>
<tr><td>Clear upper buffer chamber (new model
</td><td>560 mL of deionized water.</td></tr>
</table></td></tr>
<tr><td>3</td><td>Add 1X TTE to the overflow line of the lower buffer chamber (approximately 600 mL).

Note To make 600 mL of 1X TTE, in a 1-L graduated cylinder combine 60 mL of 10X TTE with 540 mL of deionized water and mix well.</td></tr>
<tr><td>4</td><td>Start the prerun and immediately pause it.

Note Once started and paused, the module continues to bring the gel temperature up to 51 °C. No electrophoresis takes place.</td></tr>
<tr><td>5</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 disturb the comb when rinsing the loading wells.</td></tr>
</table>

Resuspend and Load the Samples, and Start the Run

To resuspend and load the samples, and start the run:

Step	Action
1	Resuspend and denature the samples as recommended by the protocol that you are following.
2	Load the odd-numbered lanes.
3	Resume the prerun and pause it after 2 minutes.
4	Load the even-numbered lanes.
5	Resume the prerun for 1 minute, then cancel.
6	If you are using a paper comb, remove the comb.

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To resuspend and load the samples, and start the run: *(continued)*

Step	Action
7	If you have a clear buffer chamber **only**, remove 32 mL of deionized water from the upper buffer chamber. **IMPORTANT** If the water is not removed, the buffer chamber overflows. This results in an incorrect buffer concentration and possible arcing.
8	Add the following amount of 10X TTE carefully to the upper buffer chamber to get a final concentration of 2X TTE: **IMPORTANT** Use the following amount only if you started with the volumes of water stated in “Prepare the Buffer Chambers” on page 11.

If you have a...	Add...	To get...
White upper buffer chamber **Note** This chamber can hold a total volume of 600 mL.	120 mL of 10X TTE	600 mL of 2X TTE.
Clear upper buffer chamber **Note** This chamber can hold total volume of 660 mL.	132 mL of 10X TTE	660 mL of 2X TTE.

9	Mix the contents of the upper buffer chamber.
10	Start the run using the modified run module.

Analyzing the Data

Software Required

Conduct data analysis using the following:

- DNA Sequencing Analysis software
- Long Read Basecaller v3.3.1b2

To obtain this software see “Downloading Required Software” on page 1.

Conduct Data Analysis

Refer to the *ABI PRISM DNA Sequencing Analysis Software version 3.4 User’s Manual* (P/N 4306158) for instructions on how to analyze data from a sequencing run.

Expected Data

Data collected using this protocol will have larger base spacing values than gels run according to traditional protocols.

- Base spacing using this protocol is 14–15.
- Base spacing using traditional protocols is 9–11.

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