TransPrep Chemistry

Purification of gDNA from Filtrates Obtained After the Isolation of RNA from Homogenized Animal or Plant Tissue Samples

Protocol

Applied Biosystems

For Research Use Only. Not for use in diagnostic procedures.

Part Number 4326965 Rev. B
7/2003

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Preface

This preface covers:

Safety

Safety Alert Words

Four safety alert words appear in Applied Biosystems user documentation. Each word implies a particular level of observation or action, as described below:

IMPORTANT! Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

CAUTION Indicates a potentially hazardous situation that, if not avoided, can result in minor or moderate injury. It can also alert against unsafe practices, damage to an instrument, or loss of data.

WARNING Indicates a potentially hazardous situation that, if not avoided, can result in serious injury or death.

DANGER Indicates an imminently hazardous situation that, if not avoided, will result in serious injury or death. This signal word is to be limited to the most extreme situations.

Chemical Hazard Warning

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

Chemical Safety Guidelines

To minimize the hazards of chemicals:

- Read and understand the MSDSs provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. See “About MSDSs.”

- Minimize contact with chemicals. When handling chemicals, wear appropriate personal protective equipment such as safety glasses, gloves, and protective clothing. For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, a fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the cleanup procedures recommended in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to *new* customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

You can obtain from Applied Biosystems the MSDS for any chemical supplied by Applied Biosystems. This service is free and available 24 hours a day.

To obtain MSDSs:

1. Go to **https://docs.appliedbiosystems.com/msdssearch.html**
2. In the Search field, type in the chemical name, part number, or other information that appears in the MSDS of interest. Select the language of your choice, then click **Search**.
3. Find the document of interest, right-click the document title, then select any of the following:
   - **Open** – To view the document
   - **Print Target** – To print the document
   - **Save Target As** – To download a PDF version of the document to a destination that you choose

4. To have a copy of a document sent by fax or e-mail, select **Fax** or **Email** to the left of the document title in the Search Results page, then click **RETRIEVE DOCUMENTS** at the end of the document list.
5. After you enter the required information, click **View/Deliver Selected Documents Now**.

Chemical Waste Hazard

WARNING **CHEMICAL WASTE HAZARD.** Some wastes produced by the operation of the instrument or system are potentially hazardous and can cause injury, illness, or death.

Chemical Waste Guidelines

To minimize the hazards of chemical waste:

- Read and understand the MSDSs for the chemicals in a waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers
- Minimize contact with and inhalation of chemical waste. When handling chemicals, wear appropriate personal protective equipment such as safety glasses, gloves, and protective clothing.
- Handle chemical wastes in a fume hood.
- After you empty a chemical waste container, seal it with the cap provided.
- Dispose of the contents of a waste container in accordance with good laboratory practices and local, state/provincial, and/or national environmental and health regulations.

Site Preparation and Safety Guide

A site preparation and safety guide is a separate document sent to all customers who have purchased an Applied Biosystems instrument. Refer to the guide written for your instrument for information on site preparation, instrument safety, and chemical safety.

Waste Disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.

- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

Note: Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological Hazard Safety

BIOHAZARD. Biological samples such as tissues, body fluids, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective eyewear, clothing, and gloves. Read and follow the guidelines in these publications:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4, http://bmbl.od.nih.gov)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR §1910.1030, http://www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html).

Additional information about biohazard guidelines is available at: **http://www.cdc.gov**

How to Obtain Services and Support

For the latest services and support information for all locations, go to **http://www.appliedbiosystems.com**, then click the link for **Services and Support**.

At the Services and Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

In addition, the Services and Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

Introduction to TransPrep Chemistry

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The TransPrep chemistry consists of a purification tray and associated liquid reagents, as well as protocols to enable the isolation of genomic DNA (gDNA) using Applied Biosystems nucleic acid purification platforms.

This chapter covers:

About This Protocol

This protocol provides the following information:

- Safety measures to be aware of
- An overview of the process for producing gDNA on either instrument
- A list of the materials and equipment needed for each instrument
- Procedures for lysing tissue samples
- Procedures for defining new protocols for the 6700 workstation
- Procedures for preparing reagents
- Procedures for performing two runs on the 6700 workstation or the 6100 prepstation:
  - One to collect filtrate from RNA in a deep-well plate
  - One to use the filtrate from the first run to precipitate and archive the gDNA

The TransPrep process is discussed in a separate chapter for each instrument.

About the TransPrep Chemistry

Purpose of the TransPrep Chemistry

This chemistry enables you to isolate both RNA and gDNA from the same homogenized tissue sample. The gDNA is isolated by a precipitation technique from flow-through filtrate collected after RNA has been purified from animal or plant tissue homogenate. This chemistry is designed to be used with Applied Biosystems reagents and plastic consumables and with either of the following instruments:

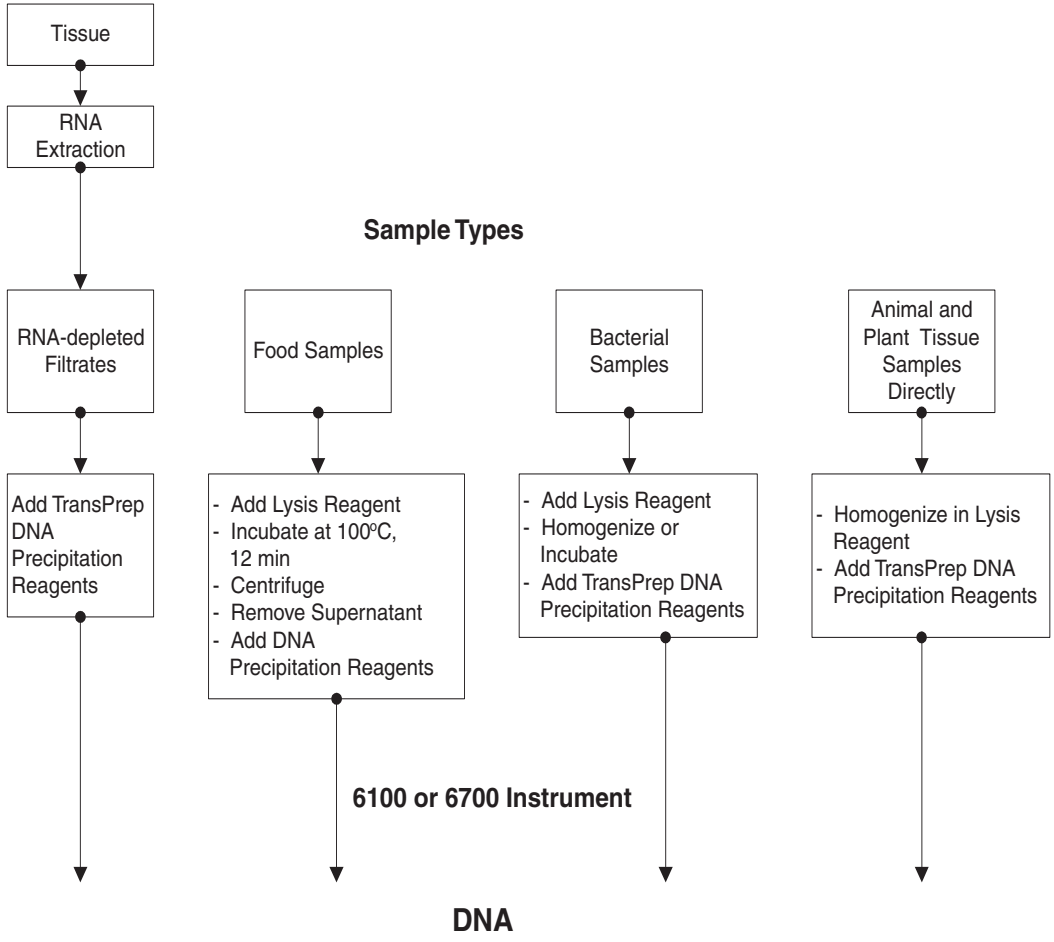
- ABI PRISM™ 6700 Automated Nucleic Acid Workstation (6700 workstation)
- ABI PRISM™ 6100 Nucleic Acid PrepStation (6100 prepstation)

DNA Sources for the TransPrep Chemistry

The TransPrep chemistry is based on alcohol precipitation of DNA from lysed biological samples. DNA may be obtained from:

- Filtrates from RNA extraction from tissue samples, using Applied Biosystems RNA chemistry
- Food samples, where direct detection of DNA is required
- Animal and plant tissue samples directly
- DNA directly from bacterial samples

Overview of the TransPrep Process



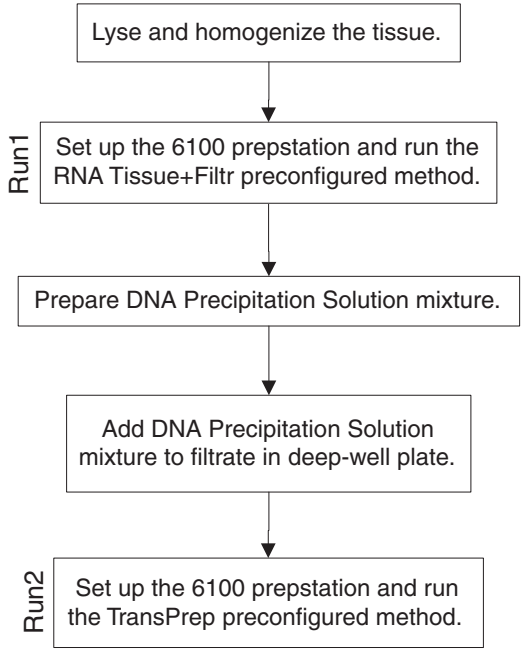
Chemistry on the 6100 PrepStation

2

This chapter covers:

Trans Prep Chemistry Overview

The following diagram shows the TransPrep Process on the 6100 Instrument.



Purified gDNA is left in the collection position.

Materials and Equipment

Equipment and Materials Required But Not Supplied

The following tables list the equipment and materials required for the TransPrep Chemistry.

Equipment	Source
ABI PRISM 6100 Nucleic Acid PrepStation	Applied Biosystems
Microcentrifuge	Major laboratory supplier (MLS)
Pipettors	MLS
Vortexer	MLS

Applied Biosystems Materials	Part Number
6100 Splash Guards	4311758
96-Well Optical Reaction Plate with Barcode	4306737
RNA Purification Wash Solution 1	4305891
RNA Purification Wash Solution 2	4305890
Total RNA Purification Tray	4305673
Nucleic Acid Purification Lysis Solution	4305895
Nucleic Acid Purification Elution Solution	4305893
DNA Precipitation Solution 1	4325962
DNA Precipitation Solution 2	4325964
DNA Wash Solution 1	4325958
DNA Wash Solution 2	4325960
DNA Elution Solution	4325956
gDNA Purification Tray 1	4318641
Deep-Well Plate	4308641

Reagent Storage and Stability

All reagents, when stored individually and correctly, are guaranteed to be stable for 1 year from the date of purchase. The mixture of DNA Precipitation Solutions 1 and 2 should not be stored for more than 4 hours.

Sample Preparation

About Sample Types

Applied Biosystems developed this procedure for sheared, macerated, or homogenized animal or plant tissue samples. Shearing or maceration of these samples reduces the molecular weight of the DNA contained in the samples from approximately 40–50 kilobases (kb) to approximately 10 kb in length. Precipitated gDNA of 10 kb in length is easily resolubilized with a short incubation period. However, precipitated high-molecular-weight gDNA requires prolonged exposure to high temperatures (1 hour at 65 °C) for resolubilization. Therefore, this procedure is not applicable to sample types such as cell culture, whole blood, or primary cell isolates because they contain high-molecular- weight gDNA (40–50 kb). You can expect yields of greater than 50% of the available gDNA from sheared or macerated sample types.

About Lysing Samples

Tissue samples should be lysed and homogenized. Refer to the *Tissue RNA Isolation* protocol (PN 4330252) for further information about lysing samples.

For details on lysing food samples, refer to the Application Note *Isolation, Detection, and Quantitation of Genetically Modified DNA in Food Materials*.

For bacterial samples, homogenize thesample directly in 1× lysis reagent using 100-µm glass beads. Consult the literature for details for specific bacterial types.

BIOHAZARD. Biological samples such as tissues, body fluids, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective eyewear, clothing, and gloves. Read and follow the guidelines in these publications:

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- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR §1910.1030, http://www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html).

Additional information about biohazard guidelines is available at: **http://www.cdc.gov**

First Run on the 6100 PrepStation

Overview

The first run on the 6100 prepstation purifies RNA and captures filtrate containing gDNA. The run consists of:

- Accessing the preconfigured method
- Loading disposables
- Replacing the plate

Accessing the Preconfigured Method

RNA Tissue+Filtr Preconfigured Method

Note: See the Tissue RNA Isolation protocol (PN 4330252) for further details.

Table 2-1 Steps in the RNA Tissue+Filtr Preconfigured Method

Step	Description	Volume (µL)	Position	Time (sec)	Vacuum (%)
—	Pre-Wet all Wells with Wash Solution 1	40	Waste	—	—
1	Load Samples	10–650	Waste	180	80
2	Add Wash Solution 1	500	Waste	180	80
3	Add Wash Solution 2	400	Waste	180	80
4	Add Wash Solution 2	300	Waste	120	60
5	Add Wash Solution 2	300	Waste	120	60
6	Pre-Elution Vacuum	—	Waste	300	90
7	Touch Off at Waste	—	Touch Off	—	—
8	Elution Solution	150	Collection	120	40
9	Touch Off at Collection	—	Touch Off	—	—

Loading Disposables

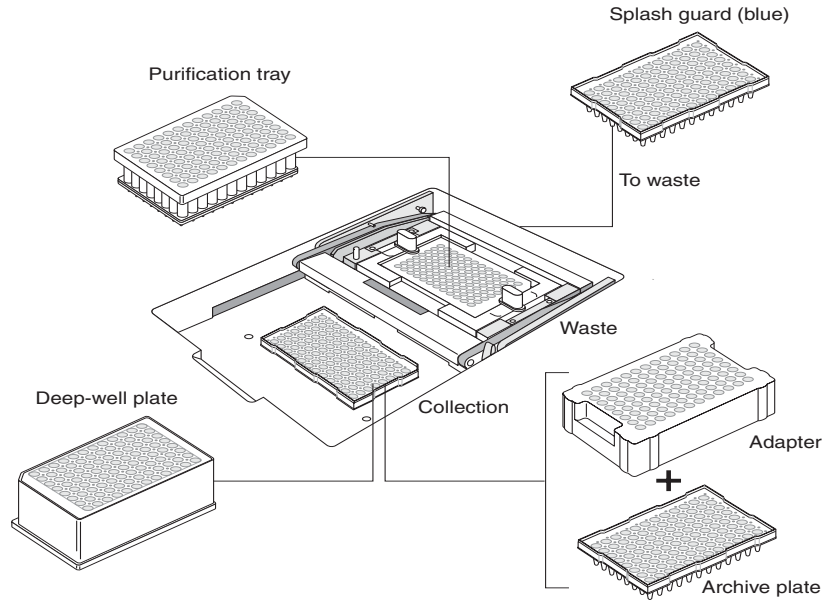


Figure 2-1 Tray positions on the 6100 prepstation.

Replacing the Plate

To replace the deep-well plate:

1.	Release the carriage handle and move the carriage to the waste position.
2.	Remove the deep-well plate from the collection position and replace it with an adapter and a 96-well optical reaction plate with barcode.
3.	Save the deep-well plate with filtrate.
4.	Choose one of the following:

If you want to...	Then...
Continue with RNA purification	See “Running the RNA Tissue+Filtr Method” on page 2-9
Proceed with the TransPrep chemistry	See “Reagent and Filtrate Preparation” on page 2-9

Running the RNA Tissue+Filtr Method

See Table 2-1, “Steps in the RNA Tissue+Filtr Preconfigured Method,” on page 2-6.

Reagent and Filtrate Preparation

About Reagents

Mix the DNA Precipitation Solutions 1 and 2 for use in the second run on the 6100 prepstation.

IMPORTANT! These reagents should be mixed immediately before use and should not be stored as a mixture.

Mixing DNA Precipitation Solutions 1 and 2

DANGER **CHEMICAL HAZARD. DNA Precipitation Solution 1 containing guanidine thiocyanate** causes eye burns and can cause skin and respiratory tract irritation. It is harmful if absorbed through the skin or swallowed. Contact with acids and bleach liberates toxic gases. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

WARNING **CHEMICAL HAZARD. DNA Precipitation Solution 2 containing ethanol** is a flammable liquid and vapor. It may cause eye, skin, and respiratory tract irritation. Prolonged or repeated contact may dry the skin. Exposure may cause central nervous system depression and liver damage. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To prepare the DNA Precipitation Solution mixture, pipette the components listed in the table below into a 120-mL reagent reservoir.

Component	Volume for One Well (µL)†	Volume for 96 Wells (mL)†
DNA Precipitation Solution 1	100	9.6
DNA Precipitation Solution 2	300	28.8
Total Volume	400	38.4

†Volumes given are for 200 µL of filtrate per well collected in a deep-well plate during the first run on the 6100 prepstation.

Adding Precipitation Solution to Filtrate

To add the DNA Precipitation Solution mixture to the filtrate, pipette 400 µL of DNA Precipitation Solution mixture into each well of the deep-well plate (see "Replacing the Plate" on page 2-8). Pipette up and down to mix thoroughly.

DANGER **CHEMICAL HAZARD. DNA Precipitation Solution mixture containing ethanol and guanidine thiocyanate** is a flammable liquid and vapor. Exposure causes eye burns and can cause skin and respiratory tract irritation. It is harmful if absorbed through the skin or swallowed, and may cause central nervous system depression and liver damage. Keep away from heat, sparks, and flame. Contact with acids and bleach liberates toxic gases. DO NOT ADD acids or bleach to any liquid waste containing DNA Precipitation Solution mixture. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Second Run on the 6100 PrepStation

Overview

The second run on the 6100 prepstation allows gDNA to be purified from RNA-depleted filtrates. The run consists of:

- Accessing the preconfigured method
- Loading disposables
- Loading the gDNA purification tray
- Running the TransPrep method

Accessing the Preconfigured Method

TransPrep Preconfigured Method

Table 2-2 Steps in the TransPrep Preconfigured Method

Step	Description	Volume (µL)	Position	Time (sec)	Vacuum (%)
—	Pre-Wet Wells with DNA Wash Solution 1	40	Waste	—	—
1	Load Samples	600†	Waste	120	20
2	Add DNA Wash Solution 1	650	Waste	90	20
3	Add DNA Wash Solution 2	650	Waste	90	20
4	Pre-Elution Vacuum	—	Waste	30	30
5	Touch Off at Waste	—	Touch Off	—	—
6	Add DNA Elution Solution and Incubate	150	Collection	120	0
7	Final Elution Step	—	Collection	120	20
8	Touch Off Collection	—	Touch Off	—	—

†200 µL of RNA-depleted filtrate and 400 µL of a 1:3 mixture of DNA Precipitation Solution 1 and DNA Precipitation Solution 2.

Loading Disposables

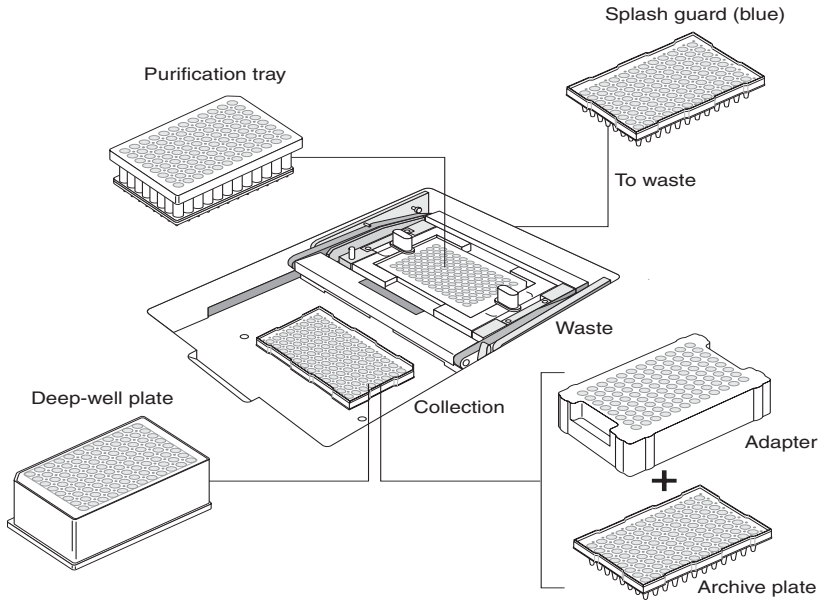


Figure 2-2 Tray positions on the 6100 prepstation.

Loading gDNA Purification Tray

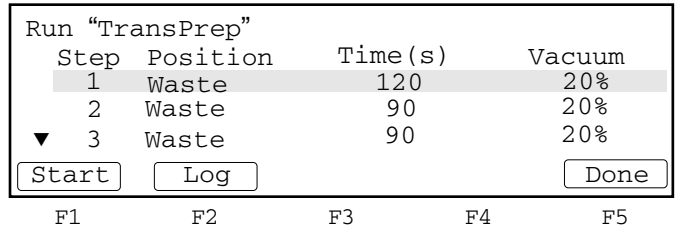
WARNING CHEMICAL HAZARD. DNA Wash Solution 1 containing ethanol is a flammable liquid and vapor. It may cause eye, skin, and respiratory tract irritation. Prolonged or repeated contact may dry the skin. Exposure may cause central nervous system depression and liver damage. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To load the gDNA purification tray:

1.	Pre-wet the purification tray by pipetting 40 µL of DNA Wash Solution 1 over each well of the purification tray.
2.	Pipette 600 µL of sample from the deep-well plate in "Adding Precipitation Solution to Filtrate" on page 2-10 into each corresponding well of the gDNA purification tray.

To load the gDNA purification tray: *(continued)*

3.	Ensure that the highlighter is at step 1 of the TransPrep method, then press **F1** (Start).



Running the TransPrep Method

WARNING CHEMICAL HAZARD.

DNA Wash Solution 1 containing ethanol is a flammable liquid and vapor. It may cause eye, skin, and respiratory tract irritation. Prolonged or repeated contact may dry the skin. Exposure may cause central nervous system depression and liver damage. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

DNA Wash Solution 2 containing ethanol is a flammable liquid and vapor. It may cause eye, skin, and respiratory tract irritation. Prolonged or repeated contact may dry the skin. Exposure may cause central nervous system depression and liver damage. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Table 2-3 Steps in the TransPrep Method

Step	Description	Volume (µL)	Position	Time (sec)	Vacuum (%)
—	Pre-Wet Wells with DNA Wash Solution 1	40	Waste	—	—
1	Load Samples	600†	Waste	120	20
2	Add DNA Wash Solution 1	650	Waste	90	20
3	Add DNA Wash Solution 2	650	Waste	90	20
4	Pre-Elution Vacuum	—	Waste	30	30
5	Touch Off at Waste	—	Touch Off	—	—
6	Add DNA Elution Solution and Incubate	150	Collection	120	0
7	Final Elution Step	—	Collection	120	20
8	Touch Off Collection	—	Touch Off	—	—

†200 µL of RNA-depleted filtrate and 400 µL of a 1:3 mixture of DNA Precipitation Solution 1 and DNA Precipitation Solution 2.

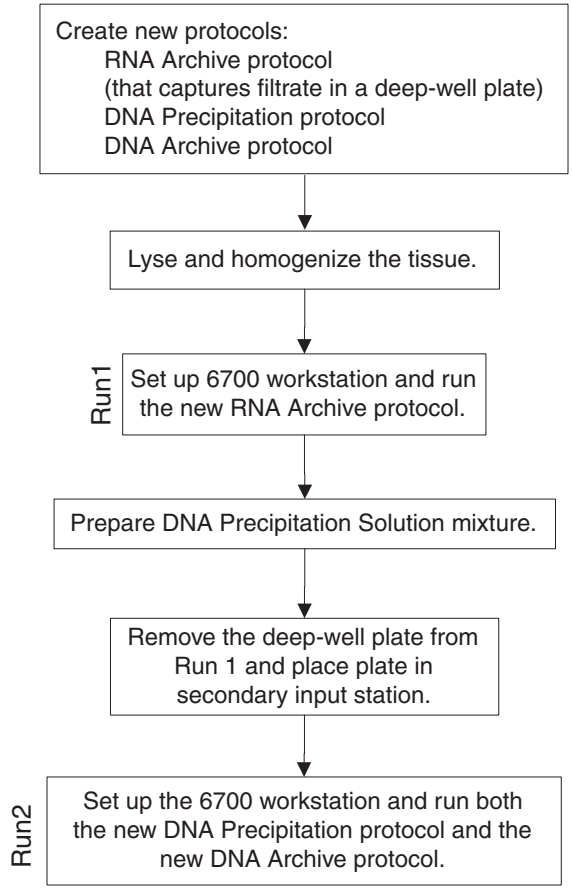
Chemistry on the 6700 Workstation

3

This chapter covers:

TransPrep Chemistry Overview

The following diagram shows the TransPrep Process on the 6700 Instrument.



Alternatively, you can manually pipette 400 µL of DNA Precipitation Solution mixture into each well, then run only the new DNA Archive protocol.

The 6700 workstation transfers the solution of precipitated gDNA from the deep-well plate to a gDNA purification tray. It then adds wash reagents. Finally it adds elution solution. Purified gDNA is left in the primary archive position.

Materials and Equipment

Equipment and Materials Required But Not Supplied

The following tables list the equipment and materials required for the TransPrep Chemistry.

Equipment	Source
ABI PRISM 6700 Automated Nucleic Acid Workstation	See your Applied Biosystems sales representative
Microcentrifuge	Major laboratory supplier (MLS)
Pipettors	MLS
Vortexer	MLS

Applied Biosystems Materials	Part Number
6700 Splash Guards	4311758
96-Well Optical Reaction Plate with Barcode	4306737
Archive Covers	4306286
Conductive Pipette Tips, 1000-µL	4306377
Conductive Pipette Tips, 200-µL	4306375
Reagent Reservoirs, 120-mL This product comes with a sheet of barcode labels for Applied Biosystems nucleic acid purification reagents.	4304831
RNA Purification Wash Solution 1	4305891
RNA Purification Wash Solution 2	4305890
Total RNA Purification Tray	4305673
Nucleic Acid Purification Lysis Solution	4305895
Nucleic Acid Purification Elution Solution	4305893

Applied Biosystems Materials	Part Number
DNA Precipitation Solution 1	4325962
DNA Precipitation Solution 2	4325964
DNA Wash Solution 1	4325958
DNA Wash Solution 2	4325960
DNA Elution Solution	4325956
gDNA Purification Tray 1	4318641
Deep-Well Plate	4308641

Reagent Storage and Stability

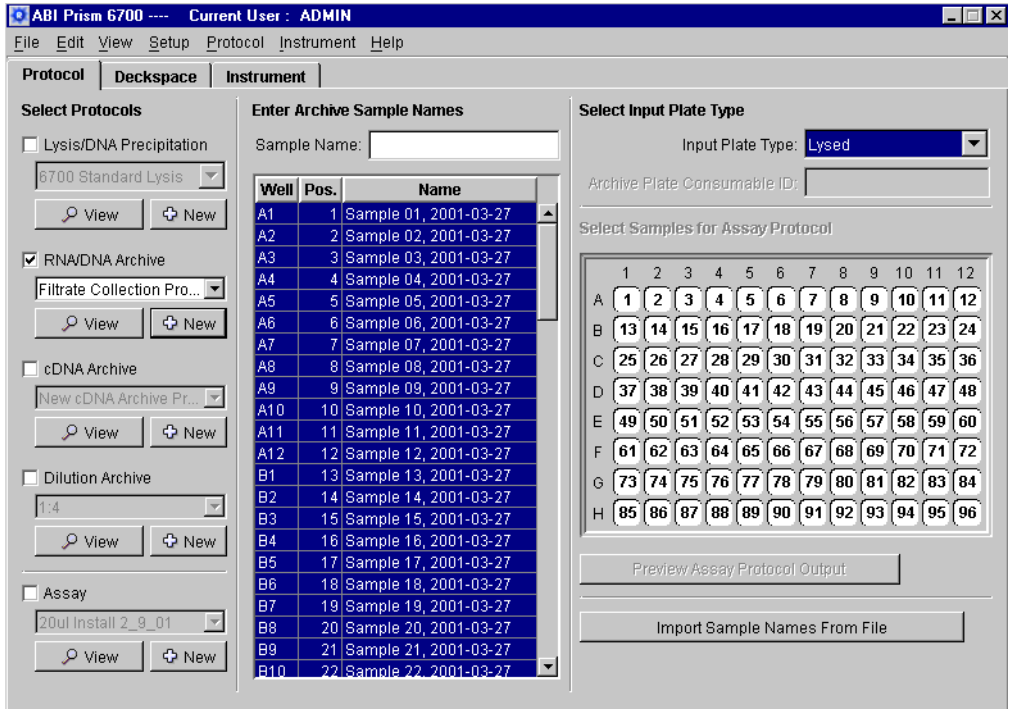
All reagents, when stored individually and correctly, are guaranteed to be stable for 1 year from the date of purchase. The mixture of DNA Precipitation Solutions 1 and 2 should not be stored for more than 4 hours.

Two Runs on the 6700 Workstation

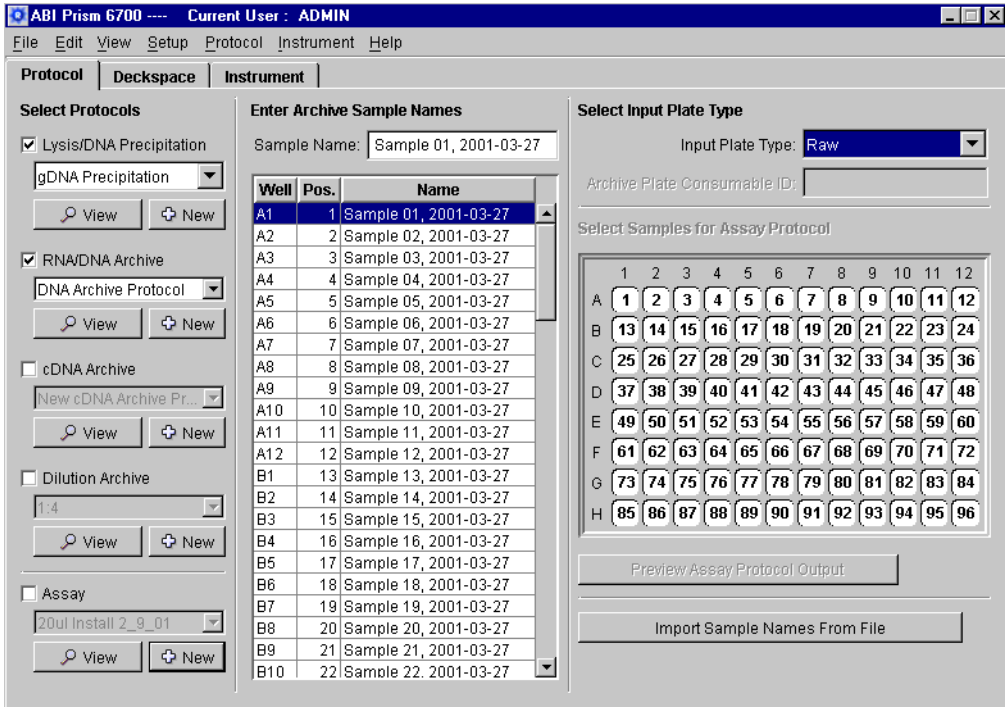
Note: Please refer to the *ABI PRISM™ 6700 Automated Nucleic Acid Workstation User Guide* (PN 4304309) for detailed information on setting up and performing runs.

Run 1

The first run on the 6700 workstation purifies RNA and captures filtrate containing gDNA.



Run 2 The second run on the 6700 workstation allows gDNA to be purified from RNA-depleted filtrates.



Protocol Definition

Creating Two New Protocols

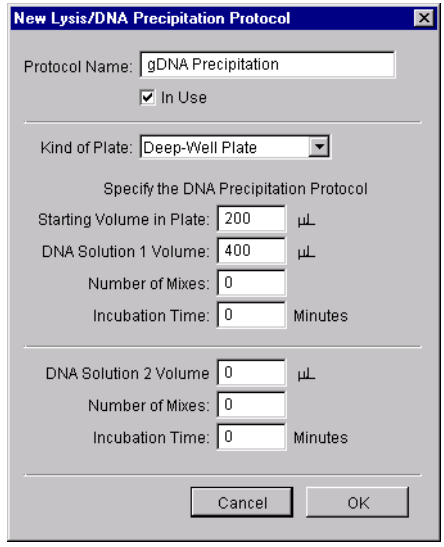
This section describes how to create two new protocols for the 6700 workstation.

- DNA Precipitation protocol
- DNA Archive protocol

Note: To create a new protocol, you must have Scientist or Administrator login access on the 6700 workstation.

Creating a New DNA Precipitation Protocol

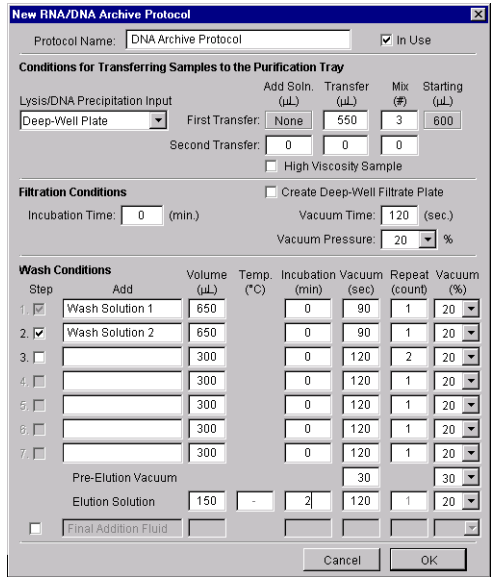
To create a new DNA Precipitation protocol:

1.	Select the **Protocol** tab, then click **New** in the Lysis/DNA Precipitation section.
2.	Enter the values as shown in the example and click **OK**. 

Creating a New DNA Archive Protocol

To create a new DNA Archive protocol:

1.	Select the **Protocol** tab, then click **New** in the RNA/DNA Archive section.
2.	Enter the values as shown in the example and click **OK**.



Sample Preparation

About Sample Types

Applied Biosystems developed this procedure for sheared, macerated, or homogenized animal or plant tissue samples. Shearing or maceration of these samples reduces the molecular weight of the DNA contained in the samples from approximately 40–50 kilobases (kb) to approximately 10 kb in length. Precipitated gDNA of 10 kb in length is easily resolubilized with a short incubation period. However, precipitated high-molecular-weight gDNA requires prolonged exposure to high temperatures (1 hour at 65 °C) for resolubilization. Therefore, this procedure is not applicable to sample types such as cell culture, whole blood, or primary cell isolates because they contain high-molecular-weight gDNA (40–50 kb). You can expect yields of greater than 50% of the available gDNA from sheared or macerated sample types.

About Lysing Samples

Tissue samples should be lysed and homogenized. Refer to the *Tissue RNA Isolation* protocol (PN 4330252) for further information about lysing samples.

BIOHAZARD. Biological samples such as tissues, body fluids, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective eyewear, clothing, and gloves. Read and follow the guidelines in these publications:

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Reagent Preparation

About Reagents

Mix the DNA Precipitation Solutions 1 and 2 for use in the second run on the 6700 workstation.

IMPORTANT! These reagents should be mixed immediately before use and should not be stored as a mixture.

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To prepare the DNA precipitation solution mixture, pipette the components listed in the table below into a 120-mL reagent reservoir.

Component	Volume for One Well (µL)†	Volume for 96 Wells (mL)[a]
DNA Precipitation Solution 1	100	9.6
DNA Precipitation Solution 2	300	28.8
Total Volume	400	38.4

†Volumes given are for 200 µL of filtrate per well collected in a deep-well plate during the first run on the 6700 workstation.

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Printed in USA, 7/2003
Part Number 4326965 Rev. B

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