

DNA Isolation from Fresh and Frozen Blood, Tissue Culture Cells, and Buccal Swabs

Protocol

© Copyright 2004, Applied Biosystems. All rights reserved.

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

Information in this document is subject to change without notice. Applied Biosystems assumes no responsibility for any errors that may appear in this document. This document is believed to be complete and accurate at the time of publication. In no event shall Applied Biosystems be liable for incidental, special, multiple, or consequential damages in connection with or arising from the use of this document.

TRADEMARKS:

Applied Biosystems is a registered trademark and AB (Design), ABI PRISM, Applera, and BloodPrep are trademarks of Applera Corporation or its subsidiaries in the U.S. and/or certain other countries.

All other trademarks are the sole property of their respective owners.

Part Number 4343586 Rev. C
08/2004

Contents

Chapter 1 Introduction

Overview	1-1
In This Chapter	1-1
About This Protocol	1-1
How It Works	1-1
Instrument Systems	1-2
Materials and Equipment	1-4
Equipment and Materials Required But Not Supplied	1-4
Safety	1-6
Safety Alert Words	1-6
Chemical Hazard Warning	1-6
Chemical Safety Guidelines	1-6
About MSDSs	1-7
Obtaining MSDSs	1-7
Chemical Waste Hazard	1-8
Chemical Waste Safety Guidelines	1-8
Waste Disposal	1-8
Biological Hazard Safety	1-9
How to Obtain Services and Support	1-9
Chemical Warnings	1-10

Chapter 2 Isolating and Purifying DNA from Blood

Introduction	2-1
In This Chapter	2-1
Isolating DNA from Fresh or Frozen Whole Blood	2-2
Anticoagulants	2-2
Input Volume	2-3
DNA Yields and Purity	2-4
Inhibitors in Isolated DNA Using BloodPrep Chemistry	2-6
Absence of Hemoglobin	2-6
Absence of Particulates	2-7

Sample Extraction	2-7
Blood Volumes and Incubation Times for Animal DNA Isolation .	2-10
Purifying DNA from Blood on ABI PRISM™ Sample Prep Stations ..	2-12
Purification	2-12
Evacuation Time	2-12
6100 PrepStation Purification of DNA	2-13
6700 Workstation Purification of DNA	2-16
Preventing Membrane Clogging for Blood Samples	2-19
Factors that Contribute to Membrane Clogging	2-19
Guidelines for Preventing Membrane Clogging	2-19

Chapter 3 Isolating and Purifying DNA from Tissue Culture Cells and Buccal Swabs

Introduction	3-1
In This Chapter	3-1
Isolating DNA from Tissue Culture Cells	3-2
Adherent and Suspension Cells	3-2
Input Range	3-2
DNA Yields and Purity	3-2
Sample Extraction	3-4
Isolating DNA from Buccal Swabs	3-6
Buccal Swab DNA	3-6
DNA Yields and Purity	3-6
Sample Extraction	3-7
Purifying DNA from Tissue Culture Cells and Buccal Swabs on ABI PRISM Sample Prep Stations	3-9
Purification	3-9
6100 PrepStation Purification of DNA	3-11
6700 Workstation Purification of DNA	3-12

Index

Introduction

Overview

In This Chapter This chapter covers:

Materials and Equipment1-4

Safety.1-6

About This Protocol This protocol describes techniques for isolating and purifying DNA from the following biological sources.

- Fresh or frozen human whole blood, fresh or frozen animal blood, and blood cell isolates
- Adherent and suspension tissue cultured cells
- Buccal swabs
- Pathogen DNA from serum, plasma, cerebrospinal fluid or cell culture supernatant

How It Works The BloodPrep™ chemistry protocol isolates DNA from 150 µL (or less) of fresh or frozen human whole blood, up to 10⁶ tissue culture cells, or buccal swab material. No separation of the red blood cells is necessary when isolating from whole blood.

BloodPrep chemistry is designed to quickly and efficiently purify DNA from a wide variety of sample materials including fresh and frozen whole blood from human and other animal species, isolated blood cells such as buffy coat or leukocyte preparations, tissue culture cells, buccal swabs, plasma, and serum.

Fresh and frozen whole blood or buffy coat preparations are purified in a two-step process. The blood is digested at elevated temperatures with Proteinase K Solution and BloodPrep™ PK Digestion Buffer. The cell lysis is completed by adding BloodPrep™ DNA Purification Solution. The digested lysates are applied to a 96-well purification tray containing a glass fiber membrane. The membrane is then washed and high quality DNA is eluted in a two-step elution buffer system.

Blood samples that are less than one day old can be lysed directly in the BloodPrep DNA Purification Solution. Tissue culture cells and buccal swabs require only a simple addition of BloodPrep DNA Purification Solution to complete cell lysis.

For the process overview, see the flow chart in Figure 1-1, “The process of isolation of DNA from blood, tissue culture cells, and buccal swabs” on page 1-3.

**Instrument
Systems**

The lysis and purification procedures discussed in this protocol can be performed on the ABI PRISM™ 6100 Nucleic Acid PrepStation and the ABI PRISM™ 6700 Nucleic Acid Workstation.

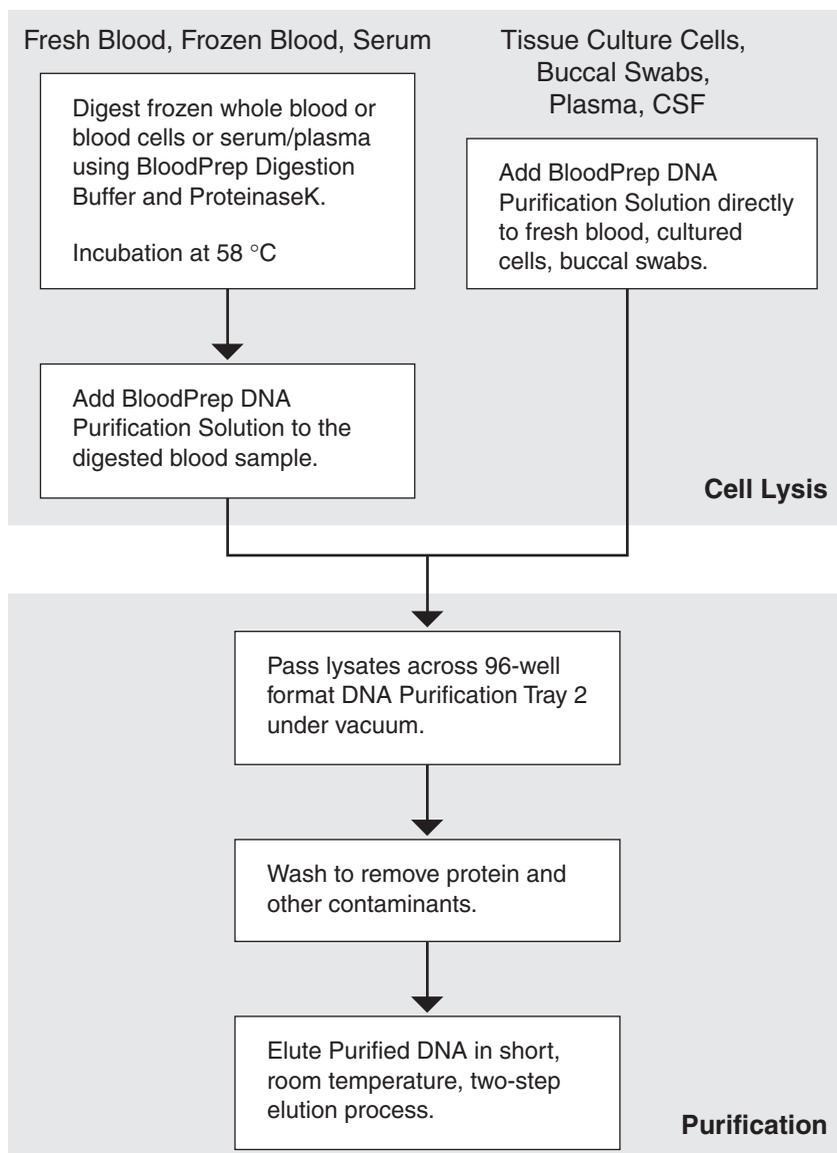


Figure 1-1 The process of isolation of DNA from blood, tissue culture cells, and buccal swabs

Materials and Equipment

Equipment and Materials Required But Not Supplied

The following tables list the equipment, accessories, and chemicals required for DNA isolation.

Table 1-1 Equipment required but not supplied:

Equipment	Source
ABI PRISM™ 6100 Nucleic Acid PrepStation	See your Applied Biosystems sales representative
ABI PRISM™ 6700 Automated Nucleic Acid Workstation	See your Applied Biosystems sales representative
Pipettors	MLS*
Vortexer	MLS
Water Bath	MLS

*MLS: Major Laboratory Supplier

Table 1-2 Accessories required but not supplied:

Accessories	Source
96-Well Deep Well Plate	Applied Biosystems (PN 4308641)
96-Well Optical Reaction Plate with Barcode*	Applied Biosystems (PN 4306737)
200 μ L Conductive pipette tips (for the 6700 only)	Applied Biosystems (PN 4306375)
1000 μ L Conductive pipette tips (for the 6700 only)	Applied Biosystems (PN 4306377)
6700 Reagent Reservoir, 120 mL	Applied Biosystems (PN 4304831)
Archive Tray Covers	Applied Biosystems (PN 4306286)
Genomic DNA Purification Tray II	Applied Biosystems (PN 4330172)
Splash guards	Applied Biosystems (PN 4311758)

Table 1-2 Accessories required but not supplied: (continued)

Accessories	Source
15- and/or 50-mL sterile tubes	MLS†
300- μ L, flat-bottom, 96-well cell culture plate	MLS
Microcentrifuge tubes	MLS

*These plates are called “archive plate” and “PCR plate” in other protocols.

†MLS: Major Laboratory Supplier.

Table 1-3 Chemicals required but not supplied:

Required Chemicals	Source
BloodPrep™ DNA Elution Solution 1	Applied Biosystems (PN 4342951)
BloodPrep™ DNA Elution Solution 2	Applied Biosystems (PN 4342950)
BloodPrep™ DNA Purification Solution	Applied Biosystems (PN 4342775)
BloodPrep™ DNA Wash Solution	Applied Biosystems (PN 4342949)
BloodPrep™ PK Digestion Buffer	Applied Biosystems (PN 4342777)
Proteinase K Solution, 20 mg/mL	Applied Biosystems (PN 4333793)
Ethanol, 70%	MLS*
Water, molecular biology grade	MLS

*Major Laboratory Supplier

Safety

Safety Alert Words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT**, **CAUTION**, **WARNING**, **DANGER**—implies a particular level of observation or action, as defined 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, may result in minor or moderate injury. It may also be used to alert against unsafe practices.



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



DANGER – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. 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 Material Safety Data Sheets (MSDS) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See “About MSDSs.”)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or 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, fume hood). For additional safety guidelines, consult the MSDS.

- 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.

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.htm>
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 Safety Guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or 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, fume hood). For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

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.

IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological Hazard Safety



WARNING 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://bmbi.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.

Chemical Warnings

Read the following chemical warnings before proceeding with the protocols in Chapters 2 and 3.



WARNING BLOODBORNE/INFECTIOUS WASTE HAZARD. Discard the supernatants following recognized disinfection procedures and in accordance with all local, state, and national bloodborne/infection regulations.



WARNING CHEMICAL HAZARD. BloodPrep™ DNA Elution Solution 1. Exposure causes eye, skin, and respiratory tract irritation. Avoid breathing the vapor. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.



DANGER CHEMICAL HAZARD. BloodPrep™ DNA Purification Solution. Exposure causes eye burns. Solution is harmful if swallowed or absorbed through the skin. Exposure causes skin and respiratory tract irritation. Avoid breathing the vapor. Contact with acids or bleach liberates toxic gases. **DO NOT ADD**

acids or bleach to any liquid waste containing BloodPrep DNA Purification Solution. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

 **WARNING CHEMICAL HAZARD. BloodPrep™ DNA Wash Solution** is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation, and may cause liver damage and central nervous system depression. Avoid breathing the vapor. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

 **WARNING CHEMICAL HAZARD. BloodPrep™ PK Digestion Buffer** is harmful if swallowed. Exposure causes eye, skin, and respiratory tract irritation. Contact with acids or bleach liberates toxic gases. DO NOT ADD acids or bleach to any liquid waste containing BloodPrep PK Digestion Buffer. Read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

 **WARNING CHEMICAL HAZARD. EDTA.** Exposure causes eye irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and

 **WARNING CHEMICAL HAZARD. Ethanol** is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation and may cause central nervous system depression and liver damage. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

 **WARNING CHEMICAL HAZARD. Proteinase K** causes eye, skin, and respiratory tract irritation. Exposure may cause allergic reactions. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

 **DANGER CHEMICAL HAZARD. Sodium hydroxide (NaOH)** causes severe eye, skin, and respiratory tract burns. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Isolating and Purifying DNA from Blood

2

Introduction

In This Chapter This chapter covers:

Isolating DNA from Fresh or Frozen Whole Blood	2-2
Purifying DNA from Blood on ABI PRISM™	
Sample Prep Stations.	2-12
Preventing Membrane Clogging for Blood Samples.	2-19

This chapter describes how to isolate and purify intact high molecular weight DNA from fresh or frozen whole blood, blood cell isolates, plasma, h and serum.

Isolating DNA from Fresh or Frozen Whole Blood

Anticoagulants Commonly used anticoagulants such as EDTA, CPD, ACD, Citrate, or Heparin are compatible with this protocol and will not inhibit PCR. As shown in Figure 2-1, this procedure gives reproducible results with various anticoagulants.

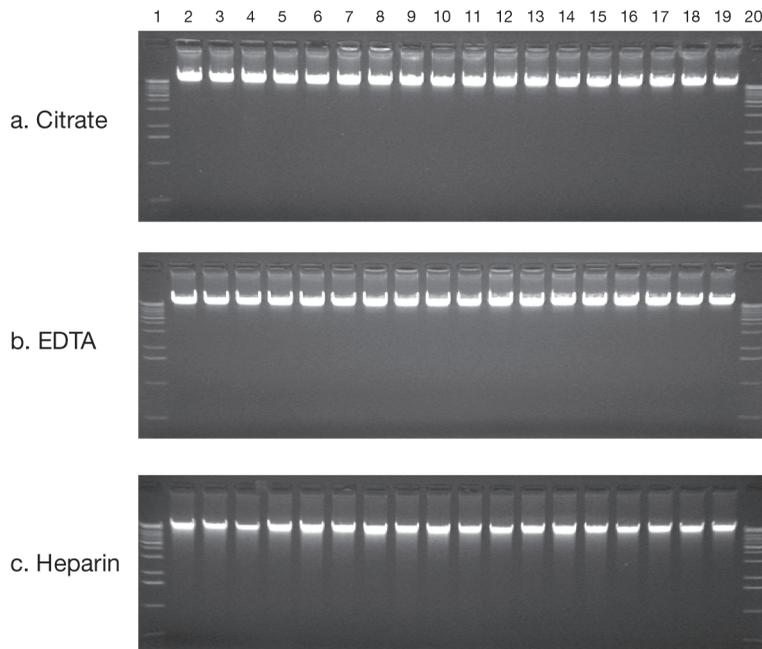


Figure 2-1 1% TBE agarose gel images of gDNA isolated from human blood in a variety of common anticoagulants. Lanes 1 and 20 show a gDNA molecular weight ladder with the largest band at 40kb.

Input Volume In most cases, 150 μL is the recommended maximum input volume for fresh and frozen human whole blood. The maximum volume for blood from most animal species is 50 μL .

IMPORTANT! Volumes greater than 150 μL clog the purification tray wells and do not increase the yield of recovered DNA.

Table 2-1 Input volumes for fresh and frozen blood:

Sample	Maximum Input Volume
Human Buffy Coat/PBMC	50 μL (1×10^6 cells per well)
Human Fresh or Frozen	150 μL
Chicken	20 μL
Cow	50 μL
Dog	50 μL
Horse	50 μL
Monkey (Macaque)	50 μL
Pig	50 μL
Rabbit	50 μL
Rat	50 μL
Sheep	50 μL

DNA Yields and Purity

150 μL of human whole blood is expected to yield 3–8 μg of human DNA depending on the white blood cell count of the sample. Yields of DNA from animals are listed in Table 2-2, “Input volumes and expected yields for animal blood DNA:” on page 2-10. For volumes less than 150 μL , the yield of DNA is 70–90% on a per cell basis. As shown in Figure 2-2 and Figure 2-3, this procedure using the BloodPrep™ chemistry gives adequate yields of gDNA.

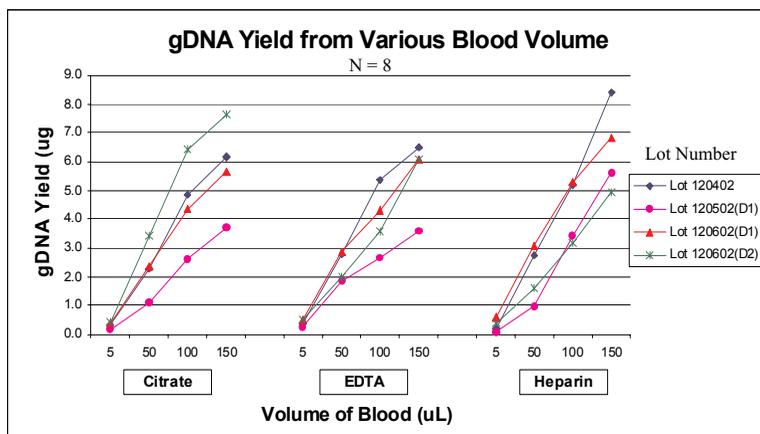


Figure 2-2 Yields of gDNA from BloodPrep™ chemistry at various input volumes of human whole blood in different anticoagulants

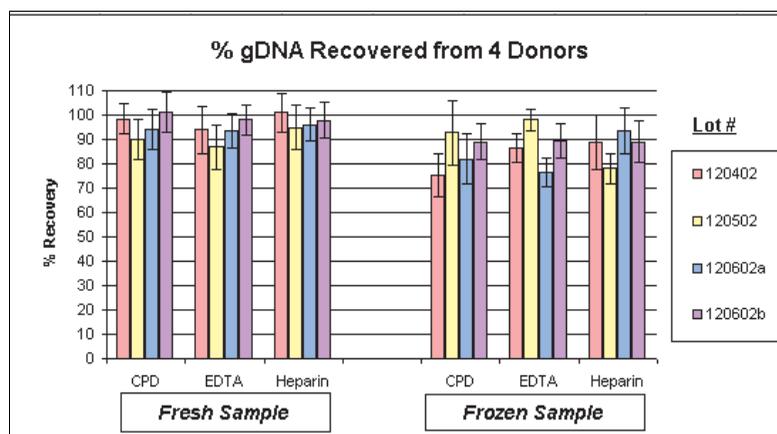


Figure 2-3 gDNA yields from 150 μL of human blood from four donors and extracted using the BloodPrep chemistry. Each bar represents the average from 32 samples.

For all types of fresh and frozen blood samples, the A_{260}/A_{280} ratios for DNA purity should be approximately 1.7–1.8 provided that volume limits and the incubation time and temperatures are adhered to.

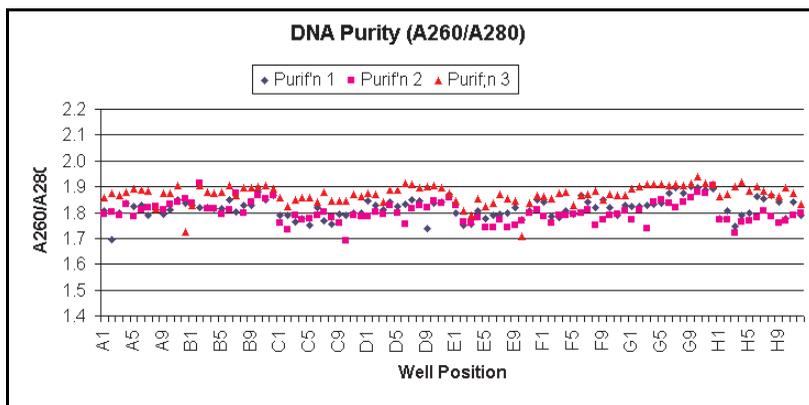


Figure 2-4 Purity of DNA isolated from 3 × 96 well purifications

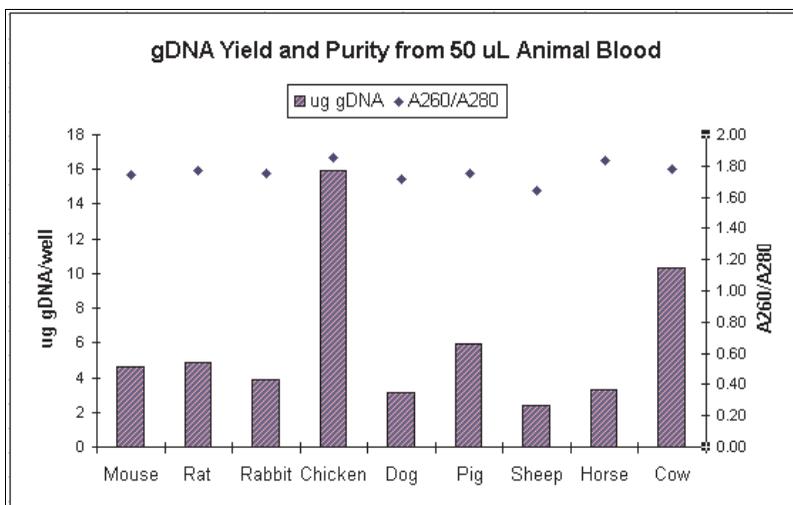


Figure 2-5 Blood gDNA yields and purity from nine different animal species

Inhibitors in Isolated DNA Using BloodPrep Chemistry

Isolated DNA is free from contaminating protein such as heme and other cellular macromolecules when you use the BloodPrep chemistry. Inhibitors do not carryover into the DNA even from samples that are anticoagulated with heparin.

Figure 2-6 shows the inhibition plot for frozen CPD anticoagulated blood and a real time PCR amplification for the 18S rRNA amplicon run using the 5' nuclease TaqMan assay on a dilution series (no dilution, 1:4, 1:16, 1:64, 1:256 and 1:1024) of gDNA isolated from 50 µL of whole blood. The no dilution point represents the addition of 1 µg of DNA to a 50 µL PCR reaction. The plot of logarithm of dilution versus threshold cycle number is linear if no inhibitors are present in the sample.

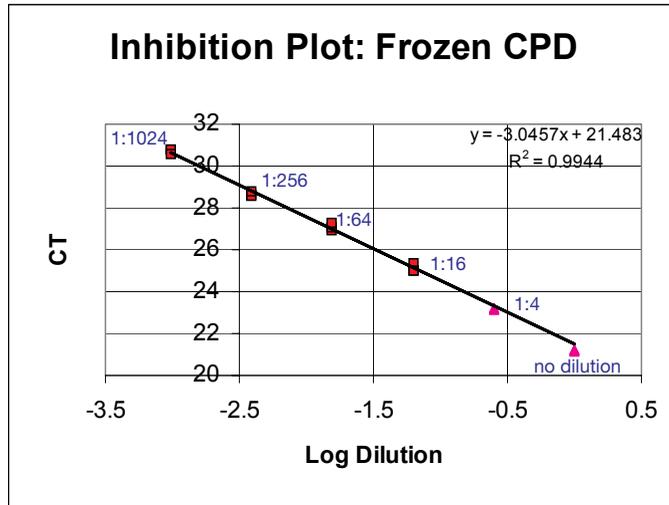


Figure 2-6 Inhibition assay plot for gDNA isolated from frozen CPD anticoagulated human blood

Absence of Hemoglobin

Heme, the non-protein iron component of hemoglobin, is a primary contaminant of DNA from blood preparations and is detected by absorption at 410nm. BloodPrep chemistry isolates DNA with $A_{410\text{nm}} < 0.05$ indicating no heme carryover. The maximum input volume and cell number limits should be adhered to to achieve the level of purity required.

Absence of Particulates	Light-scattering particulates are easily observed as an absorption at 320nm. The BloodPrep chemistry achieves A_{320} values at <0.05 units, which indicate no particulate carryover.
Sample Extraction	<p>Follow the procedure below to extract and isolate DNA from blood for purification on the ABI PRISM™ 6100 Nucleic Acid PrepStation or the ABI PRISM™ 6700 Automated Nucleic Acid Workstation.</p> <p>IMPORTANT! Blood samples should be at ambient laboratory temperature. Processing of very cold, just thawed or recently thawed samples may result in clogging of the purification tray. Clogging results from highly viscous samples due to a decrease in performance of the Proteinase K digestion step.</p> <p>Note: Thawing frozen blood at elevated temperatures is not advised as it may also initiate precipitation of proteins.</p> <p>Read the following chemical warnings before proceeding with the sample extraction protocol.</p>



WARNING 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.



WARNING CHEMICAL HAZARD. Proteinase K causes eye, skin, and respiratory tract irritation. Exposure may cause allergic reactions. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.



WARNING CHEMICAL HAZARD. BloodPrep™ PK Digestion Buffer is harmful if swallowed. Exposure causes eye, skin, and respiratory tract irritation. Contact with acids or bleach liberates toxic gases. DO NOT ADD acids or bleach to any liquid waste containing BloodPrep PK Digestion Buffer. Read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.



DANGER CHEMICAL HAZARD. BloodPrep™ DNA

Purification Solution. Exposure causes eye burns. Solution is harmful if swallowed or absorbed through the skin. Exposure causes skin and respiratory tract irritation. Avoid breathing the vapor. Contact with acids or bleach liberates toxic gases. **DO NOT ADD** acids or bleach to any liquid waste containing BloodPrep DNA Purification Solution. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To extract and isolate DNA from fresh or frozen whole blood:

1.	<p>Place 15 μL of Proteinase K Solution (20 mg/mL) in 2-mL microcentrifuge tubes or deep well plates.</p> <p>Note: If your blood samples are <24 hrs old, you may add them directly to the microcentrifuge tubes or deep well plates and proceed directly to step 6.</p>
2.	<p>Add 85 μL of PK Digestion Buffer.</p>
3.	<p>Add 150 μL of fresh or frozen whole human blood to the mixture of BloodPrep PK Digestion Buffer and Proteinase K Solution.</p> <p>Note: If you are using animal blood, use the suggested volumes in “Blood Volumes and Incubation Times for Animal DNA Isolation” on page 2-10.</p>
4.	<p>Mix thoroughly by pumping the mixture with a pipette 3 times.</p>
5.	<p>Incubate the mixture at 58 $^{\circ}\text{C}$ for 10 min.</p> <p>IMPORTANT! Temperatures in excess of 60 $^{\circ}\text{C}$ may cause degradation of the isolated DNA and reduce the activity of the Proteinase K solution.</p> <p>Note: Blood of other animal species may require longer incubation times or increased volumes of Proteinase K solution.</p>

To extract and isolate DNA from fresh or frozen whole blood:

6.	<p>Add 500 μL of BloodPrep DNA Purification Solution to generate a total volume of 750 μL.</p> <p>IMPORTANT! The BloodPrep DNA Purification Solution may require gentle heating to 37 °C for 5–10 min to dissolve precipitated salts.</p>
7.	<p>Mix the blood and BloodPrep DNA Purification Solution thoroughly by pipetting up and down five times.</p> <p>Note: It is very important that the blood and purification solution are thoroughly mixed before proceeding to the purification steps! Vortexing does not effectively mix the viscous samples and the Proteinase K solution, no matter how long you vortex.</p>
8.	<p>Read “Factors that Contribute to Membrane Clogging” on page 2-19.</p>
9.	<p>Set up the 6100 PrepStation or 6700 Workstation for a purification run. See “6100 PrepStation Purification of DNA” on page 2-13 or “6700 Workstation Purification of DNA” on page 2-16 for appropriate settings.</p> <p>Note: If you cannot proceed with purification immediately, store the lysed samples at 4 °C. Before proceeding to step 10, these samples must be gently heated for 5–10 min to bring the sample temperature up to room temperature and then gently vortexed.</p>
10.	<p>Go to “Purifying DNA from Blood on ABI PRISM™ Sample Prep Stations” on page 2-12.</p>

Blood Volumes and Incubation Times for Animal DNA Isolation

To isolate DNA from animal blood samples, refer to Table 2-2 below for the maximum input volume of blood. Follow the procedure “Isolating DNA from Fresh or Frozen Whole Blood” on page 2-2 and substitute the sample volumes listed below for step 3 of the procedure “To extract and isolate DNA from fresh or frozen whole blood:” on page 2-8.

Table 2-2 Input volumes and expected yields for animal blood DNA:

Animal Type	Max Volume Input (μL)	gDNA Yield (μg)	Incubation Time (mins)
Chicken	20	2–6	10
Cow	50	8–12	20
Dog	50	2–4	10
Horse	50	2–4	10
Monkey (Macaque)	50	2–5	30
Mouse	50	2–6	10
Pig	50	3–6	10
Rabbit	50	3–5	10
Rat	50	3–6	10
Sheep	50	2–3	30

Troubleshooting Tips

1. Sheep, cow, and monkey blood require prolonged incubation for best results.
2. Avian blood has nucleated red blood cells giving high yields of DNA. Input volumes should be lowered to 25 μL or lower.

As shown in Figure 2-7 below, you can use the BloodPrep chemistry across a wide variety of species to generate high-quality, intact DNA. The largest band in the molecular weight marker lane is 40kb.

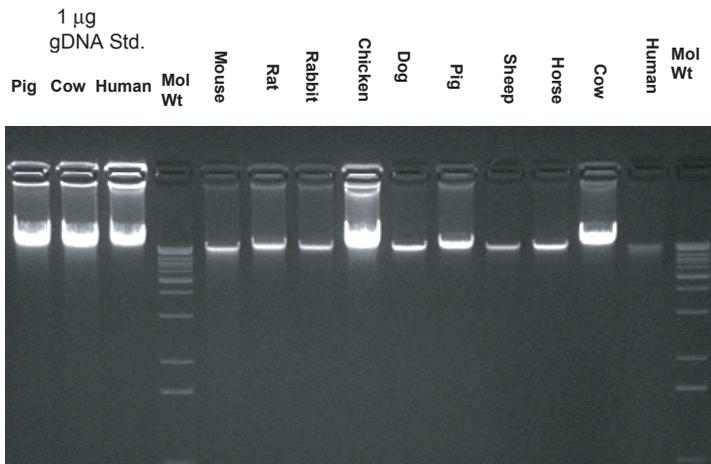


Figure 2-7 1% TBE agarose gel image of gDNA isolated from 50 μ L of frozen animal blood of various species. 20 μ L of eluate (total 200 μ L) was loaded into the gel showing relative yields of gDNA from different animal species.

Purifying DNA from Blood on ABI PRISM™ Sample Prep Stations

Purification This section describes the DNA purification procedure to use after the DNA is extracted from your sample (see “Sample Extraction” on page 2-7). Purification is accomplished by loading the samples on an ABI PRISM™ Sample Prep Station and using the settings listed in the “6100 PrepStation Purification of DNA” table on page 2-13 or the “6700 Workstation Purification of DNA” table on page 2-16. Read the biohazard warning below before proceeding with the purification process.



WARNING 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.

Evacuation Time Evacuation time is defined as the amount of time required for the blood to pass across the purification tray membrane the first time. The viscosity of blood, which is determined by the number of leukocytes, amount of lipids, and other blood cell components, influences evacuation time. If the viscosity increases, the evacuation time increases.

Healthy human patients typically have 4×10^6 – 7×10^6 leukocytes per mL of whole blood. Blood samples from patients with disease, which increases leukocyte counts or lipid content, may require you to repeat step 1 of the 6100 or 6700 instrument procedure or lower the sample volume to enable the sample to pass across the purification tray membrane. Additionally, steps 3–5, washing the DNA with the BloodPrep DNA Purification Solution, is the slowest part of the purification process and requires the most evacuation time. See “6100 PrepStation Purification of DNA” on page 2-13 and “6700 Workstation Purification of DNA” on page 2-16.

6100 PrepStation Purification of DNA

Use Table 2-3 below to set up and run the ABI PRISM™ 6100 Nucleic Acid PrepStation for sample purification. To prevent clogging the membrane with sample material, see “Factors that Contribute to Membrane Clogging” and “Guidelines for Preventing Membrane Clogging” on page 2-19.

Read the following chemical warnings before proceeding with the instrument protocol.

 **DANGER** **CHEMICAL HAZARD. BloodPrep™ DNA Purification Solution.** Exposure causes eye burns. Solution is harmful if swallowed or absorbed through the skin. Exposure causes skin and respiratory tract irritation. Avoid breathing the vapor. Contact with acids or bleach liberates toxic gases. **DO NOT ADD** acids or bleach to any liquid waste containing BloodPrep DNA Purification Solution. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

 **WARNING** **CHEMICAL HAZARD. BloodPrep™ DNA Wash Solution** is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation, and may cause liver damage and central nervous system depression. Avoid breathing the vapor. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

 **WARNING** **CHEMICAL HAZARD. BloodPrep™ DNA Elution Solution 1.** Exposure causes eye, skin, and respiratory tract irritation. Avoid breathing the vapor. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Table 2-3 6100 instrument settings for purification of DNA from blood:

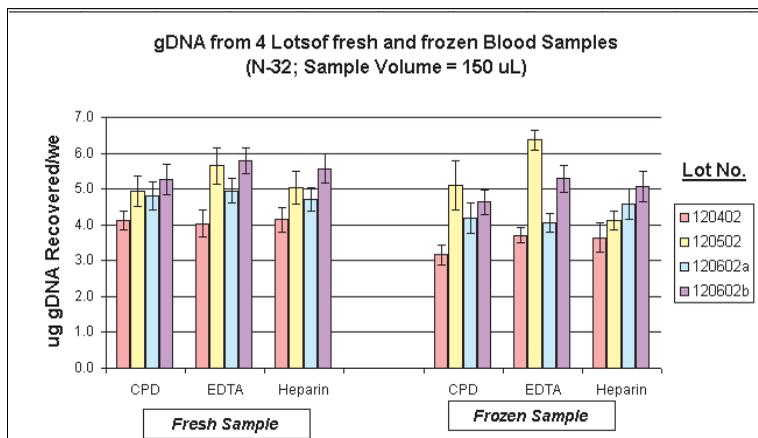
Step	Description	Volume (μL)	Position	Incubation (sec)	Vacuum (%)	Time (sec)
Note: Tape over empty wells of the purification tray with adhesive tape or an adhesive tray cover, or pre-wet all empty wells with 50 μL of BloodPrep DNA Purification Solution to ensure even vacuum. Position the carriage with the purification tray over the 6100 waste station.						
1	Load Samples ^a	650	Waste	0	80	300
2	Add BloodPrep DNA Purification Solution ^a	650	Waste	0	80	400
3	Add BloodPrep DNA Wash Solution	650	Waste	0	80	60
4	Add BloodPrep DNA Wash Solution	600	Waste	0	80	60
5	Add BloodPrep DNA Wash Solution	300	Waste	0	80	60
6	Pre-Elution Vacuum	–	Waste	0	100	120
7	Touch Off	–	Waste	–	–	–
8	Elution Solution 1 ^b	100	Collection	180	60	120
9	Elution Solution 2 ^c	100	Collection	0	60	120
10	Touch Off	–	Collection	–	–	–

a. If the well evacuates slowly, increase the vacuum setting to 100% and repeat the step.

b. Elution volume is between 75 μL and 200 μL in a standard PCR microplate. Elution volumes below 75 μL result in lowered yields of DNA. The total elution volume may be lowered to 75 μL, but the volumes of Elution Solution 1 and 2 must remain equal.

c. It is very important that the elution solutions are used in the correct order. Elution Solution 1 must be incubated with the DNA on the membrane for 3 minutes to ensure maximum yield. Then an equal volume of Elution Solution 2 must follow to give the correct pH for DNA storage.

As shown in Figure 2-8, the BloodPrep chemistry gives adequate yields for purification of gDNA in blood on the 6100 PrepStation



Lot	CPD	% CV of isolated gDNA			CPD	EDTA	Heparin
		Fresh	EDTA	Heparin			
120402	0.26	0.38	0.33	0.28	0.21	0.40	
120502	0.42	0.51	0.47	0.68	0.27	0.26	
120602a	0.40	0.35	0.32	0.43	0.25	0.43	
120602b	0.43	0.36	0.41	0.35	0.37	0.43	

Figure 2-8 Yield and %CV of gDNA isolated from four lots of human whole blood using BloodPrep chemistry and the 6100 PrepStation

6700 Workstation Purification of DNA

Use Table 2-4 below to set up and run the ABI PRISM 6700 Nucleic Acid Workstation for sample purification. To prevent clogging the membrane with sample material, see “Factors that Contribute to Membrane Clogging” and “Guidelines for Preventing Membrane Clogging” on page 2-19.

Note: See the DNA archive protocol settings on the ABI PRISM 6700 Nucleic Acid Workstation in Figure 2-9 on page 2-18.

Read the following chemical warnings before proceeding with the instrument protocol.



DANGER CHEMICAL HAZARD. BloodPrep™ DNA

Purification Solution. Exposure causes eye burns. Solution is harmful if swallowed or absorbed through the skin. Exposure causes skin and respiratory tract irritation. Avoid breathing the vapor. Contact with acids or bleach liberates toxic gases. **DO NOT ADD** acids or bleach to any liquid waste containing BloodPrep DNA Purification Solution. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.



WARNING CHEMICAL HAZARD. BloodPrep™ DNA

Wash Solution is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation, and may cause liver damage and central nervous system depression. Avoid breathing the vapor. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.



WARNING CHEMICAL HAZARD. BloodPrep™ DNA

Elution Solution 1. Exposure causes eye, skin, and respiratory tract irritation. Avoid breathing the vapor. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Table 2-4 6700 instrument settings for purification of DNA from blood:

Step	Description	Volume (μL)	Position	Incubation (sec)	Vacuum (%)	Time (sec)
1	Load Samples ^a	650	Waste	0	80	500
2	Add BloodPrep DNA Purification Solution ^a	650	Waste	0	80	500
3	Add BloodPrep DNA Wash Solution	650	Waste	0	80	60
4	Add BloodPrep DNA Wash Solution	600	Waste	0	80	60
5	Add BloodPrep DNA Wash Solution	300	Waste	0	80	60
6	Pre-Elution Vacuum	–	Waste	0	100	120
7	Touch Off	–	Waste	–	–	–
8	Elution Solution 1 ^b	100	Collection	180	60	120
9	Elution Solution 2 ^c	100	Collection	0	60	120
10	Touch Off	–	Collection	–	–	–

a.If the well evacuates slowly, increase the vacuum setting to 100% and repeat the step.

b.Elution volume is between 75 μL and 200 μL in a standard PCR microplate. Elution volumes below 75 μL result in lowered yields of DNA. The total elution volume may be lowered to 75 μL, but the volumes of Elution Solution 1 and 2 must remain equal.

c.It is very important that the elution solutions are used in the correct order. Elution Solution 1 must be incubated with the DNA on the membrane for 3 minutes to ensure maximum yield. Then an equal volume of Elution Solution 2 must follow to give the correct pH for DNA storage.

New RNA/DNA Archive Protocol

Protocol Name: In Use

Conditions for Transferring Samples to the Purification Tray

Lysis/DNA Precipitation Input: Add Soln. (μL): Transfer (μL): Mix (#): Starting (μL):

First Transfer:

Second Transfer:

High Viscosity Sample

Filtration Conditions Create Deep-Well Filtrate Plate

Incubation Time: (min.) Vacuum Time: (sec.)

Vacuum Pressure: %

Wash Conditions

Step	Add	Volume (μL)	Temp. (°C)	Incubation (min)	Vacuum (sec)	Repeat (count)	Vacuum (%)
1. <input checked="" type="checkbox"/>	DNA Purification Soln	650		0	500	1	80
2. <input checked="" type="checkbox"/>	DNA Wash Solution	650		0	60	1	80
3. <input checked="" type="checkbox"/>	DNA Wash Solution	600		0	60	1	80
4. <input checked="" type="checkbox"/>	DNA Wash Solution	300		0	60	1	80
5. <input type="checkbox"/>		300		0	120	1	20
6. <input type="checkbox"/>		300		0	120	1	20
7. <input type="checkbox"/>		300		0	120	1	20
Pre-Elution Vacuum					120		100
Elution Solution		100	-	3	120	1	60
<input checked="" type="checkbox"/>	Elution Solution 2	100		0	120	1	60

Figure 2-9 ABI Prism™ 6700 Nucleic Acid Workstation settings for the purification of DNA from blood

Preventing Membrane Clogging for Blood Samples

Factors that Contribute to Membrane Clogging

Several factors can contribute to clogging the purification tray membrane and thereby obstructing sample filtration. These include:

- Blood clots (coagulated blood)
- Microclots (small coagulated blood clots)
- Blood frozen with a citrate anticoagulant
- Sample viscosity
- Too many leukocytes (samples from patients with disease)
- Sample input volume too large
- Insufficient digestion time
- Insufficient amounts of Proteinase K

Guidelines for Preventing Membrane Clogging

Follow these guidelines to prevent membrane clogging:

- Use fresh blood or blood that has been frozen for short periods of time.

Blood that has been frozen for long periods of time (>1 year) can contain microclots of material, which form viscous, stringy red blobs of coagulated blood.

- Thoroughly mix blood with anticoagulants before freezing.
- For 6700 Workstation samples, lower the input volume of frozen whole blood to 125 μL or 100 μL to help decrease clogging frequency.

Irreversible clogging of purification tray wells can occur at input levels above 150 μL . Clogging occurs most frequently during sample evacuation and the BloodPrep DNA Purification Solution wash steps described in steps 1–7 in “6100 PrepStation Purification of DNA” on page 2-13 and “6700 Workstation Purification of DNA” on page 2-16.

- Increase the amount of Proteinase K Solution from 15 μL to 20 μL or 30 μL (see step 1 in “Sample Extraction” on page 2-7).
- Ensure the incubation temperature and time are set to 58 $^{\circ}\text{C}$ for 10 min.

- Thoroughly mix the BloodPrep PK Digestion Buffer, Proteinase K Solution, and blood sample before incubation. After digestion, thoroughly mix the digested blood with BloodPrep DNA Purification Solution as described in step 4–7 in “Sample Extraction” on page 2-7.
- If microclots (seen as small red colored patches on the membrane surface) can be seen after addition of DNA wash solution, repeat steps 3–5 adding 100–300 μL of BloodPrep DNA Purification Solution.

Isolating and Purifying DNA from Tissue Culture Cells and Buccal Swabs

3

Introduction

In This Chapter This chapter covers:

Isolating DNA from Tissue Culture Cells3-2

Isolating DNA from Buccal Swabs3-6

Purifying DNA from Tissue Culture Cells and
Buccal Swabs on ABI PRISM Sample Prep Stations3-9

This chapter describes how to isolate and purify intact high molecular weight DNA from tissue culture cells and buccal swabs.

Isolating DNA from Tissue Culture Cells

Adherent and Suspension Cells

The following procedure is suitable for isolating DNA from tissue culture cells, plasma, serum, and cerebrospinal fluid. Both adherent and suspension cells are isolated using this procedure.

Input Range

The input number of cells should be from 1×10^6 to 1×10^7 cells per well.

DNA Yields and Purity

DNA yields from tissue culture cell lines vary depending on the ploidy (number of chromosomes per cell) of the cell line. Raji cells (diploid) yield approximately 5–6 μg DNA per 1×10^6 cells. PC3 cells (near triploid) yield approximately 8 μg per 1×10^6 cells. HeLa cells (100% aneuploid, near tetraploid) yield over 15 μg of DNA per 1×10^6 cells.

Because of the large amount of DNA per cell in a tetraploid cell line such as HeLa, flow rates of the lysed samples can slow dramatically as the purification membrane comes close to saturation. Lower input levels (e.g. 1×10^5 cells/well) are recommended for such cell lines. The samples have good purity, giving $A_{260/280}$ ratios of 1.8.

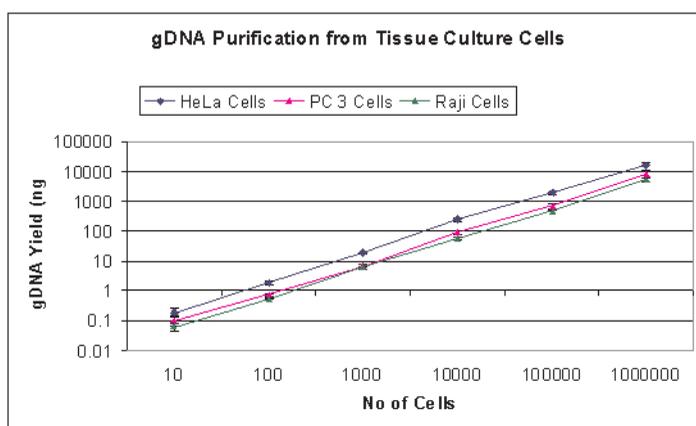


Figure 3-1 Yields of gDNA from tissue culture cells at input levels of 10 to 1×10^6 cells. Each point is in quadruplicate. Results show that the purification process recovers gDNA linearly in these input ranges.

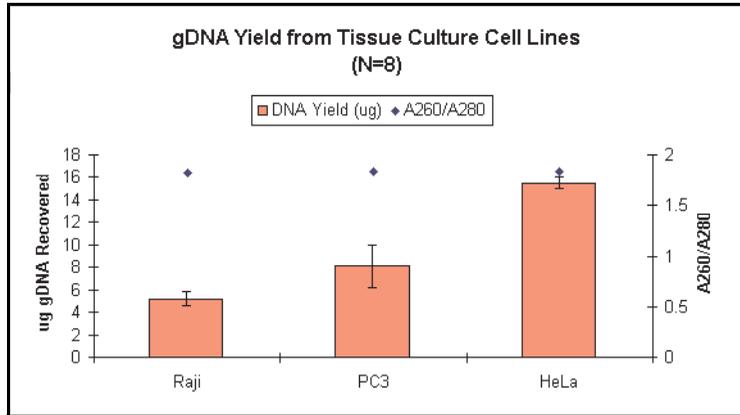


Figure 3-2 Yields and purity of DNA from tissue culture cell lines

Sample Extraction

Follow the steps below to extract DNA from tissue culture cells for purification on the ABI PRISM™ 6100 Nucleic Acid PrepStation or the ABI PRISM™ 6700 Automated Nucleic Acid Workstation.

Read the following chemical warnings before proceeding with the sample extraction protocol.



WARNING

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.



DANGER

CHEMICAL HAZARD. BloodPrep™ DNA Purification Solution. Exposure causes eye burns. Solution is harmful if swallowed or absorbed through the skin. Exposure causes skin and respiratory tract irritation. Avoid breathing the vapor. Contact with acids or bleach liberates toxic gases. **DO NOT ADD** acids or bleach to any liquid waste containing BloodPrep DNA Purification Solution. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To extract and isolate DNA from tissue culture cells:

- | | |
|----|---|
| 1. | <p>Remove the cells from the tissue culture media.</p> <ul style="list-style-type: none">• For adherent cells, remove the tissue culture media from all wells by aspiration.• For suspension cells, pellet the media by centrifugation and remove the supernatant by aspiration. |
|----|---|

Note: For plasma, serum, and cerebrospinal fluid samples, pellet the cells by centrifugation following your standard laboratory procedures, and remove the supernatant by aspiration.

To extract and isolate DNA from tissue culture cells: (continued)

2.	<p>Add 200–650 μL of BloodPrep™ DNA Purification Solution into each well of a 96-well tissue culture plate or a 96-well deep well plate.</p> <p>Note: Do not exceed 650 μL in volume unless the sample is extremely viscous. If the sample is extremely viscous, reduce the sample input volume or increase the volume of BloodPrep DNA Purification Solution appropriately.</p> <p>IMPORTANT! The BloodPrep DNA Purification Solution may require gentle heating to 37 °C for 5–10 min to dissolve precipitated salts.</p>
3.	<p>Mix the cells and BloodPrep DNA Purification Solution thoroughly by vortexing for 20 sec to 1.0 min, or by pumping the mixture with a pipette at least 5 times.</p>
4.	<p>Set up the 6100 PrepStation or the 6700 Workstation for a purification run. See “6100 PrepStation Purification of DNA” on page 3-11 or “6700 Workstation Purification of DNA” on page 3-12 for the appropriate settings.</p> <p>IMPORTANT! The purification tray should be located at the waste position.</p> <p>Note: If you cannot proceed with purification immediately, store the lysed samples at 4 °C. Before proceeding to step 5, these samples must be gently heated for 5–10 min to room temperature and vortexed.</p>
5.	<p>Go to “Purifying DNA from Tissue Culture Cells and Buccal Swabs on ABI PRISM Sample Prep Stations” on page 3-9.</p>

Isolating DNA from Buccal Swabs

Buccal Swab DNA The following procedure is suitable for the isolation of DNA from buccal swabs.

DNA Yields and Purity Yields of DNA from swabs are highly variable depending on the swab type, the person being swabbed, the swabbing technique, and the number of cells captured on the swab. The expected yield using this protocol is 200 ng to 5 μ g per swab. As shown in Figure 3-3, the BloodPrep™ chemistry gives adequate yields of buccal swab gDNA.

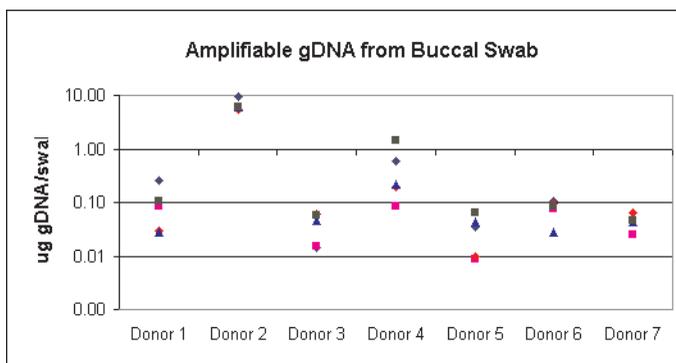


Figure 3-3 gDNA isolation from buccal swabs. Cotton swabs were used on seven different donors. Yields in this study were measured by 18S gDNA TaqMan PCR assay on the ABI PRISM® 7700 Sequence Detection System.

Sample Extraction

Follow the steps below to extract and isolate DNA from buccal swabs for purification on the ABI PRISM™ 6100 Nucleic Acid PrepStation or the ABI PRISM™ 6700 Automated Nucleic Acid Workstation.

Read the following chemical warnings before proceeding with the sample extraction protocol.



WARNING 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.



DANGER CHEMICAL HAZARD. BloodPrep™ DNA Purification Solution. Exposure causes eye burns. Solution is harmful if swallowed or absorbed through the skin. Exposure causes skin and respiratory tract irritation. Avoid breathing the vapor. Contact with acids or bleach liberates toxic gases. **DO NOT ADD** acids or bleach to any liquid waste containing BloodPrep DNA Purification Solution. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

To extract and isolate DNA from buccal swabs:

1.	Perform the buccal scrape by following your standard laboratory procedure.
2.	Swirl the swab in a 2-mL microcentrifuge tube containing 800 μ L of BloodPrep DNA Purification Solution for 30 sec to 1 min. IMPORTANT! The BloodPrep DNA Purification Solution may require gentle heating to 37 °C for 5–10 min to dissolve precipitated salts.
3.	Remove the swab, retaining as much liquid as possible in the microcentrifuge tube.

To extract and isolate DNA from buccal swabs: (continued)

4.	<p>Setup the 6100 PrepStation or the 6700 Workstation for a purification run. See “6100 PrepStation Purification of DNA” on page 3-11 or “6700 Workstation Purification of DNA” on page 3-12 for the appropriate settings.</p> <p>Note: If you cannot proceed with purification immediately, store the lysed samples at 4 °C. Before proceeding to step 5, these samples must be gently heated for 5–10 min to room temperature and vortexed.</p>
5.	<p>Go to “Purifying DNA from Tissue Culture Cells and Buccal Swabs on ABI PRISM Sample Prep Stations” on page 3-9.</p>

Purifying DNA from Tissue Culture Cells and Buccal Swabs on ABI PRISM Sample Prep Stations

Purification This section describes the DNA purification procedures to use after the DNA is extracted from your sample (see “Sample Extraction” on page 3-4 and “Sample Extraction” on page 3-7). To purify, load the samples on an ABI PRISM Sample PrepStation. Use the settings and procedures listed in the “6100 PrepStation Purification of DNA” table on page 3-11 or the “6700 Workstation Purification of DNA” table on page 3-12.

Read the following chemical warnings before proceeding with the instrument protocols.



WARNING 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.



DANGER CHEMICAL HAZARD. BloodPrep™ DNA Purification Solution. Exposure causes eye burns. Solution is harmful if swallowed or absorbed through the skin. Exposure causes skin and respiratory tract irritation. Avoid breathing the vapor. Contact with acids or bleach liberates toxic gases. **DO NOT ADD** acids or bleach to any liquid waste containing BloodPrep DNA Purification Solution. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.



WARNING CHEMICAL HAZARD. BloodPrep™ DNA Wash Solution is a flammable liquid and vapor. Exposure causes eye, skin, and respiratory tract irritation, and may cause liver damage and central nervous system depression. Avoid breathing the vapor. Keep away from heat, sparks, and flame. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.



WARNING CHEMICAL HAZARD. BloodPrep™ DNA Elution Solution 1. Exposure causes eye, skin, and respiratory tract irritation. Avoid breathing the vapor. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

6100 PrepStation Purification of DNA Use Table 3-1 below to set up and run the ABI PRISM™ 6100 Nucleic Acid PrepStation to purify the DNA samples you prepared.

Table 3-1 6100 settings for purification of DNA from tissue culture cells and buccal swabs:

Step	Description	Volume (μL)	Position	Incubation (sec)	Vacuum (%)	Time (sec)
Note: Tape over empty wells of the purification tray with adhesive tape or an adhesive tray cover, or pre-wet all empty wells with 50 μL of BloodPrep DNA Purification Solution to ensure even vacuum. Position the carriage with the purification tray over the waste station..						
1	Load Samples ^a	650	Waste	0	80	300
2	Add BloodPrep DNA Purification Solution	650	Waste	0	80	300
3	Add BloodPrep DNA Wash Solution	650	Waste	0	80	60
4	Add BloodPrep DNA Wash Solution	600	Waste	0	80	60
5	Add BloodPrep DNA Wash Solution	300	Waste	0	80	60
9	Pre-Elution Vacuum	–	Waste	0	100	120
10	Touch Off	–	Waste	–	–	–
11	Elution Solution 1 ^b	100	Collection	180	60	120
12	Elution Solution 2 ^c	100	Collection	0	60	120
13	Touch Off	–	Collection	–	–	–

a. For lysates with volumes in excess of 650 μL, use the Quick Run feature to pull 650 μL aliquots of lysate across the purification tray membrane, operating vacuum at 80% for 180 secs. Repeat until one aliquot remains to be added and then proceed to step 1 of the purification protocol. If the well starts to evacuate slowly after a number of additions, increase the vacuum setting to 100% and repeat the step.

b. Elution volume is between 75 μL and 200 μL in a standard PCR microplate. Elution volumes below 75 μL result in lowered yields of DNA. The total elution volume may be lowered to 75 μL, but the volumes of Elution Solution 1 and 2 must remain equal.

c. It is very important that the elution solutions are used in the correct order. Elution Solution 1 must be incubated with the DNA on the membrane for 3 minutes to ensure maximum yield. Then an equal volume of Elution Solution 2 must follow to give the correct pH for DNA storage.

6700 Workstation Purification of DNA

Use Table 3-2 below to set up and run the ABI PRISM 6700 Nucleic Acid Workstation to purify the DNA samples you prepared.

Note: See the settings on the ABI PRISM 6700 Nucleic Acid Workstation in Figure 3-4 on page 3-13.

Table 3-2 6700 settings for purification of DNA from tissue culture cells and buccal swabs:

Step	Description	Volume (μL)	Position	Incubation (sec)	Vacuum (%)	Time (sec)
1	Load Samples ^a	650	Waste	0	80	300
2	Add BloodPrep DNA Purification Solution ^b	650	Waste	0	80	300
3	Add BloodPrep DNA Wash Solution	650	Waste	0	80	60
4	Add BloodPrep DNA Wash Solution	600	Waste	0	80	60
5	Add BloodPrep DNA Wash Solution	300	Waste	0	80	60
9	Pre-Elution Vacuum	–	Waste	0	100	120
10	Touch Off	–	Waste	–	–	–
11	Elution Solution 1 ^c	100	Collection	3	60	120
12	Elution Solution 2 ^d	100	Collection	0	60	120
13	Touch Off	–	Collection	–	–	–

a. If the well evacuates slowly, increase the vacuum setting to 100% and repeat the step.

b. If the well evacuates slowly, increase the vacuum setting to 100% and repeat the step.

c. Elution volume is between 75 μL and 200 μL in a standard PCR microplate. Elution volumes below 75 μL result in lowered yields of DNA. The total elution volume may be lowered to 75 μL, but the volumes of Elution Solution 1 and 2 must remain equal.

d. It is very important that the elution solutions are used in the correct order. Elution Solution 1 must be incubated with the gDNA on the membrane for 3 minutes to ensure maximum yield. Then an equal volume of Elution Solution 2 must follow to give the correct pH for DNA storage.

New RNA/DNA Archive Protocol

Protocol Name: In Use

Conditions for Transferring Samples to the Purification Tray

Lysis/DNA Precipitation Input: Add Soln. (μL): Transfer (μL): Mix (#): Starting (μL):

First Transfer:

Second Transfer:

High Viscosity Sample

Filtration Conditions Create Deep-Well Filtrate Plate

Incubation Time: (min.) Vacuum Time: (sec.)

Vacuum Pressure: %

Wash Conditions

Step	Add	Volume (μL)	Temp. (°C)	Incubation (min)	Vacuum (sec)	Repeat (count)	Vacuum (%)
1. <input checked="" type="checkbox"/>	DNA Purification Soln	650		0	300	1	80
2. <input checked="" type="checkbox"/>	Wash Solution	650		0	60	1	80
3. <input checked="" type="checkbox"/>	Wash Solution	600		0	60	2	80
4. <input checked="" type="checkbox"/>	Wash Solution	300		0	60	1	80
5. <input type="checkbox"/>		300		0	120	1	20
6. <input type="checkbox"/>		300		0	120	1	20
7. <input type="checkbox"/>		300		0	120	1	20
Pre-Elution Vacuum					120		100
Elution Solution		100	-	3	120	1	60
<input checked="" type="checkbox"/>	Elution Solution 2	100		0	120	1	60

Figure 3-4 ABI PRISM™ 6700 Nucleic Acid Workstation settings for the purification of DNA from tissue culture cells and buccal swabs

Index

Numerics

- 6100 PrepStation, purification of DNA 2-13, 3-11
- 6700 Workstation, purification of DNA 2-16, 3-12

A

- accessories 1-4
- aneuploid 3-2
- anticoagulants 2-2

B

- blood
 - anticoagulants 2-2
 - evacuation time on sample prep stations 2-12
 - input volume 2-3
 - isolating and purifying DNA 2-1
 - purification on sample prep stations 2-12
 - purity 2-5
 - sample yields and purity 2-4
 - viscosity 2-12
 - yields and purity 2-4
- buccal swabs
 - 6100 prep station 3-11
 - 6700 prep station 3-12
 - isolation of DNA 3-6
 - purification on sample prep stations 3-9
 - yields and purity 3-6

C

- cell lines
 - HeLa 3-2
 - ploidy 3-2
 - tetraploid 3-2
- chemical warnings

- bloodborne/infectious waste hazard 1-9, 1-10
- EDTA 1-11
- ethanol 1-11
- Proteinase K 1-11, 2-7
- sodium hydroxide 1-11
- chemicals required 1-5

D

- diploid 3-2
- DNA
 - about the protocol 1-1
 - absence of heme 2-6
 - absence of particulates 2-7
 - absorbance ratios for blood purity 2-5
 - blood on sample prep stations 2-12
 - buccal swabs on sample prep stations 3-9
 - evacuation time for blood 2-12
 - gel images from blood 2-2
 - inhibitors 2-6
 - isolation flow chart 1-3
 - isolation from blood 2-1
 - isolation from buccal swabs 3-6
 - isolation from tissue cells 3-2
 - protocol for blood 2-2
 - purification 2-12
 - tetraploid cell lines 3-2
 - tissue culture cells on sample prep stations 3-9
 - yields and purity for blood 2-4
 - yields and purity for buccal swabs 3-6

E

- equipment 1-4
- evacuation time for blood samples 2-12

F

flow chart of DNA isolation 1-3
flow rates of lyzed samples 3-2

H

HeLa cells 3-2
heme, absence 2-6

I

input range, tissue culture cells 3-2
input volume, blood 2-1, 2-3
instrument systems 1-2

L

lysate digestion 1-1

M

membrane
 clogging factors 2-19
 saturation 3-2

P

ploidy 3-2
purification membrane
 clogging factors 2-19
 clogging prevention 2-19
purification of DNA
 blood 2-12
 tissue culture cells and buccal swabs 3-9
purity, blood 2-4
purity, tissue culture cells and buccal
 swabs 3-2

R

Raji cells 3-2

S

safety alerts
 biohazard 2-7, 2-12, 3-4, 3-7, 3-9

sample lysis, blood 2-7
sample lysis, buccal swabs 3-7
sample lysis, tissue culture cells 3-4
sample viscosity 2-12

T

tetraploid 3-2
tissue culture cells
 6100 prep station 3-11
 6700 workstation 3-12
 DNA yield and purity 3-2
 input range 3-2
 isolation of DNA 3-2
 purification 3-9
 purification on sample prep stations 3-9
triploid 3-2
troubleshooting animal blood samples 2-10

V

viscosity of blood 2-12
volumes required for blood samples 2-3

Y

yields and purity
 blood 2-4
 buccal swabs 3-6
 tissue culture cells 3-2



iScience. To better understand the complex interaction of biological systems, life scientists are developing revolutionary approaches to discovery that unite technology, informatics, and traditional laboratory research. In partnership with our customers, Applied Biosystems provides the innovative products, services, and knowledge resources that make this new, **Integrated Science** possible.

Worldwide Sales and Support

Applied Biosystems vast distribution and service network, composed of highly trained support and applications personnel, reaches 150 countries on six continents. For sales office locations and technical support, please call our local office or refer to our Web site at www.appliedbiosystems.com.

Applera is committed to providing the world's leading technology and information for life scientists. Applera Corporation consists of the Applied Biosystems and Celera Genomics businesses.

Headquarters

850 Lincoln Centre Drive
Foster City, CA 94404 USA
Phone: +1 650.638.5800
Toll Free (In North America): +1 800.345.5224
Fax: +1 650.638.5884

08/2004

