NucPrep® Chemistry: Isolation of Genomic DNA from Animal and Plant Tissue

Protocol
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Section 1  Introduction

Overview

About This Protocol
This protocol describes isolation of DNA from animal and plant tissue using Applied Biosystems NucPrep® chemistry and nucleic acid purification platforms.

Isolating DNA from Animal Tissue
This protocol is designed to isolate DNA directly from whole animal tissue, including rodent tails, without maceration. There are four processes involved:

1. Obtaining an appropriate tissue sample from the animal

Note: This document discusses techniques for isolating DNA from animal tissues. Techniques for surgically removing animal tissue are beyond the scope of this document and will not be discussed here.

2. Performing a digestion reaction on the whole tissue sample
3. Pre-filtering the digested tissue lysate
4. Purifying DNA from the filtered, digested tissue lysate

Isolating DNA from Plant Tissue
This protocol is also designed to isolate DNA directly from plant tissue. There are four processes involved:

1. Obtaining the plant tissue sample
2. Homogenizing the plant tissue
3. Pre-filtering the homogenized plant tissue
4. Purifying DNA from the homogenized, filtered tissue lysate
By using Applied Biosystems NucPrep chemistry, Tissue Pre-Filter Tray II, DNA Purification Tray II, and instrument systems, you may significantly streamline these processes. You can obtain 96 samples of highly pure gDNA in approximately 2.5 to 3 hours. The time required for each process is as follows:

<table>
<thead>
<tr>
<th>Process</th>
<th>Time Required</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Animal Tissue</td>
</tr>
<tr>
<td>Digestion</td>
<td>1 hour</td>
</tr>
<tr>
<td>Homogenization</td>
<td>N/A</td>
</tr>
<tr>
<td>Pre-filtration</td>
<td>~0.5 hour</td>
</tr>
<tr>
<td>Purification</td>
<td>~1 to 1.5 hours</td>
</tr>
</tbody>
</table>

Applied Biosystems NucPrep chemistry uses a unique formulation of digestion buffer in combination with proteinase K to effectively and rapidly digest the tissue and release the nucleic acid.

The digested material is pre-filtered to remove hair and bone fragments or plant particulates using Tissue Pre-Filter Tray II. The digested, pre-filtered material is then transferred to DNA Purification Tray II, where it is passed across the tray under controlled vacuum conditions.

Pre-filtration using Tissue Pre-Filter Tray II is designed to prevent clogging of DNA Purification Tray II. Tissue Pre-Filter Tray II:
- Removes bone and hair fragments
- Reduces protein burden on the DNA Purification Tray II membrane
- Removes particulates left after the plant tissue is homogenized

**IMPORTANT!** All tissue samples must be pre-filtered before being transferred to the purification tray.

Care should be taken not to greatly exceed the recommended pre-filtration times for each tissue type (see page 36). This may cause excessive foaming in the deep-well plate and increase the risk of cross-contamination. Stop the vacuum after the liquid has been removed from all wells.

Due to the viscosity of the digested tissue lysate, you may need wide-bore pipette tips when transferring the lysate to Tissue Pre-Filter Tray II.
**Instrument Systems**

The pre-filtration and purification techniques discussed in this document can be performed on the following Applied Biosystems instrument systems:

- **ABI PRISM™ 6700 Automated Nucleic Acid Workstation (6700 Workstation)**
  
The 6700 Workstation is an automated nucleic acid purification platform. Once you place the lysate into the 6700 Workstation, the 6700 Workstation performs all other operations automatically.

- **ABI PRISM™ 6100 Nucleic Acid PrepStation (6100 PrepStation)**
  
The 6100 PrepStation is a semi-automated nucleic acid purification platform. The firmware and an integrated vacuum system perform all vacuum operations automatically. You must add all samples and wash reagents.

**Summary Diagram**

The procedures required to isolate gDNA from whole animal tissue are summarized in the diagram below.

1. **Remove tissue from the organism.**
   - **Optional.** Store the tissue sample:
     - Store at 4 °C for short-term storage (<12 h)
     - Freeze at -20 to -80 °C for long-term storage

2. Perform a digestion reaction on the whole animal tissue.

3. Perform pre-filtration using the:
   - 6100 PrepStation OR
   - 6700 Workstation

4. Perform purification using the:
   - 6100 PrepStation OR
   - 6700 Workstation
Yields and Purity of gDNA Derived from NucPrep Chemistry

Overview
The graphs shown below should be regarded only as guidelines for the yield of gDNA that can be obtained using this protocol. Actual amounts of tissue passed across DNA Purification Tray II and the yields obtained from these tissues can vary. You should ensure that a test is performed before committing to a full run of 96 samples.

Yields of gDNA
When using Applied Biosystems NucPrep chemistry, different tissue types will give significantly different yields of gDNA. Figure 1 (below) through Figure 3 (on page 5) show several tissue samples.

- For rodent tails, you may expect between 10 and 15 µg of gDNA per tail at A$_{260/280}$ ratios of 1.7 or greater. This indicates that the material is highly pure and free of protein contamination. The gDNA obtained will likely show no PCR inhibition.
- Yields of DNA from plant tissue vary considerably from species to species. Some representative yields are shown in Figure 3 on page 5.

Figure 1  Yield and purity of gDNA obtained from rodent tissues (10 mg each); N = 4
Figure 2  Yield of gDNA obtained from rodent tissues (10, 5, and 2.5 mg of tissue per well); N = 4

Figure 3  Yield of gDNA obtained from plant leaves (20 and 40 mg of plant tissue per well); N = 4
Purity of gDNA: RNA and Protein Contamination

By using Applied Biosystems NucPrep chemistry and this protocol, you can obtain gDNA that is highly pure and contains only low amounts of RNA and protein contamination. For animal tissues, no RNA bands should be visible on an agarose gel (see Figure 4 and Figure 5 below). Plant tissues may show very small quantities of RNA contamination (see Figure 6 on page 7).

![Image of agarose gel with DNA samples]

**Figure 4** gDNA from rat tissues (duplicate pairs of each organ sample)

Lung  Brain  Liver  Pancreas  Kidney  Sm Int  Lrg Int  Muscle  Heart

Rat Tail  Mouse Tail

**Figure 5** gDNA from rodent tails
Purity of DNA: DNase Activity

DNA isolated using Applied Biosystems NucPrep chemistry is free from residual DNase activity. In the experiment shown in Figure 7 (below), rat liver DNA was incubated at 37 °C for 30 minutes in the presence of magnesium and calcium (key metal ions for DNase activity). No noticeable degradation was observed at the 30-minute timepoint. DNA incubated with DNase was highly degraded at this timepoint.
## Section 2 Materials, Safety, and Technical Support

### Materials Required but Not Supplied

#### Overview
The following tables list the equipment, accessories, and chemicals required to perform this protocol. Unless otherwise noted, many items listed can be obtained from a major laboratory supplier (MLS).

#### Equipment

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABI Prism™ 6700 Automated Nucleic Acid Workstation OR</td>
<td>See your Applied Biosystems sales representative</td>
</tr>
<tr>
<td>ABI Prism™ 6100 Nucleic Acid PrepStation</td>
<td></td>
</tr>
<tr>
<td>Microcentrifuge</td>
<td>MLS</td>
</tr>
<tr>
<td>Pipettors</td>
<td>MLS</td>
</tr>
<tr>
<td>Vortexer</td>
<td>MLS</td>
</tr>
<tr>
<td>Heater/shaker</td>
<td>MLS</td>
</tr>
</tbody>
</table>

#### Accessories

<table>
<thead>
<tr>
<th>Accessories</th>
<th>Supplier</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>15- and 50-mL sterile tubes (e.g., BD Falcon)</td>
<td>MLS</td>
<td>—</td>
</tr>
<tr>
<td>Wide-bore pipette tips</td>
<td>MLS</td>
<td>—</td>
</tr>
<tr>
<td>Deep-well plate</td>
<td>Applied Biosystems</td>
<td>4308641</td>
</tr>
<tr>
<td>96-Well Optical Reaction Plate with Barcode (also called “archive plate” and “PCR plate”)</td>
<td>Applied Biosystems</td>
<td>4306737</td>
</tr>
<tr>
<td>Splash Guard</td>
<td>Applied Biosystems</td>
<td>4311758</td>
</tr>
<tr>
<td>Tissue Pre-Filter Tray II</td>
<td>Applied Biosystems</td>
<td>4330688</td>
</tr>
<tr>
<td>DNA Purification Tray II</td>
<td>Applied Biosystems</td>
<td>4330172</td>
</tr>
<tr>
<td><strong>Additional Materials for Purification on 6700 Workstation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Archive Covers</td>
<td>Applied Biosystems</td>
<td>4306286</td>
</tr>
<tr>
<td>Conductive Pipette Tips, 200-µL</td>
<td>Applied Biosystems</td>
<td>4306375</td>
</tr>
</tbody>
</table>
## Chemicals

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Supplier</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>NucPrep® Proteinase K Solution</td>
<td>Applied Biosystems</td>
<td>4333793</td>
</tr>
<tr>
<td>NucPrep® Digestion Buffer</td>
<td>Applied Biosystems</td>
<td>4333800</td>
</tr>
<tr>
<td>NucPrep® DNA Purification Solution</td>
<td>Applied Biosystems</td>
<td>4333802</td>
</tr>
<tr>
<td>NucPrep® DNA Wash Solution</td>
<td>Applied Biosystems</td>
<td>4333796</td>
</tr>
<tr>
<td>NucPrep® DNA Elution Solution 1</td>
<td>Applied Biosystems</td>
<td>4333794</td>
</tr>
<tr>
<td>NucPrep® DNA Elution Solution 2</td>
<td>Applied Biosystems</td>
<td>4333798</td>
</tr>
</tbody>
</table>

## Documentation

<table>
<thead>
<tr>
<th>Document</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABI PRISM™ 6100 Nucleic Acid PrepStation User’s Manual</td>
<td>4326242</td>
</tr>
<tr>
<td>ABI PRISM™ 6700 Automated Nucleic Acid Workstation User’s Manual</td>
<td>4304309</td>
</tr>
<tr>
<td>Tissue RNA Isolation: Isolation of Total RNA from Plant and Animal Tissue Protocol</td>
<td>4330252</td>
</tr>
</tbody>
</table>
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 described below.

Note: Calls attention to useful information.

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 which, 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 which, if not avoided, could result in death or serious injury.

⚠️ DANGER Indicates an imminently hazardous situation which, 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.

- Read and understand the material safety data sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (e.g., 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 (e.g., 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.
CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

- 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.
- Handle chemical wastes in a fume hood.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (e.g., 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 (e.g., fume hood). For additional safety guidelines, consult the MSDS.
- 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.

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, chemical safety, and waste profiles.

About MSDSs
Some of the chemicals used with this instrument may be listed as hazardous by their manufacturer. When hazards exist, warnings are prominently displayed on the labels of all chemicals.

Chemical manufacturers supply a current material safety data sheet (MSDS) before or with shipments of hazardous chemicals to new customers and with the first shipment of a hazardous chemical after an MSDS update. MSDSs provide you with the safety information you need to store, handle, transport and dispose of the chemicals safely.

We strongly recommend that you replace the appropriate MSDS in your files each time you receive a new MSDS packaged with a hazardous chemical.

CHEMICAL HAZARD. Be sure to familiarize yourself with the MSDSs before using reagents or solvents.
### Ordering MSDSs

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

1. From the U.S. or Canada, dial **1.800.487.6809**.

2. Follow the voice instructions to order documents (for delivery by fax).

   **Note:** There is a limit of five documents per fax request.

<table>
<thead>
<tr>
<th>In the U.S.</th>
<th>1.800.345.5224, and press 1.</th>
</tr>
</thead>
<tbody>
<tr>
<td>In Canada</td>
<td>1.800.668.6913, and press 1 for English or 2 for French.</td>
</tr>
</tbody>
</table>

3. Go to [http://www.appliedbiosystems.com](http://www.appliedbiosystems.com)

4. Click **SERVICES & SUPPORT** at the top of the page, click **Documents on Demand**, then click **MSDS**.

5. Click **MSDS Index**, search through the list for the chemical of interest to you, then click on the MSDS document number for that chemical to open a PDF version of the MSDS.

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

For services and support, access the Applied Biosystems Web site:
http://www.appliedbiosystems.com

At the Applied Biosystems Web site, 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 Applied Biosystems Web site provides a list of telephone and fax numbers that can be used to contact Technical Support.
**Section 3   Tissue Storage and Preparation**

**Safety Warnings**

**Biohazard Warning**

The procedures in this section discuss handling of tissue samples. Before performing these procedures, please read and follow the biohazard warning below.

**WARNING BIOHAZARD.** Biological samples such as tissues and blood have the potential to transmit infectious diseases. Follow the U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4) and in *Occupational Safety and Health Standards, Toxic and Hazardous Substances* (29 CFR §1910.1030) concerning the principles of risk assessment, biological containment, and safe laboratory practices for activities involving clinical specimens. You can obtain additional information by connecting to the government Web site [http://www.cdc.gov](http://www.cdc.gov).

**Freezing and Storing the Tissue Sample**

**Overview**

As soon as animal tissue is isolated from the whole live organism, the quality of nucleic acid in the tissue sample may begin to degrade if it is not stored under the appropriate conditions.
DNA is less susceptible to degradation than RNA, but precautions should be taken to ensure that the sample is stored correctly. For prolonged periods, tissue samples should be stored frozen at -20 to -80 °C.

The tissue sample can be stored as follows:

<table>
<thead>
<tr>
<th>If you will be storing the lysate...</th>
<th>Then store at...</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 12 h</td>
<td>4 °C</td>
</tr>
<tr>
<td>&gt;12 h</td>
<td>-20 to -80 °C.</td>
</tr>
</tbody>
</table>

Note: We recommend -80 °C for extended storage periods or archival purposes (i.e., >1 month).

Homogenizing Plant Tissue

See Tissue RNA Isolation Protocol

For procedures on homogenizing plant tissue, please refer to Tissue RNA Isolation: Isolation of Total RNA from Plant and Animal Tissue Protocol (P/N 4330252).

Performing the Digestion Reaction on Animal Tissue

Overview

A digestion reaction is performed on the animal tissue to release nucleic acids into solution. Applied Biosystems NucPrep Digestion Buffer contains a unique formulation to allow digestion of whole tails or pieces of tissue from any animal in approximately 1 hour.

To perform the digestion reaction, you:

- Add a digestion buffer and enzyme to the whole tissue.
- Use a heater/shaker device to release the nucleic acid into the digestion buffer.
- After the digestion reaction is complete, add NucPrep® DNA Purification Solution.
Performing the Digestion Reaction

To perform the digestion reaction:

1. Add 5 to 50 mg of solid animal tissue (0.5 to 1.5 cm of rodent tail) to a microcentrifuge tube or deep-well plate.

   **Note:** While more than 50 mg of tissue can be added to the digestion reaction, you should not exceed the recommended input quantities for either Tissue Pre-Filter Tray II or DNA Purification Tray II. If you exceed the recommended amounts, the trays are likely to clog.

   If you need to digest a whole, large piece of tissue to obtain a representative DNA selection:
   a. Increase the volume of the digestion reaction appropriately.
   b. Take a smaller aliquot of the digested tissue to add to DNA Purification Tray II. The mass of tissue in the aliquot should not exceed the recommended input quantities.

2. To the solid animal tissue, add:
   - 150 µL of NucPrep Digestion Buffer
     **WARNING** CHEMICAL HAZARD. NucPrep Digestion Buffer causes eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.
   - 50 µL of NucPrep Proteinase K Solution
     **WARNING** CHEMICAL HAZARD. NucPrep Proteinase K Solution causes eye, skin, and respiratory tract irritation, and may cause allergic respiratory and skin reactions. Contact with acids or bleach liberates toxic gases. DO NOT ADD acids or bleach to any liquid waste containing NucPrep Proteinase K Solution. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

3. Using a heater/shaker device (e.g., an Eppendorf ThermoShaker), incubate the sample with shaking as follows:

<table>
<thead>
<tr>
<th>Speed</th>
<th>Temperature</th>
<th>Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>~500 rpm</td>
<td>65 °C</td>
<td>30 to 60 min</td>
</tr>
</tbody>
</table>

   **IMPORTANT!** Shaking is necessary to dissolve the tissue. If you do not have a heater/shaker device, vortex or mix the sample every 5 min, until the tissue pieces dissolve.
To perform the digestion reaction: (continued)

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>4.</strong></td>
<td>At the end of the incubation period, add 600 µL per well of DNA Purification Solution.</td>
</tr>
</tbody>
</table>
| **5.** | Mix the DNA Purification Solution and tissue lysate as follows:  
  - Vortex or mix for 30 sec to 1 min.  
  OR  
  - Pipette at least three times with wide-bore pipette tips until the reagents are thoroughly mixed.  
  **IMPORTANT!** The digested tissue may be extremely viscous at this point. It is important that the DNA Purification Solution and the digested tissue lysate are thoroughly mixed before proceeding to the pre-filtration process. Wide-bore pipette tips may be necessary for this step. |
Section 4  Pre-Filtration and Purification on the ABI PRISM™ 6100 Nucleic Acid PrepStation

Safety Warnings

Biohazard Warning

The procedures in this section discuss handling of tissue samples. Before performing these procedures, please read and follow the biohazard warning below.

⚠️ WARNING BIOHAZARD. Biological samples such as tissues and blood have the potential to transmit infectious diseases. Follow the U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories (stock no. 017-040-00547-4) and in Occupational Safety and Health Standards, Toxic and Hazardous Substances (29 CFR §1910.1030) concerning the principles of risk assessment, biological containment, and safe laboratory practices for activities involving clinical specimens. You can obtain additional information by connecting to the government Web site http://www.cdc.gov.
Performing Pre-Filtration

Overview

The digested tissue lysate/NucPrep® DNA Purification Solution is passed across Tissue Pre-Filter Tray II to remove undigested tissue debris (such as hair and bone) or unhomogenized plant particulates. The filtrate is recovered in a deep-well plate.

Pre-Filtration Times for a Variety of Rodent Tissues

The viscosity of digested rodent tissue lysates or homogenized plant tissue lysates differs significantly from tissue type to tissue type. This difference affects the amount of time required for the pre-filtration process. Figure 8 below provides pre-filtration times for a variety of rodent tissues. As each tissue is essentially unique, these times are given as guidance only and may be expected to vary.

**IMPORTANT!** Care should be taken not to greatly exceed the recommended pre-filtration times for each tissue type. This may cause excessive foaming in the deep-well plate and increase the risk of cross-contamination. As soon as all wells are clear of lysate, stop the vacuum.

**Note:** Increases in the amount of tissue being filtered will cause a corresponding increase in the pre-filtration time.

![Figure 8](image)

*Figure 8 Suggested pre-filtration times for a variety of rodent tissues (10, 5, and 2.5 mg of tissue per well)*
To load the disposables on the 6100 PrepStation:

1. Place the following disposables on the 6100 PrepStation as indicated:
   a. Deep-well plate in the collection compartment
   b. New Tissue Pre-Filter Tray II in the purification tray carriage. Turn the two knobs to secure the tray in place.

2. Move the carriage to the collection position. Push the carriage handle down until the carriage locks into position (seals).

3. **IMPORTANT!** If you have any unused wells, you must tape over them with an adhesive tape cover. If you do not do this, the vacuum may not be able to achieve the correct setpoint.
Performing Pre-Filtration with Quick Run

To perform pre-filtration as a Quick Run:

1. Pipette 750 µL of digested tissue lysate/NucPrep DNA Purification Solution into each well of Tissue Pre-Filter Tray II.

   IMPORTANT! Overloading Tissue Pre-Filter Tray II may cause the tray to clog. If this happens, you will not be able to complete the protocol.

2. From the main menu, press F1 (Quick).

   The Quick Run screen opens.

   Quick Run

   Position Collection Time(s) Vacuum

   Start Log F1 F2 F3 F4 F5

   999 100%

3. Enter the values shown below, then press F1 (Start).

<table>
<thead>
<tr>
<th>Position</th>
<th>Time</th>
<th>Vacuum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collection</td>
<td>500 sec</td>
<td>60%</td>
</tr>
</tbody>
</table>

   Note: The time will vary depending on the type of tissue you are using. See page 20 for suggested pre-filtration times.

To load the disposables on the 6100 PrepStation: (continued)

4. You are now ready to perform pre-filtration. You may use either of the following procedures:
   - “Performing Pre-Filtration with Quick Run” on page 22
   - “Performing Pre-Filtration with the Preconfigured Method” on page 24
To perform pre-filtration as a Quick Run: *(continued)*

<table>
<thead>
<tr>
<th>4.</th>
<th>Observe Tissue Pre-Filter Tray II to see if all material has passed through the filter:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a. Repeat step 3 until all material has passed through.</td>
</tr>
<tr>
<td></td>
<td>b. Stop the vacuum after the liquid has been removed from all wells.</td>
</tr>
<tr>
<td>5.</td>
<td>Set the carriage handle to the touchoff position and perform touchoff.</td>
</tr>
<tr>
<td>6.</td>
<td>Clear the instrument:</td>
</tr>
<tr>
<td></td>
<td>a. Use the release lever to release the purification tray handle.</td>
</tr>
<tr>
<td></td>
<td>b. Move the carriage to the waste position.</td>
</tr>
<tr>
<td></td>
<td>c. Remove Tissue Pre-Filter Tray II and the deep-well plate from the instrument.</td>
</tr>
<tr>
<td>7.</td>
<td>Save the deep-well plate for purification. If necessary, store as follows:</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>If this is for...</th>
<th>Then...</th>
</tr>
</thead>
<tbody>
<tr>
<td>short-term storage before purification (up to a maximum of 12 h)</td>
<td>store at 4 °C.</td>
</tr>
<tr>
<td>long-term storage</td>
<td>store at −20 to −80 °C.</td>
</tr>
</tbody>
</table>
Performing Pre-Filtration with the Preconfigured Method

Accessing the Preconfigured Method

To access the TissueFilter preconfigured method:

1. In the main menu, press F3 (User).
   The Select User Name screen opens.

   Select User Name
   <ABI> markh
   <ALL> markr
   andy peterh
   markb

   [Select] [New] [Edit] [Delete] [Cancel]

   F1   F2   F3   F4   F5

2. Use the arrow keys to highlight user ABI.

3. Press F1 (Select).
   The main menu displays ABI as the user.

   ABI PRISM™ 6100 PrepStation
   Version 01.01
   User: <ABI>

   [Quick] [Method] [User] [Log] [Util]

   F1   F2   F3   F4   F5

4. Press F2 (Method).
   The Method Select 1 screen opens.

   Method User Steps LastUsed
   Pre-Filter ABI  3  01/16/01
   RNA Blood ABI  9  01/15/01
   RNA Cell ABI  9  01/04/01
   RNA Tissue-Filtr ABI  7  01/17/01
   TissueFilter ABI  7  01/17/01

   [Run] [New] [Edit] [More] [Done]

   F1   F2   F3   F4   F5

5. Use the up and down arrow keys to highlight the TissueFilter method.
Running the TissueFilter Method

To run the TissueFilter method:

1. Pipette 750 µL of digested tissue lysate/DNA Purification Solution into each well of Tissue Pre-Filter Tray II.

   **IMPORTANT!** Overloading Tissue Pre-Filter Tray II may cause the tray to clog. If this happens, you will not be able to complete the protocol.

2. Ensure that the highlighter is at step 1 of the TissueFilter method, then press F1 (Start).

<table>
<thead>
<tr>
<th>Step</th>
<th>Position</th>
<th>Time(s)</th>
<th>Vacuum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Collection</td>
<td>500</td>
<td>60%</td>
</tr>
<tr>
<td>2</td>
<td>Touchoff</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

   **Note:** The time will vary depending on the type of tissue you are using. See page 20 for suggested pre-filtration times.

3. Observe Tissue Pre-Filter Tray II to see if all material has passed through the filter:
   a. Repeat step 2 until all material has passed through.
   b. Stop the vacuum after the liquid has been removed from all wells.
4. Perform touchoff:
   a. Set the carriage handle to the Touchoff position.
   b. Ensure that the highlighter is at step 3 of the TissueFilter method, then press F1 (Start).

<table>
<thead>
<tr>
<th>Step</th>
<th>Position</th>
<th>Time(s)</th>
<th>Vacuum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Collection</td>
<td>500</td>
<td>60%</td>
</tr>
<tr>
<td>2</td>
<td>Touchoff</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

5. Clear the instrument:
   a. Use the release lever to release the purification tray handle.
   b. Move the carriage to the Waste position.
   c. Remove Tissue Pre-Filter Tray II and deep-well plate from the instrument.

6. Save the deep-well plate for purification. If necessary, store as follows:

<table>
<thead>
<tr>
<th>If this is for...</th>
<th>Then...</th>
</tr>
</thead>
<tbody>
<tr>
<td>short-term storage before purification (up to a maximum of 12 h)</td>
<td>store at 4 °C.</td>
</tr>
<tr>
<td>long-term storage</td>
<td>store at −20 to −80 °C.</td>
</tr>
</tbody>
</table>
Performing Purification

Overview

The pre-filtered, digested tissue lysate/NucPrep DNA Purification Solution is passed across DNA Purification Tray II. The gDNA is washed and eluted in a 96-well reaction plate format (P/N 4306737). A two-step elution protocol is used.

**IMPORTANT!** It is important that NucPrep DNA Elution Solution 1 is incubated with the sample and that equal volumes of NucPrep DNA Elution Solution 1 and NucPrep DNA Elution Solution 2 are used.

Evacuation Times for a Variety of Rodent Tissues

The viscosity of digested rodent tissue lysates or homogenized plant tissue lysates differs significantly from tissue type to tissue type. This difference affects the evacuation time required for the purification process. (The evacuation time is the first time the pre-filtered, digested tissue lysate is passed across DNA Purification Tray II).

Figure 9 below provides evacuation times for a variety of rodent tissues. As each tissue is essentially unique, these times are given as guidance only and may be expected to vary.

**Note:** Increases in the amount of pre-filtered, digested tissue lysate or pre-filtered, homogenized tissue lysate added to DNA Purification Tray II will cause a corresponding increase in the evacuation time.

![Figure 9](image-url)

**Figure 9** Evacuation times of pre-filtered tissue lysates (10, 5, and 2.5 mg of tissue per well)
For the pre-filtered, digested tissue lysate/NucPrep DNA Purification Solution, you perform purification on the 6100 PrepStation as follows:

- Create a new method
- Perform the run, which includes:
  - Loading the disposables
  - Loading DNA Purification Tray II
  - Running the method

The parameters and reagents specific to this protocol are provided in the procedures below and on page 30.

The procedures that follow provide a broad overview of the steps required to perform a purification run on the 6100 PrepStation. If you need more detailed procedures, refer to the ABI PRISM™ 6100 Nucleic Acid PrepStation User’s Manual (P/N 4326242).

**Creating a New Method**

To create a new method:

1. In the main menu, press F3 (User).
   
   The **Select User Name** screen opens.

<table>
<thead>
<tr>
<th>Select User Name</th>
<th>markh</th>
<th>markr</th>
<th>peterh</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;ABI&gt; markh</td>
<td>markr</td>
<td>peterh</td>
<td></td>
</tr>
<tr>
<td>&lt;ALL&gt; markh</td>
<td>markr</td>
<td>peterh</td>
<td></td>
</tr>
<tr>
<td>andy markh</td>
<td>markr</td>
<td>peterh</td>
<td></td>
</tr>
<tr>
<td>markb</td>
<td>markr</td>
<td>peterh</td>
<td></td>
</tr>
</tbody>
</table>

   F1 F2 F3 F4 F5

2. Use the arrow keys to highlight the user.
To create a new method: (continued)

3. Press F1 (Select).
   The main menu displays the user.

4. Press F2 (Method).
   The Method Select 1 screen opens.

5. Press F2 (New) and enter parameters for the NucPrep chemistry method. The parameters are provided in the table on page 32.

6. Save the new method as NucPrep, then continue with “Performing a Purification Run for the Digested Tissue Lysate” on page 30.
Performing a Purification Run for the Digested Tissue Lysate

To perform a purification run:

**Load the Disposables**

1. Place the following disposables on the 6100 PrepStation as indicated:
   a. 96-Well Optical Reaction Plate with Barcode in the collection compartment
   b. Splash guard in the waste compartment
   c. DNA Purification Tray II in the purification tray carriage. Turn the two knobs to secure the tray in place.

2. Move the carriage to the waste position. Push the carriage handle down until it locks into position (seals).
To perform a purification run: (continued)

### Load DNA Purification Tray II

3. Pre-wet the purification tray by pipetting 40 µL of NucPrep DNA Purification Solution over each well of the purification tray.

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

   **IMPORTANT!** If you have any unused wells, you must pre-wet them or tape over them with an adhesive tape cover. If you do not do this, the vacuum may not be able to achieve the correct setpoint.

4. Pipette a 650-µL aliquot of the pre-filtered, digested tissue lysate/NucPrep DNA Purification Solution into each well of the purification tray.

### Run the Method

5. Ensure that the highlighter is at step 1 of the NucPrep method, then press F1 (Start).

6. Perform run steps 2 to 9, as described under “NucPrep Chemistry Method Steps” on page 32.

   **Note:** For further information on running protocols on the 6100 PrepStation, please refer to the ABI PRISM™ 6100 Nucleic Acid PrepStation User’s Manual (P/N 4326242).
NucPrep Chemistry Method Steps

The NucPrep chemistry method may be used for isolation of gDNA from pre-filtered, digested tissue lysate. This method includes the steps listed below.

**IMPORTANT!** Please refer to the reagent warnings on page 33 before performing the NucPrep chemistry method.

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
<th>Volume per Well (µL)</th>
<th>Position</th>
<th>Incubation (sec)</th>
<th>Vacuum (%)</th>
<th>Time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>—</td>
<td>Pre-wet all wells with NucPrep DNA Purification Solution</td>
<td>40</td>
<td>Waste</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>Load samples</td>
<td>650</td>
<td>Waste</td>
<td>0</td>
<td>100</td>
<td>180</td>
</tr>
<tr>
<td>2</td>
<td>Add NucPrep DNA Wash Solution</td>
<td>650</td>
<td>Waste</td>
<td>0</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>3</td>
<td>Add NucPrep DNA Wash Solution</td>
<td>650</td>
<td>Waste</td>
<td>0</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>4</td>
<td>Add NucPrep DNA Wash Solution</td>
<td>650</td>
<td>Waste</td>
<td>0</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>5</td>
<td>Perform pre-elution vacuum</td>
<td>—</td>
<td>Waste</td>
<td>—</td>
<td>100</td>
<td>180</td>
</tr>
<tr>
<td>6</td>
<td>Touchoff at Waste</td>
<td>—</td>
<td>Waste</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>Add NucPrep DNA Elution Solution 1*</td>
<td>100</td>
<td>Collection</td>
<td>180</td>
<td>60</td>
<td>180</td>
</tr>
<tr>
<td>8</td>
<td>Add NucPrep DNA Elution Solution 2†</td>
<td>100</td>
<td>Collection</td>
<td>0</td>
<td>60</td>
<td>180</td>
</tr>
<tr>
<td>9</td>
<td>Touchoff at Collection</td>
<td>—</td>
<td>Collection</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* Failure to perform the 180-second incubation step at this point will result in low yields of gDNA.
†In order to obtain the correct pH for the final sample, equal volumes of NucPrep DNA Elution Solution 1 and NucPrep DNA Elution Solution 2 MUST be used.
Reagent Warnings

Please read the reagent warnings below before performing the NucPrep chemistry method.

⚠️ DANGER CHEMICAL HAZARD. NucPrep DNA Purification Solution contains guanidine thiocyanate. Exposure causes eye burns, and can cause skin and respiratory tract irritation. It is harmful if absorbed through the skin or swallowed. Contact with acids or bleach liberates toxic gases. DO NOT ADD acids or bleach to any liquid waste containing NucPrep DNA Purification Solution. Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

⚠️ WARNING CHEMICAL HAZARD. NucPrep DNA Wash Solution 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.
Section 5  Pre-Filtration and Purification on the ABI PRISM 6700 Automated Nucleic Acid Workstation

Safety Warnings

**Biohazard Warning**

The procedures in this section discuss handling of tissue samples. Before performing these procedures, please read and follow the biohazard warning below.

⚠️ **WARNING BIOHAZARD.** Biological samples such as tissues and blood have the potential to transmit infectious diseases. Follow the U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4) and in *Occupational Safety and Health Standards, Toxic and Hazardous Substances* (29 CFR §1910.1030) concerning the principles of risk assessment, biological containment, and safe laboratory practices for activities involving clinical specimens. You can obtain additional information by connecting to the government Web site [http://www.cdc.gov](http://www.cdc.gov).

**Laser Hazard Warning**

The procedures in this section require the use of a barcode reader to load the consumables and reagents onto the deckspace. Please read and follow the laser hazard warning below.

⚠️ **WARNING LASER HAZARD.** Exposure to direct or reflected laser light can burn the retina and leave permanent blind spots. Never look into the laser beam. Remove jewelry and anything else that can reflect the beam into your eyes. Protect others from exposure to the beam.
Performing Pre-Filtration

Overview
The digested tissue lysate/NucPrep® DNA Purification Solution is passed across Tissue Pre-Filter Tray II to remove undigested tissue debris such as hair and bone or unhomogenized plant particulates. The filtrate is recovered in a deep-well plate.

Pre-Filtration Times for a Variety of Rodent Tissues

The viscosity of digested rodent tissue lysates or homogenized plant tissue lysates differs significantly from tissue type to tissue type. This difference affects the amount of time required for the pre-filtration process. Figure 10 below provides pre-filtration times for a variety of rodent tissues. As each tissue is essentially unique, these times are given as guidance only and may be expected to vary.

IMPORTANT! Care should be taken not to greatly exceed the recommended pre-filtration times for each tissue type. This may cause excessive foaming in the deep-well plate and increase the risk of cross-contamination. As soon as all wells are clear of lysate, stop the vacuum.

Note: Increases in the amount of tissue being filtered will cause a corresponding increase in the pre-filtration time.

Figure 10  Suggested pre-filtration times for a variety of rodent tissues (10, 5, and 2.5 mg of tissue per well)
Preparation of the 6700 Workstation

To prepare the 6700 Workstation for pre-filtration:

1. Place a deep-well plate in the filtrate position of the 6700 Workstation.

2. Place a new Tissue Pre-Filter Tray II into the purification tray carriage of the 6700 Workstation and lock it into position. Close the instrument door.

3. Locate the carriage over the deep-well plate.

4. Load the samples:
   a. Pipette 650 µL of digested tissue lysate/DNA Purification Solution into each well of Tissue Pre-Filter Tray II.

   **IMPORTANT!** Overloading Tissue Pre-Filter Tray II may cause the tray to clog. If this happens, you will not be able to complete the protocol.

   b. Close the instrument door.

5. Launch the ABI PRISM™ 6700 Automated Nucleic Acid Workstation software and log in at the Administrator level.

   **Note:** For more information on using the 6700 software, see the *ABI PRISM™ 6700 Automated Nucleic Acid Workstation User’s Manual* (P/N 4304309).

Performing Pre-Filtration

To perform pre-filtration on the 6700 Workstation:


2. Check the Perform 'Vacuum' Test checkbox.

   This function test allows you to turn on the vacuum at a specified location for a specified time/pressure. This feature is used to pass the lysate through Tissue Pre-Filter Tray II and capture the cleared lysate in a deep-well plate.
To perform pre-filtration on the 6700 Workstation: (continued)

3. a. Specify the following:
   - **Vacuum Location** as Filtrate
   - **Carriage Location** as Filtrate
   - **Vacuum Intensity** as 60%
   - **Time/secs.** as 500

   **Note:** The time will vary depending on the type of tissue you are using. See page 36 for suggested pre-filtration times.

   b. Click **Start**.

   ![Function Tests Image]

4. Observe Tissue Pre-Filter Tray II to see if all material has passed through the filter. Repeat step 3 until all material has passed through.

5. a. Ensure that the **Perform 'Vacuum' Test** checkbox is cleared.
   b. Check the **Perform 'Move Vacuum Station' Test** checkbox.
   This function test moves the vacuum station to the archive position.
To perform pre-filtration on the 6700 Workstation: (continued)

6.  a. Specify the following:
    – **First Destination** as Filtrate
    – **Second Destination** as None
    – **Repeat** as 1
  b. Check the **Touch Off** checkbox.
  c. Click **Start**.

7.  When the **Move Vacuum Station Test** is completed, remove the deep-well plate containing the filtered tissue lysate.

8.  Save the deep-well plate for purification. If necessary, store as follows:

<table>
<thead>
<tr>
<th>If this is for...</th>
<th>Then...</th>
</tr>
</thead>
<tbody>
<tr>
<td>short-term storage before purification (up to a maximum of 12 h)</td>
<td>store at 4 °C.</td>
</tr>
<tr>
<td>long-term storage</td>
<td>store at –20 to –80 °C.</td>
</tr>
</tbody>
</table>
Performing Purification

Overview

The pre-filtered, digested tissue lysate/NucPrep DNA Purification Solution is passed across DNA Purification Tray II. The gDNA is washed and eluted in a 96-well reaction plate format (P/N 4306737). A two-step elution protocol is used.

IMPORTANT! It is important that NucPrep DNA Elution Solution 1 is incubated with the sample and that equal volumes of NucPrep DNA Elution Solution 1 and NucPrep DNA Elution Solution 2 are used.

Evacuation Times for a Variety of Rodent Tissues

The viscosity of digested rodent tissue lysates or homogenized plant tissue lysates differs significantly from tissue type to tissue type. This difference affects the evacuation time required for the purification process. (The evacuation time is the first time the pre-filtered, digested tissue lysate is passed across DNA Purification Tray II).

Figure 11 below provides evacuation times for a variety of rodent tissues. As each tissue is essentially unique, these times are given as guidance only and may be expected to vary.

Note: Increases in the amount of pre-filtered, digested tissue lysate or pre-filtered, homogenized tissue lysate added to DNA Purification Tray II will cause a corresponding increase in the evacuation time.

Figure 11  Evacuation times of pre-filtered tissue lysates (10, 5, and 2.5 mg of tissue per well)
For the pre-filtered, digested tissue lysate/NucPrep DNA Purification Solution, you perform purification on the 6700 Workstation as follows:

- Create a new protocol
- Perform the run, which includes:
  - Loading the samples
  - Making selections on the Protocol tab
  - Setting up the deckspace
  - Starting the run

The parameters and reagents specific to this protocol are provided in the procedures below and on page 43.

The procedures that follow provide a broad overview of the steps required to perform a purification run on the 6700 Workstation. If you need more detailed procedures, refer to the *ABI PRISM™ 6700 Automated Nucleic Acid Workstation User’s Manual* (P/N 4304309).

Creating a New gDNA Purification Protocol

To create a new gDNA purification protocol:

1. Log in to the 6700 Workstation and select the Protocol tab.

2. In the Protocol section, click the New button under the RNA/DNA Archive protocol.

   The New RNA/DNA Archive Protocol dialog box opens.
To create a new gDNA purification protocol: (continued)

3. Enter the parameters shown below.

<table>
<thead>
<tr>
<th>Step</th>
<th>Add</th>
<th>Volume (μL)</th>
<th>Tempo (°C)</th>
<th>Incubation Vacuum (min)</th>
<th>Repeat count</th>
<th>Vacuum (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DNA Purification Soln</td>
<td>50</td>
<td>0</td>
<td>240</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>DNA Wash Solution</td>
<td>300</td>
<td>0</td>
<td>120</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>DNA Wash Solution</td>
<td>300</td>
<td>0</td>
<td>120</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>DNA Wash Solution</td>
<td>300</td>
<td>0</td>
<td>120</td>
<td>1</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>DNA Elution Solution</td>
<td>100</td>
<td>0</td>
<td>180</td>
<td>1</td>
<td>60</td>
</tr>
<tr>
<td>6</td>
<td>DNA Elution Soln 2</td>
<td>100</td>
<td>0</td>
<td>180</td>
<td>1</td>
<td>60</td>
</tr>
</tbody>
</table>

**Note:** An extra wash step is included because DNA Purification Tray II must be pre-wet with NucPrep DNA Purification Solution.

4. Click OK to save the protocol, then continue with “Performing a Purification Run for the Digested Tissue Lysate” below.
Performing a Purification Run for the Digested Tissue Lysate

To perform a purification run:

**Load the Samples**

1. If it is not already in a deep-well plate, pipette the pre-filtered, digested tissue lysate/NucPrep DNA Purification Solution into a deep-well plate.
   
   **IMPORTANT!** The maximum volume that DNA Purification Tray II can accommodate is 650 µL. Overloading DNA Purification Tray II may cause the tray to clog. If this happens, you will not be able to complete the protocol.

2. Place the deep-well plate at the secondary input position, as shown below. The deep-well plate is designed to sit on three of the four pins at the secondary input position. The top right corner of the plate is notched to fit AGAINST the top right pin, not OVER it.

   ![Diagram of secondary input position]

   Crosshairs designate orientation.

   **Note:** If the pipette tips do not enter the wells of the deep-well plate correctly, remove the two captive screws from the plate and rotate the plate 180 degrees. Reattach the captive screws firmly before continuing.

**Make Selections on the Protocol Tab**

3. Log in to the 6700 Workstation and select the **Protocol** tab.

4. Check the box next to the new gDNA protocol, which was created as described on page 41.

5. Select **Lysed** from the **Input Plate Type** pop-up menu.
To perform a purification run: (continued)

6. Populate the sample list to show which samples are to be run.

7. In the Deckspace tab, verify protocol setup.
   If the Deckspace tab becomes active, the protocols are set up properly. Otherwise, resolve any errors before proceeding. Refer to the ABI PRISM™ 6700 Automated Nucleic Acid Workstation User’s Manual (P/N 4304309) if necessary.

Set Up the Deckspace

8. In the Instrument tab, click the Cool Peltiers button.

9. Assemble the consumables and reagents required, as listed below.

   **Consumables and Reagents**
   - Splash guard
   - 96-Well Optical Reaction Plate with Barcode
   - Conductive pipette tips, 1000-µL
   - Conductive pipette tips, 200-µL
   - NucPrep DNA Purification Solution
   - Reagent reservoirs, 120-mL
   - NucPrep DNA Wash Solution
   - NucPrep DNA Elution Solution 1
   - NucPrep DNA Elution Solution 2
   - DNA Purification Tray II
   - Deep-well plate
To perform a purification run: (continued)

10. In the **Deckspace** tab, load the plates on the deckspace.

<table>
<thead>
<tr>
<th>Deckspace Location</th>
<th>Plate Required</th>
</tr>
</thead>
<tbody>
<tr>
<td>Input 1</td>
<td>Placeholder 96-well plate</td>
</tr>
<tr>
<td>Archive</td>
<td>96-Well Optical Reaction Plate with Barcode</td>
</tr>
<tr>
<td>Purification</td>
<td>DNA Purification Tray II</td>
</tr>
<tr>
<td>Filtrate</td>
<td>Deep-well plate</td>
</tr>
<tr>
<td>Dilution 1</td>
<td>Placeholder 96-well plate</td>
</tr>
<tr>
<td>Dilution 2</td>
<td>Placeholder 96-well plate</td>
</tr>
<tr>
<td>Output 1</td>
<td>Placeholder 96-well plate</td>
</tr>
<tr>
<td>Output 2</td>
<td>Placeholder 96-well plate</td>
</tr>
<tr>
<td>Output 3</td>
<td>Placeholder 96-well plate</td>
</tr>
<tr>
<td>Output 4</td>
<td>Placeholder 96-well plate</td>
</tr>
</tbody>
</table>

11. Load the tips on the deckspace.

<table>
<thead>
<tr>
<th>Deckspace Location</th>
<th>Tip Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tips 1–4</td>
<td>200-µL disposable</td>
</tr>
<tr>
<td>Tip 5–8</td>
<td>1000-µL or 200-µL disposable</td>
</tr>
</tbody>
</table>
To perform a purification run: *(continued)*

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.</td>
<td>Fill the reagent reservoirs with the reagents listed below and load them on the deckspace.</td>
</tr>
<tr>
<td></td>
<td>- NucPrep DNA Elution Solution 1</td>
</tr>
<tr>
<td></td>
<td>- NucPrep DNA Elution Solution 2</td>
</tr>
<tr>
<td></td>
<td>- NucPrep DNA Wash Solution</td>
</tr>
<tr>
<td></td>
<td><strong>WARNING</strong> CHEMICAL HAZARD. NucPrep DNA Wash Solution 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.</td>
</tr>
<tr>
<td></td>
<td>- NucPrep DNA Purification Solution</td>
</tr>
<tr>
<td></td>
<td><strong>WARNING</strong> CHEMICAL HAZARD. NucPrep DNA Purification Solution contains guanidine thiocyanate. Exposure causes eye burns, and can cause skin and respiratory tract irritation. It is harmful if absorbed through the skin or swallowed. Contact with acids or bleach liberates toxic gases. <strong>DO NOT ADD acids or bleach to any liquid waste containing NucPrep DNA Purification Solution.</strong> Read the MSDS, and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.</td>
</tr>
<tr>
<td>13.</td>
<td>Load the splash guard on the deckspace.</td>
</tr>
<tr>
<td>14.</td>
<td>Verify the deckspace, close the instrument door, and start the purification run.</td>
</tr>
</tbody>
</table>
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