

NucPrep[®] Chemistry: DNA Isolation from Plant and Animal Tissues

- **Rapid digestion and purification of DNA from any animal tissue, and rapid purification of DNA from plant tissue increases throughput in your genetic studies**
- **Isolate high-quality, fully intact, high-molecular-weight DNA free from PCR inhibitors, and suitable for any downstream application**
- **Streamlined protocol without centrifugation steps saves time and effort**
- **The “whole-solution;” reagents, disposables, and instrument systems provides time-saving and cost-effective purification technologies**

Streamline Your Genotyping and Genetic Studies with NucPrep[®] Chemistry

NucPrep[®] chemistry is designed to rapidly, economically, and efficiently isolate DNA from a wide variety of plant or animal tissues. Protocols for the isolation of DNA from animal tissue typically require an overnight digestion with Proteinase K or homogenization and Proteinase K treatment to lyse the tissue and release nucleic acid. Subsequently, simple precipitation protocols usually yield protein-contaminated DNA with poor $A_{260/280}$ ratios that often inhibit downstream PCR enzymes.

NucPrep chemistry provides you with a newly developed, uniquely formulated, fast-acting digestion buffer that completely dissolves whole pieces of animal tissue. The NucPrep chemistry system approach uses the 96-well DNA Purification Tray 2 with an application-specific membrane, the 96-well Tissue Pre-Filter Tray 2, NucPrep Digestion Buffer, Proteinase K, NucPrep DNA Purification Solution, NucPrep DNA Wash Solution, and NucPrep DNA Elution Solution 1 and 2 compatible with all PCR-based downstream applications.

These disposables and reagents are optimized for use on both Applied Biosystems nucleic acid purification platforms—the ABI PRISM[™] 6100 Nucleic Acid PrepStation and the ABI PRISM[™] 6700 Automated Nucleic Acid Workstation.



NucPrep Chemistry System

A specifically designed digestion buffer digests whole animal tissues (including samples from rodent and pig tails, skin, tumors, semen, cartilage, fish tissue, tissue culture cells, and blood spots on cloth or paper) in one hour or less. Combined with a fast 96-well-format purification process, the extremely rapid digestion protocol provides you with up to 96 high-quality DNA samples in only 2–2.5 hours, making DNA isolation from your valuable samples easier.

Hair and bone fragments from animal tissue are removed by filtration through a dedicated filtration consumable if required. A purification solution is then added to the filtrate. The digested and lysed tissue filtrate is added to the purification tray, where the DNA is

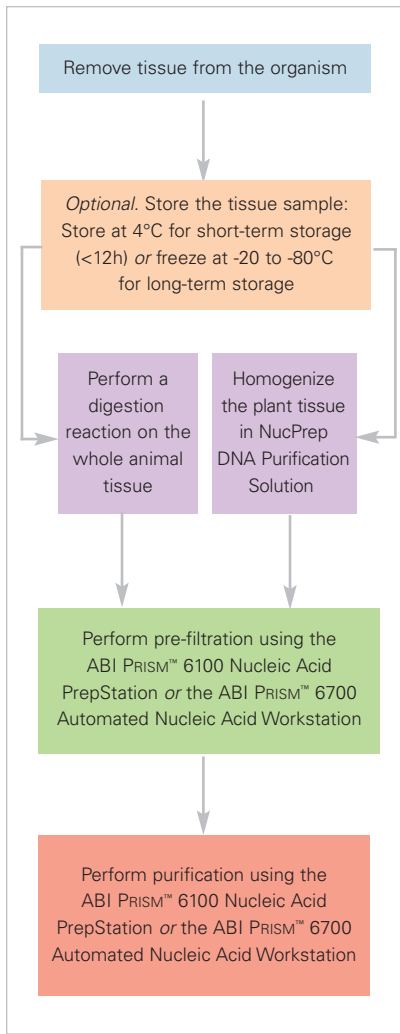


Figure 1. DNA isolation processes using NucPrep chemistry.

captured on the tray membrane. The captured DNA is then washed with purification and alcohol-based wash solutions to remove protein and other contaminants before being solubilized in a two-step elution process. The isolated high-molecular-weight DNA should be free of protein

and RNA contaminants as well as inhibitors of downstream PCR-based processes (Figures 2–4).

Plant materials such as leaves or roots must be homogenized directly in the NucPrep DNA Purification Solution, and then filtered to remove plant

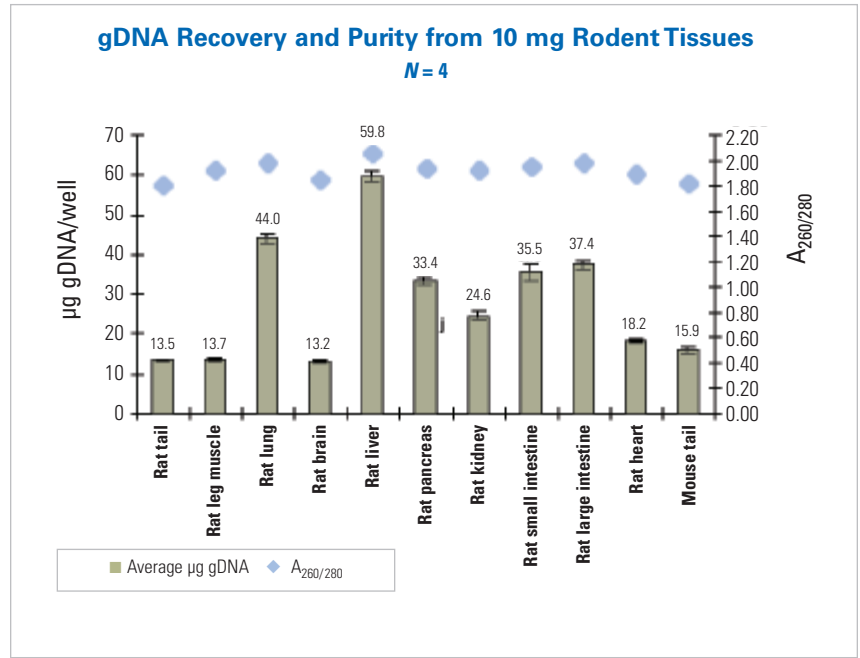


Figure 2. DNA yield and purity from 10 mg samples of rodent tissue (number of samples = 4).

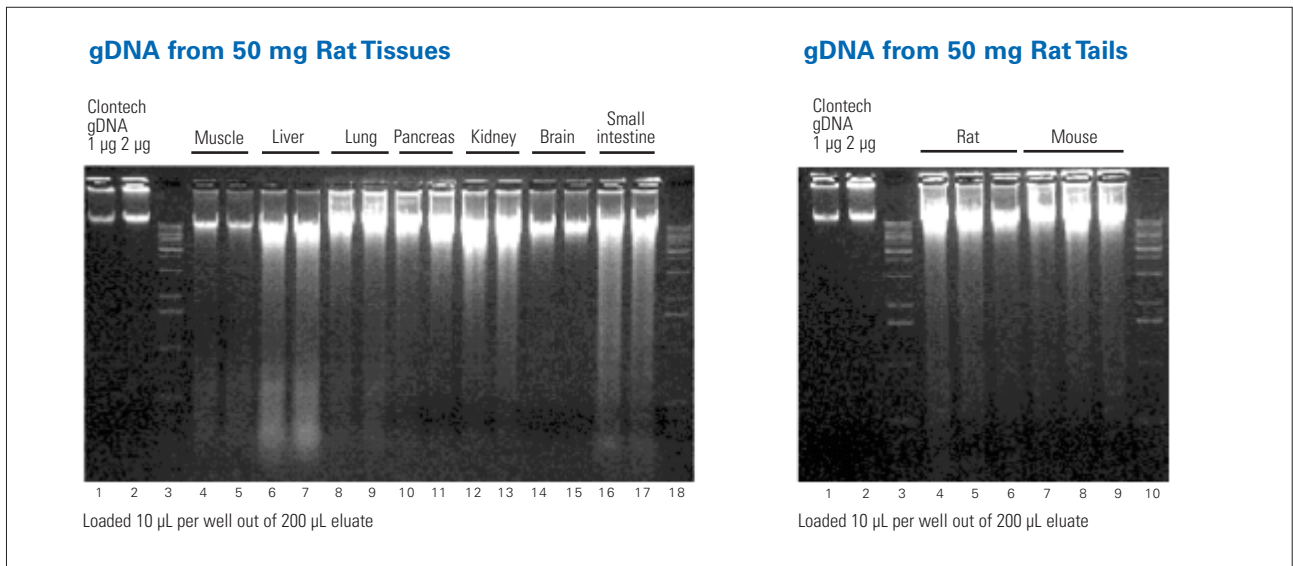


Figure 3. Agarose gel electrophoresis of DNA isolated by using NucPrep chemistry. Duplicate samples from each tissue type are run. Lanes 1 and 2 show control DNA at 1 µg and 2 µg per lane, respectively. Lane 3 shows a molecular weight marker. The isolated DNA is shown to be intact, double stranded, and > 50 kb in length.

fragments left over from the homogenization step. The filtered homogenate is then added to the purification tray. The plant DNA is captured on the membrane and washed to remove polyphenols, polysaccharides, and cell wall components before being resolubilized in a two-step elution process. This procedure isolates intact high-molecular-weight DNA that is free of protein contaminants and PCR inhibitors and is suitable for use in any application.

NucPrep chemistry is not applicable to the isolation of DNA from larger quantities of whole blood (> 50 µL).

Yields and Purity of DNA Obtained by Using NucPrep Chemistry

Animal Tissue

DNA yields vary considerably with the type of tissue and with the conditions in which the isolated material was stored. The DNA yields shown in Figure 2 are general guidelines. For example, a 10 mg sample of rat muscle tissue yields approximately 12 µg of DNA, whereas a 10 mg sample of rat liver tissue yields approximately 60 µg of DNA. Rodent (rat and mouse) tails yield 10–20 µg of DNA per 0.5–1.0 cm, and pig tails yield 10–15 µg of DNA per 0.5 cm. The yields from other rodent tissues vary widely, as shown in Figure 2.

Plant Tissue

The yields of DNA from plant tissues also vary considerably depending on the type of plant, its age, and the part of the plant being isolated (examples are shown in Figure 5). In general, NucPrep chemistry isolates high-quality DNA that is intact and free of protein and PCR inhibitors.

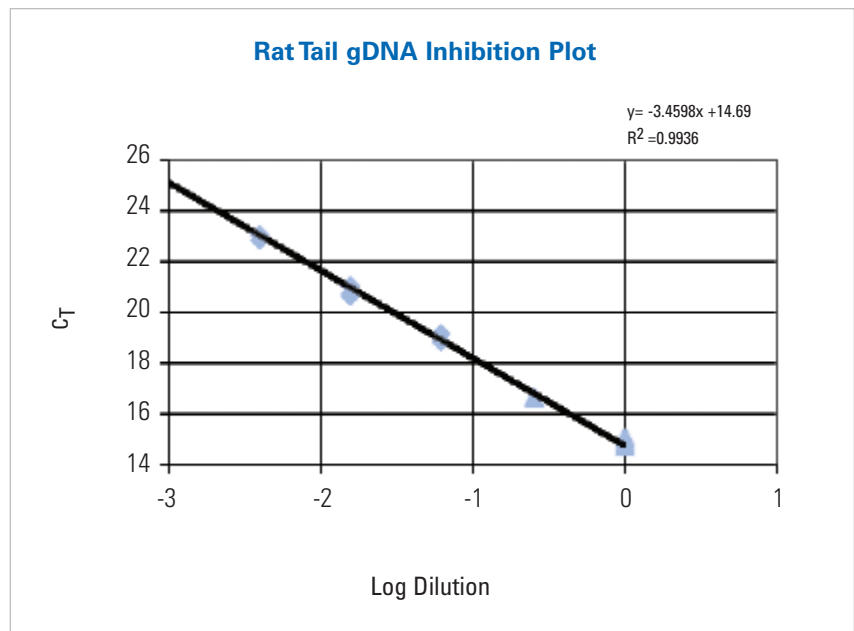


Figure 4. Results of an inhibition assay based on real-time PCR. In this assay, a DNA sample isolated from rodent tail is assayed for inhibitors of the PCR process. A dilution series was created as follows: no dilution, 1:4, 1:16, 1:64, 1:256, and 1:1024. For each triplicate set of dilution points, a 5' nuclease real-time PCR assay was performed for the 18S rRNA amplicon on an ABI PRISM® 7700 Sequence Detection System. If the isolated DNA sample is free of PCR inhibitors, the plot of the threshold cycle versus the logarithm of the dilution is a straight line. The presence of inhibitors is shown by a pronounced curve at the 1:4 and no-dilution points.

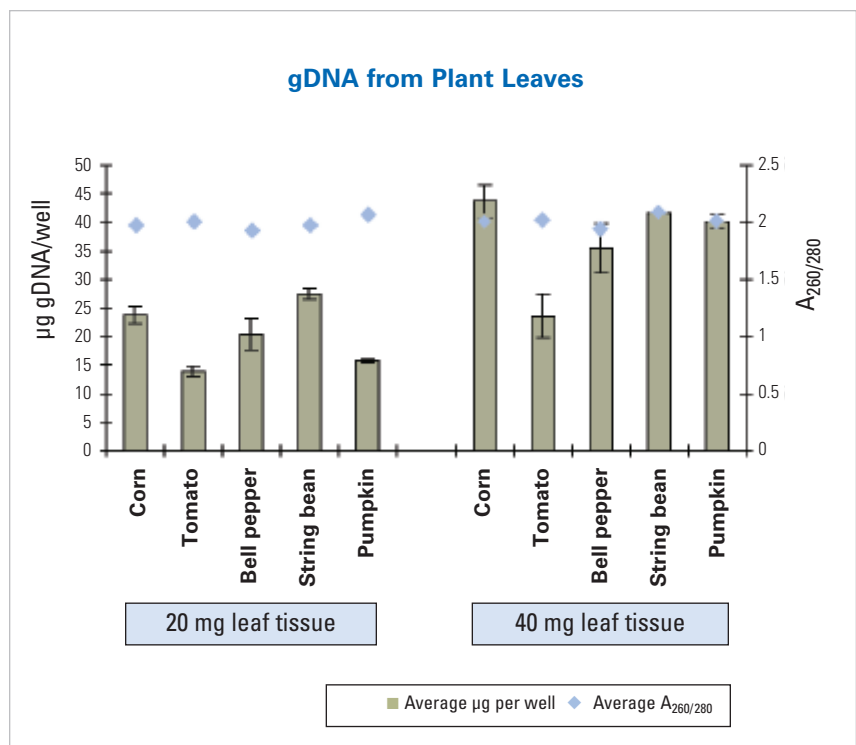


Figure 5. DNA yields from plant materials (*N* = 4). This data shows that high-quality DNA and high yields can be obtained from a variety of leaf tissues. Slightly elevated A_{260/280} ratios indicate the presence of small amounts of RNA (< 10% by weight of the DNA sample).

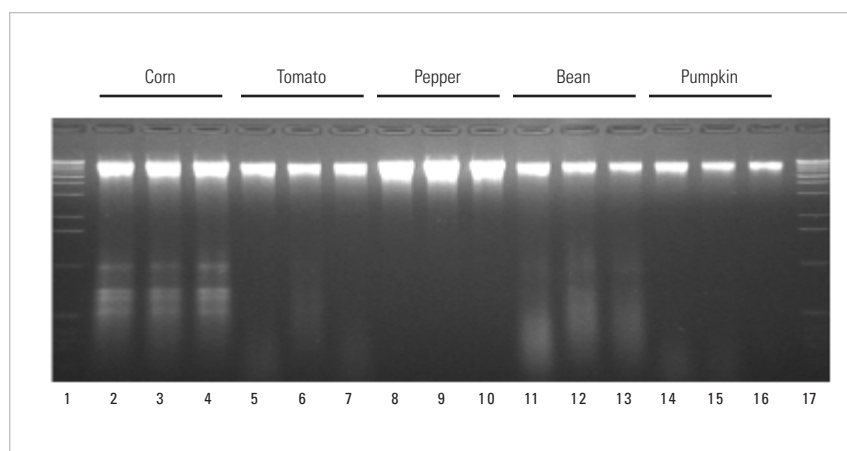


Figure 6. Agarose gel image of DNA isolated from a variety of plant tissues. Lanes 1 and 17 contain molecular weight markers and each sample is shown in triplicate.

Ordering Information

Description	Quantity	Part Number
NucPrep® DNA Digestion Buffer	250 mL	4333800
Proteinase K	5 mL	4333793
NucPrep® DNA Purification Solution	900 mL	4333802
NucPrep® DNA Wash Solution	975 mL	4333796
NucPrep® DNA Elution Solution 1	200 mL	4333794
NucPrep® DNA Elution Solution 2	200 mL	4333798
DNA Purification Tray 2	10 per box	4330172
Tissue Pre-Filter Tray 2	10 per box	4330688

Other Disposables

96-Well Barcoded PCR Microplate	20 per box	4306737
Deep Well Plates	10 per box	4308641
Splash Guards	20 per box	4311758
Archive Tray Covers	10 per box	4306286

Instrument Systems

ABI PRISM™ 6100 Nucleic Acid PrepStation	6100-01
ABI PRISM™ 6700 Automated Nucleic Acid Workstation	6700-01



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.

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