Applied Biosystems

BloodPrep™ Chemistry: DNA Isolation from Whole Blood, Tissue-Cultured Cells, Buccal Swabs, and Other Materials

- **Isolates DNA from up to 96 samples of fresh or frozen blood from any animal species, tissue-cultured cells, or buccal swab samples in one hour or less**
- **Provides high quality, high molecular weight genomic DNA, free from inhibitors of PCR and suitable for any DNA-based assay application**
- **Offers streamlined protocols without centrifugation, novel reagents to prevent clogging, and fast DNA elution at room temperature**
- **Part of an integrated solution including reagents, disposables, and instruments to provide time-saving and cost-effective purification**

Streamline and Improve Your Genotyping and Genetic Studies

Applied Biosystems has designed BloodPrep™ Chemistry for quick and efficient DNA purification from many sample matrices, including: fresh and frozen whole blood from various animal species in all common anticoagulants, isolated blood cells such as "buffy coat" or leukocyte fractions, cultured cells, and buccal swabs. Novel reagent formulations greatly reduce the risk of clogging in purification membranes. They also enhance purity and remove contaminating protein and PCR inhibitors, thereby improving the performance of your DNA-based assays.

Chemistry Components

Each BloodPrep Chemistry reagent set can process approximately 600 samples, and includes the following components:

- Proteinase K and Digestion Buffer for blood-protein digestion
- DNA Purification Solution to lyse cells and retain DNA on a glass fiber membrane
- Wash Solution
- A unique two-step elution buffer system that resolubilizes very high molecular DNA in only 3 minutes at room temperature
- 96-well DNA Purification Tray with an application-specific glass fiber membrane for capturing, washing, and eluting DNA

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

Overcoming the Difficulties of DNA Isolation from Whole Blood and Isolated Blood Cells

Isolating DNA from both fresh and particularly from frozen, whole blood samples is very difficult. Normal human blood consists of 45% erythrocytes and 54% thrombocytes, which contain no DNA. The DNA-containing leukocytes consist of only 1–2% of all blood cells at 6.6 pg of DNA per cell (5×10^6 to 10^7 cells/mL).

In addition, blood typically contains 150 mg/mL of hemoglobin and 60–80

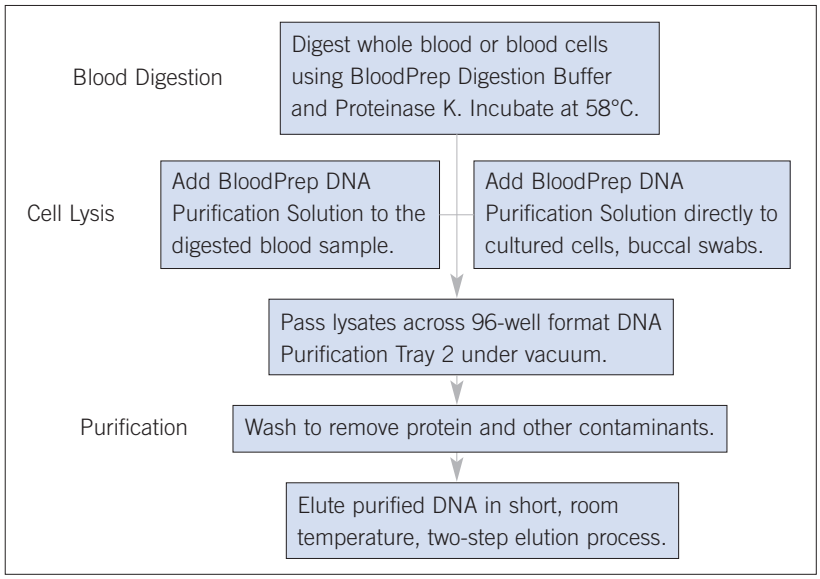


Figure 1. BloodPrep Chemistry DNA Isolation Process

mg/mL of plasma protein. These proteins bind strongly to purification materials such as glass fiber membranes during the purification process. Protein binding—even at low (<150 µL) input volumes of blood—can be so extensive as to prevent the lysates from flowing across the purification tray and cause clogging.

This is particularly true of non-human blood samples such as those from sheep, cow, and chicken (avian nucleated red blood cells yield a very large amount of DNA per unit volume of sample). Protein contamination of the final product can cause difficulty in downstream nucleic acid-based assays such as SNP, OLA, STR, or other forms of genotyping or DNA sequencing.

Changes in protein structure caused by freezing and freeze-thaw cycles also appear to exacerbate the difficulty of DNA isolation from whole blood. Improper mixing of blood with the anticoagulant at initial drawing can leave strands of clotted blood, which can also contribute to clogging. Furthermore, changes in blood chemistry that result from disease or treatment can be profound, raising or lowering components such as lipids and triglycerides which interact strongly with nucleic acid purification membranes and processes. Applied Biosystems has designed BloodPrep Chemistry to specifically combat those difficulties.

BloodPrep Chemistry Purification

Up to 150 µL of fresh or frozen human whole blood (less material for other animal species is recommended) is added along with a small amount of Proteinase K to BloodPrep Digestion Buffer. BloodPrep Digestion Buffer contains components that protect the integrity of DNA during the subsequent incubation at elevated temperatures. The digestion buffer is incubated at 58°C for 10 minutes to allow degradation of blood proteins while retaining intact DNA (Figure 1).

Following incubation, BloodPrep DNA Purification Solution is then added to the digest to complete cell lysis. BloodPrep DNA Purification Solution contains a novel detergent formulation, which helps to overcome clogging and prevent blood proteins and heme from contaminating the eluted DNA. The blood lysate is then passed across a glass fiber membrane in a 96-well format purification tray (max volume 700 µL) under vacuum, where DNA is retained on the membrane.

The membrane is then washed to remove protein and other contaminants, and finally eluted at room temperature in a simple, two-step process. This isolates very high molecular weight DNA—typically >50 kb—with little to none of the shortened DNA degradation products common to other isolation chemistries. Such products are visible as streaks at

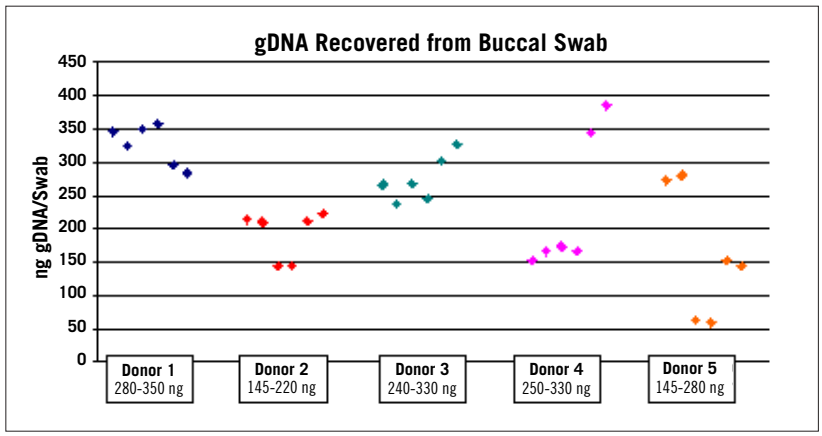
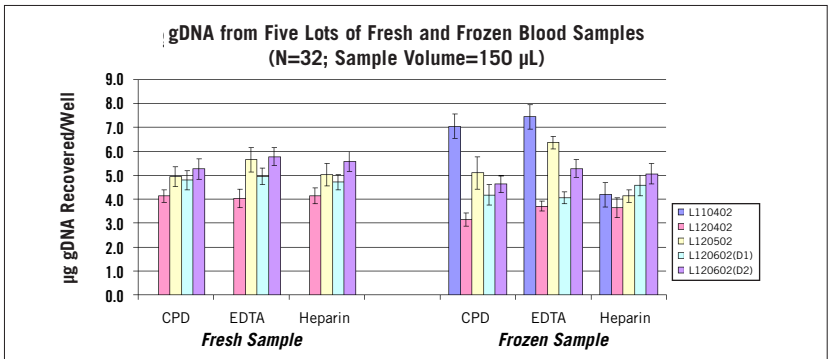


Figure 2. DNA isolation from buccal swabs. Cotton swabs were used on five different donors. Buccal swabs give varying amounts of genomic DNA depending on the person being swabbed, and the swab type. Yields in this study were measured by 18S rRNA PCR assays on real-time PCR instrument systems.



Lot	Fresh (%CV)			Frozen (%CV)		
	CPD	EDTA	Heparin	CPD	EDTA	Heparin
L110402				7.21	6.46	11.21
L120402	6.29	9.51	7.93	8.75	5.76	11.08
L120502	8.43	9.04	9.26	13.27	4.24	6.32
L120602(D1)	8.30	7.06	6.74	10.36	6.15	9.46
L120602(D2)	8.21	6.30	7.42	7.51	6.99	8.52

Figure 3. Yield and %CV of DNA isolated from five lots of human whole blood using BloodPrep Chemistry and the 6100 PrepStation.

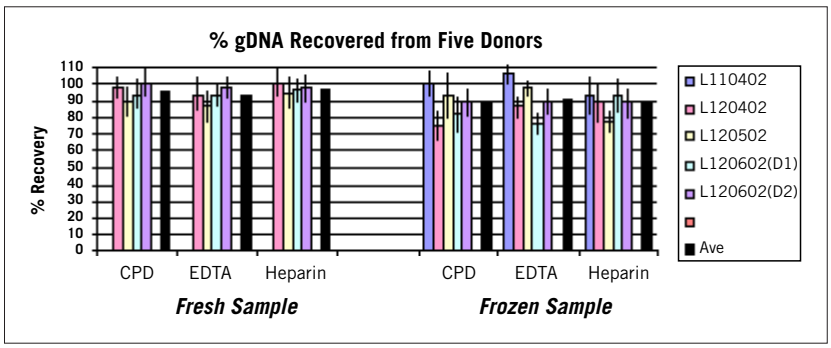


Figure 4. Yield of DNA from five lots of human blood isolated from 150 µL of human whole blood using BloodPrep Chemistry. Recovery percentage calculated by measuring white blood cell counts prior to purification.

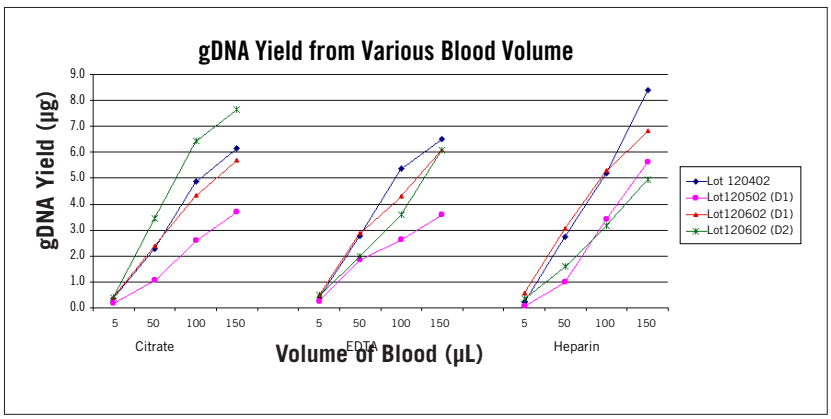


Figure 5. Yields of DNA from BloodPrep Chemistry at various input volumes from four human whole blood donors in different anticoagulants.

lower molecular weights on agarose gel pictures after electrophoresis.

Isolation of DNA from Tissue-Cultured Cells and Buccal Swabs

DNA isolation from the sample types mentioned above presents much less of a challenge than isolation from whole blood. Overall protein levels are much lower and the complexity of the sample is greatly reduced. Tissue-cultured cells and buccal swabs (Figure 2) are simply lysed by addition of BloodPrep DNA Purification Solution.

Performance Specifications

BloodPrep Chemistry is specified to isolate >70% of the available DNA from normal human blood samples at volumes of 150 µL and typically isolates greater than 90% of the available DNA based on white blood cell counts.

Yields are 2–8 µg (typically >4 µg from normal human blood) of DNA at input volumes of 150 µL. The value varies, depending on the leukocyte count of the sample (see Figures 3, 4, and 5). DNA yields can vary considerably and are especially dependent on the storage conditions of the isolated blood.

Coefficients of variation (%CV) are expected to be less than 30% (typically <15%) for purification of the same sample indicating that the purification process is highly reproducible both well-to-well and plate-to-plate.

DNA yields from other animal species vary widely, but with typically larger amounts of DNA. This is due to increased leukocyte counts per unit volume of blood—or as in avian, reptile, or camelid samples, the presence of nucleated red blood cells (Figures 6 and 6a).

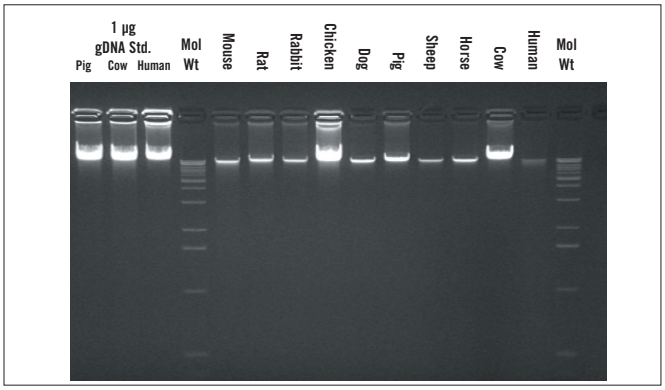


Figure 6. 1% TBE agarose gel image of DNA isolated from 50 µL of frozen animal blood of various species. 10 µL of eluate (total 200 µL) was loaded into the gel, which indicates the relative yields of DNA from different animal species.

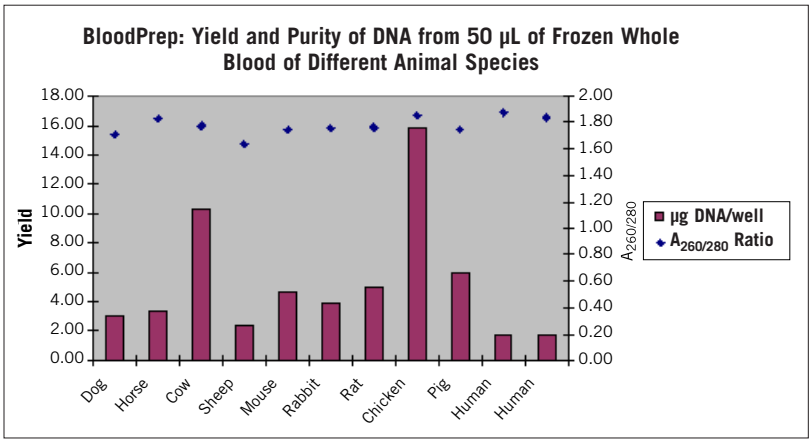


Figure 6a. Yield and purity of DNA from 50 µL of various animal species blood.

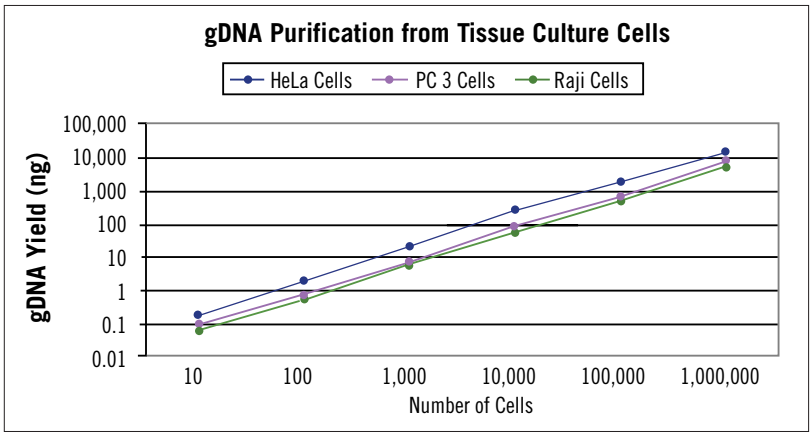


Figure 7. Yields of DNA from tissue-culture cells at input levels of 10 to 1 x 10^6 cells. Each point is in quadruplicate. Results show that the purification process recovers DNA linearly at these input ranges.

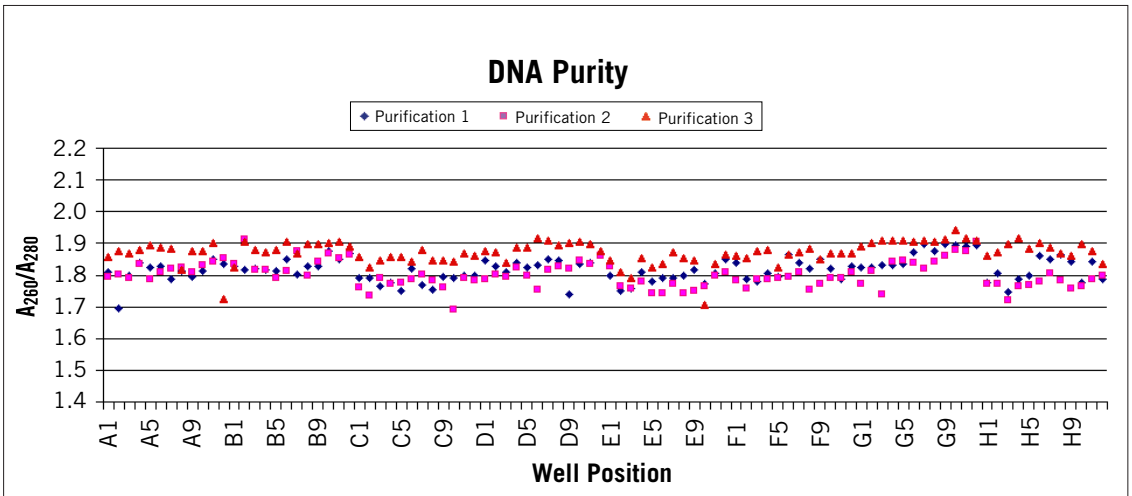
BloodPrep Chemistry can also isolate DNA from isolated leukocyte ("buffy coat"), or other nucleated cell fractions at input volumes of up to 1 x 10^6 cells per well. Recovery of DNA appears to be linear at input ranges from between 10 and 1 x 10^6 cells per well (Figure 7).

Yield of DNA from tissue-cultured cells varies depending on the ploidy (number of chromosomes per cell) of the cell line. Human Raji (diploid) cells yield approximately 5 µg of DNA per 1 x 10^6 cells, PC3 (near triploid) cells yield approximately 8 µg, while HeLa cells (100% aneuploid, near tetraploid) yield almost 15 µg of DNA per 1 x 10^6 cells. Because of the large amount of DNA per cell for HeLa cells, flow-rates of the lysed sample can slow dramatically as the purification membrane comes close to saturation. Lower input numbers of cells (e.g. 1 x 10^5 cells per well) are recommended for this type of cell line (Figure 7).

Purity and Inhibition

Purity as measured by $A_{260/280}$ ratios is expected to be greater than 1.7, indicating that very little protein or other cellular macromolecule contamination is present in the eluted DNA. A_{320} and A_{410} values are expected to be less than 0.05 absorbance units, indicating that the sample is free of particulates and heme, respectively (Figure 8).

There are expected to be no inhibitors of the PCR process. Assessment of the level of inhibitors is performed using a 5' nuclease assay with TaqMan® probes for the 18S rRNA amplicon on a real-time PCR platform. A dilution series of isolated DNA is created in the following manner: no dilution; 1:4; 1:16; 1:64; 1:256; and 1; 1,024. Each point on the dilution curve is analyzed in the 5' nuclease assay in triplicate. The no-dilution point represents addition of approximately 1 µg of DNA to a 50 µL PCR reaction. A logarithm of dilution versus threshold cycle (the PCR cycle number at which fluorescence from the 5' nuclease assay is detected above background) will be plotted as a straight line if the PCR process is uninhibited (Figure 9).



Purification Run	*Count*	*Ave $A_{260/280}$*	*StDev*	*%CV*
1	96	1.82	(+/- 0.04)	2.09
2	96	1.80	(+/- 0.04)	2.22
3	96	1.87	(+/- 0.04)	1.93
Inter-run Ave	3 Plates	1.83	(+/- 0.04)	2.19

Figure 8. Purity of DNA isolated from 3 x 96-well purifications.

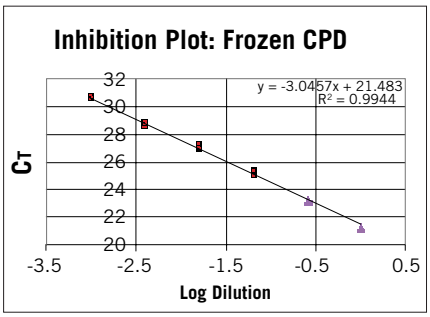


Figure 9. Inhibition assay for DNA isolated from frozen CPD anticoagulated human blood. A real-time PCR amplification for the 18S rRNA amplicon is performed using the 5' nuclease assay with TaqMan probes on a dilution series (no dilution; 1:4; 1:16; 1:64; 1:256; and 1:1,024) of DNA isolated from 50 µL of whole blood. The no-dilution point represents the addition of 1 µg of DNA to a 50 µL PCR reaction. A plot of logarithm of dilution versus threshold cycle number is linear if no inhibitors are present in the sample.

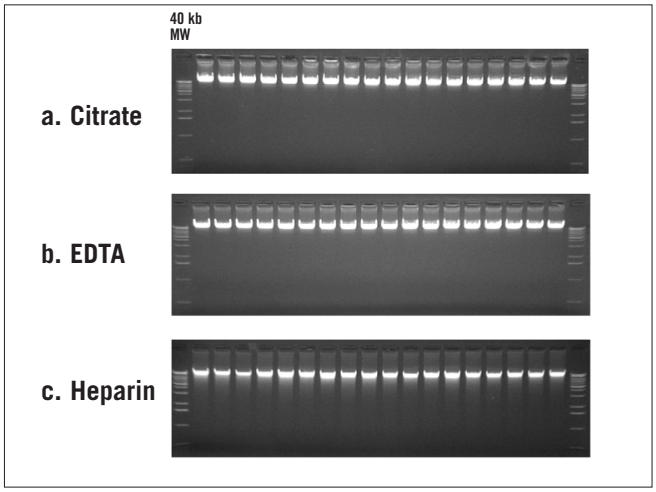


Figure 10. 1% TBE agarose gel images of DNA isolated from human blood in a variety of common anticoagulants. Lanes 1 and 20 show a molecular weight ladder with the largest band at 40 kb.

No RNA contamination is expected on agarose gel images of isolated DNA. The material is expected to be above 40 kb and intact without streaking that results from degradation during incubation at elevated temperatures (Figure 10).

Purification process time is expected to be one hour or less using either the ABI PRISM™ 6100 Nucleic Acid PrepStation or ABI PRISM™ 6700 Automated Nucleic Acid Workstation for human blood, buffy coat, buccal swab, or tissue-cultured cell samples. Blood from other animal species may require prolonged digestion times to help lyse thicker cell walls.

Ordering Information

Description	Quantity	Part Number
Proteinase K	5 mL	4333793
BloodPrep Digestion Buffer	250 mL	4342777
BloodPrep DNA Purification Solution	1,000 mL	4342775
BloodPrep DNA Wash Solution	975 mL	4342949
BloodPrep DNA Elution Solution 1	200 mL	4342951
BloodPrep DNA Elution Solution 2	200 mL	4342950
Genomic DNA Purification Tray 2	10 per box	4330172
Instrument Systems		
ABI PRISM™ 6100 Nucleic Acid PrepStation		6100-01
ABI PRISM™ 6700 Nucleic Acid Workstation		6700-01
Other Disposables		
96-Well Barcoded PCR Microplate	20 per box	4306737
Deep Well Plates	10 per box	4306841
Splash Guards	20 per box	4311758
Archive Tray Covers	10 per box	4306286

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