promega Application Notes

Genomic DNA Purification from Blood

Wizard® SV Genomic DNA Purification Systems

Abstract

The Wizard® SV and SV 96 Genomic DNA Purification Systems (Cat.# A2360, A2361, A2370, A2371) have been developed for the fast, simple purification of genomic DNA from sample types such as mouse-tail clippings, animal tissue, and tissue culture cells. In this article we demonstrate how purification of genomic DNA from blood can be accomplished using the Wizard® SV and SV 96 Systems by making a few simple modifications to the protocol.

Procedure

Genomic DNA can be purified either from whole blood or from leukocytes. Purification from leukocytes is recommended when highly pure genomic DNA (A_{260}:A_{280} ≥1.7) is essential in downstream applications. Otherwise, genomic DNA can be purified directly from as much as 200µl of whole blood without requiring laborious leukocyte preparation steps. Genomic DNA purified directly from whole blood using the Wizard® SV Genomic DNA Purification Systems has a reduced A_{260}:A_{280} ratio relative to DNA purified from leukocytes, but both preparations perform equally well in downstream applications such as single-target or multiplex PCR amplification. Genomic DNA prepared from either leukocytes or whole blood is of high molecular weight (>23kb) when visualized by agarose gel electrophoresis (Figure 1).

Genomic DNA Purification from Whole Blood

Sample Lysate Preparation:

1. Place up to 200µl of whole blood into a sterile 1.5ml microcentrifuge tube. **Note:** The maximum volume of whole blood that can be processed is 200µl. Use of more than 200µl will result in clogging of the SV columns.
2. Prepare 400µl of Whole Blood Lysis Buffer (Wizard® SV Lysis Buffer + 1% Triton® X-100 [Cat.# H5142]) for every 200µl of whole blood.
3. Prepare Proteinase K Solution (20mg/ml) by resuspending 100mg Proteinase K (Cat.# V3021) in 5ml nuclease-free water.
4. Add 40µl Proteinase K (20mg/ml) to the 200µl of whole blood in the microcentrifuge tube. Pipet to mix. Incubate at room temperature for 10 minutes. Invert the tube occasionally to mix.

Table 1. Equipment and Reagents Required for Genomic DNA Purification from Whole Blood.

Equipment/Reagent	Cat.#
Wizard® SV Genomic DNA Purification System	A2360, A2361
Proteinase K (100mg)	V3021
Triton® X-100	H5142
1.5–2ml microcentrifuge tubes	
Table top centrifuge capable of 13,000 × *g*.	

5. Add 400µl of prepared Whole Blood Lysis Buffer to the Proteinase K-treated whole blood sample. Vortex briefly to mix. Incubate at room temperature for 10 minutes, vortexing occasionally to mix. (It is important that the Whole Blood Lysis Buffer contains 1% Triton® X-100. Failure to add the Triton® X-100 may result in clogging of the column).

DNA Purification from Whole Blood Lysate:

6. The *Wizard® SV Genomic DNA Purification System Technical Bulletin* #TB302, which is supplied with the system, provides complete instructions for genomic DNA purification from whole blood. Step-by-step instructions for DNA purification using either "spin" (microcentrifuge) or vacuum formats are given in Sections IV.C and IV.D of the Technical Bulletin, respectively.
7. Elute the genomic DNA in 75–250µl nuclease-free water. The optimal elution volume depends on the volume of the original whole blood sample and the desired concentration of genomic DNA for downstream applications. Elution in smaller volumes will concentrate the DNA but may lower total yield. Larger elution volumes will give optimal yields but a more dilute final DNA preparation. We recommend eluting in 100µl of nuclease-free water and adjusting from there based on need.

Genomic DNA Purification from Leukocytes

Sample Lysate Preparation:

1. Place up to 1ml of whole blood in a sterile 1.5–2ml microcentrifuge tube. **Note:** The maximum volume of blood that can be processed for genomic DNA purification from leukocytes is 1ml. Use of more than 1ml will exceed the capacity of the Spin Basket.
2. Collect blood cells by centrifugation at 3,000rpm (400 × *g*) for 5 minutes to generate a relatively clear supernatant (approximately 30% of the volume) and a large cell pellet (approximately 60–70% of the total

volume. Remove the supernatant by carefully pipeting from the top of the sample. Do not disturb the cell pellet.

3. Add 1ml of Red Blood Cell Lysis Solution (Cat.# Z3141) and resuspend the cell pellet by carefully pipeting 4–5 times.
4. Centrifuge at 3,000rpm for 5 minutes. **Note:** A distinct pellet may not be observed at this point. Remove and discard 1ml of supernatant by pipeting from the top. Leave the remaining supernatant and cell pellet in the tube.
5. Repeat steps 3 and 4 two to three more times (for a total of 3–4 red blood cell lysis steps). **Note:** Because leukocytes constitute approximately 1% of total blood volume, the size of the cell pellet will decrease significantly during the red blood cell lysis procedure. Exercise caution to avoid loss of the white blood cell pellet in steps 3–5. After red blood cell lysis steps, a mostly white cell pellet should be visible. This pellet will not be completely white, but some white should be visible.
6. Remove all but 100µl of the supernatant from the tube, being careful to avoid the cell pellet.
7. Add 150µl of Wizard® SV Lysis Buffer to the cells. Mix lysate by pipetting.

Table 2. Equipment and Reagents Required for Genomic DNA Purification from Leukocytes.

Equipment/Reagent	Cat.#
Wizard® SV Genomic DNA Purification System	A2360, A2361
Red Blood Cell Lysis Solution	Z3141
1.5–2ml microcentrifuge tubes	
Table-top centrifuge capable of 13,000 × *g*.	

DNA Purification from Cell Lysate:

8. The *Wizard® SV Genomic DNA Purification System Technical Bulletin* #TB302, which is supplied with the system, provides complete instructions for genomic DNA purification from leukocytes. Step-by-step instructions for DNA purification using either "spin" (microcentrifuge) or vacuum formats are given in Sections IV.C and IV.D of the Technical Bulletin, respectively.
9. Elute the genomic DNA in 75–250µl nuclease-free water. The optimal elution volume depends on the volume of whole blood from which leukocytes were prepared and the desired concentration of genomic DNA for downstream applications. Elution in smaller volumes will concentrate the DNA but may lower total yield. Larger elution volumes will give optimal yields but a more dilute final DNA preparation. We recommend eluting in 100µl of nuclease-free water and adjusting from there based on need.

Table 3. Equipment and Reagents Required for High-Throughput Genomic DNA Purification from Whole Blood.

Equipment/Reagent	Cat.#
Wizard® SV 96 Genomic DNA Purification System	A2370, A2371
Proteinase K (100mg)	V3021
Triton® X-100	H5142
Vac-Man® 96 Vacuum Manifold	A2291
Vacuum trap for waste collection	
Vacuum pump capable of 15–20 inches of Hg	
Vacuum tubing	
Orbital shaker	
96-well deep well plate for Proteinase K digestion	
Adhesive plate sealers (foil)	

High-Throughput Genomic DNA Purification

High-throughput purification of genomic DNA from whole blood can be performed using the Wizard® SV 96 Genomic DNA Purification System (Cat.# A2370, A2371). This system enables simultaneous purification of up to 96 samples using the SV 96 Binding Plate. We have found that the SV 96 format is most suitable for purification of genomic DNA from whole blood. While preparation of leukocytes should be possible in a 96-well, deep well plate, we have had difficulty performing the the red blood cell lysis procedure in the 96-well format. Simultaneous visualization of cell pellets is difficult, causing significant variability in DNA yield from leukocytes. Therefore, we recommend the Wizard® SV 96 Genomic DNA Purification System for direct purification of genomic DNA from whole blood. The SV 96 format reduces purification time and complexity and generates DNA that performs well in single and multiplex amplification reactions.

The maximum volume of whole blood that can be processed in the SV 96 format is 200µl per well. Exceeding 200µl of whole blood will result in clogging of the columns in the SV 96 Binding Plate. The procedure for genomic DNA purification from whole blood is identical to the purification procedure using SV Spin Baskets, except that sample lysates are prepared in a 96-well, deep-well plate and mixing steps are performed using an orbital shaker.

Once sample lysates are prepared, genomic DNA purification and elution are performed as described in Section IV.B of the *Wizard® SV 96 Genomic DNA Purification System Technical Bulletin* #TB303. DNA is eluted in 75–250µl nuclease-free water. The optimal elution volume of purified genomic DNA will depend on the desired concentration of genomic DNA for downstream applications. We recommend eluting in 100µl of nuclease-free water and adjusting from there based on need.

Analysis of Purified Genomic DNA

Yield, Purity and Integrity:

Genomic DNA yields from a specific volume of blood will vary depending on the number of nucleated white blood cells present. The greater the number of white blood cells, the greater the yield. Table 4 shows the average yield and purity of DNA isolated using the three different genomic DNA purification procedures described here.

Genomic DNA purified from leukocytes shows greater yield and purity than genomic DNA purified from whole blood. Greater yield is observed because a larger volume of blood can be processed when purifying genomic DNA from leukocytes (1ml maximum). Purification of DNA from leukocytes results in improved purity because a large amount of potential carryover is eliminated by the red blood cell lysis procedure. However, the procedure for isolation of DNA from leukocytes is more labor-intensive.

Often, the downstream application for purified genomic DNA is amplification. The fast and simple purification directly from 200µl of whole blood generates amplifiable genomic DNA without the labor-intensive and time-consuming process of leukocyte preparation. Both purification procedures purify high quality, intact, genomic DNA (Figure 1).

Amplification:

Genomic DNA purified directly from 200µl of whole blood is amplifiable in both single-target and multiplex amplification reactions (Figure 2). Therefore, even though this method results in DNA with a lower A_{260}:A_{280} ratio than DNA isolated from leukocytes (Table 4), the DNA performs well in PCR, including sensitive multiplex amplification reactions.

Table 4. Average Yield and Purity of Genomic DNA Isolated from Leukocytes or Whole Blood Using the Wizard® SV and SV 96 Genomic DNA Purification Systems.

Purification Method	Starting Material	Average Yield (µg)	Average Purity (A_{260}/A_{280})
Wizard® SV Genomic System	leukocytes (from 1ml whole blood)	11 +/- 2	1.9
Wizard® SV Genomic System	200µl whole blood	6.2 +/- 1	1.3
Wizard® SV 96 Genomic System	200µl whole blood	5.7 +/- 1.3	1.3

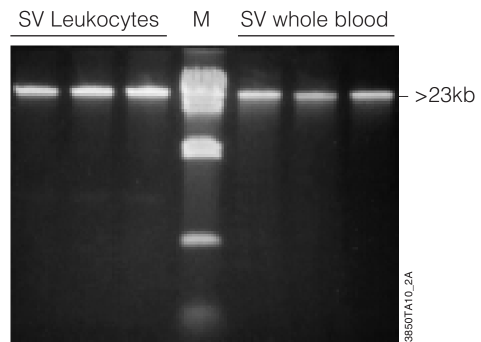

Figure 1. Visualization of purified genomic DNA by agarose gel electrophoresis. Genomic DNA purified from leukocytes or whole blood using the Wizard® SV Genomic DNA Purification System was run on a 1% agarose gel and stained with ethidium bromide. Twenty microliters of purified genomic DNA was loaded in each lane. M = Lambda DNA/*Hin*d III Markers (Cat.# G1711).

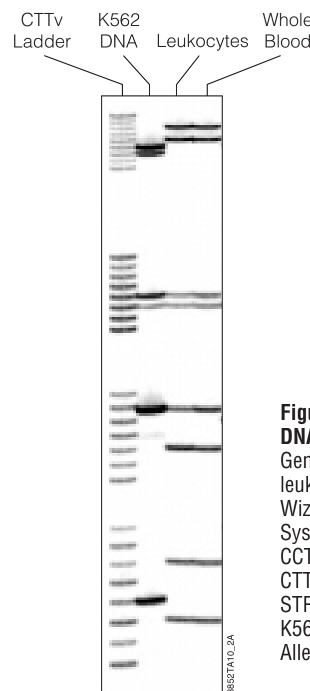

Figure 2. Performance of purified genomic DNA in multiplex PCR amplification. Genomic DNA (1ng) isolated from leukocytes or whole blood using the Wizard® SV Genomic DNA Purification System was amplified using the *GenePrint*® CCTv Multiplex System (Cat.# DC6301). The CTTv Multiplex amplifies alleles from four STR loci—CSF1PO, TPOX, TH01 and vWA. K562 DNA was used as a positive control. Allelic ladder markers are shown on the left.

Purification and Amplification from Whole Blood Collected in the Presence of Anticoagulant:

Purification of genomic DNA from whole blood collected into four commonly used collection tubes was compared. Genomic DNA was purified directly from 200µl of whole blood collected in: Sodium Heparin, Lithium Heparin, Buffered Citrate, and K_3EDTA Vacutainer® tubes. Purified DNA was tested in PCR to assay for carryover of amplification inhibitors (such as heparin) introduced during sample collection. Figure 3 shows that efficient amplification of all genomic DNA preparations was achieved, regardless of the type of collection tube used. This indicates that potential PCR inhibitors, such as heparin, introduced during sample collection are removed during the purification procedure.

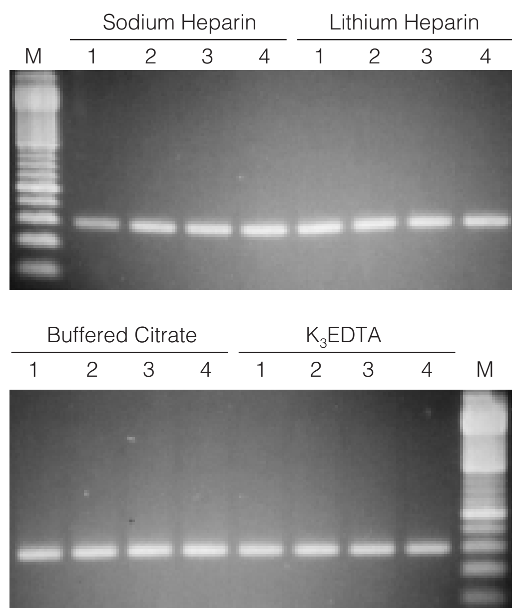

Figure 3. Amplification of a 297bp fragment of exon 13 of CFTR from 1µl of purified genomic DNA isolated from 200µl of whole blood collected into tubes containing different anticoagulants. Amplification products were visualized on 2% agarose gels stained with ethidium bromide. M = 100bp DNA Ladder (Cat.# G2101).

Purification Protocol Notes

Age of Blood Samples:

For the experiments described here, blood samples for genomic DNA purification from prepared leukocytes were less than one week old and were stored at 4°C after blood draw. We have also purified genomic DNA from frozen blood samples, and have seen no significant difference in terms of yield or functionality. DNA prepared from leukocytes from blood samples more than one week old showed some degradation characteristic of DNA from apoptotic cells (data not shown). Therefore, we do not recommend purification of genomic DNA from blood samples stored at 4°C for more than one week. Additionally, we observed a significant increase in the incidence of column clogging with whole blood processed more than one week after blood draw.

Column Clogging:

Column clogging may occur during purification under the following conditions:

1. Proteinase K treatment is omitted.
2. 1% v/v Triton® X-100 is not added to the Wizard® SV Lysis Buffer.
3. Blood samples are more than one week old.
4. More than 1ml whole blood is used for preparation of leukocytes.

Column clogging can result in lower than expected yield and purity and may cause cross-contamination between samples during the SV 96 purification process. When genomic DNA is purified from blood samples less than one week old, and the procedures given here are followed, we have not observed column clogging or cross-contamination of samples (data not shown).

Summary

This article demonstrates the utility of the Wizard® SV and SV 96 Genomic DNA Purification Systems for purification of genomic DNA from whole blood. Genomic DNA can be purified from prepared leukocytes or directly from a limited volume of whole blood. Purified genomic DNA is intact and suitable for downstream amplification reactions.

Ordering Information

Product	Size	Cat.#
Wizard® SV Genomic DNA Purification System	50 preps	A2360
	250 preps	A2361
Wizard® SV 96 Genomic DNA Purification System	1 × 96	A2370
	4 × 96	A2371
SV RNA Red Blood Cell Lysis Solution*	200ml	Z3141
Proteinase K*	100mg	V3021
Triton® X-100, Molecular Grade*	100ml	H5142
Vac-Man® 96 Vacuum Manifold	1	A2291

*For Laboratory Use.

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