

PureGenome Blood gDNA Protocol

(Blood gDNA Isolation Kit)

Introduction

The ALINE PureGenome Blood gDNA Isolation Kit utilizes ALINE propriatory paramagnetic bead technology to isolate genomic DNA from fresh or frozen whole blood containing various anticoagulants such as Citrate, EDTA, and Heparin. The protocol can be performed in both 96-well and single tube formats. The protocol consists of the following step: lysis, binding, washing and elution. Contaminants are removed during the binding and washing steps. The ALINE PureGenome Blood gDNA kit is adaptable to various automation platforms commercially available.

Applications:

- Restriction enzyme digestion
- · Human Identity resting
- PCR amplification
- Membrane hybridizations (e.g., Southern and dot/slot blots).
- AFLP, RFLP, RAPD, microsatellite and SNP analyses (for genotyping, fingerprinting,etc.)

Process Overview:

- 1. Lyse whole blood or serum in Lysis Buffer and Proteinase K
- 2. Bind genomic DNA to paramagnetic beads
- 3. Separate beads from contaminants
- 4. Wash the magnetic beads with Wash 1 Buffer to remove contaminants
- 5. Wash the magnetic beads with Wash 2 Buffer to remove contaminants
- 6. Elute DNA from magnetic particles
- 7. Transfer to new plate



The reagents have a shelf life of 6 months if stored as directed.

| Reagents description | storage condition |
|----------------------|---|
| Lysis Buffer (clear) | Room Temperature |
| Bind Buffer | 4℃ |
| Wash 1 (clear) | Room Temperature DO NOT REFRIGERATE DO NOT HEAT |
| Wash 2 (clear) | Add 100% ethanol as specified on the bottle before use. Room Temperature |

User supplied Consumables and Hardware:

| Name | Recommended Vendor and P/N |
|------------------------|--|
| Magnetic Separator: | For 96 well format: 96 well ring stand, Ambion Inc., (acquired by Applied Biosystems), # AM10050, www.appliedbiosystems.com For single tube format: Ambion Inc (acquired by Applied Biosystems), # AM10026, Single Place Magnetic Stand, www.appliedbiosystems.com |
| Reaction Plate: | -For 96 well format: 96 well 1.2 mL magnet compatible deepwell block [ABGene #AB-1127; http://www.abgene.com] -For single tube format: 2.0 mL microcentrifuge tubes; [ABGene #T5022; http://www.abgene.com] |
| Pipette Tips: | 100μL–1000 μL pipette tip [Rainin #GPSL1000;http://www.rainin.com] 5 μL – 30 μL pipette tip [ABGene #AB-0652;http://www.abgene.com] |

Reagents:

100% Ethanol

Proteinase K at 96 μ g/ μ L in storage buffer (20 mM TrisHCl, pH 8.0, 1 mM CaCl₂, 50% glycerol).

Elution buffer 10 mM TrisHCl pH8 (or Reagent Grade Water)

Plate Purification Procedure (For up to 200 µL of Blood/Serum):



Starting Material: ALINE PureGenome Blood gDNA Kit can be used with fresh or frozen whole blood containing Citrate, EDTA, or Heparin anticoagulants. Frozen samples should be thawed at room temperature or 37oC then mixed well before beginning the protocol.

The 96 well plate format can purify up to 200 μ L of blood/serum per well. The protocol below lists reagent additions based on a 200 μ L starting volume. The reagent volumes should be scaled linearly if starting with smaller sample volumes.

Please note that modification to this protocol is necessary for automated processing with the ALINE PureGenome gDNA methods for various automation platforms.

<u>Aerosol-barrier (filter) pipette tips are strongly recommended when performing the ALINE</u> PureGenome purification.

1. Mix the blood gently by inverting the stock tube several times. Tipmixing or vortexing is not recommended. Aliquot 200 μ L of fresh or frozen blood into a 1.2 mL 96-well plate (AB-1127 from ABGene).

Mixing blood thoroughly before aliquoting helps to increase yield. For a plate to be 'magnet compatible', the bottom of each well should directly contact each ring magnet. Ideally, the wells should have a round bottom without any plastic extrusions.

2. Add 400 μ L (2 x sample volume) Lysis Buffer and 9 μ L (0.045x sample volume) of 96 μ g/ μ L Proteinase K to the samples. Gently pipette mix 10 times or until well mixed.

When lysing the samples, use a mix volume that is less than the total volume in the well and pipette slowly to minimize the formation of air bubbles.

3. Incubate the samples at 37° C for 10 minutes, or room temperature for 30 minutes, to lyse.

For lysis at 37 °C, samples can be placed in an incubator/warm room or in a water bath.

4. IMPORTANT: <u>Invert the Bind Buffer bottle 20 times to ensure complete</u> resuspension of magnetic particles before using. Add 300 μL (1.5x sample volume) Bind Buffer to the samples and gently pipette mix 10 times or until well mixed.

During this step, DNA binds to the magnetic particles. When mixing, use a mix volume that is less than the total volume in the well and pipette slowly to minimize the formation



of air bubbles. Air bubbles can trap magnetic beads and prevent them from being pulled to the bottom of the plate, thus decreasing yield.

5. Place the sample plate on a magnetic plate for 15 minutes to separate.

The solution will be very dark in color and it will be difficult to see the ring of beads form at the bottom of the plate. As long as the samples have been allowed to separate for the specified time, it can be assumed that a complete ring has formed.

6. Aspirate off the supernatant and discard while the plate is situated on the magnet.

Due to the large volume of supernatant, this step may require multiple aspirations to remove all the liquid. It will be difficult to see the ring of beads at the bottom of the well until the liquid level gets low. When aspirating, place the pipette at the center of the well to avoid touching the magnetic beads.

7. <u>Take the plate off the magnet</u>. Add 800 μ L (4x sample volume) of Wash 1 and pipette mix at least 10 times (with a 1mL pipette set to 0.8 mL) or until the magnetic beads are resuspended from the bottom of the well.

Pipette mix until most of the magnetic beads are back in suspension. A few beads may still stick to the bottom of the well, and some of the resuspended beads may form clumps. If a white precipitate has formed in the Wash 1 Buffer prior to use, gently shake or stir at room temperature until the solids dissolve. DO NOT HEAT to recombine.

- 8. Place the plate back on the magnet for 10 minutes, or until the solution clears. The supernatant may be brownish in color due to residual blood components.
- **9.** Aspirate and discard the supernatant while the plate is situated on the magnet. Avoid disrupting the ring of beads.

10. Repeat steps 7-9 for a second wash with the Wash 1.

During the second wash, the beads will not clump as much as in the first wash. Mixing well is critical at this step as the Wash Buffer helps to rinse away digested protein. Incomplete resuspension may cause beads to clump together during the final elution step, which can make transfer of the eluant difficult.



- 11. Dispense 800 μ L (4x sample volume) of Wash 2 into each well while the plate is on the magnet. Wait 30 seconds, then remove and discard the supernatant. Repeat once for a total of 2 washes.
- 12. Remove as much of the liquid as possible. Add $200\mu L$ (at least = sample volume) of elution buffer to each sample to elute.

For blood samples, use of elution volumes less than 200 μ L may result in decreased recovery. Drying samples is not suggested for this protocol as over-drying the DNA onto the beads makes it difficult to fully elute the samples. If the beads appear very wet, a conservative dry time of 5 minutes at room temperature could be used.

- 13. Remove the plate from the magnet and resuspend the beads by gently pipette mixing. Incubate the plate for 2 minutes at room temperature, and then pipette mix again to complete the elution.
- 14. Place the plate back on the magnet for 10 minutes, or until the supernatant clears. Transfer the supernatant to a clean plate for storage (-20_oC).

If beads are being aspirated during the transfer you can dispense the sample back into the well and let the plate sit for an additional 10 minutes to better compact the bead ring. During the transfer, place the pipette tip in the center of the bead ring and aspirate slowly.

Tube Purification Procedure (For up to 400 µL of Blood/Serum):

Starting Material: ALINE PureGenome Blood Kit can be used with fresh or frozen whole blood containing Citrate, EDTA, or Heparin anticoagulants. Frozen samples should be thawed at room temperature or 37 °C then mixed well before beginning the protocol.

The tube format can purify up to 400 μ L of blood per 2 mL tube. The protocol below lists reagent additions based on a 400 μ L starting volume. The reagent volumes can be scaled linearly if starting with smaller or larger sample volumes.

If you are purifying 400 μ L of blood/serum, be sure to use a 2mL microcentrifuge tube (i.e. ABGene #T5022). Smaller tubes will not be able to accommodate the reagent



volumes necessary for purification of 400 µL blood/serum. If only 1.7 mL microcentrifuge tubes are available, decrease the starting blood volume to 300 µL.

Aerosol-barrier (filter) pipette tips are strongly recommended when performing the ALINE PureGenome purification.

1. Mix the blood gently by inverting the stock tube several times. Tipmixing or vortexing is not recommended. Aliquot 400 μ L of fresh or frozen blood into a 2 mL microcentrifuge tube.

Mixing blood thoroughly before aliquoting helps to increase yield.

2. Add 800 μ L (2 x sample volume) Lysis Buffer and 18 μ L (0.045 x sample volume) of 96 μ g/ μ L Proteinase K to the samples. Gently pipette tipmix 10 times or until well mixed.

Add the 800 µL in two portions to avoid wetting the filter in the filter tip. When lysing the samples, use a mix volume that is less than the total volume in the well and pipette slowly to minimize the formation of air bubbles.

3. Incubate the samples at 37° C for 10 minutes, or room temperature for 30 minutes, to lyse.

For lysis at 37°C, samples can be placed in an incubator/warm room, water bath or a thermal cycler that is compatible with microcentrifuge tubes.

4. IMPORTANT: Invert the Bind Buffer bottle 20 times to ensure complete resuspension of magnetic particles before using. Add 600 µL (1.5 x sample volume) Bind Buffer to the samples and gently pipette mix 10 times or until well mixed.

During this step, DNA binds to the magnetic particles. When mixing, use a mix volume that is less than the total volume in the well and pipette slowly to minimize the formation of air bubbles. Air bubbles can trap magnetic beads and prevent them from being pulled to the magnet, thus decreasing yield.

5. Place the tube on the single Place Magnetic Stand for 15 minutes to separate.



The solution will be very dark in color and it will be difficult to see the beads separate on the side of the tube. As long as the samples have been allowed to separate for the specified time, it can be assumed that the beads have separated completely.

6. Aspirate off the supernatant and discard while the tube is situated on the magnet.

Due to the large volume of supernatant, this step may require multiple aspirations to remove all the liquid. It will be difficult to see the beads on the side of the tube until the liquid level gets low. When aspirating, place the pipette on the side the tube opposite the magnet to avoid touching the magnetic beads.

7. <u>Take the tube off the magnet.</u> Add 1.6 mL (4x sample volume) of Wash 1 and pipette mix at least 10 times (with a 1mL pipette set to 0.8 mL) or until the magnetic beads are resuspended from the side of the tube.

Mix until the magnetic beads are back in suspension. A few beads may still stick to the side of the tube, and some of the resuspended beads may form clumps. If a white precipitate has formed in the Wash Buffer prior to use, gently shake or stir at room temperature until the solids dissolve. DO NOT HEAT to recombine.

- **8. Place the tube back on the magnet for 10 minutes, or until the solution clears.** The supernatant may be brownish in color due to residual blood components.
- 9. Aspirate and discard the supernatant while the tube is situated on the magnet.
- 10. Repeat steps 7-9 for a second wash with Wash 1.

During the second wash, the beads will not clump as much as in the first wash. Mixing well is critical at this step as the Wash Buffer helps to rinse away digested protein. Incomplete resuspension may cause beads to clump together during the final elution step which can make transfer of the eluant difficult.

11. Dispense 1.6 mL (4 x sample volume) of Wash 2 into each tube while the tube is on the magnet. Wait 30 seconds, then remove and discard the supernatant. Repeat for a total of 2 washes.

Washing with ethanol removes traces of the Wash Buffer and salts that could inhibit downstream PCR applications.



12. Remove as much of the final wash as possible. Add 400 μ L (at least 1x sample volume) of elution buffer to each sample to elute.

For blood samples, use of elution volumes less than 200 μ L may result in decreased recovery. Drying samples is not suggested for this protocol as over-drying the DNA onto the beads makes it difficult to fully elute the samples. If the beads appear very wet, a conservative dry time of 5 minutes at room temperature could be used.

- 13. Resuspend beads off the magnet by gently pipette mixing. Incubate the tube for 2 minutes at room temperature, then pipette mix again to complete the elution.
- 14. Place the tube back on the magnet for 10 minutes, or until the supernatant clears. Transfer the supernatant to a clean plate or clean tubes for storage (20° C).

If beads are being aspirated during the transfer you can dispense the sample back into the tube and let the tube incubate for an additional 10 minutes to better compact the bead pellet. During the transfer, place the pipette tip on the side of the tube opposite the bead pellet and aspirate slowly.

Warnings and Precautions

- 1. ALINE Bioscience Corp. kits are intended *For Laboratory Use* only.
- 2. The U.S. Centers for Disease Control, the Food and Drug Administration, and the American Hospital Association recommend applying "universal precautions" when handling blood and body fluids to protect health care and laboratory workers.

Purified DNA may contain blood-borne pathogens, so effective barrier protections should be used throughout all stages of this procedure.