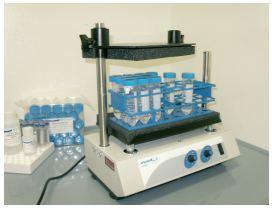
PreAnalytiX
A QIAGEN / BD Company

Supplementary Protocol for Manual, High-Throughput Genomic DNA Purification Using the PAXgene™ Blood DNA System

IMPORTANT: Please read the PAXgene Blood DNA Kit Handbook, paying careful attention to the safety information, before following this supplementary protocol. For further safety information, please consult the appropriate material safety data sheets (MSDSs). These are available online at **www.preanalytix.com/DNA_MSDS.asp** for each kit component.

Equipment recommendations

Equipment	Supplier	Model	
Multitube vortexer	VWR International	Multitube Vortexer	
Centrifuge	QIAGEN	Centrifuge 4-15C	
Centrifuge rotor and buckets	Sigma	Sigma Laboratory Centrifuge Rotor and Bucket for 7 x 50 ml processing tubes	
Bottle-top dispenser	Brand	Seripettor®	
Test tube racks for 24 x 50 ml tubes	Nalgene	Unwire™ Test Tube Racks	
Hand-dispenser pipet	Eppendorf	Multipette® Plus	

For further information call QIAGEN Technical Services or your local QIAGEN distributor.

General recommendations

- Use bottle-top dispensers to dispense Buffer BG2 (wash buffer), isopropanol, and ethanol.
- Use a hand-dispenser pipet to dispense Buffer BG3 (digestion buffer that has PreAnalytiX Protease added) and Buffer BG4 (resuspension buffer).
- Always work with 24 samples at any one time.
- During incubation and centrifugation steps, work with another batch of 24 samples.
- Process multiples of 24 samples (e.g., 72, 96, or 120 samples).
- Invert a rack of 24 tubes in parallel.
- Use a centrifuge rotor capable of holding 24–28 tubes at the same time.
- Use a multitube vortexer.
- DNA samples are stable when stored in Buffer BG3 (digestion buffer) and after the addition of isopropanol and ethanol. Therefore, the procedure can be interrupted and samples left at room temperature (18–25ºC) after steps 7, 8, 10, and 13 for at least 24 hours.

Preparation of reagents

Buffer BG3/PreAnalytiX Protease mixture

For every sample to be processed, mix 5 ml Buffer BG3 (digestion buffer) and 50 µl reconstituted PreAnalytiX Protease (see “Preparation of reagents” in the *PAXgene Blood DNA Kit Handbook*). For example, if 96 samples are to be processed, mix 500 ml Buffer BG3 and 5 ml PreAnalytiX Protease (each PAXgene Blood DNA Kit [25] contains enough Buffer BG3 and PreAnalytiX Protease to process 28 samples).

The Buffer BG3/PreAnalytiX Protease mix should not be prepared more than 2 hours before use.

High-throughput manual protocol

1. **Pour blood from 24 PAXgene Blood DNA Tubes into 24 Processing Tubes already containing 25 ml Buffer BG1 and standing upright in a rack. Mix by inverting the rack 5 times.**
   If the blood in the PAXgene Blood DNA Tubes have separated into plasma and red blood cells, invert the tubes carefully 10 times to homogenize the samples.
2. **Centrifuge for 5 min at 2500 x *g* in a swing-out rotor.**
   During this centrifugation step you may work with a second batch of 24 samples.
3. **Carefully discard the supernatant and add 5 ml Buffer BG2 with a dispenser.**
   In rare cases the pellet may be loose, so pour slowly.
4. **Wash the pellets by vortexing on a multitube vortexer vigorously for 5 s.**
5. **Centrifuge for 3 min at 2500 x *g* in a swing-out rotor.**
   During this centrifugation step you may work with a third batch of 24 samples or the second batch from step 2.
6. **Carefully discard the supernatant and place tubes back in the rack.**
   In rare cases the pellet may be loose, so pour slowly.

7. **Add 5 ml Buffer BG3/PreAnalytiX Protease (see "Preparation of reagents") with a dispenser or hand-dispenser pipet, close tubes, and vortex on a multitube vortexer for 20 s at high speed.**
   Vortexing for 20 s is essential to dissolve the pellet completely. Shorter vortexing times may lead to incomplete resuspension of the pellet and reduced DNA yield or purity. After resuspension, samples can be left at room temperature (18–25°C) for up to 24 h or at 2–8ºC for up to 7 days.
8. **Place the tubes in a heating block or water bath and incubate at 65°C for 10 min.**
   The sample changes color from light red to light green, indicating that protein digestion has occurred. After the incubation, samples can be left at room temperature (18–25°C) for up to 24 h or at 2–8ºC for up to 7 days. During this incubation step you may work with a fourth batch of 24 samples or a previous batch from step 2 or 5.
9. **Vortex again on a multitube vortexer for 5 s at high speed.**
10. **Add 5 ml isopropanol (100%) and mix by inverting the rack at least 20 times until the white DNA strands clump visibly together.**
    Complete mixing with isopropanol is essential to precipitate the DNA and should be checked by inspection. Only tightly clumped DNA strands can be efficiently pelleted by centrifugation. Do not vortex as this might reduce DNA yield. After precipitation samples can be stored for at least 24 h at room temperature (18–25ºC).
11. **Centrifuge for 3 min at 2500 x *g*.**
    During this centrifugation step you may work with a fifth batch of 24 samples or a previous batch from step 2, 5, or 8.
12. **Discard the supernatant and add 5 ml of 70% (v/v) ethanol.**
    In rare cases the pellet may be loose, so pour slowly. Inverting the tubes onto absorbent paper minimizes backflow of isopropanol from the rim and sides of the tubes onto the pellets.
13. **Wash the pellets briefly by gently shaking the rack.**
    Samples can be stored for at least 24 h at room temperature (18–25ºC).
14. **Centrifuge for 3 min at 2500 x *g*.**
    During this centrifugation step you may work with a sixth batch of 24 samples or a previous batch from step 2, 5, 8, or 11.
15. **Discard the supernatant and leave the tubes inverted on a clean piece of absorbent paper for at least 5 min.**
    In rare cases the pellet may be loose, so pour slowly. Inverting the tubes onto absorbent paper minimizes backflow of ethanol from the rim and sides of the tubes onto the pellets.
16. **Carefully dab the tubes onto absorbent paper to remove ethanol from the rim and leave them inverted for a further 5 min to allow the DNA pellet to dry.**
    Avoid over-drying the pellet, since over-dried DNA is very difficult to dissolve.

17. **Add 1 ml Buffer BG4 with a hand-dispenser pipet and dissolve the DNA by incubating for 1 h at 65°C in a heating block or water bath, followed by incubation overnight at room temperature (18–25ºC).**
Highly concentrated, high-molecular-weight genomic DNA samples may not redissolve completely after an incubation of 1 h at 65°C. We therefore recommend an additional overnight (12 h) incubation at room temperature.