



High quality genomic DNA extraction from whole blood stored in PAXgene™ Blood DNA Tube

Introduction

Whole blood samples are critical for disease prediction and diagnosis. The PAXgene™ Blood DNA Tube (PreAnalytiX) is intended for the collection of whole blood for genomic DNA (gDNA) isolation. Typically, before gDNA purification, scientists need to centrifuge whole blood specimens to pellet down the cell nuclei and mitochondria. This centrifuge step can be labor intensive and increases hands on time. Here we show that using the GenFind V2 protocol, researchers can process whole blood directly from PAXgene™ Blood DNA Tubes with no centrifugation step required. By using GenFind V2, isolation from 200 μ L whole blood yielded about 6 μ g of high purity gDNA. This method is also automated and demonstrated on Biomek liquid handler for limited hands on time.

Workflow



Method

1. Thaw PAXgene™ Blood DNA Tubes by incubating the tubes in a water bath at RT or 55°C for 30 minutes. After thawing, carefully invert the tubes 10 times as per the PAXgene™ Blood DNA Tubes protocol.
2. Transfer out 200 μ L blood from the PAXgene™ Blood DNA Tubes into a magnet-compatible 1.2 mL 96-well plate.
3. Add 100 μ L detergent buffer (6.7 % SDS / 7.1 % Triton X-100) to samples and mix well.
4. Lyse whole blood in Lysis Buffer and Proteinase K.
5. Bind gDNA to paramagnetic beads.
6. Separate beads from contaminants.
7. Wash the magnetic beads with Wash Buffer 1 to remove contaminants.
8. Wash the magnetic beads with Wash Buffer 2 to remove contaminants.
9. Elute DNA from magnetic particles.
10. Transfer to new plate.

Result

gDNA was extracted from 200 μ L frozen blood collected in the PAXgene™ Blood DNA tubes. DNA yield (Figure 1) and purity (Figure 2) were measured using NanoDrop (Thermo Fisher Scientific). The average DNA yield was about 6 μ g. Increasing lysis temperature to 55°C did not significantly increase DNA yield; however, it improved the DNA purity: both 260/280 and 260/230 were above 1.8.

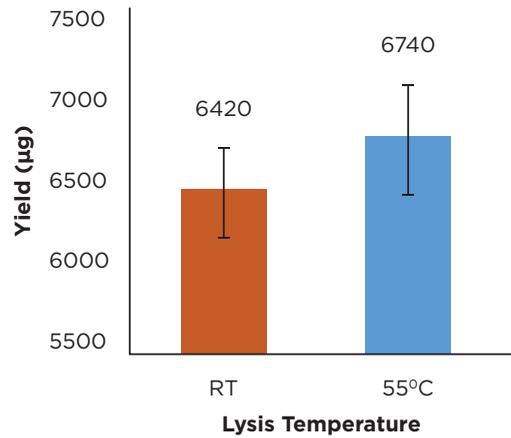


Figure 1. The DNA yield from 200 μ L blood.

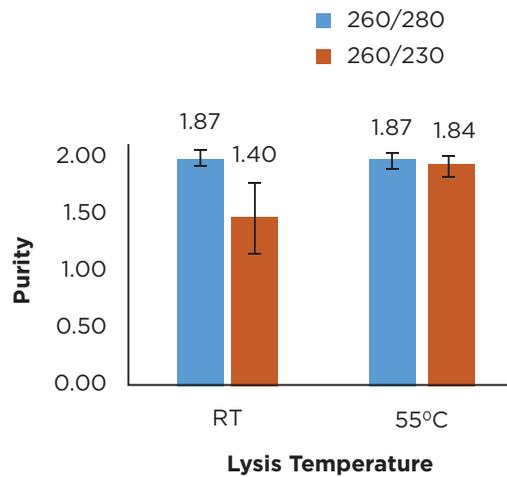


Figure 2. The DNA purity from 200 μ L blood.

Conclusion

The GenFind V2 chemistry offers robust DNA extraction. Whole blood can be used as input material. The workflow is simple with no centrifugation needed. It can also be automated to decrease hands on time further.

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