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GenSolve User Manual:
Whole Blood DNA Recovery from
FTA paper, Guthrie cards, and GenPlate
elements

GENOMIC DNA ARCHIVING

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For use with:

- GVR-100, GenSolve for DNA recovery from whole blood spotted paper, 100 recoveries
- GVBIOSTART-1

Summary

The GenSolve kit recovers double stranded DNA from paper spotted with blood or other biological material, regardless of storage time, for subsequent purification via Qiagen QIAamp Blood DNA Mini Kit. DNA can be efficiently recovered from treated paper, i.e. FTA, and untreated paper, i.e. Guthrie cards. The FTA chemistry, available in a 384 well format as GenPlates, lyses blood cells upon contact and binds DNA, stabilizing it over the long term at room temperature.

The Protease component of GenSolve coupled with recovery solution enhances digestion of dried biological material at an elevated temperature, liberating bound DNA from the paper. The resulting lysate, which is a mixture of DNA, cellular debris and FTA chemicals, if applicable, is then applied directly onto a purification membrane. Subsequent washing steps separate the DNA from cellular material and a final elution step results in purified DNA ready for most genetic analysis techniques.

Recovered DNA is at a concentration that is too low to be measured by traditional spectrophotometry. A fluorometric method such as PicoGreen or quantitative PCR is recommended for quantitation.

Expected Results

GenSolve is designed to meet certain performance specifications for DNA yield and quality when recovering from whole-blood spotted 6mm disks of FTA® paper (i.e. GenPlate elements).

- Yield – Average of 130ng per disk with a maximum yield of about 350ng/disk and a minimum yield of about 50ng/disk depending on the White Blood Cell content of the original sample
- Concentration – Ranges from 0.5 to 2 ng/µl
- Size – Majority of fragments are at least 35kb
- Reproducibility – <20%CV between triplicate samples and between assays
- PCR Amplification – Successful amplification with primers used in both research and clinical laboratories

Safety Information

- Kit is intended *For Laboratory Use.*
- Certain chemicals used in GenSolve reagents may be hazardous: lithium dodecyl sulfate.
- Certain chemicals present in FTA paper may be hazardous: sodium dodecyl sulfate, uric acid and ethylenediaminetetraacetic acid.
- GenVault Protease contains subtilism which is a sensitizer and an irritant.
- Care should be taken when working with hazardous chemicals, such as minimizing contact; wearing appropriate personal protective equipment (safety glasses, gloves and a lab coat). Avoid contact with eyes and ingestion.

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Storage

- Protease should be stored at 2-8°C
- After resuspension and addition of Protease, Recovery Solution A should be used within 2-3 hours for maximum DNA yield (Discard after 24 hours)

GenSolve Kit Components

- Recovery Solution A, 4 vials
- 1.0% LiDS Solution (Lithium Dodecyl Sulfate), 66ml
- Protease, ≥16 U/g, 2.5ml *(Note this reagent is typically yellow, brown, or orange in color)*
- Recovery Solution B, 2.5ml

Additional Materials Required

- GenVault Incubator/Shaker (Catalog #GVMXR-1)
- GenVault Spin Basket/Tube Assembly (Catalog #GVSPIN-250)
- 100% Ethanol
- Qiagen QIAamp DNA Blood Mini Kit (50 preps, Catalog #51104)
- Qiagen 2.0ml collection tubes (Catalog #19201)
- Microfuge Tubes, 1.7ml and 2.0ml
- P200 and P1000 pipets, pipet tips
- Microcentrifuge

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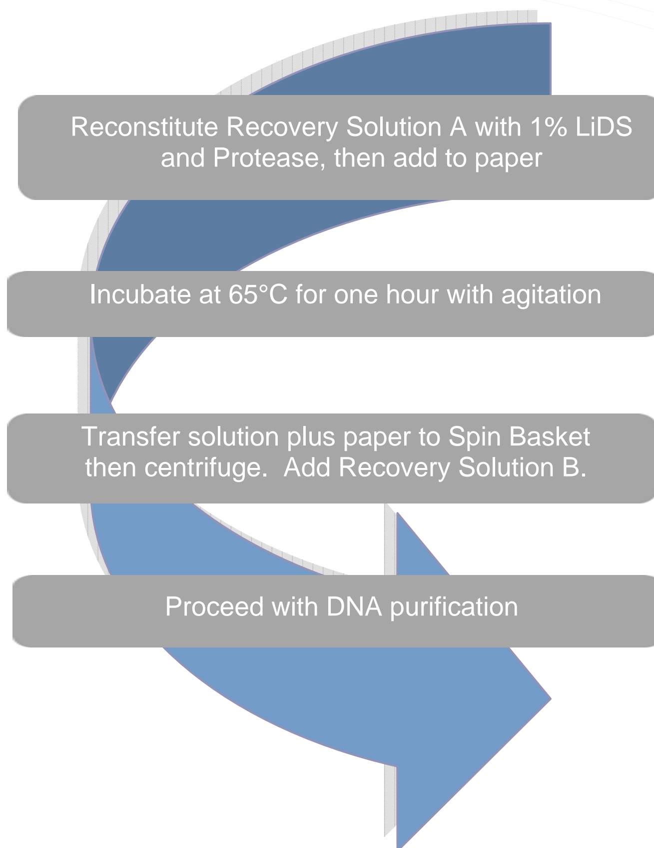
Overview of Procedure



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DNA Recovery

Notes: Protocol can efficiently process GenPlate elements, and 6mm FTA or Guthrie card punches. For maximum DNA yield ensure that spotted paper has been cured for at least two weeks. If higher concentrations or more DNA is desired, up to six elements (6mm disks) can be processed simultaneously without changing protocol.

1. Reconstitute Recovery Solution A with 16ml of 1.0% LiDS solution provided. Vortex to completely re-suspend the lyophilized reagent. One vial is sufficient for 25 recoveries.
2. Pre-heat Incubator/Shaker to 65°C.
3. Add 535µl of Protease into the vial from step 1 and vortex briefly. *(After addition of Protease, Solution A should be used within 2-3 hours for maximum recovery. Protease is typically yellow, brown, or orange in color)*
4. Punch element(s) into one 2ml microtube for each unique sample.
5. Add 620µl of Recovery Solution A/Protease mix.
6. Place the tube in the Incubator/Shaker pre-heated to 65°C. Vortex ~1 min at 1,400 rpm and inspect to make sure that each element is completely submerged in the solution; repeat until all elements are submerged. Continue vortexing for one hour. It is important to vortex at 1,400 rpm for maximum DNA recovery. If speed <1,400 rpm, vortex for 2 hours, speed < 700 rpm is not recommended.
7. Centrifuge at 16,300 x g for 0.5 minutes to collect liquid off cap.
8. Add 20µl of Recovery Solution B to a new microtube and insert a spin basket. Transfer the solution from

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step 7 into the Spin Basket making sure to transfer along the element(s) by scooping it with pipet tip.

9. Centrifuge at 16,300 x g for two minutes.
10. Discard Spin Basket and element.
11. Pulse vortex each microtube.
12. Proceed directly to DNA purification.

DNA Purification

Note: 1 set of spin column/2ml collection tube, 4 sets of 2ml collection tubes and 1 set of microtubes per recovery will be needed for this procedure. Make certain buffers AW1 and AW2 from Qiagen (51104) kit have ethanol added per Qiagen's protocol before starting this procedure.

1. Add 600µl of 100% Ethanol to each microtube containing recovered DNA.
2. Pulse-vortex each sample. Centrifuge briefly
3. Load 600µl of the sample onto a spin column/collection tube. Close the cap and centrifuge at 6,000 x g for 1 minute. Place the spin column in a new 2ml collection tube and discard the tube containing the filtrate.
4. Repeat step 3 until the entire sample has been applied onto the spin column.
5. Add 500µl of AW1 Buffer onto the spin column and centrifuge at 6,000 x g for 1 minute. Discard the collection tube containing the filtrate and place the spin column in a new 2ml collection tube.
6. Add 500µl of AW2 Buffer onto the spin column and centrifuge at 16,300 x g for 4 minutes. Discard the collection tube containing the filtrate and place the spin column in a new 2ml collection tube.
7. Centrifuge at 16,300 x g for 1.5 minutes.

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8. Place the spin column in a new 1.7ml tube.
9. Elute the DNA sample by adding 200 µl of AE Buffer to spin column. Incubate the sample at room temperature for 5 minutes and centrifuge at 6000 x g for 1 minute.

THE ELUATE IS READY FOR QUANTITATION AND DOWNSTREAM PROCESSING.

DNA Concentration

This addendum will assist in concentrating recovered DNA for use in highly-multiplexed genotyping assays.
Note: protocol requires Millipore Microcon YM-100 (Catalog #42413) columns. Do not allow Microcon membrane to dry with sample on it. Do not touch the membrane with pipet tip during sample addition or wash steps. When washing and concentrating gDNA samples, do not spin at more than 500xg (2400 rpm).

1. Insert MICROCON-YM-100 sample reservoir into a microfuge tube.
2. Add 50 µL of water and spin at 12,300 rpm (14,000 x g) for 3 minutes.
3. Apply up to 500µl of SAMPLE onto MICROCON-YM-100.
4. Spin 2,400 rpm (500 x g) for 15 minutes (LOW SPEED SPIN).
5. Decant microfuge tube and repeat steps 3 and 4 until the entire SAMPLE has been applied.
6. Transfer MICROCON-YM-100 to a new microtube.
7. Add 250 µL of water to MICROCON-YM-100.
8. Spin at 2,400 rpm for 15 minutes (LOW SPEED SPIN).
9. Optional: decant microtube and repeat steps 7-8.

10. Continue to centrifuge until ~25 µL remains on the column. For maximum recovery, do not spin to dryness. If processing multiple MICROCON-YM-100 units, independently monitor each unit, as they concentrate at different rates.
11. Gently pipet mix the ~ 25ul being careful not to touch the filter at the bottom of the filtration unit.
12. Invert MICROCON-YM-100 and transfer into a new microtube.
13. Spin at 3,500 rpm (1,100 x g) for 3 minutes to collect the DNA.

Frequently Asked Questions (FAQs)

HOW CAN I QUANTITATE MY RECOVERED DNA?

GenVault recommends a PicoGreen assay, as spectrophotometric analysis using Optical Density (OD) at 260nm is not sensitive enough. PicoGreen assay uses an intercalating dye to specifically quantitate only double-stranded DNA.

HOW CAN I CONVERT RPM TO G (RCF)?

Incorrect spinning can lead to low yields and purity. Check your centrifuge setting using the following equation: $g(rcf) = 1.12*r*(rpm/1000)^2$ where r is the radius of the rotor in mm.

I LEFT MY PROTEASE OUT AT ROOM TEMPERATURE. CAN I STILL USE IT?

Yes, up to 18-24 hours. Store the Protease at 4°C. Refer to page 4 for storage information.

HOW DO I GET A MATERIAL SAFETY DATA SHEET (MSDS)?

MSDS documents are available on GenVault's website: www.genvault.com

Notes

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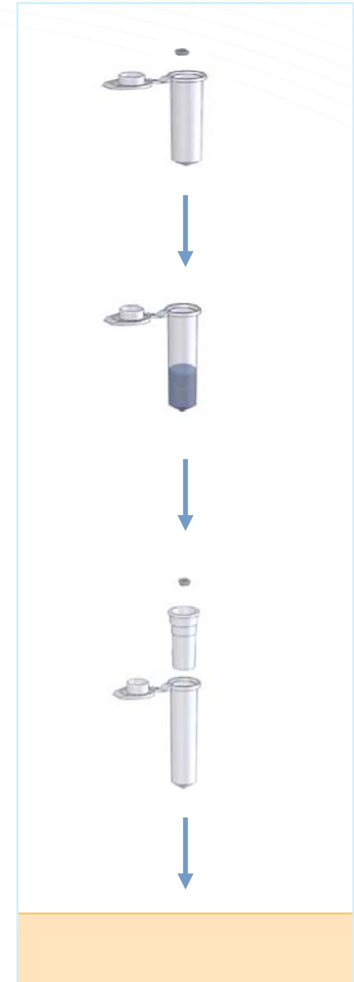
Quick Protocol



1. Remove element(s) from GenPlate and place into a 2.0ml microcentrifuge tube.
2. Add 620µl of fresh Recovery Solution A + Protease, and incubate at 65°C for one hour with agitation.
3. Add Spin Basket to a new 2.0ml microcentrifuge tube containing 20µl of Recovery Solution B, transfer solution + element(s) from step 2 into Spin Basket and Centrifuge.
4. Proceed with DNA purification.

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