Product Application

DNA Extraction from Whole Blood Spotted on FTA® Cards Using the ReliaPrep™ FFPE gDNA Miniprep System

Purify gDNA from whole blood collected on FTA® cards using the ReliaPrep™ FFPE gDNA Miniprep System coupled with Incubation Buffer

Kit: ReliaPrep™ FFPE gDNA Miniprep System (Cat. A2351)

Analyses: UV absorbance and QuantiFluor® quantitation, qPCR amplification

Sample Type(s): 100ul of Human Whole Blood spotted on FTA cards

Input: One to f 5mm punches from FTA cards

This protocol was developed by Promega Applications Scientists and is intended for research use only.

The user is responsible for determining its suitability in the user's application.

Further information can be found in Technical Manual #TM352, available at: **www.promega.com/protocols**

Materials Required:

- ReliaPrep™ FFPE gDNA Miniprep System (A2351)
- Incubation Buffer (D920B)
- DNA IQ™ Spin Baskets (V1221)
- FTA Classic Cards (GE Healthcare #WB120205)

Protocol

1. Add 200µl of Incubation Buffer to up to five 5mm punches of blood spotted on FTA cards.
2. Add 20µl of Proteinase K and mix by pipetting.
3. Incubate at 56°C for 30 minutes
4. Add entire contents of tube to a DNA IQ™ Spin Baskets placed in a clean 1.5ml microtube
5. Spin the sample at maximum speed for 2 minutes.
6. Transfer the flowthrough to a clean 1.5ml microtube.
7. Add 220µl of BL Buffer and 240µl of ethanol (95–100%) and vortex briefly to mix.
8. Transfer the sample to a Binding Column/Collection Tube assembly, and cap the column.

Proceed with the protocol in the technical manual (TM352) to purify the DNA using the ReliaPrep™ minicolumn.

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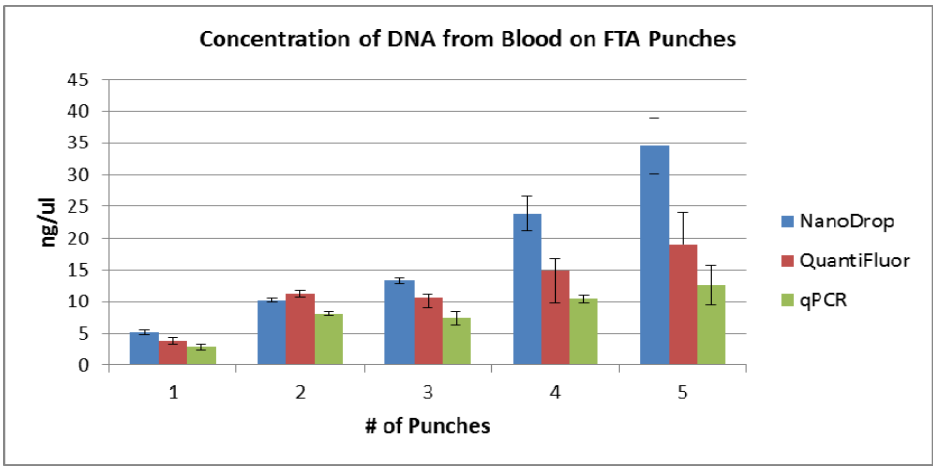
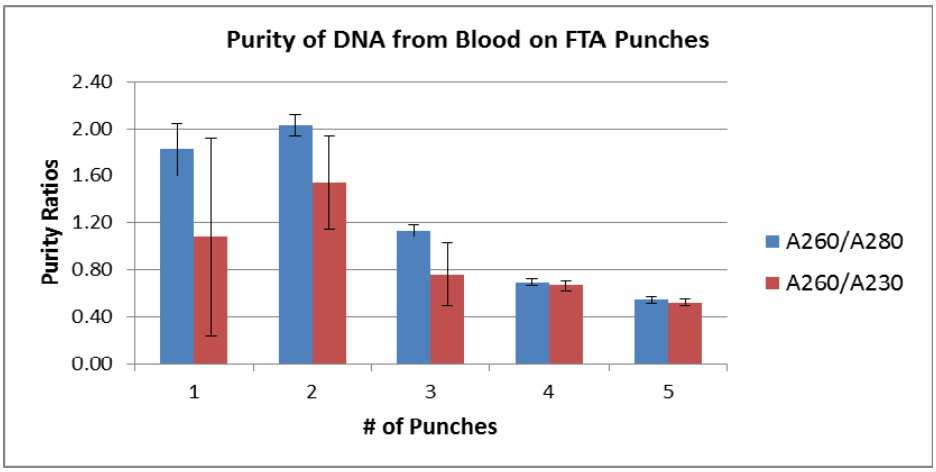
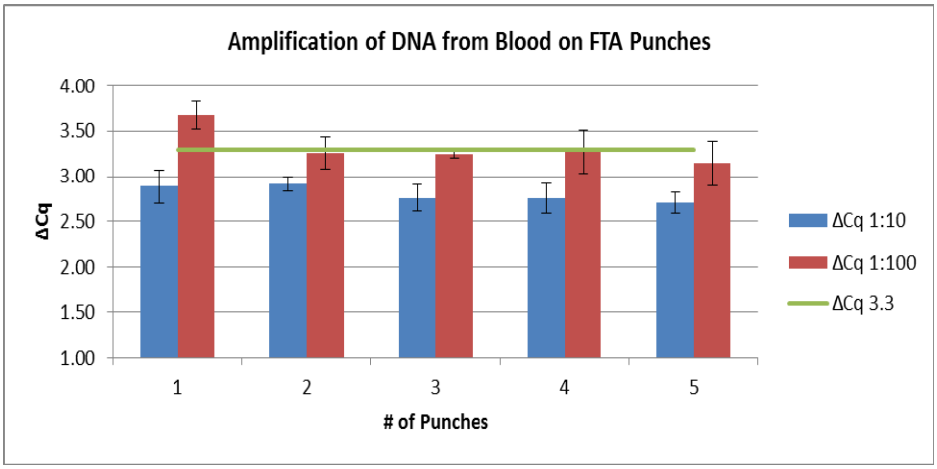
Results:



Top: Average concentration of DNA purified from whole blood on multiple 5mm FTA punches using the ReliaPrep™ FFPE gDNA Miniprep System as measured by NanoDrop, Quantifluor™, and qPCR. N=3. Standard deviations are shown.



Middle: Purity ratios of DNA from whole blood on multiple 5mm FTA punches using the ReliaPrep™ FFPE gDNA Miniprep System. N=3. Standard deviations are shown.



Bottom: qPCR analysis of DNA from whole blood on multiple 5mm FTA punches using the ReliaPrep™ FFPE gDNA Miniprep System and the GoTaq qPCR Master Mix. The average ΔCq of undiluted and 1:10 diluted samples as well as the average ΔCq of 1:10 and 1:100 diluted samples are shown. A ΔCq of 3.3 would suggest that no PCR inhibition is evident. N=3. Standard deviations are shown.