Promega

Product Application

Automated DNA Purification from Blood on FTA Cards

Purify DNA from whole blood spotted on FTA® Classic cards using the Maxwell® RSC FFPE Plus DNA Kit and the Maxwell® RSC Instrument.

Kit: Maxwell® RSC FFPE Plus DNA Kit (Cat.# AS1720)

Analyses: qPCR, Next Generation Sequencing (NGS)

Sample Type: 100µl of human whole blood spotted on FTA® Classic cards

Input: One to five 5mm punches

Materials Required:

- Maxwell® RSC FFPE Plus DNA Kit (Cat.# AS1720)
- DNA IQ™ Spin Baskets (Cat.# V1225)
- Maxwell® RSC Instrument (Cat.# AS4500)
- Heat block set to 56°C

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

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM574, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Protocol:

1. Place one to five 5mm punches from whole blood spotted on FTA® Classic cards in a clean 1.5ml tube.
2. Add 180µl of Incubation Buffer and 20µl prepared Proteinase K. Vortex.
3. Ensure punches are fully submerged, then incubate at 56°C for 30 minutes.
4. Transfer the entire contents to a DNA IQ™ Spin Basket placed in a clean 1.5ml tube.
5. Centrifuge at maximum speed for 2 minutes.
6. Add 400µl of Lysis Buffer to the lysate. Vortex.
7. Transfer the full lysate to well #1 of the Maxwell® RSC FFPE Plus DNA Kit cartridge.
8. Place an elution tube with 50µl of Nuclease-Free Water onto the sample rack.
9. Place a plunger in well #8 of the cartridge.
10. Select the Maxwell® RSC FFPE Plus DNA method and run.

Results:

Amplifiable DNA was purified from one to five 5mm punches of blood spotted on FTA® Classic cards using the method described above (Figure 1). Libraries were prepared from the purified DNA with the AmpliSeq™ for Illumina Cancer HotSpot Panel v2. Cleanup steps were performed using the ProNex® Size-Selective Purification System (Cat.# NG2001). Libraries were quantified with the ProNex® NGS Library Quant Kit (Cat.# NG1201) and run on a MiniSeq instrument with a MiniSeq High Output Reagent Kit for 150bp paired end sequencing. Sequencing data were analyzed using the DNA Amplicon module on Local Run Manager. Trends were similar among libraries prepared with DNA from 3 individuals, results for 1 representative individual is shown for clarity (Figures 2 and 3). DNA purified according to the method described above was compatible with the NGS workflow and produced high quality sequencing data.

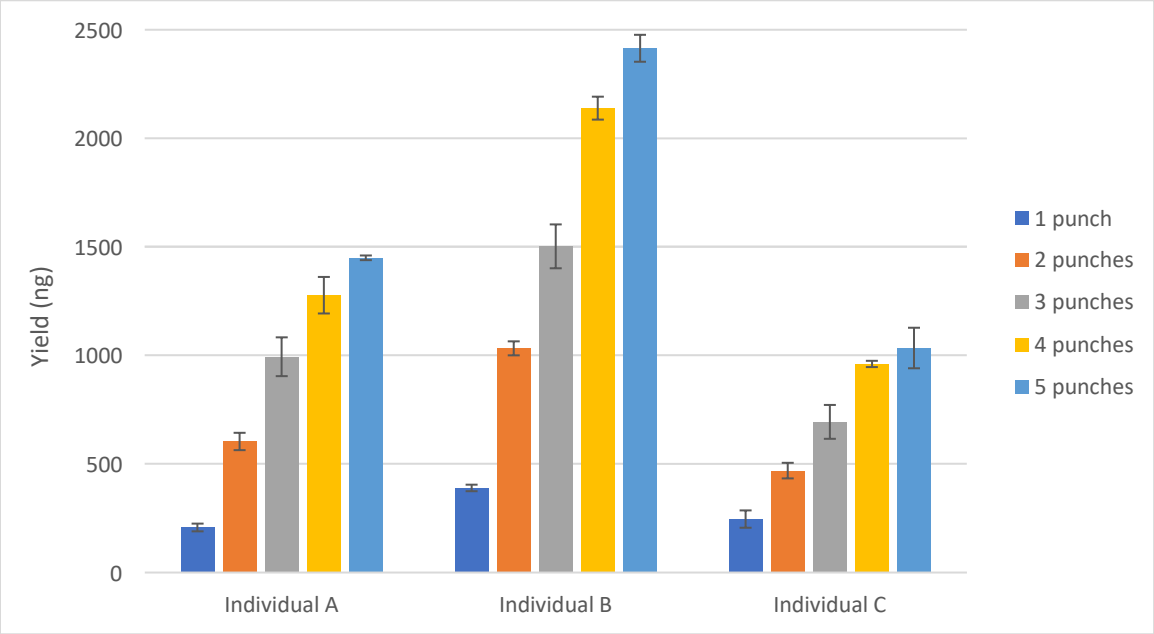

Figure 1. Increasing yield of amplifiable DNA purified from one to five 5mm punches of whole blood spotted on FTA® Classic cards. 100µl of fresh whole blood from three individuals was spotted on FTA® Classic cards (Cat.# WB120205). One to five 5mm punches were taken from blood spots and DNA was purified using the method described above. DNA was quantified by qPCR with the 75bp target of the ProNex® DNA QC Assay (Cat.# NG1004). Mean and standard deviation of triplicate purifications amplified in duplicate is shown.

Metric	Value
Cluster Density	128 ± 26 K/mm²
Total Reads	43,097,358
Reads Passing Filter	40,348,770
% Reads Passing Filter	93.62%
% Reads Identified	96.17%
Average % Q30 score	95.93%

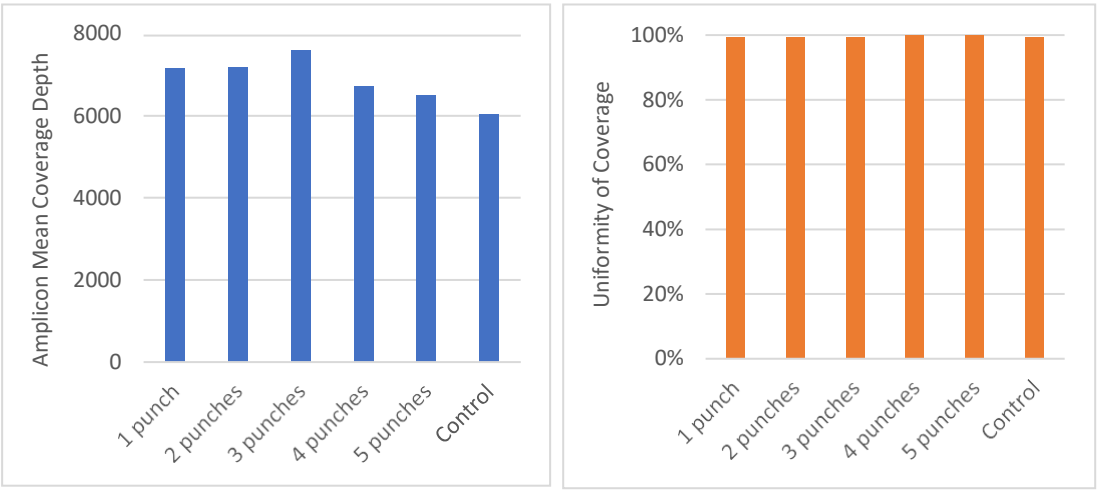

Figure 2. High quality sequencing data was produced from libraries prepared from DNA purified from blood spotted on FTA® Classic cards. DNA samples for Individual A or Tru-Q 2 reference standard (Horizon) were used as input for the library preparation workflow. **Left.** MiniSeq instrument quality metrics for the sequencing run. **Center.** Average mean coverage depth across all 207 amplicons for individual A and the reference standard (control). **Right.** Coverage uniformity across all 207 amplicons for individual A. Uniformity of coverage describes the percentage of amplicons with a sequencing depth greater than 0.2 times the mean coverage depth.

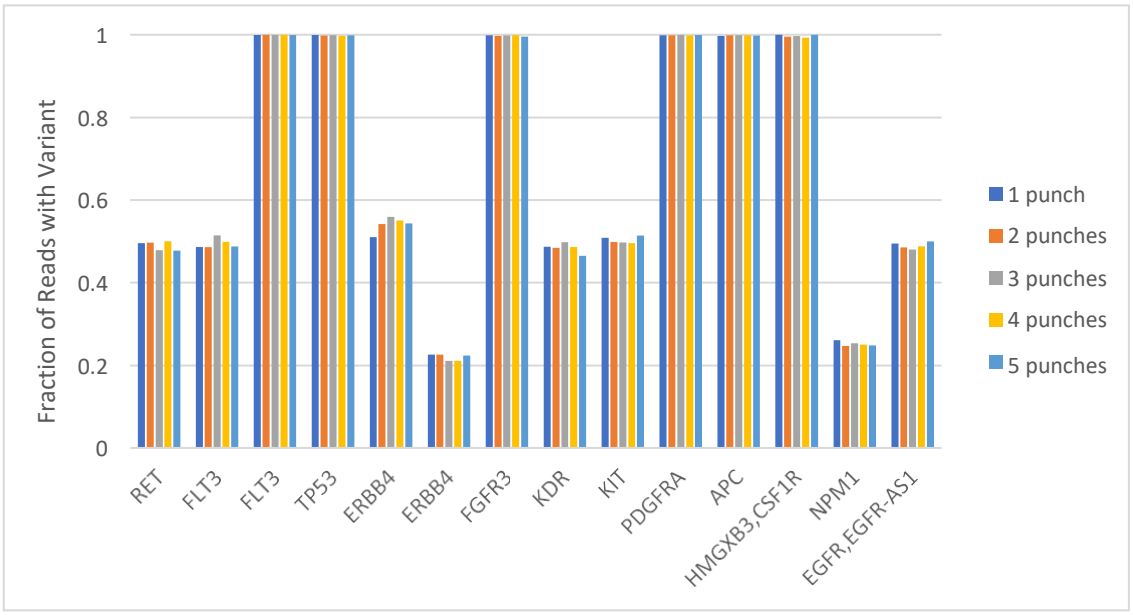

Figure 3. Detected variants are consistent across samples purified from different numbers of punches. Sequencing data was analyzed with the following parameters: somatic setting, 3.5% allele frequency cutoff, 100 read depth requirement for variant calling, indel correction on. Samples with strand bias > -100 were omitted. Though blood represents a sample type that would display germline mutations, data were analyzed with the somatic setting in order to detect all variants present in the Tru-Q 2 reference standard. Variant frequency for variants identified in individual A are shown plotted as the fraction of identified reads with the variation relative to the targeted gene.