

Gene expression and genomic DNA stabilization in whole blood stored at room temperature

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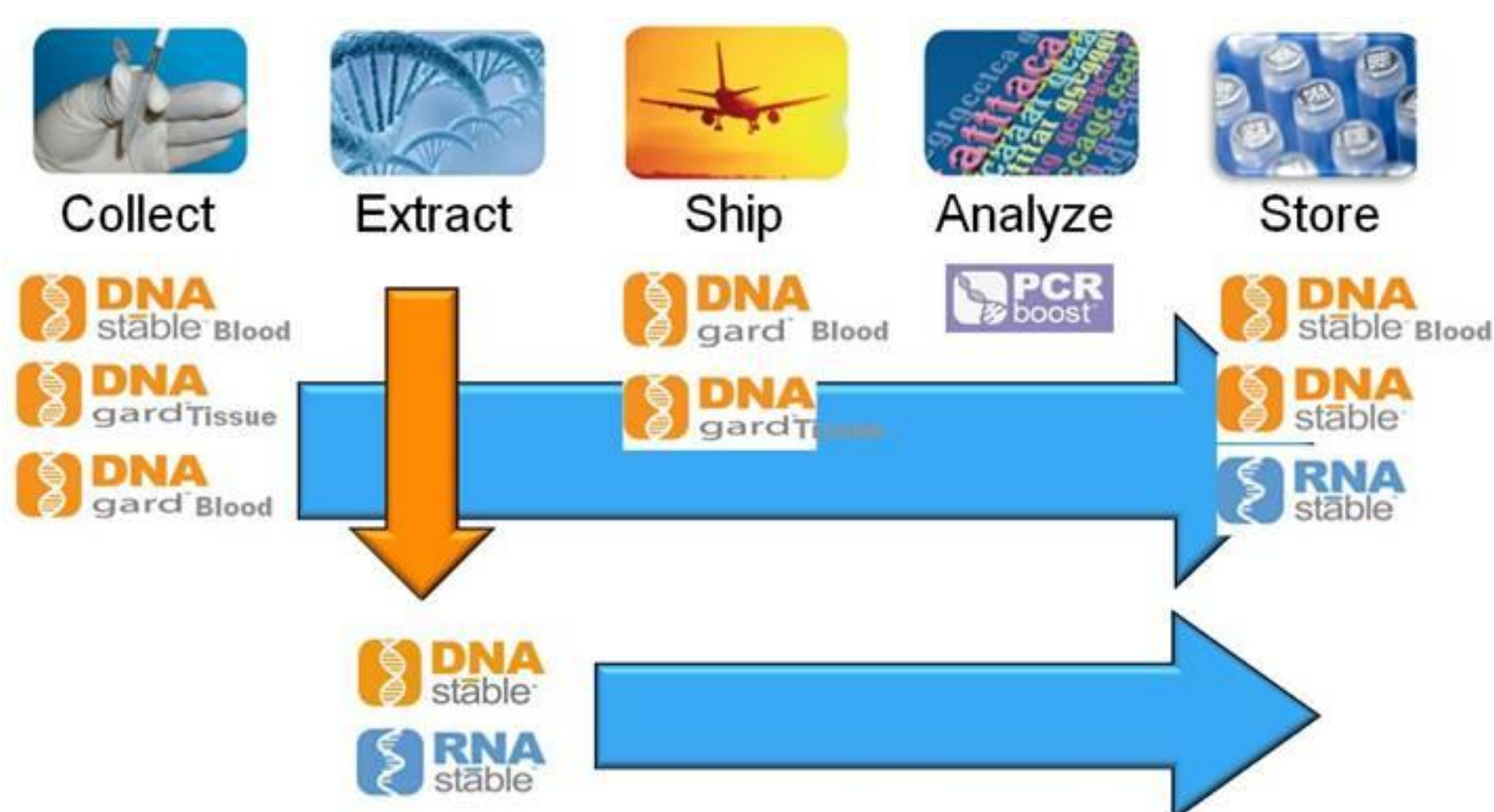
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Abstract

Genomic and mitochondrial DNA and gene expression profiles from blood samples are increasingly being used to diagnose specific diseases, monitor disease progression and assess patient responses to medical treatment. However, such applications require reliable preservation of total RNA and DNA in patient samples during collection, transport and storage. Numerous agents threaten DNA and RNA profiles in collected blood samples, including nucleolytic attack, oxidative damage and hydrolysis. Such damage can severely disrupt genotyping and DNA sequencing efforts. Transcription profiles are highly dynamic and can change rapidly during and after blood collection using current methods, potentially affecting interpretation of the expression analysis. In this study, we compare freezer storage with room temperature stabilization formulations, DNAgard Blood and PAXgene Blood DNA, in their capacities to preserve genomic DNA integrity in human whole blood for 8 months. In a separate analysis, we compare freezer storage with room temperature stabilization formulations, PAXgene Blood RNA and Biomatrix formulation RGB, in their capacities to stabilize RNA in whole blood samples. We assess changes in the relative expression of a panel of 89 genes in whole blood specimens over the course of 7 days using RT-qPCR and the Human Common Cytokines PCR Array (SABiosciences). Our results demonstrate that room temperature blood storage is a valid alternative to cold-storage for preserving gDNA for at least 8 months and RNA for at least 7 days.

"Gard" stabilization technologies



Biomatrix's "Gard" technologies are designed for the immediate stabilization of DNA and RNA from mammalian whole blood with the convenience of room temperature shipping, processing and storage. The liquid storage reagent rapidly permeates cell membranes to stabilize and protect genomic DNA and RNA. The use of Biomatrix's "Gard" technologies allows a streamlined workflow from blood collection in the field to sample processing in the laboratory.

Results -- genomic DNA stability

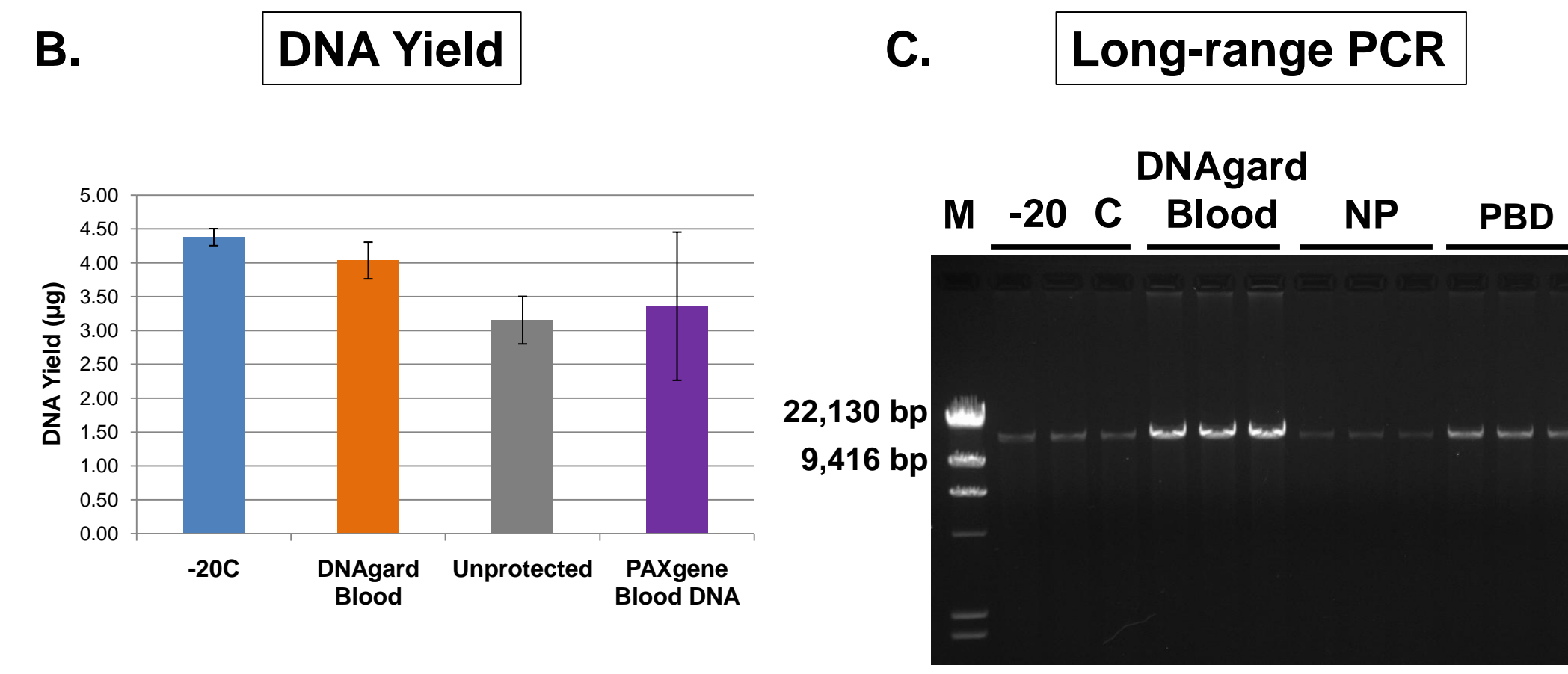
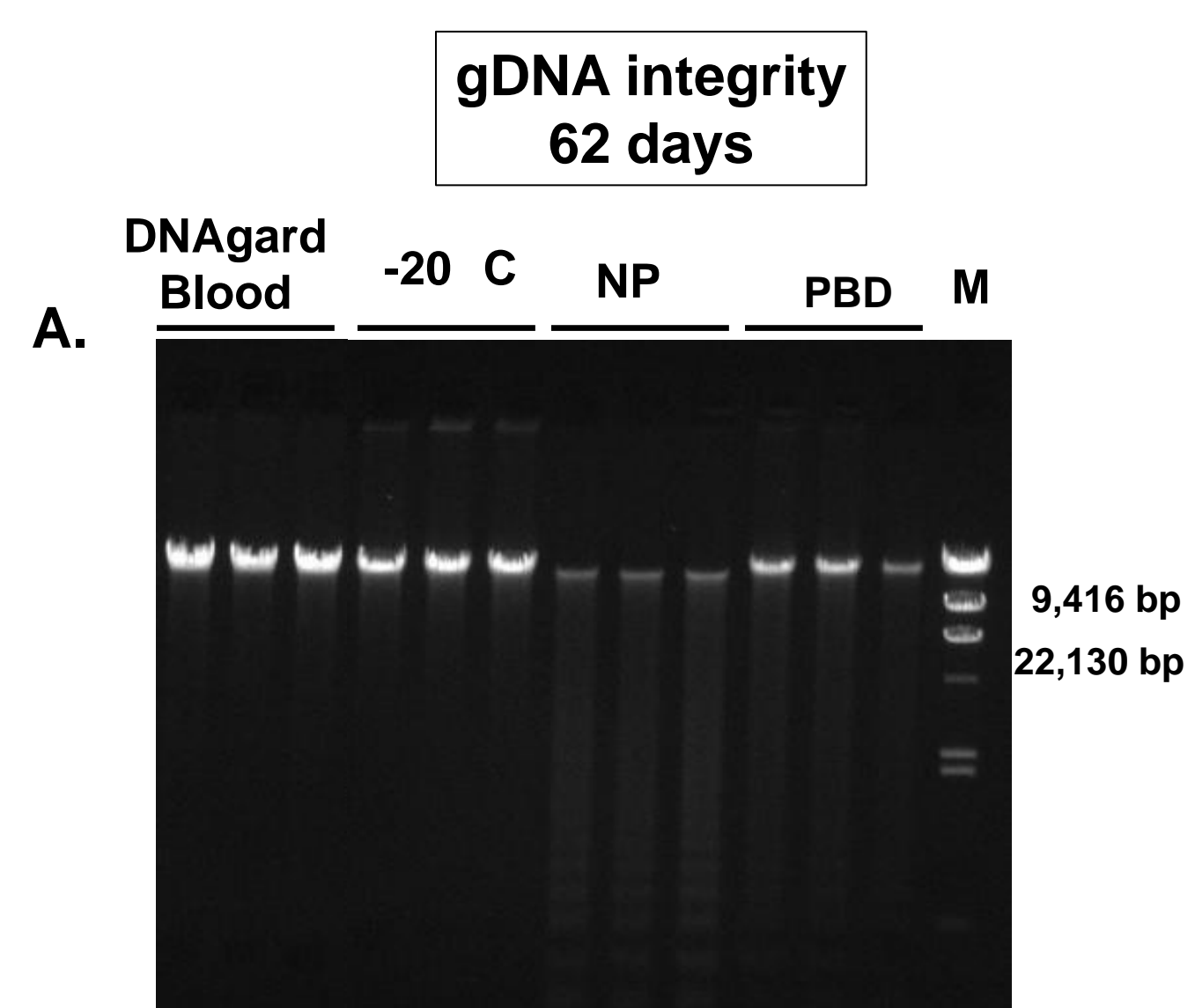


Figure 1. Long-term genomic DNA stabilization in blood at room temperature. Human blood from a single donor was collected in K₂-EDTA Vacutainers™ and in PAXgene Blood DNA tubes (PBD). Within 1 hour of collection, aliquots (100 µl) of blood from the K₂-EDTA Vacutainers™ were mixed with 25 µl DNAgard Blood stabilization formulation and stored at room temperature. Aliquots were also stored in the absence of stabilizer at room temperature (non-protected; NP) or frozen at -20 °C. PBD tubes were stored at room temperature. **A. Genomic DNA integrity at 62 days.** After 62 days of storage, total DNA was extracted from triplicate DNAgard Blood, NP and -20 °C samples using the FlexiGene DNA Kit (QIAGEN). An equivalent volume of blood was drawn from PBD tubes and extracted using the PAXgene Blood DNA Kit. 5% of the recovered DNA was analyzed by agarose gel electrophoresis (0.8%; 1xTAE). M = Lambda DNA-HindIII Digest. **B. Picogreen quantification of recovered DNA from the 62 day samples.** **C. Long-range PCR.** 100 ng of DNA from each of the 62 day samples was used as a template for PCR amplification of a 22 kbp fragment incorporating the *PLAT* gene using the Manual PCR Extender System (5 Prime). 20% of each amplification was analyzed via agarose gel electrophoresis (0.8%, 1xTAE). M = Lambda DNA-HindIII Digest.

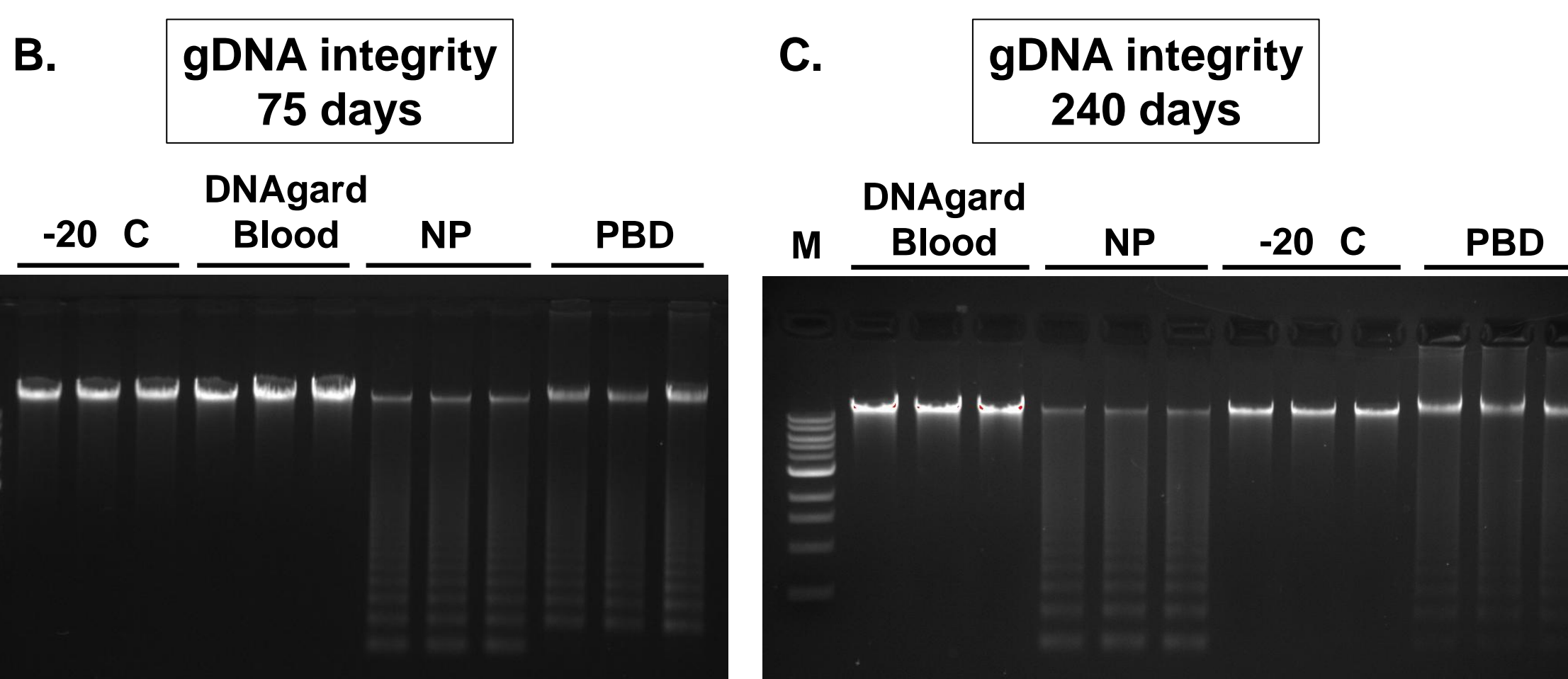
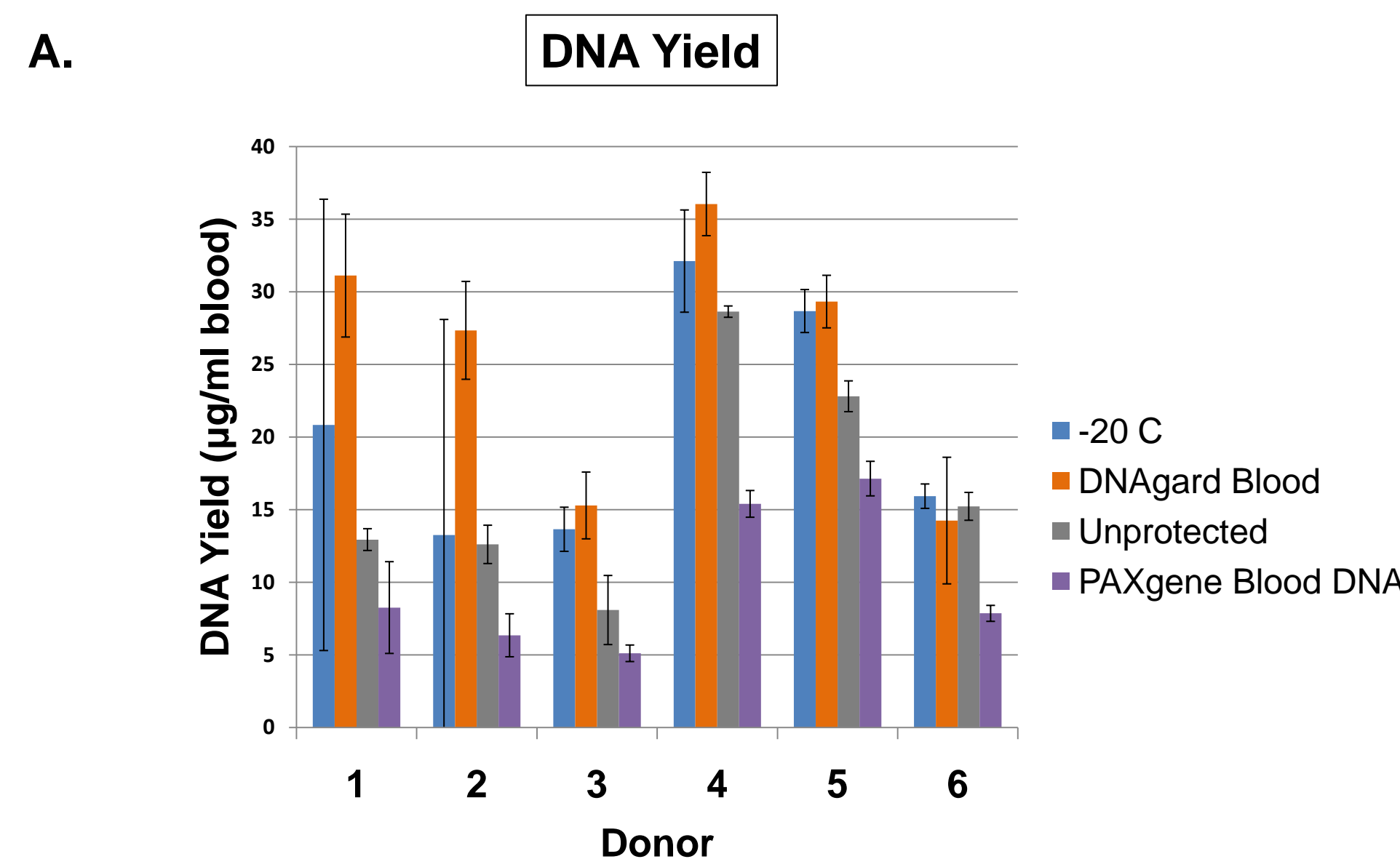


Figure 2. Multi-donor analysis of genomic DNA stabilization in blood at room temperature. Blood from six human donors was collected in K₂-EDTA Vacutainers™ and in PAXgene Blood DNA tubes (PBD). Within 30 minutes of collection, DNAgard Blood stabilization formulation was added to specified K₂-EDTA Vacutainers™ at a 4:1 (blood:formulation) ratio and stored at room temperature. K₂-EDTA Vacutainers™ to which no stabilizer was added were stored at room temperature (non-protected; NP) or frozen at -20 °C. PBD tubes were stored at room temperature. **A. DNA yield after 1 month room temperature storage.** After 30 days of storage, total DNA was extracted from triplicate 1 ml blood samples from each donor. DNAgard Blood, -20 °C and NP samples were extracted using the FlexiGene DNA Kit (QIAGEN). PBD samples were extracted using the PAXgene Blood DNA Kit. DNA yield in µg is presented standard deviation. **B. Genomic DNA integrity after 75 days room temperature storage.** Triplicate 0.1 ml blood samples from DNAgard Blood, -20 °C and NP conditions were processed for DNA extraction using the QIAamp Blood Mini kit with QIAcube automation. PBD samples were extracted as described above. 5% of the recovered DNA was analyzed by agarose gel electrophoresis (0.8%; 1xTAE). Results from donor #2 are shown. M = 1 kb DNA ladder (New England Biolabs). **C. Genomic DNA integrity after 240 days room temperature storage.** Samples were processed as described above (section B). Results from donor #1 are shown.

Results -- stabilization of gene expression

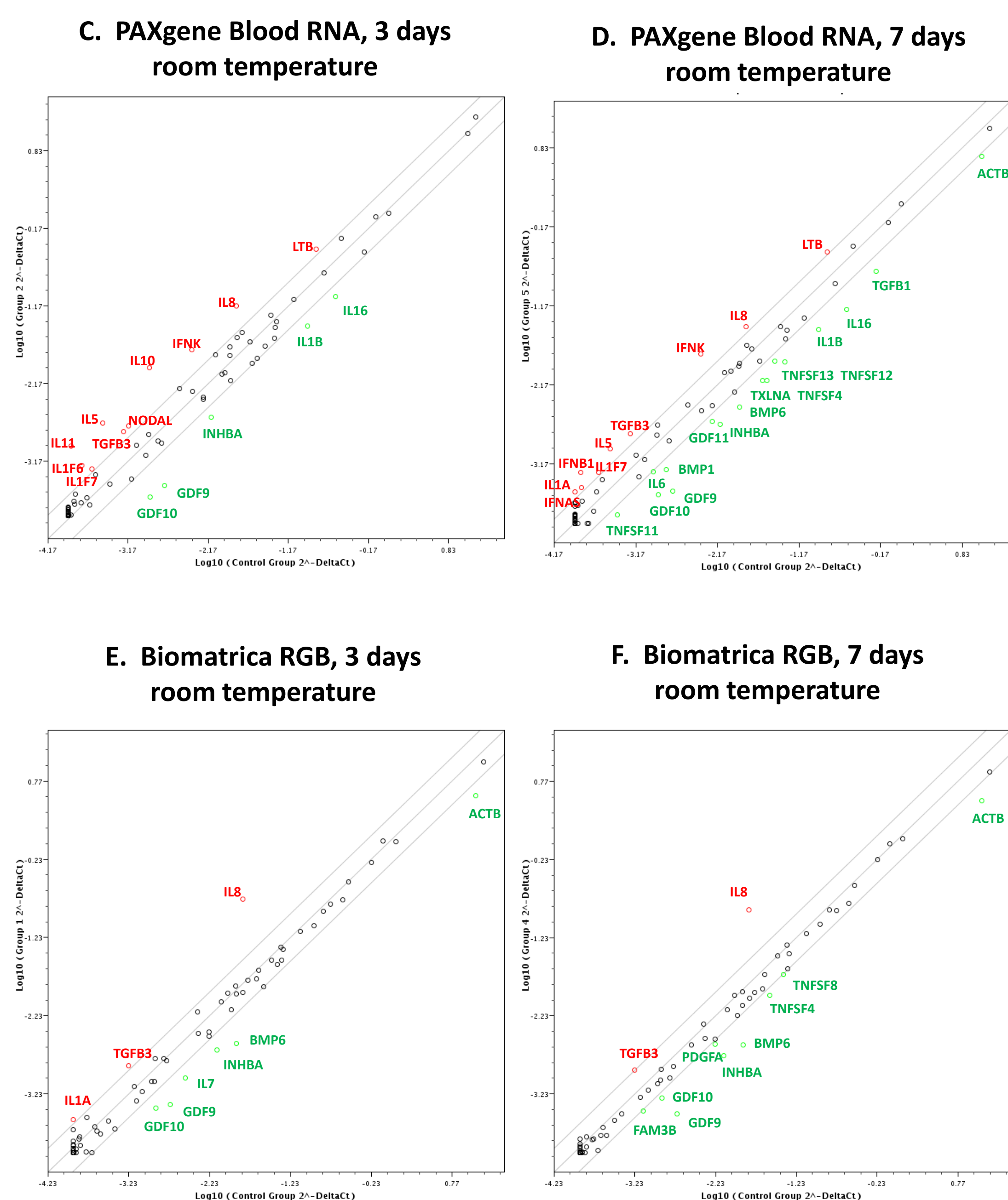
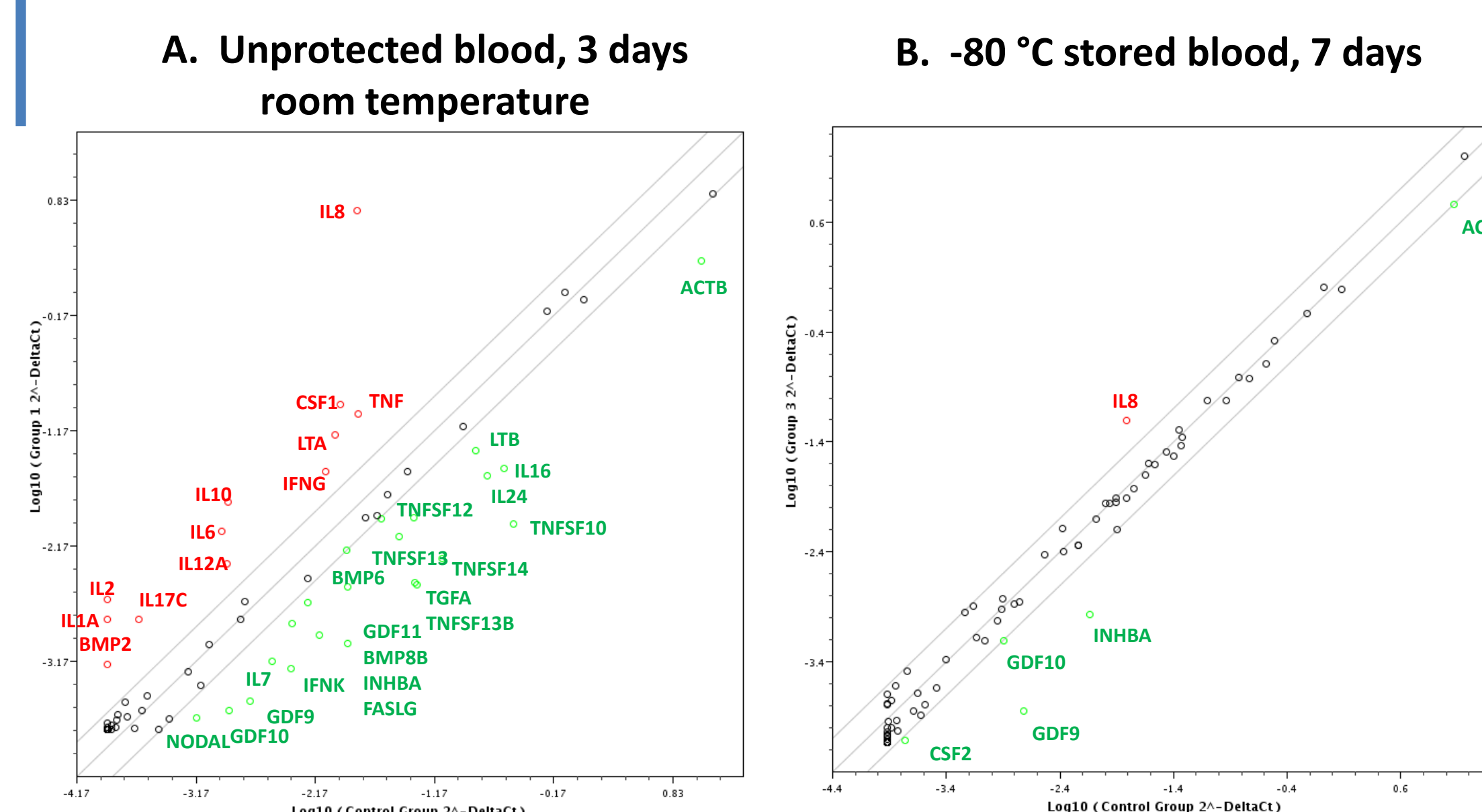


Figure 3. RT-qPCR analysis of gene expression stability in whole blood at room temperature. Human whole blood from a single donor was collected in K₂-EDTA Vacutainers™ (BD) and in PAXgene Blood RNA (PBR) tubes. Blood aliquots from K₂-EDTA Vacutainers™ were then transferred to microfuge tubes containing Biomatrix "Gard" formulation for RNA stabilization in blood (Biomatrix RGB) at a 3:1 (formulation: blood) ratio and stored at room temperature. Control blood aliquots were stored without stabilizer at room temperature (unprotected; NP) or frozen at -20 °C. Total RNA was extracted from unstabilized blood immediately after collection (Time 0) and from RGB, NP and -20 °C samples at specified time points using the RiboPure Blood Kit (Ambion). RNA was extracted from PBR samples using the PAXgene Blood RNA Kit. Triplicate samples from each group were processed. 500 ng total RNA was reverse transcribed using the RT² First Strand Kit (SABiosciences). Gene expression of a panel of 89 genes was quantified by SYBR Green real-time PCR analysis using the Human Common Cytokines RT² Profiler PCR Array (SABiosciences) using an Applied Biosystems 7300 Real-Time PCR System. Gene expression levels were normalized using RPL13A and GAPDH as reference genes. Fold changes in gene expression were quantified relative to transcript levels at the time of blood collection (Time 0) using the ΔΔC_q method. Data are presented as scatter plots, plotting log₁₀ (2^{-ΔΔC_q}) for each gene in the test group on the y-axis against the log₁₀ (2^{-ΔΔC_q}) for each gene in the Time 0 sample on the x-axis. (ΔΔC_q values are the average of the triplicate biological replicates). Boundaries on the scatter plots are set at 2-fold, such that genes upregulated more than 2-fold relative to the Time 0 group lie above the linear plot and are colored red; genes downregulated more than 2-fold lie below the linear plot and are colored green. Gene abbreviations are indicated.

Summary

Genomic DNA integrity in blood stored at room temperature:

- DNAgard Blood was equivalent to freezer storage in maintaining intact, high molecular weight gDNA for at least 8 months (Fig. 1A and 2 B,C). Samples stored unprotected or in PAXgene Blood DNA resulted in partially degraded DNA.
- gDNA recovered from blood stored in DNAgard Blood was of high integrity as demonstrated by long-range PCR amplification of a 22 kbp fragment of the genome (Fig. 1C).

Gene transcript analysis in blood stored at room temperature:

- Freezer storage does not prevent changes in gene expression levels (Fig. 3B)
- A number of interleukins, interferons, bone morphogenic proteins, TGF-β family proteins, TNF proteins and other genes were up- or down-regulated in blood stored unprotected at room temperature (as much as 362-fold after 3 days).
- The gene expression profile in blood stored for 3 days at room temperature in Biomatrix RGB is highly similar to blood stored frozen and outperforms PAXgene Blood RNA (number of genes up- or down-regulated > 2-fold: 9, Biomatrix; 6, frozen storage; 15, PAXgene)
- The gene expression profile is highly stable in blood stored in Biomatrix RGB for 7 days in terms of the number and magnitude of genes up- or down-regulated.

Storage	Time	# genes upregulated	# genes downregulated
Unprotected	3 days	12	22
		IL8 (362-fold)	TNFSF10 (-29-fold)
-80 °C	7 days	1	5
		IL8 (4-fold)	GDF9 (-14-fold)
PAXgene Blood RNA	3 days	10	5
		IL10 (9-fold)	GDF9 (-6-fold)
PAXgene Blood RNA	7 days	9	16
		IFNK (4-fold)	GDF9 (-6-fold)
Biomatrix RGB	3 days	3	6
		IL8 (12-fold)	BMP6 (-5-fold)
Biomatrix RGB	7 days	2	9
		IL8 (9-fold)	GDF9 (-6-fold)