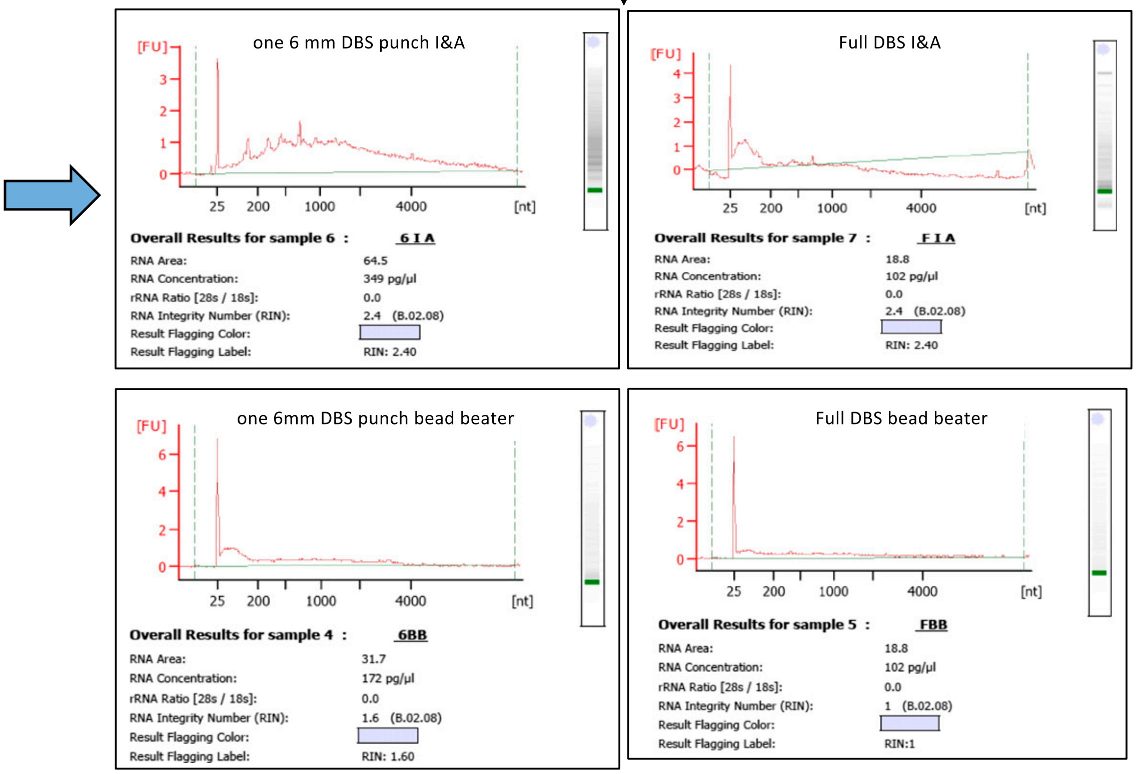
DBS RNA Extraction Testing Flowchart

1. Test different DBS punch sizes and two sample disruption methods
   A. Homogenizer disruption method: one 6 mm punch, one full DBS
   B. Incubation and agitation method: one 6 mm punch, one full DBS



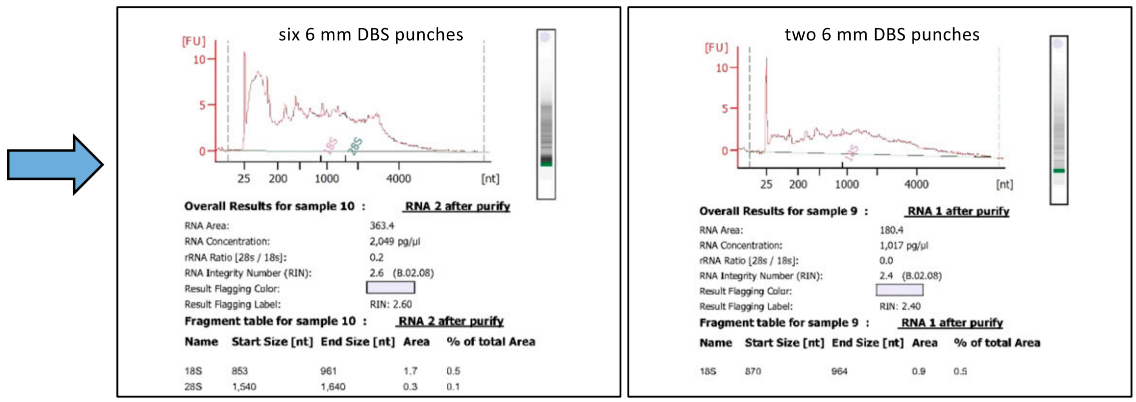
The blue arrow indicates the condition that provided the optimal results in this experiment. (Clockwise from top left): This image indicates a tube containing one 6 mm punch that was homogenized using a 1-hour incubation at room temperature with vortexing for 30 seconds every 15 minutes. The top right image indicates a full DBS that underwent the same method. The bottom right image indicates a full DBS that was homogenized using a bead beater. The sample was "beat" twice consecutively for 30 seconds at the highest setting. The bottom left image shows one 6 mm punch that was homogenized using the bead beater method. All samples were homogenized in 350 µL lysis buffer with 3.5 µL beta-mercaptoethanol. This set of conditions was determined to be most optimal based on both the Agilent image above and the NanoDrop concentration.

Supplemental Figure 1. Optimization of RNA extraction and purification steps from dried blood spots (DBS). ID numbers on the Agilent graphs were the IDs used during research and development.

2. Test optimal number of 6 mm punches per extraction tube, using optimal sample disruption method from Test 1

A. Two tubes with one 6 mm punch each, pooled after sample disruption

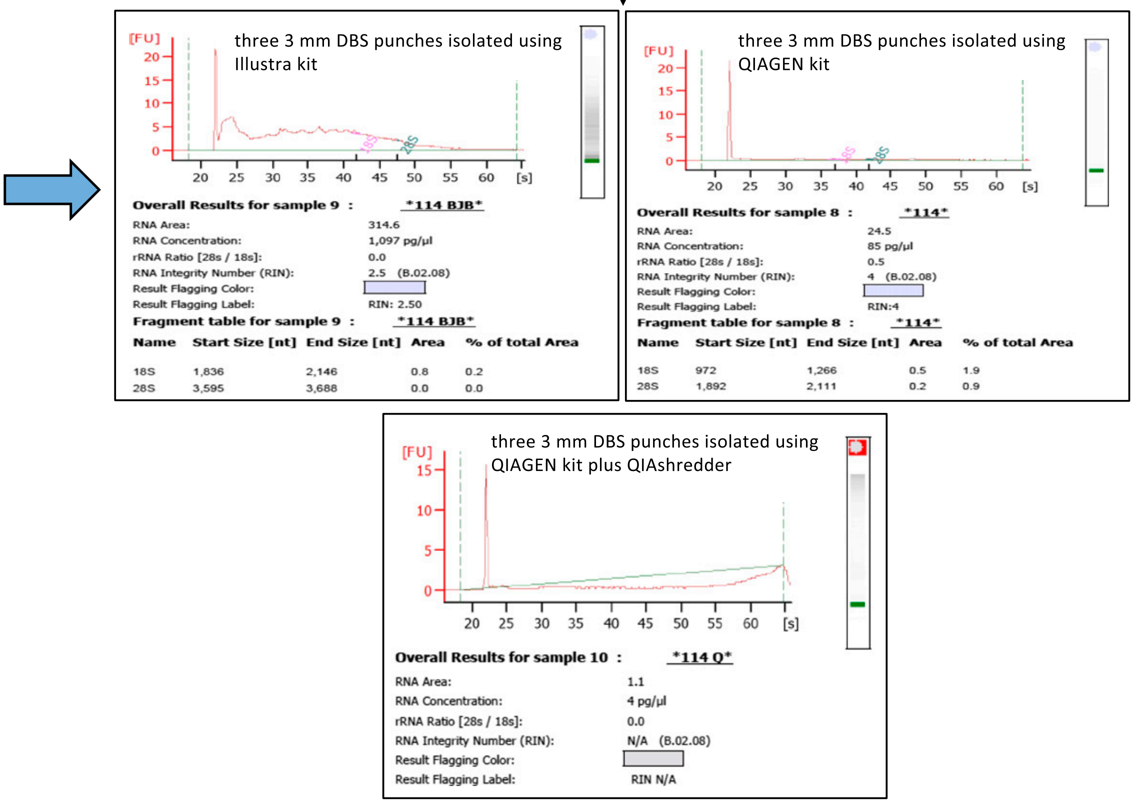
B. Three tubes with two 6 mm punches each, pooled after sample disruption



The blue arrow indicates the condition that provided the optimal results in this experiment. The left image indicates a tube that contained six 6 mm punches that were homogenized in three different tubes (each containing two punches) and then pooled into one tube after homogenization for the RNA isolation. The right image indicates a tube that contained two 6 mm punches that were homogenized individually and then pooled for the RNA isolation procedure. All samples were homogenized using the incubation and agitation method as described in the previous experiment. This set of conditions was determined to be most optimal based on both the Agilent image above and the NanoDrop concentration.

Supplemental Figure 1. (Continued)

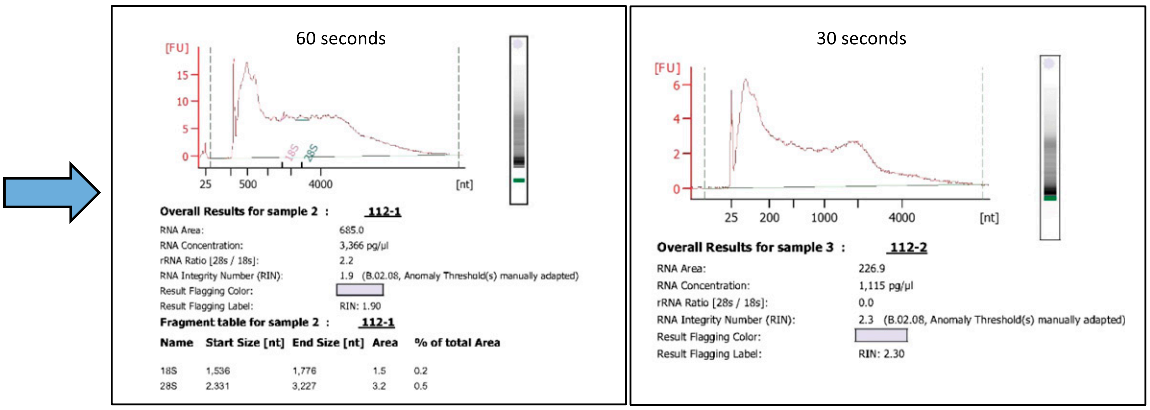
3. Test optimal RNA extraction method
A. Three 3 mm punches using Rneasy Micro Kit (QIAGEN)
B. Three 3 mm punches using Illustra Kit (GE Healthcare)



The blue arrow indicates the condition that provided the optimal results in this experiment. The top left image indicates three 3 mm DBS punches extracted using the Illustra RNA Isolation Kit (GE Healthcare). The top right image was three 3 mm DBS punches extracted using the RNeasy micro kit (QIAGEN), and the bottom image was three 3 mm DBS punches extracted using the RNeasy micro kit (QIAGEN), in addition to a pass through a QIAshredder column. All tubes were homogenized using the incubation and agitation method as described in experiment 1. This set of conditions was determined to be most optimal based on both the Agilent image above and the NanoDrop concentration.

SUPPLEMENTAL FIGURE 1. (Continued)

4. Test bead beater sample disruption method
A. Three 3 mm punches in bead beater 30 seconds at max speed (5 m/s)
B. Three 3 mm punches in bead beater 60 seconds at max speed (5 m/s)

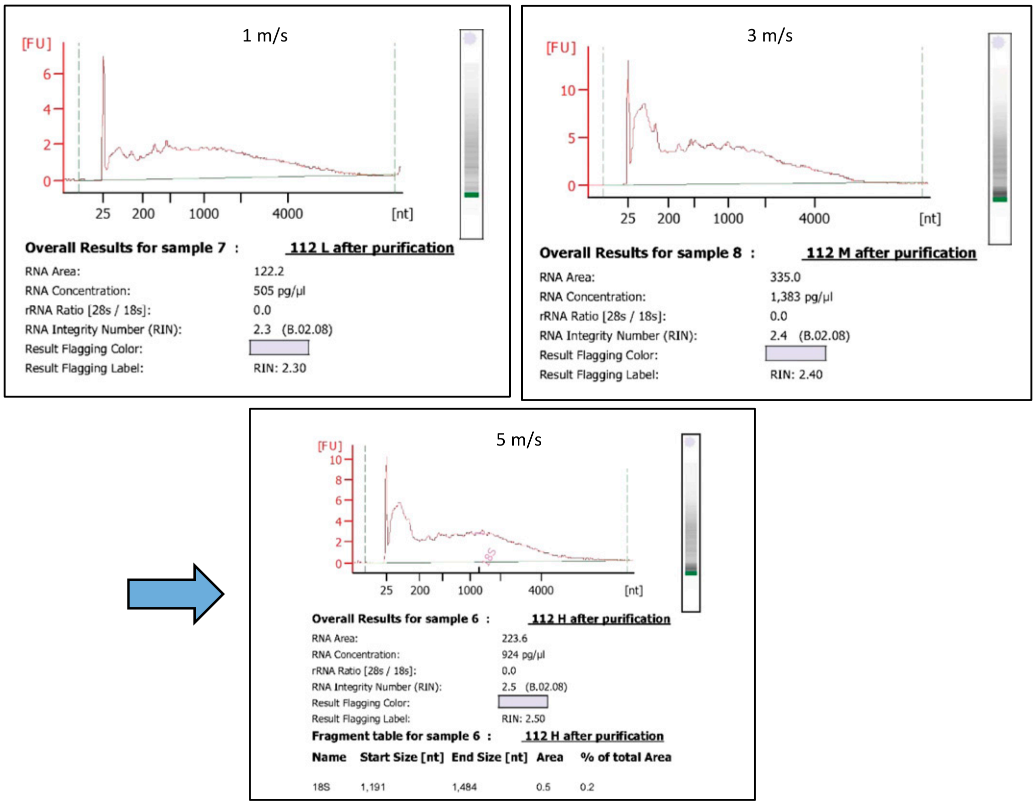


The blue arrow indicates the condition that provided the optimal results in this experiment. The image on the left indicates a tube with DBS that was homogenized using the Bead Mill 4 Bead Beater (Fisher Scientific) for 60 seconds, and the image on the right indicates a tube with DBS was homogenized using the Bead Mill 4 Bead Beater for 30 seconds. All tubes contained three 3 mm punches from the same patient, and were homogenized in a solution of 350 µL lysis buffer and 35 µL beta-mercaptoethanol. This set of conditions was determined to be most optimal basedon both the Agilent image above and the NanoDrop concentration.

↓

Supplemental Figure 1. (Continued)

5. Determination of optimal bead beater speed for disruption
A. Three 3 mm punches in bead beater 60 seconds at minimum speed (1 m/s)
B. Three 3 mm punches in bead beater 60 seconds at medium speed (3 m/s)
C. Three 3 mm punches in bead beater 60 seconds at maximum speed (5 m/s)



The blue arrow indicates the condition that provided the optimal results in this experiment. All tubes contained three 3 mm punches from the same patient. The punches were homogenized in tubes also containing 350 µL lysis buffer and 3.5 µL beta-mercaptoethanol. This set of conditions was determined to be most optimal based on both the Agilent image above and the NanoDrop concentration.

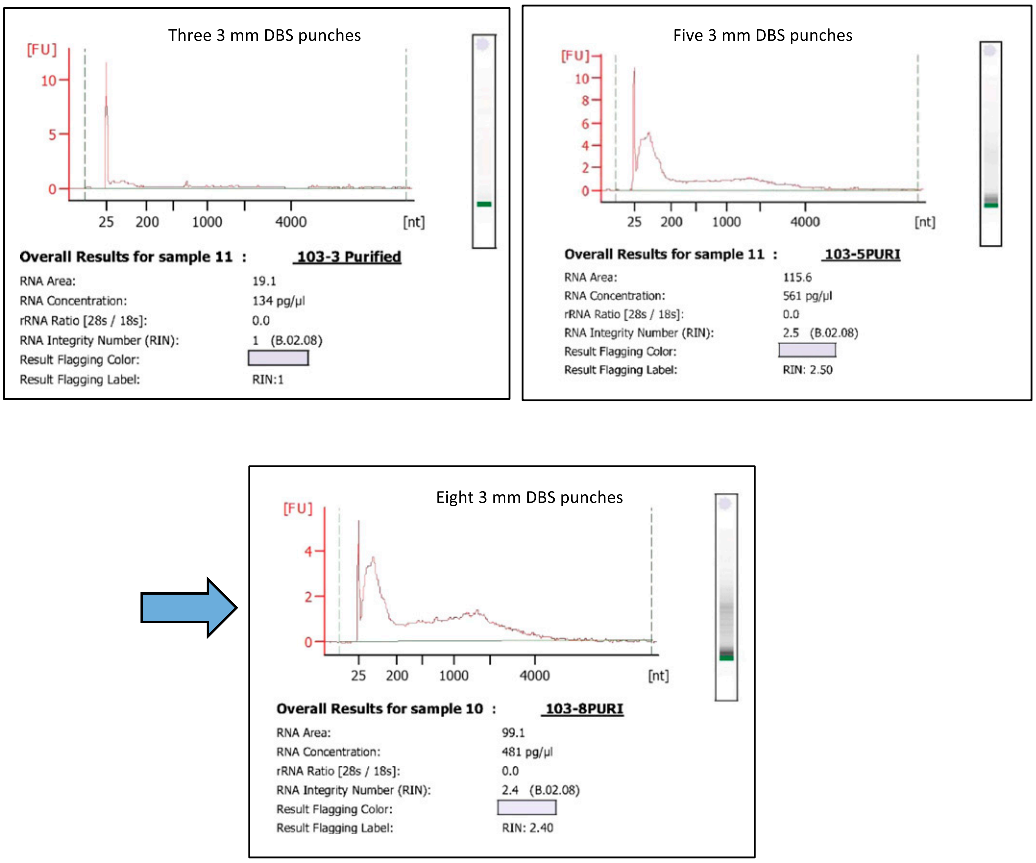
SUPPLEMENTAL FIGURE 1. (Continued)

6. Test optimal number of 3 mm punches per tube using optimal bead beater speed and time

A. Three 3 mm punches

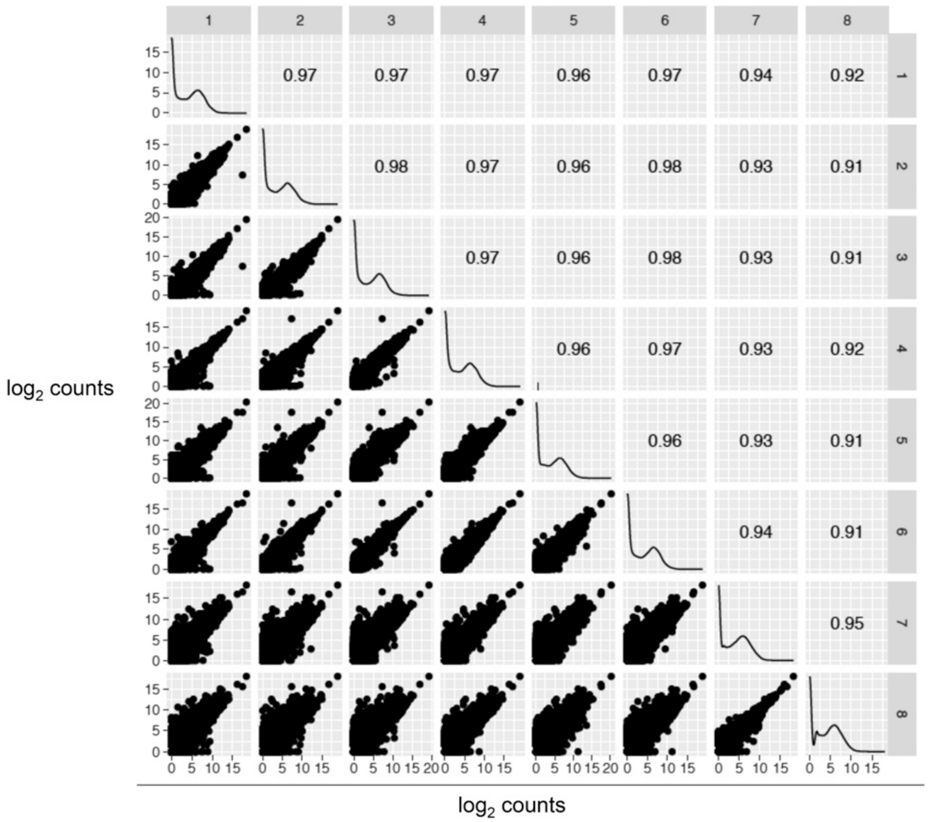
B. Five 3 mm punches

C. Eight 3 mm punches

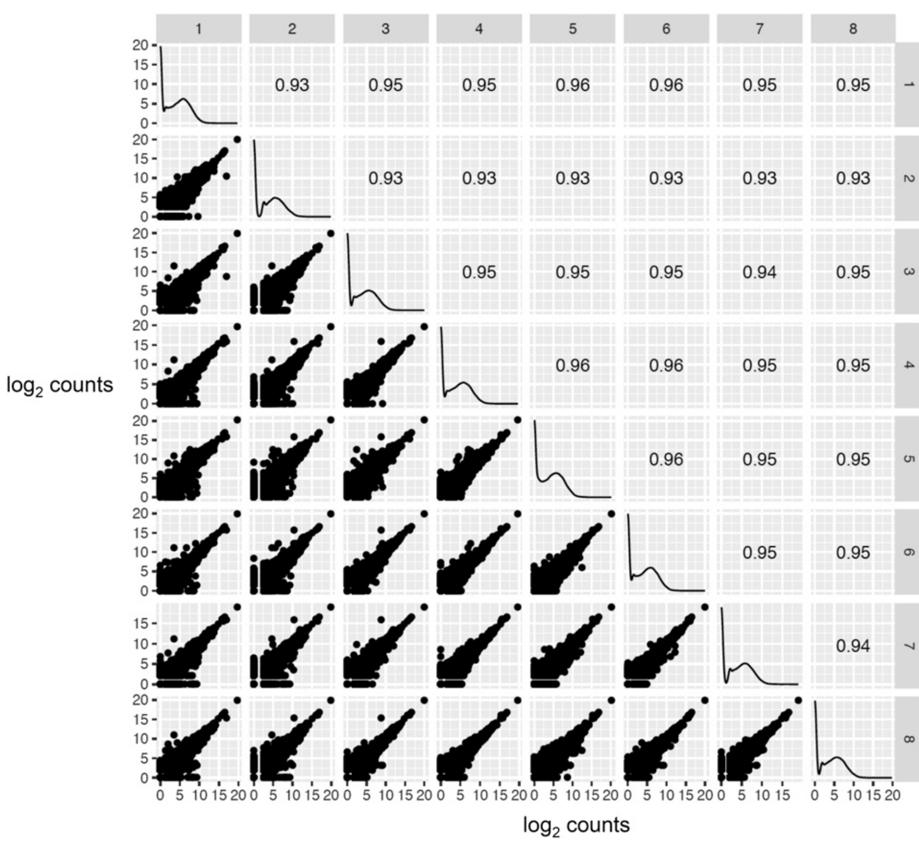


The blue arrow indicates the condition that provided the optimal results in this experiment. The optimal number of 3 mm punches proved to be eight in one tube. All tubes in this experiment were homogenized using the Bead Mill 4 bead beater for 60 seconds at 5 m/s in 350 µL lysis buffer and 3.5 µL beta-mercaptoethanol. This set of conditions was determined to be most optimal based on both the Agilent image above and the NanoDrop concentration.

Supplemental Figure 1. (Continued)



SUPPLEMENTAL FIGURE 2. Inter-sample correlations for Tempus tube transcriptomes. Left-lower triangular entries are scatter plots of $\log_2$ counts for each pair of eight samples and upper triangular entries are Spearman correlations of $\log_2$ counts from each pair.



SUPPLEMENTAL FIGURE 3. Inter-person correlation for dried blood spot transcriptomes.

Supplemental Table 1

Top 1% of genes with increased counts in transcriptome from DBS RNA

Gene ID
A2M
ALS2CR12
ANKRD36
ANKRD36B
ANKRD36BP2
APLF
ASPM
ASTN2
ATAD5
BNIP3L
CASC5
CC2D2A
CCDC141
CCDC144B
CCDC18
CDC42BPA
CDKN2B-AS1
CENPE
CEP57L1
CLEC4GP1
CNTLN
DDI2
DNAJC6
DOK6
EFCAB13
EGR1
EGR3
ERCC6L2
FAM46C
FOS
FOSB
GSG2
GYPA
HELB
HEMGN
HIST1H1E
KCNQ1OT1
KIF14
KIF20B
LNPEP
MAB21L3
MALAT1
MAN1A2
MBNL1-AS1
MBNL3
MPP6
MTMR12
MTRNR2L1
MTRNR2L10
MTRNR2L2
MTRNR2L3
MTRNR2L4
MTRNR2L6
MTRNR2L8
NBEAL1
NEIL3
NR4A2
NRSN1
NUSAP1
ORC4
PGM5P2
PGPEP1
PTGS2
PZP
RMRP
RPPH1
SCARNA10
SCARNA17

(continued)

Supplemental Table 1

Continued

Gene ID
SCARNA2
SCARNA21
SCARNA7
SHISA9
SLITRK4
SOX6
SPECC1
SSTR5-AS1
STIL
SWT1
TBCEL
TCP11L2
TMEM212
TPM4
TTN
UGDH-AS1
UGT8
VRK1
YOD1
ZBED6
ZNF460
ZNF471

DBS = dried blood spot.

Supplemental Table 2

Top 1% of genes with increased counts in transcriptome from whole blood Tempus RNA

ACAP3
APOBR
ARHGDIA
ATN1
BAX
C11orf68
C16orf13
CD7
CDK10
CDK2AP2
CIRBP
CORO1B
DBP
DENND1C
DMPK
DYRK1B
ENGASE
ERF
FBRSL1
GLTSCR1
GPAA1
GPBAR1
HAPLN3
HDAC10
IDUA
IFITM3
IGFLR1
KLF16
LENG8
LRCH4
LSM10
LSP1
LTBP3
LTBP4
LY6E
MAP1S
MAP3K10
MAPK3
MBD6
MEN1
MIB2
MLLT1
MMP9
NCDN
NDUFS7
NPDC1
OGFR
OLIG1
OSCAR
PRAM1
PRKCSH
PTP4A3
RAD9A
RFNG
RHPN1
RPS16
SAMD1
SAP25
SCAF1
SF3A2
SGSH
SH2B1
SH3BP1
SIGIRR
SLC39A13
SLC52A2
SPI1
SRM
STMN3
STUB1

(continued)

Supplemental Table 2

Continued

TAF1C
TBKBP1
TELO2
TGFB1
TMEM134
TMUB1
TNFSF12
TNK2
VAMP1
WAS
ZDHHC8
ZFPM1
ZNF385A
ZNF444
ZNF467
ZNF524
ZNF580
ZYX