Environ Health Perspect

DOI: 10.1289/EHP6262

Supplemental Material

Evaluating the Role of the Steroid and Xenobiotic Receptor (SXR/PXR) in PCB-153 Metabolism and Protection against Associated Adverse Effects during Perinatal and Chronic Exposure in Mice

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RNA-seq script

RNA quality: Bioanalyzer (Agilent Bioanalyzer 2100) results for RNA used for RNA-seq

Additional File- Excel Document

<u>Table S1</u>: Breeding Results

F1 male exposure until 4 weeks of age					
	# F0 female breeders	*# of litters*	*# pups/litter (avg ± SEM)*	*# F1 male offspring born*	*# F1 male offspring used for experiments*
WT DMSO	9	6	6.71 ± 0.29	22	19
WT PCB	9	9	6.33 ± 0.50	27	18
SXRKO DMSO	6	5	6.40 ± 0.68	17	16
SXRKO PCB	7	4	7.00 ± 0.41	13	13
F1 male exposure until 10 months of age					
	# F0 female breeders	*# of litters*	*# pups/litter (avg ± SEM)*	*# F1 male offspring born*	*# F1 male offspring used for experiments*
WT DMSO	10	6	6.50 ± 0.92	20	14
WT PCB	10	7	6.71 ± 0.42	24	23
SXRKO DMSO	17	12	6.58 ± 0.51	43	33
SXRKO PCB	18	16	6.25 ± 0.47	42	40
hSXRki DMSO	8	4	5.67 ± 0.71	15	14
hSXRki PCB	8	6	6.25 ± 0.63	18	15

Notes: Some F1 males were not used for exposure experiments because they died shortly after birth, were runts, or died at 5 weeks of age, as noted in the Results section. The numbers for the F1 SXRKO mice in the chronic exposure study (until 10 months old) are the sum between two independent experiments: 1) comparing SXRKO (n= 15 DMSO; 13 PCB) and hSXRki mice and 2) comparing SXRKO (n= 18 DMSO; 27 PCB) and WT mice.
WT= wildtype; SXRKO= SXR knockout; hSXRki= humanized SXR knock-in; DMSO= exposure to 0.1% DMSO (vehicle) via drinking water; PCB= exposure to 1 µM PCB-153 via drinking water.

Table S2: qPCR primer sequences

Gene (mouse)	**Forward primer**	**Reverse primer**
36B4/Rplp0	AAGCGCGTCCTGGCATTGTCT	CCGCAGGGGCAGCAGTGGT
Actb	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
Fech	GACCGAGACCTCATGACACTTCC	ATAACTCATCCAGCAGCTTCACCA
Klf1	GAGCCCTCCAAGAAACTTTCCT	TCAGACCGCCACCACTTGAG
Epor	CGGGATGGACTTCAACTACAGC	AACACCAGAAACGCACGGAG
Prxd2	GGACTACAGAGGGAAGTACG	TGAACTGAGAGTCCACAGAC
Gpx1	CGTTTGAGTCCCAACATCTC	ATCGTTCATCTCGGTGTAGTC
Gstp1	TCCTTTGCCGATTACAACTTGC	AGTCCACTACTGTTTGCCATTGC
Gadd45a	GCTGCTACCTCTGCTTACCTCTG	CCTTCCATTGTGATGAATGTGGGT
Rad23a	AGATGTTGAATGAGCCTCCCG	TGCCTTCAGCCTTTCTATAGCC
Blvrb	CAGGTTATGAGGTGACGGTG	CCACAGTCTTGTCCACATCGG
Gstp3	GTGACCTTGGATGTTTGGGAGC	CTTTGCCATAGAGCCCGAAGGA
Mgst3	TACAGCACAGATCCTGAGAACG	TAAACACCTCCCACCGTTAGGA
Epb42	GACAGTCCTTCACTATCACCCTG	TGGCTTGGGTCTTATTGATCTTGG
Alad	CAGTGCCTCCAACCTCATCTATCC	GACGCCAAAGATCAGGACACAG
Hbq1b	AGACTTCAGCCCAGAGATGC	GAGACTATAGAACCCTGTATCCC
Fam213a	GCAGACCTGATGTCCTTGAAGC	ATGAACATCATCTTCCGCCTCTC
Cyp2b10	GTGCCCACAGACAAATCTCCA	GGACTTCTCCTTCTCCATGCG

Notes: 36B4/Rplp0: ribosomal protein, large, P0; Actb: β-actin; Fech: ferrochelatase; Klf1: kruppel-like factor 1; Epor: erythropoietin receptor; Prxd2: peroxiredoxin 2; Gpx1: glutathione peroxidase; Gstp1: glutathione S-transferase pi 1; Gadd45a: growth arrest and DNA damage inducible alpha; Rad23a: RAD23 Homolog A; Blvrb: biliverdin B; Gstp3: glutathione S-transferase pi 3; Mgst3: microsomal glutathione S-transferase 3; Epb42: erythrocyte membrane protein band 4.2; Alad: δ-aminolevulinic acid dehydratase; Hbq1b: hemoglobin subunit theta 1B; Fam213a: peroxiredoxin-like 2A; Cyp2b10: cytochrome P450, family 2, subfamily b, polypeptide 10

Table S3: Proportion of deaths at 5 weeks of age in WT, hSXRki, and SXRKO mice exposed to PCB-153 or vehicle in chronic exposure study

		DMSO	PCB-153
COHORT 1	WT	0/6 (0%)	0/13 (0%)
	SXRKO	0/15 (0%)	**3/16 (18.8%)**
COHORT 2	WT	0/8 (0%)	0/10 (0%)
	SXRKO	0/3 (0%)	**2/11 (18.2%)**
COHORT 3	hSXRki	0/14 (0%)	0/15 (0%)
	SXRKO	0/15 (0%)	0/13 (0%)

Notes: WT= wildtype; SXRKO= SXR knockout; hSXRki= humanized SXR knock-in; DMSO= exposure to 0.1% DMSO (vehicle) via drinking water; PCB= exposure to 1 µM PCB-153 via drinking water.

<u>Table S4</u>: Wild-type blood parameters over time in chronic exposure study

	Week 6		Week 21-22		Week 30		Week 40	
	WT DMSO n= 14	**WT PCB n= 23**	**WT DMSO n= 14**	**WT PCB n= 23**	**WT DMSO n= 14**	**WT PCB n= 23**	**WT DMSO n= 14**	**WT PCB n= 23**
WBC(10^3/mm3)	14.8±2.8	14.8±3.7	22±10.4	24.1±6.1	23.5±5.5	26.7±5.5	26.5±6.2	26.3±3.6
RBC (10^6/mm3)	10.6±0.6	11.2±0.7	11.1±0.4	11.2±0.4	10.7±0.5	10.7±0.4	10.2±0.2	10.3±0.8
HGB (g/dL)	18.4±0.4	18.5±0.7	17.8±0.4	17.9±0.7	17.5±0.6	17.5±0.5	17.6±0.1	17.6±0.5
HCT (%)	58.6±2.5	61±2.6	55.8±3.4	55.8±1.9	52.8±2.8	52.7±2.1	51.4±1.4	51.1±3.5
MCV (fl)	55.1±1.9	54.4±2	49.1±0.7	49.7±0.8	48.9±0.9	49.2±0.7	50.1±1.1	49.1±1.1
MCH (pg)	17.3±1	16.5±1.3	15.9±0.4	15.9±0.5	16.3±0.6	16.3±0.5	17.1±0.4	17±0.8
MCHC (g/dL)	31.5±1.3	30.3±1.3	32.4±0.9	32.1±1.1	33.3±1.2	33.3±1	34.2±0.9	34.5±1.2
RDW (%)	15.7±1.7	14.5±1.4	13.8±0.5	13.9±0.5	13.8±0.4	13.9±0.3	14.1±0.3	14.3±0.2
PLT (10^3/mm3)	955.5±562	581.1±531.7	822±395.7	561.2±303.6	776.6±502.6	707.6±252.3	871.5±523.4	961.4±521.5
MPV (fl)	6.7±0.4	6.3±0.5	6±0.6	5.8±0.3	6.1±0.4	6.1±0.2	6.2±0.2	6.1±0.2
LYM (%)	82.2±4.3	82.9±5.1	74±3.7	74.1±6.9	71.8±6.9	71.6±6.1	72.5±5.6	71.3±4.3
MON (%)	2.9±0.4	2.9±0.4	4±0.3	3.9±0.4	3.9±0.5	4±0.3	4±0.5	4±0.3
GRA (%)	14.8±3.9	14.1±4.8	21.9±3.4	21.8±6.5	24.2±6.4	24.3±5.8	23.4±5.2	24.6±4.1

Values presented as mean±SD

WBC= white blood cell count; RBC= red blood cell count; HGB= hemoglobin; HCT= hematocrit; MCV= mean corpuscular volume; MCH= mean cell hemoglobin content; MCHC= mean corpuscular hemoglobin concentration; RDW= red blood cell distribution width; PLT= platelet count; MPV= mean platelet volume; LYM= lymphocyte count; MON= monocyte count; GRA= granulocyte count; WT= wildtype; DMSO= exposure to 0.1% DMSO (vehicle) via drinking water; PCB= exposure to 1 µM PCB-153 via drinking water.

Table S5: SXRKO blood parameters over time in chronic exposure study

	Week 6		Week 22		Week 30		Week 40	
	SXRKO DMSO n= 18	**SXRKO PCB n= 22**	**SXRKO DMSO n= 18**	**SXRKO PCB n= 22**	**SXRKO DMSO n= 18**	**SXRKO PCB n=22**	**SXRKO DMSO n= 18**	**SXRKO PCB n=22**
WBC(10^3/mm3)	14.7±5	16.3±4	21.3±3.9	19.8±6.5	25.3±5.8	21.9±4.1	22±5.8	19.1±7.4
RBC (10^6/mm3)	11.8±1	11.1±0.8	11.2±0.4	11±0.4	11.1±0.5	10.9±0.5	10.5±0.5	10.6±0.8
HGB (g/dL)	18.7±1.1	****17.8±0.7**	18.3±0.5	*****17.7±1**	18.3±0.5	*****17.7±0.4**	18±0.3	*****17.6±0.7**
HCT (%)	63.2±4.6	***58.8±4.3**	56.4±1.9	55.1±2.6	55.3±2.8	54.1±2.6	53.2±2.6	53.3±4.8
MCV (fl)	53.7±1.9	53±3	50±0.6	49.8±0.7	49.7±0.7	49.5±0.9	50.3±0.8	49.6±0.7
MCH (pg)	15.9±1.1	16.1±1.1	16.3±0.4	16±0.8	16.5±0.7	16.2±0.5	17±0.6	16.5±0.8
MCHC (g/dL)	29.5±1.1	30.3±1.5	32.5±1	32.2±1.9	33.2±1.5	32.8±1.1	33.9±1.3	33.1±1.9
RDW (%)	13.9±0.8	***15.2±1.6**	14.2±0.5	14.4±0.5	13.9±0.4	13.1±3.3	14±0.5	14.1±0.5
PLT (10^3/mm3)	335.3±384.7	432±343.2	602.4±290.9	684.5±299	646.6±377.5	751.1±366.9	1051±581	980.6±531.7
MPV (fl)	6.4±0.3	6.7±0.6	5.6±0.1	5.6±0.2	6.1±0.2	6±0.2	5.9±0.3	5.8±0.2
LYM (%)	80.8±6.5	77.5±7.7	78.6±1.5	76.1±5.4	71.3±4.9	73.9±4.9	72.5±5.5	71.2±6
MON (%)	3.4±0.8	3.5±0.7	3.9±0.1	3.9±0.4	4.1±0.4	4.9±4	4±0.4	4.1±0.4
GRA (%)	15.6±6	18.9±7.1	17.4±1.5	19.9±5.1	24.5±4.6	22.1±4.6	23.3±5.2	24.6±5.7

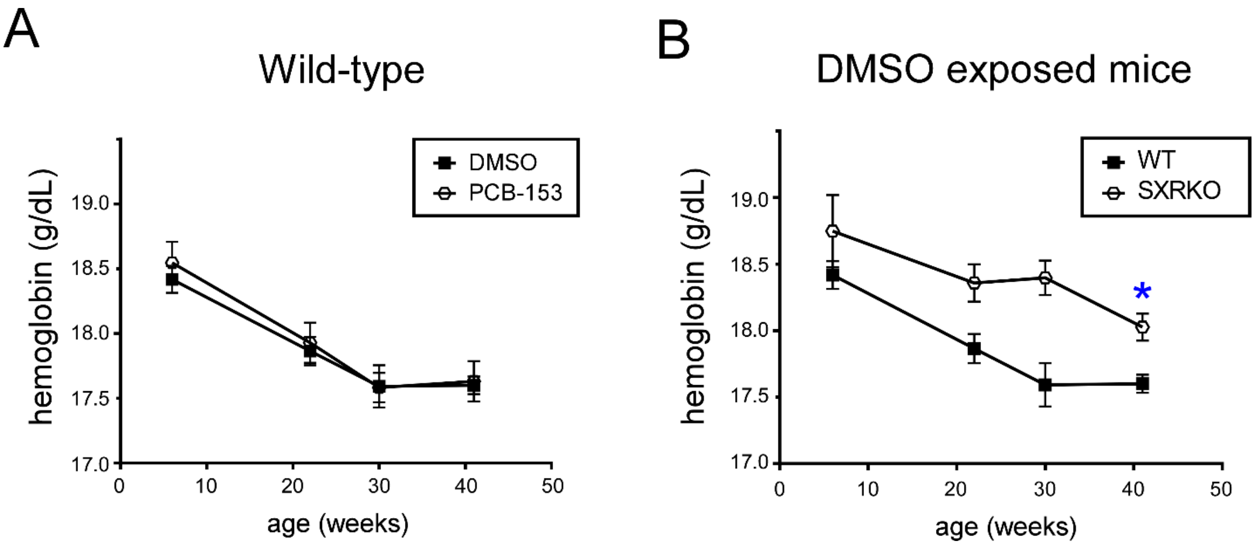
Values presented as mean±SD
asterisks indicate significance compared to vehicle treated group at the same timepoint, determined by Student's t-test. *p<0.05, **p<0.01, ***p<0.001 WBC= white blood cell count; RBC= red blood cell count; HGB= hemoglobin; HCT= hematocrit; MCV= mean corpuscular volume; MCH= mean cell hemoglobin content; MCHC= mean corpuscular hemoglobin concentration; RDW= red blood cell distribution width; PLT= platelet count; MPV= mean platelet volume; LYM= lymphocyte count; MON= monocyte count; GRA= granulocyte count; SXRKO= SXR knockout; DMSO= exposure to 0.1% DMSO (vehicle) via drinking water; PCB= exposure to 1 µM PCB-153 via drinking water..

Excel Table S1: Significant Liver DEGs in Supplemental Excel File

Excel Table S2: Significant Spleen DEGs in Supplemental Excel File

Excel Table S3: Liver GO terms in Supplemental Excel File

Excel Table S4: Spleen GO terms in Supplemental Excel File



<u>**Figure S1**</u>**: Blood hemoglobin levels throughout lifespan.** Blood hemoglobin levels of wild-type (WT) mice from 6 to 40 weeks of age exposed to either PCB-153 (n= 23) or vehicle/DMSO (n= 14) as measured by the blood analyzer and plotted as mean ± SEM (A). Blood hemoglobin values of vehicle/DMSO exposed WT (n= 14) and SXRKO (n= 18) mice from 6 to 40 weeks of age (B). Data plotted as mean ± SEM. *= p-value <0.05 determined by Student's t-test comparing to WT mice at the same timepoint in panel B.

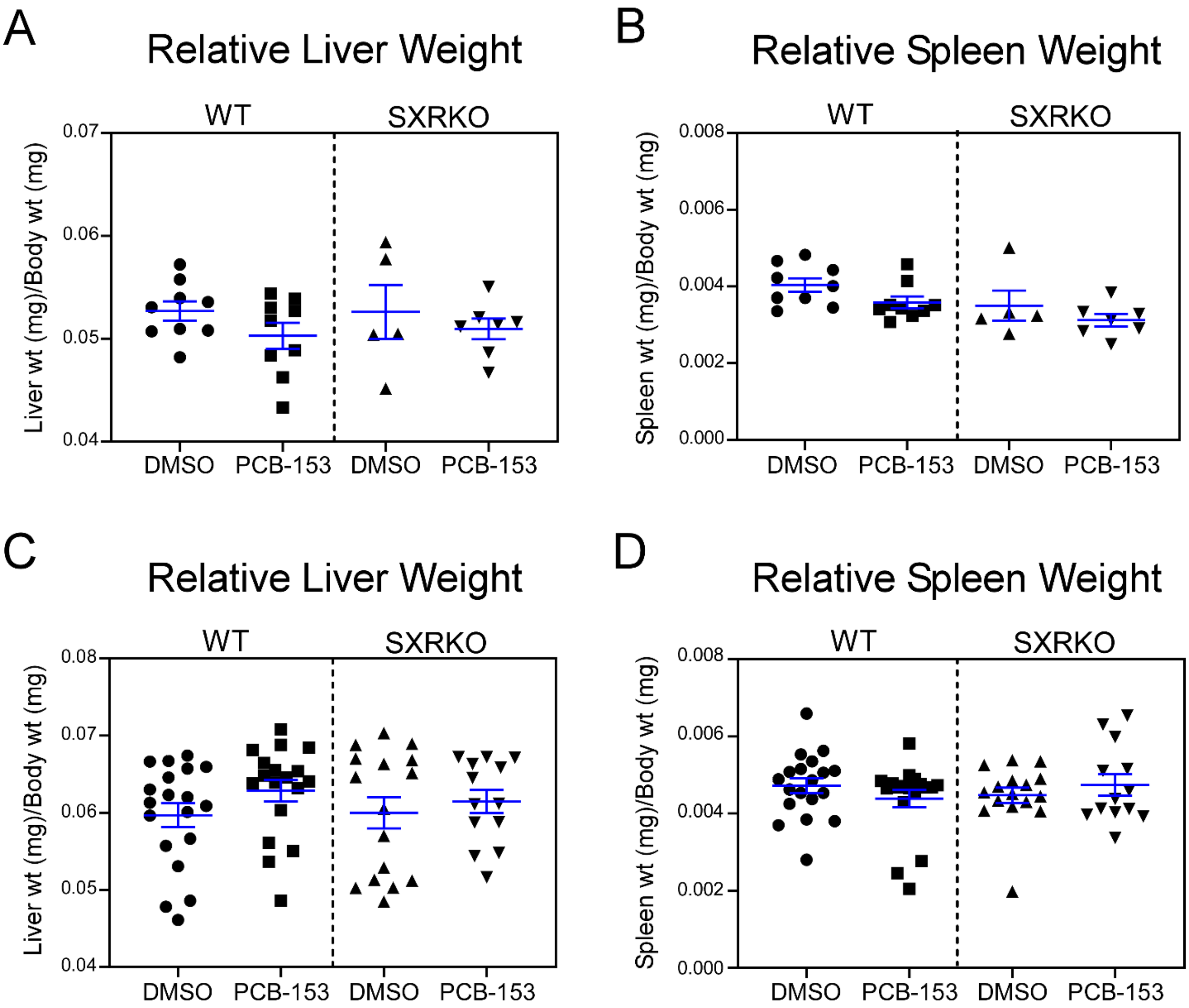
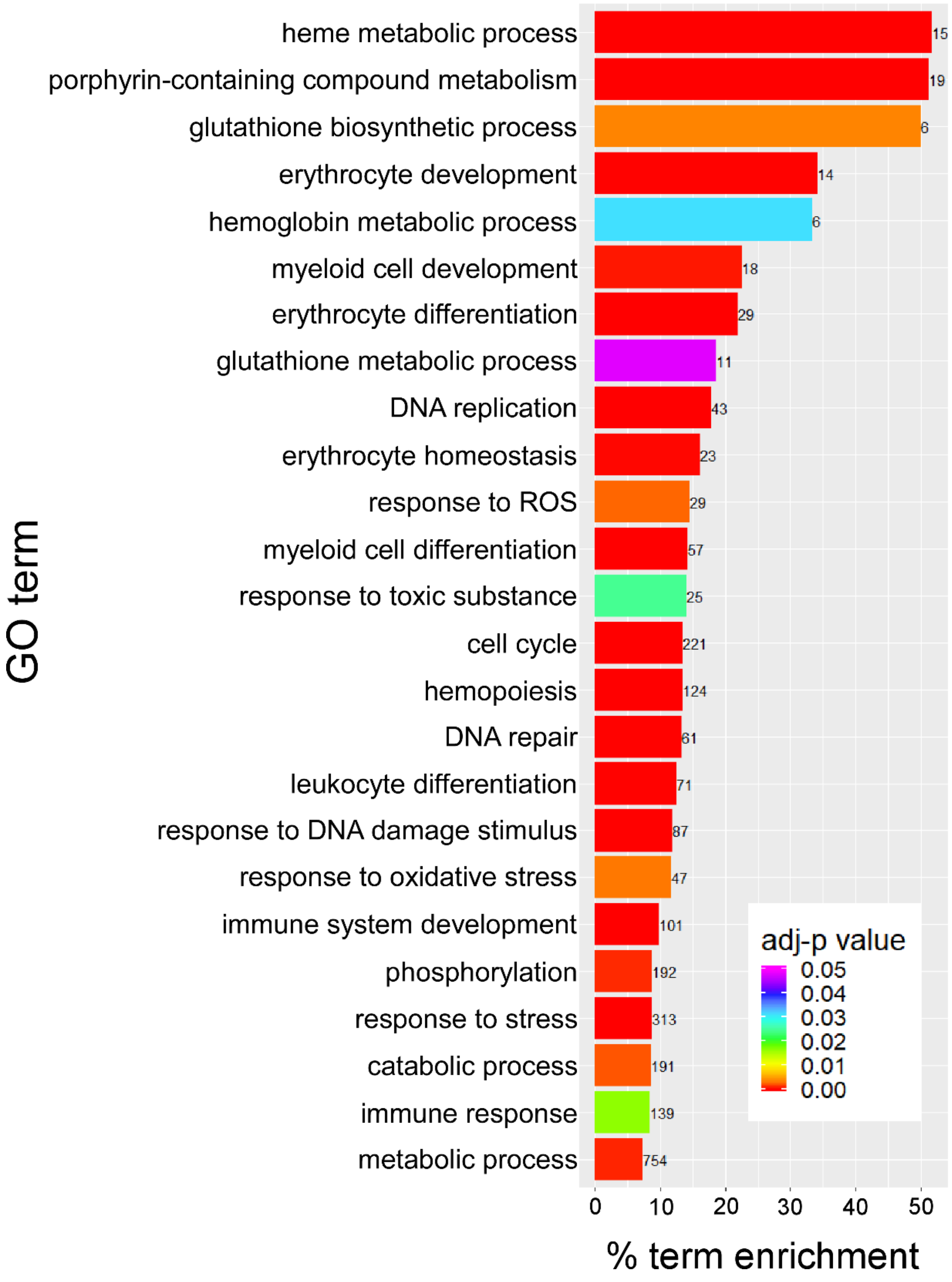
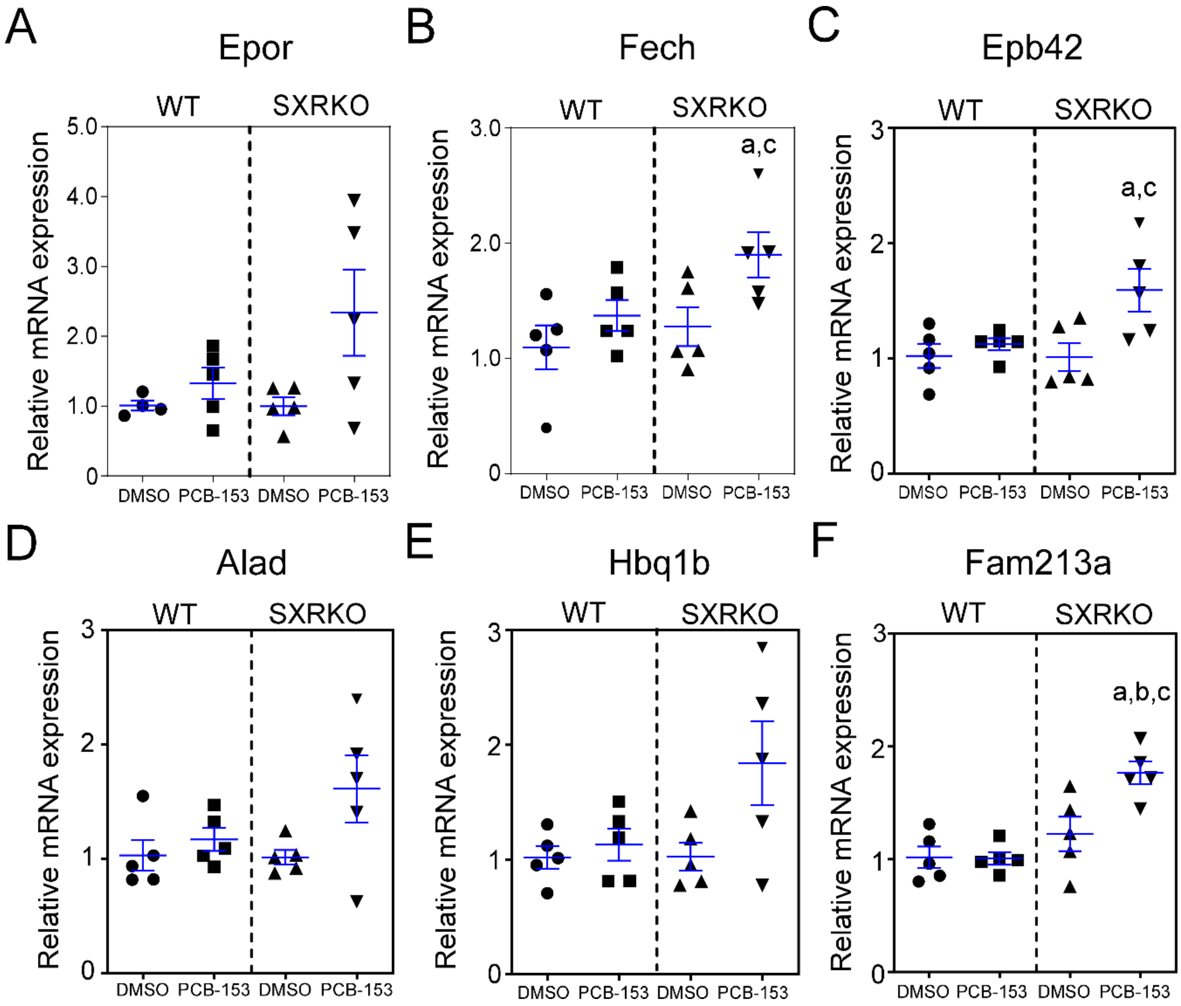


Figure S2: Relative liver and spleen weights of dams and pups from perinatal PCB-153 exposure study. Relative liver weights (A) and spleen weights (B) for DMSO and PCB-153 exposed WT and SXRKO dams (WT DMSO: n=9; WT PCB-153: n=9; SXRKO DMSO: n=5; SXRKO PCB-153: n=7). Relative liver weights (C) and spleen weights (D) for perinatally exposed WT and SXRKO mice at 4 weeks of age (WT DMSO: n= 19; WT PCB-153: n=18; SXRKO DMSO: n=16; SXRKO PCB-153: n=13). Plotted as mean ± SEM.



<u>Figure S3</u>: Gene Ontology (GO) term enrichment of spleen RNA-seq data set comparing SXRKO mice exposed to PCB-153 vs SXRKO mice exposed to vehicle (DMSO) in perinatal exposure study. GO term enrichment of select biological processes (BP) of SXRKO PCB vs SXRKO DMSO in the spleen RNA-seq dataset. Number of significant genes within each term is displayed to the right of the bar.



<u>Figure S4</u>: qPCR validation of select differentially expressed genes (DEGs) related to erythrocyte development and heme metabolism in the spleen. Relative mRNA expression of Erythropoietin receptor (Epor) (A), Ferrochelatase (Fech) (B), Erythrocyte membrane protein band 4.2 (Epb42) (C), δ-aminolevulinic acid dehydratase (Alad) (D), hemoglobin subunit theta 1B (Hbq1b) (E), and Redox-regulatory protein Fam213a (F) in the spleen (n= 5 per group) determined by qPCR analysis compared to β-actin/Actb housekeeping gene. Plotted as mean ± SEM. a= statistically significant compared to WT DMSO, b= statistically significant compared to WT PCB, c= statistically significant compared to SXRKO DMSO determined by two-way ANOVA and Tukey's multiple comparisons test.

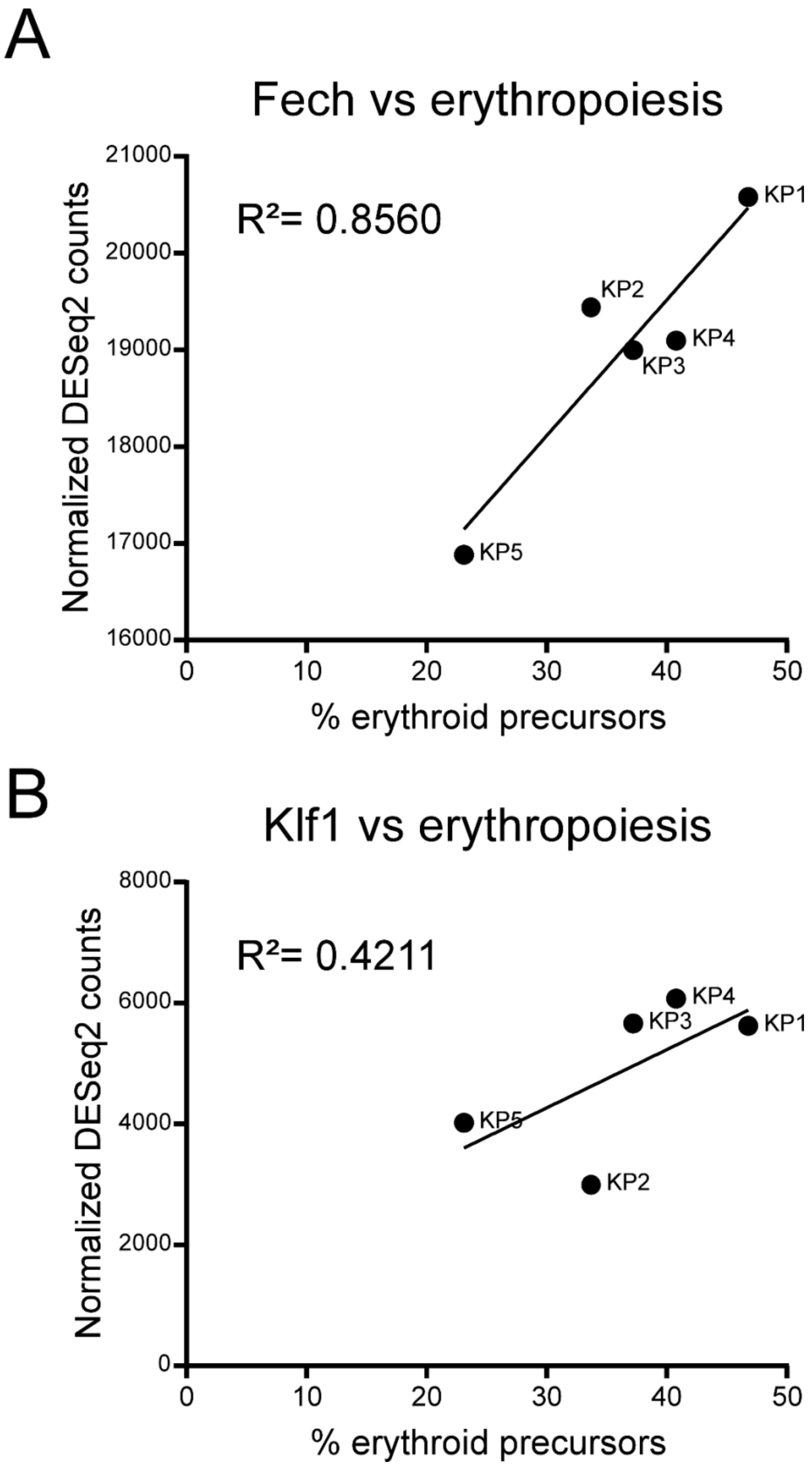
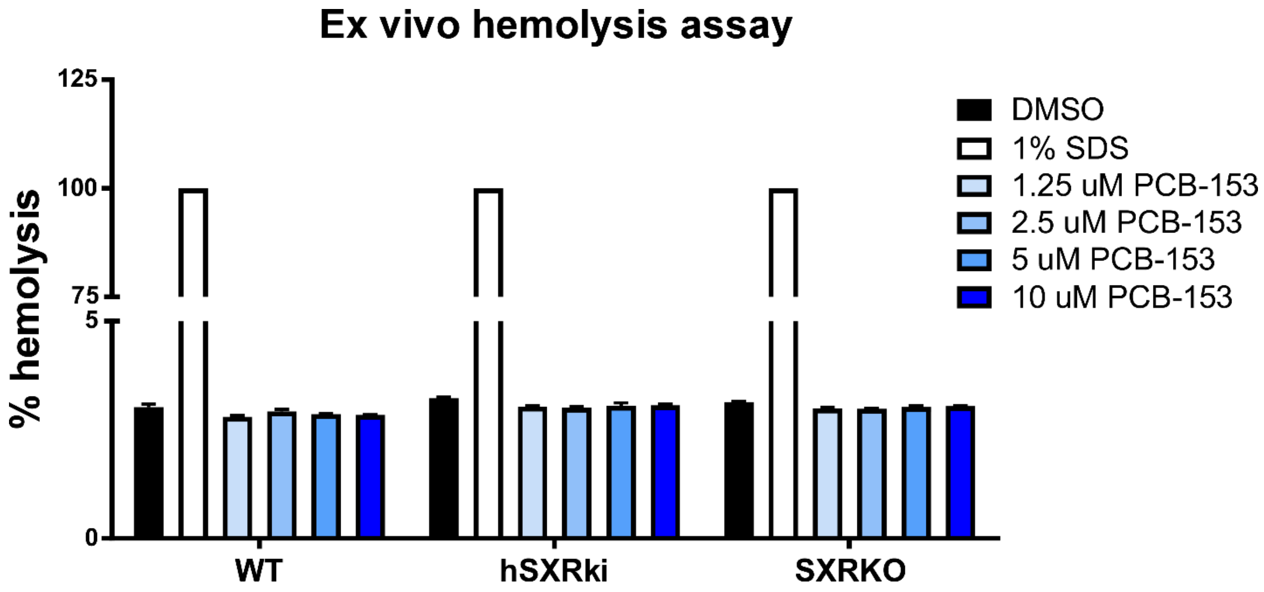


Figure S5: Correlation plots of mRNA expression of erythrocyte development and heme metabolism genes with level of erythropoiesis in the spleen of SXRKO mice exposed to PCB-153. Plots of the percentage of splenic erythroid precursors vs normalized DESeq2 counts in the spleen dataset for Fech (A) and Klf1 (B). KP= SXRKO mice exposed to PCB-153. KP#= sample ID#



<u>Figure S6</u>: PCB-153 *ex vivo* hemolysis assay. *Ex vivo* hemolysis assay of red blood cells (RBCs) from WT (n=3), hSXRki (n=3), and SXRKO (n=3) mice treated *ex vivo* to vehicle (DMSO), positive control (1% SDS), and four different concentrations of PCB-153. Results plotted as mean fold over positive control ± SEM.

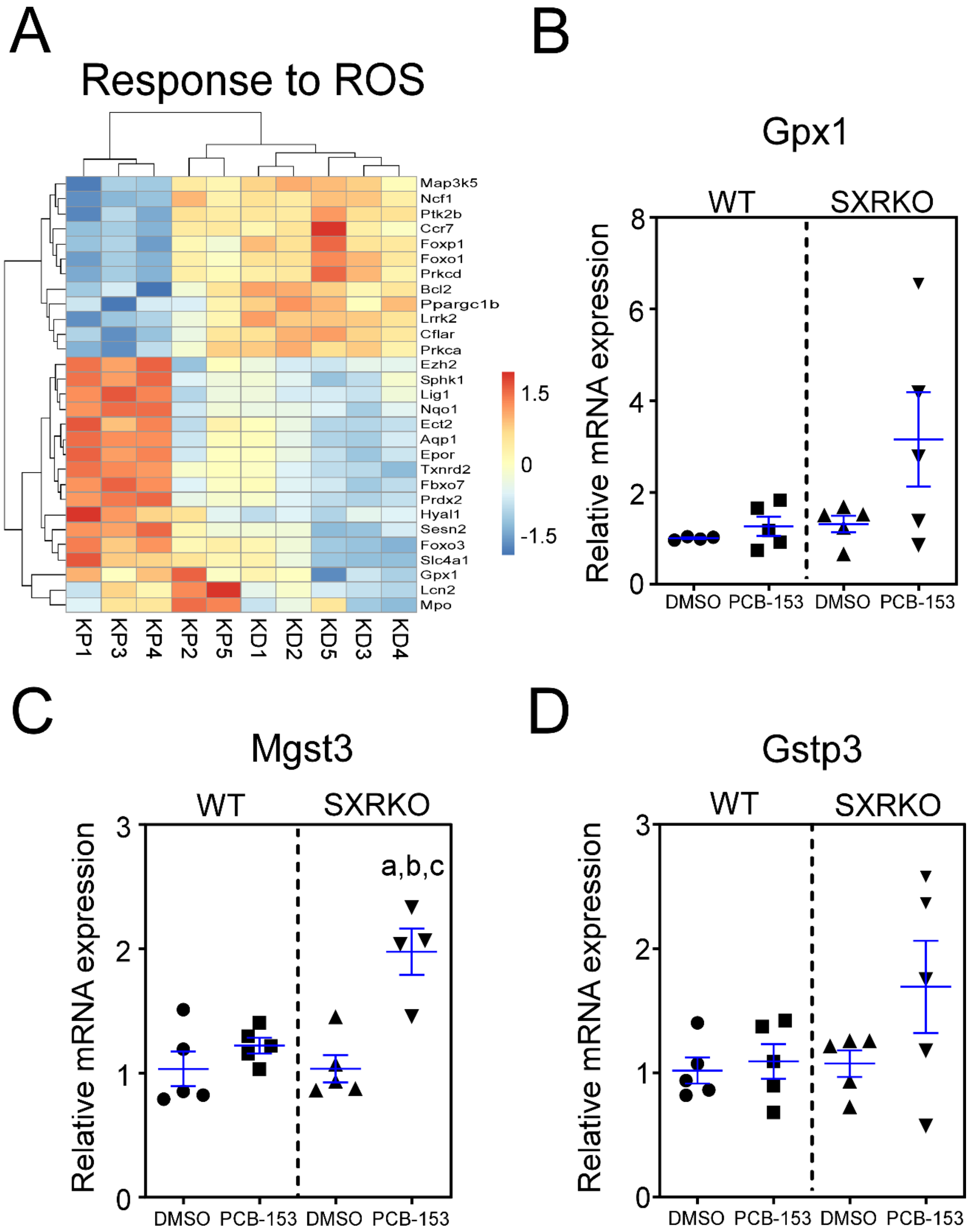


Figure S7: qPCR validation of differentially expressed genes (DEGs) related to oxidative stress in the spleen of SXRKO mice exposed to PCB-153 vs vehicle (DMSO). Heatmap of DEGs in the GO term of Response to reactive oxygen species (ROS) (A). qPCR validation of the antioxidant gene Gpx1 (B) and glutathione S-transferase genes, Mgst3 (C) and Gstp3 in the spleen (n= 5 per group) compared to Actb housekeeping gene, plotted as mean ± SEM. a= statistically significant compared to WT DMSO, b= statistically significant compared to WT PCB, c= statistically significant compared to SXRKO DMSO determined by two-way ANOVA and Tukey's multiple comparisons test.

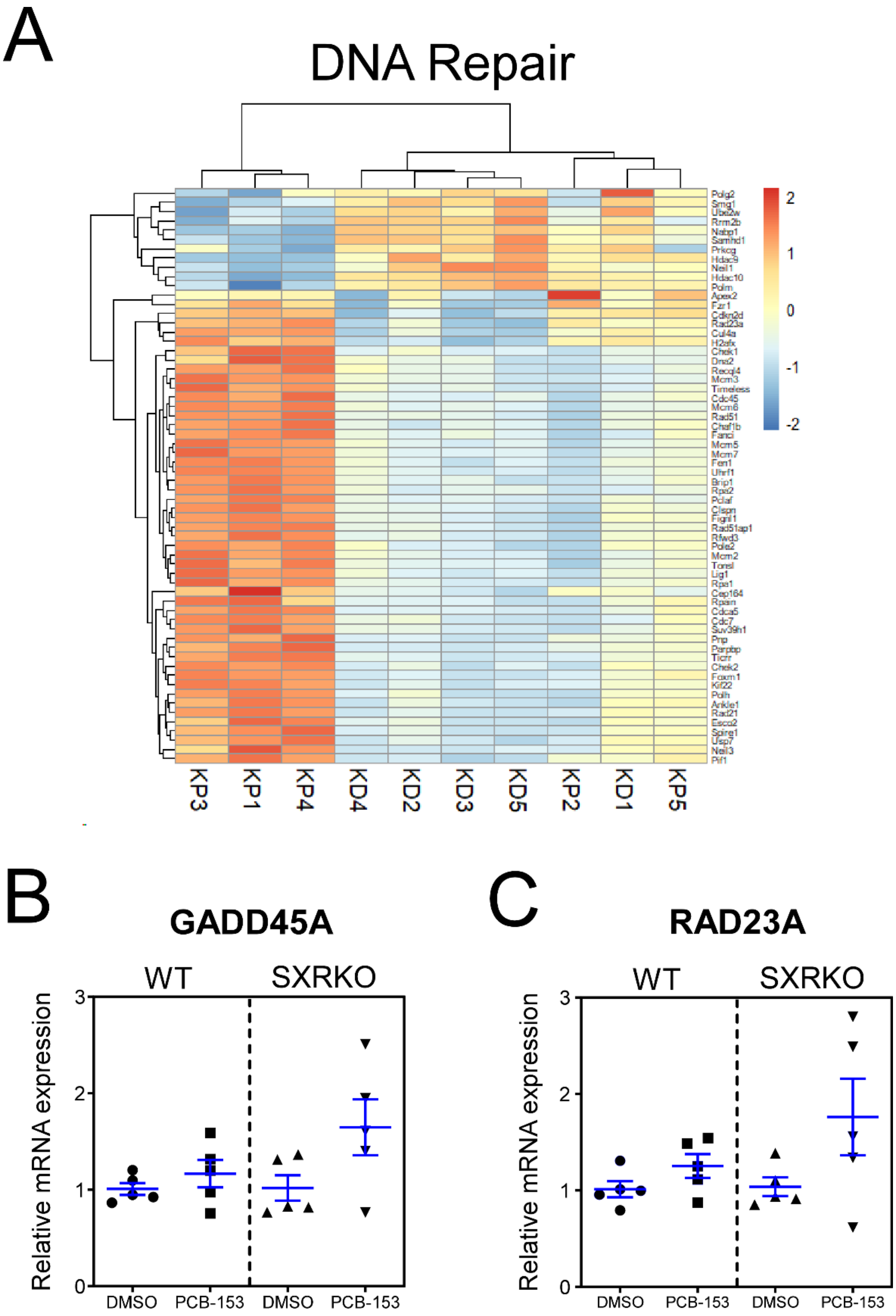
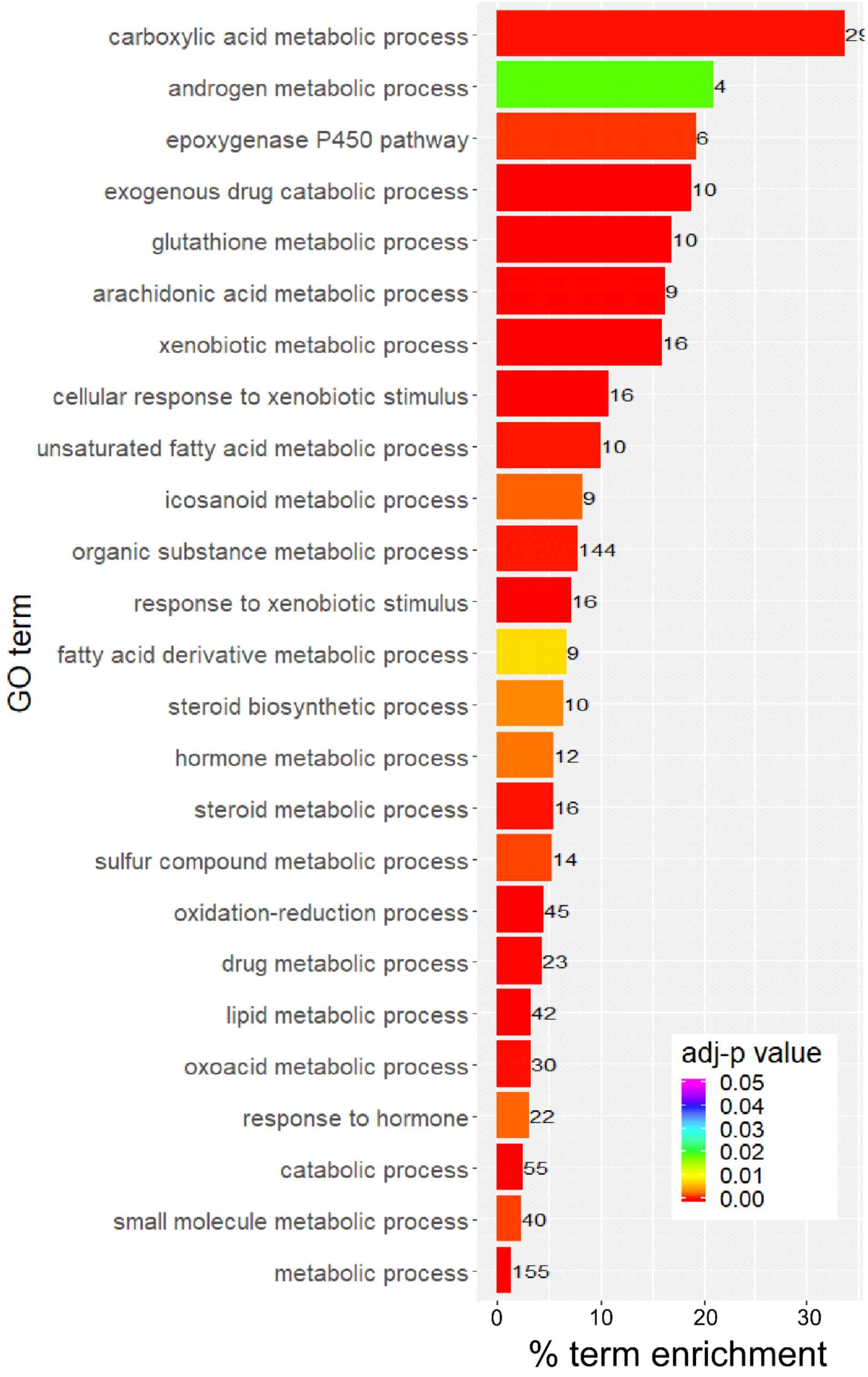


Figure S8:**DNA repair genes in the spleen of WT and SXRKO mice exposed to vehicle or PCB-153**. Heatmap of significant differentially expressed genes within the GO term of DNA repair between spleens of SXRKO mice exposed to PCB-153 vs DMSO (A). qPCR validation of Growth Arrest and DNA Damage Inducible Alpha (Gadd45a) (B) and RAD23 Homolog A (Rad23a) (C) in the spleen (n= 5 per group) compared to Actb housekeeping gene, plotted as mean ± SEM.



<u>Figure S9</u>: Gene Ontology (GO) term enrichment of liver RNA-seq dataset comparing SXRKO vs wild-type mice exposed to vehicle (DMSO). GO term enrichment of select biological processes (BP) of SXRKO DMSO vs WT DMSO in the liver RNA-seq dataset. Number of significant genes within each term is displayed to the right of the bar.

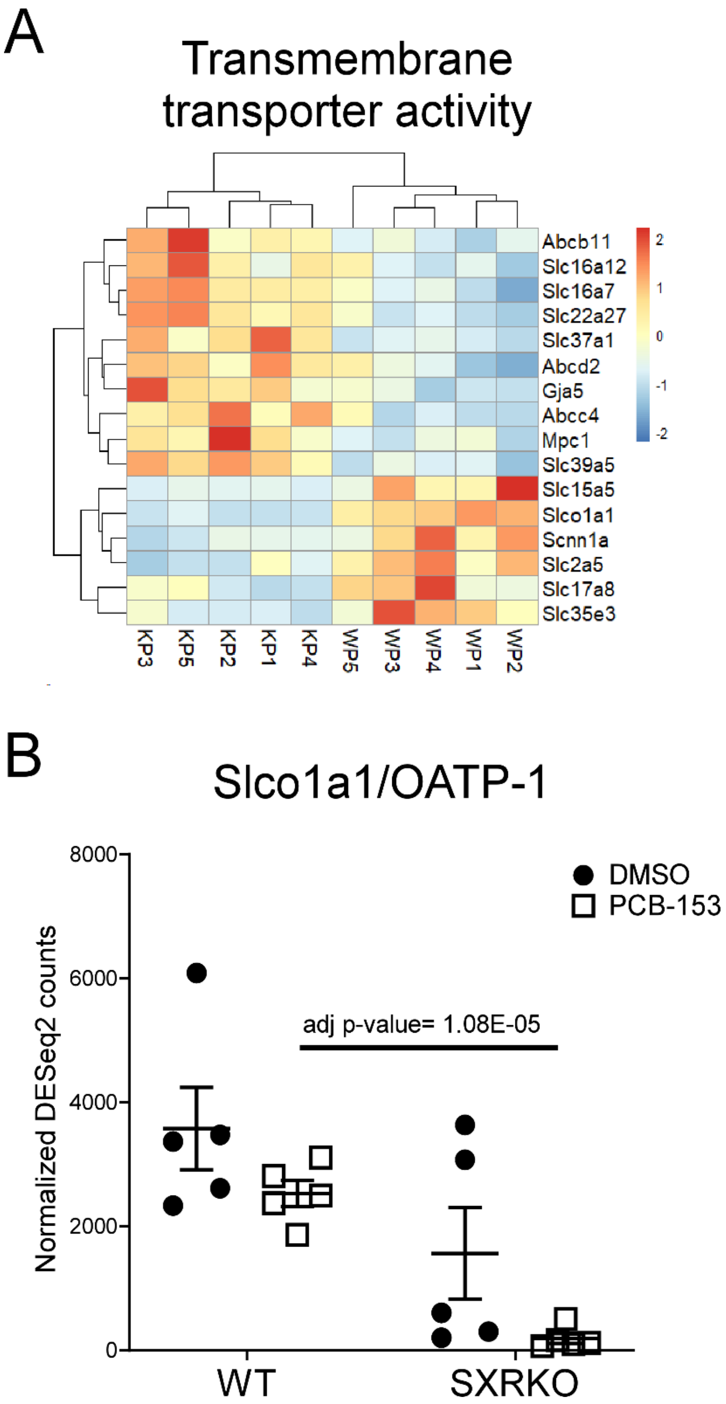
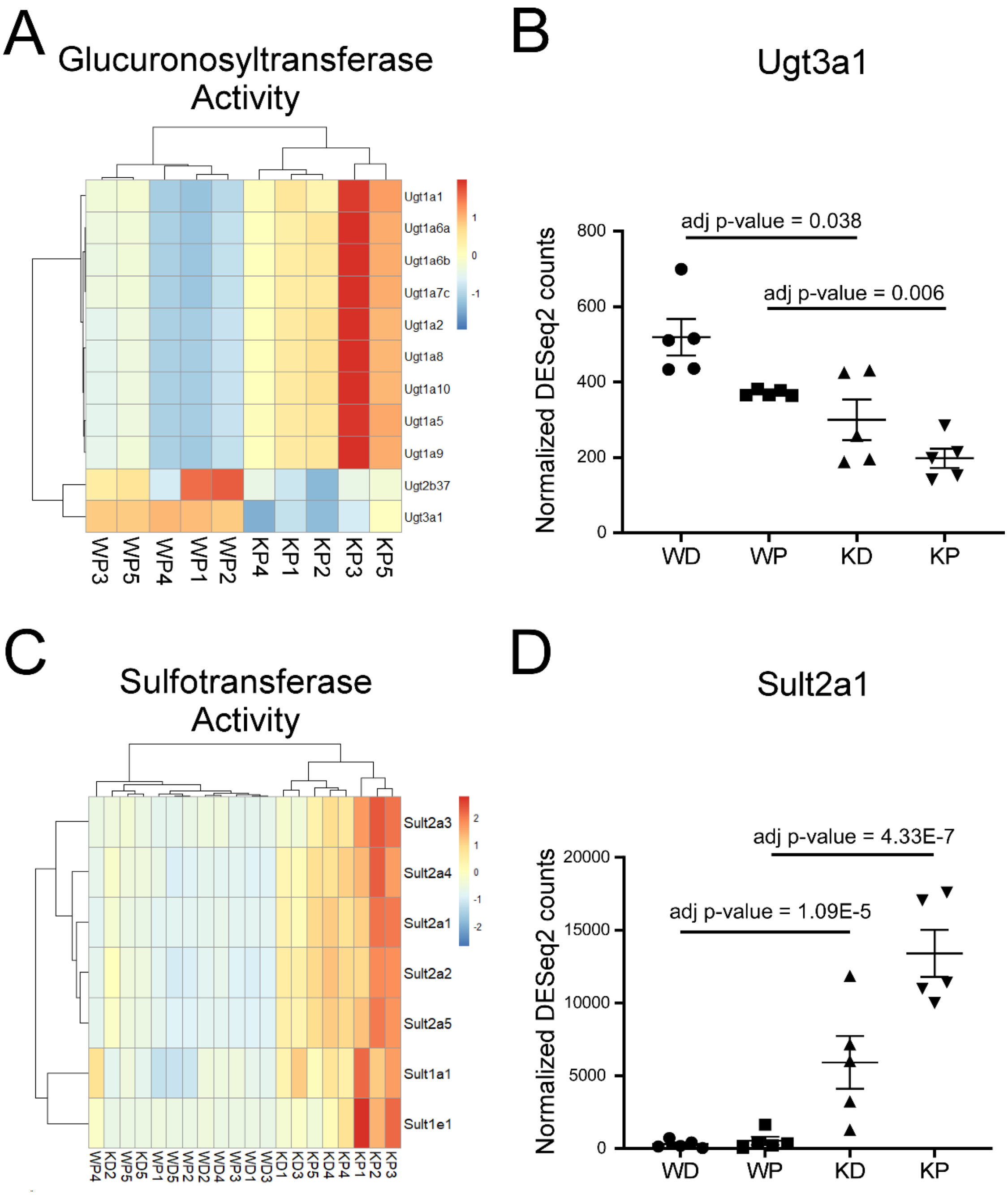
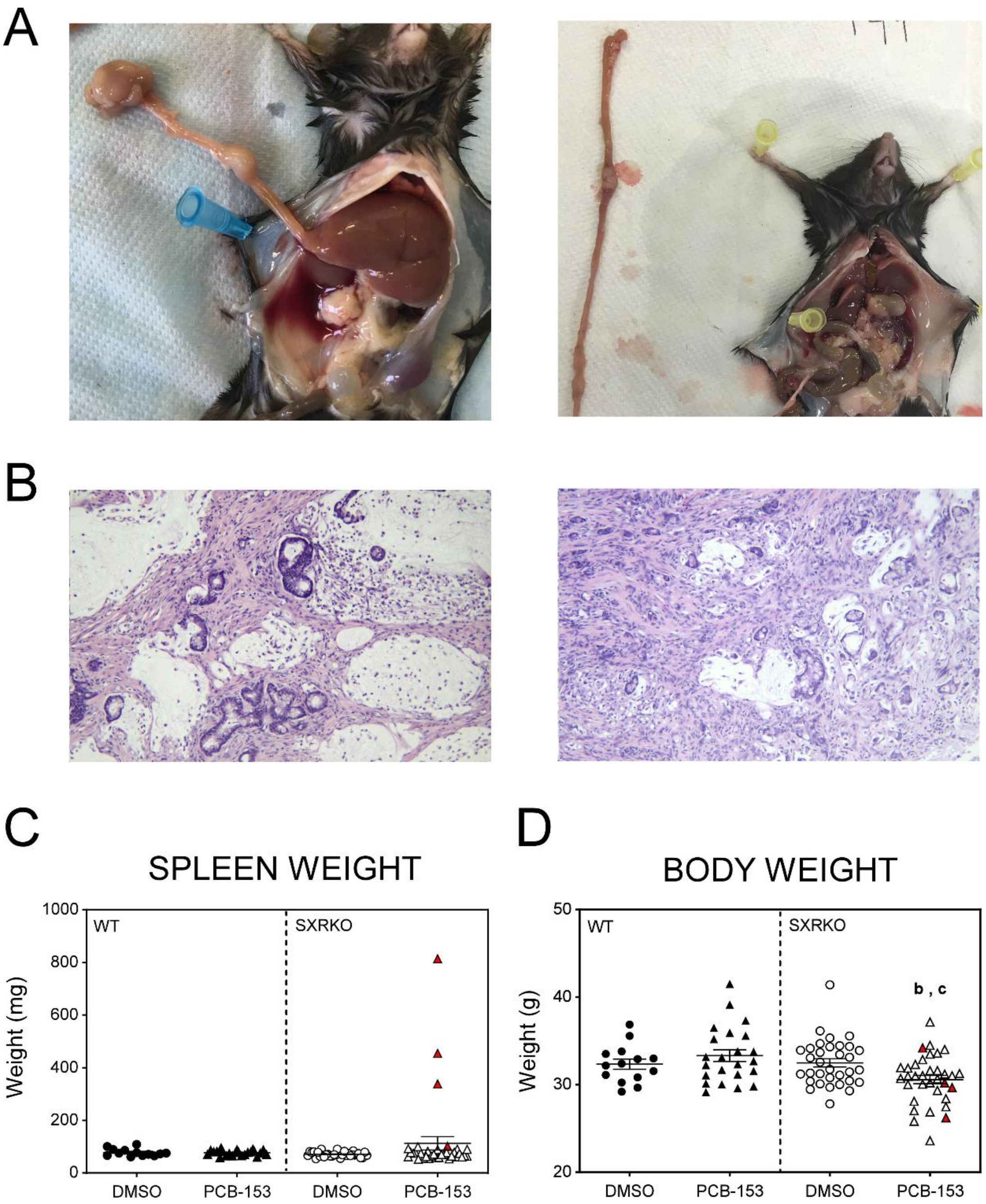


Figure S10: Differential expression of genes related to transmembrane transporter activity between WT and SXRKO mice with DMSO and PCB-153 exposure. Heatmap of DEGs related to transmembrane transporter activity in the livers between SXRKO and WT mice exposed to PCB-153 (A). Normalized counts of solute carrier organic anion transporter family member 1A1 (Sclo1a1/OATP-1) obtained from DESeq2 (B). Lines demonstrate the comparisons that were significant as determined by DESeq2 using the criteria listed in the Methods. Adjusted p-values determined by DESeq2 analysis are displayed above lines for corresponding comparisons.



<u>Figure S11</u>: Differential expression of various UGT and SULT genes between livers of WT and SXRKO mice with DMSO and PCB-153 exposure. Heatmap of DEGs related to glucuronosyltransferase activity in the livers between SXRKO and WT mice exposed to PCB-153 (A). Normalized counts of Ugt3a1 obtained from DESeq2 (B). Heatmap of DEGs related to sulfotransferase activity, comparing all groups (C). Normalized counts of Sult2a1 obtained from DESeq2 analysis (D). Lines demonstrate the comparisons that were significant as determined by DESeq2 using the criteria listed in the Methods. Adjusted p-values determined by DESeq2 analysis are displayed above lines for corresponding comparisons.



<u>Figure S12</u>: Tumor images, body weights, and spleen weights of mice chronically exposed to PCB-153. Representative images of SXRKO mice chronically exposed to PCB-153 displaying tumors in the upper small intestine near the duodenum-jejunum junction (A). Representative histology images of the intestinal tumors at 40X total magnification (B). Spleen weights (C) and body weights (D) of chronically exposed mice (WT DMSO: n= 14; WT PCB-153: n=23; SXRKO DMSO: n=33; SXRKO PCB-153: n=35). Tumor bearing mice are indicated with red shaded symbols in panel C and D. Data plotted as mean ± SEM. b= statistically significant compared to WT PCB, c= statistically significant compared to SXRKO DMSO determined by two-way ANOVA and Tukey’s multiple comparisons test.

RNA-seq script

```
#########################
#STAR HPC Cluster Script#
#########################

#generate genome index on HPC cluster
module purge
module load gcc/6.4.0 STAR/2.6.0c
STAR --runThreadN 12 \
--runMode genomeGenerate \
--genomeDir /bio/regusqui/PCB_RNAseq/mm10/STARindexENSEMBLandEncode \
--genomeFastaFiles
/bio/regusqui/PCB_RNAseq/mm10/ENSEMBLfasta/Mus_musculus.GRCm38.dna.pri
mary_assembly.fa \
--sjdbGTFfile
/bio/regusqui/PCB_RNAseq/mm10/ENCODEmouseAnnotation/gencode.vM20.annot
ation.gtf \
--sjdbOverhang 99

#Align fastq files to mouse genome (repeated for each file)
STAR --runThreadN 16 \
--genomeDir /dfs3/bio/regusqui/PCB_RNAseq/mm10/STARindexENSEMBL \
--readFilesIn
/dfs3/bio/regusqui/PCB_RNAseq/liver/fastq_files/reSequencedFiles/KP2-
GCCAAT-Sequences.txt.gz \
--readFilesCommand zcat \
--outFileNamePrefix
/dfs3/bio/regusqui/PCB_RNAseq/liver/newBAMfiles/WD1_ \
--outSAMtype BAM Unsorted SortedByCoordinate

#####################
# Rsubread R Script #
#####################

#install Rsubread
if (!requireNamespace("BiocManager", quietly = TRUE))
+ install.packages(BiocManager)
BiocManager::install("Rsubread", version = "3.8")
library("Rsubread")

#set input and output directories
dir_input <- "/dfs3/bio/regusqui/PCB_RNAseq/liver/newBAMfiles/"
dir_output <- "/dfs3/bio/regusqui/PCB_RNAseq/liver/Rsubread/"
setwd(dir_output)
```

```
#specify GTF file
gtf.file <-
"/dfs3/bio/regusqui/PCB_RNAseq/mm10/ENSEMBLannotation/Mus_musculus.GRC
m38.95.gtf"

#load STAR BAM files
WD1 <- paste0(dir_input, "WD1_Aligned.out.bam")
WD2 <- paste0(dir_input, "WD2_Aligned.out.bam")
WD3 <- paste0(dir_input, "WD3_Aligned.out.bam")
WD4 <- paste0(dir_input, "WD4_Aligned.out.bam")
WD5 <- paste0(dir_input, "WD5_Aligned.out.bam")
WP1 <- paste0(dir_input, "WP1_Aligned.out.bam")
WP2 <- paste0(dir_input, "WP2_Aligned.out.bam")
WP3 <- paste0(dir_input, "WP3_Aligned.out.bam")
WP4 <- paste0(dir_input, "WP4_Aligned.out.bam")
WP5 <- paste0(dir_input, "WP5_Aligned.out.bam")
KD1 <- paste0(dir_input, "KD1_Aligned.out.bam")
KD2 <- paste0(dir_input, "KD2_Aligned.out.bam")
KD3 <- paste0(dir_input, "KD3_Aligned.out.bam")
KD4 <- paste0(dir_input, "KD4_Aligned.out.bam")
KD5 <- paste0(dir_input, "KD5_Aligned.out.bam")
KP1 <- paste0(dir_input, "KP1_Aligned.out.bam")
KP2 <- paste0(dir_input, "KP2_Aligned.out.bam")
KP3 <- paste0(dir_input, "KP3_Aligned.out.bam")
KP4 <- paste0(dir_input, "KP4_Aligned.out.bam")
KP5 <- paste0(dir_input, "KP5_Aligned.out.bam")
bam.files <-
c(WD1,WD2,WD3,WD4,WD5,WP1,WP2,WP3,WP4,WP5,KD1,KD2,KD3,KD4,KD5,KP1,KP2,
KP3,KP4,KP5)

#Run Rsubread to obtain exon counts
counts <- featureCounts(files = bam.files,
                         annot.ext = gtf.file,
                         isGTFAnnotationFile = TRUE,
                         GTF.featureType = "exon",
                         GTF.attrType = "gene_name",
                         allowMultiOverlap = TRUE,
                         isPairedEnd = FALSE,
                         nthreads = 24,
                         countMultiMappingReads = TRUE,
                         strandSpecific = 0)

# Get counts in data frame
liver_unsorted_counts.df <- as.data.frame(counts$counts)

# Simplify column names
```

```
colnames(liver_unsorted_counts.df) <-
sub("X.dfs3.bio.regusqui.PCB_RNAseq.liver.newBAMfiles.", "",
colnames(liver_unsorted_counts.df))
colnames(liver_unsorted_counts.df) <- sub("_Aligned.out.bam", "",
colnames(liver_unsorted_counts.df))

# Save data frame file
write.table(liver_unsorted_counts.df,
file=paste0(dir_output,"liver_unsorted_counts.txt"),quote=F,col.names=
T,row.names=T,sep="\t")

###################
# DESeq2 R Script #
###################

# Load DESeq2
BiocManager::install("DESeq2", version = "3.8")
library("DESeq2")

# Set new input output and working directory
dir_input <- "/dfs3/bio/regusqui/PCB_RNAseq/liver/Rsubread/"
dir_output <- "/dfs3/bio/regusqui/PCB_RNAseq/liver/DESeq2/"
setwd(dir_output)

# Load Rsubread count file
counts <- paste0(dir_input, "liver_unsorted_counts.txt"
countData <- read.table(counts, as.is=T, header=T, row.names=1)


# Define experimental design
samples <- c("WD1", "WD2", "WD3", "WD4", "WD5", "WP1", "WP2", "WP3",
"WP4", "WP5", "KD1", "KD2", "KD3", "KD4", "KD5", "KP1", "KP2", "KP3",
"KP4", "KP5")
condition <- c("WD", "WD", "WD", "WD", "WD", "WP", "WP", "WP", "WP",
"WP", "KD", "KD", "KD", "KD", "KD", "KP", "KP", "KP", "KP", "KP")
type <- "single-read"
liver_colData <- data.frame(condition=condition, type=type,
row.names=samples)

#DEG analysis
liver_dds_unsorted <- DESeqDataSetFromMatrix(countData = countData,
colData = liver_colData, design = ~ condition)
liver_dds_unsorted <- DESeq(liver_dds_unsorted)
liver_DESeq2results_KDvsWD_alpha0.05 <- results(liver_dds_unsorted,
alpha=0.05, contrast = c("condition", "KD", "WD"))
```

```
liver_DESeq2results_KPvsKD_alpha0.05 <- results(liver_dds_unsorted,
alpha = 0.05, contrast = c("condition", "KP", "KD"))
liver_DESeq2results_KPvsWP_alpha0.05 <- results(liver_dds_unsorted,
alpha = 0.05, contrast = c("condition", "KP", "WP"))
liver_DESeq2results_WPvsWD_alpha0.05 <- results(liver_dds_unsorted,
alpha = 0.05, contrast = c("condition", "WP", "WD"))
write.table(liver_DESeq2results_KDvsWD_alpha0.05, file =
paste0(dir_output, "liver_KDvsWD_alpha0.05.txt"), quote = F, col.names
= T, sep = "\t")
write.table(liver_DESeq2results_KPvsKD_alpha0.05, file =
paste0(dir_output, "liver_KPvsKD_alpha0.05.txt"), quote = F, col.names
= T, sep = "\t")
write.table(liver_DESeq2results_KPvsWP_alpha0.05, file =
paste0(dir_output, "liver_KPvsWP_alpha0.05.txt"), quote = F, col.names
= T, sep = "\t")
write.table(liver_DESeq2results_WPvsWD_alpha0.05, file =
paste0(dir_output, "liver_WPvsWD_alpha0.05.txt"), quote = F, col.names
= T, sep = "\t")

#obtain normalized counts
liver_dds_unsorted <- estimateSizeFactors(liver_dds_unsorted)
norm_liver_counts <- counts(liver_dds_unsorted, normalized=TRUE)
write.csv(norm_liver_counts, file="norm_liver_counts.csv")
```

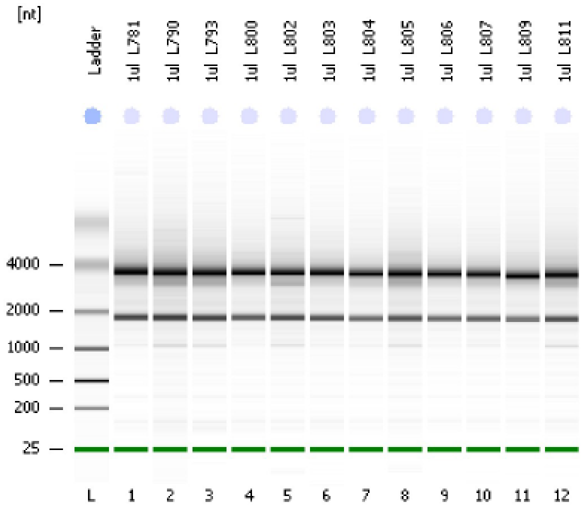
<u>RNA quality</u>: Bioanalyzer (Agilent Bioanalyzer 2100) results for RNA used for RNA-seq

<u>Liver</u>: pages S24-S34, document links on page S35

<u>Spleen</u>: pages S36-S46, document links on page S47 (ignore samples labeled as L### in these documents as these were samples that were not included in the analysis for this experiment)

Assay Class: EukaryoteTotal RNA Nano
Data Path: D:\..._EukaryoteTotal RNA Nano_DE11400731_2019-03-05_14-45-22.xad
Created: 3/5/2019 2:45:22 PM
Modified: 3/6/2019 12:05:38 PM

Electrophoresis File Run Summary



Instrument Information:

Instrument Name: DE11400731
Firmware: C.01.069
Serial#: DE11400731
Type: G2938A

Assay Information:

Assay Origin Path: C:\Program Files\Agilent\2100 bioanalyzer\2100 expert\assays\RNA\Eukaryote Total RNA Nano Series II.xsy

Title: Eukaryote Total RNA Nano Series II

Version: 2.5

Assay Comments: Copyright © 2003-2006 Agilent Technologies

Chip Information:

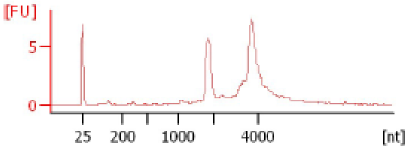
Chip Lot:

Reagent Kit Lot:

Chip Comments:

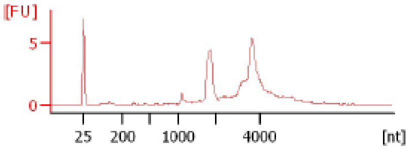
1ul_L781

RIN:10



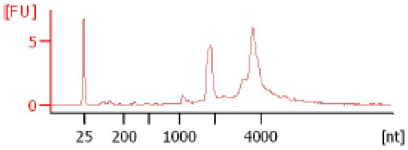
1ul_L790

RIN: 9.20



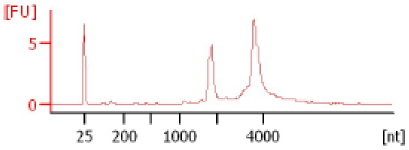
1ul_L793

RIN: 9.70



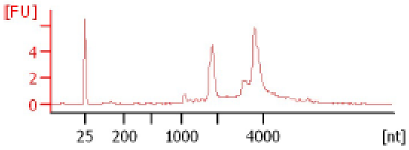
1ul_L800

RIN: 9.30



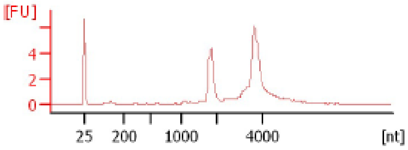
1ul_L802

RIN: 9.60



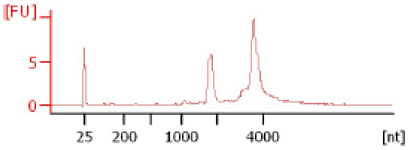
1ul_L803

RIN: 9.40



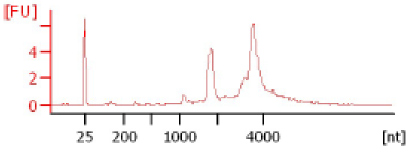
1ul_L804

RIN: 9.90



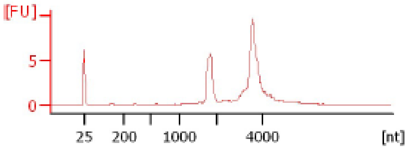
1ul_L805

RIN: 9.50



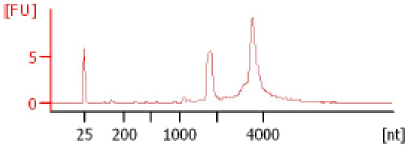
1ul_L806

RIN:10



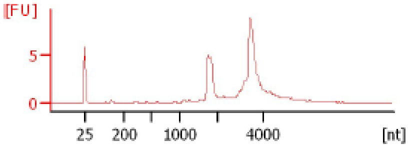
1ul_L807

RIN: 9.90



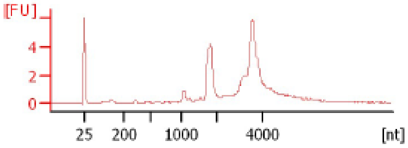
1ul_L809

RIN: 9.90



1ul_L811

RIN: 9.60



Printed: 3/6/2019 12:06:22 PM

Assay Class:	EukaryoteTotal RNA Nano	Created:	3/5/2019 2:45:22 PM
Data Path:	D:\..._EukaryoteTotal RNA Nano_DE11400731_2019-03-05_14-45-22.xad	Modified:	3/6/2019 12:05:38 PM

Electrophoresis File Run Summary (Chip Summary)

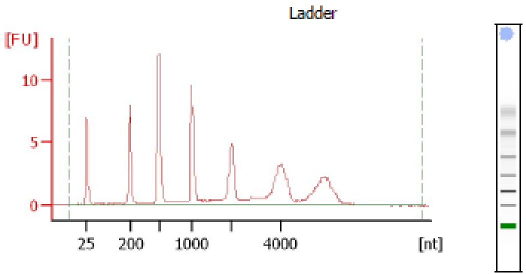
Sample Name	Sample Comment	Status	Result Label	Result Color
1ul_L781		✓	RIN:10	🟪
1ul_L790		✓	RIN: 9.20	🟪
1ul_L793		✓	RIN: 9.70	🟪
1ul_L800		✓	RIN: 9.30	🟪
1ul_L802		✓	RIN: 9.60	🟪
1ul_L803		✓	RIN: 9.40	🟪
1ul_L804		✓	RIN: 9.90	🟪
1ul_L805		✓	RIN: 9.50	🟪
1ul_L806		✓	RIN:10	🟪
1ul_L807		✓	RIN: 9.90	🟪
1ul_L809		✓	RIN: 9.90	🟪
1ul_L811		✓	RIN: 9.60	🟪

Chip Lot # **Reagent Kit Lot #**

Chip Comments :

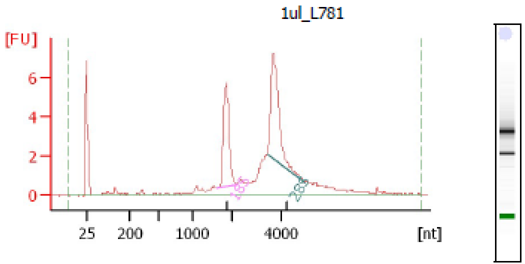
Assay Class:	EukaryoteTotal RNA Nano	Created:	3/5/2019 2:45:22 PM
Data Path:	D:\..._EukaryoteTotal RNA Nano_DE11400731_2019-03-05_14-45-22.xad	Modified:	3/6/2019 12:05:38 PM

Electropherogram Summary



Overall Results for Ladder

RNA Area:	91.4
RNA Concentration:	150 ng/µl
Result Flagging Color:	🟦
Result Flagging Label:	All Other Samples

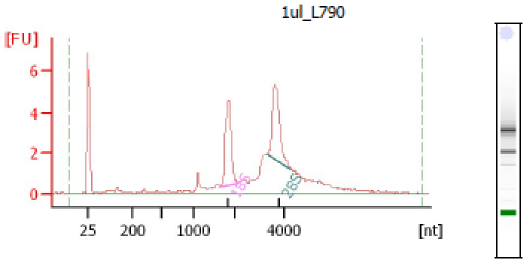


Overall Results for sample 1 : 1ul_L781

RNA Area:	62.2
RNA Concentration:	102 ng/µl
rRNA Ratio [28s / 18s]:	1.3
RNA Integrity Number (RIN):	10 (B.02.06)
Result Flagging Color:	🟦
Result Flagging Label:	RIN:10

Fragment table for sample 1 : 1ul_L781

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,624	2,188	11.1	17.8
28S	3,410	4,938	14.4	23.1

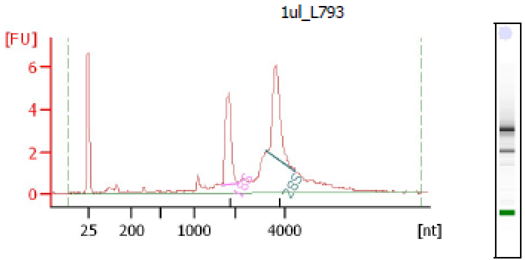


Overall Results for sample 2 : 1ul_L790

RNA Area:	56.2
RNA Concentration:	92 ng/µl
rRNA Ratio [28s / 18s]:	1.0
RNA Integrity Number (RIN):	9.2 (B.02.06)
Result Flagging Color:	🟦
Result Flagging Label:	RIN: 9.20

Fragment table for sample 2 : 1ul_L790

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,636	2,147	8.9	15.9
28S	3,313	4,257	8.8	15.7



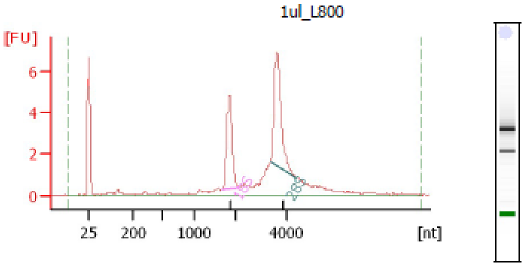
Overall Results for sample 3 : 1ul_L793

RNA Area:	57.0
RNA Concentration:	94 ng/µl
rRNA Ratio [28s / 18s]:	1.1
RNA Integrity Number (RIN):	9.7 (B.02.06)
Result Flagging Color:	🟦
Result Flagging Label:	RIN: 9.70

Fragment table for sample 3 : 1ul_L793

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,657	2,177	9.2	16.2
28S	3,253	4,412	10.3	18.1

Electropherogram Summary Continued ...

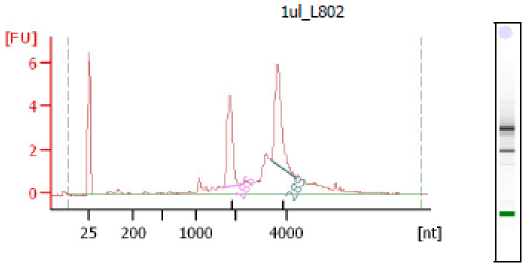


Overall Results for sample 4 : 1ul_L800

RNA Area: 51.6
RNA Concentration: 85 ng/µl
rRNA Ratio [28s / 18s]: 1.3
RNA Integrity Number (RIN): 9.3 (B.02.06)
Result Flagging Color: 🟦
Result Flagging Label: RIN: 9.30

Fragment table for sample 4 : 1ul_L800

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,667	2,149	9.2	17.8
28S	3,395	4,358	12.3	23.9

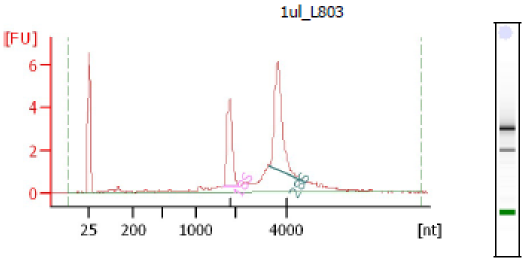


Overall Results for sample 5 : 1ul_L802

RNA Area: 47.7
RNA Concentration: 78 ng/µl
rRNA Ratio [28s / 18s]: 1.1
RNA Integrity Number (RIN): 9.6 (B.02.06)
Result Flagging Color: 🟦
Result Flagging Label: RIN: 9.60

Fragment table for sample 5 : 1ul_L802

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,634	2,208	8.5	17.8
28S	3,368	4,347	9.6	20.2

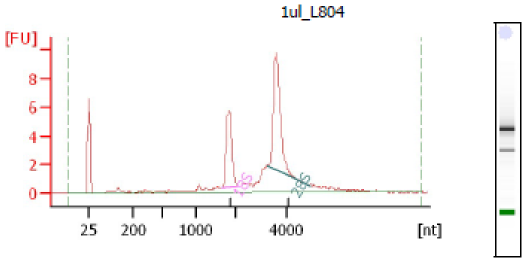


Overall Results for sample 6 : 1ul_L803

RNA Area: 44.7
RNA Concentration: 73 ng/µl
rRNA Ratio [28s / 18s]: 1.5
RNA Integrity Number (RIN): 9.4 (B.02.06)
Result Flagging Color: 🟦
Result Flagging Label: RIN: 9.40

Fragment table for sample 6 : 1ul_L803

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,695	2,122	8.4	18.7
28S	3,337	4,764	12.2	27.4



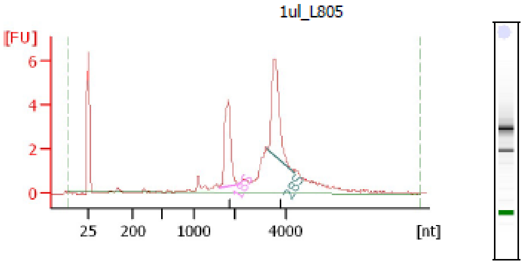
Overall Results for sample 7 : 1ul_L804

RNA Area: 62.4
RNA Concentration: 102 ng/µl
rRNA Ratio [28s / 18s]: 1.5
RNA Integrity Number (RIN): 9.9 (B.02.06)
Result Flagging Color: 🟦
Result Flagging Label: RIN: 9.90

Fragment table for sample 7 : 1ul_L804

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,660	2,136	11.3	18.2
28S	3,263	4,920	17.5	28.0

Electropherogram Summary Continued ...

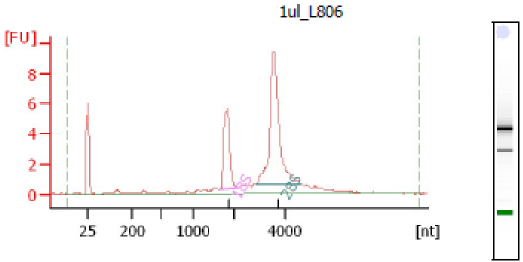


Overall Results for sample 8 : **1ul_L805**

RNA Area:	50.1
RNA Concentration:	82 ng/µl
rRNA Ratio [28s / 18s]:	1.2
RNA Integrity Number (RIN):	9.5 (B.02.06)
Result Flagging Color:	⬜
Result Flagging Label:	RIN: 9.50

Fragment table for sample 8 : **1ul_L805**

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,597	2,177	8.6	17.1
28S	3,293	4,373	10.4	20.8

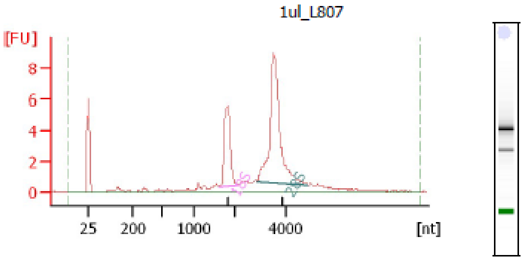


Overall Results for sample 9 : **1ul_L806**

RNA Area:	62.1
RNA Concentration:	102 ng/µl
rRNA Ratio [28s / 18s]:	2.4
RNA Integrity Number (RIN):	10 (B.02.06)
Result Flagging Color:	⬜
Result Flagging Label:	RIN:10

Fragment table for sample 9 : **1ul_L806**

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,667	2,107	11.1	17.8
28S	2,871	4,599	26.4	42.5

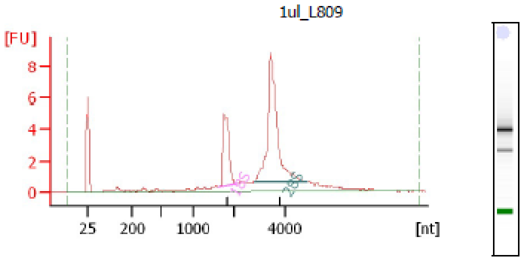


Overall Results for sample 10 : **1ul_L807**

RNA Area:	62.7
RNA Concentration:	103 ng/µl
rRNA Ratio [28s / 18s]:	2.3
RNA Integrity Number (RIN):	9.9 (B.02.06)
Result Flagging Color:	⬜
Result Flagging Label:	RIN: 9.90

Fragment table for sample 10 : **1ul_L807**

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,632	2,107	11.3	18.0
28S	2,843	4,911	26.4	42.2



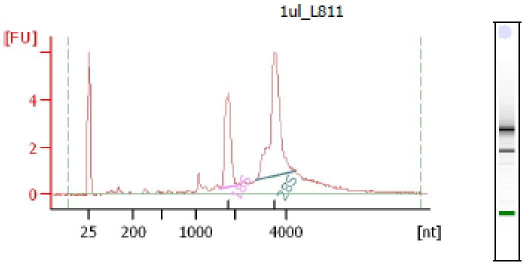
Overall Results for sample 11 : **1ul_L809**

RNA Area:	58.6
RNA Concentration:	96 ng/µl
rRNA Ratio [28s / 18s]:	2.6
RNA Integrity Number (RIN):	9.9 (B.02.06)
Result Flagging Color:	⬜
Result Flagging Label:	RIN: 9.90

Fragment table for sample 11 : **1ul_L809**

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,623	2,107	10.1	17.3
28S	2,786	4,868	26.2	44.6

Electropherogram Summary Continued ...


1ul_L811

Overall Results for sample 12 : **1ul_L811**

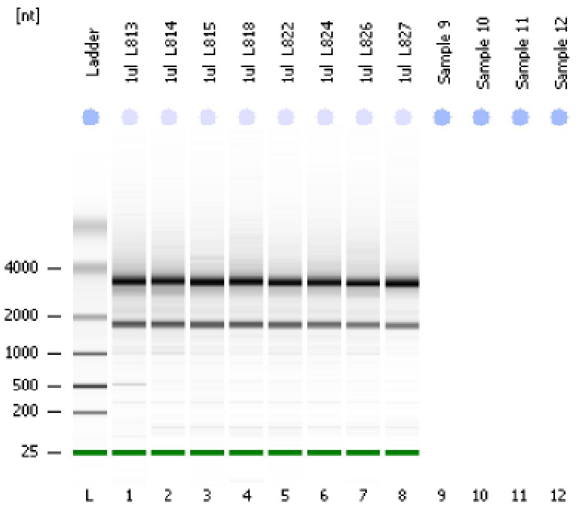
RNA Area:	50.2
RNA Concentration:	82 ng/µl
rRNA Ratio [28s / 18s]:	2.0
RNA Integrity Number (RIN):	9.6 (B.02.06)
Result Flagging Color:	▭
Result Flagging Label:	RIN: 9.60

Fragment table for sample 12 : **1ul_L811**

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,598	2,093	8.5	16.9
28S	2,786	4,312	17.0	33.9

Assay Class: EukaryoteTotal RNA Nano
Data Path: D:\..._EukaryoteTotal RNA Nano_DE11400731_2019-03-06_12-36-04.xad
Created: 3/6/2019 12:36:04 PM
Modified: 3/6/2019 12:57:59 PM

Electrophoresis File Run Summary



Instrument Information:

Instrument Name: DE11400731
Firmware: C.01.069
Serial#: DE11400731
Type: G2938A

Assay Information:

Assay Origin Path: C:\Program Files\Agilent\2100 bioanalyzer\2100 expert\assays\RNA\Eukaryote Total RNA Nano Series II.xsy

Title: Eukaryote Total RNA Nano Series II

Version: 2.5

Assay Comments: Copyright © 2003-2006 Agilent Technologies

Chip Information:

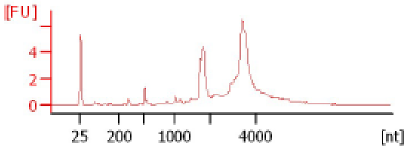
Chip Lot:

Reagent Kit Lot:

Chip Comments:

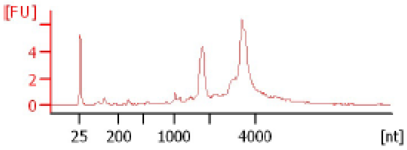
1ul_L813

RIN: 9.40



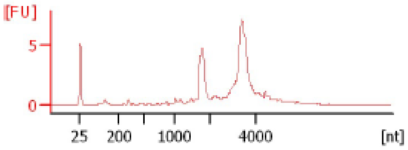
1ul_L814

RIN: 9.30



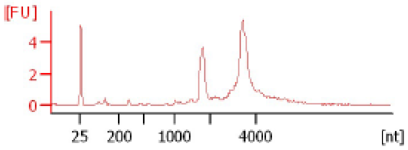
1ul_L815

RIN: 9.60



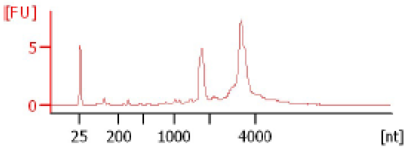
1ul_L818

RIN: 9.80



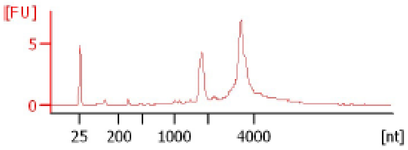
1ul_L822

RIN: 9.70



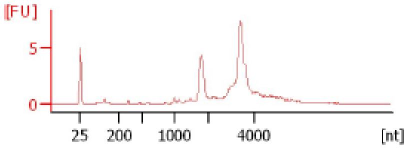
1ul_L824

RIN: 9.50



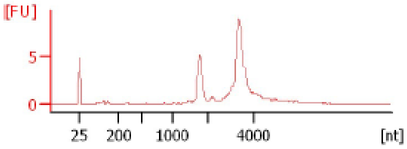
1ul_L826

RIN: 9.50



1ul_L827

RIN:10



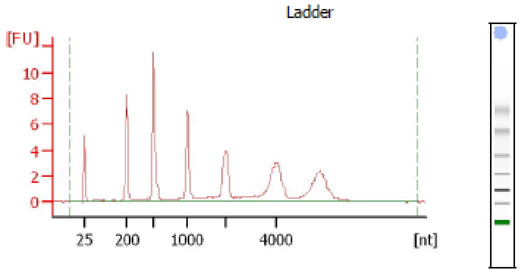
Electrophoresis File Run Summary (Chip Summary)

Sample Name	Sample Comment	Status	Result Label	Result Color
1ul_L813		✓	RIN: 9.40	▭
1ul_L814		✓	RIN: 9.30	▭
1ul_L815		✓	RIN: 9.60	▭
1ul_L818		✓	RIN: 9.80	▭
1ul_L822		✓	RIN: 9.70	▭
1ul_L824		✓	RIN: 9.50	▭
1ul_L826		✓	RIN: 9.50	▭
1ul_L827		✓	RIN:10	▭
Sample 9				🟦
Sample 10				🟦
Sample 11				🟦
Sample 12				🟦

Chip Lot # **Reagent Kit Lot #**

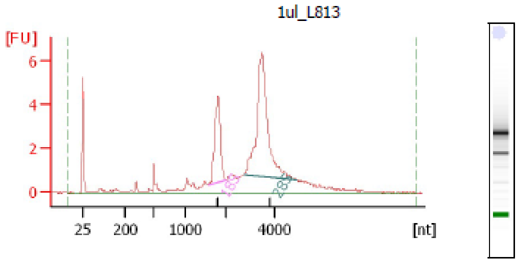
Chip Comments :

Electropherogram Summary



Overall Results for Ladder

RNA Area:	86.4
RNA Concentration:	150 ng/µl
Result Flagging Color:	🟦
Result Flagging Label:	All Other Samples

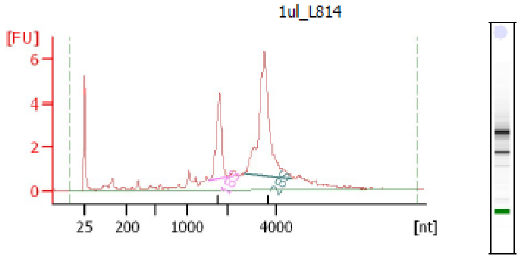


Overall Results for sample 1 : **<u>1ul_L813</u>**

RNA Area:	63.0
RNA Concentration:	109 ng/µl
rRNA Ratio [28s / 18s]:	2.3
RNA Integrity Number (RIN):	9.4 (A.01.01, Anomaly Threshold(s) manually adapted)
Result Flagging Color:	🟦
Result Flagging Label:	RIN: 9.40

Fragment table for sample 1 : **<u>1ul_L813</u>**

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,574	2,057	9.2	14.6
28S	2,751	4,845	21.2	33.7

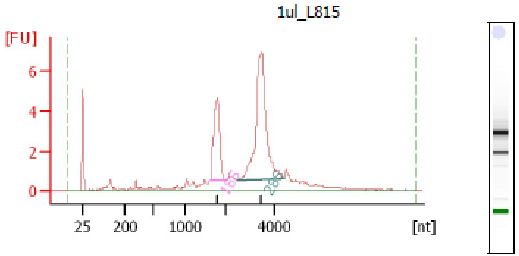


Overall Results for sample 2 : **<u>1ul_L814</u>**

RNA Area:	61.5
RNA Concentration:	107 ng/µl
rRNA Ratio [28s / 18s]:	2.3
RNA Integrity Number (RIN):	9.3 (A.01.01)
Result Flagging Color:	🟦
Result Flagging Label:	RIN: 9.30

Fragment table for sample 2 : **<u>1ul_L814</u>**

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,547	2,043	9.1	14.8
28S	2,722	4,619	20.6	33.5



Overall Results for sample 3 : **<u>1ul_L815</u>**

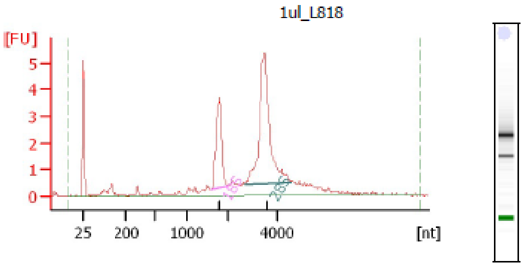
RNA Area:	60.7
RNA Concentration:	105 ng/µl
rRNA Ratio [28s / 18s]:	2.2
RNA Integrity Number (RIN):	9.6 (A.01.01)
Result Flagging Color:	🟦
Result Flagging Label:	RIN: 9.60

Fragment table for sample 3 : **<u>1ul_L815</u>**

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,637	2,015	10.1	16.6
28S	2,524	4,406	22.7	37.3

Assay Class:	EukaryoteTotal RNA Nano	Created:	3/6/2019 12:36:04 PM
Data Path:	D:\..._EukaryoteTotal RNA Nano_DE11400731_2019-03-06_12-36-04.xad	Modified:	3/6/2019 12:57:59 PM

Electropherogram Summary Continued ...

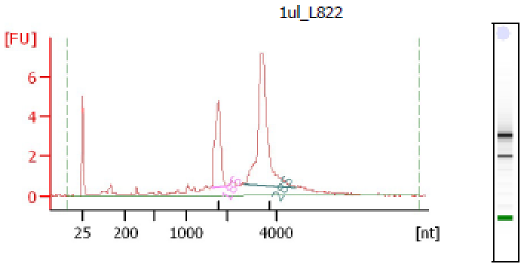


Overall Results for sample 4 : **1ul_L818**

RNA Area:	46.8
RNA Concentration:	81 ng/µl
rRNA Ratio [28s / 18s]:	2.2
RNA Integrity Number (RIN):	9.8 (A.01.01)
Result Flagging Color:	🟦
Result Flagging Label:	RIN: 9.80

Fragment table for sample 4 : **1ul_L818**

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,574	2,015	7.6	16.3
28S	2,736	4,534	16.9	36.2

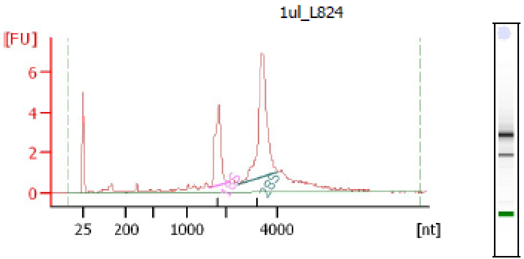


Overall Results for sample 5 : **1ul_L822**

RNA Area:	56.1
RNA Concentration:	97 ng/µl
rRNA Ratio [28s / 18s]:	2.2
RNA Integrity Number (RIN):	9.7 (A.01.01)
Result Flagging Color:	🟦
Result Flagging Label:	RIN: 9.70

Fragment table for sample 5 : **1ul_L822**

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,628	2,029	9.9	17.6
28S	2,736	4,760	21.4	38.2

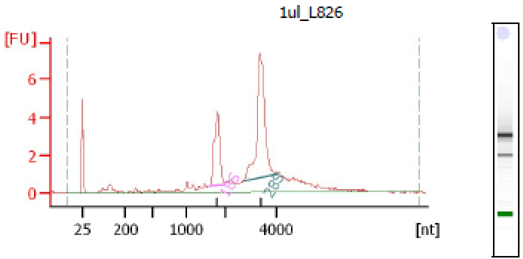


Overall Results for sample 6 : **1ul_L824**

RNA Area:	56.1
RNA Concentration:	97 ng/µl
rRNA Ratio [28s / 18s]:	2.0
RNA Integrity Number (RIN):	9.5 (A.01.01)
Result Flagging Color:	🟦
Result Flagging Label:	RIN: 9.50

Fragment table for sample 6 : **1ul_L824**

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,545	2,028	8.8	15.7
28S	2,496	4,014	17.8	31.8



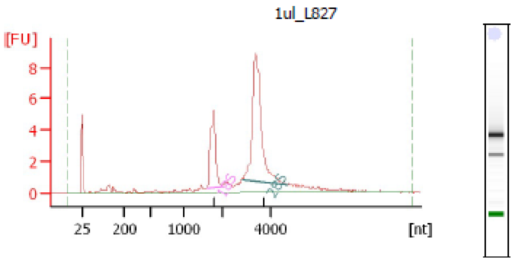
Overall Results for sample 7 : **1ul_L826**

RNA Area:	53.3
RNA Concentration:	93 ng/µl
rRNA Ratio [28s / 18s]:	2.1
RNA Integrity Number (RIN):	9.5 (A.01.01)
Result Flagging Color:	🟦
Result Flagging Label:	RIN: 9.50

Fragment table for sample 7 : **1ul_L826**

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,545	1,990	8.3	15.6
28S	2,709	4,070	17.8	33.3

Electropherogram Summary Continued ...



Overall Results for sample 8 : **1ul_L827**

RNA Area:	59.7
RNA Concentration:	104 ng/µl
rRNA Ratio [28s / 18s]:	2.6
RNA Integrity Number (RIN):	10 (A.01.01)
Result Flagging Color:	🟪
Result Flagging Label:	RIN:10

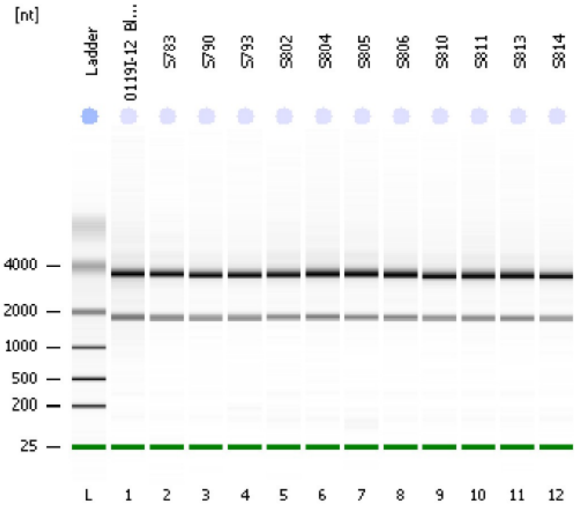
Fragment table for sample 8 : **1ul_L827**

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,616	1,999	10.1	16.9
28S	2,809	4,643	25.9	43.3

Acrobat Document Acrobat Document

Assay Class: EukaryoteTotal RNA Nano
Data Path: D:\..._EukaryoteTotal RNA Nano_DE11400731_2019-01-08_08-36-54.xad
Created: 1/8/2019 8:36:54 AM
Modified: 1/8/2019 9:04:26 AM

Electrophoresis File Run Summary



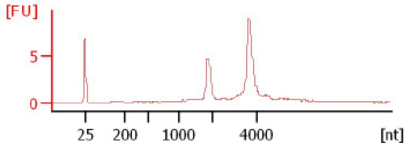
Instrument Information:
Instrument Name: DE11400731
Serial#: DE11400731
Firmware: C.01.069
Type: G2938A

Assay Information:
Assay Origin Path: C:\Program Files\Agilent\2100 bioanalyzer\2100 expert\assays\RNA\Eukaryote Total RNA Nano Series II.xsy
Title: Eukaryote Total RNA Nano Series II
Version: 2.5
Assay Comments: Copyright © 2003-2006 Agilent Technologies

Chip Information:
Chip Lot:
Reagent Kit Lot:
Chip Comments:

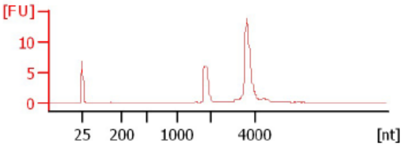
0119I-12_BlumB1_S781

RIN: 9.40



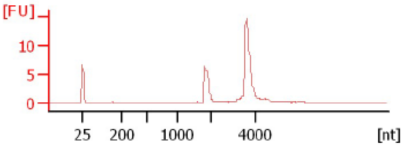
S783

RIN: 9.90



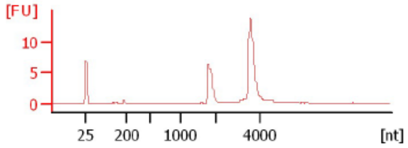
S790

RIN: 9.90



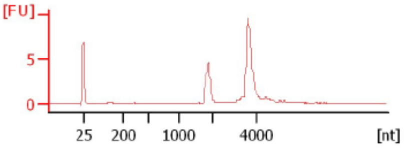
S793

RIN:10



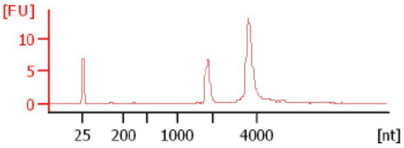
S802

RIN:10



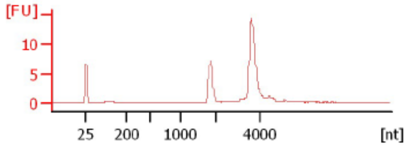
S804

RIN:10



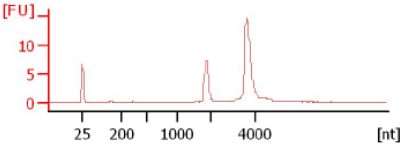
S805

RIN:10



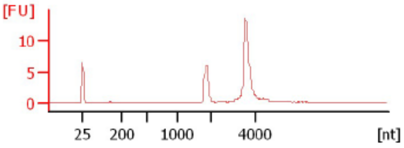
S806

RIN:10



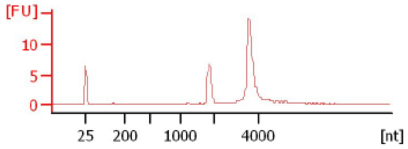
S810

RIN:10



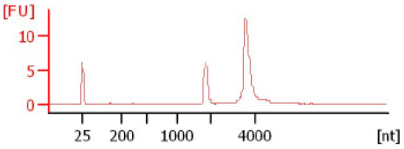
S811

RIN:10



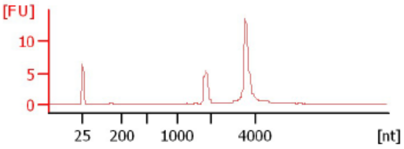
S813

RIN:10



S814

RIN: 9.60



Assay Class:	EukaryoteTotal RNA Nano	Created:	1/8/2019 8:36:54 AM
Data Path:	D:\..._EukaryoteTotal RNA Nano_DE11400731_2019-01-08_08-36-54.xad	Modified:	1/8/2019 9:04:26 AM

Electrophoresis File Run Summary (Chip Summary)

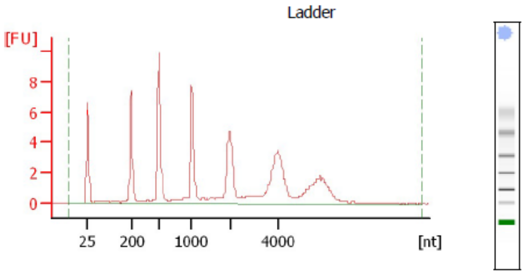
Sample Name	Sample Comment	Status	Result Label	Result Color
0119I-12_BlumB1_S781		✓	RIN: 9.40	🟪
S783		✓	RIN: 9.90	🟪
S790		✓	RIN: 9.90	🟪
S793		✓	RIN:10	🟪
S802		✓	RIN:10	🟪
S804		✓	RIN:10	🟪
S805		✓	RIN:10	🟪
S806		✓	RIN:10	🟪
S810		✓	RIN:10	🟪
S811		✓	RIN:10	🟪
S813		✓	RIN:10	🟪
S814		✓	RIN: 9.60	🟪

Chip Lot # **Reagent Kit Lot #**

Chip Comments :

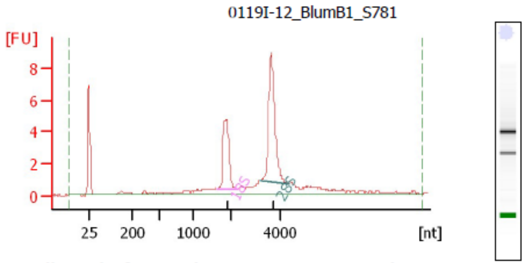
Assay Class:	EukaryoteTotal RNA Nano	Created:	1/8/2019 8:36:54 AM
Data Path:	D:\..._EukaryoteTotal RNA Nano_DE11400731_2019-01-08_08-36-54.xad	Modified:	1/8/2019 9:04:26 AM

Electropherogram Summary



Overall Results for Ladder

RNA Area:	85.1
RNA Concentration:	150 ng/µl
Result Flagging Color:	🟦
Result Flagging Label:	All Other Samples

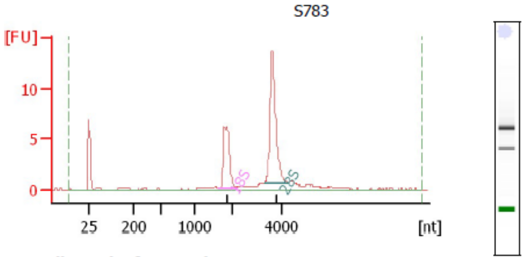


Overall Results for sample 1 : 0119I-12_BlumB1_S781

RNA Area:	53.4
RNA Concentration:	94 ng/µl
rRNA Ratio [28s / 18s]:	1.7
RNA Integrity Number (RIN):	9.4 (A.01.01)
Result Flagging Color:	🟦
Result Flagging Label:	RIN: 9.40

Fragment table for sample 1 : 0119I-12_BlumB1_S781

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,626	2,224	10.4	19.4
28S	3,197	4,333	17.1	32.1

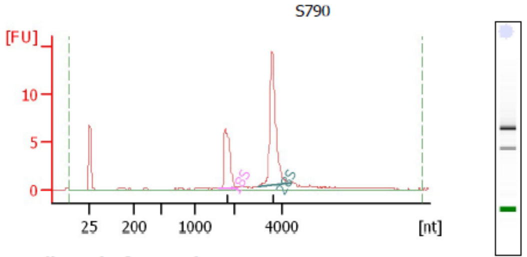


Overall Results for sample 2 : S783

RNA Area:	55.6
RNA Concentration:	98 ng/µl
rRNA Ratio [28s / 18s]:	1.8
RNA Integrity Number (RIN):	9.9 (A.01.01)
Result Flagging Color:	🟦
Result Flagging Label:	RIN: 9.90

Fragment table for sample 2 : S783

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,611	2,196	13.8	24.9
28S	3,283	4,267	24.7	44.5



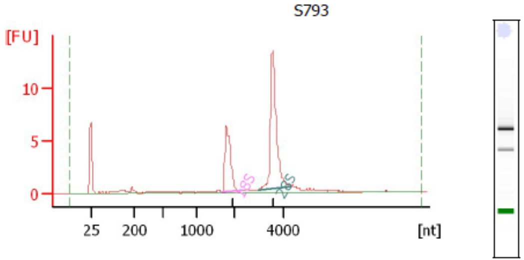
Overall Results for sample 3 : S790

RNA Area:	53.5
RNA Concentration:	94 ng/µl
rRNA Ratio [28s / 18s]:	2.1
RNA Integrity Number (RIN):	9.9 (A.01.01, Anomaly Threshold(s) manually adapted)
Result Flagging Color:	🟦
Result Flagging Label:	RIN: 9.90

Fragment table for sample 3 : S790

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,631	2,154	13.2	24.6
28S	2,974	4,336	27.8	51.9

Electropherogram Summary Continued ...

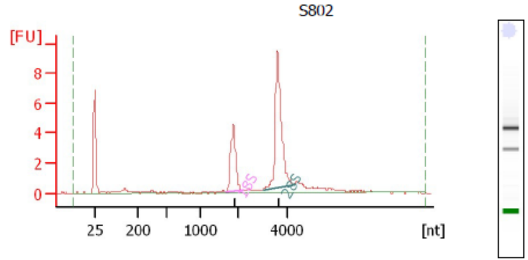


Overall Results for sample 4 : **S793**

RNA Area: 53.4
RNA Concentration: 94 ng/µl
rRNA Ratio [28s / 18s]: 2.1
RNA Integrity Number (RIN): 10 (A.01.01)
Result Flagging Color: 🟪
Result Flagging Label: RIN:10

Fragment table for sample 4 : **S793**

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,689	2,330	12.6	23.7
28S	2,988	4,333	26.1	48.8

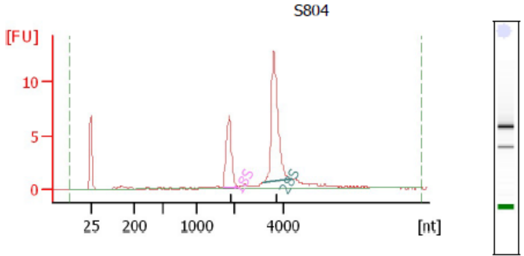


Overall Results for sample 5 : **S802**

RNA Area: 41.7
RNA Concentration: 73 ng/µl
rRNA Ratio [28s / 18s]: 2.1
RNA Integrity Number (RIN): 10 (A.01.01)
Result Flagging Color: 🟪
Result Flagging Label: RIN:10

Fragment table for sample 5 : **S802**

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,663	2,200	8.9	21.3
28S	3,002	4,256	18.6	44.7

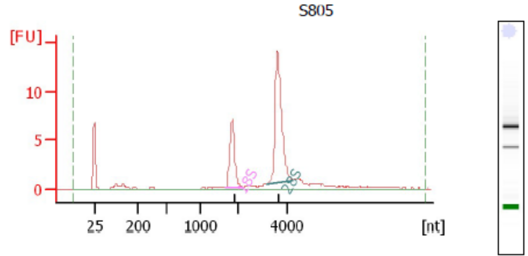


Overall Results for sample 6 : **S804**

RNA Area: 60.5
RNA Concentration: 107 ng/µl
rRNA Ratio [28s / 18s]: 2.1
RNA Integrity Number (RIN): 10 (A.01.01)
Result Flagging Color: 🟪
Result Flagging Label: RIN:10

Fragment table for sample 6 : **S804**

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,726	2,169	13.0	21.5
28S	3,193	4,398	26.8	44.3



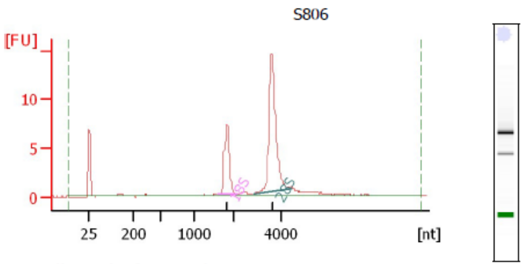
Overall Results for sample 7 : **S805**

RNA Area: 60.1
RNA Concentration: 106 ng/µl
rRNA Ratio [28s / 18s]: 2.2
RNA Integrity Number (RIN): 10 (A.01.01)
Result Flagging Color: 🟪
Result Flagging Label: RIN:10

Fragment table for sample 7 : **S805**

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,680	2,228	12.9	21.5
28S	3,178	4,230	28.8	47.9

Electropherogram Summary Continued ...

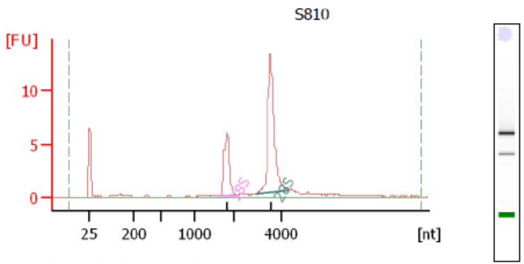


Overall Results for sample 8 : S806

RNA Area: 64.0
RNA Concentration: 113 ng/µl
rRNA Ratio [28s / 18s]: 2.3
RNA Integrity Number (RIN): 10 (A.01.01)
Result Flagging Color: 🟪
Result Flagging Label: RIN:10

Fragment table for sample 8 : S806

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,613	2,168	13.8	21.5
28S	2,886	4,424	31.6	49.4

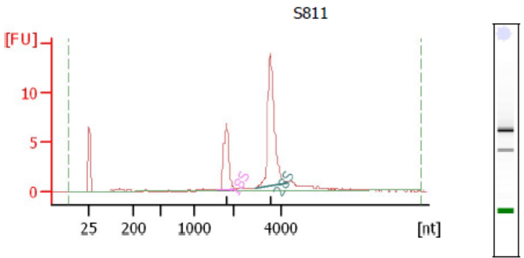


Overall Results for sample 9 : S810

RNA Area: 54.1
RNA Concentration: 95 ng/µl
rRNA Ratio [28s / 18s]: 2.3
RNA Integrity Number (RIN): 10 (A.01.01, Anomaly Threshold(s) manually adapted)
Result Flagging Color: 🟪
Result Flagging Label: RIN:10

Fragment table for sample 9 : S810

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,631	2,154	11.8	21.8
28S	2,930	4,278	26.8	49.6

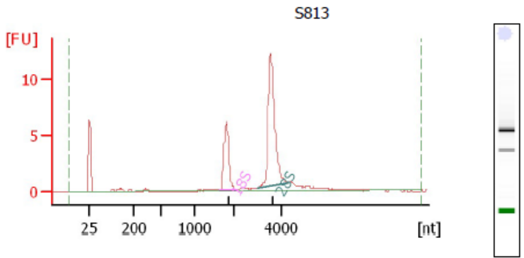


Overall Results for sample 10 : S811

RNA Area: 63.5
RNA Concentration: 112 ng/µl
rRNA Ratio [28s / 18s]: 2.2
RNA Integrity Number (RIN): 10 (A.01.01)
Result Flagging Color: 🟪
Result Flagging Label: RIN:10

Fragment table for sample 10 : S811

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,631	2,110	13.0	20.4
28S	2,945	4,249	28.9	45.5



Overall Results for sample 11 : S813

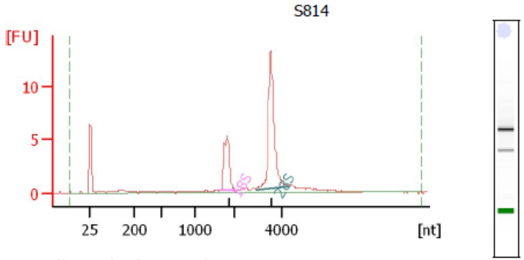
RNA Area: 51.2
RNA Concentration: 90 ng/µl
rRNA Ratio [28s / 18s]: 2.2
RNA Integrity Number (RIN): 10 (A.01.01)
Result Flagging Color: 🟪
Result Flagging Label: RIN:10

Fragment table for sample 11 : S813

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,678	2,154	11.4	22.3
28S	2,974	4,278	25.6	50.0

Assay Class:	EukaryoteTotal RNA Nano	Created:	1/8/2019 8:36:54 AM
Data Path:	D:\..._EukaryoteTotal RNA Nano_DE11400731_2019-01-08_08-36-54.xad	Modified:	1/8/2019 9:04:26 AM

Electropherogram Summary Continued ...



Overall Results for sample 12 : **S814**

RNA Area:	51.7
RNA Concentration:	91 ng/µl
rRNA Ratio [28s / 18s]:	2.3
RNA Integrity Number (RIN):	9.6 (A.01.01)
Result Flagging Color:	▭
Result Flagging Label:	RIN: 9.60

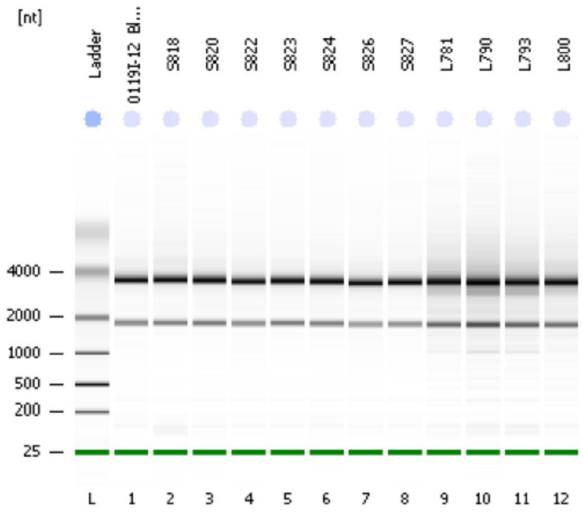
Fragment table for sample 12 : **S814**

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,669	2,168	10.9	21.1
28S	2,915	4,234	24.7	47.7

Assay Class: EukaryoteTotal RNA Nano
Data Path: D:\..._EukaryoteTotal RNA Nano_DE11400731_2019-01-08_10-06-07.xad

Created: 1/8/2019 10:06:07 AM
Modified: 1/8/2019 3:03:10 PM

Electrophoresis File Run Summary



Instrument Information:

Instrument Name: DE11400731
Serial#: DE11400731
Firmware: C.01.069
Type: G2938A

Assay Information:

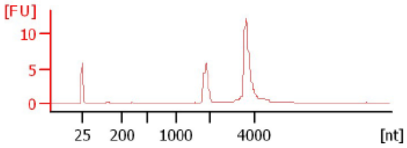
Assay Origin Path: C:\Program Files\Agilent\2100 bioanalyzer\2100 expert\assays\RNA\Eukaryote Total RNA Nano Series II.xsy
Title: Eukaryote Total RNA Nano Series II
Version: 2.5
Assay Comments: Copyright © 2003-2006 Agilent Technologies

Chip Information:

Chip Lot:
Reagent Kit Lot:
Chip Comments:

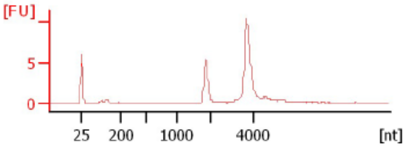
0119I-12_BlumB2_S815

RIN: 9.90



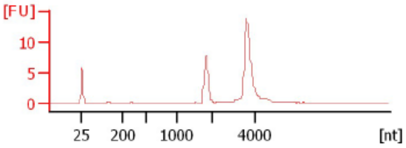
S818

RIN:10



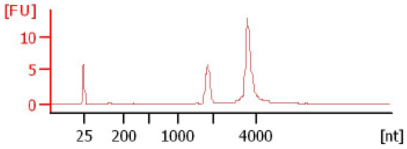
S820

RIN:10



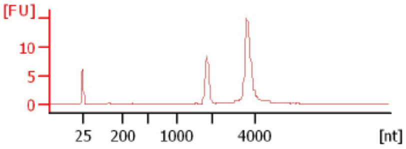
S822

RIN: 9.90



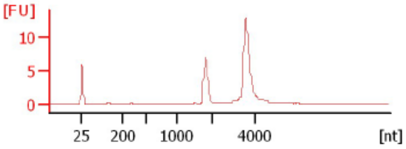
S823

RIN:10



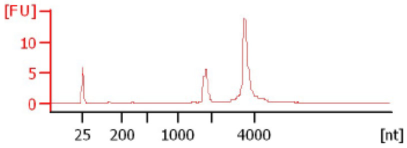
S824

RIN:10



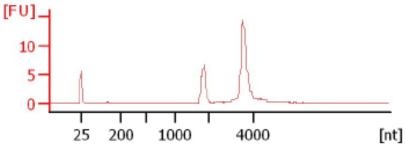
S826

RIN: 9.50



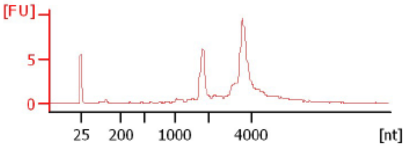
S827

RIN:10



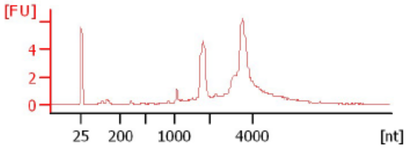
L781

RIN: 9.80



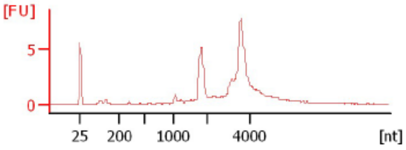
L790

RIN: 9.50



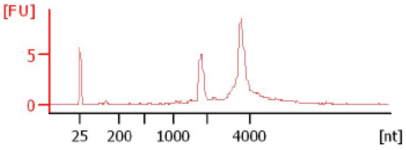
L793

RIN: 9.70



L800

RIN: 9.80



Assay Class:	EukaryoteTotal RNA Nano	Created:	1/8/2019 10:06:07 AM
Data Path:	D:\..._EukaryoteTotal RNA Nano_DE11400731_2019-01-08_10-06-07.xad	Modified:	1/8/2019 3:03:10 PM

Electrophoresis File Run Summary (Chip Summary)

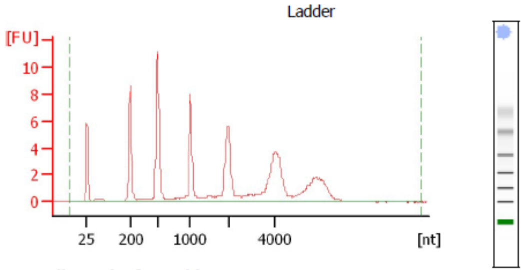
Sample Name	Sample Comment	Status	Result Label	Result Color
0119I-12_BlumB2_S815		✓	RIN: 9.90	🟪
S818		✓	RIN:10	🟪
S820		✓	RIN:10	🟪
S822		✓	RIN: 9.90	🟪
S823		✓	RIN:10	🟪
S824		✓	RIN:10	🟪
S826		✓	RIN: 9.50	🟪
S827		✓	RIN:10	🟪
L781		✓	RIN: 9.80	🟪
L790		✓	RIN: 9.50	🟪
L793		✓	RIN: 9.70	🟪
L800		✓	RIN: 9.80	🟪

Chip Lot # **Reagent Kit Lot #**

Chip Comments :

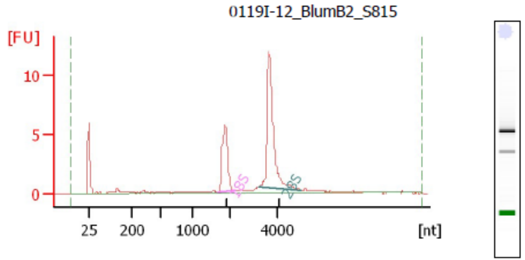
Assay Class:	EukaryoteTotal RNA Nano	Created:	1/8/2019 10:06:07 AM
Data Path:	D:\..._EukaryoteTotal RNA Nano_DE11400731_2019-01-08_10-06-07.xad	Modified:	1/8/2019 3:03:10 PM

Electropherogram Summary



Overall Results for Ladder

RNA Area:	91.2
RNA Concentration:	150 ng/µl
Result Flagging Color:	🟦
Result Flagging Label:	All Other Samples

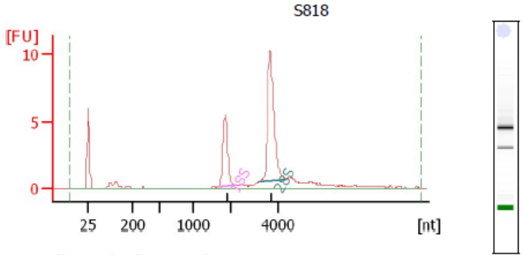


Overall Results for sample 1 : 0119I-12_BlumB2_S815

RNA Area:	52.4
RNA Concentration:	86 ng/µl
rRNA Ratio [28s / 18s]:	2.2
RNA Integrity Number (RIN):	9.9 (A.01.01)
Result Flagging Color:	🟦
Result Flagging Label:	RIN: 9.90

Fragment table for sample 1 : 0119I-12_BlumB2_S815

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,685	2,199	12.0	23.0
28S	3,179	5,019	25.9	49.4

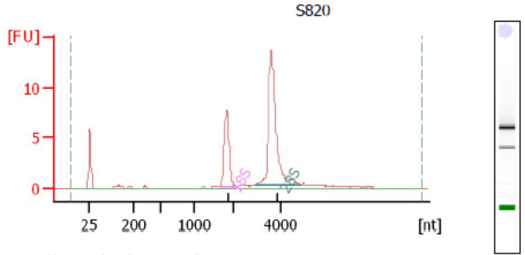


Overall Results for sample 2 : S818

RNA Area:	49.7
RNA Concentration:	82 ng/µl
rRNA Ratio [28s / 18s]:	2.1
RNA Integrity Number (RIN):	10 (A.01.01)
Result Flagging Color:	🟦
Result Flagging Label:	RIN:10

Fragment table for sample 2 : S818

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,697	2,216	9.8	19.7
28S	3,192	4,288	20.7	41.5



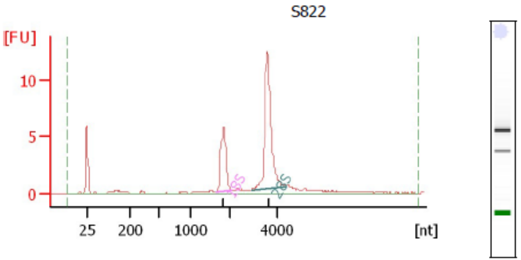
Overall Results for sample 3 : S820

RNA Area:	66.0
RNA Concentration:	109 ng/µl
rRNA Ratio [28s / 18s]:	2.3
RNA Integrity Number (RIN):	10 (A.01.01)
Result Flagging Color:	🟦
Result Flagging Label:	RIN:10

Fragment table for sample 3 : S820

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,662	2,142	14.4	21.8
28S	2,936	4,868	33.4	50.5

Electropherogram Summary Continued ...

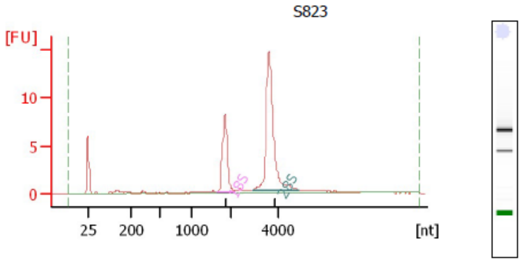


Overall Results for sample 4 : S822

RNA Area:	51.0
RNA Concentration:	84 ng/µl
rRNA Ratio [28s / 18s]:	2.1
RNA Integrity Number (RIN):	9.9 (A.01.01)
Result Flagging Color:	▭
Result Flagging Label:	RIN: 9.90

Fragment table for sample 4 : S822

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,655	2,098	11.8	23.2
28S	2,935	4,355	25.0	49.0

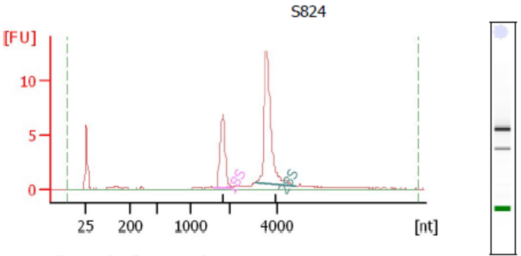


Overall Results for sample 5 : S823

RNA Area:	62.8
RNA Concentration:	103 ng/µl
rRNA Ratio [28s / 18s]:	2.3
RNA Integrity Number (RIN):	10 (A.01.01, Anomaly Threshold(s) manually adapted)
Result Flagging Color:	▭
Result Flagging Label:	RIN:10

Fragment table for sample 5 : S823

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,645	2,143	15.1	24.0
28S	2,920	4,863	35.1	55.8

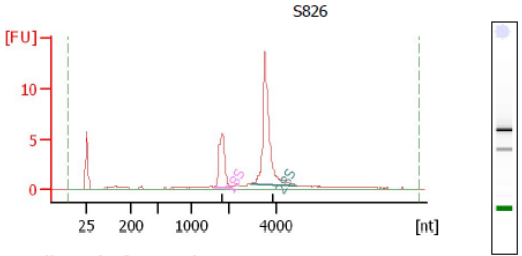


Overall Results for sample 6 : S824

RNA Area:	58.7
RNA Concentration:	97 ng/µl
rRNA Ratio [28s / 18s]:	2.2
RNA Integrity Number (RIN):	10 (A.01.01)
Result Flagging Color:	▭
Result Flagging Label:	RIN:10

Fragment table for sample 6 : S824

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,625	2,127	13.0	22.1
28S	3,130	4,793	28.2	47.9



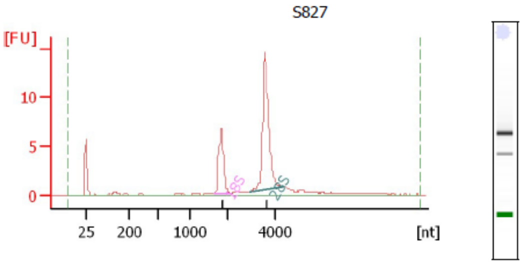
Overall Results for sample 7 : S826

RNA Area:	59.4
RNA Concentration:	98 ng/µl
rRNA Ratio [28s / 18s]:	2.6
RNA Integrity Number (RIN):	9.5 (A.01.01, Anomaly Threshold(s) manually adapted)
Result Flagging Color:	▭
Result Flagging Label:	RIN: 9.50

Fragment table for sample 7 : S826

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,577	2,095	11.6	19.6
28S	2,906	4,843	29.9	50.3

Electropherogram Summary Continued ...

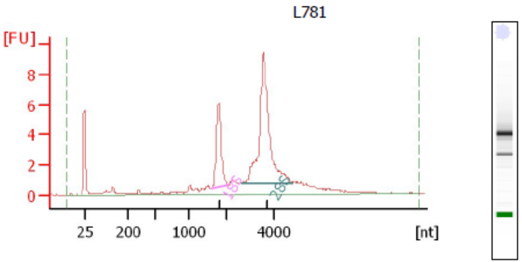

S827

Overall Results for sample 8 : S827

RNA Area:	63.4
RNA Concentration:	104 ng/µl
rRNA Ratio [28s / 18s]:	2.3
RNA Integrity Number (RIN):	10 (A.01.01)
Result Flagging Color:	🟪
Result Flagging Label:	RIN:10

Fragment table for sample 8 : S827

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,638	2,138	13.2	20.8
28S	2,938	4,325	30.3	47.8

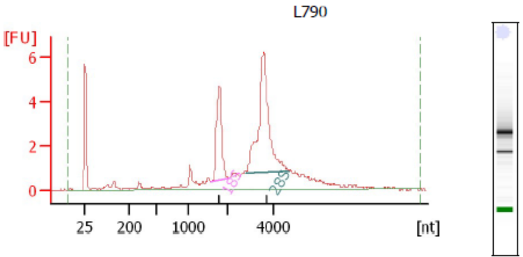

L781

Overall Results for sample 9 : L781

RNA Area:	72.7
RNA Concentration:	120 ng/µl
rRNA Ratio [28s / 18s]:	2.6
RNA Integrity Number (RIN):	9.8 (A.01.01)
Result Flagging Color:	🟪
Result Flagging Label:	RIN: 9.80

Fragment table for sample 9 : L781

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,618	2,092	11.2	15.5
28S	2,681	4,813	29.7	40.8

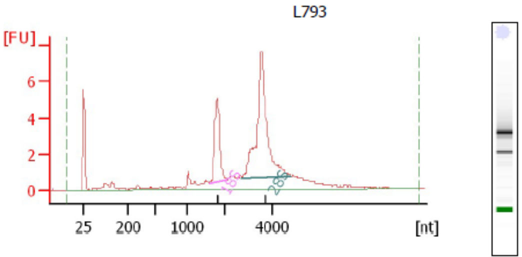

L790

Overall Results for sample 10 : L790

RNA Area:	61.4
RNA Concentration:	101 ng/µl
rRNA Ratio [28s / 18s]:	2.2
RNA Integrity Number (RIN):	9.5 (A.01.01)
Result Flagging Color:	🟪
Result Flagging Label:	RIN: 9.50

Fragment table for sample 10 : L790

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,579	2,106	8.9	14.5
28S	2,833	4,636	19.6	31.9


L793

Overall Results for sample 11 : L793

RNA Area:	63.7
RNA Concentration:	105 ng/µl
rRNA Ratio [28s / 18s]:	2.6
RNA Integrity Number (RIN):	9.7 (A.01.01)
Result Flagging Color:	🟪
Result Flagging Label:	RIN: 9.70

Fragment table for sample 11 : L793

Name	Start Size [nt]	End Size [nt]	Area	% of total Area
18S	1,577	2,074	9.4	14.7
28S	2,712	4,838	24.2	38.0

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