GTEx – Sample Molecular Quality and Data Production

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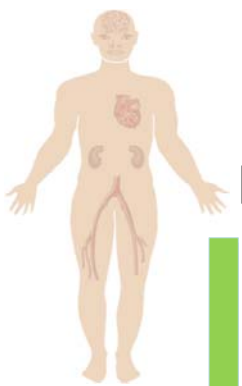
23 February, 2012

BROAD INSTITUTE

GTEx Sample Workflow



LDACC

BSS

Bloods & Fresh Skin

1. Whole Blood → DNA
2. Whole Blood → Cell Culture (EBV)
3. Fresh Skin → Fibroblast Culture
4. PAXgene Tube → RNA

Genotyping

Analysis

Donor

Tissue Samples

CBR

PAXgene Preserved Tissues → RNA

Brain

Brain Bank

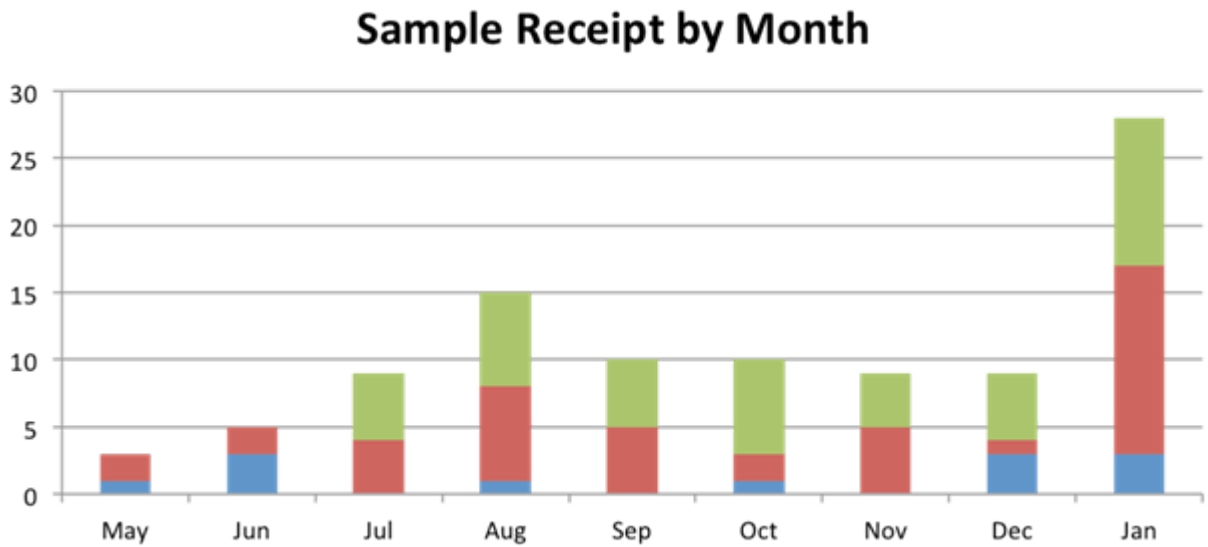
Snap Frozen Tissues → RNA

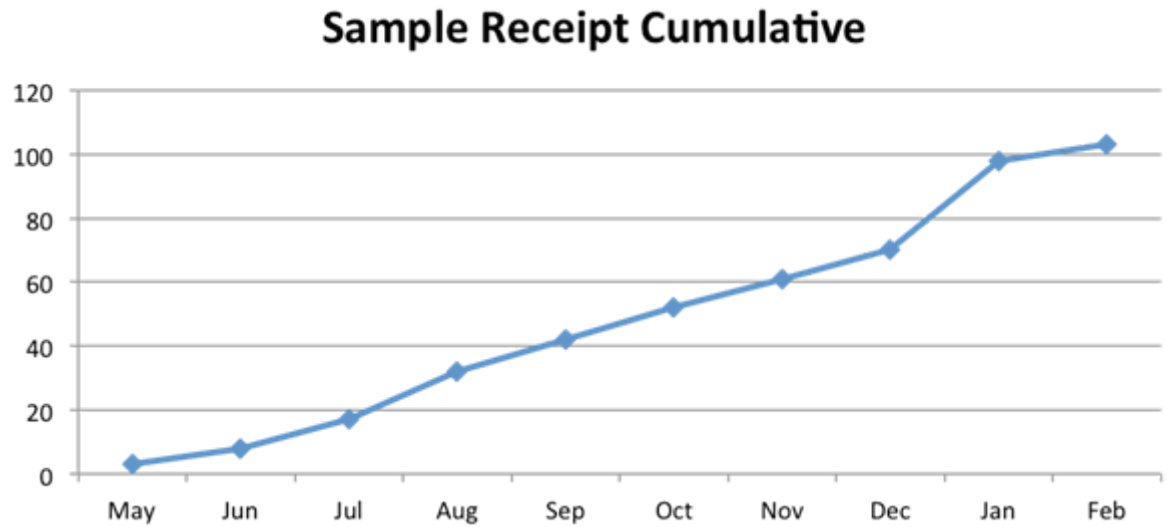
Expression arrays and RNA Seq

Analysis

dbGAP
R01 Analysis Centers

Sample Receipt by LDACC

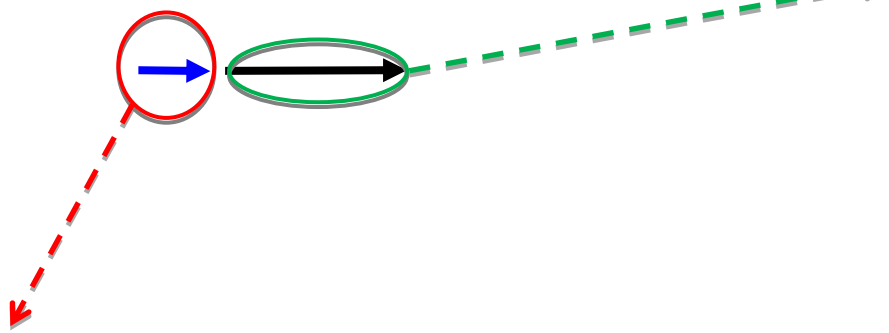




N=105 donors

GTEx Project Current Status

Base Pilot, Period-1 (12 months)	Base Pilot, Period-2 (18 months)



May onwards

GTEx Beta & Accrual Phase

105 Donors (x 19-30 tissues each)

* PaxGene Preservative only

Bloods → Rcvd - 105 Donors
Processed DNA - 104
QC'd – 100
→ Genotyped – 64

Tissues → Rcvd – 104 Donors PAX (+29 Brains)
Processed RNA – 91 (+25)
QC'd – 80 (+20)
→ Expression Arrays - 4 batches of 93-95
→ RNA SEQ –290 samples

Jan to April

GTEx Pilot Experiment

3 Donors (x 20-25 tissues each)

* Test of 3 tissue preservation methods
  - Snap-freezing
  - RNAlater
  - PaxGene preservative

Target Tissues

Paxgene Preserved Tissues

- Adipose
- Adrenal Gland
- Artery – Aorta
- Artery – Coronary
- Artery – Tibial
- Bladder
- Breast - Mammary Tissue
- Cervix – Ecto & Endocervix
- Colon
- Esophagus – Mucosa & Muscularis
- Fallopian Tube
- Heart
- Kidney – Cortex & Medulla
- Liver
- Lung
- Muscle – Skeletal
- Nerve – Tibial
- Ovary
- Pancreas
- Pituitary
- Prostate
- Skin
- Spleen
- Stomach
- Testis
- Thyroid
- Uterus
- Vagina
- Brain – Cerebellum
- Brain – Cortex

Fresh Frozen Brains

- Cerebellar Hemisphere
- Frontal Cortex (BA9)
- Hippocampus
- Substantia nigra
- Anterior cingulate cortex (BA24)
- Amygdala
- Caudate (basal ganglia)
- Nucleus accumbens (basal ganglia)
- Putamen (basal ganglia)
- Hypothalamus
- Spinal cord (cervical c-1)

Currently collecting 9-30 tissues per donor

Target Tissues – High Priority

Paxgene Preserved Tissues

- **Adipose**
- Adrenal Gland
- Artery – Aorta
- Artery – Coronary
- **Artery – Tibial**
- Bladder
- Breast - Mammary Tissue
- Cervix – Ecto & Endocervix
- Colon
- Esophagus – Mucosa & Muscularis
- Fallopian Tube
- **Heart**
- Kidney – Cortex & Medulla
- Liver
- **Lung**
- **Muscle – Skeletal**
- **Nerve – Tibial**
- Ovary
- Pancreas
- Pituitary
- Prostate
- **Skin**
- Spleen
- Stomach
- Testis
- **Thyroid**
- Uterus
- Vagina
- **Brain – Cerebellum**
- **Brain – Cortex**

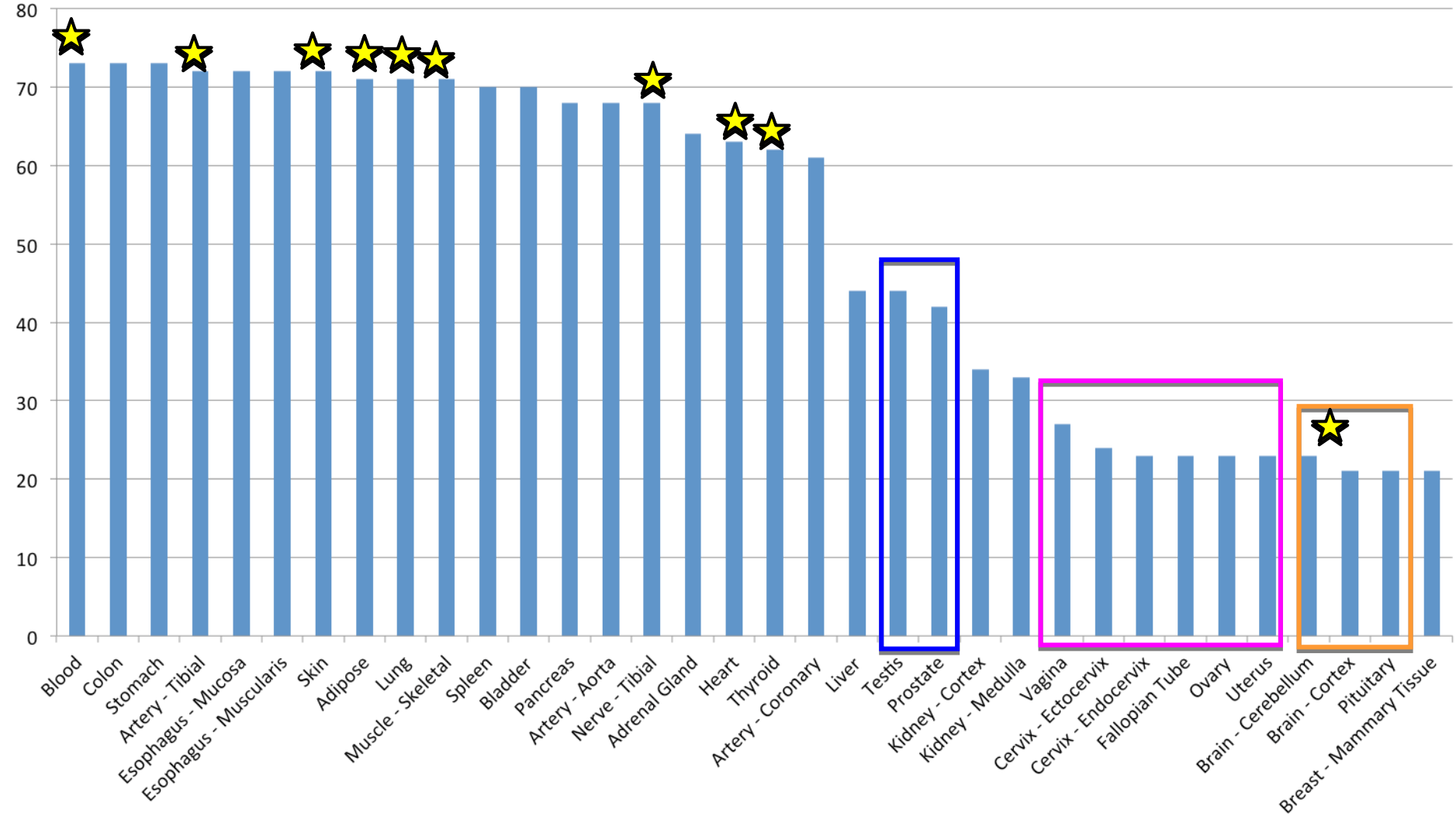
- **Fibroblast Cell Line**
- **Blood**
- **Lymphoblastoid Cell Line**

Fresh Frozen Brains

- **Cerebellar Hemisphere**
- **Frontal Cortex (BA9)**
- **Hippocampus**
- **Substantia nigra**
- **Anterior cingulate cortex (BA24)**
- **Amygdala**
- **Caudate (basal ganglia)**
- **Nucleus accumbens (basal ganglia)**
- **Putamen (basal ganglia)**
- **Hypothalamus**
- **Spinal cord (cervical c-1)**

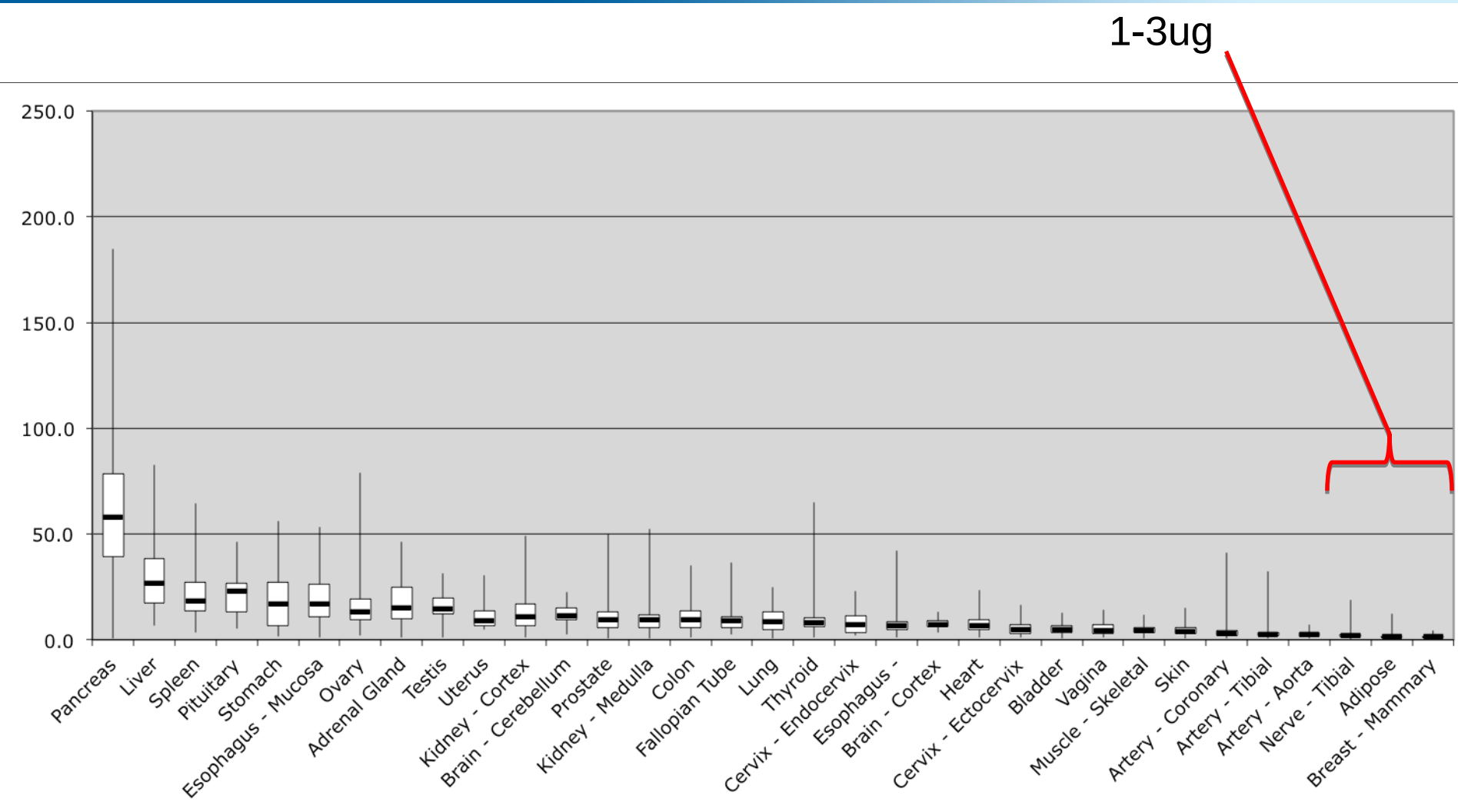
21 Tissues + 3 = 24 Priority

Tissue Distribution



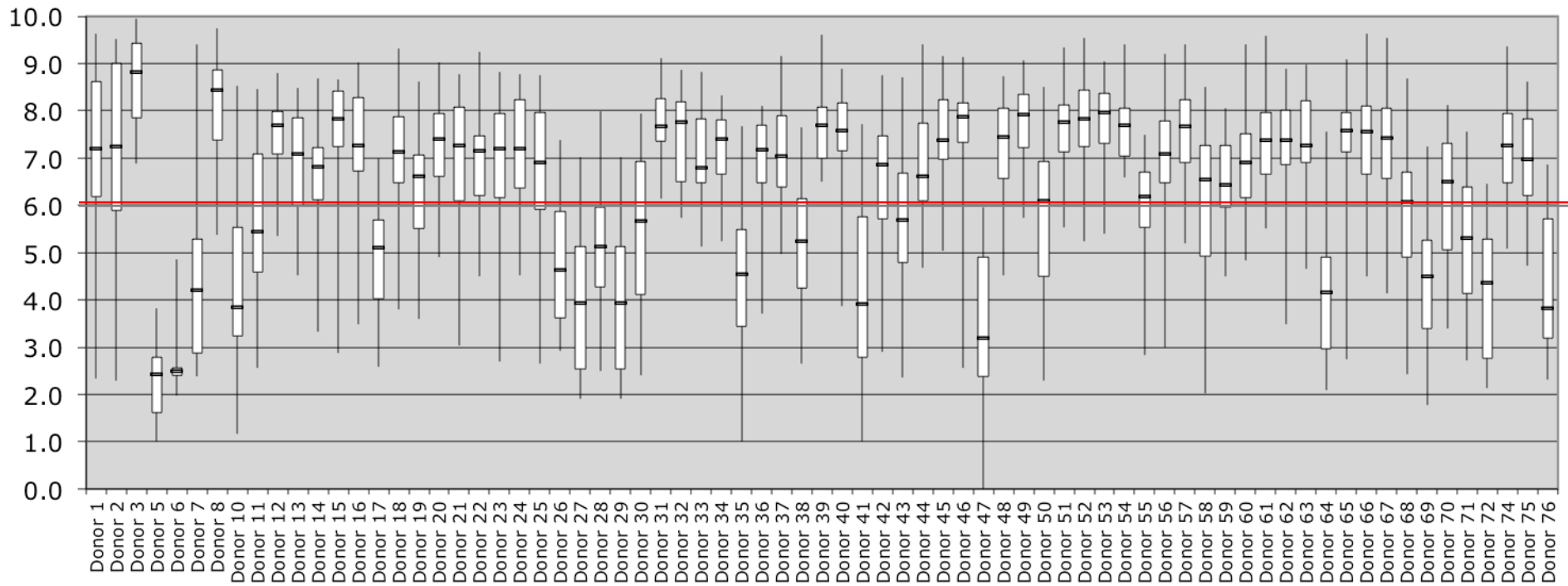
N = 75 Donors

RNA Yields



N = 1667 Paxgene Tissues

RNA Quality – Paxgene Tissues



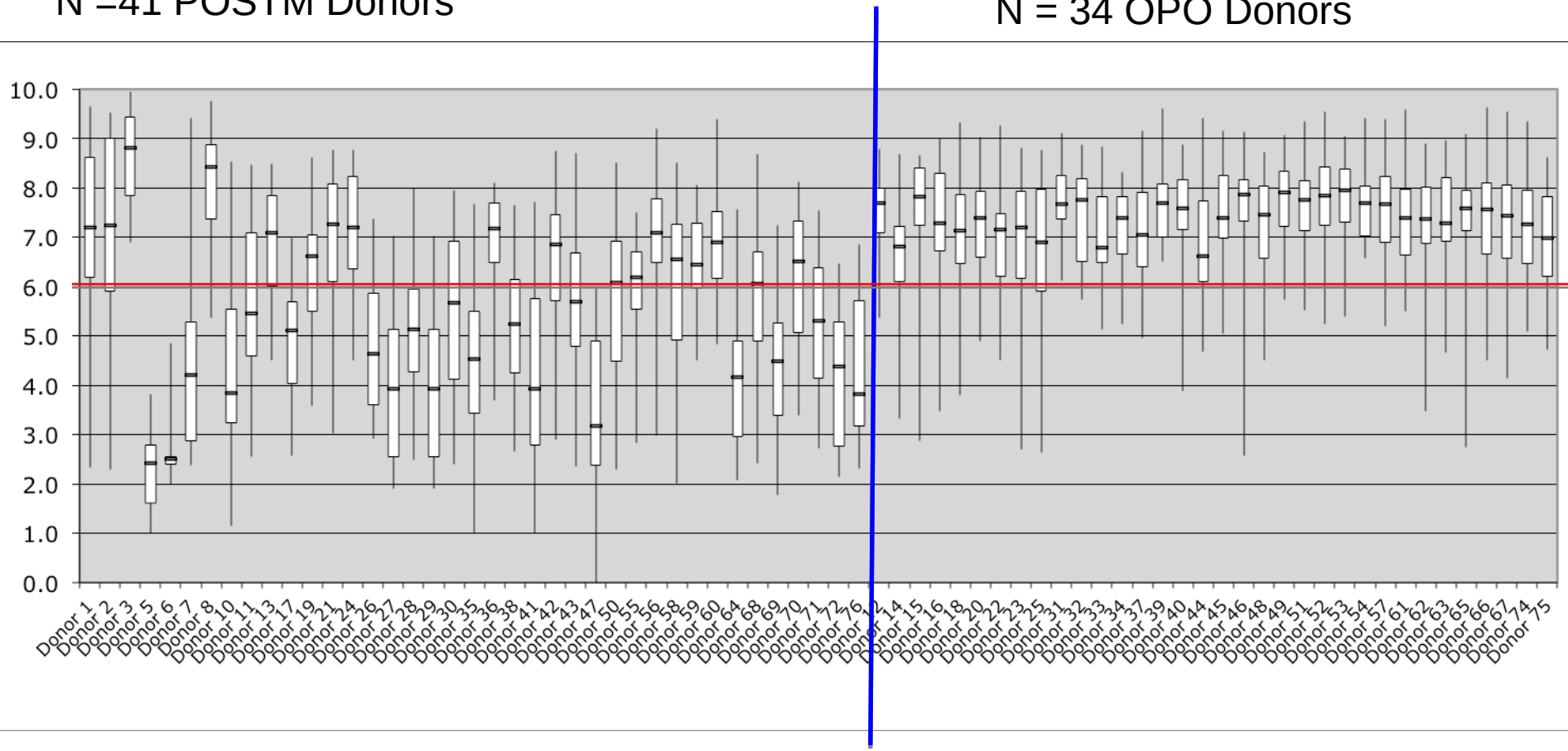
N=75 donors

RNA Quality varies by Donor

RNA Quality – Paxgene Tissues

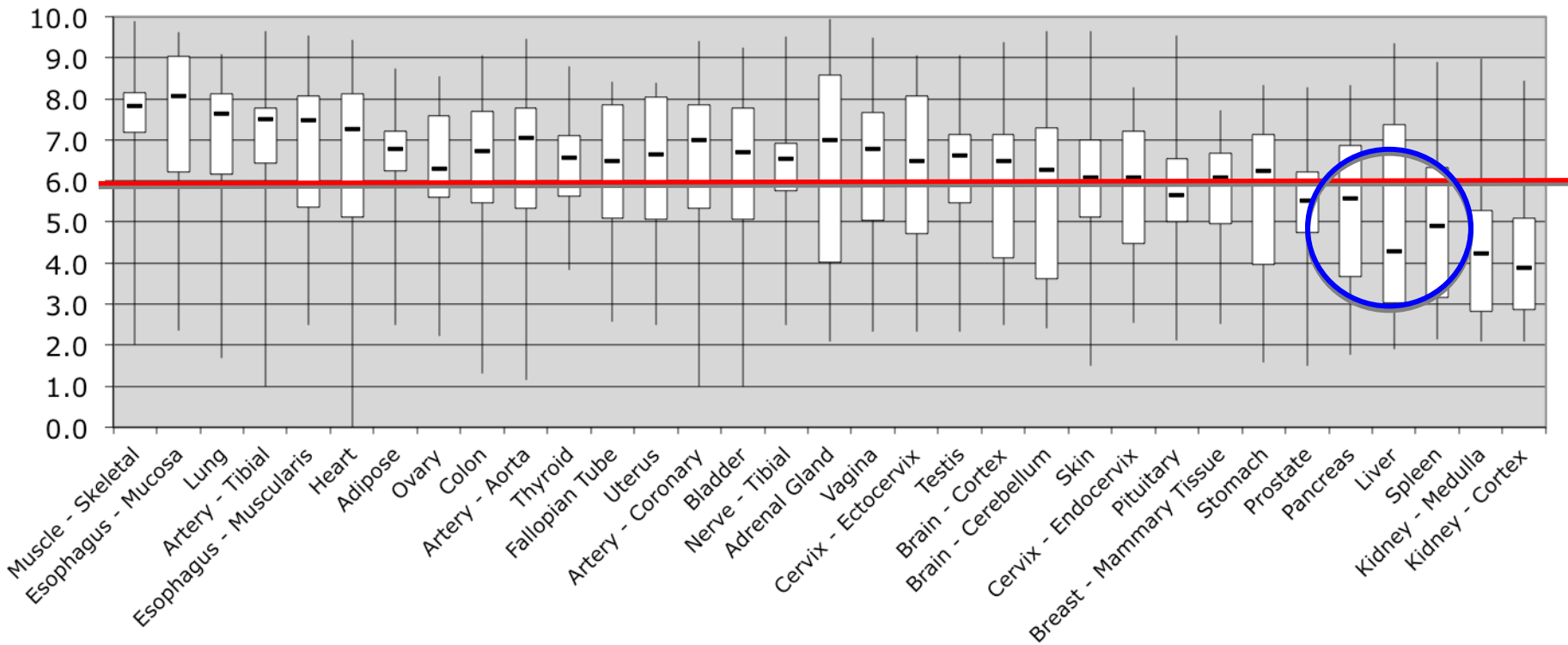
N =41 POSTM Donors

N = 34 OPO Donors



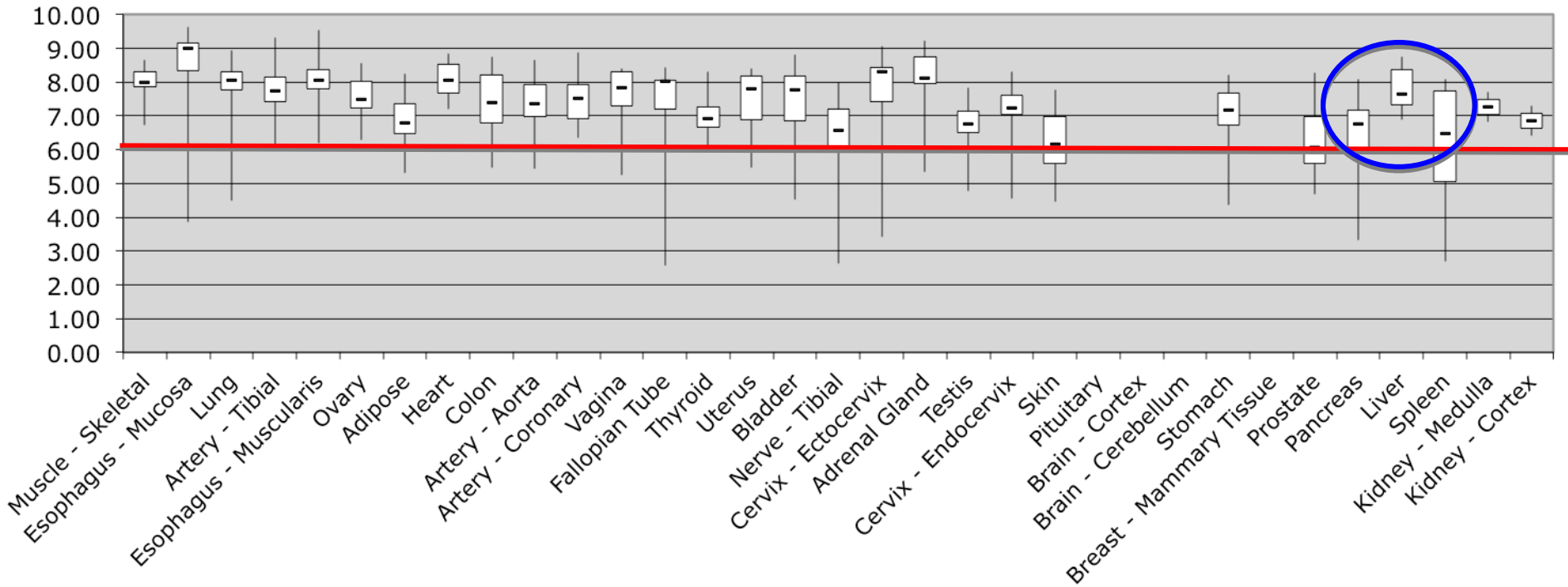
Donor RNA variation influenced by collection type

RNA Quality by Tissue Type



N=1667 tissues

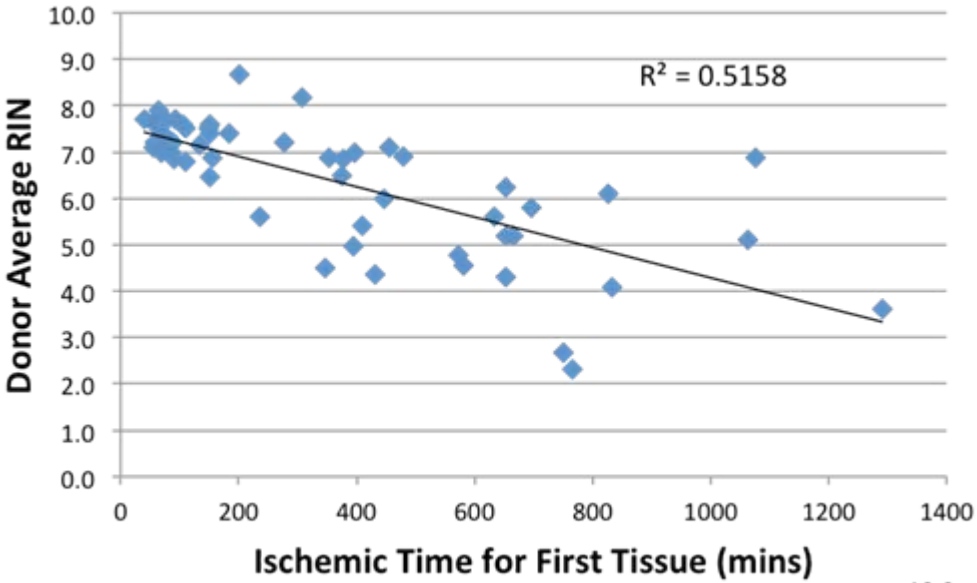
RNA Quality by Tissue Type



OPO donors only

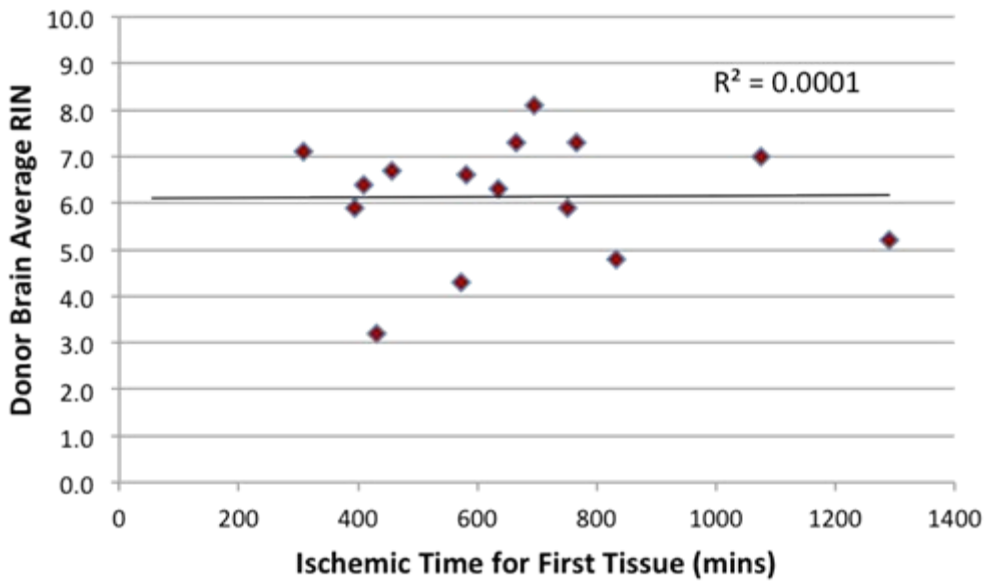
Tissue-specific RNA quality also donor-driven

RNA Quality and PMI



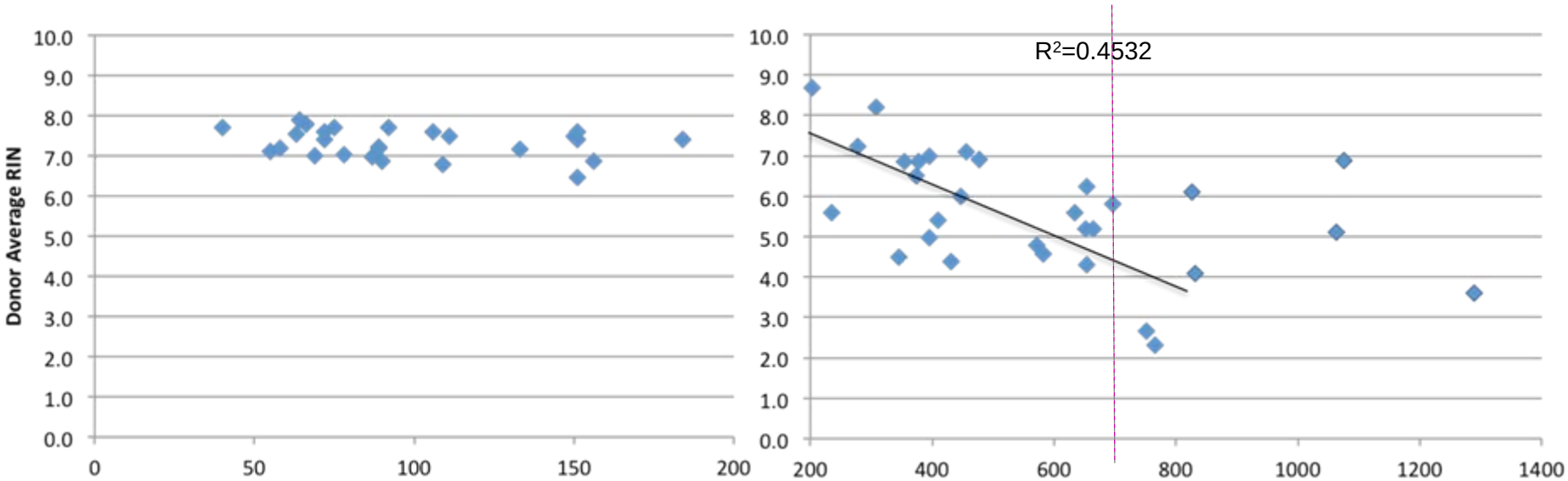
N=56 donors

N=15 donors



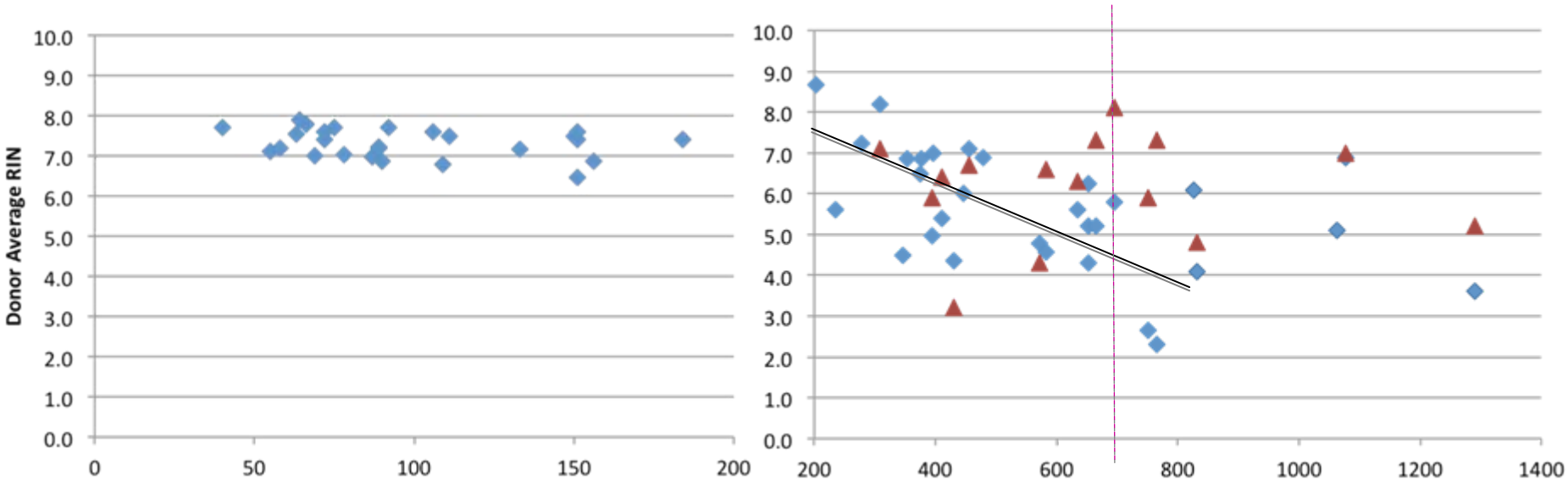
RIN decreases with increasing PMI for PAXgene Tissues, but not for Fresh Frozen Brains

RNA Quality declines with PMI



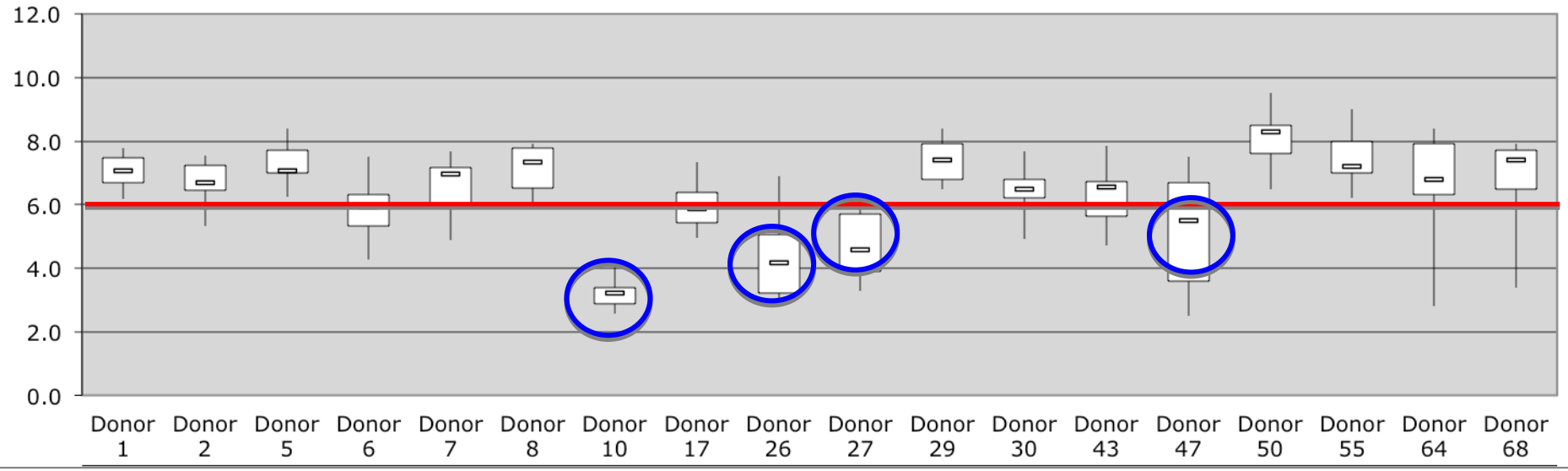
RINs are largely stable out to about 3 hours, then decline from 3- ~12 hours. More variable thereafter.

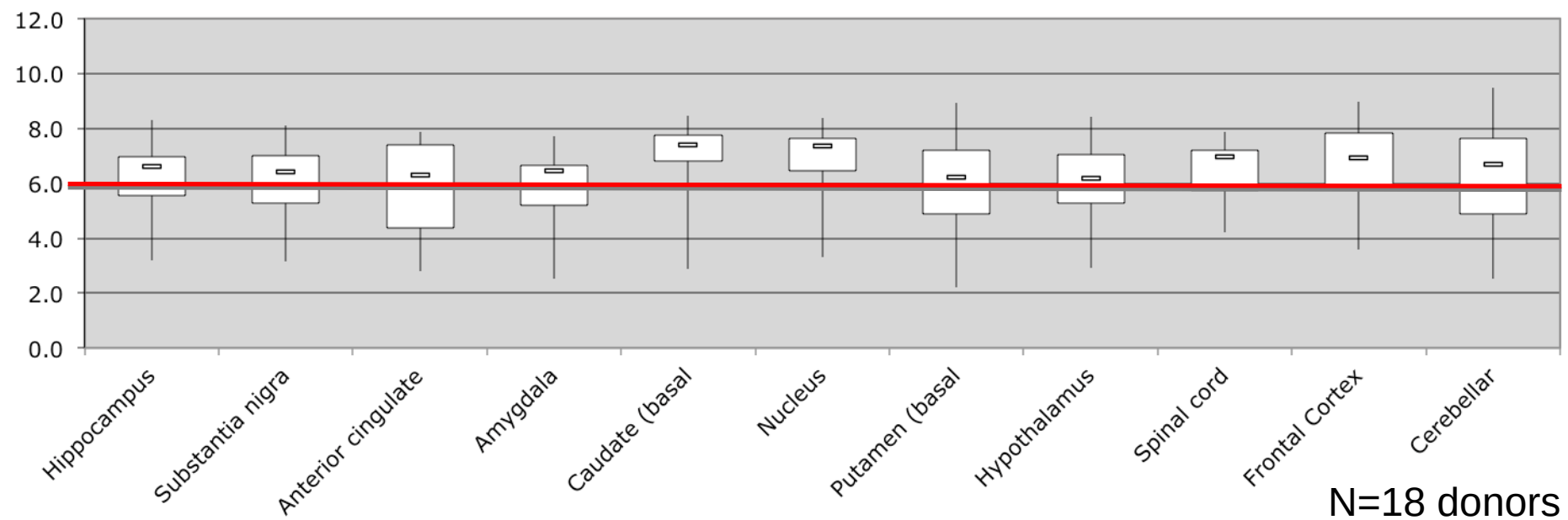
RNA Quality declines with PMI



Brain RINs less correlated with PMI, and not always the same as mean PAXgene RIN from same donor

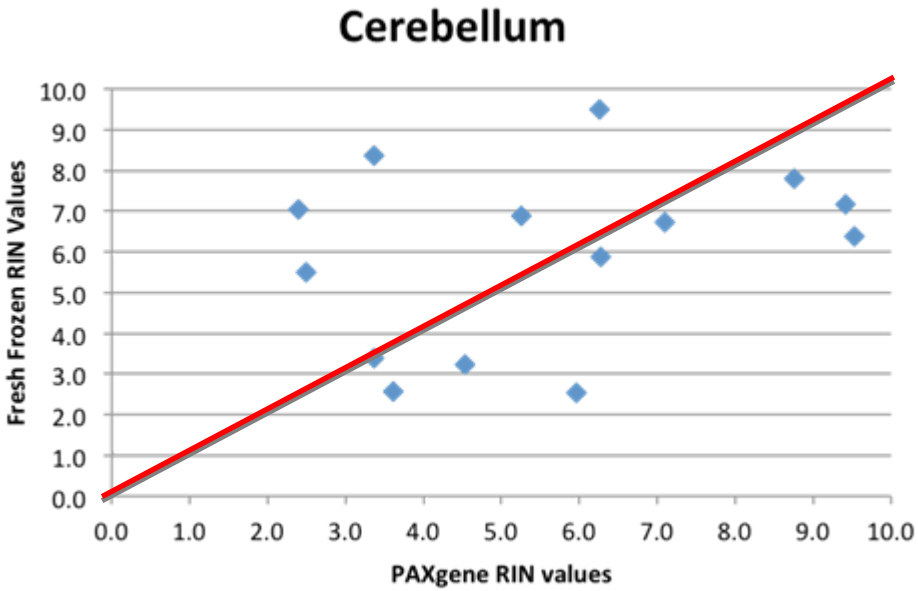
Fresh Frozen Brains



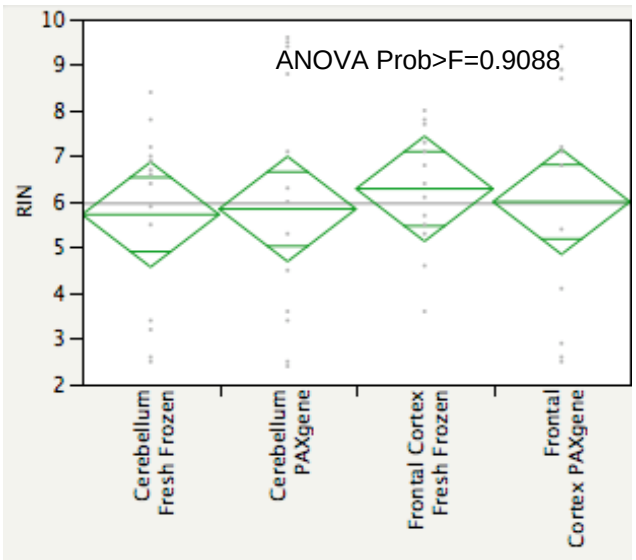


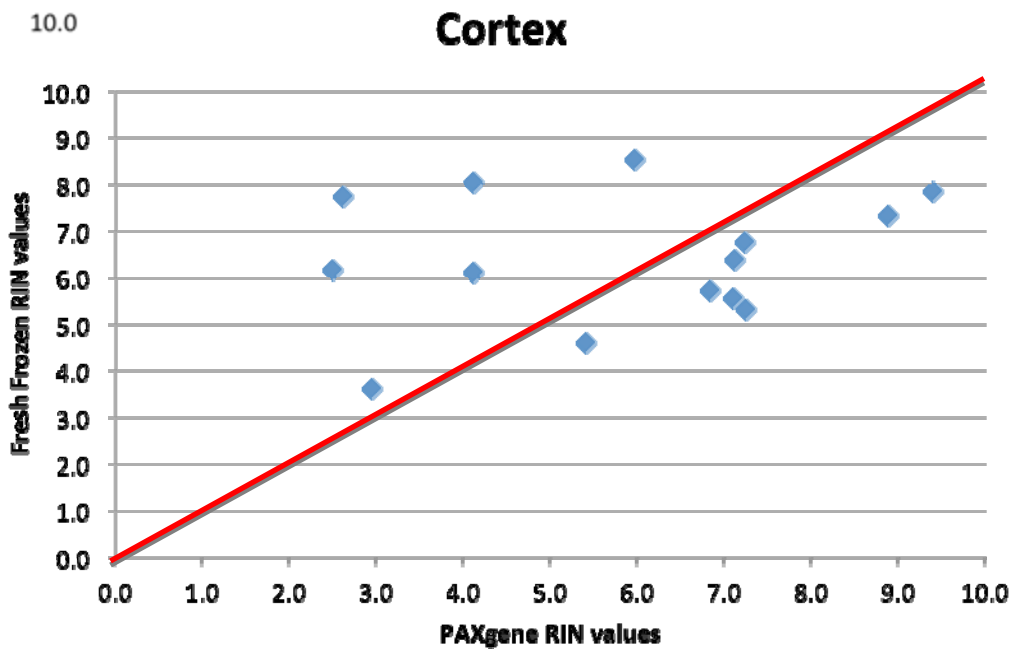
N=18 donors

PAXgene vs FF RNA Quality - Brains

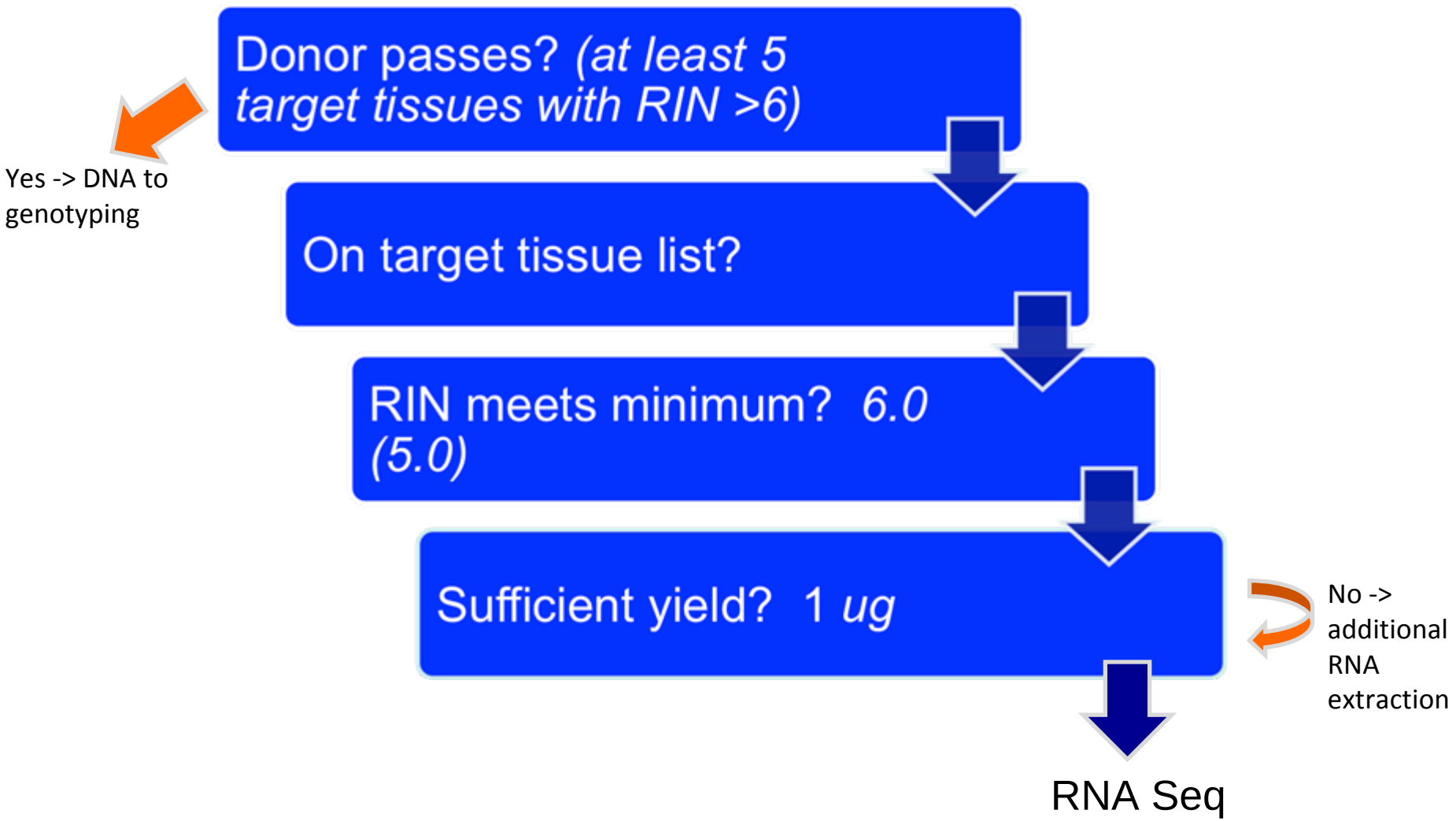


N=15 donors

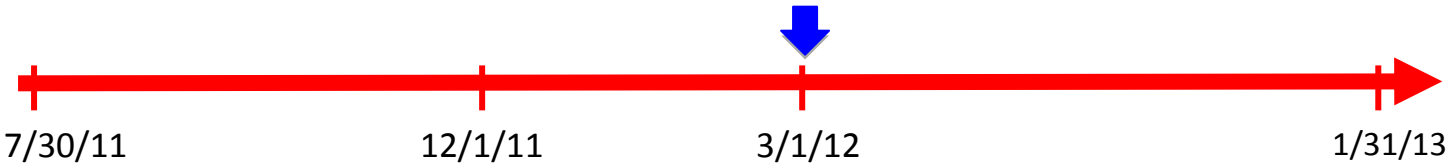




Sample Qualification

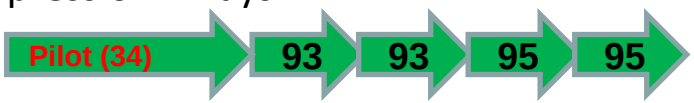


Molecular Analysis Current Status

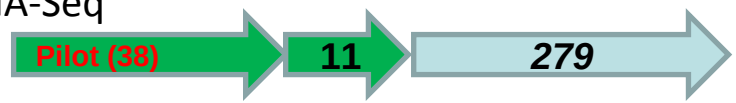


Genotyping Arrays

Expression Arrays



RNA-Seq



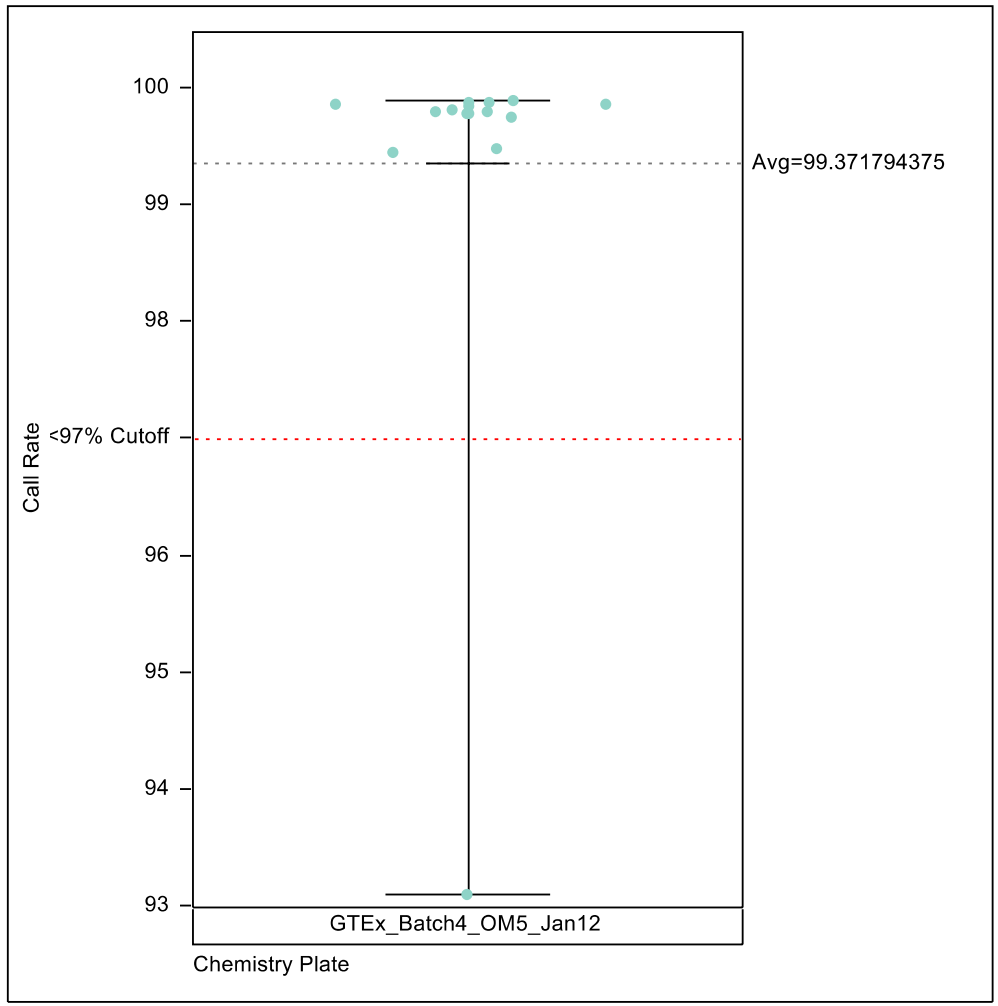
Completed by 3/1/12 Data freeze	Pilot Goal
64	300
376 (34)	1000
290 (38)	1500

Illumina 5M Genotyping

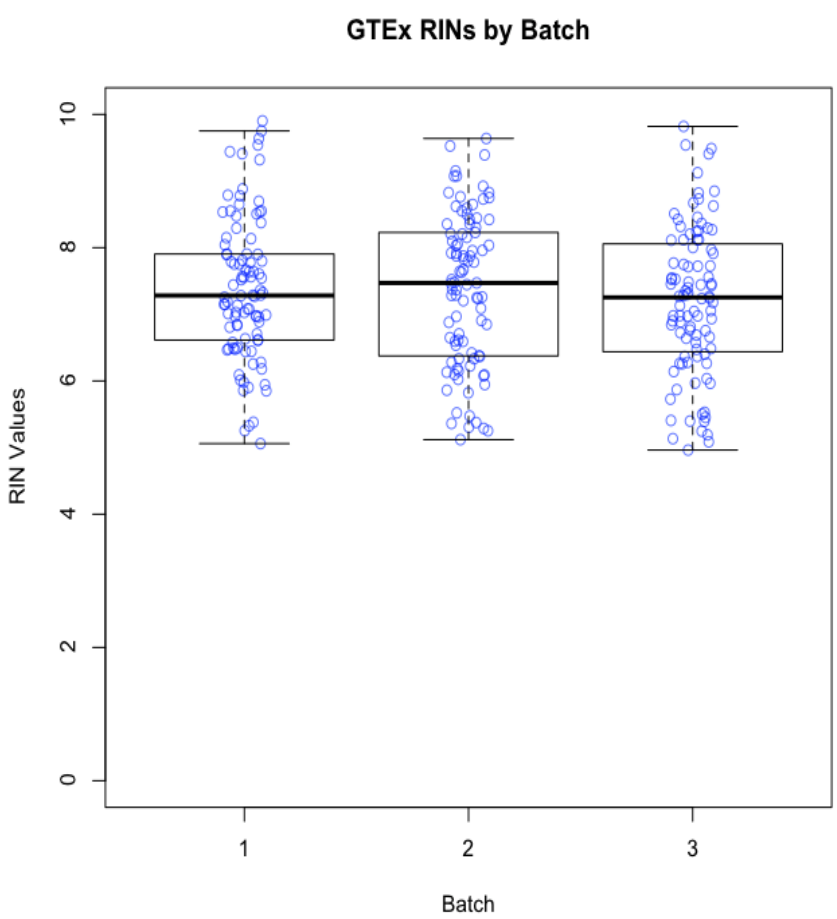
- Allele Frequency Distribution of ~5 million SNPs in GTEx 5M Batch 1
  - 100% pass rate
  - 99.35% average call rate
  - ~3.1 million polymorphic sites

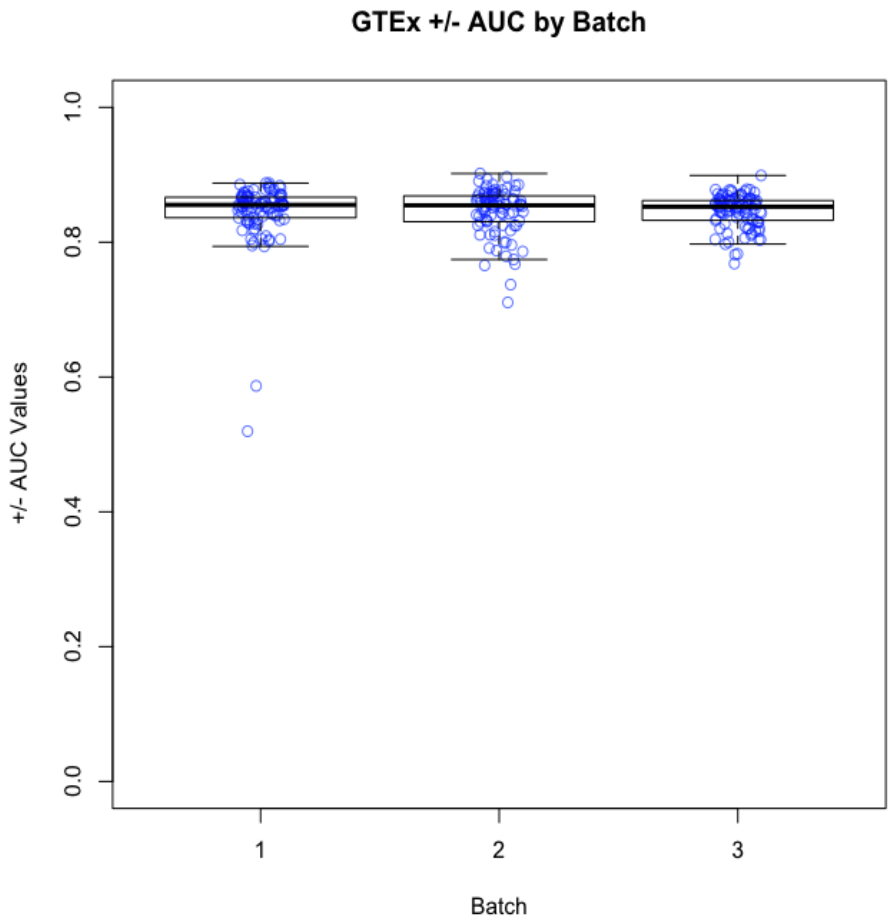
MAF	SNP Count
0	1156894
0.03-0.1	1615589
0.1-0.2	530866
0.2-0.3	362103
0.3-0.4	337539
0.4-0.5	298341

Batch 4



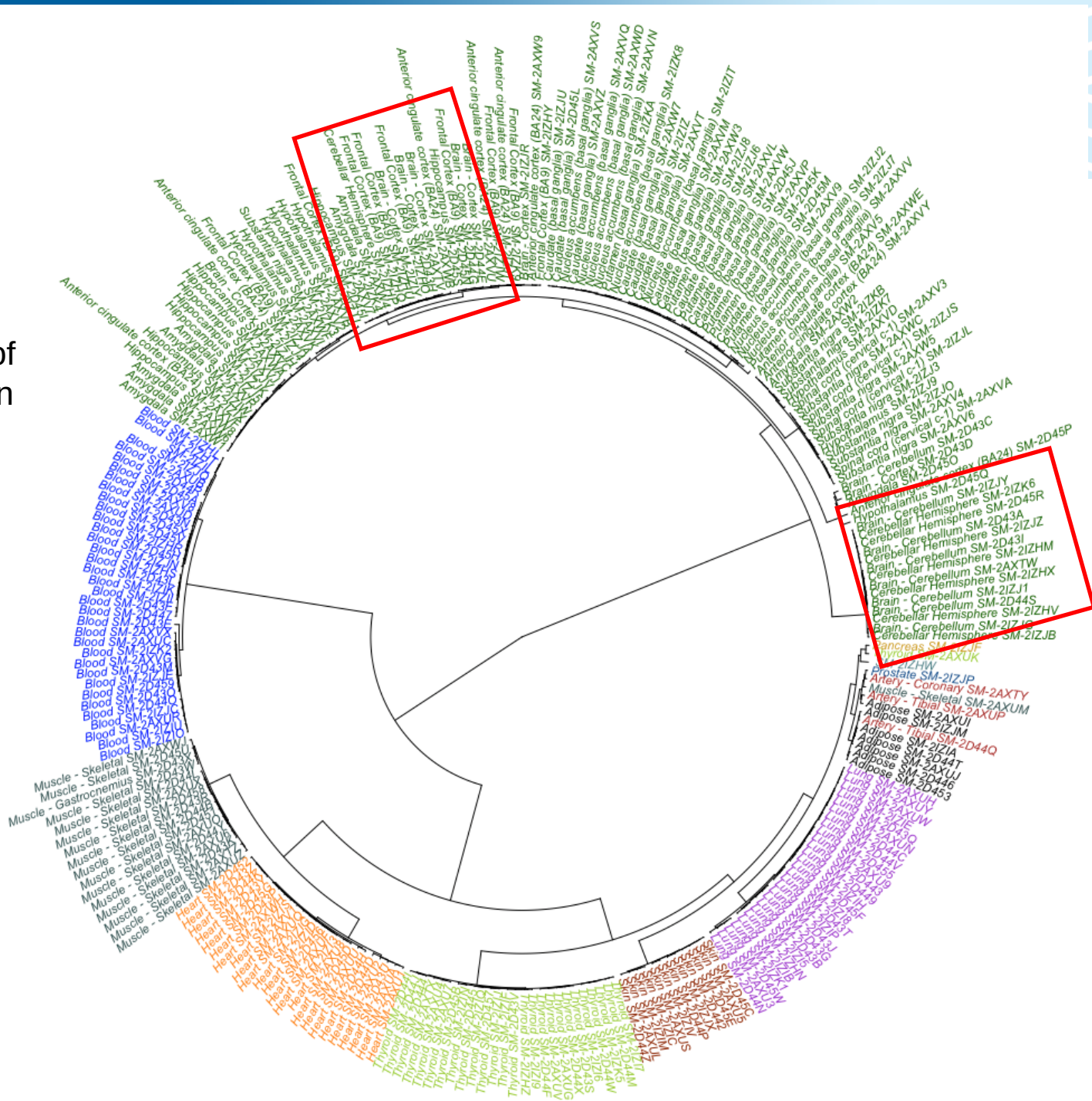
Expression Array QC by Batch





Hierachical Clustering

Distance measure:
Spearman Correlation of all Affymetrix expression levels



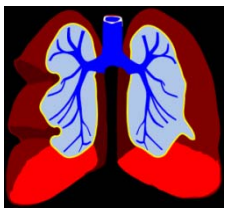
RNA Sequencing

- Tech dev efforts have focused on identifying a cDNA library construction method that provides high quality transcript profiles and:
  - Uses < 1ug total RNA input
  - Works with degraded RNA
  - Can be readily scaled to high-throughput
  - Works with preserved tissues (e.g. Paxgene Tissue)

Poly-A method (Tru-Seq, dUTP) vs non-poly-A DSN

GTEx Pilot RNA Experiments

GOAL → Select tissues for which the 3 methods produced similar, and at least mid-range, RIN scores for all samples, and for which there was sufficient RNA yield for both Affymetrix GeneArrays and RNASeq experiments.



Fresh Frozen, Liquid N2

→ RNA

RNAlater

→ RNA

→ RNA seq w/ DSN

PAXgene Tissue

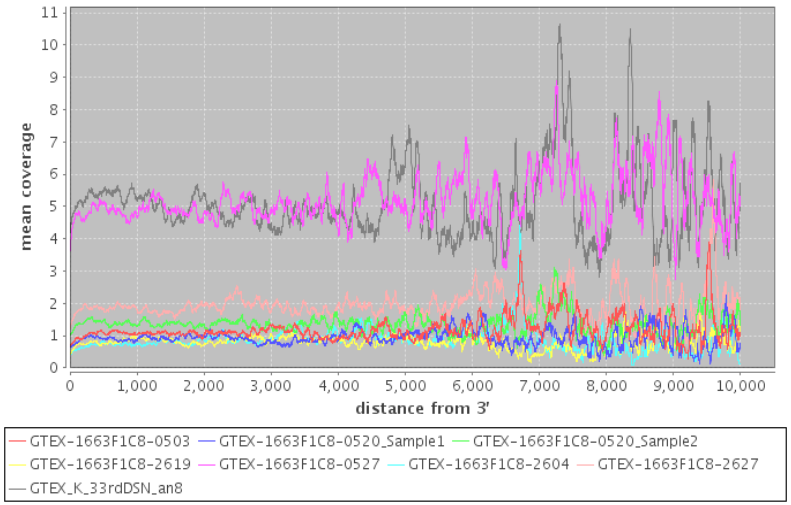
→ RNA

RNA Stable

RNA stable

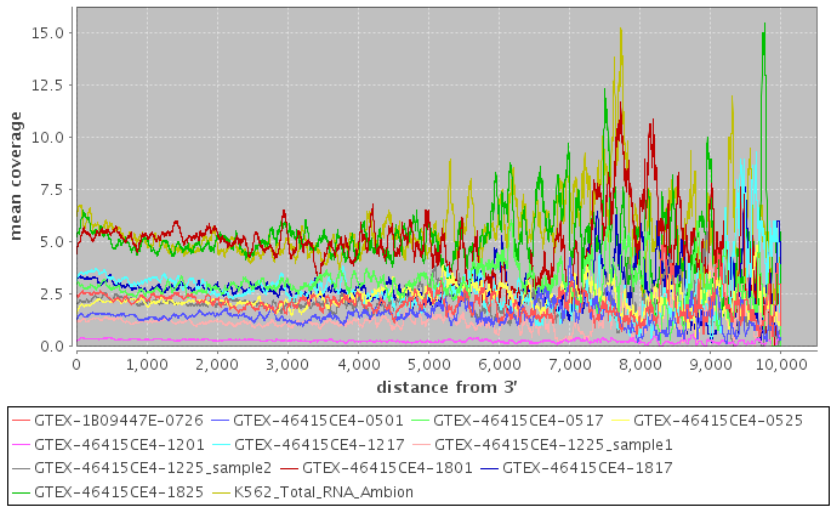
GTEx Pilot Tissue experiment results

*Based on 1000 middle expressed transcripts



Donor 2

Mean dup. rate: 73%
Mean CV: 1.23
Mean Gap %: 41%

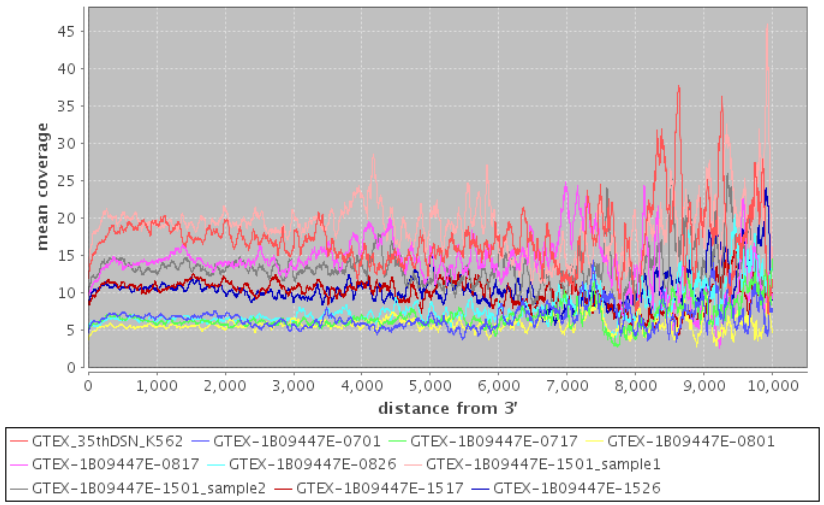


Donor 4

Mean dup. rate: 64%
Mean CV: 1.00
Mean Gap %: 19%

Donor 3

Mean dup. rate: 54%
Mean CV: 0.93
Mean Gap %: 11%



No evident difference between preservation methods.

RNA Seq First Production Batch

Donor	Tissue	RIN
GTEX-N7MS	Blood	8.2
GTEX-N7MS	Brain	8.7
GTEX-N7MS	Brain	9.6
GTEX-N7MT	Lung	9.1
GTEX-N7MT	Brain	9.5
GTEX-NPJ8	Blood	5.5
GTEX-NPJ8	Lung	8.8
GTEX-NPJ8	Brain	8.8
GTEX-NPJ8	Brain	9.4
GTEX-O5YV	Lung	6.1
GTEX-O5YV	Muscle	7.1

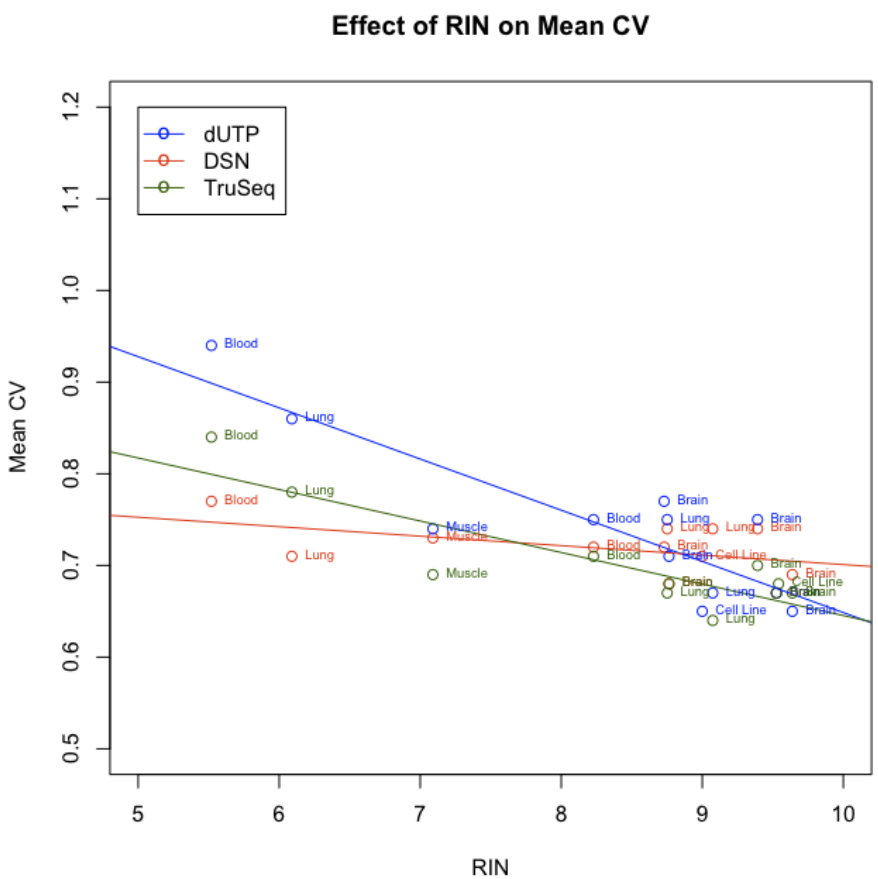
4 ug polyA+ LC
(2 methods)

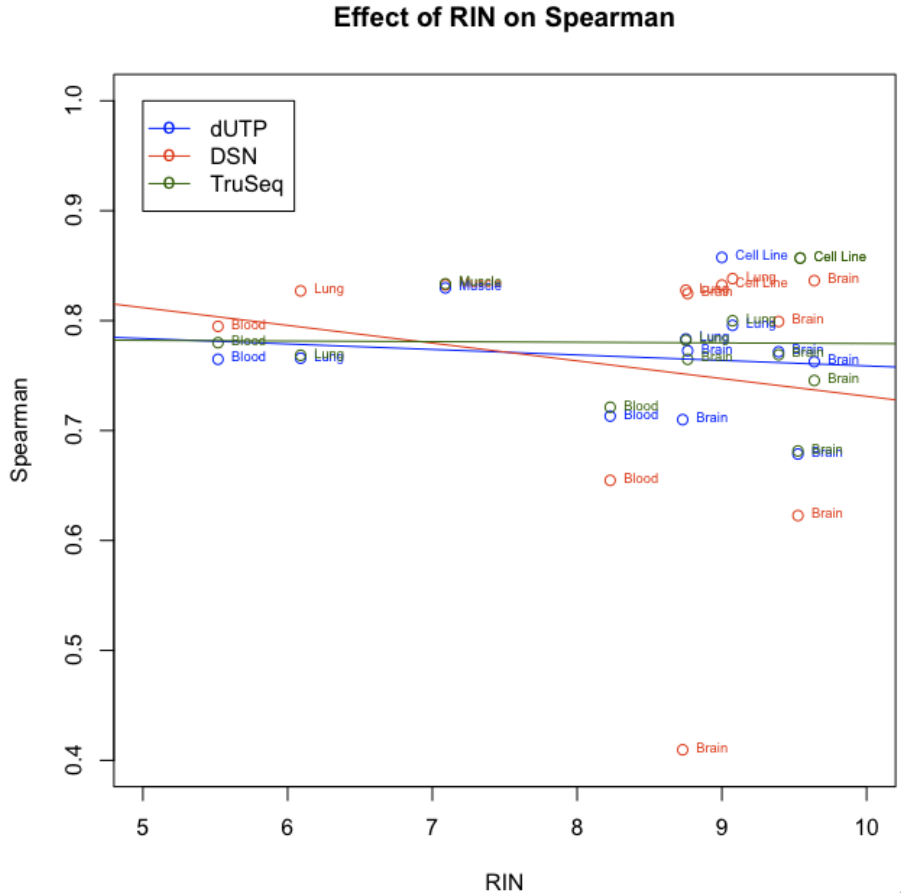
1 ug DSN LC

1/6th lane, 101 PE

FIREHOSE
Genotype-Tissue Expression

Coverage metrics





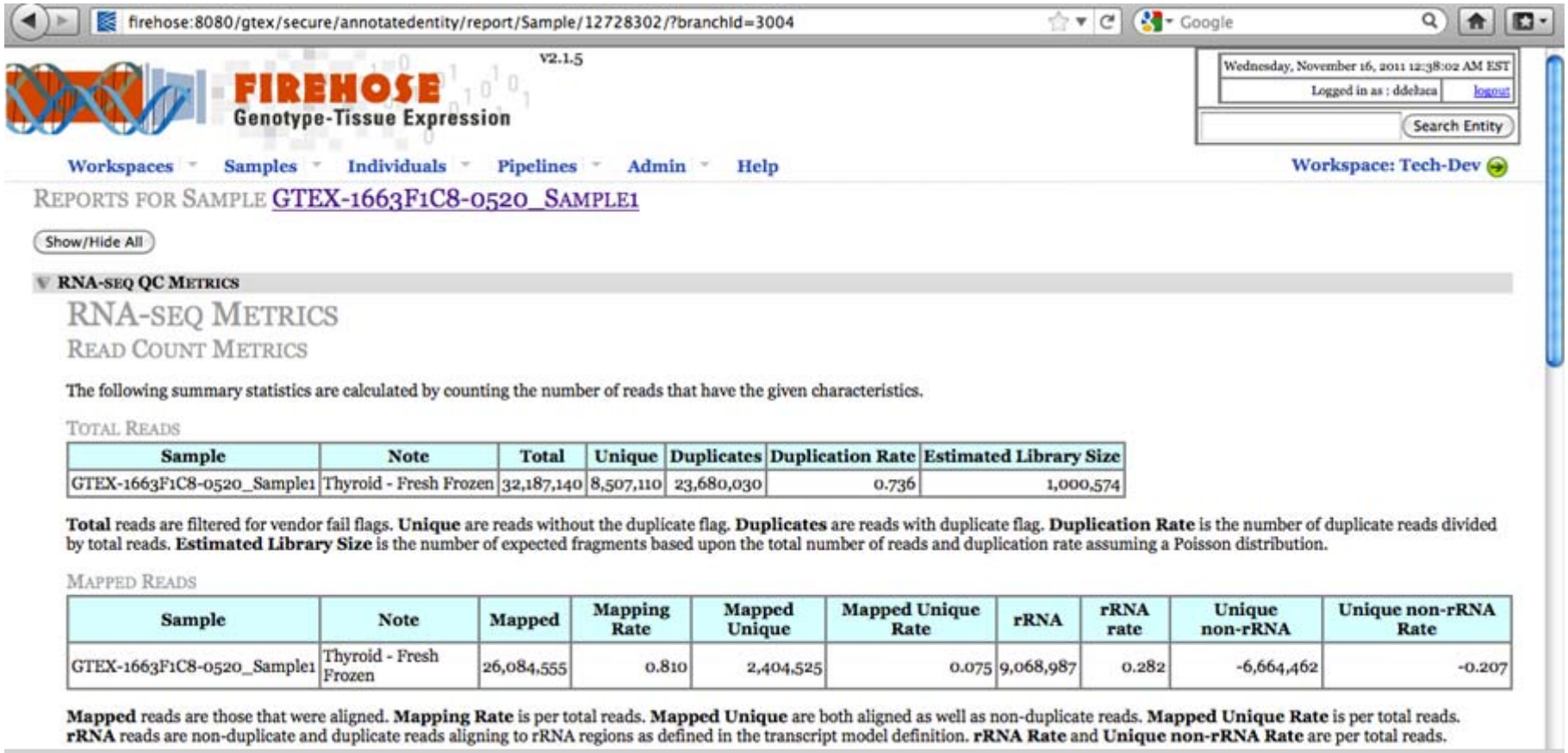
For middle expressed 1000 transcripts, downsampled to 20m reads.

LC Protocol Summary

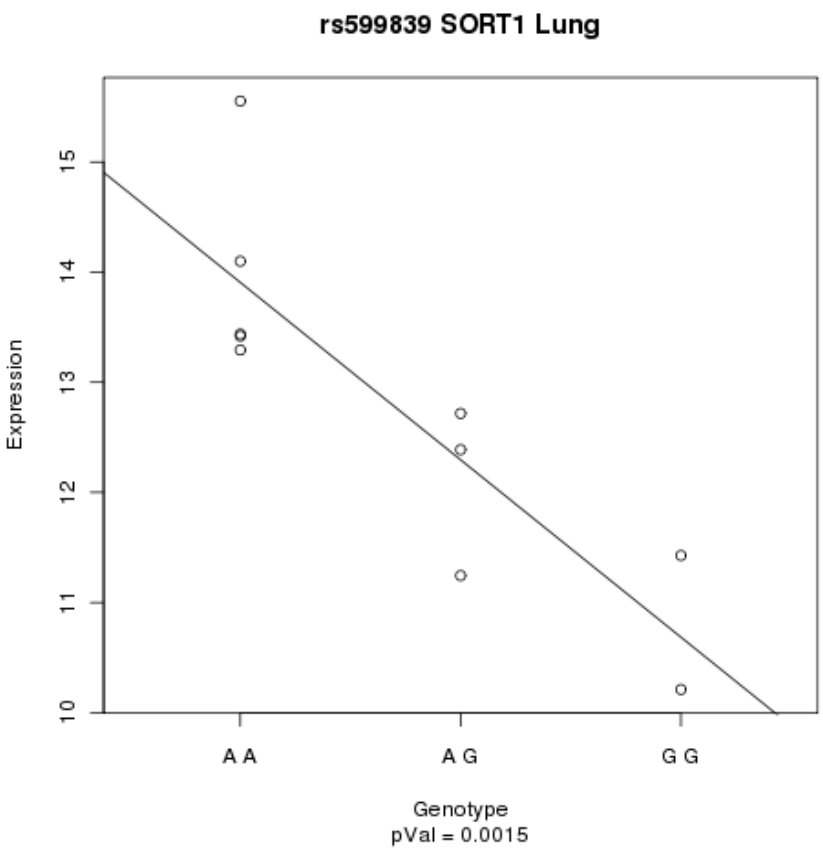
- For RINs >=7, polyA+ and DSN perform similarly
  - polyA does seem to be adversely affected by RINs <6
- BUT, DSN has significant disadvantages compared to polyA+ in terms of protocol robustness, GC bias, and cost
- polyA+ protocol currently requires 1-5ug RNA, but ongoing testing aims to reduce that to 0.1-1ug

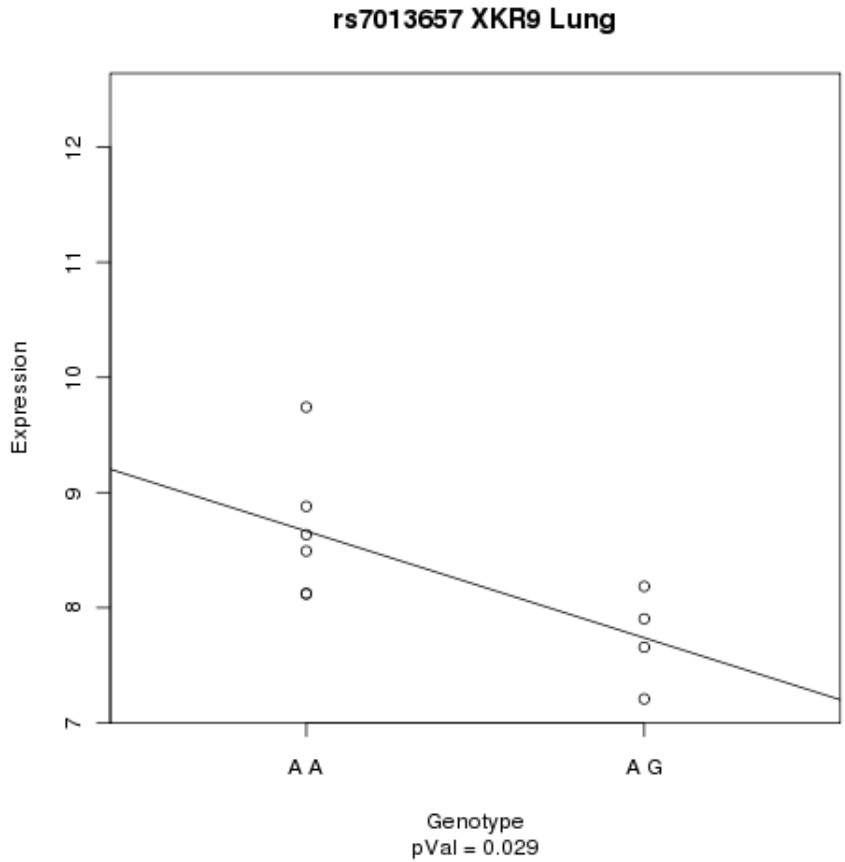
CURRENT PRODUCTION – polyA+ protocol, with 1ug RNA input and RIN>=6

RNA Seq Analysis Pipeline

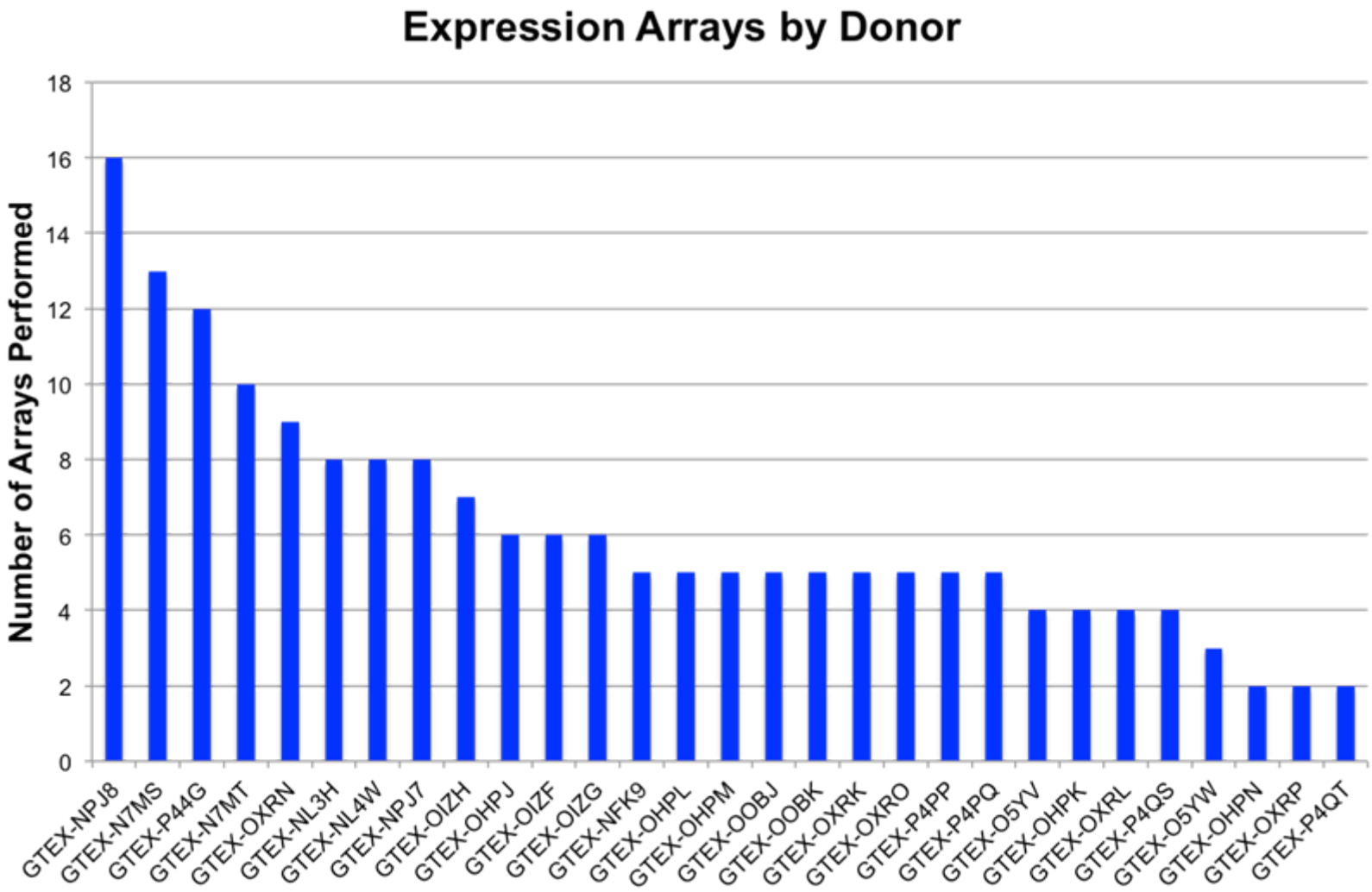


Known Liver eQTLs found in Lung





Current Power Limitations



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