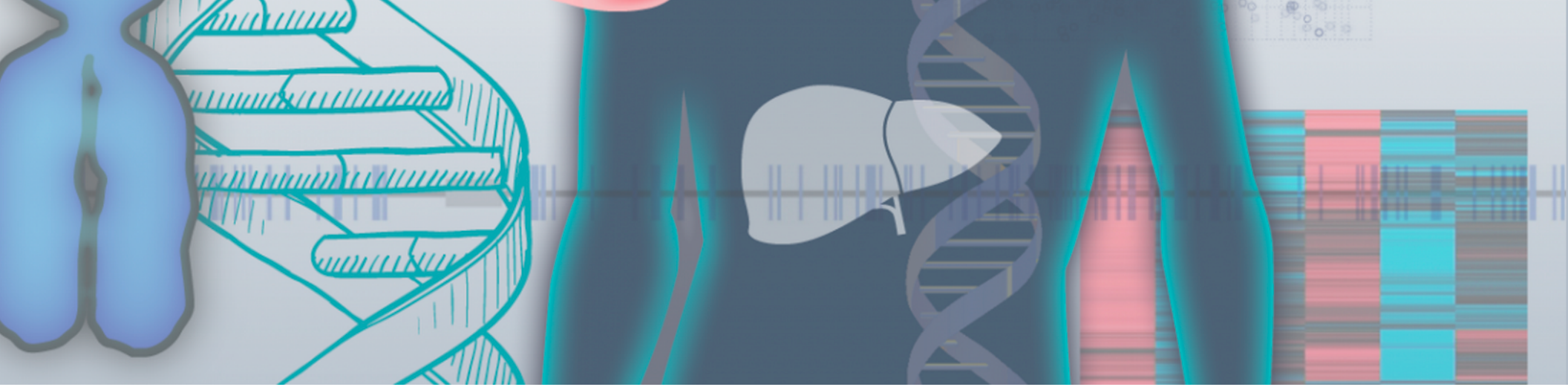


TOPMed multi-omics: transcriptomics in the MESA cohort

François Aguet

MESA Steering Committee Meeting :: 03/29/2018

BROAD INSTITUTE

NHLBI TOPMed

MESA

UNIVERSITY OF WASHINGTON 1861

The TOPMed MESA RNA-seq pilot

Exam 1 (baseline)
July 2000 - July 2002
1163 participants

- PBMCs

Exam 5
2010 - 2012
966 participants

- PBMCs
- Sorted cells (subset)
  - T cells
  - Monocytes

The TOPMed RNA-seq pilot:

Establish robust, multi-center transcriptomics pipeline and assess feasibility in the MESA cohort, with the goal to reproducibly detect changes in gene expression that are correlated with other phenotypes.

The TOPMed MESA RNA-seq pilot

Cohort	Broad	NWGC	Total
PBMC (Exam 1)	580	583	1163
PBMC (Exam 5)	498	468	966
T cell (Exam 5)	201	204	405
Monocyte (Exam 5)	202	199	401
PBMC (Control)	15	15	30
Whole Blood (Exam 5)	4	4	8
Total	1500	1473	**2973**

- **Pre-pilot:** 55 samples at each sequencing center
  - MESA samples: 20 Exam 5 PBMC, 16 Exam 1 PBMC, 4 Exam 1 Whole Blood
  - 15 control samples, replicating Exam 1 conditions
- **MESA cohort:** 1349 participants
  - 1195 with genotype in Freeze 5 VCF

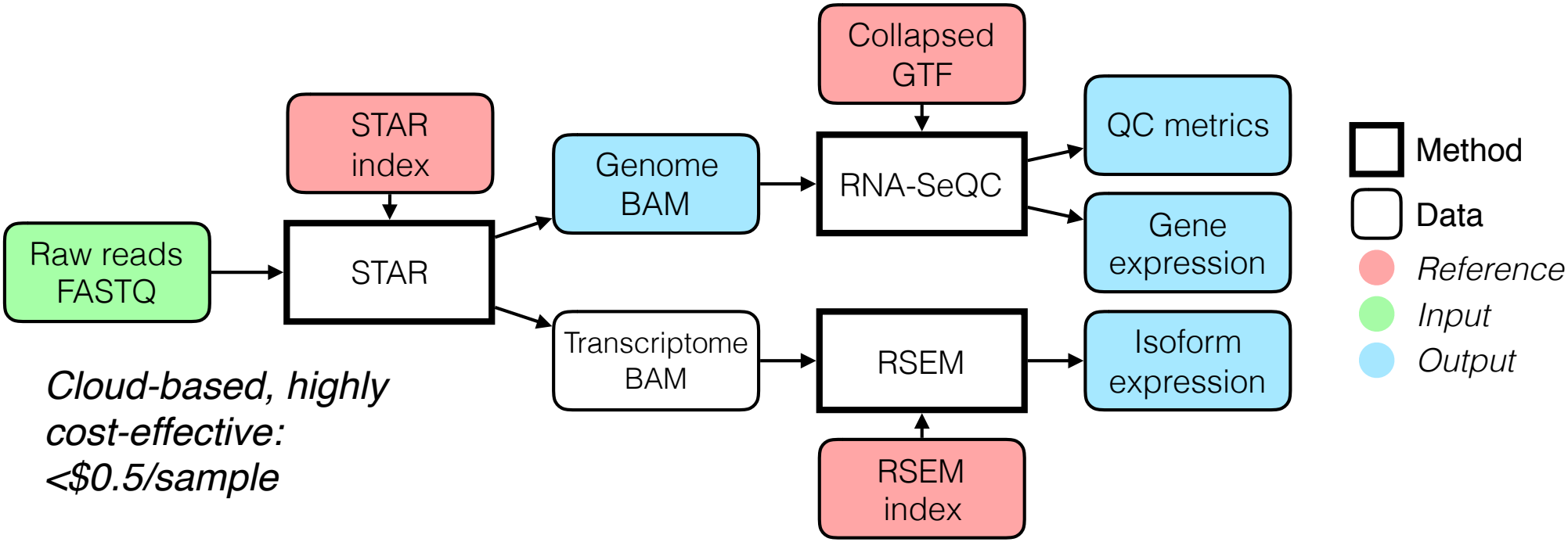
Harmonized pipelines to minimize batch effects

- **RNA-seq is highly sensitive to technical variation**
  - Experimental design: samples from the same participant sequenced at same center; within- and cross-center replicates were included
- **Sequencing protocols matched to the extent possible**
  - All samples were sequenced on HiSeq 4000 to ≥ 40M reads
- **Identical data processing pipelines**
  - Based on methods and guidelines initially developed for the GTEx Consortium
  - Implemented at both sequencing centers

’t Hoen et al., *Nat. Biotech.*, 2013

Harmonized processing pipeline

Developed and based on benchmarks for the GTEx Consortium



Cloud-based, highly cost-effective: <$0.5/sample

Pipeline components

Alignment	STAR v2.5.3a
Gene expression	RNA-SeQC v1.1.9
Transcript expression	RSEM v1.3.0
QC metrics	RNA-SeQC v1.1.9

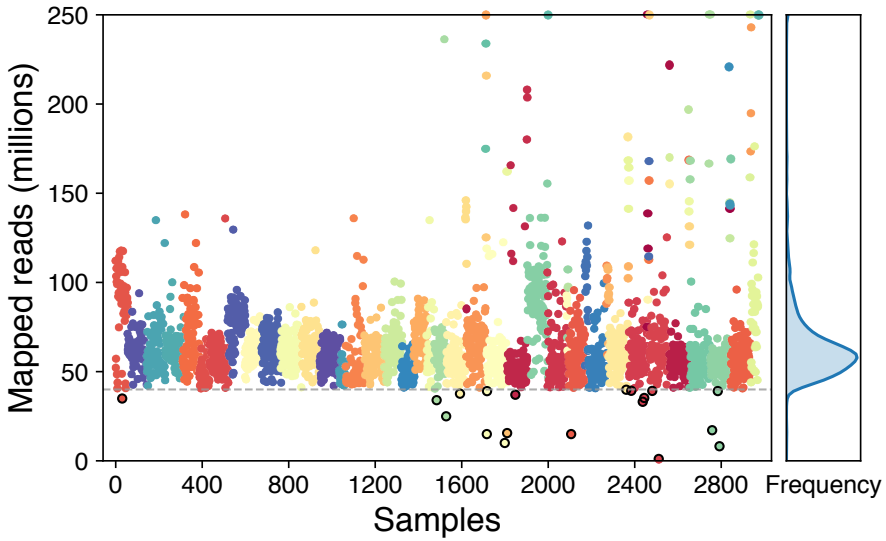
References

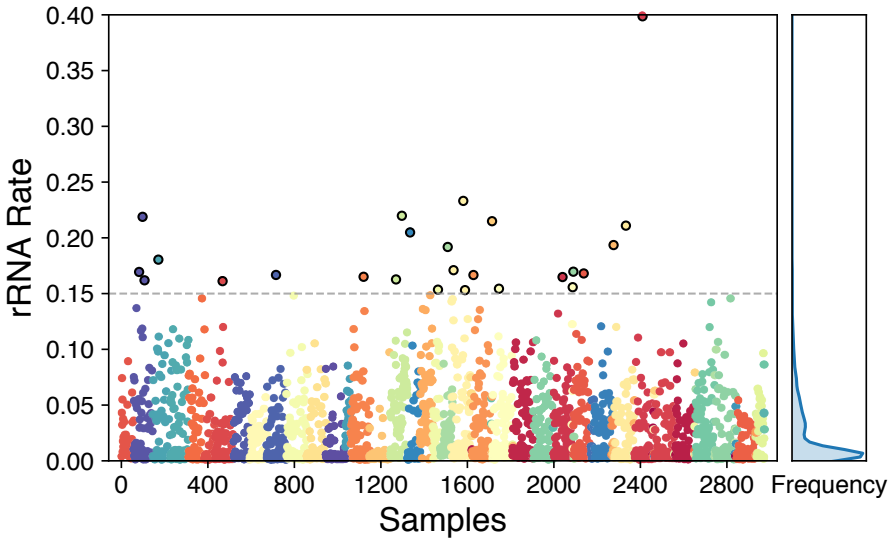
- GRCh38
- GENCODE v26

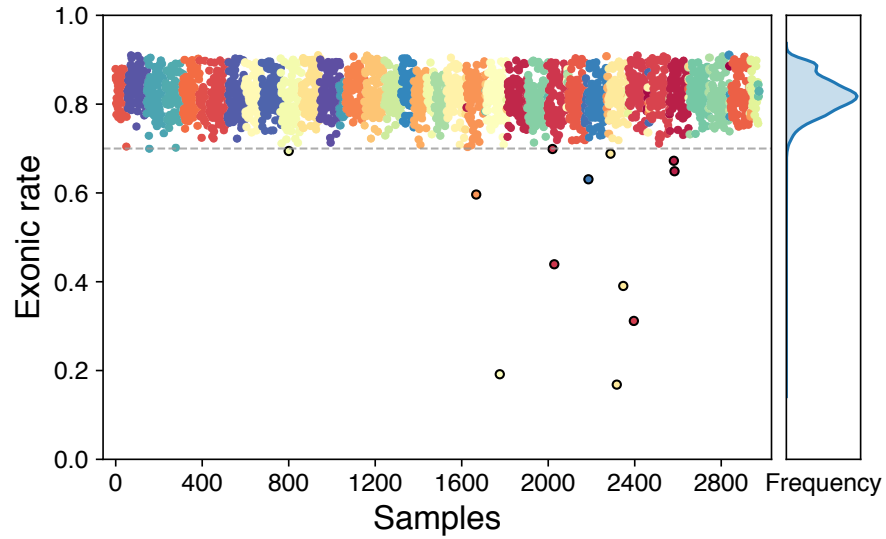
www.firecloud.org : namespace broadinstitute_gtex
https://github.com/broadinstitute/gtex-pipeline
- see TOPMed_RNAseq_pipeline.md

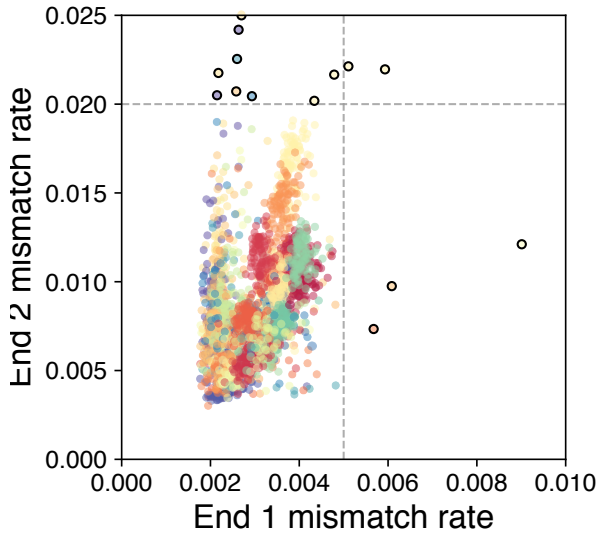
STAR: Dobin et al., *Bioinformatics*, 2013
RSEM: Li et al., *Bioinformatics*, 2010
RNA-SeQC: DeLuca et al., *Bioinformatics*, 2012

Post-sequencing quality control of RNA-seq samples



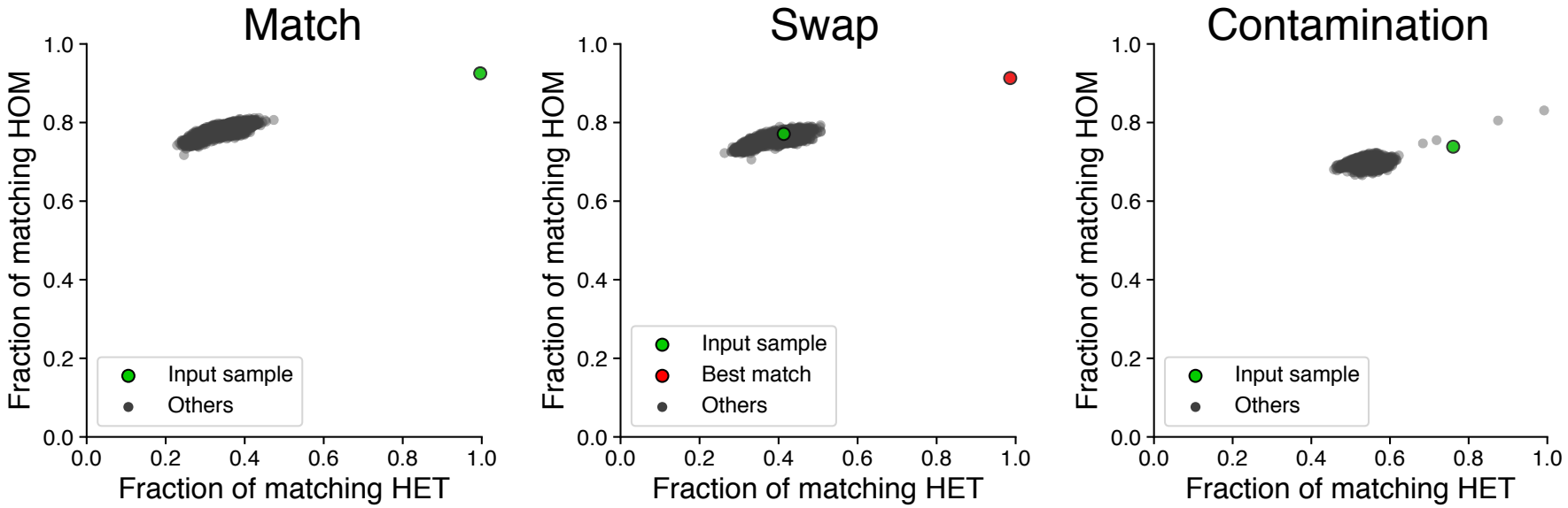






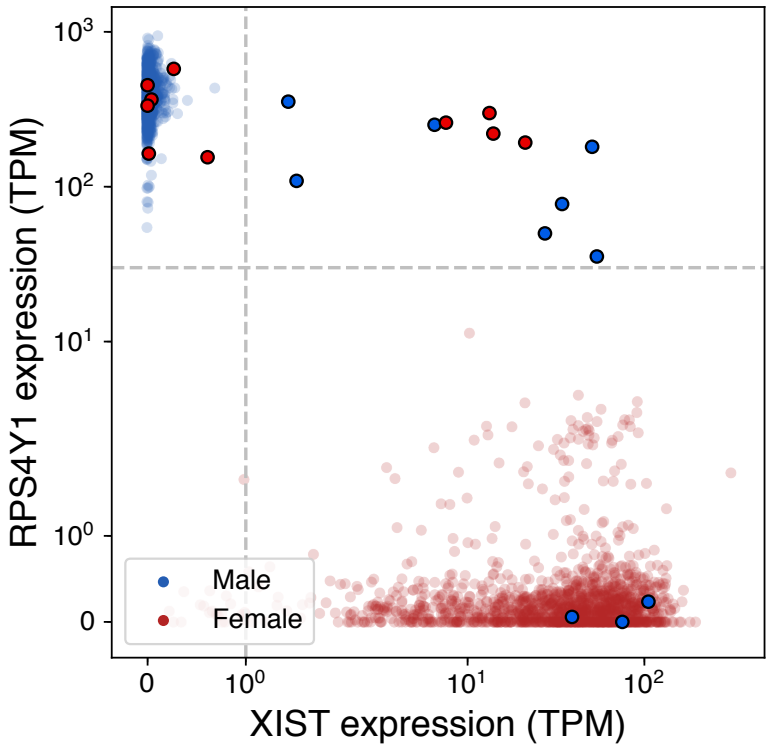
- Highly similar across sequencing centers
- Coverage goal of 40M reads exceeded for most samples

Post-sequencing quality control of RNA-seq samples



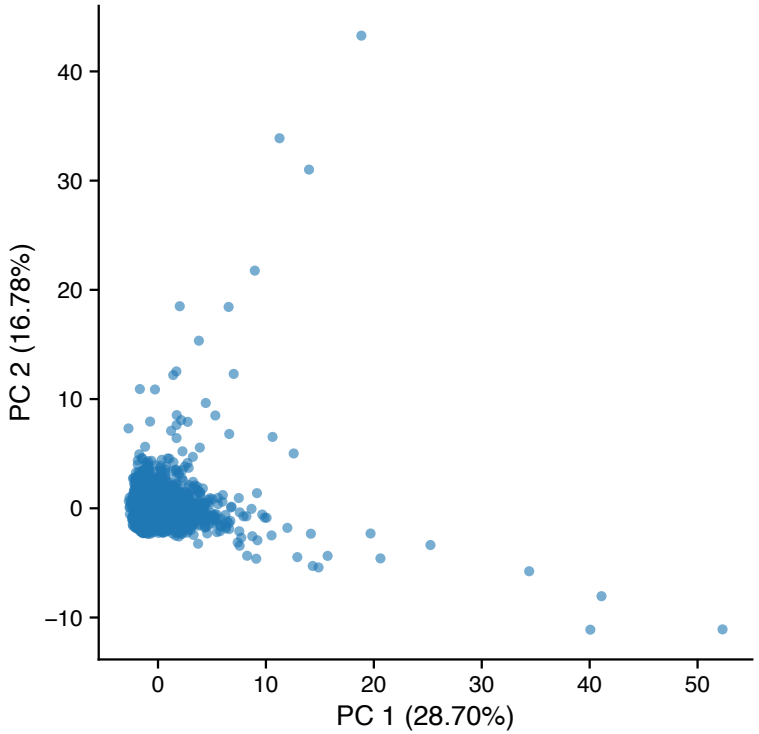
Sample swap detection

- **Fingerprinting:** concordance of heterozygous and homozygous sites between RNA-seq samples and VCF (43 mismatches)
- Expression-based sex check (20 mismatches)

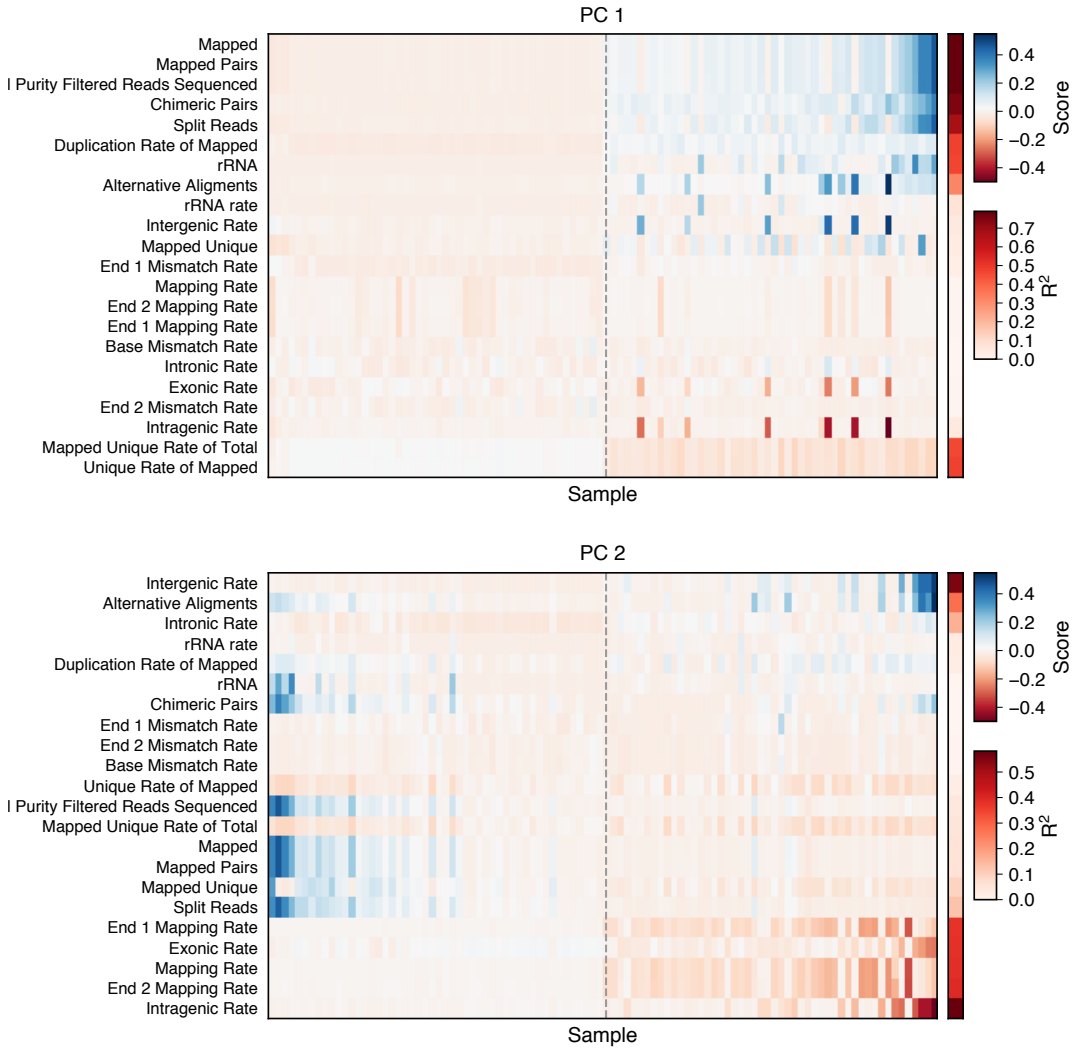


Identification of low-quality samples based on sequencing metrics

Normalized metrics

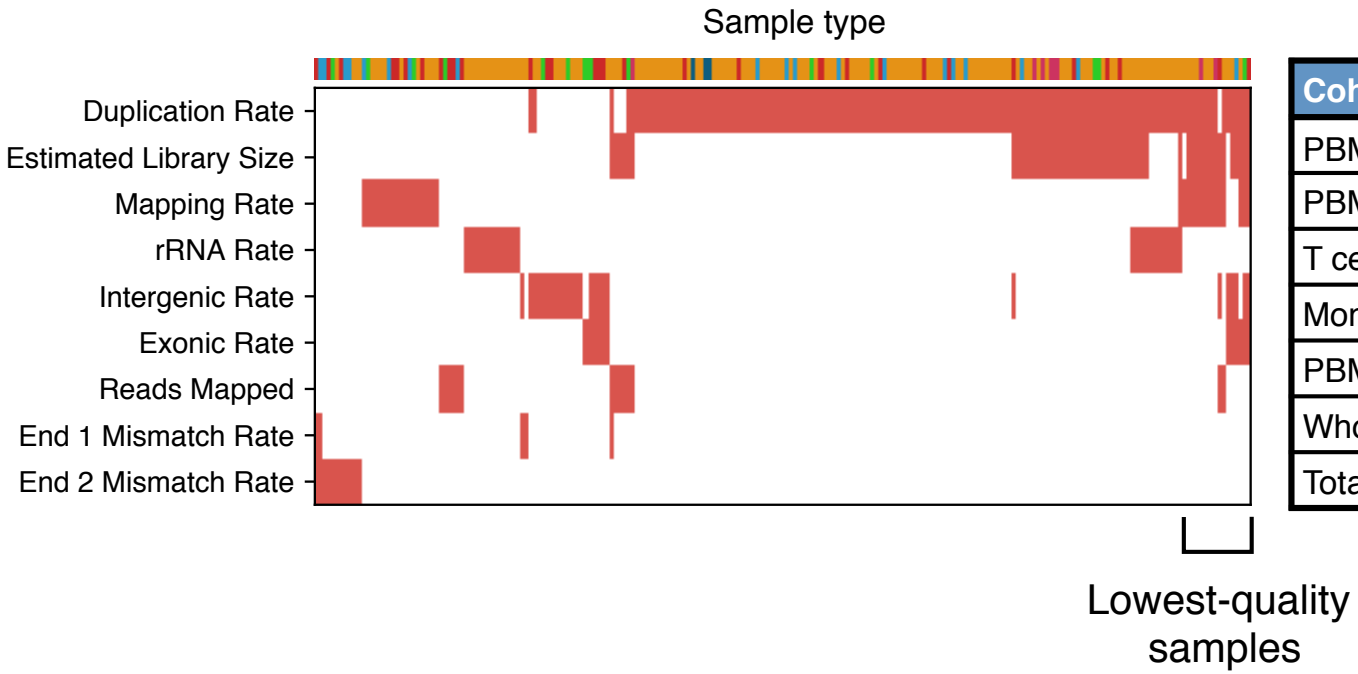


Association of PCs with metrics



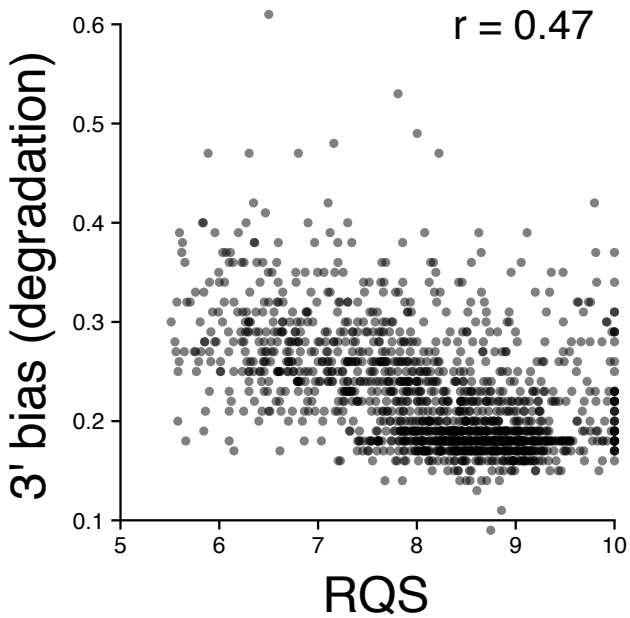
- Lowest-quality samples are outliers for multiple QC metrics

Identification of low-quality samples based on sequencing metrics



Cohort	Samples	Failed	%
PBMC (Exam 1)	1163	145	12.5
PBMC (Exam 5)	966	31	3.2
T cell (Exam 5)	405	14	3.5
Monocyte (Exam 5)	401	19	4.7
PBMC (Control)	30	3	10.0
Whole Blood (Exam 5)	8	7	87.5
Total	**2973**	**219**	**7.4**

- Small set of poor quality samples failed multiple metrics
- RNA quality: lab metrics weakly correlated with sequencing metrics
- Stringent thresholds applied to define high-quality analysis set
- Recommendation to exclude 219 samples



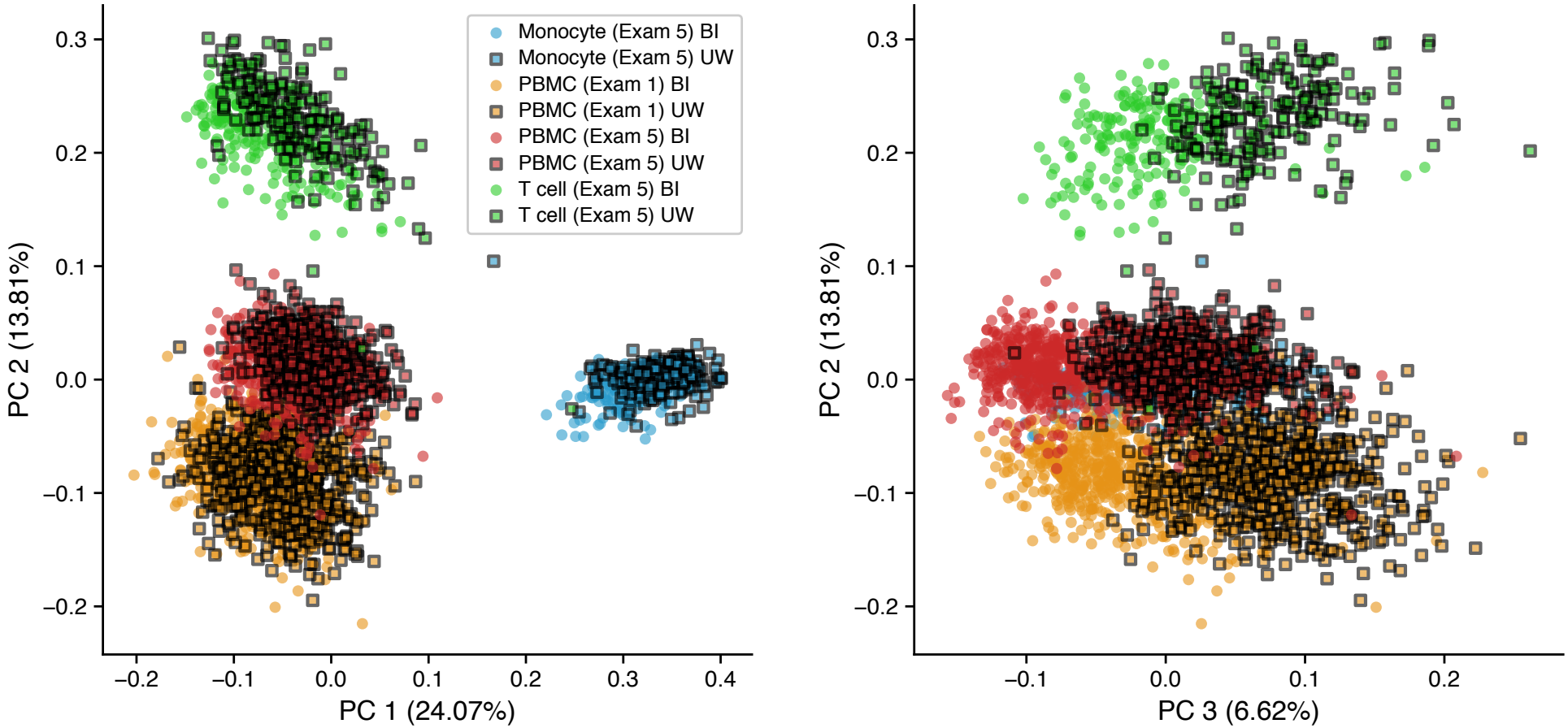
Analysis freeze (preliminary)

Cohort	PBMC Exam 1	PBMC Exam 5	T cell Exam 5	Monocyte Exam 5	Total
All samples	**972**	**916**	**385**	**375**	**2648**
With genotype (freeze 5)	863	811	343	336	2353

PBMC Exam 1	PBMC Exam 5	T cell Exam 5	Monocyte Exam 5	Total
●	●	●	●	**198**
	●	●	●	**331**
●	●			**608**

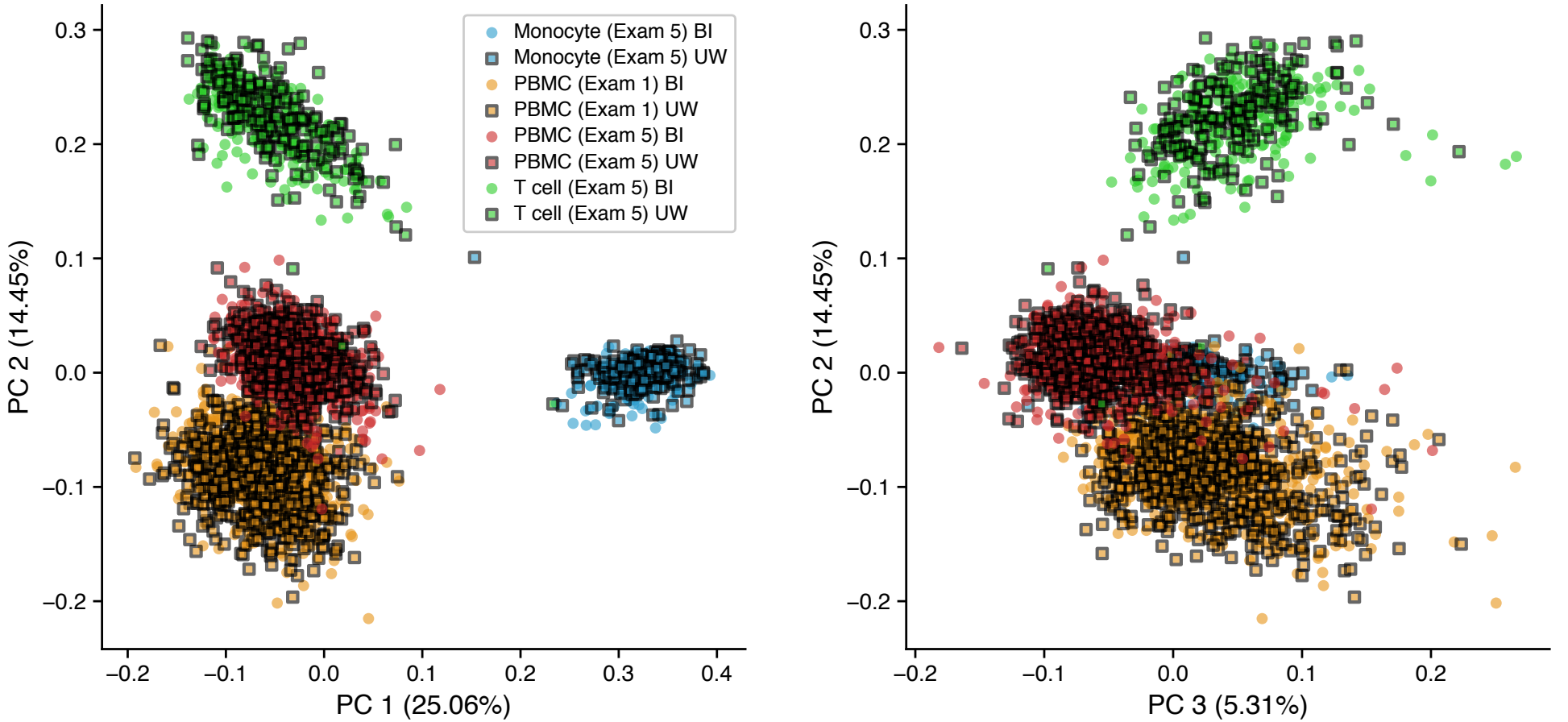
- Combination of QC steps:
  - Technical (QC metrics)
  - Expression outliers (sex check)
  - Unresolved swaps
- Freeze 5: 1149 participants with genotype data

Expression variation across sample types



- Sequencing center batch effect observed, but weak as a result of harmonization
- **Confounders: RNA isolation method, blood draw tube, and exam are linked:**
  - Trizol / Citrate CPT / Exam 1
  - All prep / Heparin CPT / Exam 5

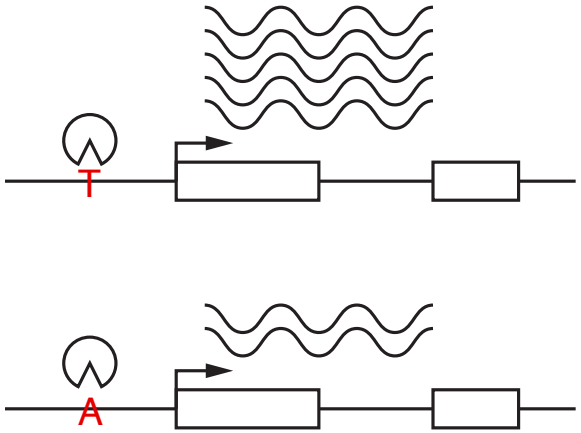
Expression variation across sample types

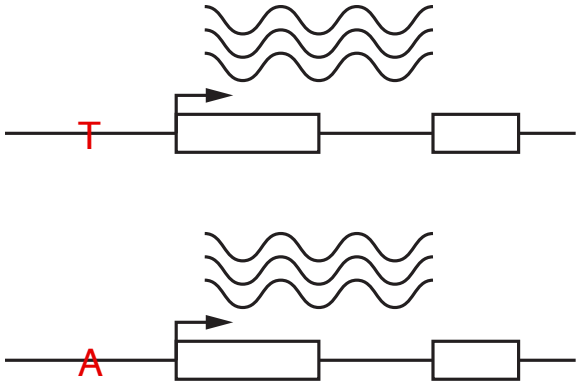


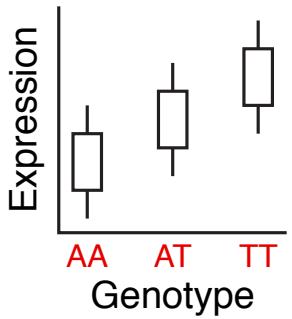
Corrected for sequencing center

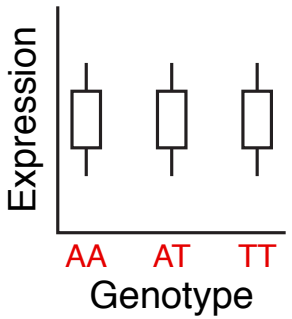
- Batch effects between Exam 1 and 5 hinder direct comparison of gene expression
- Comparisons can be made through relative measurements in each cohort
  - *Do regulatory effects on gene expression replicate between Exam 1 and Exam 5?*

Expression quantitative trait loci



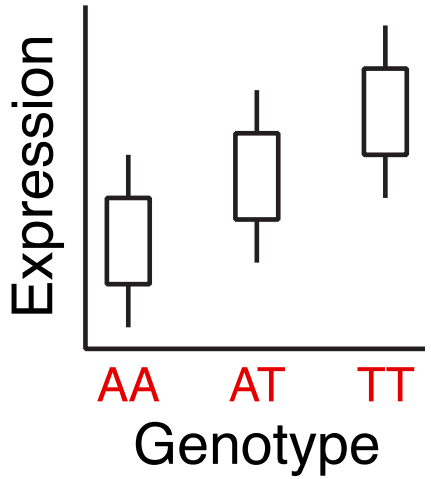






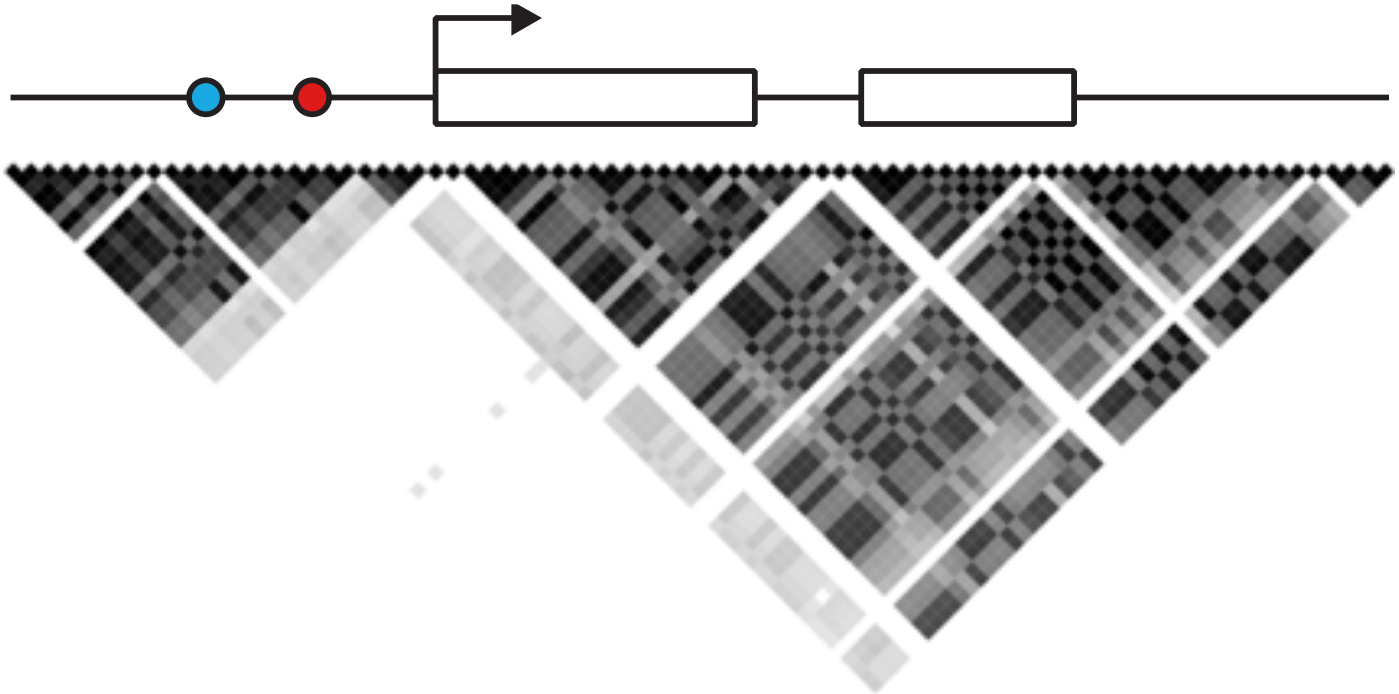
Regulatory variation is measured as expression quantitative trait loci (eQTLs)

Definition of *cis*-eQTLs



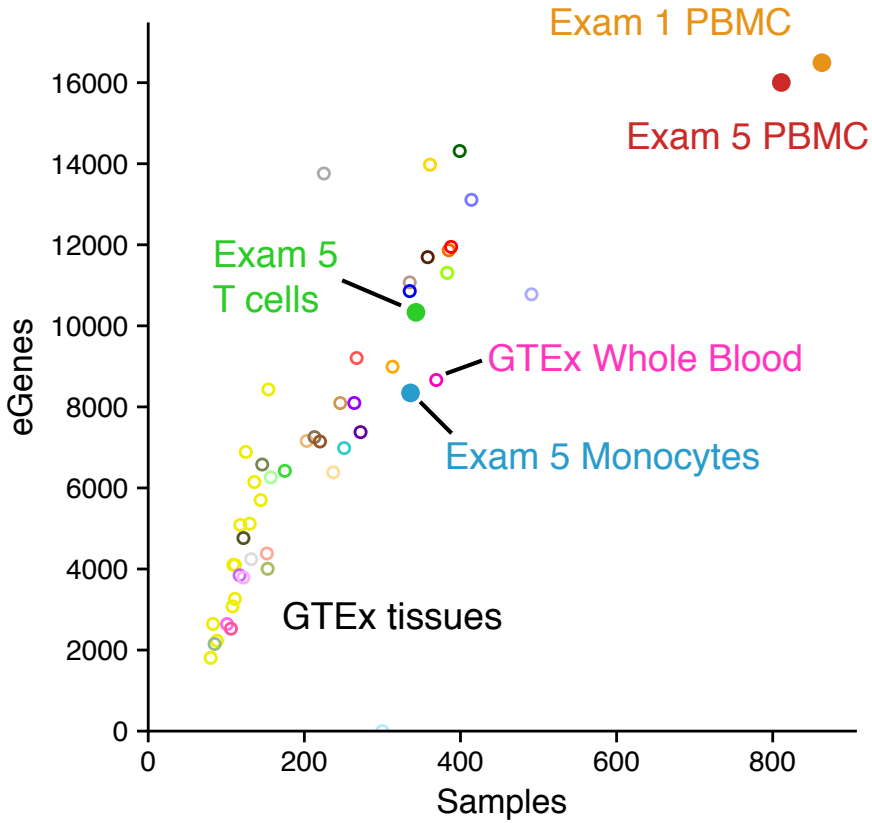
- ***cis*-eQTL**: genome-wide significant association between ≥ 1 eVariant and eGene, with associations tested within ±1Mb *cis*-window around TSS. Does not imply evidence of allelic effects at each locus.
- **eGene**: gene with at least one significant eQTL (at 5% FDR).
- **eVariant**: variant with a significant association to ≥1 eGene.
- **Effect allele**: ALT allele (not necessarily the minor allele).

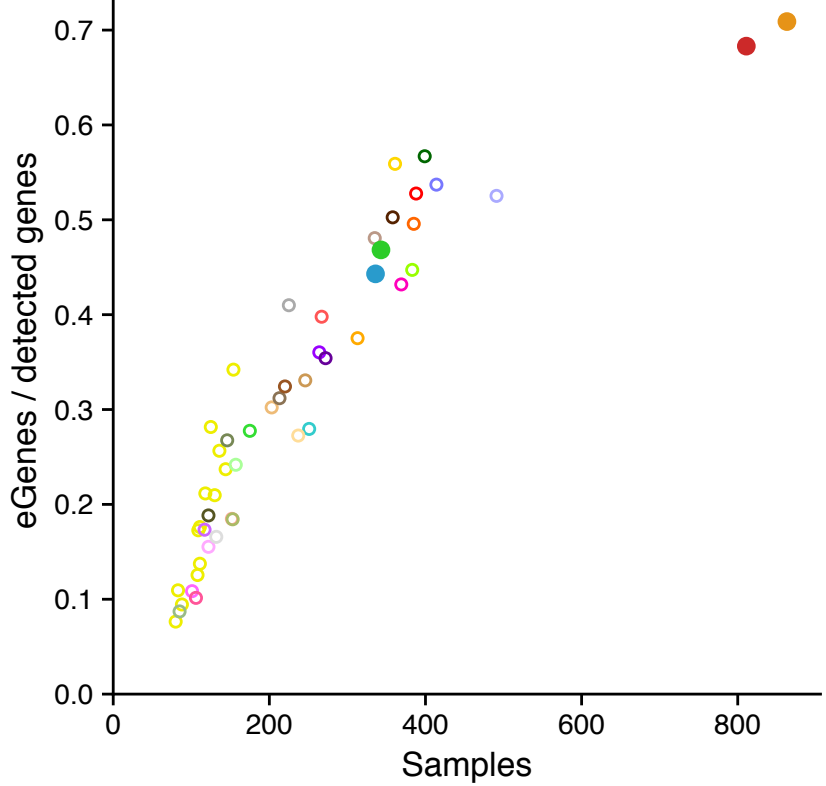
eQTL mapping and eGene discovery



- Variants in *cis*-window (±1Mb from TSS) correlated due to linkage disequilibrium (LD)
- LD must be incorporated in multiple hypothesis testing correction for establishing genome-wide significance
  - Empirical p-values from permutation of genotypes
- Methods developed for GTEx (GTEx Consortium, 2017)

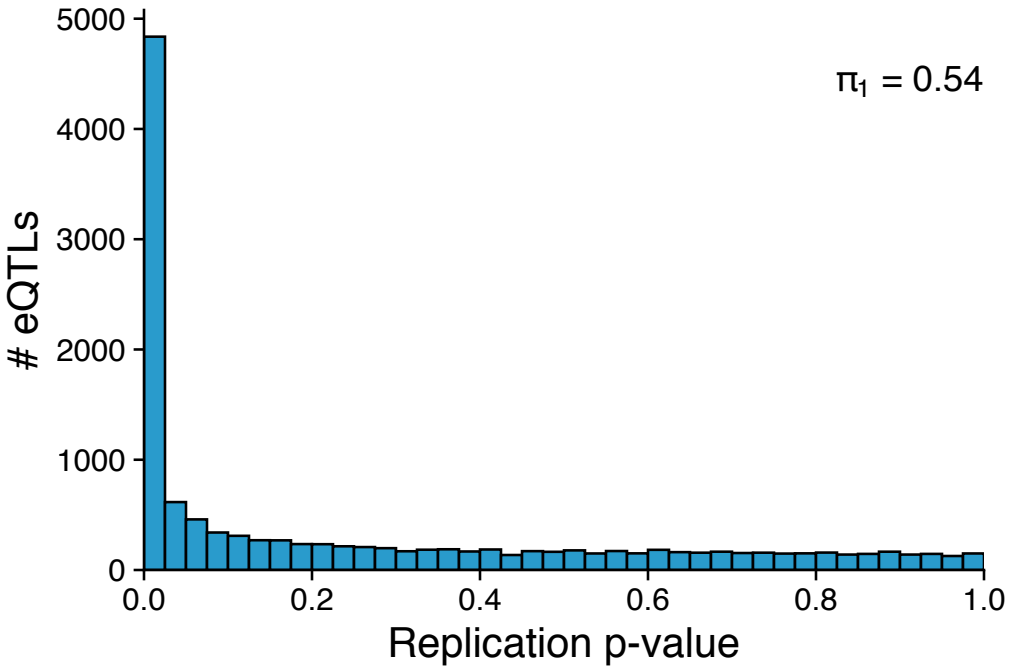
Expression quantitative trait loci detected in MESA





- *cis*-eQTL: variants within ±1Mb of TSS
- eGene: gene with ≥ 1 significant eQTL at ≤ 0.05 FDR
- GTEx data: V7

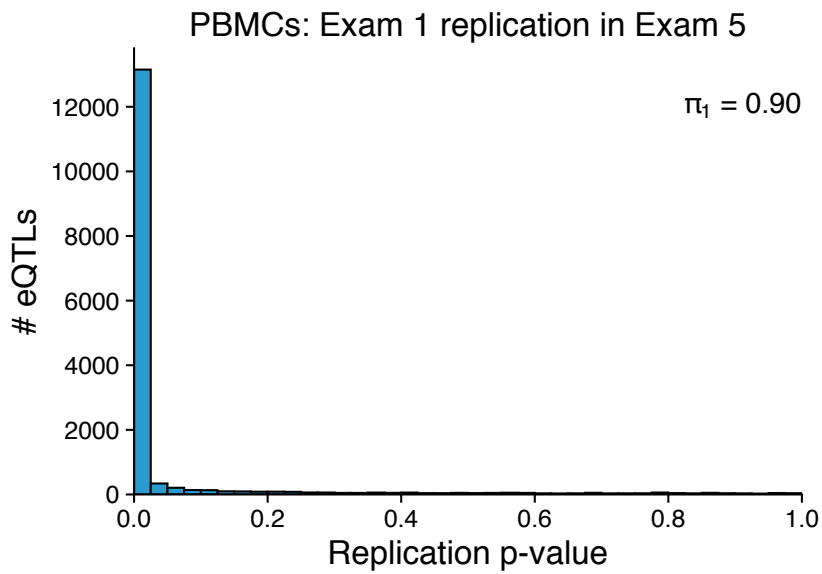
Replication of eQTLs between cohorts

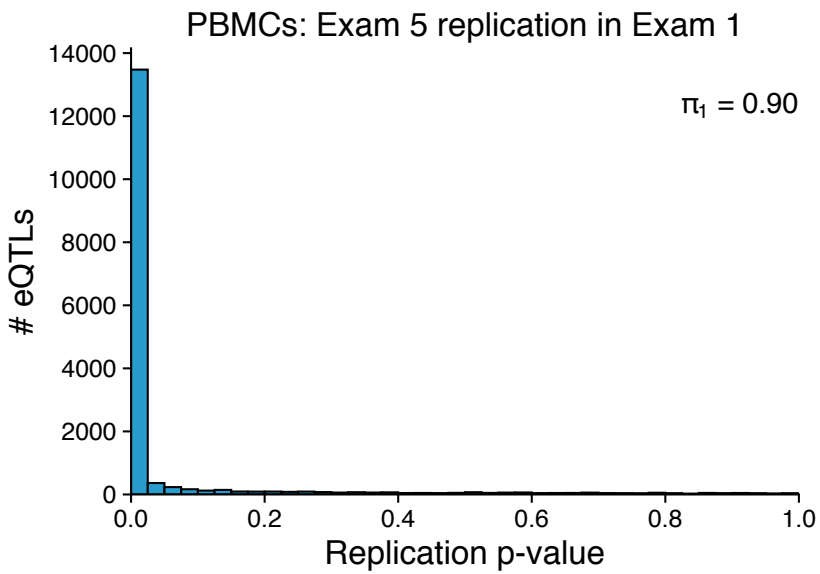


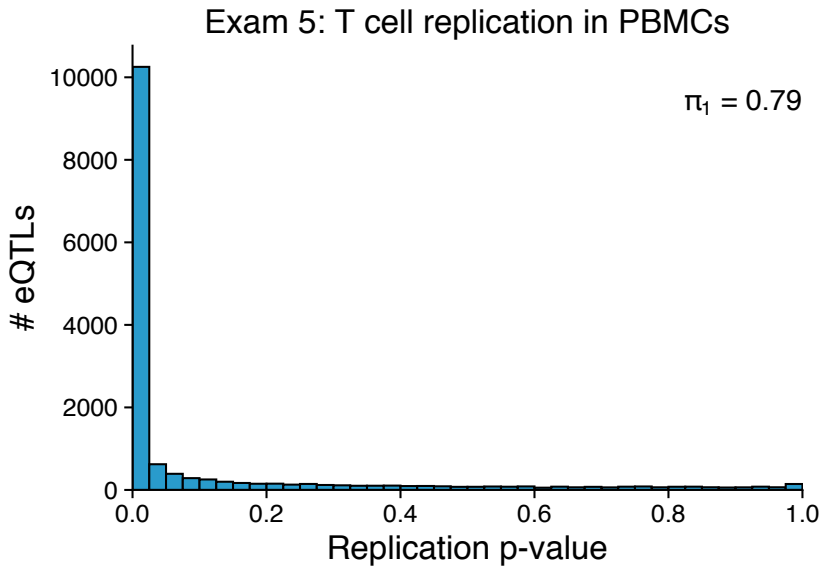
- For all genome-wide significant eQTLs in the discovery cohort, association p-values are calculated in the replication cohort
- An enrichment for small p-values indicates replication
- The proportion of replicating eQTLs is measured using the $\pi 1$ statistic, which measures the proportion of true positives

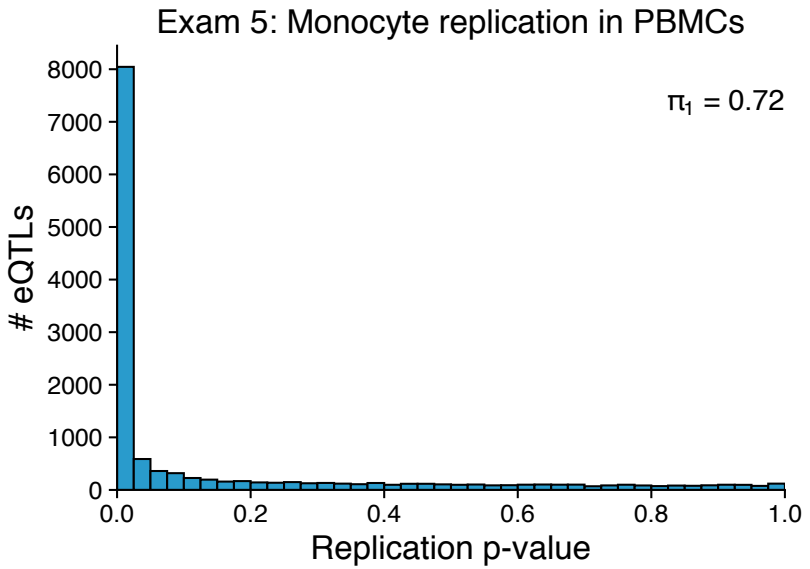
Storey & Tibshirani, *PNAS*, 2003

eQTL replication across exams and sample types



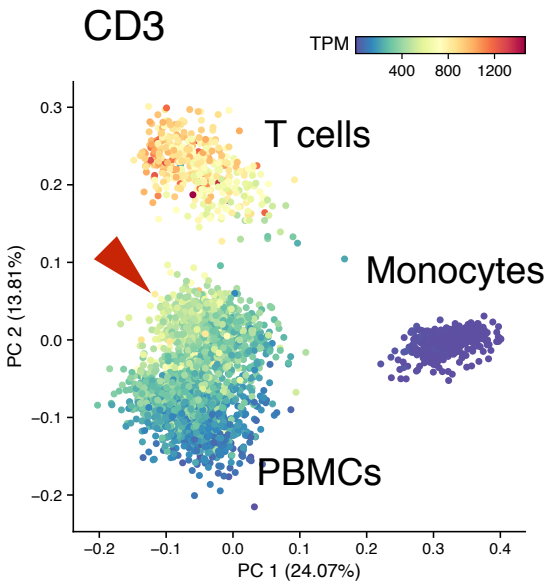


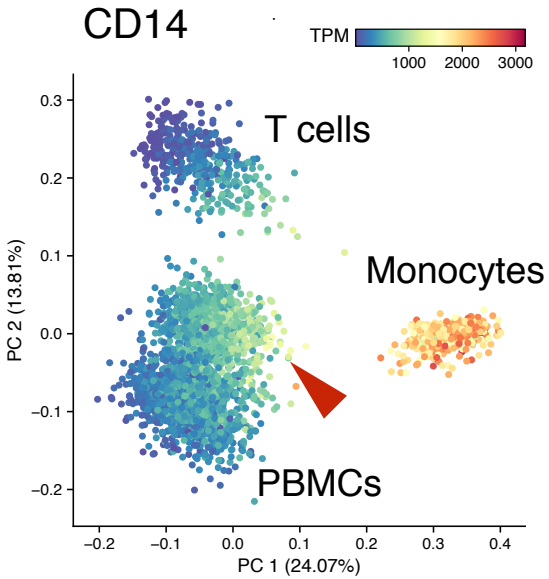


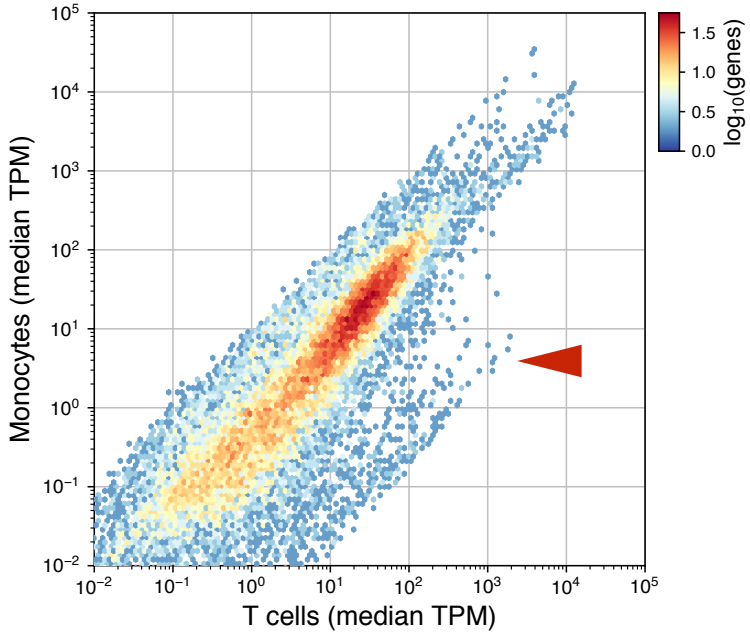


π_1: proportion of true positives

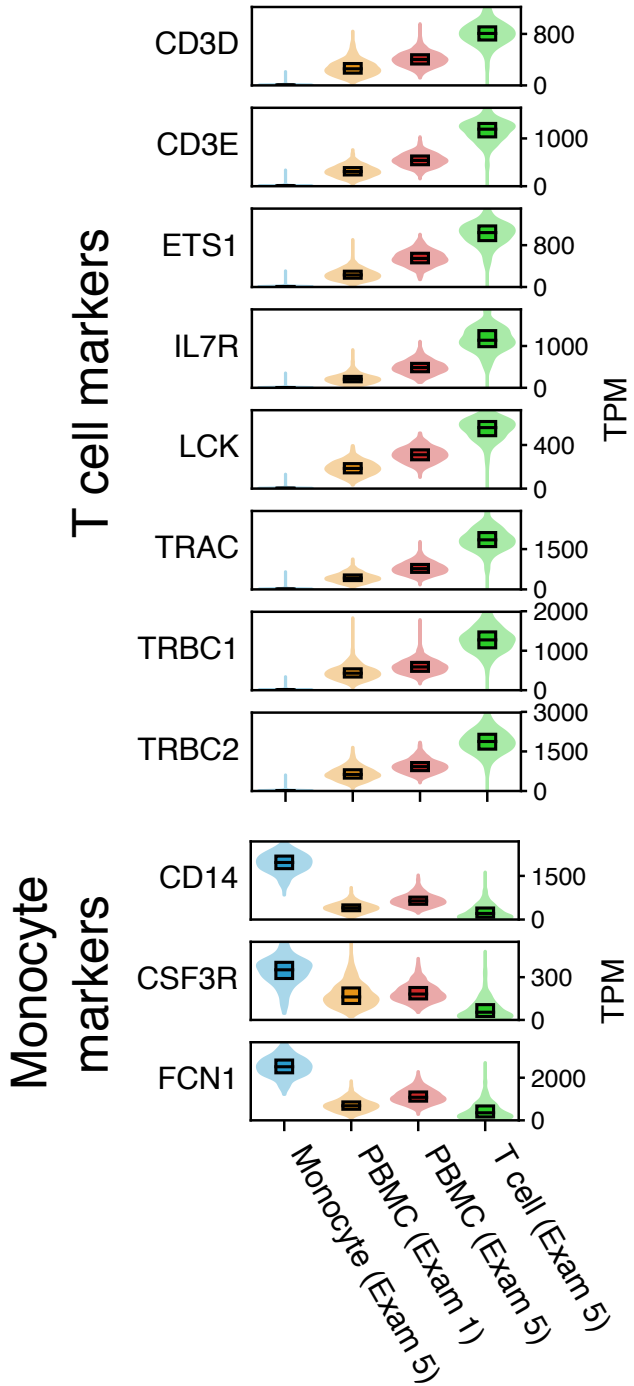
Towards identification of cell-type specific effects







Multiple highly specific (unique) markers for T cells; no unique markers for monocytes



Summary and outlook

- MESA RNA-seq data is of overall excellent quality, across exams and sample types
- Additional data being generated:
  - Splicing QTLs
  - Allele-specific expression
- Planned analyses:
  - Integration with methylation data (methylQTLs), other omics
  - Deconvolution approaches to detect cell type-specific effects in PBMCs and compare these between exams and omics data types
  - Identification of changes as a function of time and participant age (QTLs and expression)

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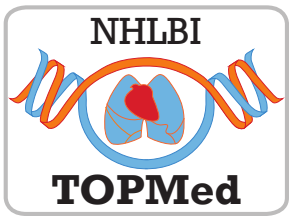

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