

DNA Methylation QC and Analyses—A Story of Invariance

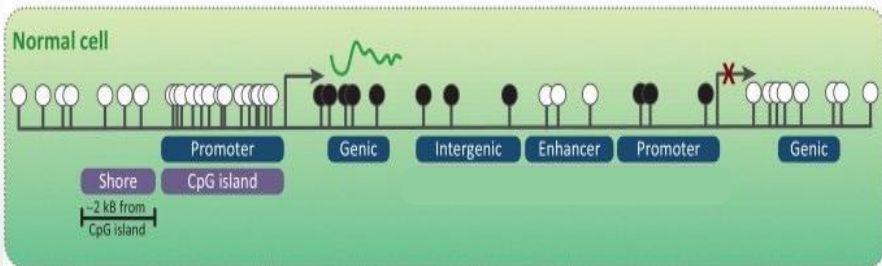
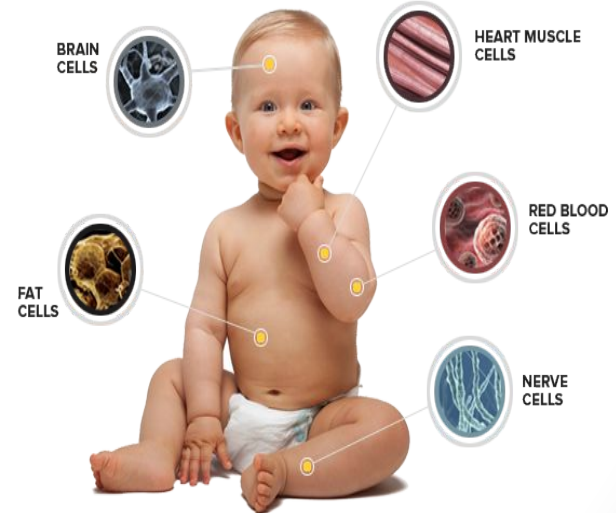
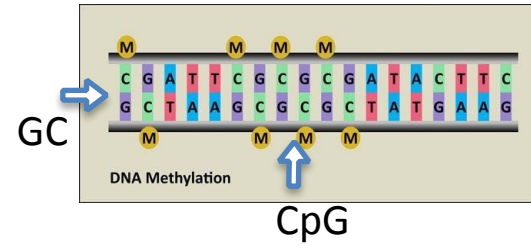
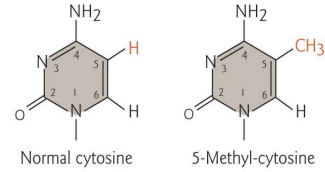
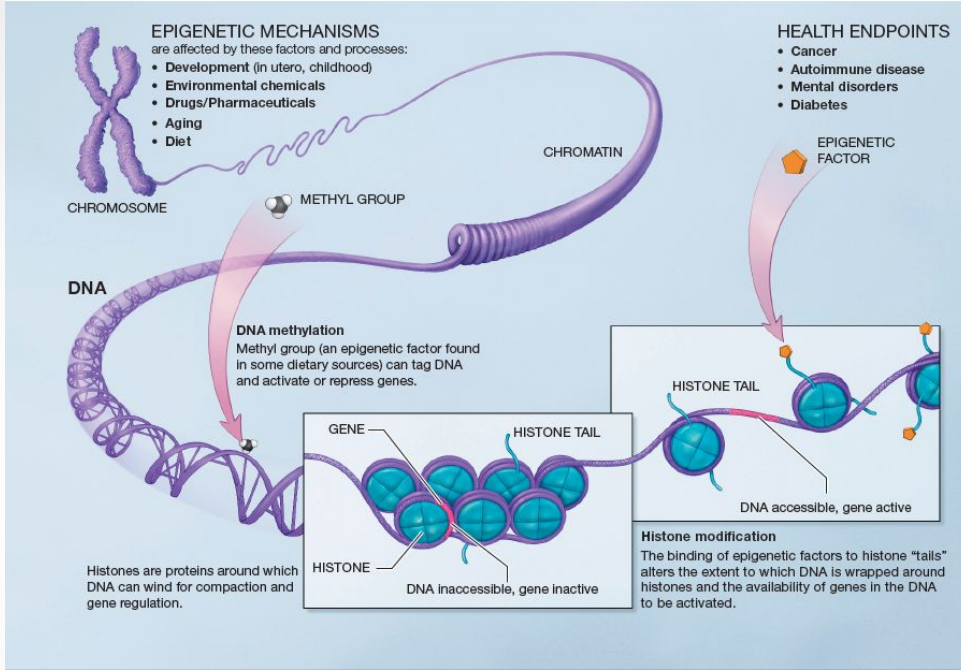
April 11, 2023

NIA Biomarker Network Meeting

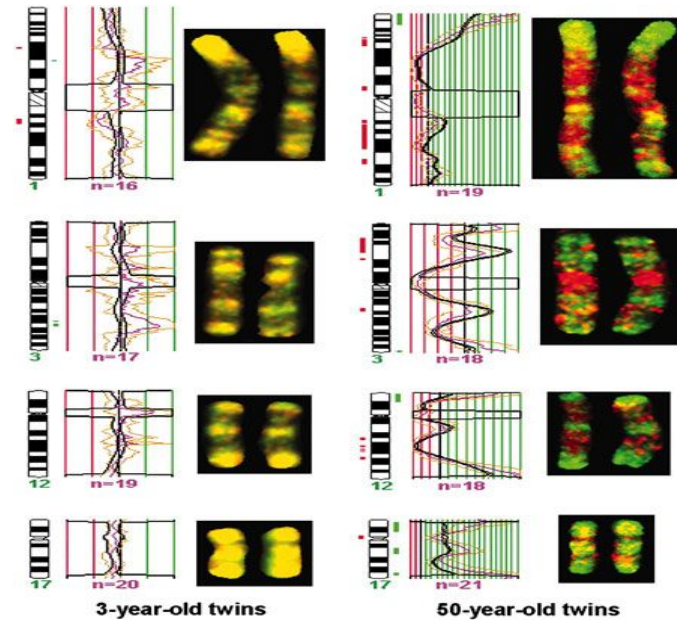
Colter Mitchell



DNA Methylation



Methylation of Changes with Exposure/Time

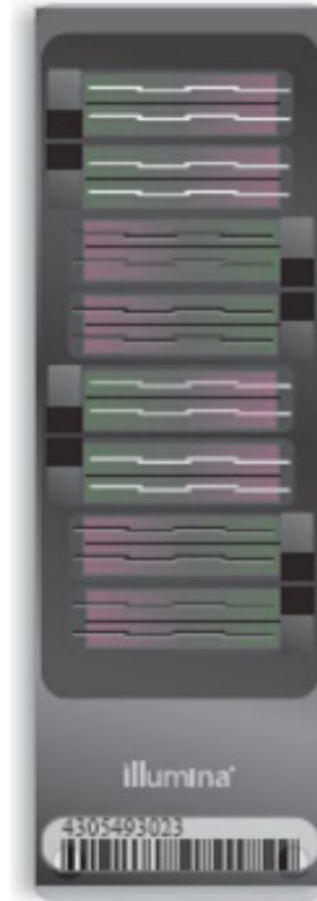


Yellow=same, Green & Red
=hyper or hypomethylated

450k



EPIC

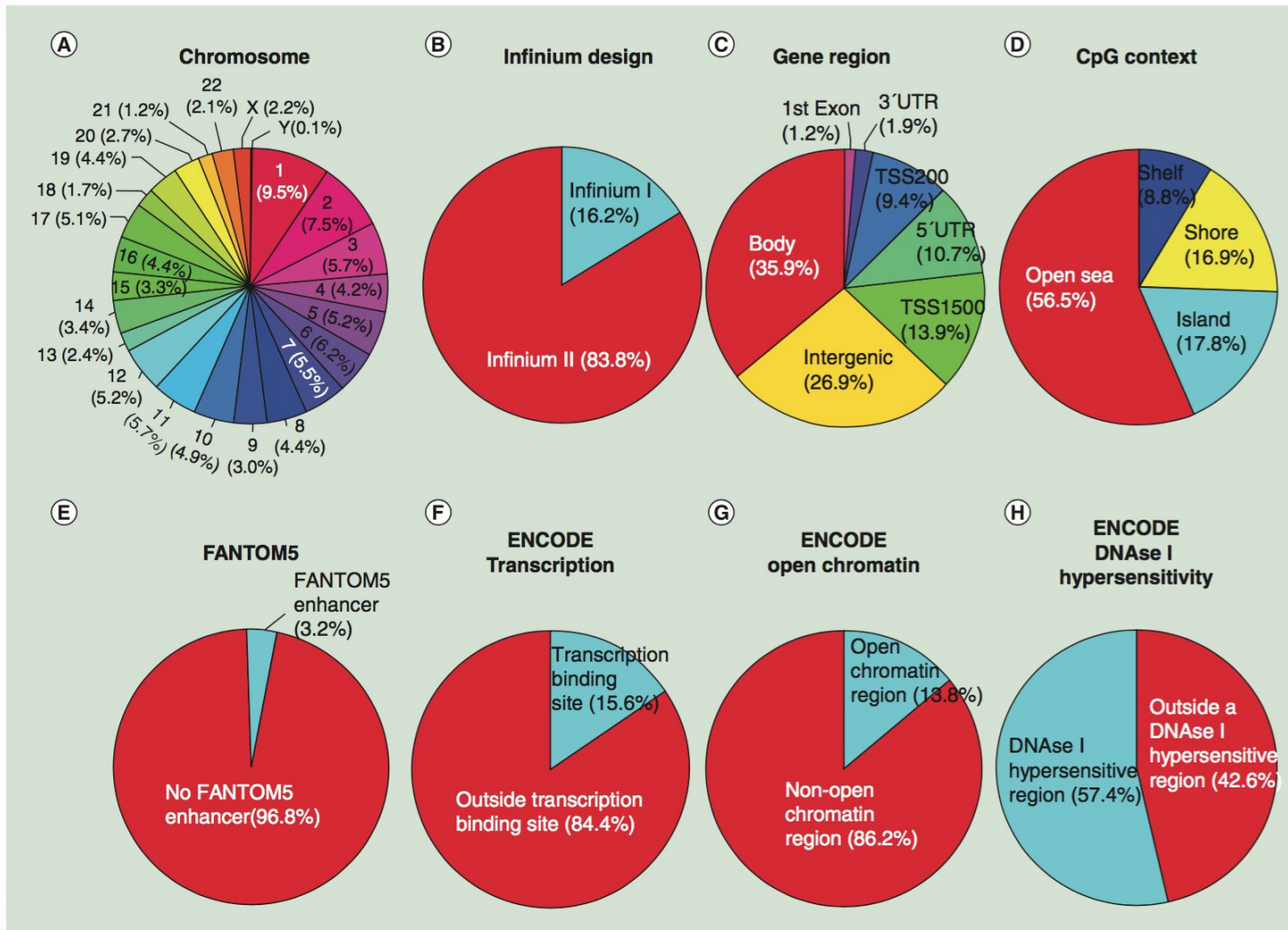


<http://www.illumina.com/content/dam/illumina-marketing/documents/products/datasheets/humanmethylationepic-data-sheet-1070-2015-008.pdf>

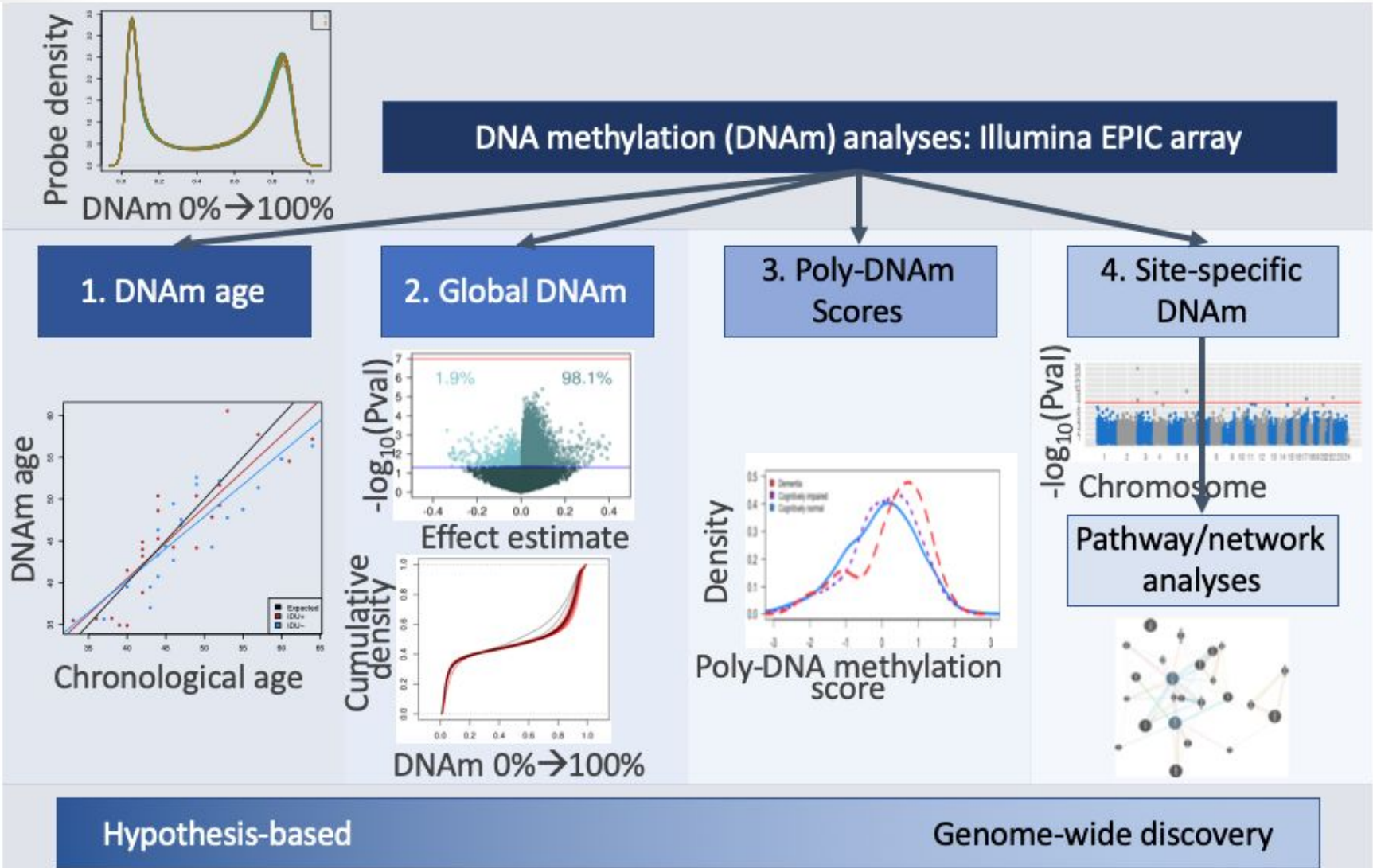
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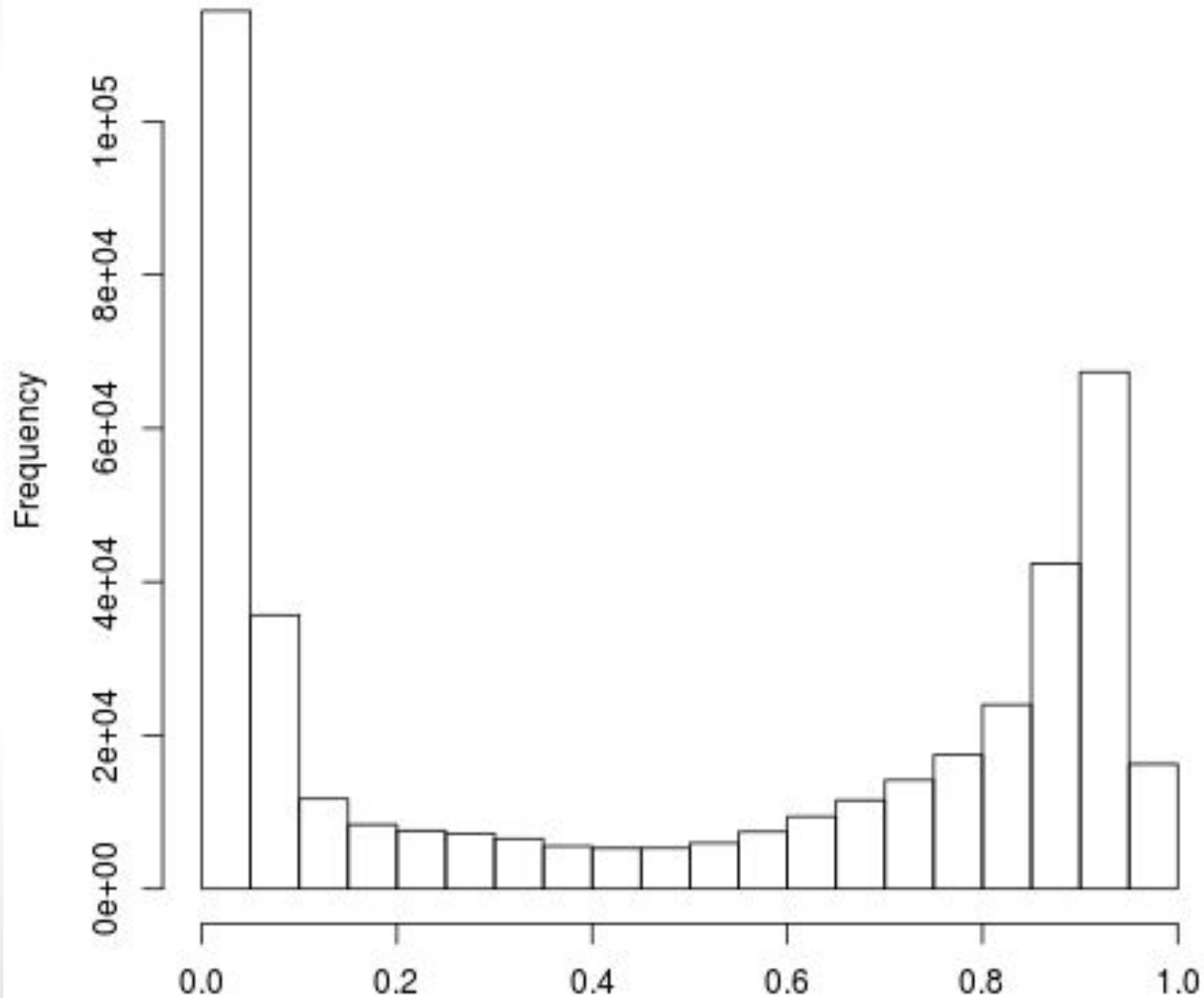
Genome coverage, EPIC



Epigenetic Methods

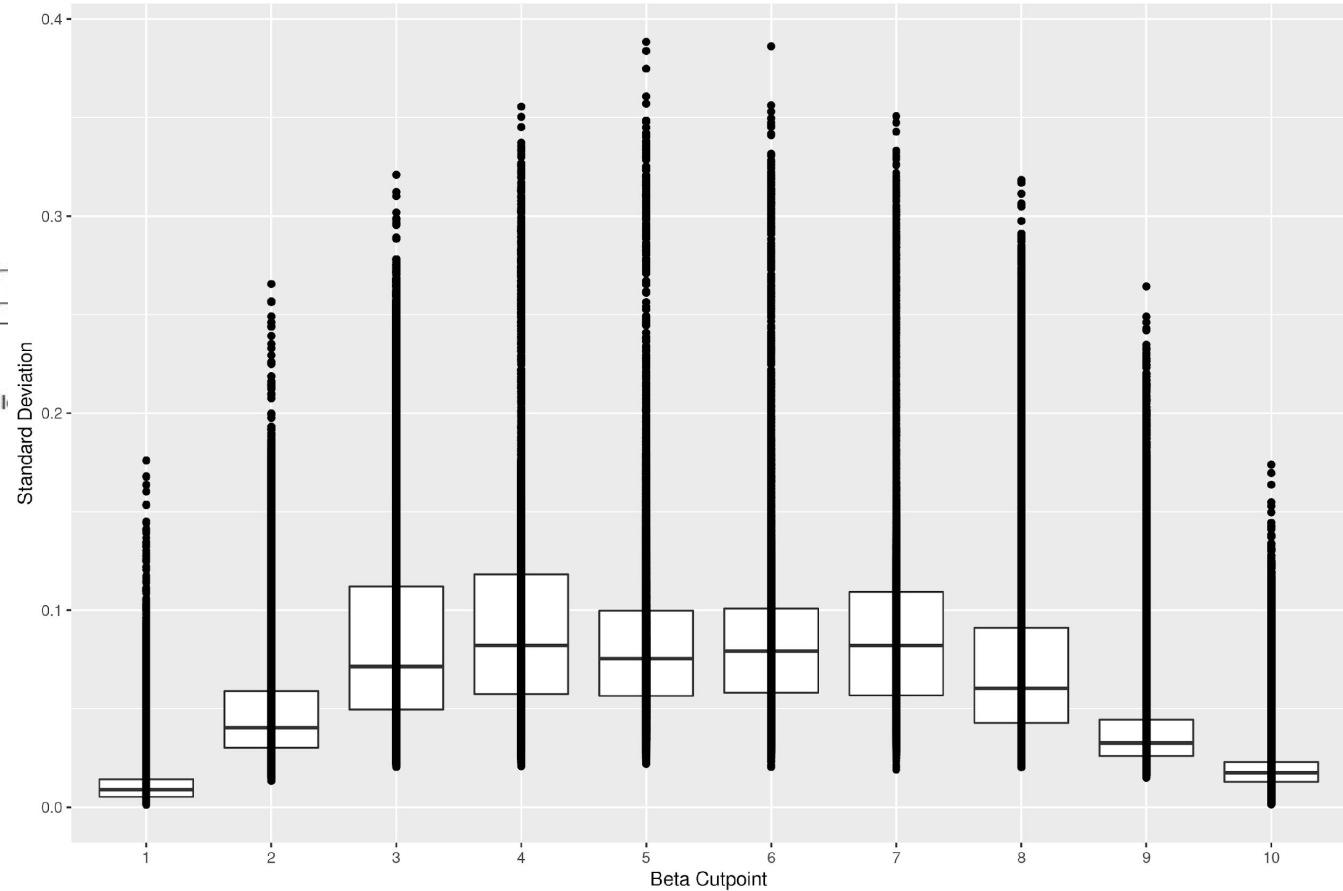
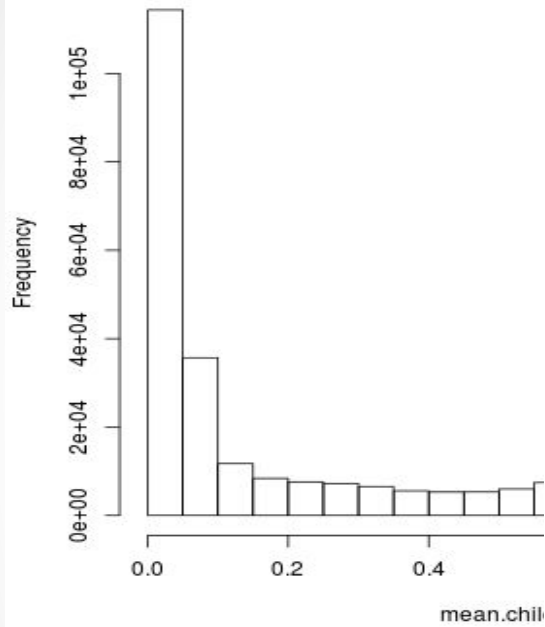


Number of Probes by Mean Methylation

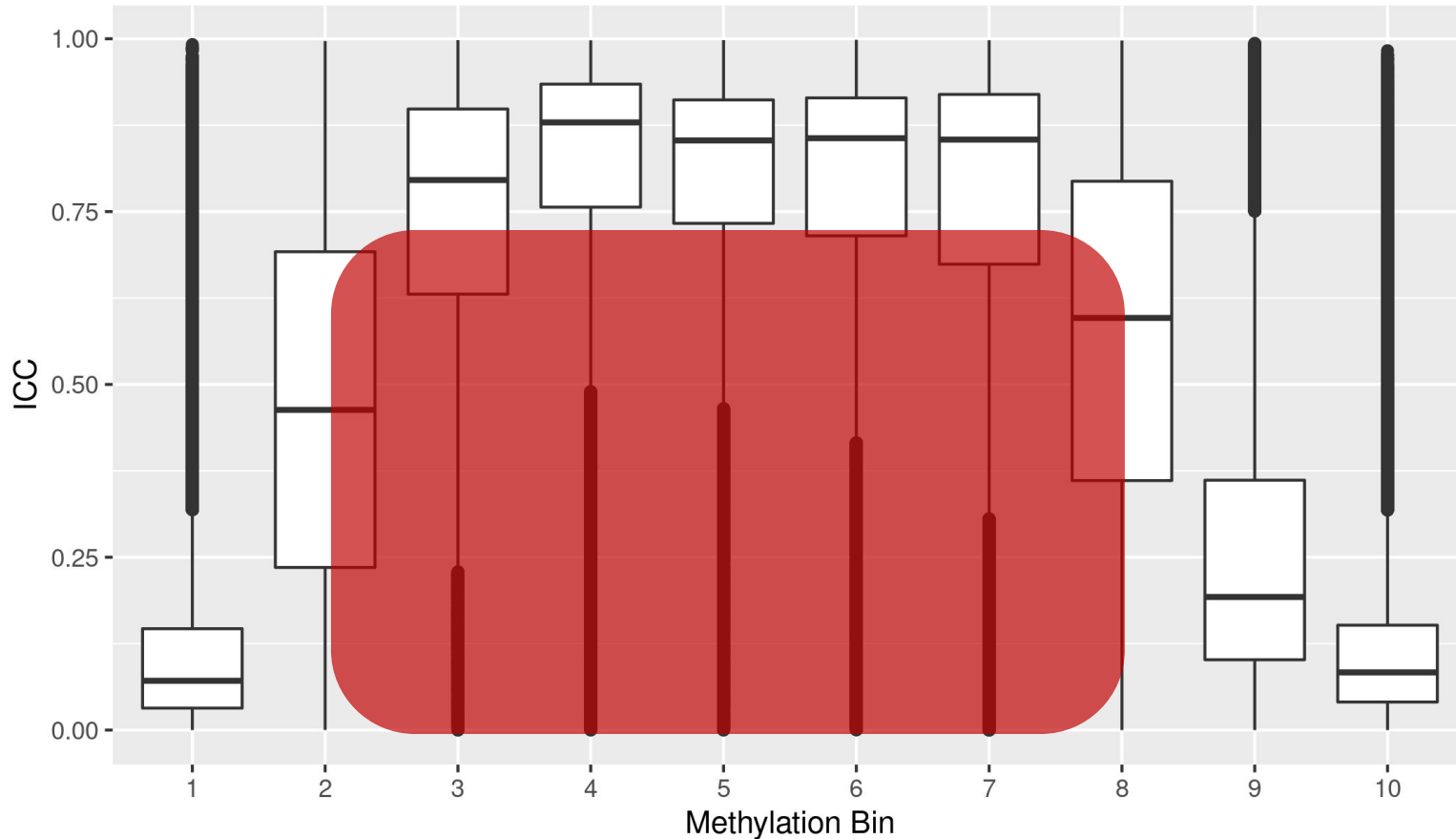


Distribution of probe variation

Decile frequency Y9



Reliability by probe mean

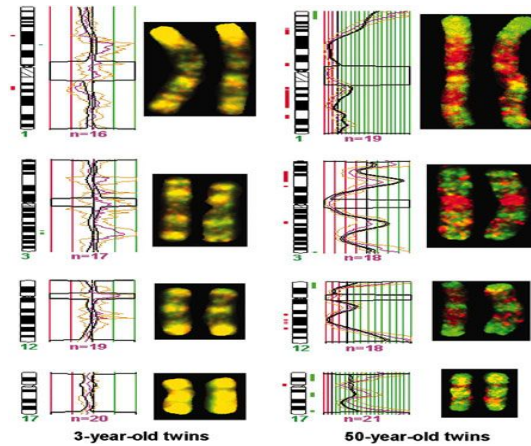
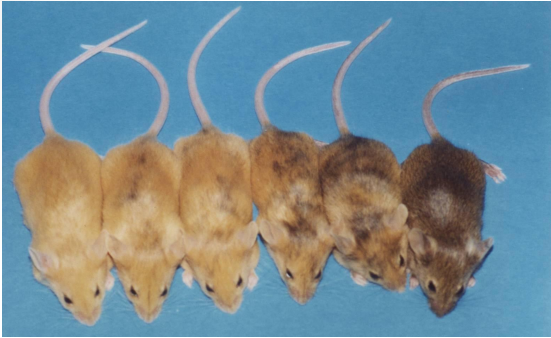


Invariant probes are highly consistent

10-30% of probes variant and low reliability

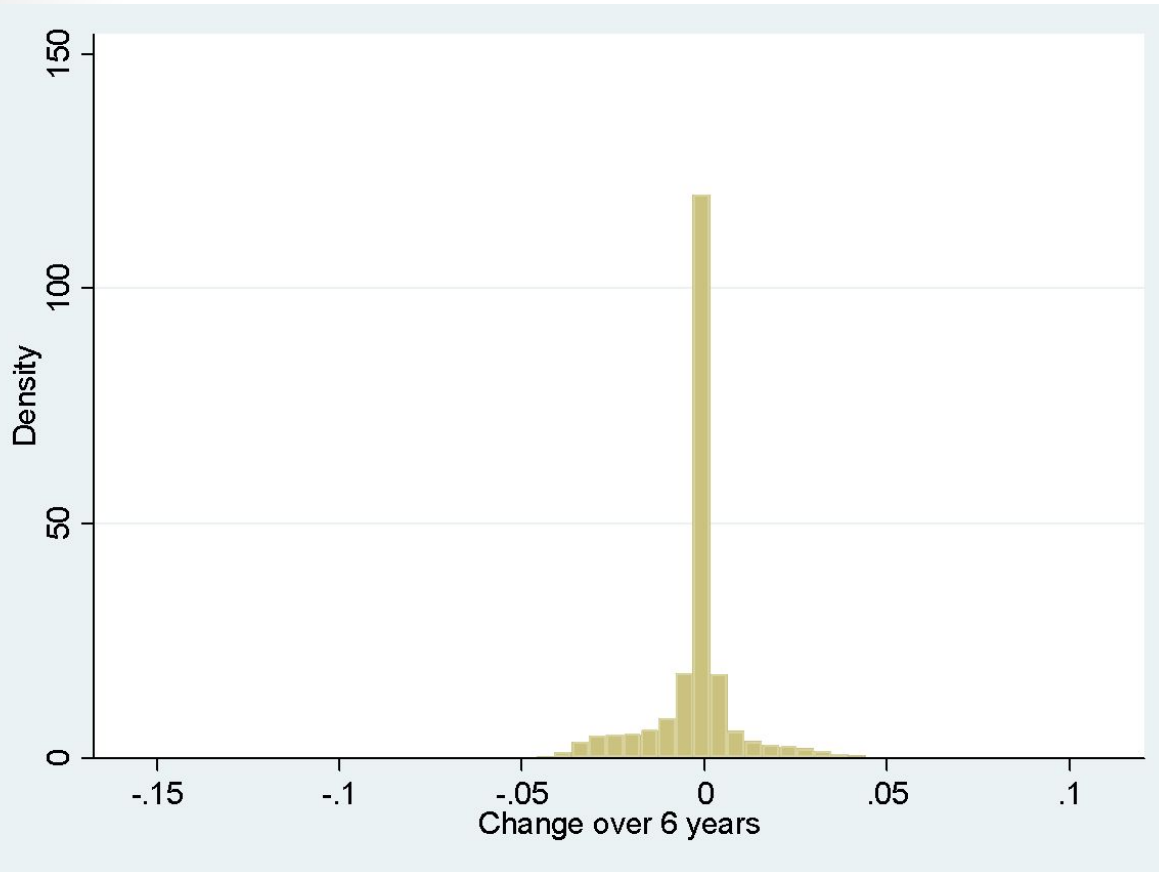
Low reliability probes rarely overlap

Methylation of Changes with Exposure/Time



- Future of Families and Child Wellbeing
- 2,020 children between at ages 9 and 15
- 450K and EPIC
- Plated together—**no longitudinal batch effect**

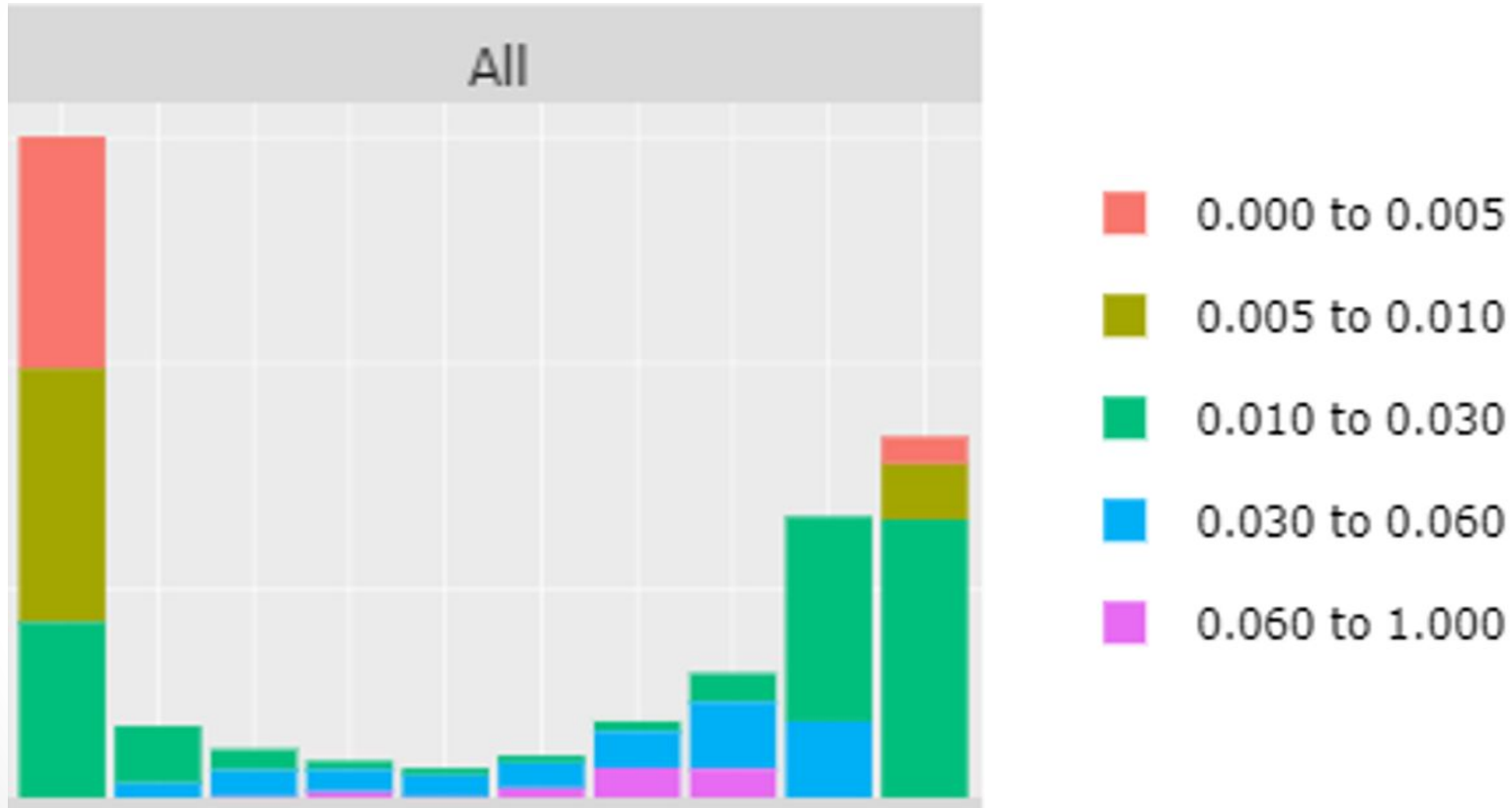
Change over time



Probe changes

- 2/3 < 0.006
- 78.2% < 0.01
- **21.8% > 0.01**
- **4.9% > 0.03**
- **0.2% > 0.05**

How is change distributed?



Epigenome Wide Longitudinal Change

Traits-matching probes

Aging-1,859

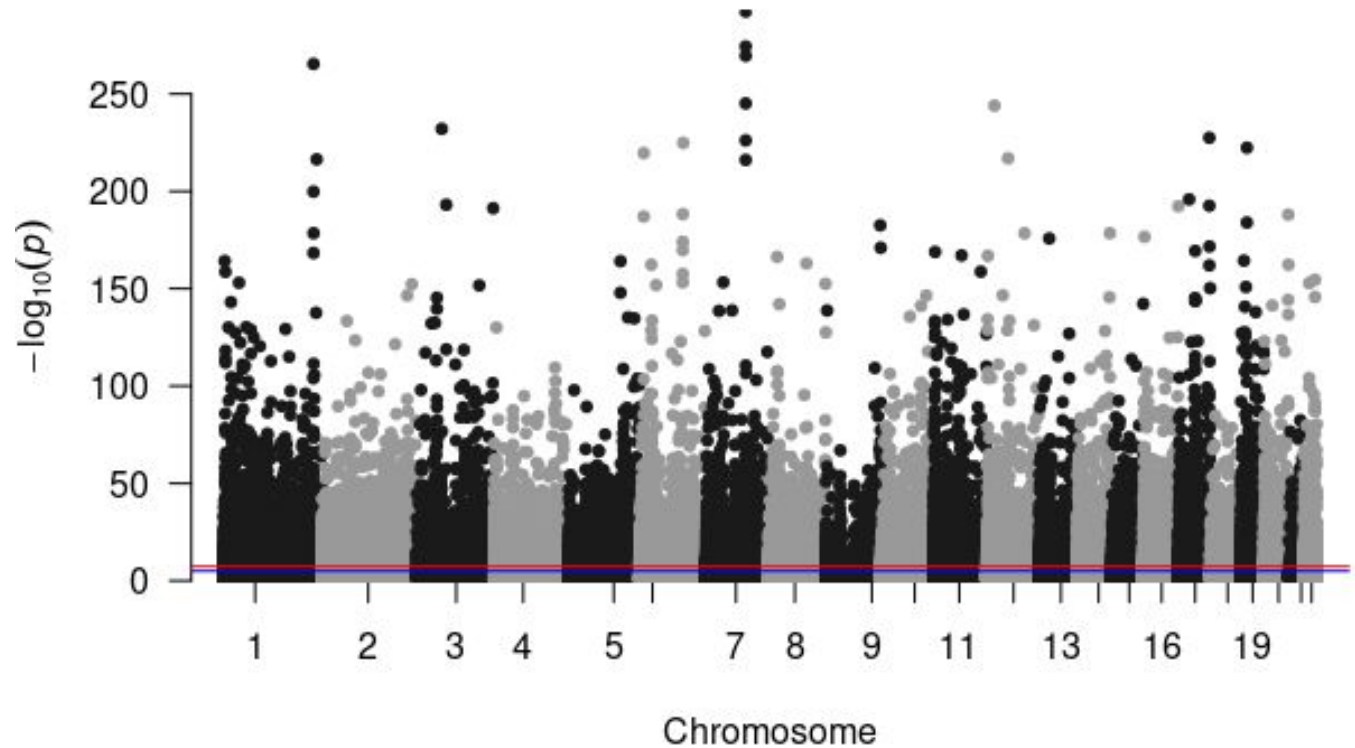
BMI-334

Puberty-209

Smoking-352

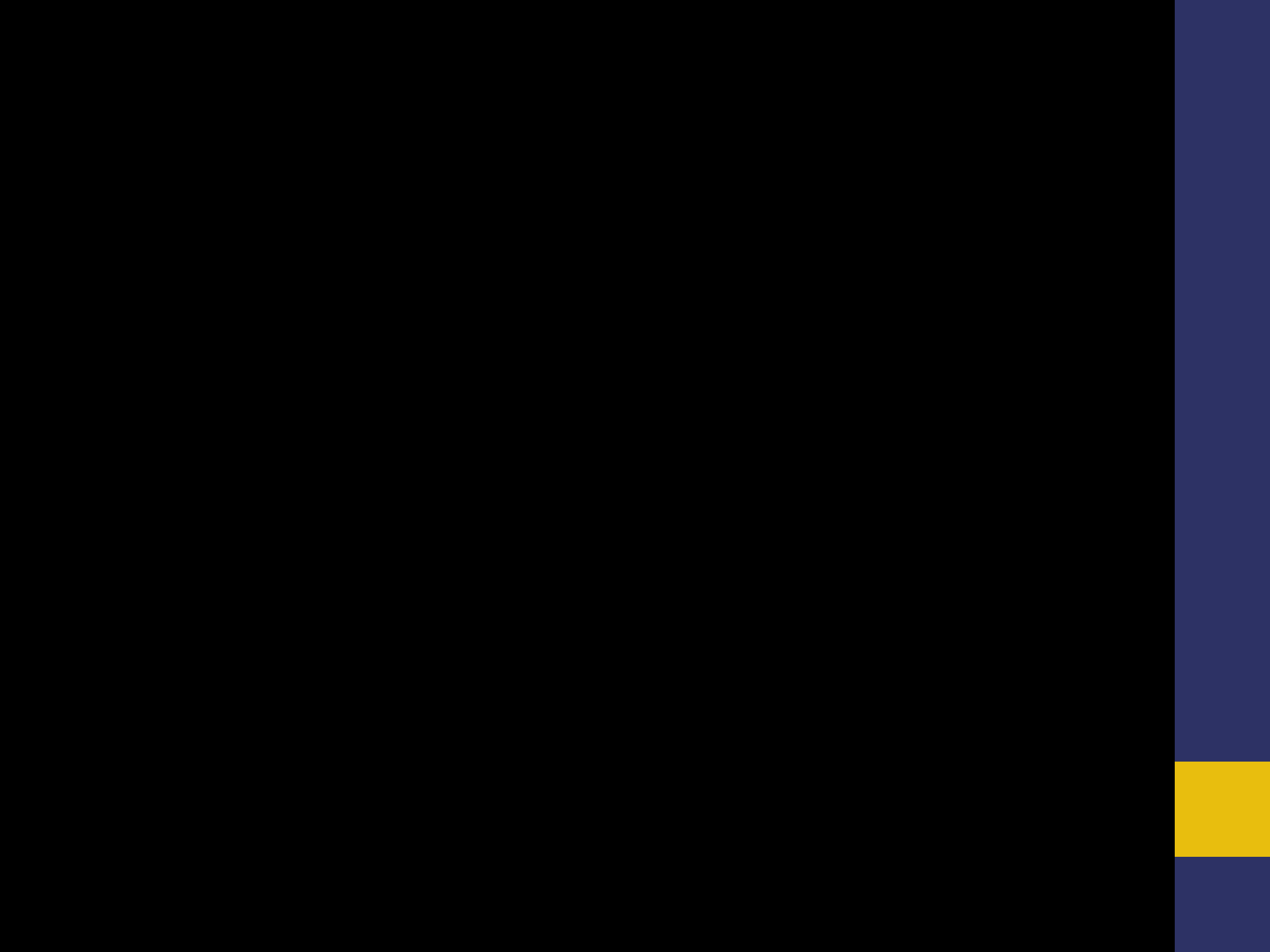
Preterm birth- 176

Asthma-152

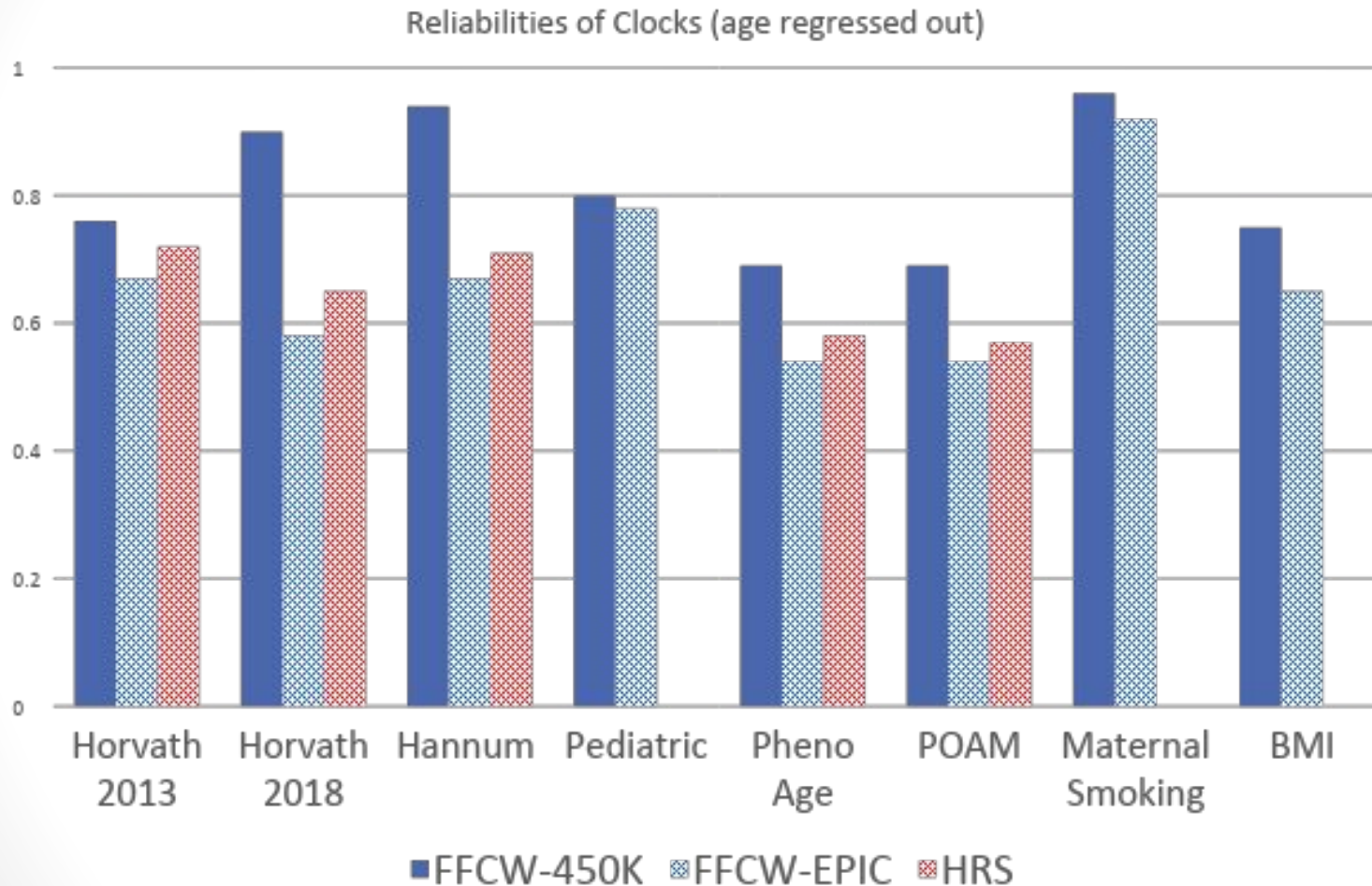


Discussion

- Most Illumina DNAm probes (450K, EPIC, EPIC 2) are invariant in human immune cells
- Studies need to include many duplicates to determine reliability of variant probes
- Include in a QC step
- These need to be reported more consistently
- Likely may not overlap with other studies
- Most probes are likely invariant over time
- Many probes may not change more than measurement error
- Less than 5% of probes change more than 3%
- Longitudinal Change probes may be a good avenue for future focus
- Dealing with longitudinal batch effects is a major focus this year



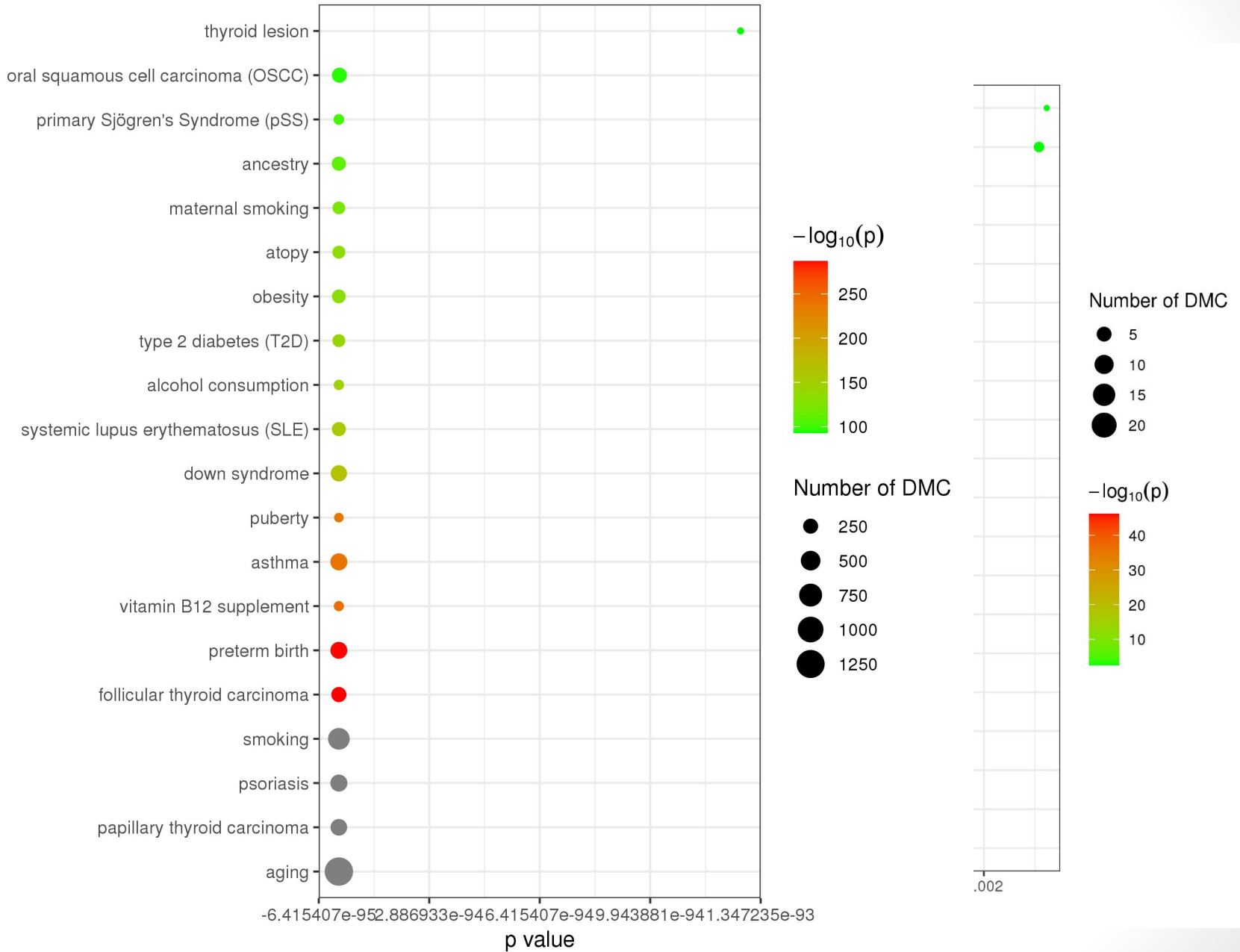
Reliabilities of Scores



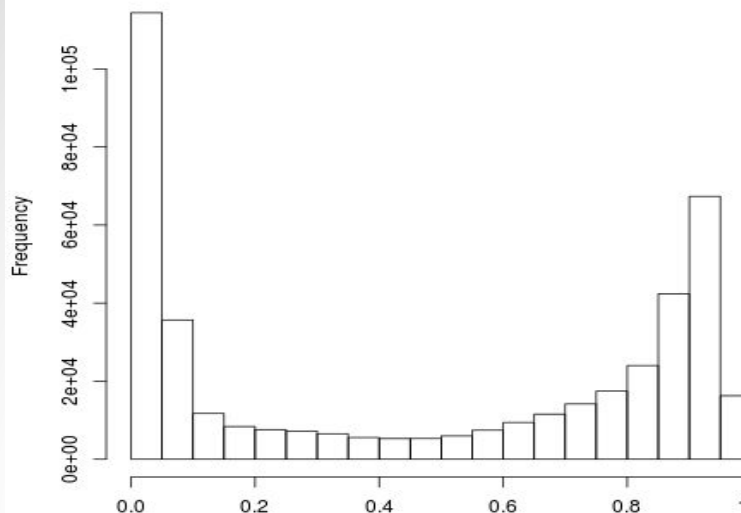
Duplicate Study DNA Methylation

- Fragile Families and Child Wellbeing
 - Current n=2,020 at age 9 and 15 using Illumina 450K (n=780) and EPIC (n=1,240)
 - Age 9 and age 15 plated together
 - Duplicates n=54 (450K) and n=164 (EPIC)
- HRS
 - Current n=4,018 all EPIC
 - Duplicates n=39

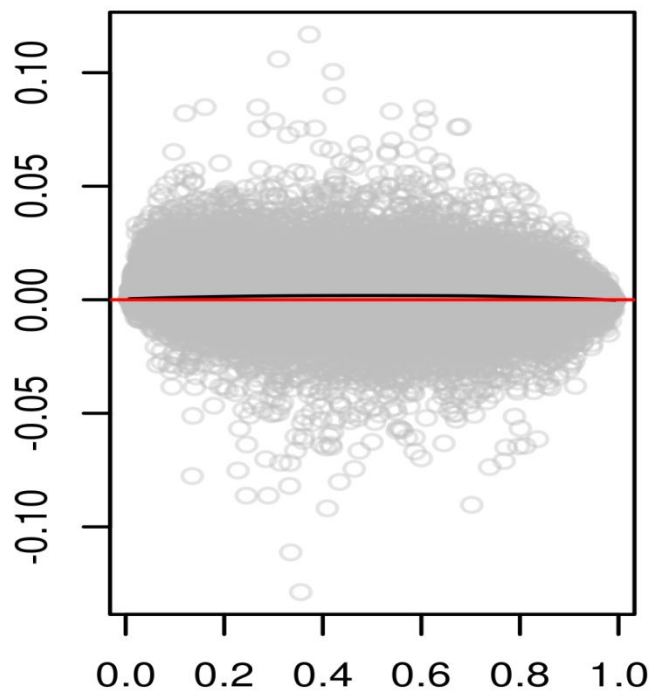
Trait enrichment



Decile frequency Y9



NHW Beta Change

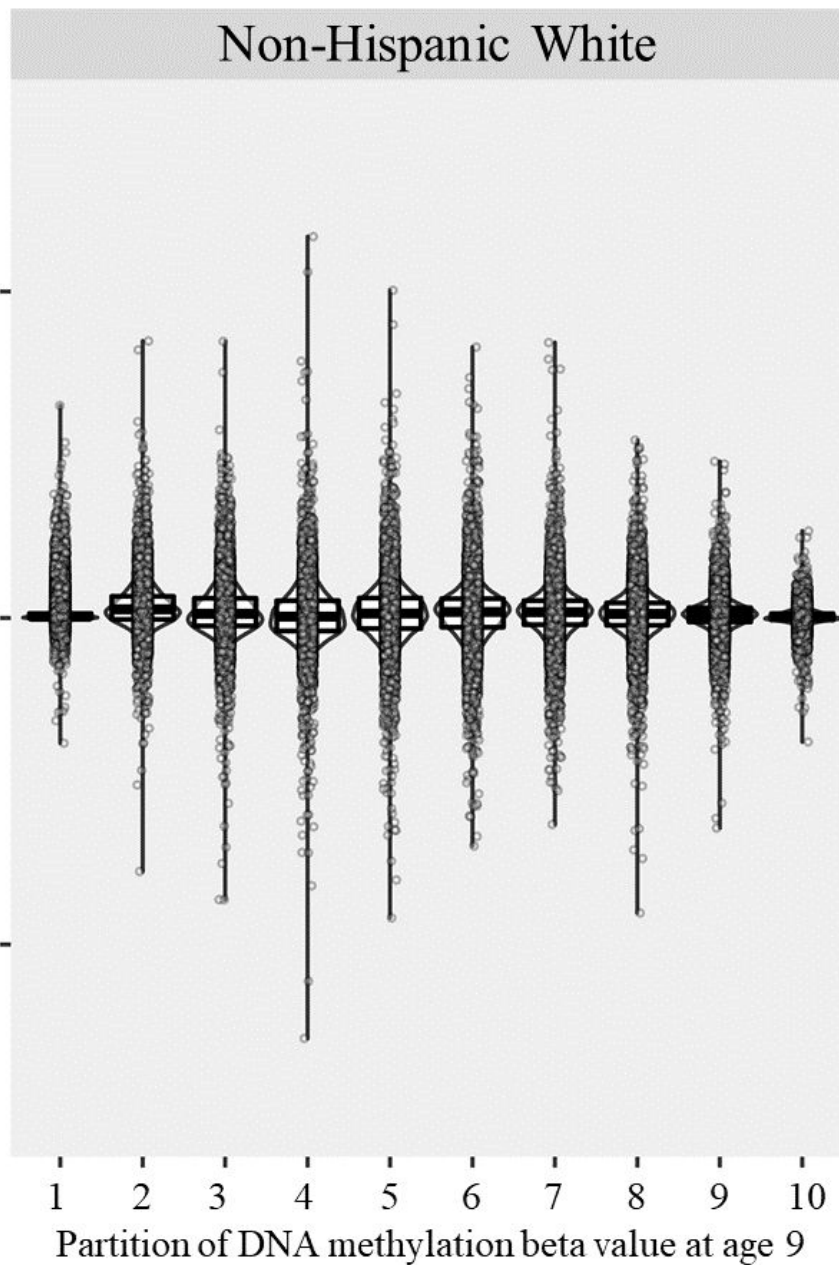


NHW Y9 Beta

Non-Hispanic White

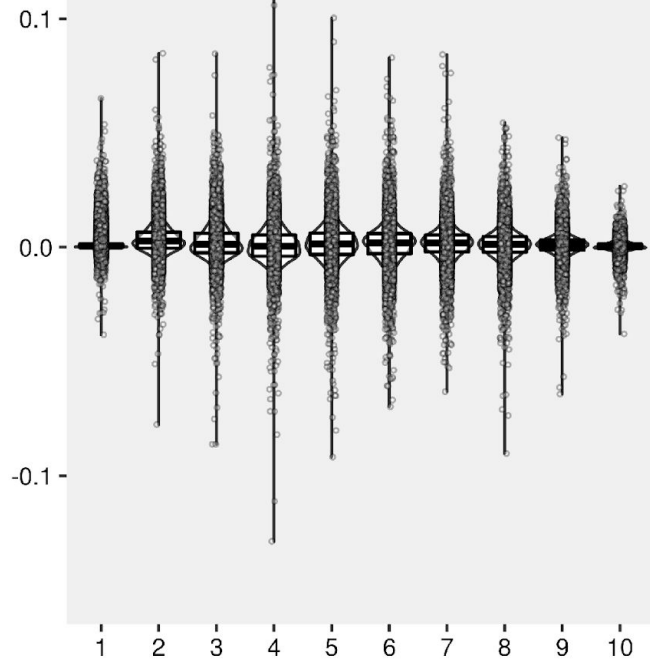
Change in DNA methylation beta value from age 9 to 15

0.1
0.0
-0.1

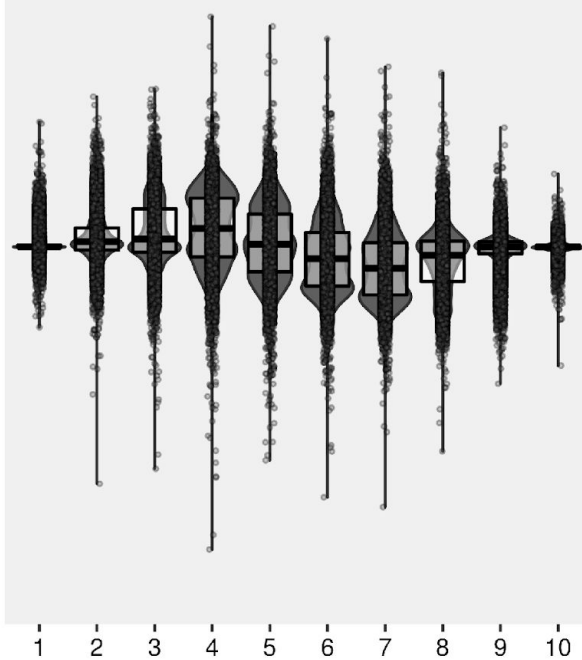


Partition of DNA methylation beta value at age 9

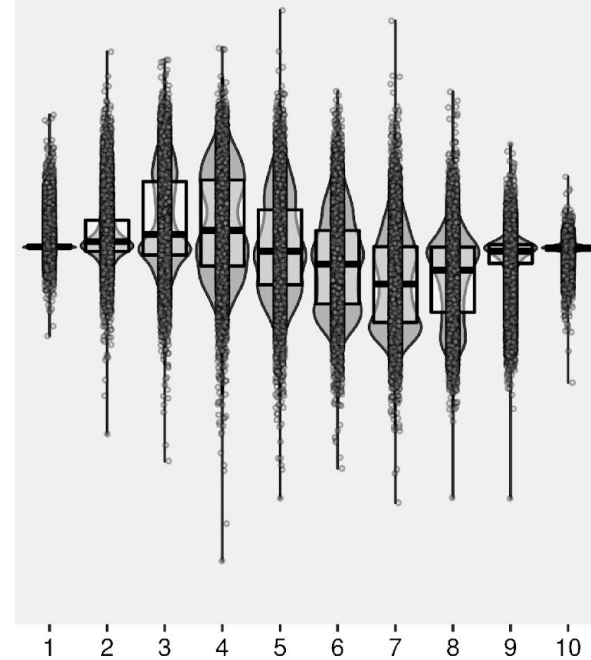
Non-Hispanic White



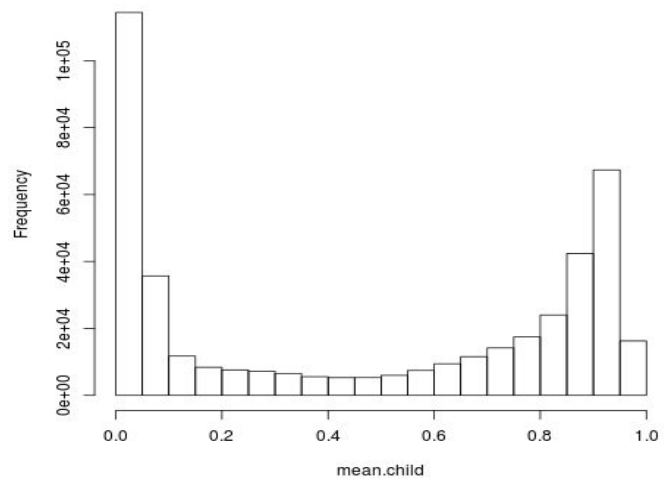
Non-Hispanic Black



Hispanic

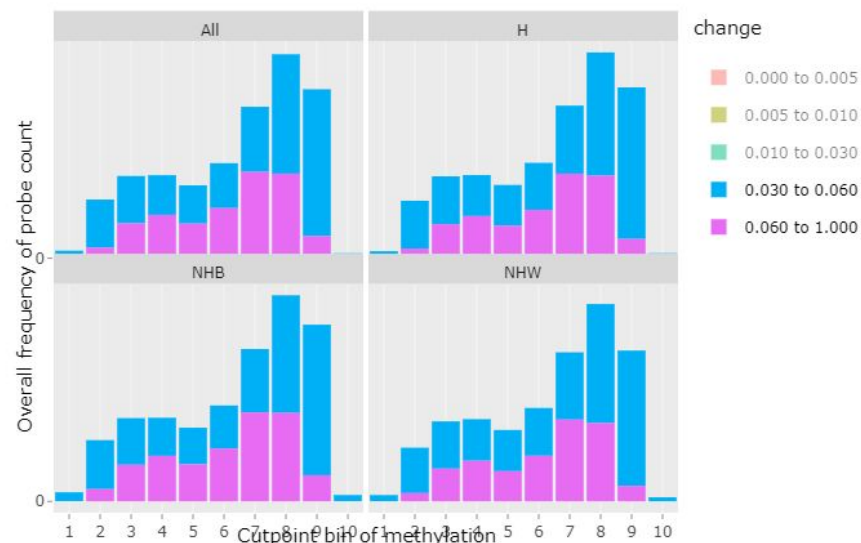


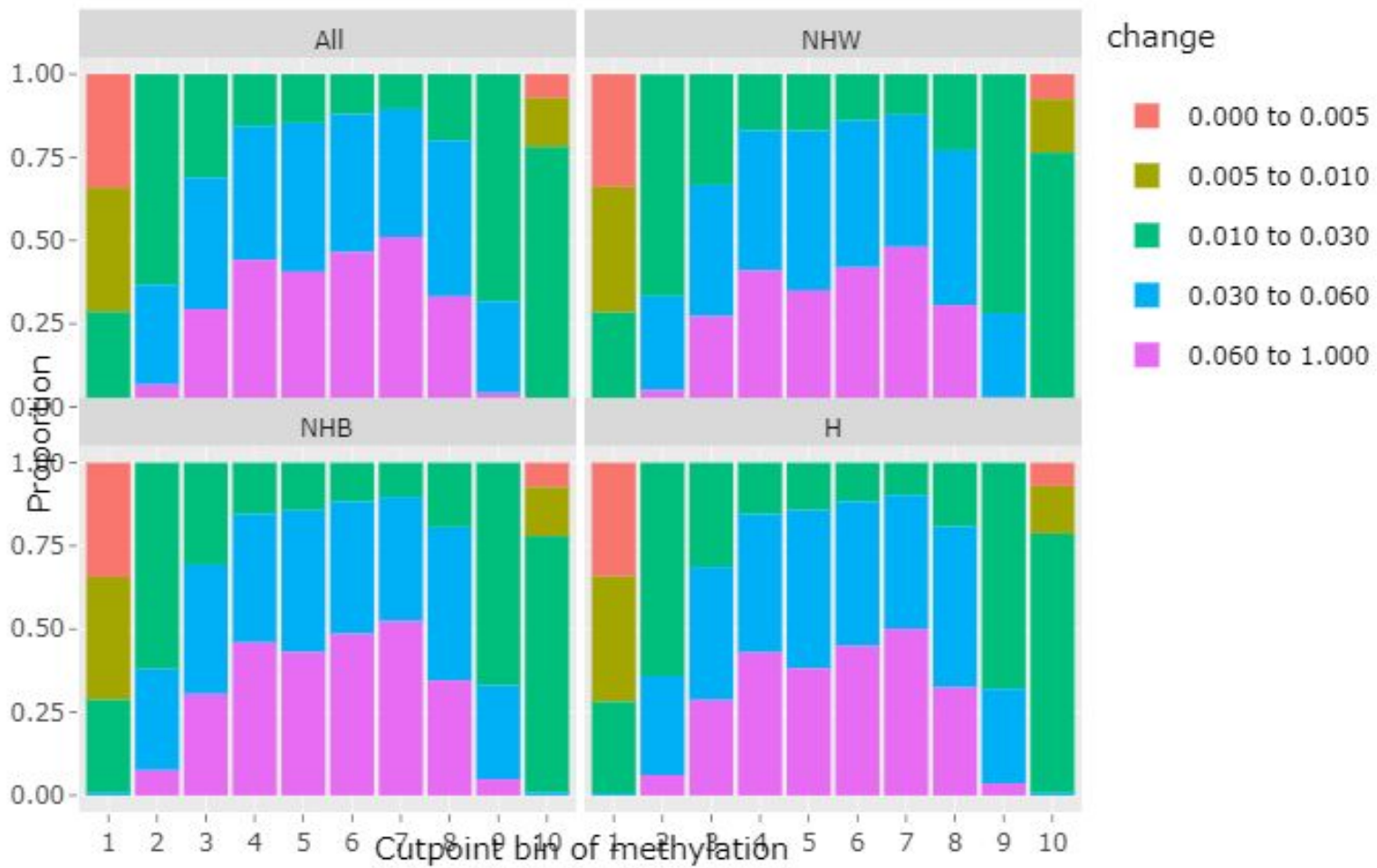
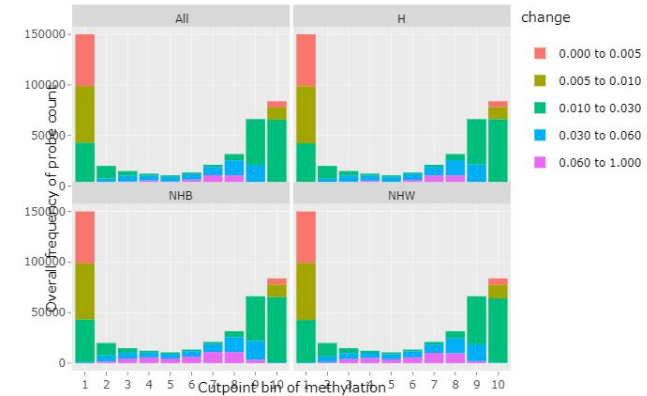
Decile frequency Y9



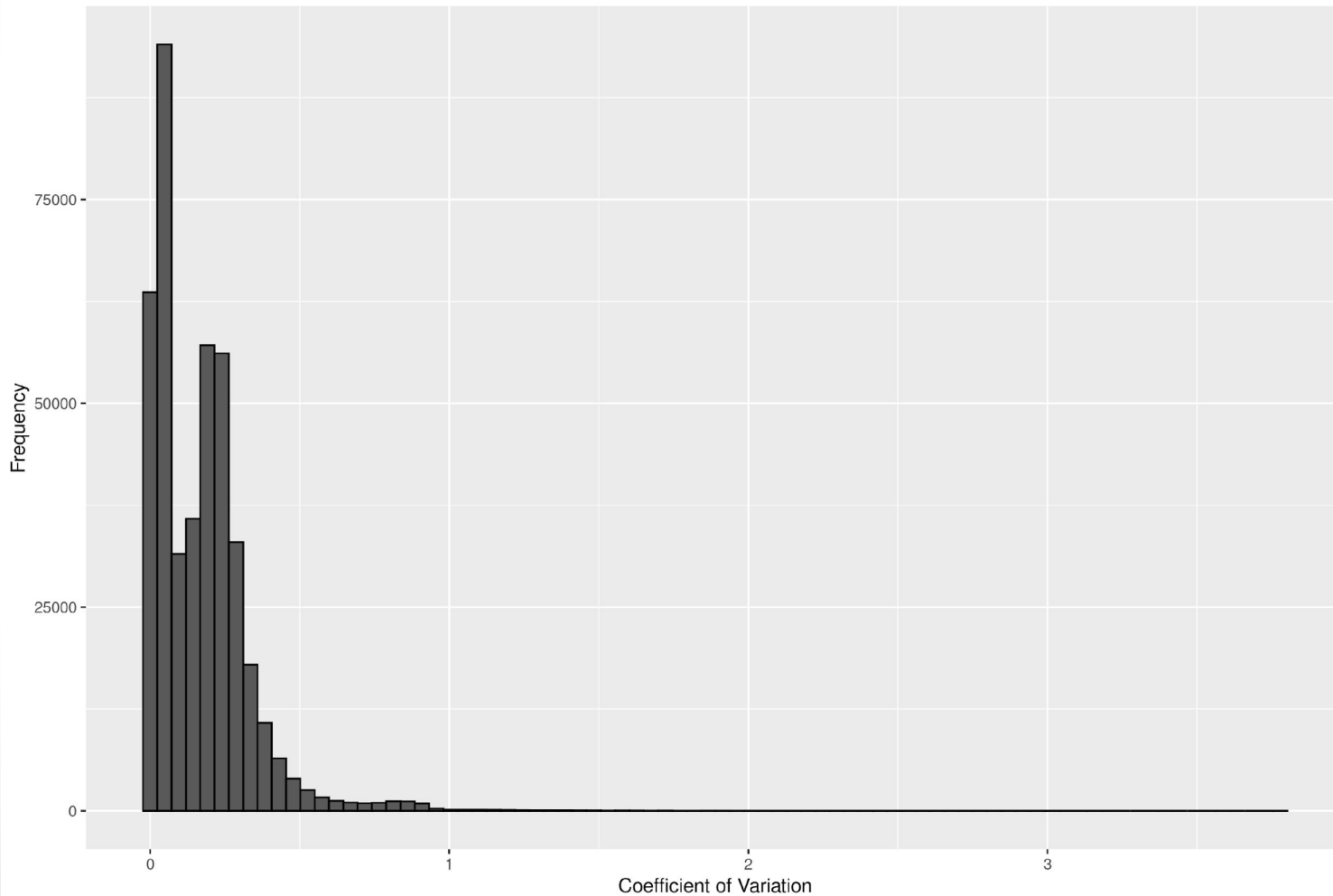
Duplicate Analyses

- 54 duplicates (same person at same age)-split by age
 - 29 Black, 13 White, 12 Hispanic
 - 29 Female, 25 male
 - 27 >INR of 1.5
- Reliability is higher for extremes-0.992 for all groups
- Reliability of middle is lower (average is 0.96):
 - 0.98 for Hispanic
 - 0.97 for white & female
 - 0.96 for black and male
- 25% of all probes change more than measurement error



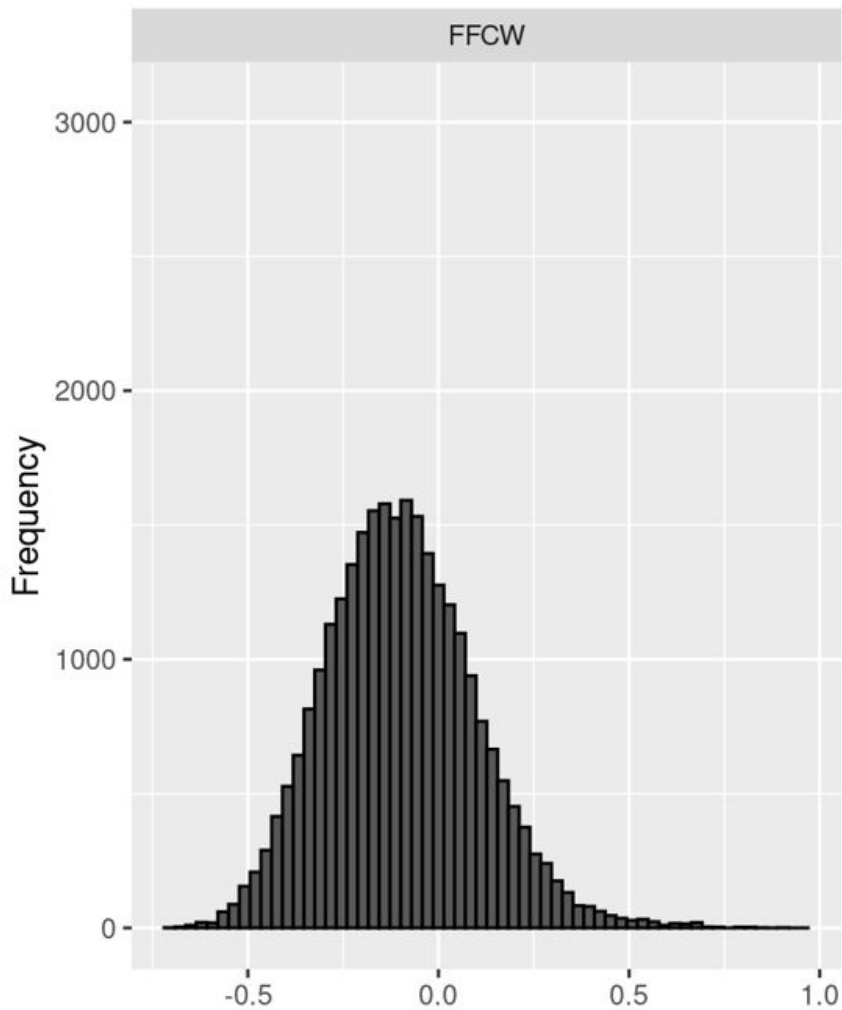


Probe Absolute CV (using M-values)

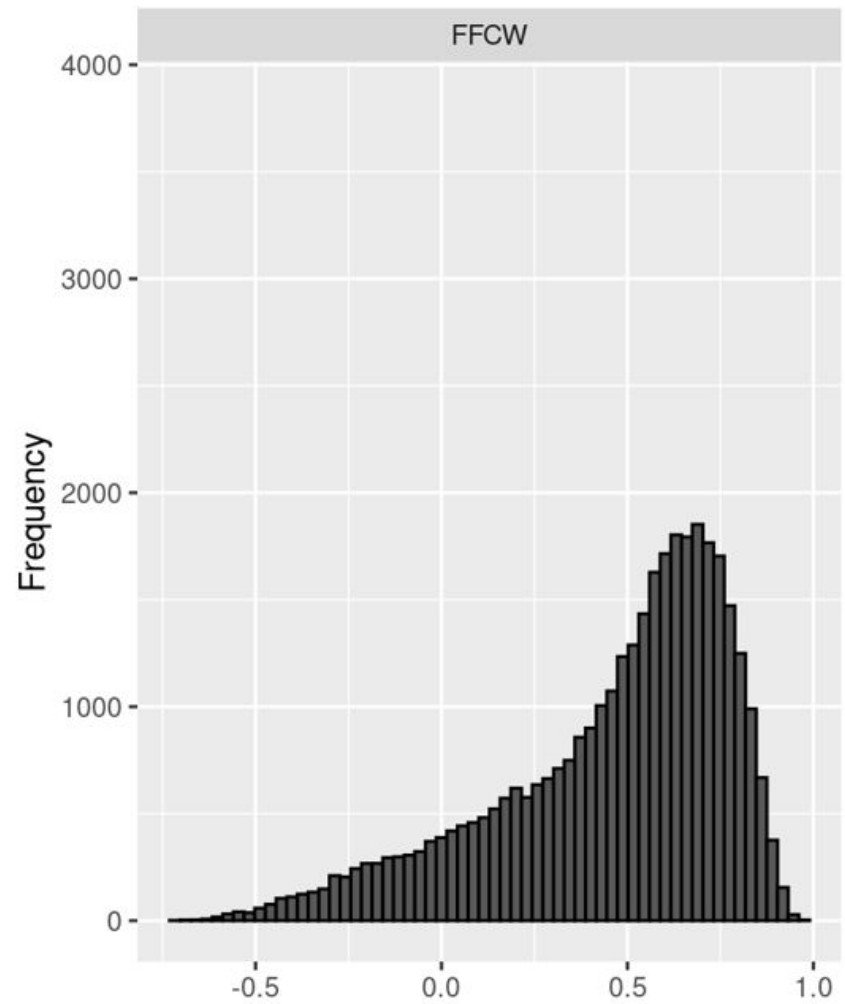


Reliability by CV

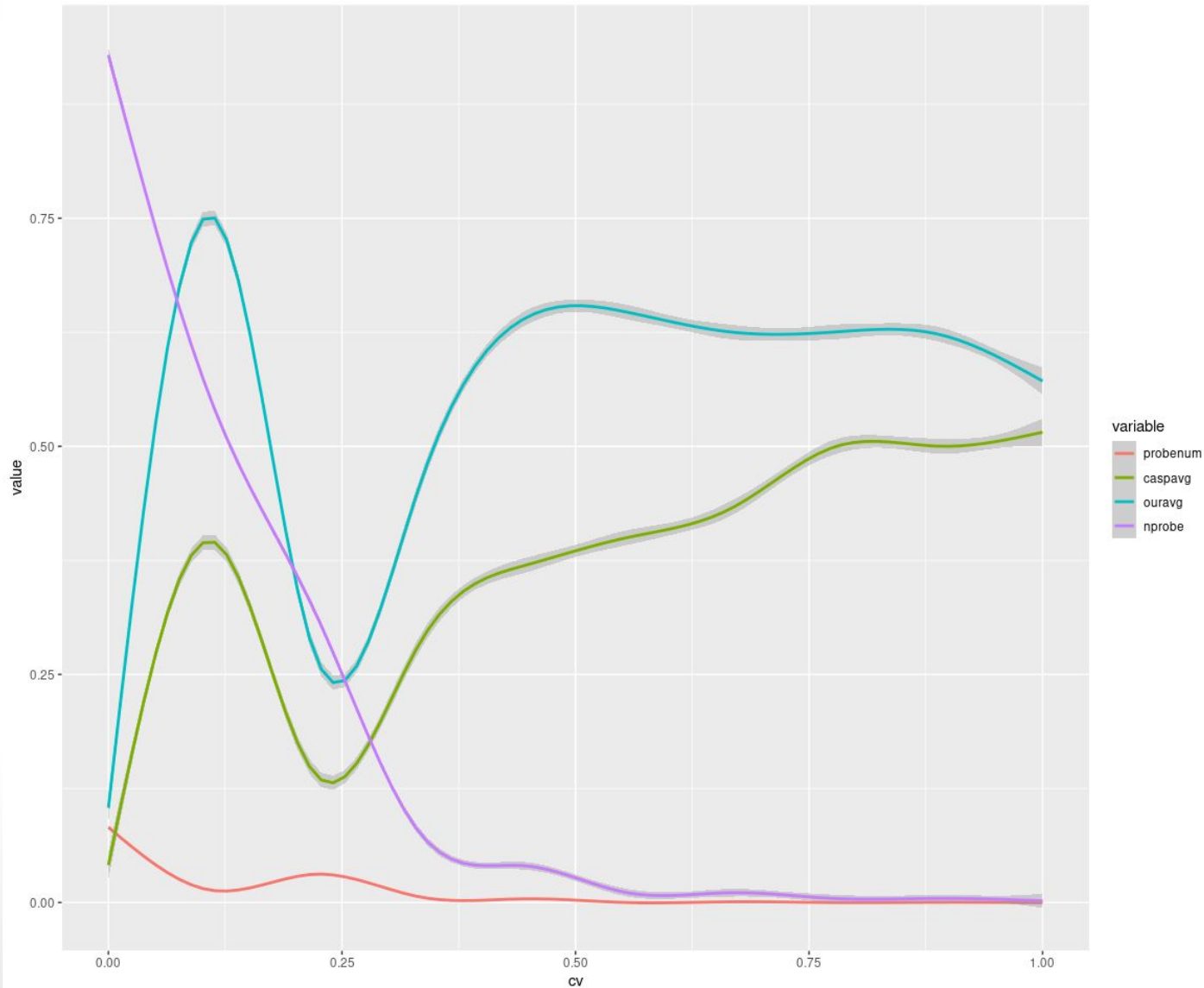
$0.000 < cv < 0.050$



$0.150 < cv < 0.200$

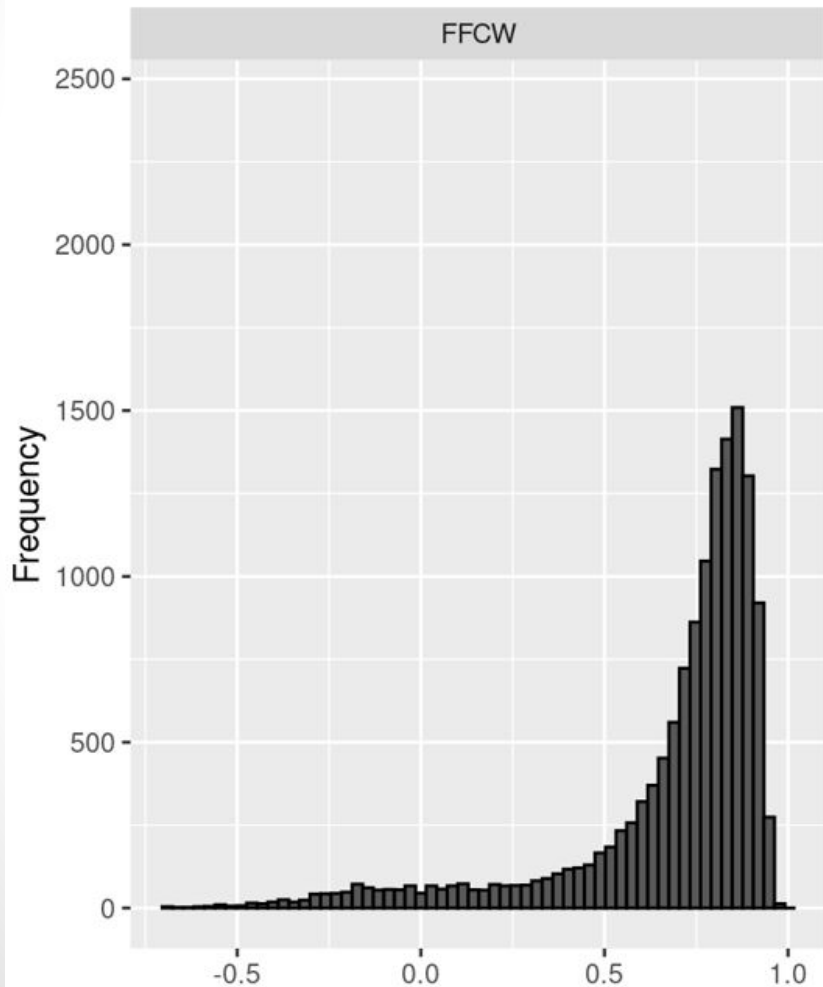


Reliability by CV

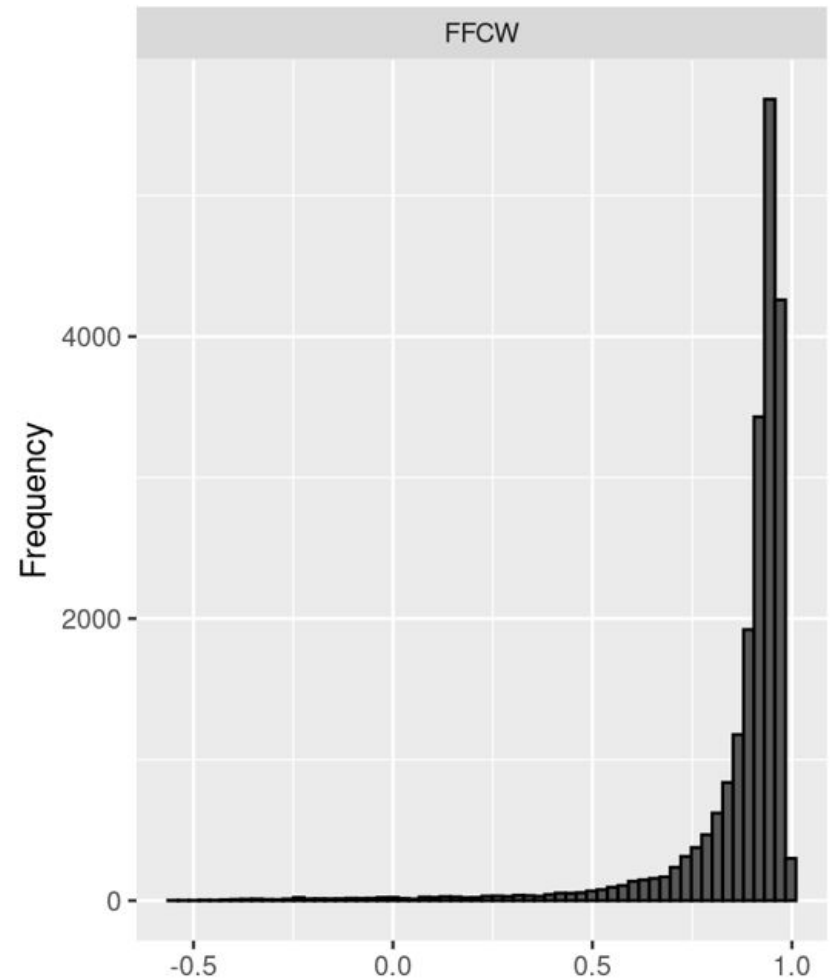


Reliability by CV

$0.250 < cv < 0.300$

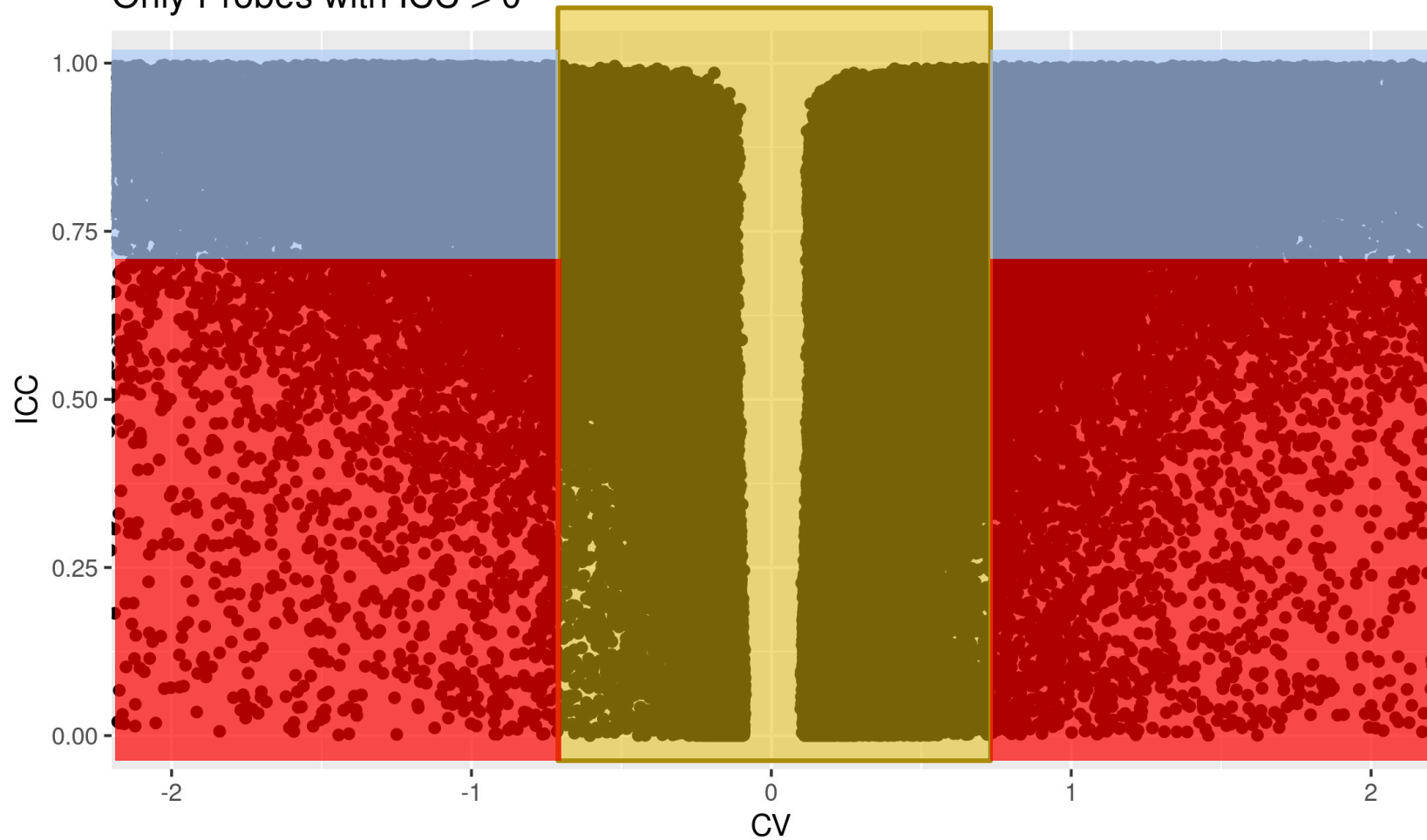


$0.500 < cv < 0.750$



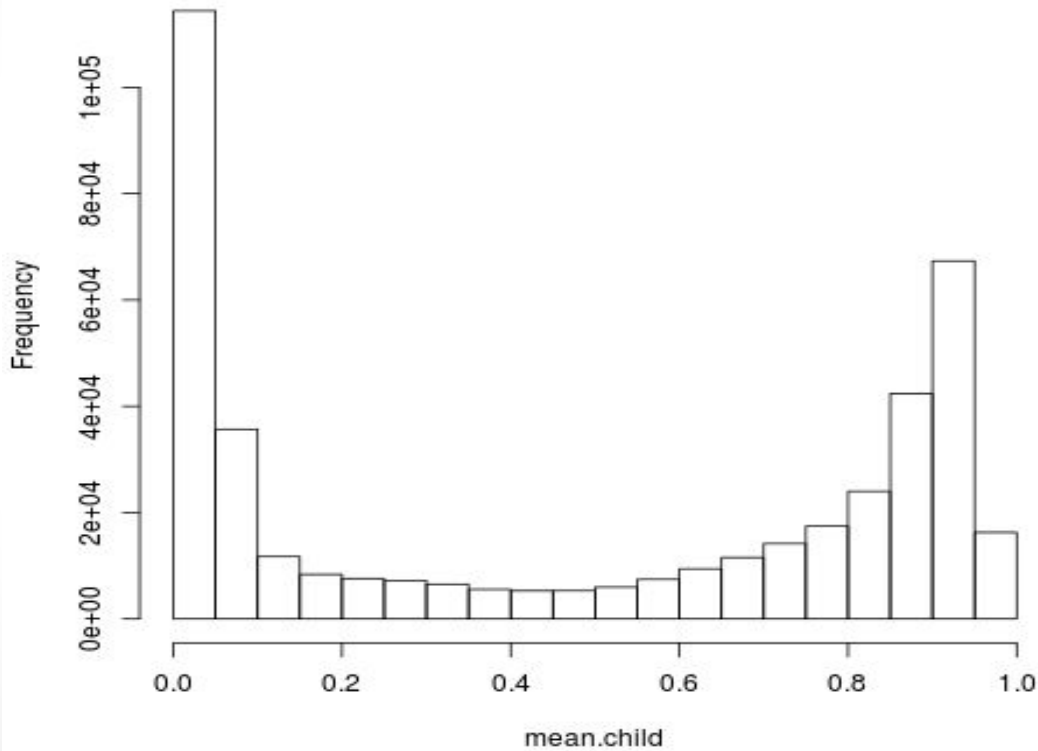
Reliability CV

Only Probes with ICC > 0

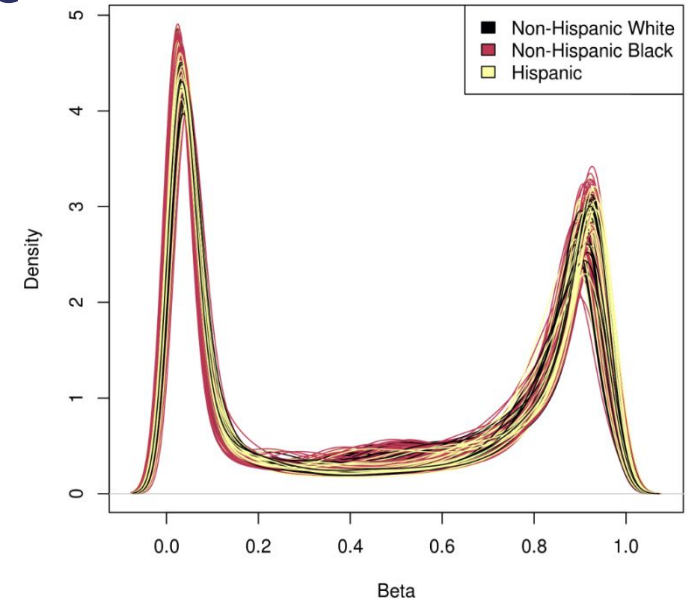


FFCW Methylation Data

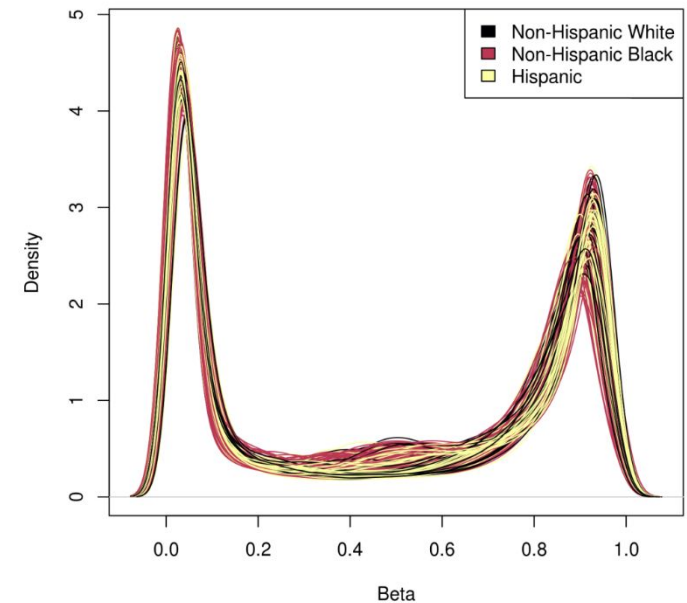
Decile frequency Y9



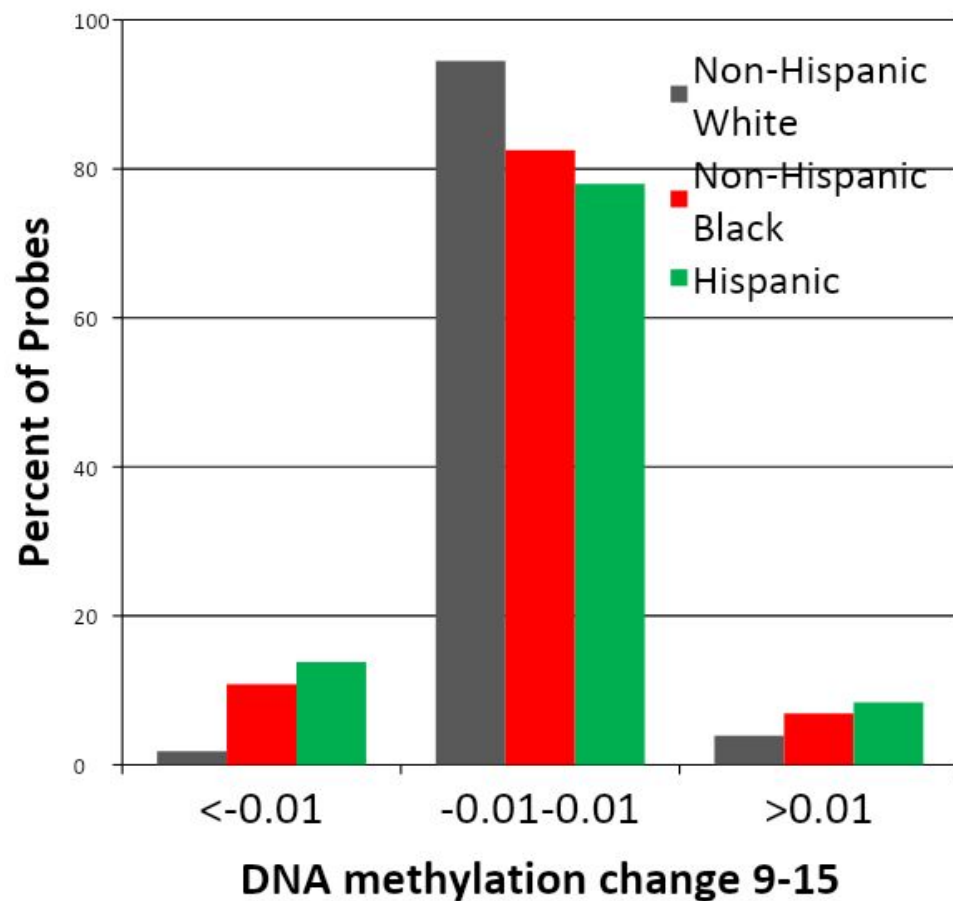
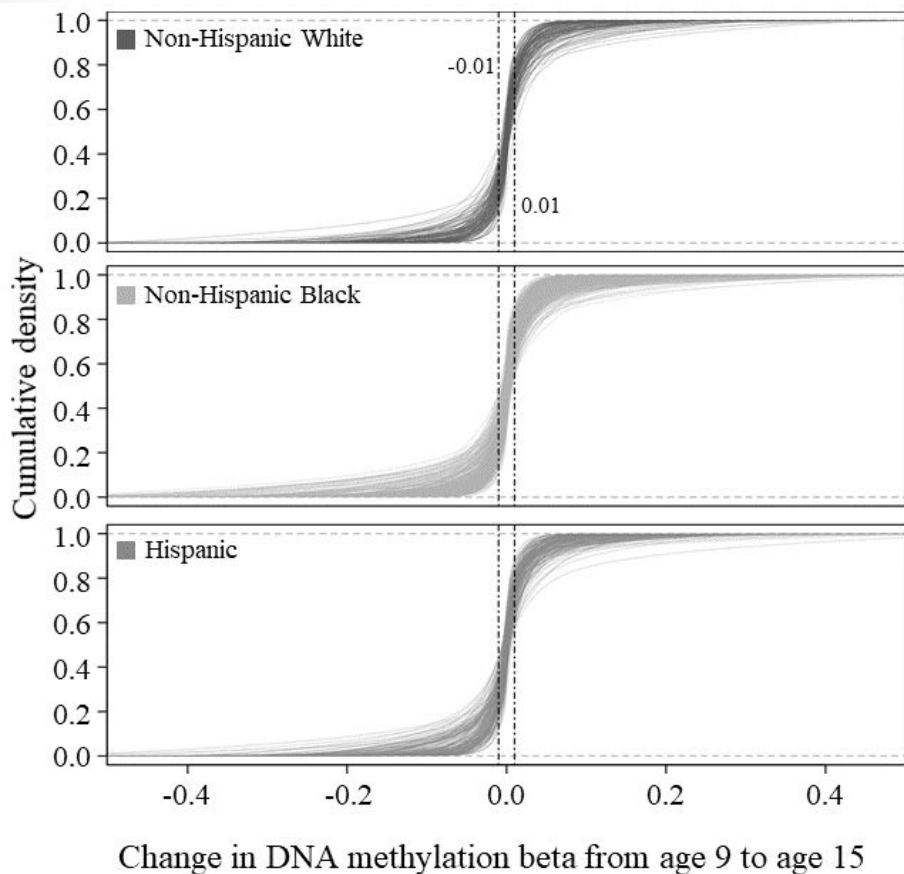
Beta distributions by ancestry for Y9

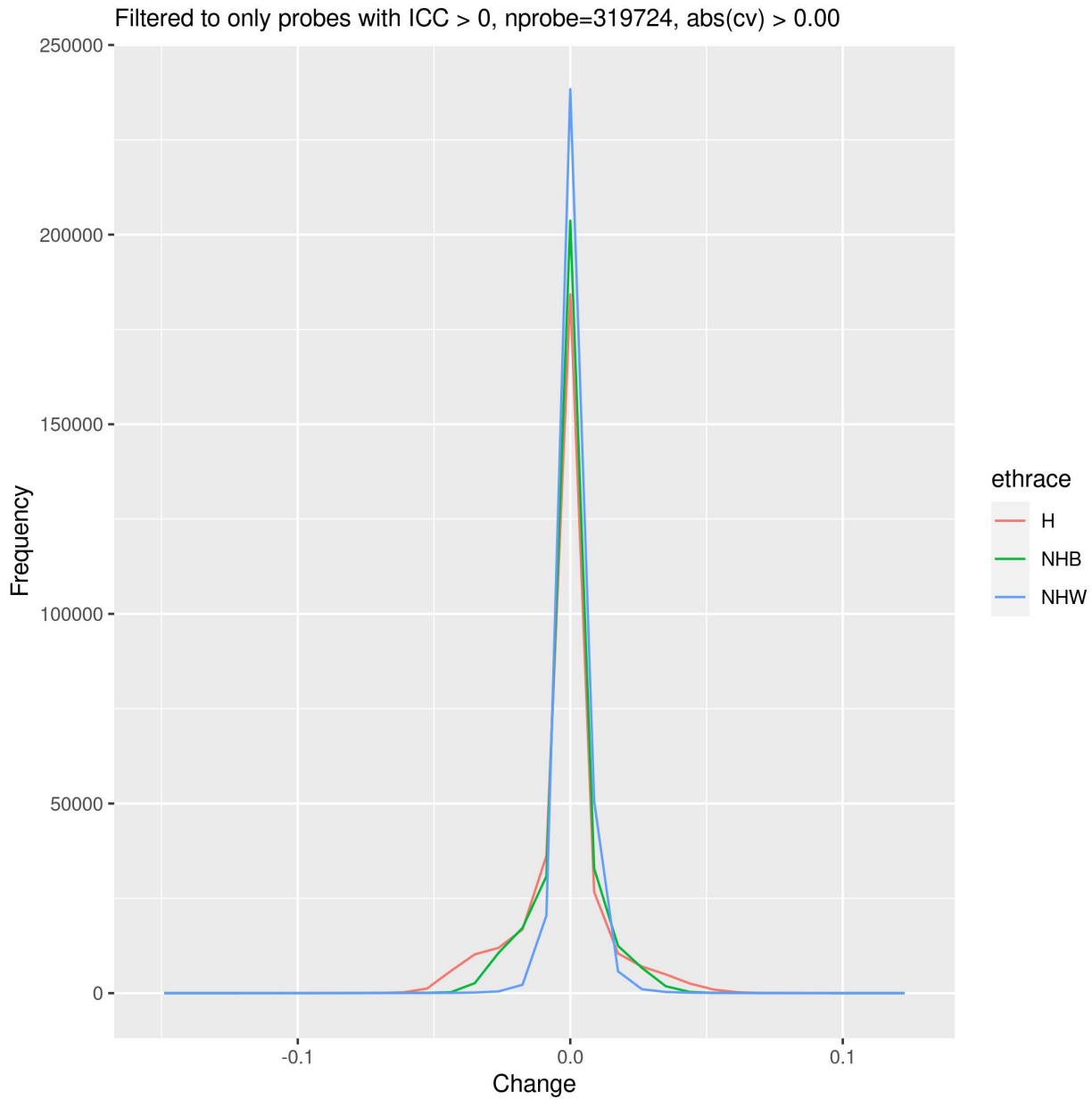


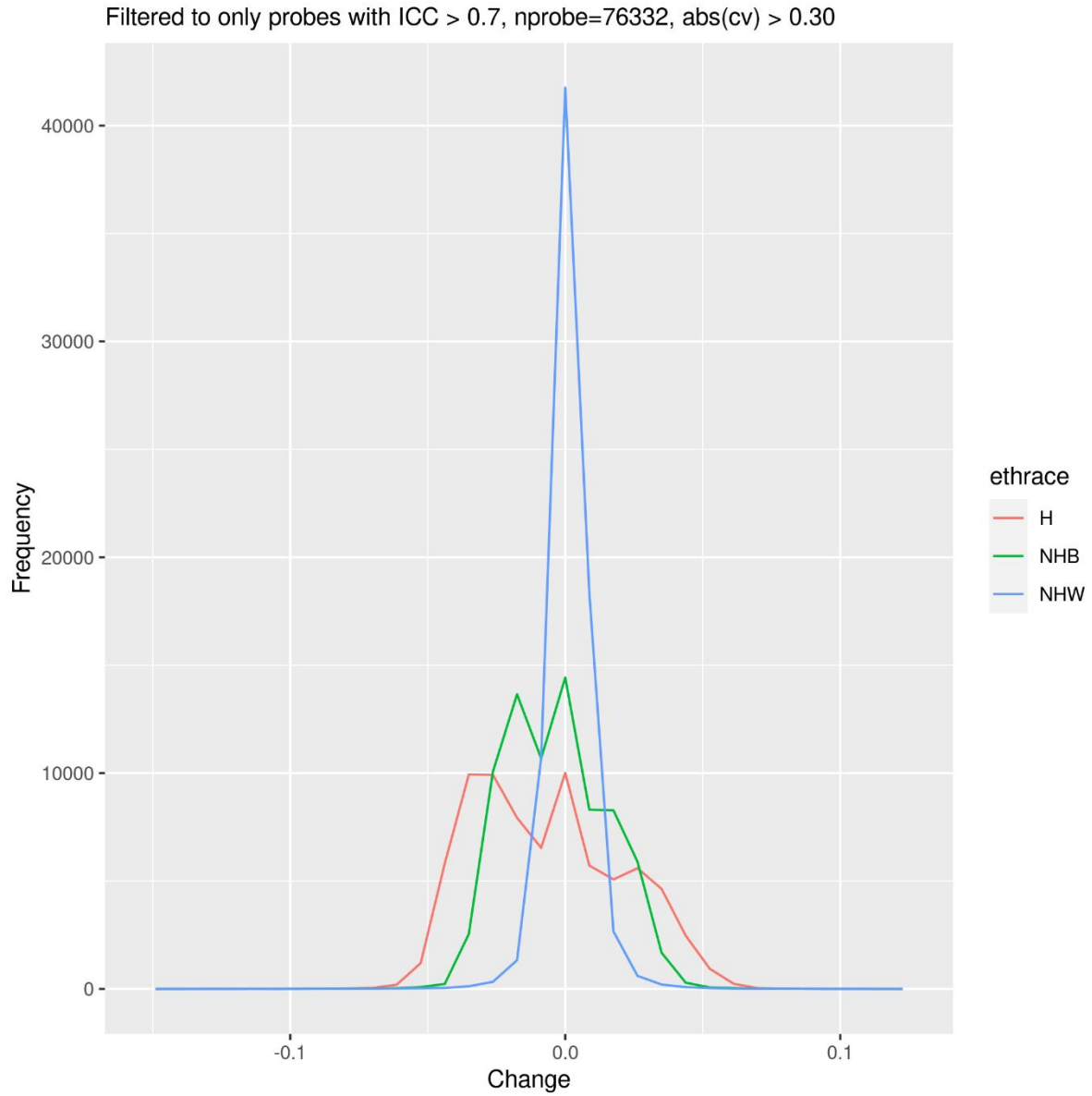
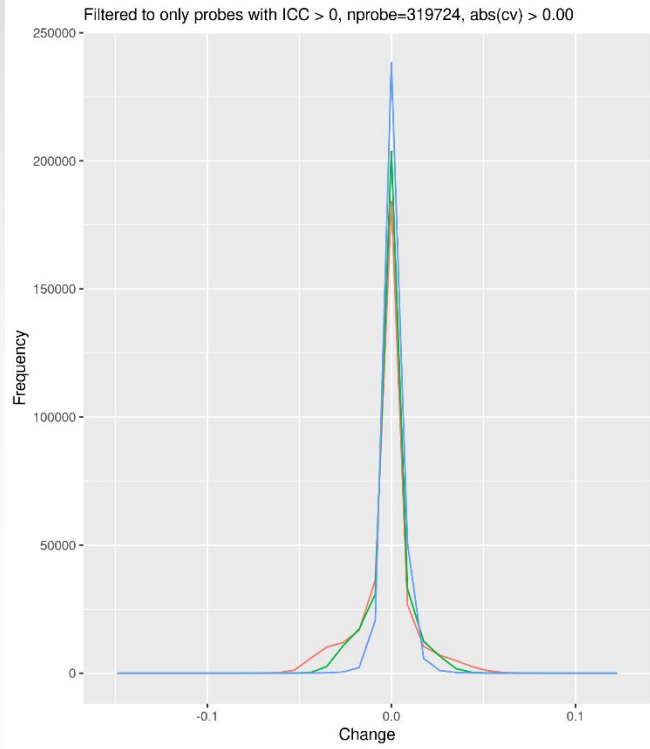
Beta distributions by ancestry for Y15



How is DNA Methylation change distributed?

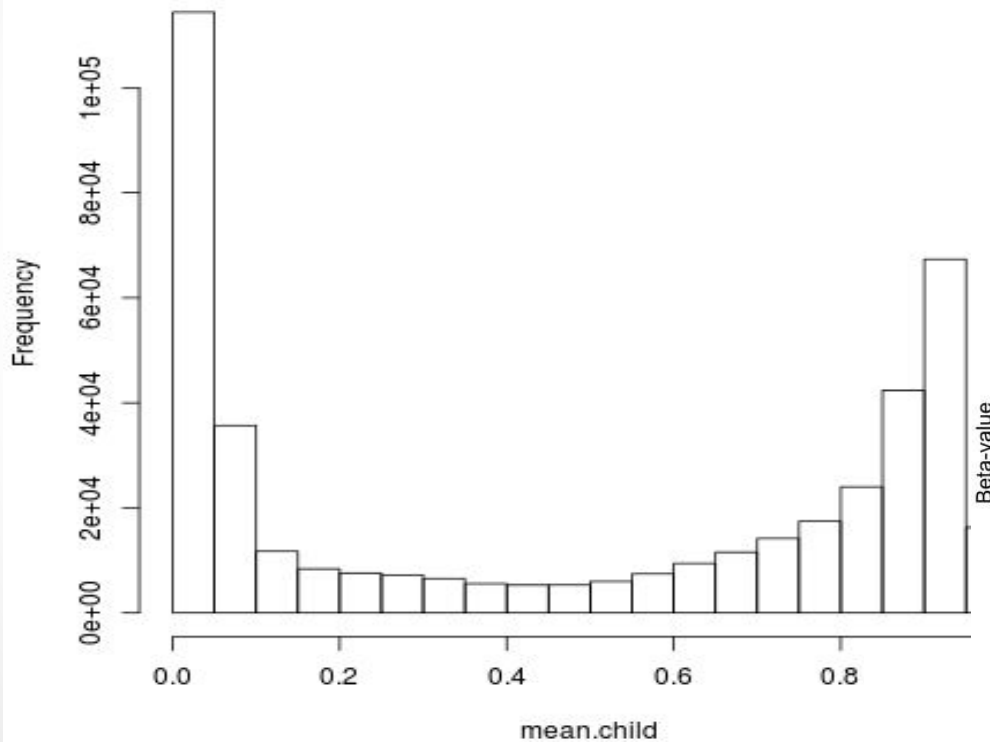




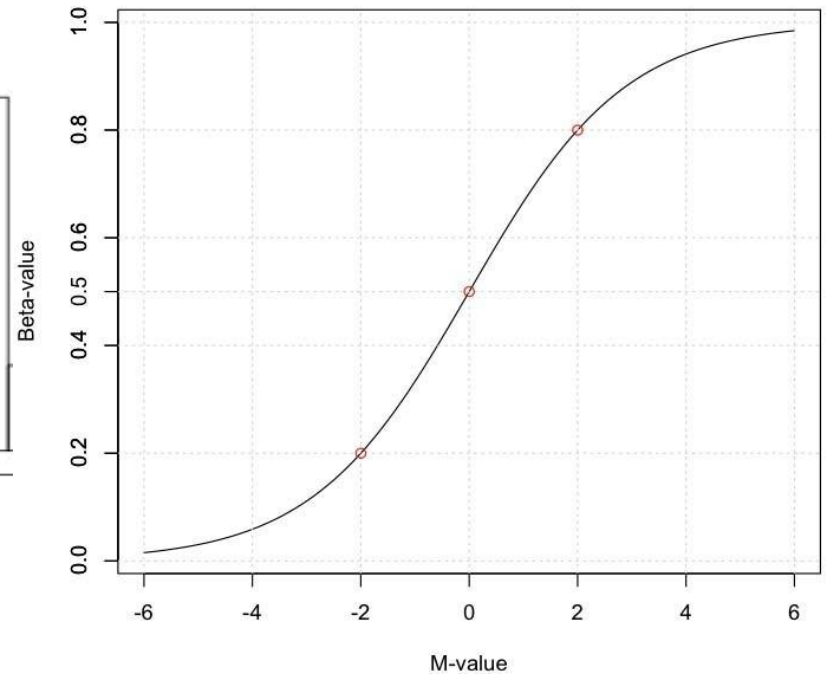


Distribution of probe mean methylation

Decile frequency Y9



$$M_i = \log_2 \left(\frac{Beta_i}{1 - Beta_i} \right)$$



Longitudinal Change in Probes by Race/Ethnicity

Filtered to only probes with ICC > 0.7, nprobe=76332, abs(cv) > 0.30

