

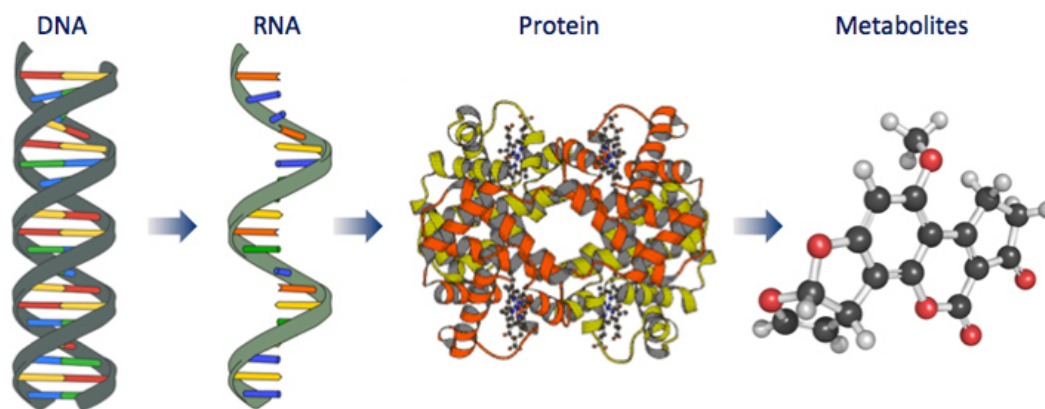
# Epigenetic modifications and resistance to TB

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Oct 11, 2019

## Central dogma



# Central dogma

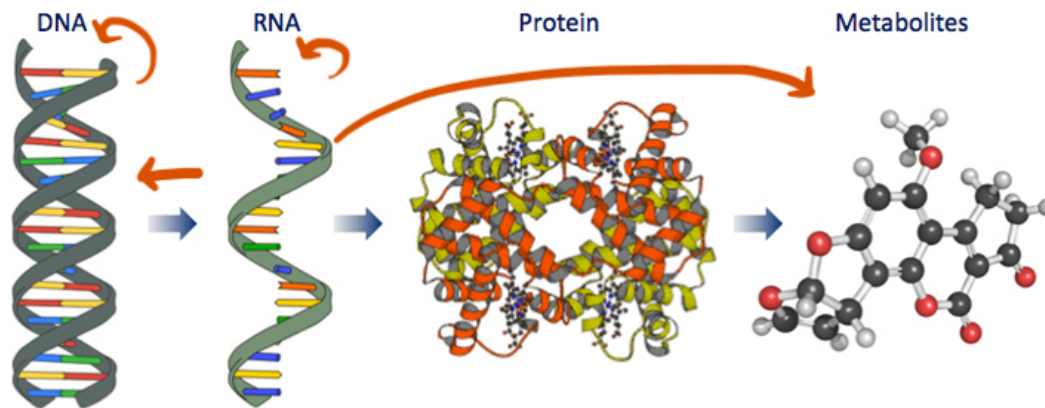
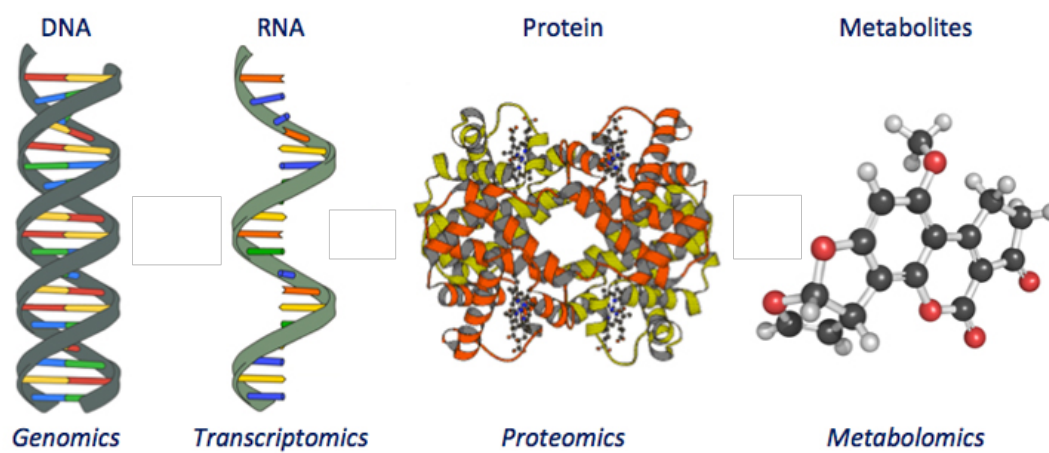


image: CHROMAcademy

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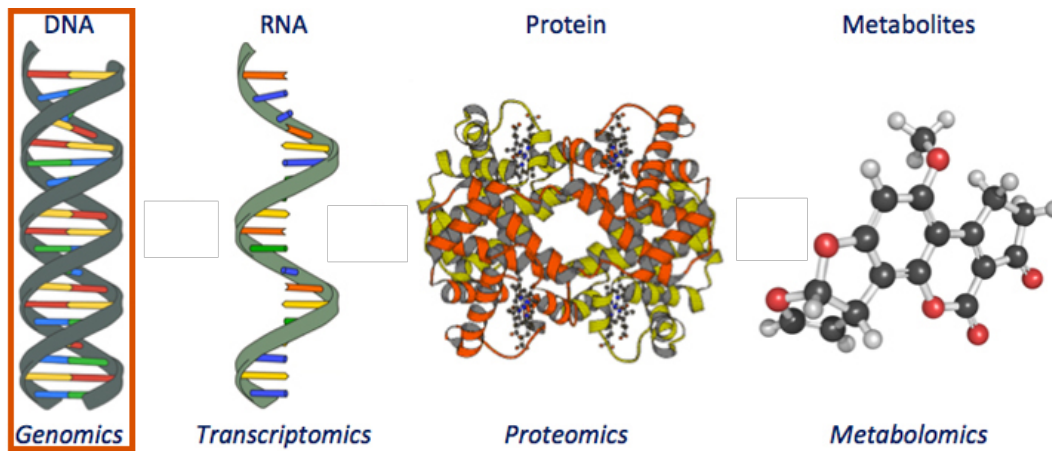
## The 'omics world



- Snapshots in time

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# The 'omics world



- Snapshots in time

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## DNA is not wholly fixed

- The DNA you're born with is not the DNA you die with
- **Local mutations**
  - Changes in DNA sequence at the cell or tissue level
  - Not heritable
- **Epigenetics**
  - Changes in DNA that do not involve sequence
  - Heritable

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# Epigenetics

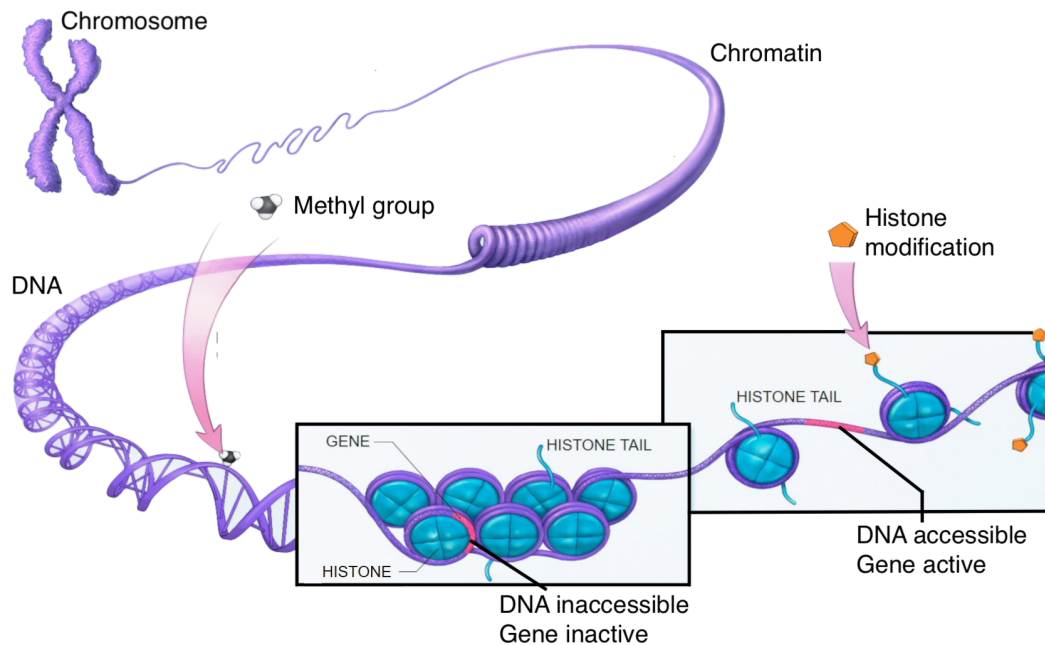


image modified from National Institutes of Health

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## Genomics: the whole picture

- DNA sequence
- DNA methylation
- Chromatin accessibility
  - Sum of all histone modifications
- = Predict gene expression

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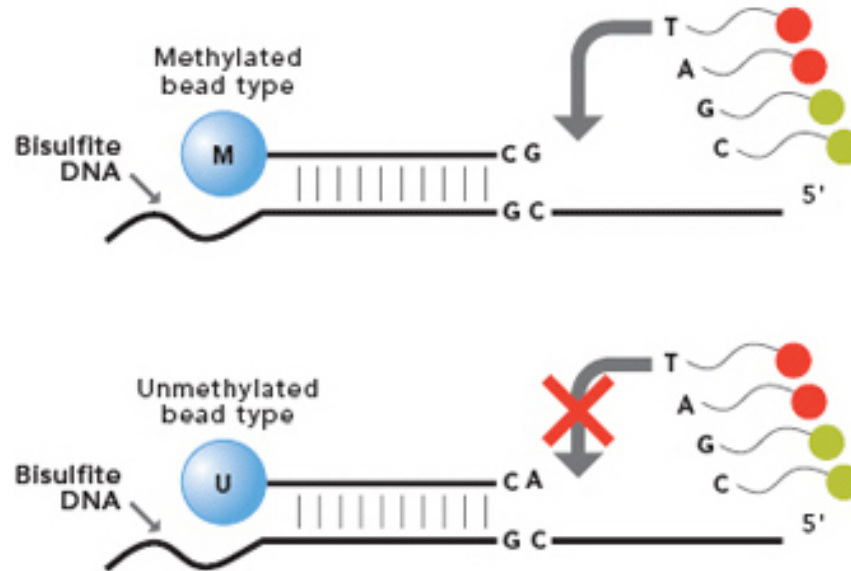
# Methylation methods

## Measuring methylation

- Only occurs at CpG sites
- Array-type (as opposed to whole genome)
- Illumina Infinium MethylationEPIC arrays I and II
- 865,918 probes across genome

# MethylationEPIC arrays

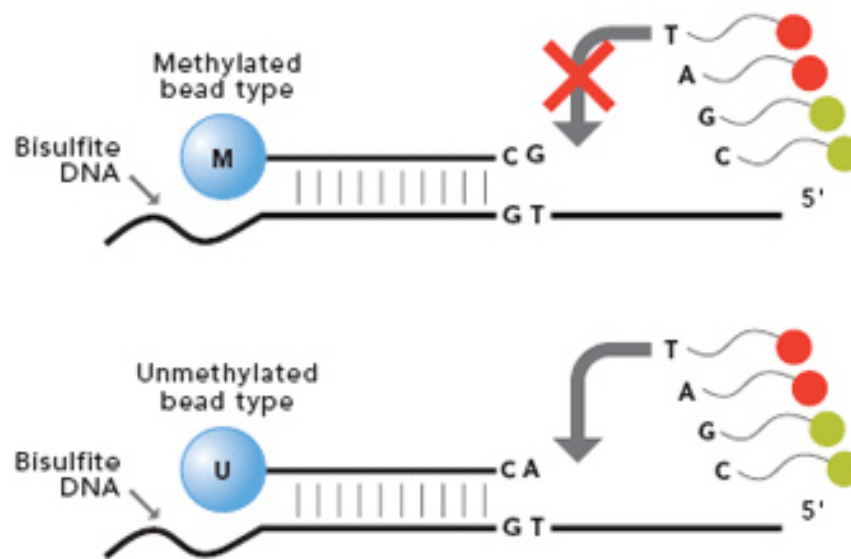
Methylated CG sites protected during bisulfite treatment



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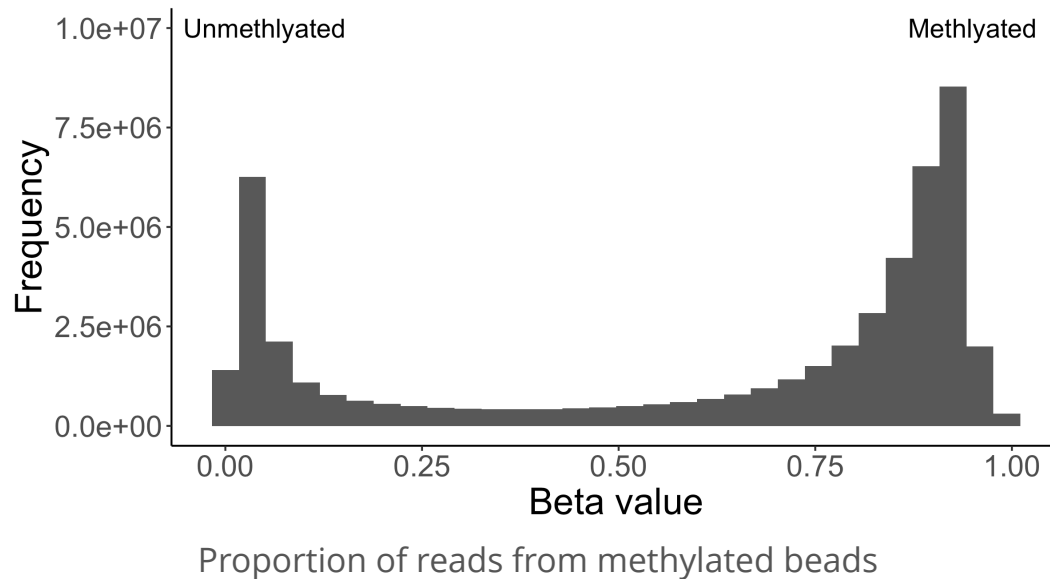
# MethylationEPIC arrays

Unmethylated CG sites converted to TG during bisulfite treatment



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# MethylationEPIC arrays



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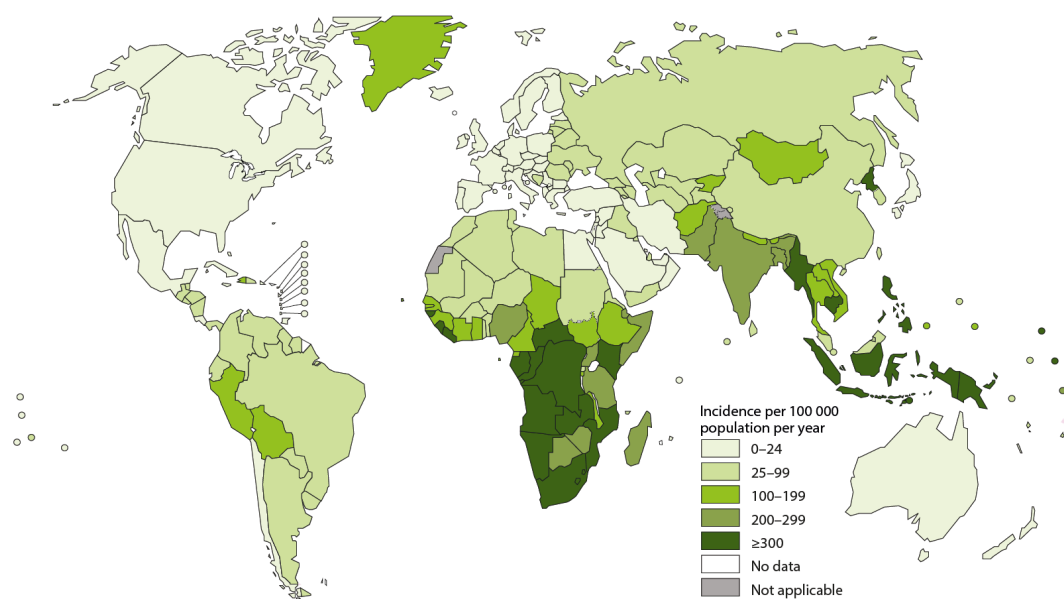
## Why isn't it 0:1?

- **Technical**
  - 1 bp mismatch doesn't 100% prevent extension
  - Incomplete bisulfite conversion
- **Biological**
  - Heterogeneous genes
  - Heterogeneous cell population

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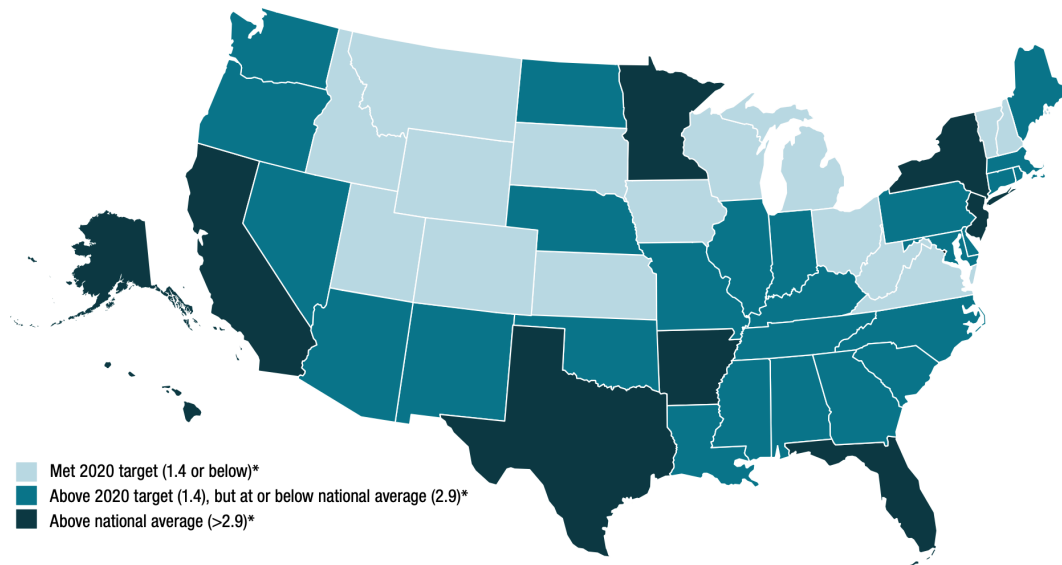
# Methylation results

## Estimated TB incidence rates, 2017





## Estimated TB incidence rates, 2016



\*Cases per 100,000 persons

image: CDC National TB Surveillance Systems

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## Uganda household contact study

- 2002 – 2012 in Kampala, Uganda
- 872 Index Cases with culture confirmed pulmonary TB
- 2585 contacts of Index Cases



Henry Boom



Cathy Stein



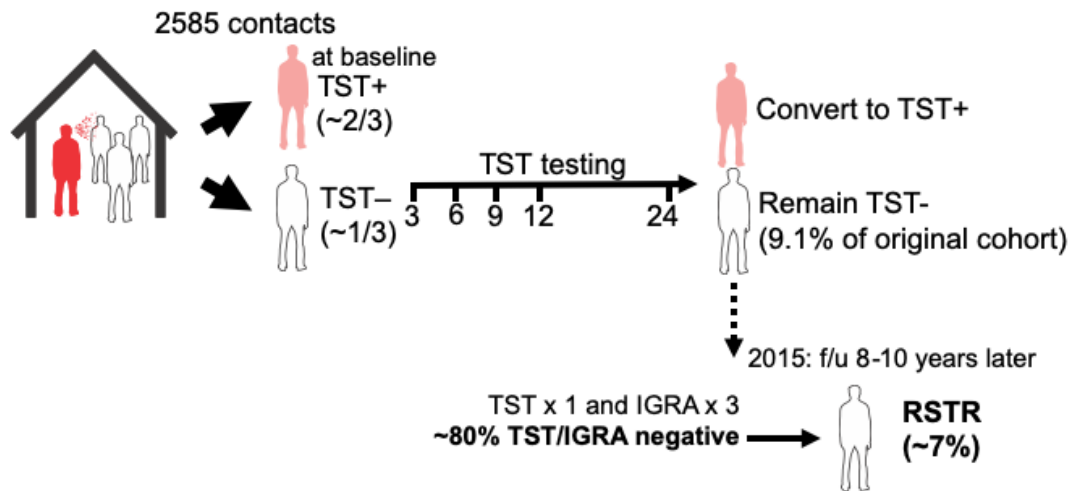
Harriet Mayanja-Kizza

Ma N *et al* 2014 doi: [10.1186/1471-2334-14-352](https://doi.org/10.1186/1471-2334-14-352)

- Stein C *et al* 2018 doi: [10.1093/aje/kwx380](https://doi.org/10.1093/aje/kwx380)

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# Uganda household contact study



Slide courtesy T Hawn

- TST: Tuberculin skin test
- IGRA: Interferon-gamma release assay

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## RSTR: Resister

- highly exposed to TB but by all available measures, remains uninfected for many years

## LTBI: latent TB infection

- infected with TB but not actively manifesting symptoms

## TB: pulmonary TB

- infected with TB in lungs and actively manifesting symptoms

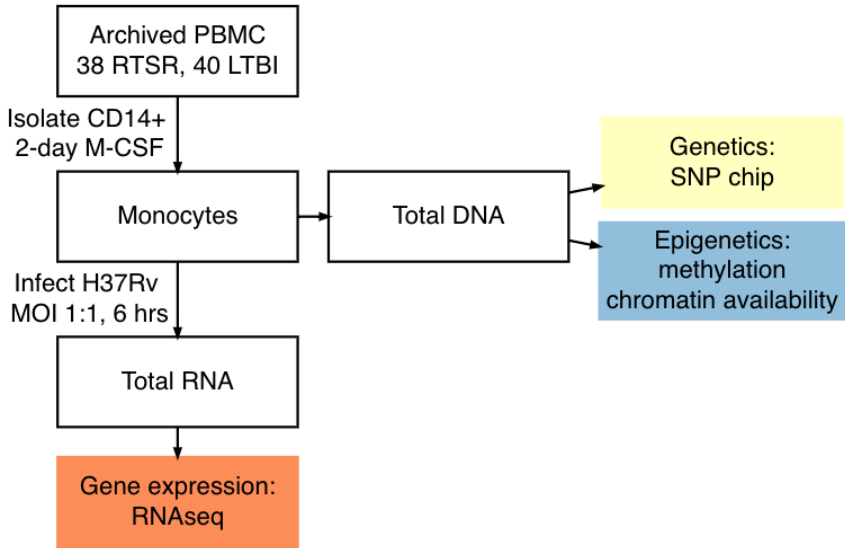
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# What makes resisters resistant?

## Why epigenetics in RSTR?

- Transcription of histone deacetylase (HDAC) genes associated with RSTR-derived PBMCs (monocytes) infected with Mtb
- Genetic variation in HDAC genes associated with Ugandan RSTRs
- HDAC3 inhibitor treatment decreased Mtb replication in macrophages

# Experimental design



Jason  
Simmons



Glenna  
Peterson

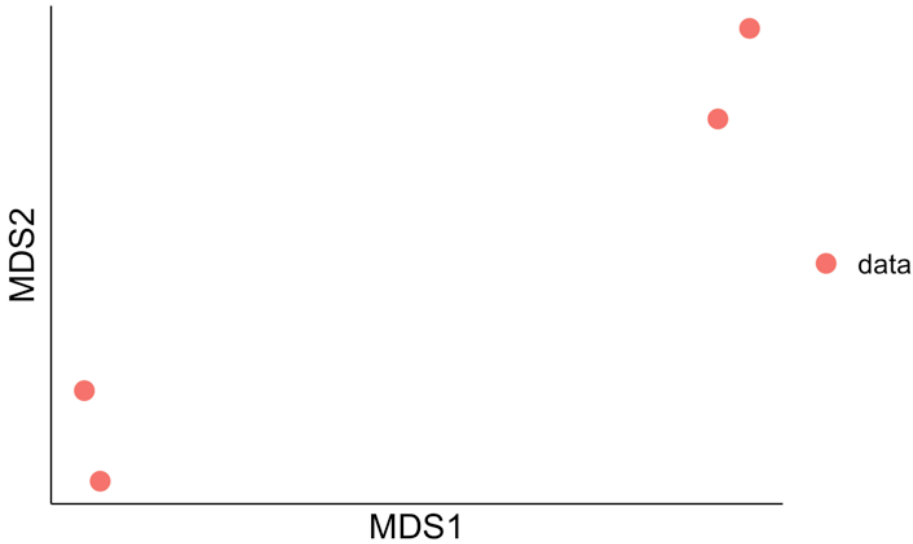
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# Cohort metrics

	RSTR	LTBI
N	31.0	30.0
Age at enroll, mean	15.7	18.7
Female, %	50.0	50.0
TB risk exposure score, mean	6.1	6.2
HIV+	0.0	0.0

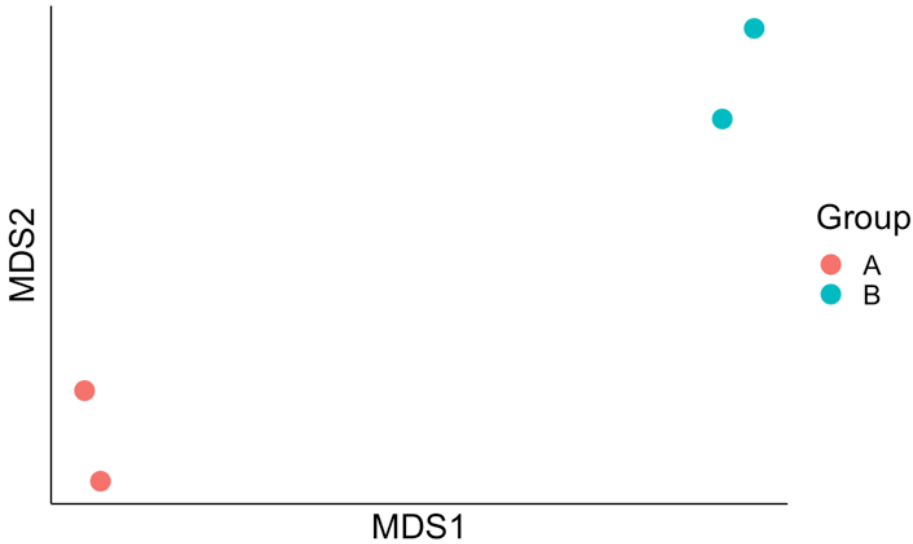
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# Overall methylation profiles



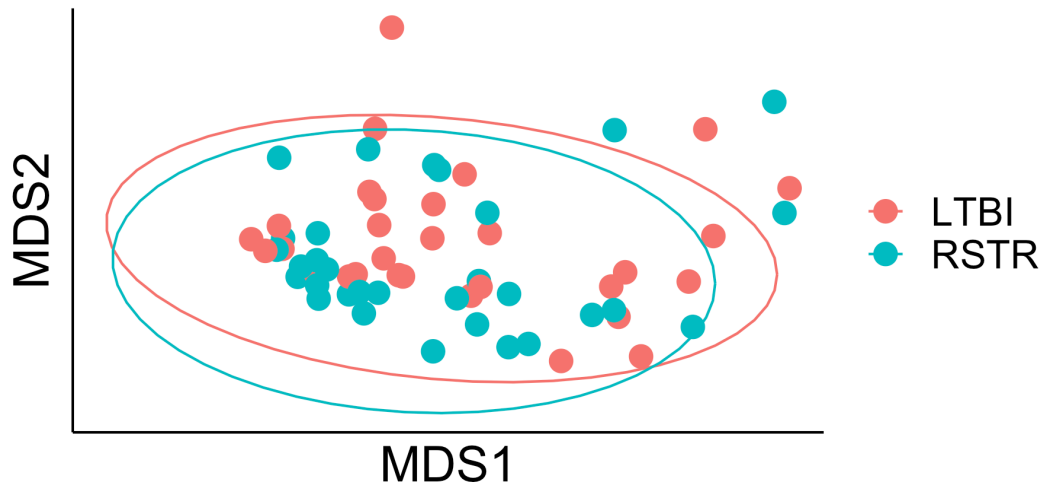
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# Overall methylation profiles



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# Overall methylation profiles



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## Cautions for MDS (and PCA)

- Coerces multi-dimensional data into 2D
- Does not capture full variability of the data
- Can only identify very large, global differences
- *Not seeing clear separation in MDS/PCA **does not** indicate that no differences exist.*

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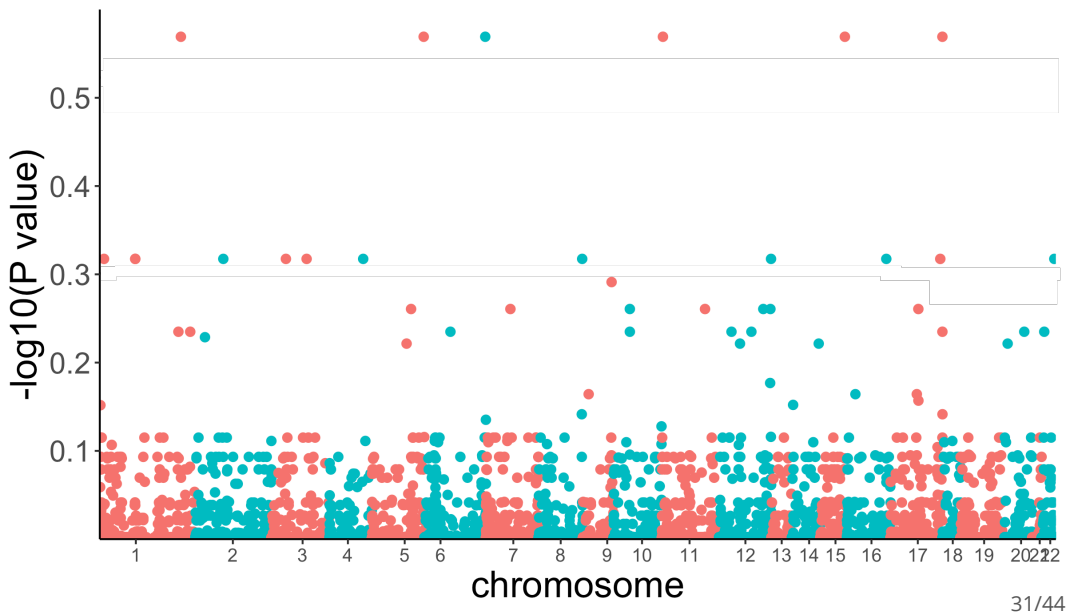
# Probe-level analysis

## Differentially methylated probes

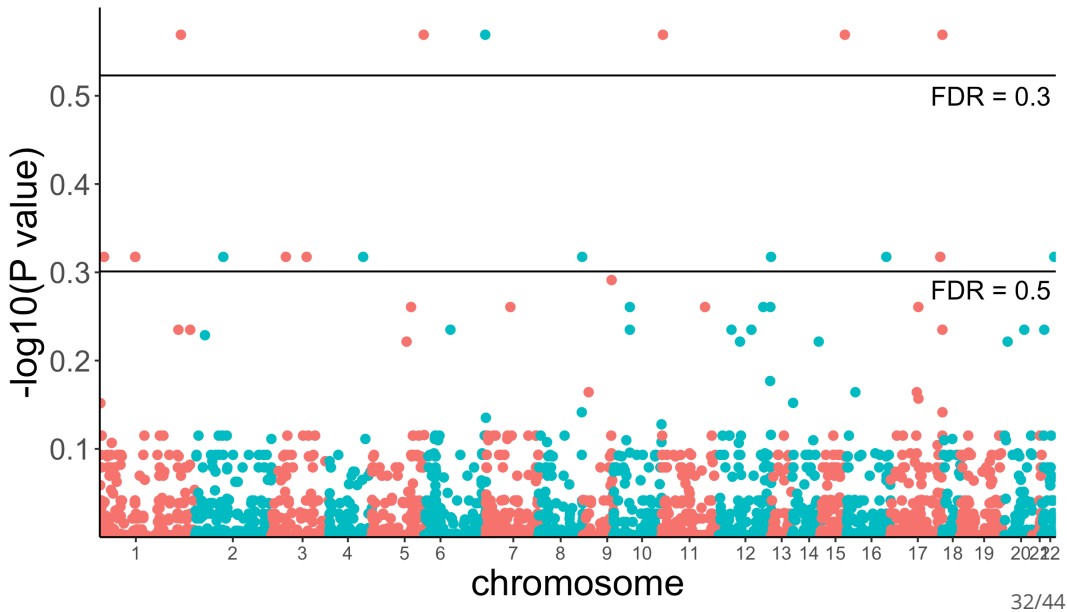
- Linear regression of each probe (R package `limma`)
- Multiple comparison correction (FDR)

```
##           Contrasts
## Levels RSTR-LTBI
##   LTBI      -1
##   RSTR       1
```

# Differentially methylated probes



# Differentially methylated probes





# Cautions for DM probes

- Multiple comparison correction
  - $P\text{-value} \leq 0.05 = 33113$
  - $FDR \leq 0.05 = 0$
  - $FDR \leq 0.5 = 18$
- How biologically relevant is a single methylation site?
- How biologically relevant are sites in the middle of genes?

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## Region-level analysis

# Differentially methylated regions

	Small effect size		Large effect size		Speed	Co-variates	R
	Precision	Power	Precision	Power			
bumphunter	-	-	+	-	+	Y	Y
combp	++	-	++	++	+	Y	N
<b>DMRcate</b>	<b>++</b>	<b>--</b>	<b>++</b>	<b>+</b>	<b>++</b>	<b>Y</b>	<b>Y</b>
Probe Lasso	+	--	++	+	++	N	Y

- Mallik *et al* 2018 doi: [10.1093/bib/bby085](https://doi.org/10.1093/bib/bby085)
- Peters *et al* 2015 doi: [10.1186/1756-8935-8-6](https://doi.org/10.1186/1756-8935-8-6)

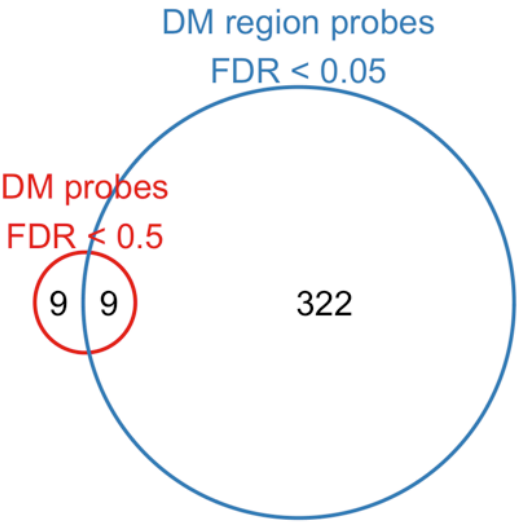
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## DMRcate

1. Define significant probes from linear models ( $\text{FDR} \leq 0.5$ )
2. Estimate methylation of other probes in sample using a Kernel smoother for **DMP weighted** and **unweighted** models
3. Keep estimates from best fit for each sample
4. Re-estimate P-values for DMP using new estimated methylation values
5. Multiple comparison correction (FDR)
6. Group significant probes within 500 bp into regions

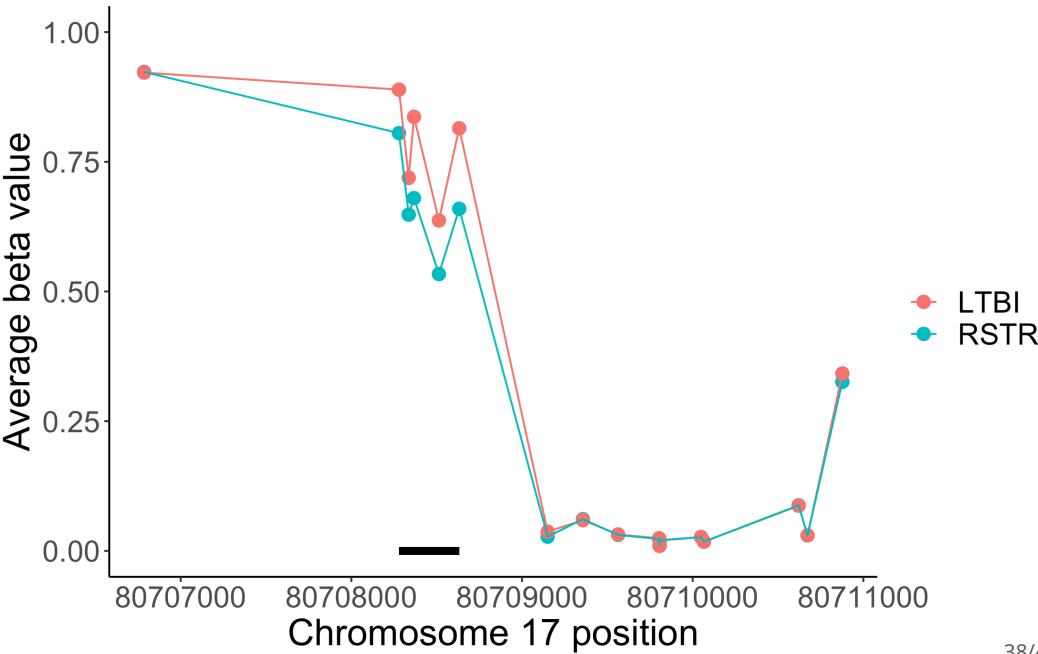
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# Probes in regions



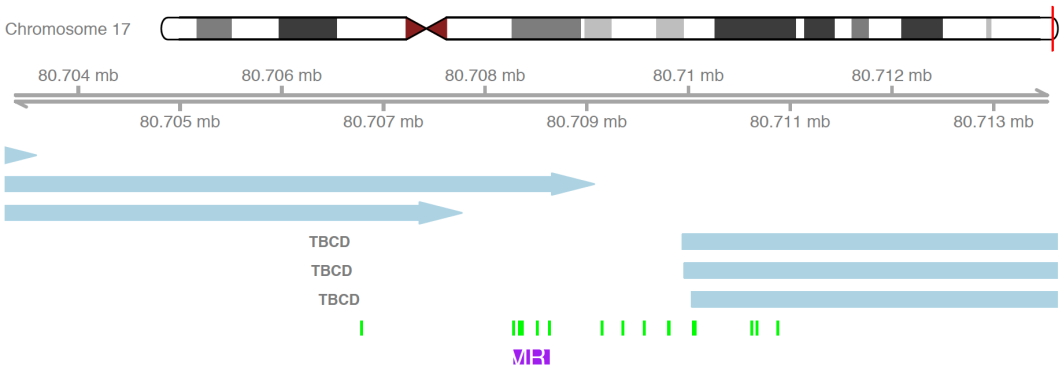
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## Example region: DMR\_2



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# Example region: DMR\_2



- TBCD: tubulin folding cofactor D

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## Identified regions

FDR cutoff	Total regions	Total genes	Total probes in regions
1e-05	4	4	17
1e-04	5	5	20
1e-03	11	10	49
1e-02	37	32	178
5e-02	92	69	331

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# Gold standard genes of interest in RSTR

- Lower methylation
- Higher chromatin availability
- Higher RNA expression
- Or vice versa

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## Future directions

- More complex models including demographics, exposure risk score, BCG vaccination, etc.
- Inclusion of SNP probes
- Compare to RNA-seq and chromatin availability

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# Acknowledgements

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Case Western
- Henry Boom, MD,  
Case Western
- Harriet Mayanja-Kizza, MD,  
Makerere University
- Chetan Seshadri, MD,  
UW-Medicine

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# Bioinformatics Interest Group at SLU

- Monthly paired seminar and workshop series
- Coming Winter 2019
- Recruiting executive team members!

For more info:

- UW mail list: [slu\\_bioinformatics](mailto:slu_bioinformatics)
- Website: <https://big-slu.github.io/>
- Email me: [kadm@uw.edu](mailto:kadm@uw.edu)

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