All the ”-omics”

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Overview

Today's Goals

- What other questions can genomics answer?
- What technologies underlie these methods?

- Group dataset selection

Methods in Context

My goal is to present the methods as:

- Biological Question

  (Broad enough to apply to whatever system)
- Underlying NGS
- A paper that utilizes the method

RNAseq

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  - What genes are being expressed in a cell?

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- Faherty. 2016. Gene Expression Profiling in the Hibernating Primate, Cheirogaleus Medius.

ASE

- Allele-Specific Expression

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- Quantification of gene expression by diploid copy

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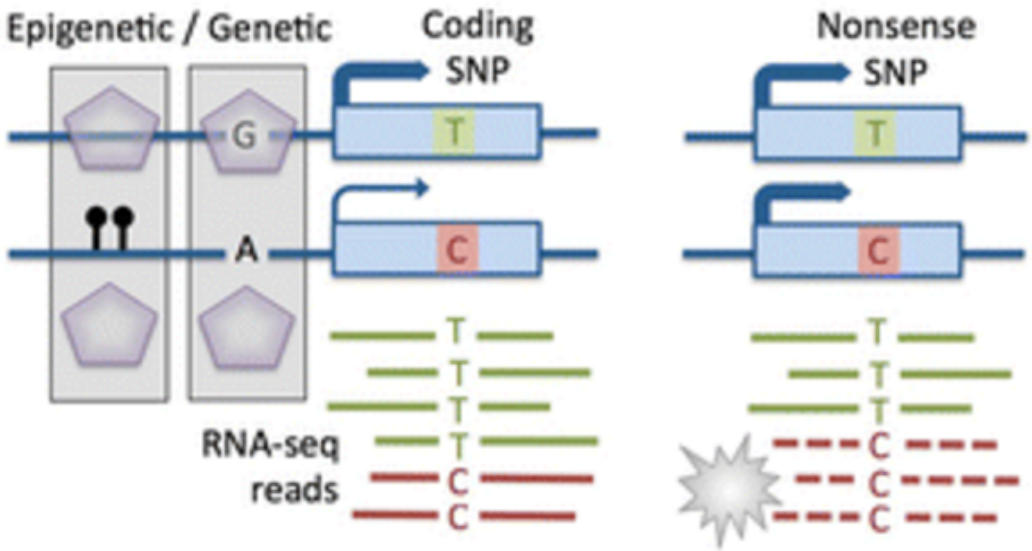
- Quantification of gene expression by diploid copy
- Can determine effect of small-scale changes on expression

ASE

- Comparing haplotypes allows for detection of differences:



ASE

- Comparing haplotypes allows for detection of differences:
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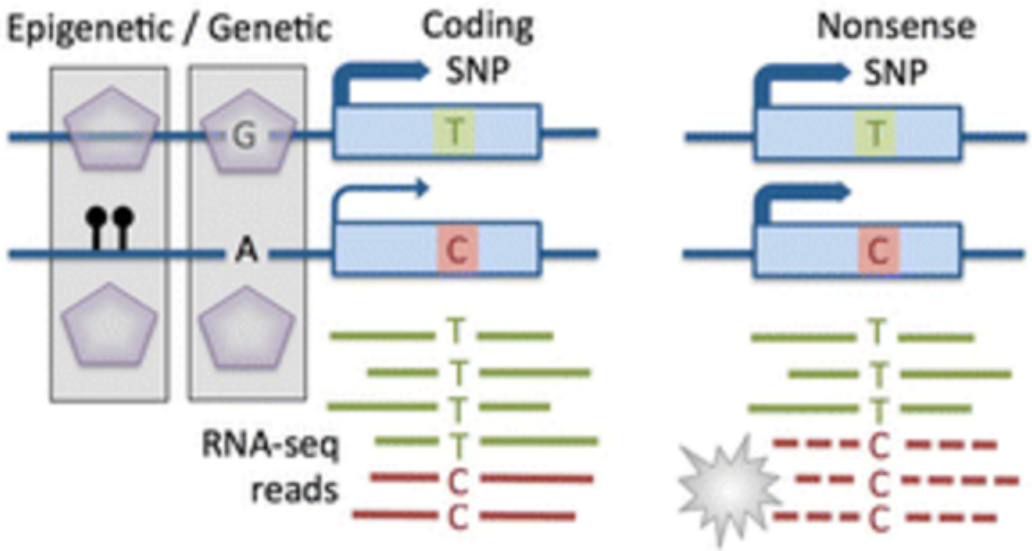

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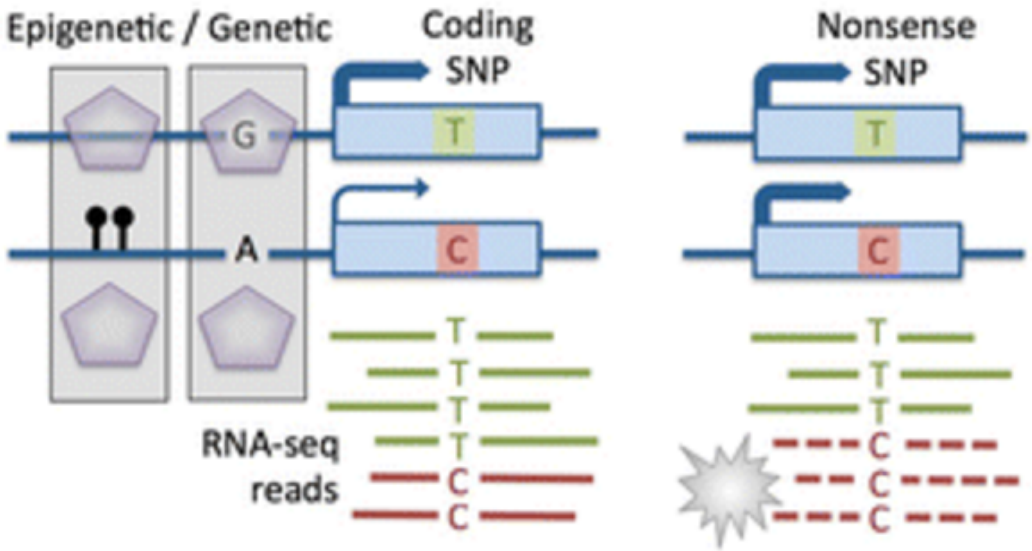

ASE

- Comparing haplotypes allows for detection of differences:
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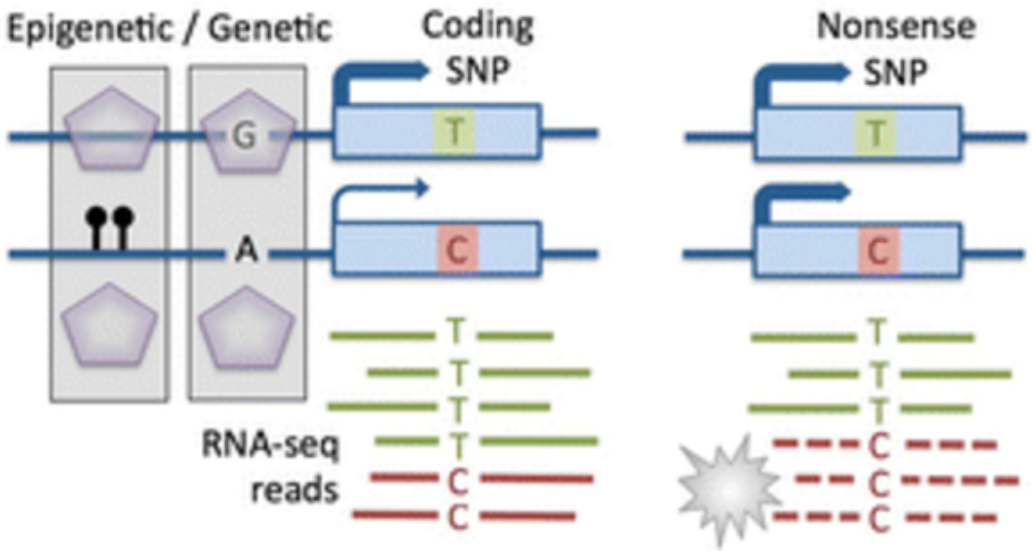
- SNPs
- TF binding sites or methylation



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- Lappalainen et al. 2013. Transcriptome and genome sequencing uncovers functional variation in humans.

Iso-Seq

- Isoform Sequencing

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- Long read RNA sequencing

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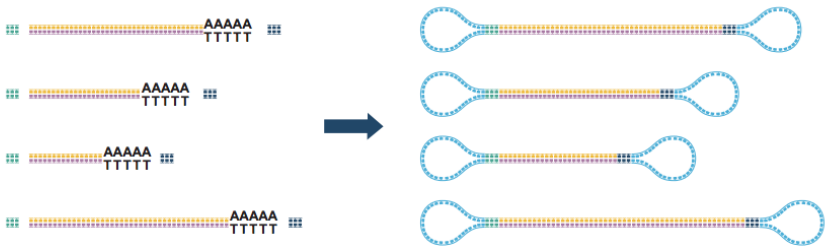
- Long read RNA sequencing
- Allows for the full transcript to be sequenced

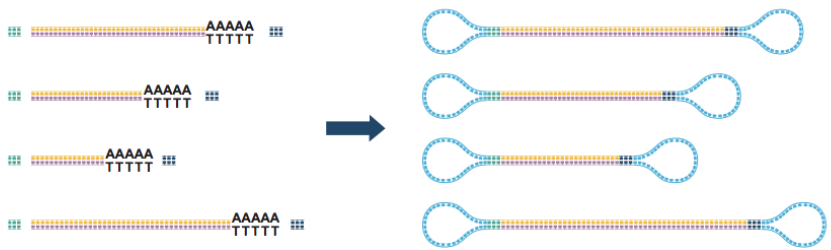
Iso-Seq

- RNAseq library preparation



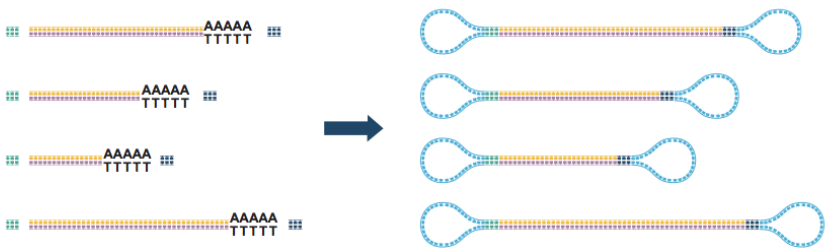
Iso-Seq

- RNAseq library preparation
- No fragmentation and keep full transcripts



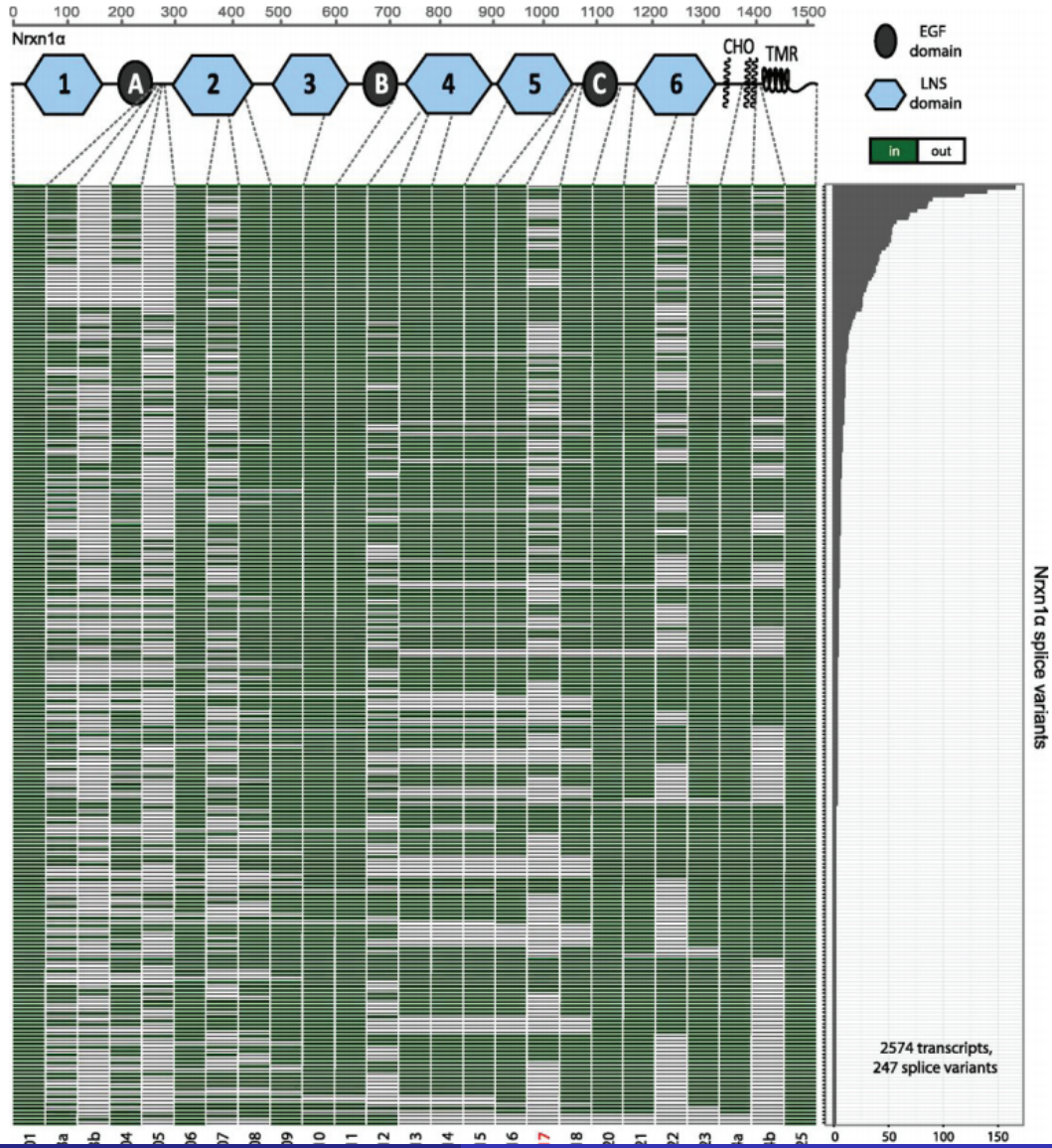
Iso-Seq

- RNAseq library preparation
- No fragmentation and keep full transcripts
- Prepare and sequence on the PacBio platform



Iso-Seq

- Get longer data



Iso-Seq

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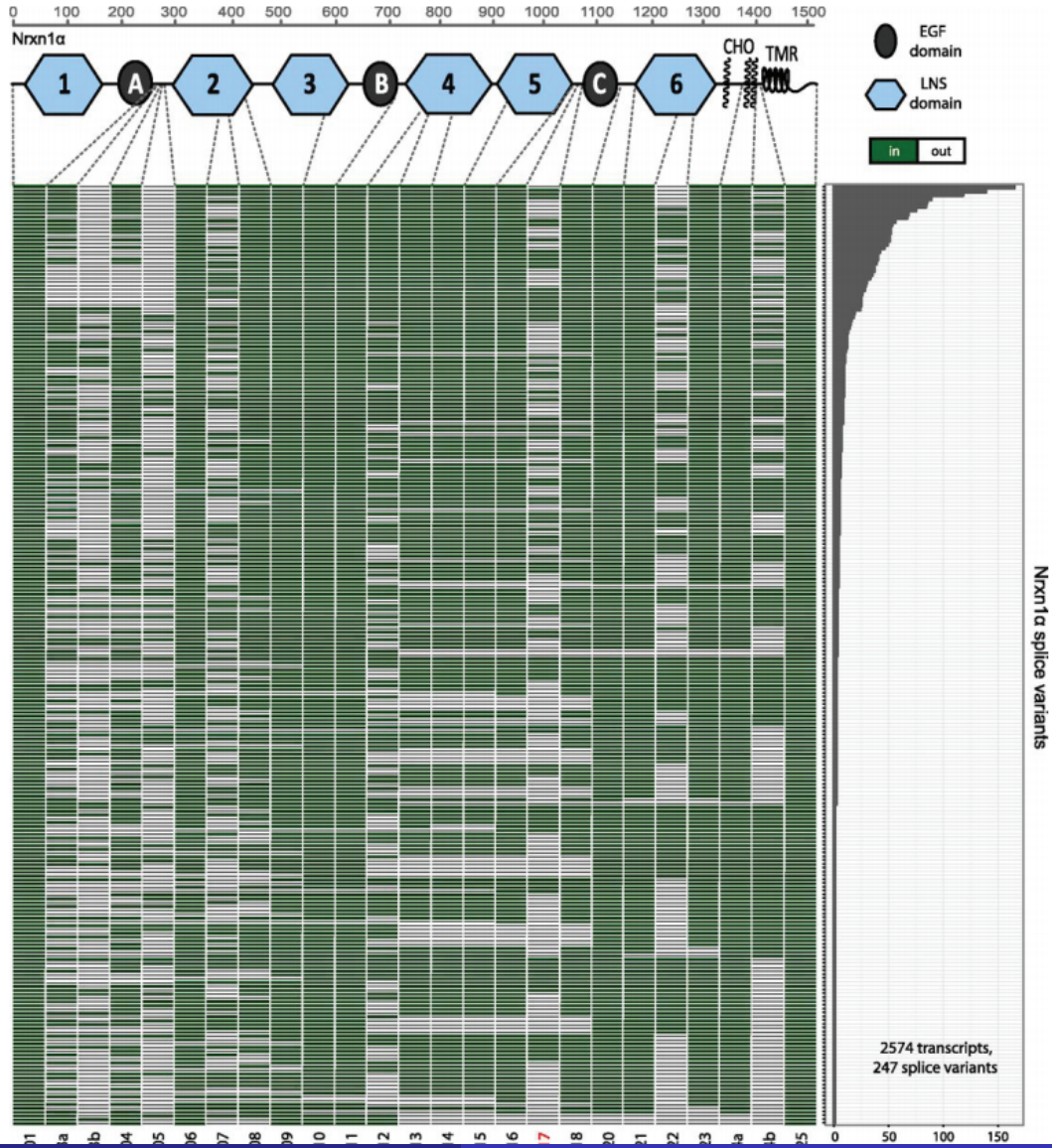
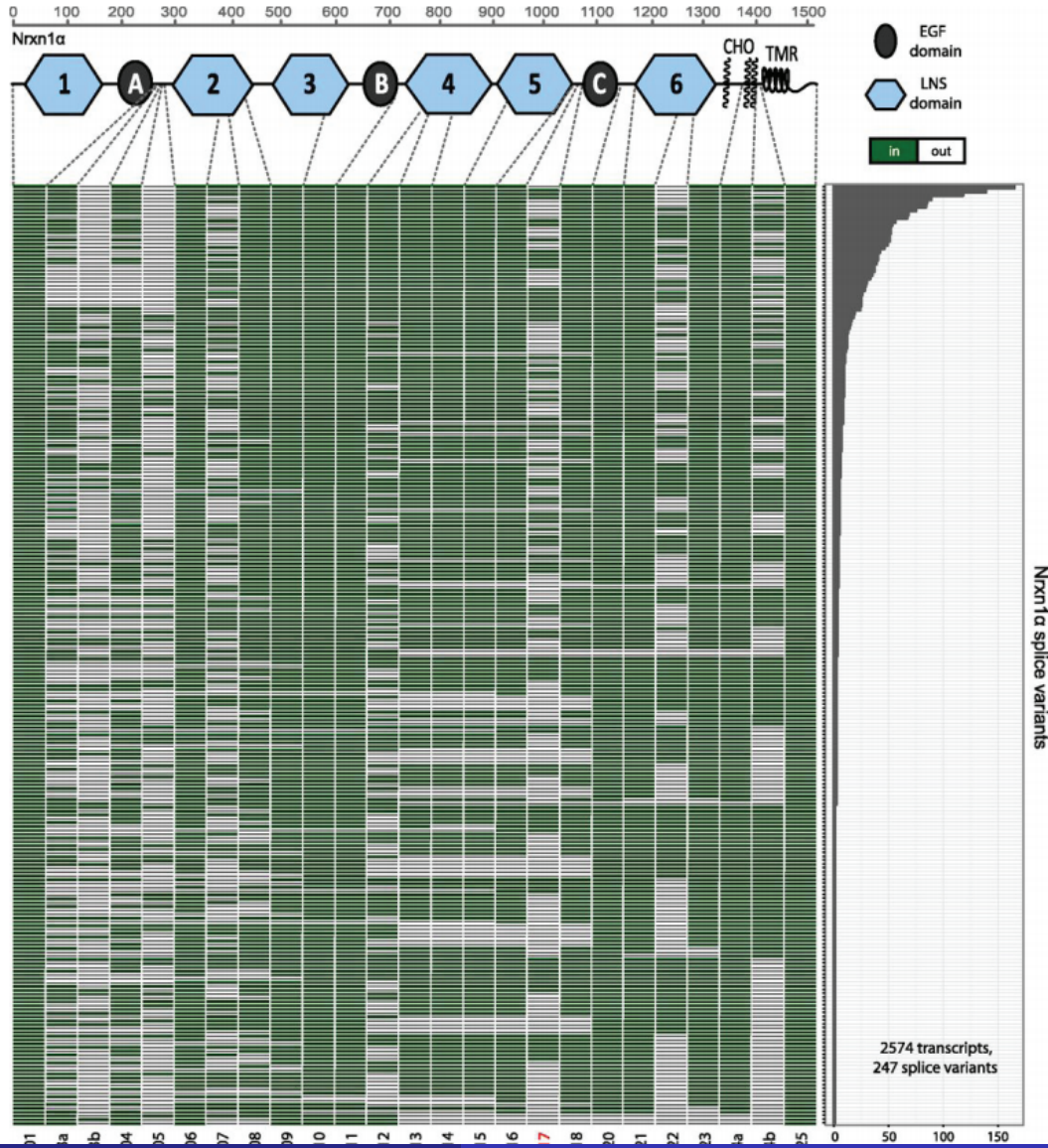
- Don't have to assemble full transcripts



Iso-Seq

- Get longer data
- Don’t have to assemble full transcripts
- Actually “see” all the isoforms



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- Cheng et al. 2017. Long-read sequencing of the coffee bean transcriptome reveals the diversity of full-length transcripts.

Microbiome

- What is the community of microbes that live within vertebrates?

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- Gut is most commonly studied, but there are plenty of other targets

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  - How do antibiotics alter this community?
- Also can compare across populations (diet effect)
- Can often tell a differnce based on lifestyle

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  - Often stable (encodes ribosomal RNA molecule)
  - Used to distinguish “species”
- The most common form of microbiome research amplifies this region
- Then sequencing is performed to assess the community

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- Gather a sample and prepare all the DNA therein for sequencing

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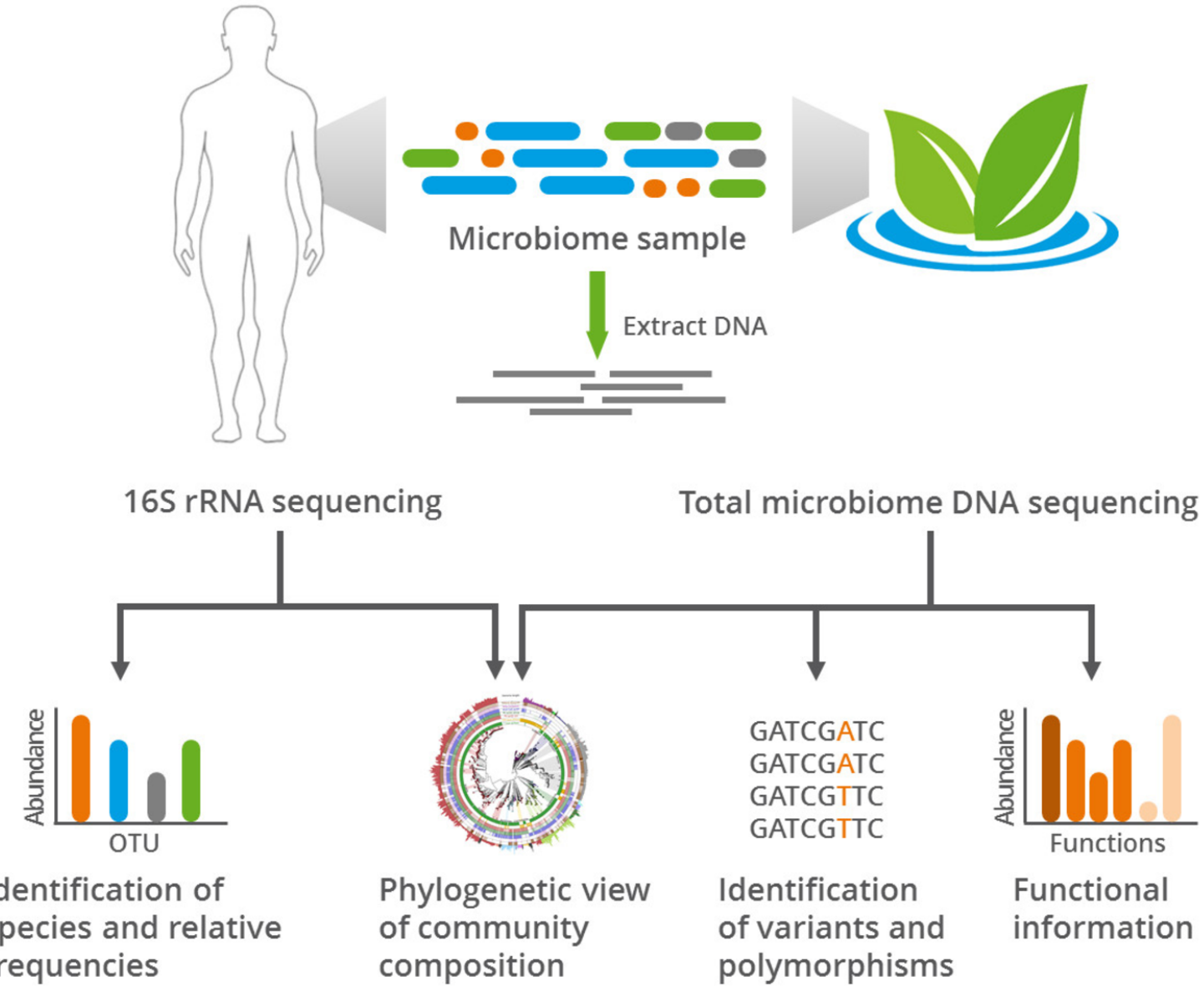
- Gather a sample and prepare all the DNA therein for sequencing
- Catches everything, unbiased
- Categorization is often difficult
- Captures functional capabilities of microbiota

16S v. Metagenomics



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- Davie et al. 2014. Diet rapidly and reproducibly alters the human gut microbiome.

Epi-

- Epi- means?

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- On, upon

Epi-

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- On, upon
- Above

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  - microRNAs

Methylation

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- (Adenine can too, just less common)

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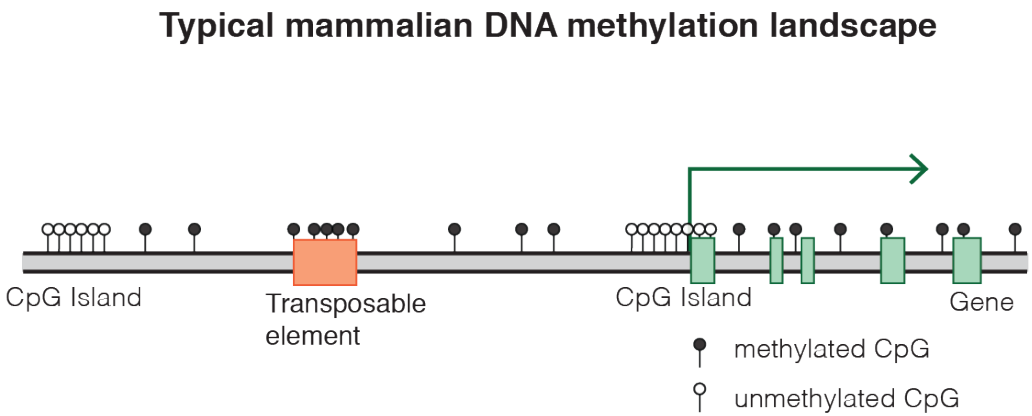
- (Adenine can too, just less common)
- Supresses transcription if it occurs in a gene promoter region

Methylation

- Often present near TE's and Genes



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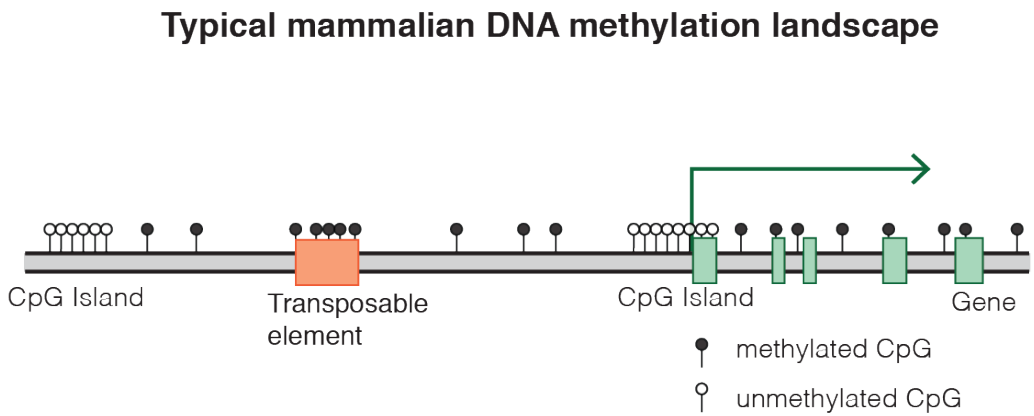

Methylation

- Often present near TE's and Genes
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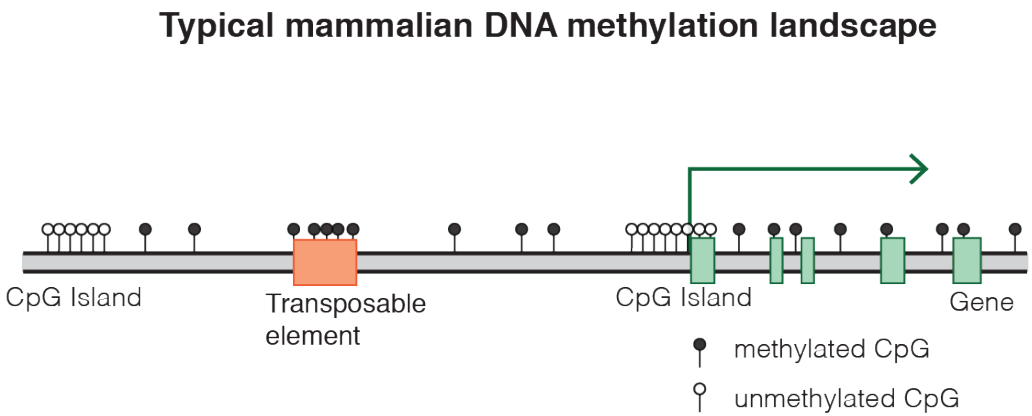
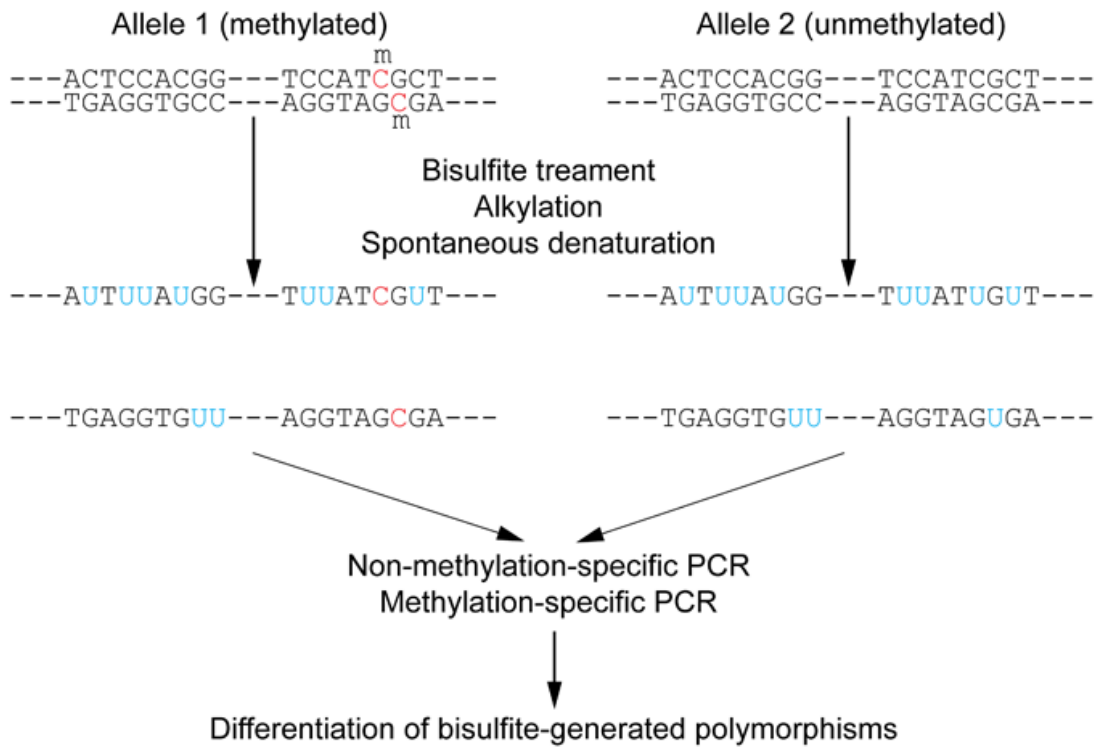
- Absence allows gene transcription



Bisulfite Sequencing

- Library prep converts unmethylated C's to U's



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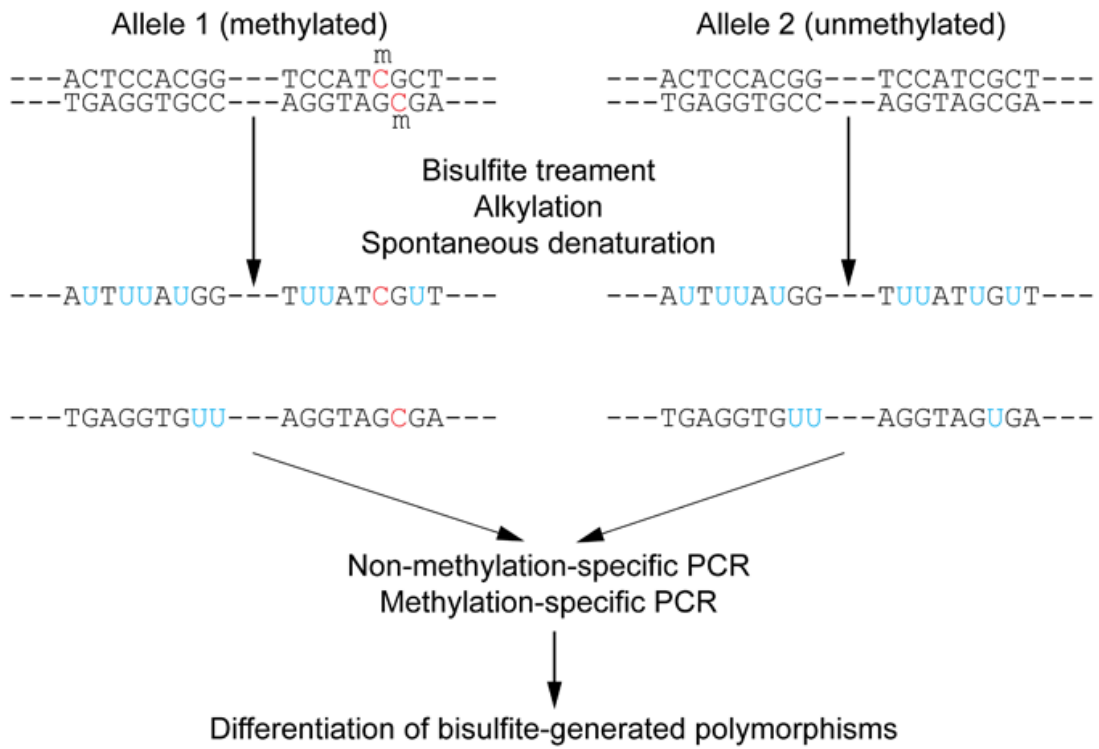

Bisulfite Sequencing

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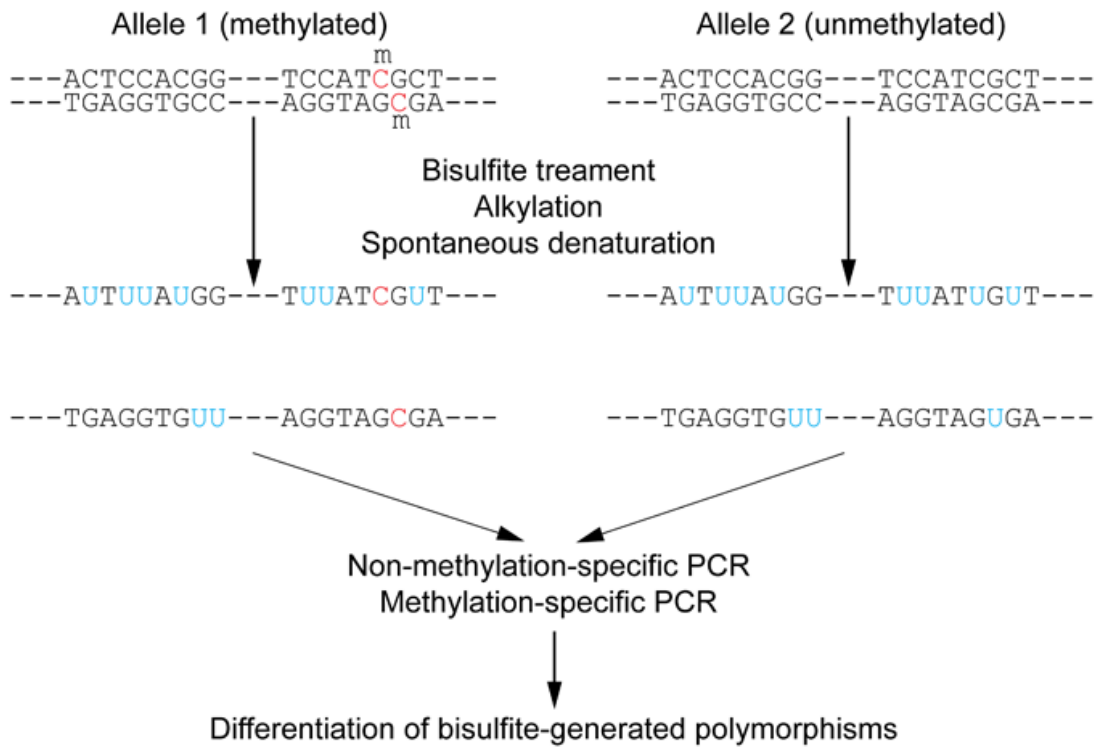
- Sequence to determine where methylated sites are



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- Lea et al. 2016. Resource base influences genomewide DNA methylation levels in wild baboons (Papio cynocephalus).

ATAC-seq

- Assay for Transposase-Accessible Chromatin using sequencing

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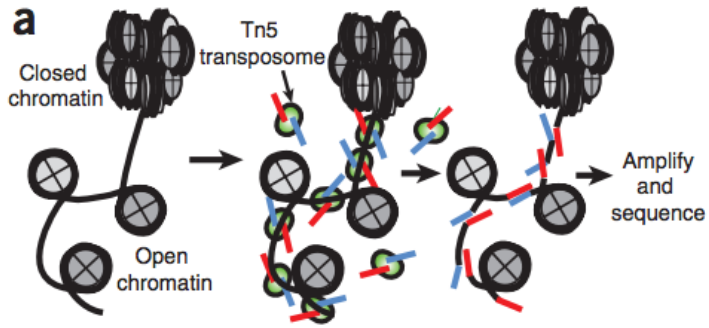
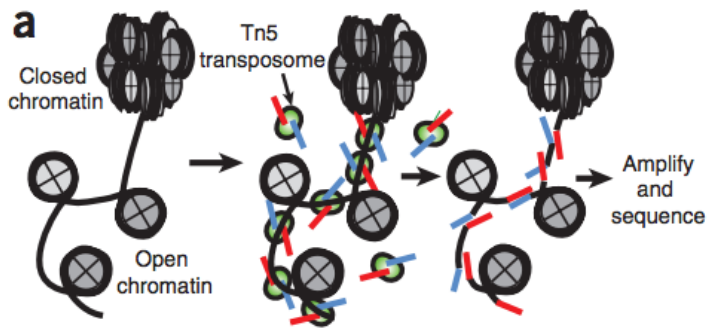
ATAC-seq

- Transposase (green)



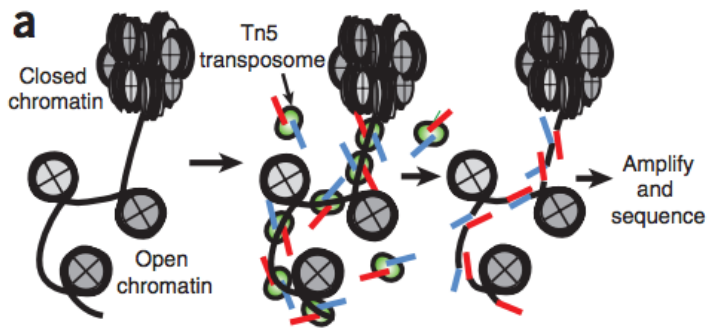
ATAC-seq

- Transposase (green)
- Sequencing adaptors (red and blue)



ATAC-seq

- Transposase (green)
- Sequencing adaptors (red and blue)
- Inserts only in regions of open chromatin, between nucleosomes



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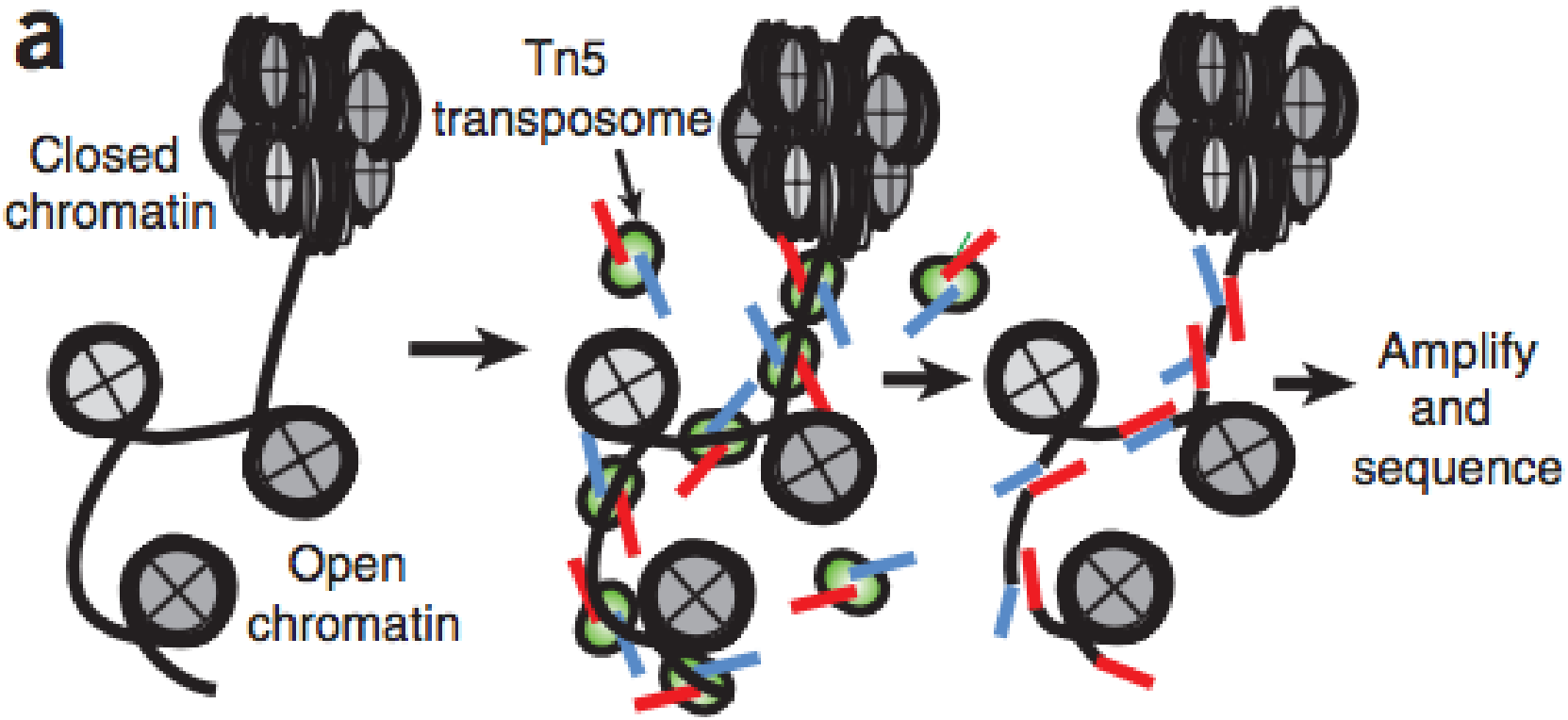
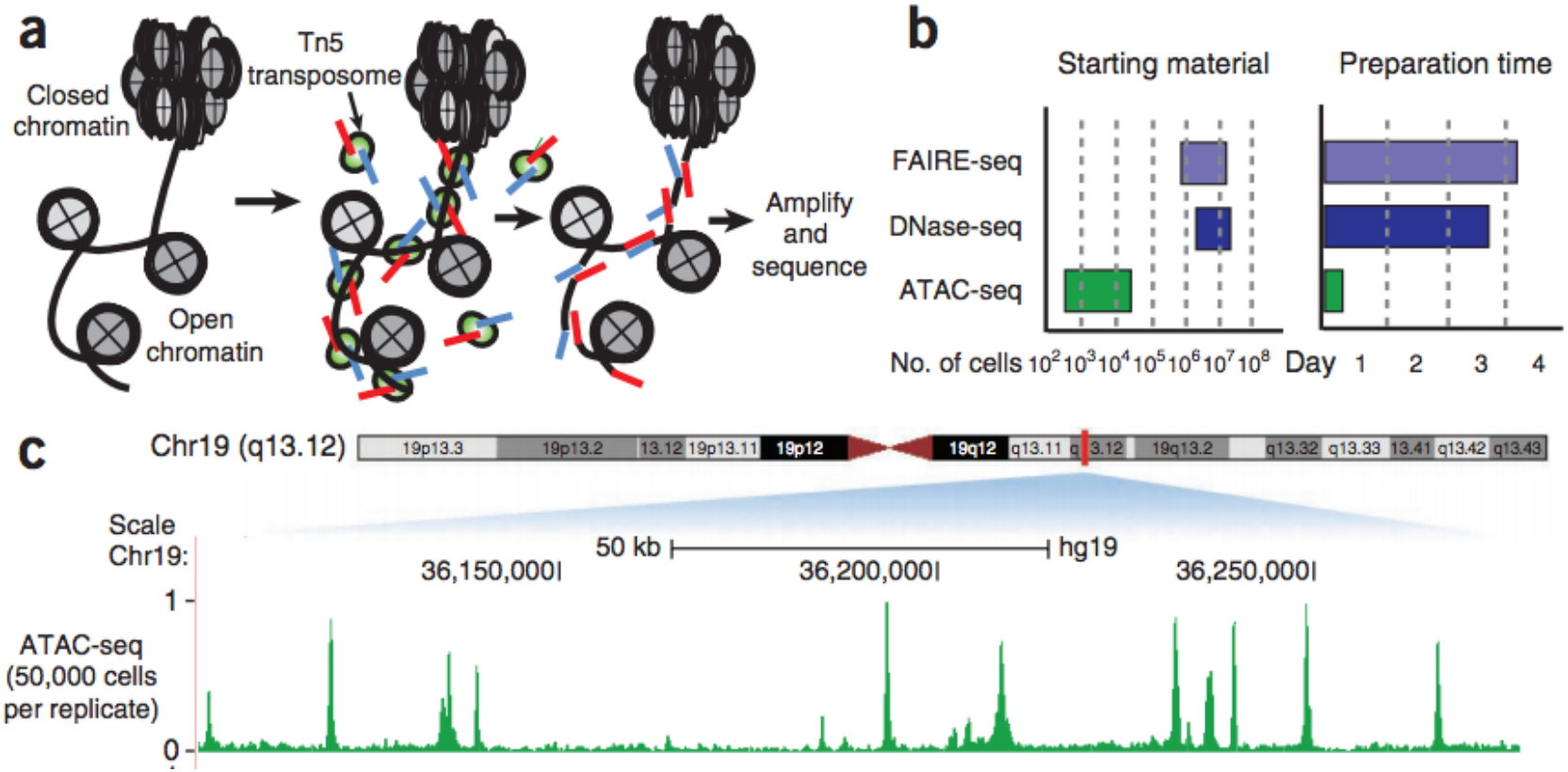

a
Closed chromatin
Tn5 transposome
Open chromatin
Amplify and sequence

ATAC-seq Results



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- Ackerman et al. 2016. Integration of ATAC-seq and RNA-seq identifies human alpha cell and beta cell signature genes.

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- (But the methods are very similar)

CHIPseq

- Chromatin Immunoprecipitation sequencing

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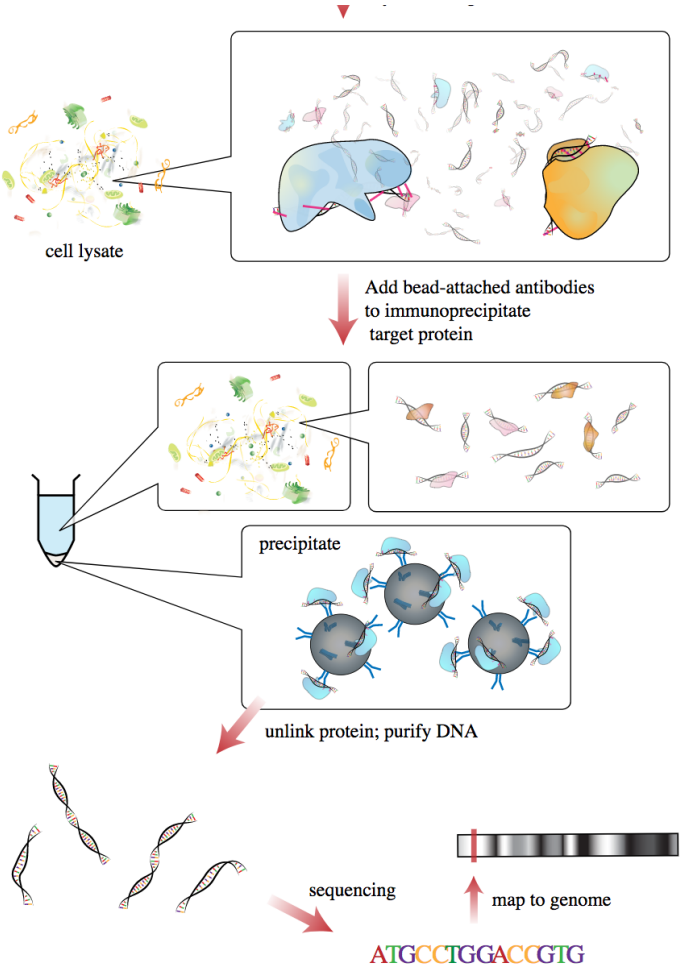
- Often used in Transcription Factor work

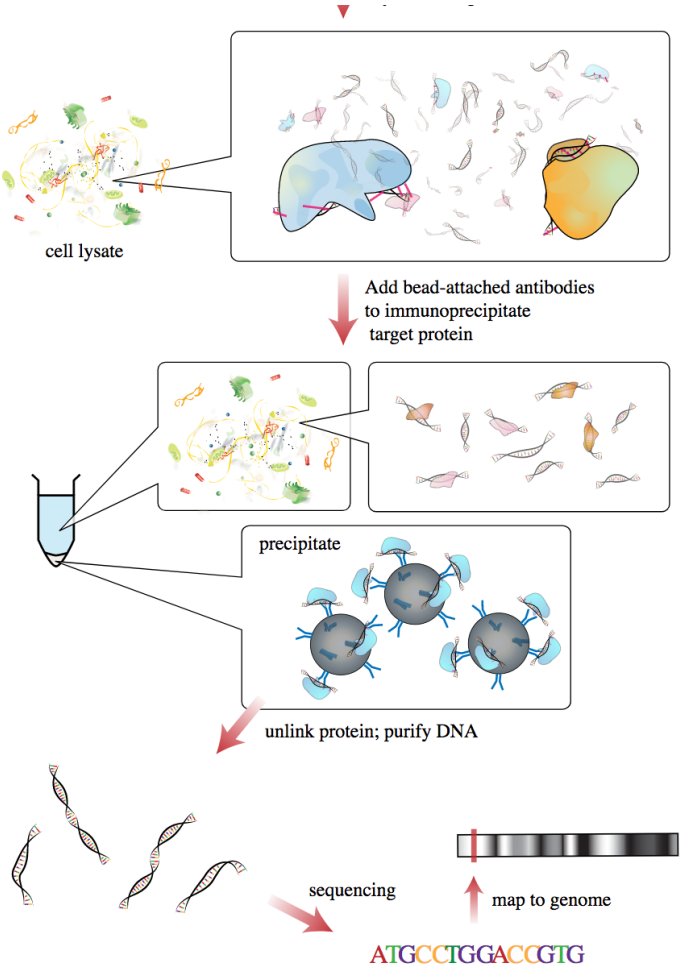
ATAC-seq

- Fix protein to DNA



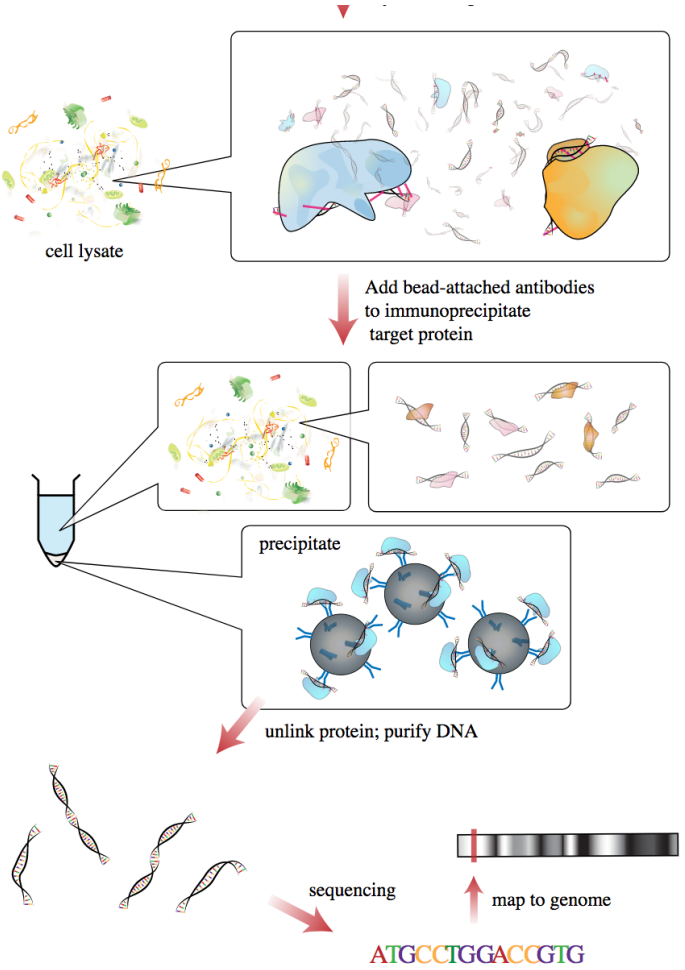
ATAC-seq

- Fix protein to DNA
- Break up genome



ATAC-seq

- Fix protein to DNA
- Break up genome
- Capture and sequence only DNA that is attached to protein



CHIPseq

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- Schmidt. 2010. Five-Vertebrate ChIP-seq Reveals the Evolutionary Dynamics of Transcription Factor Binding.

Get with your Groups:

- Jennifer, Nayib, Chris
- Austin, Alan, Raymond - flies and bacteria
- Othmane, Kevin, Alvin
- Helena, Jake, Rahul - flies and devo
- Hank, Sisi, Joy

- Today's Tasks:
- Meet with your group
- Discuss possible datasets
- Settle on a preference and clear it with me
- Find sra link for Thursday

The End