CS-E5875 High-Throughput Bioinformatics
ChIP-seq data analysis

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Contents

Transcriptional regulation

- Transcriptional regulation is largely controlled by protein-DNA interactions

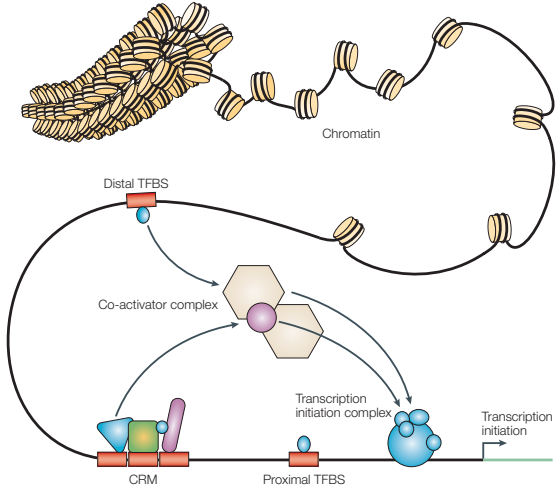

Figure from (Wasserman & Sandelin, 2004)

Transcriptional regulation

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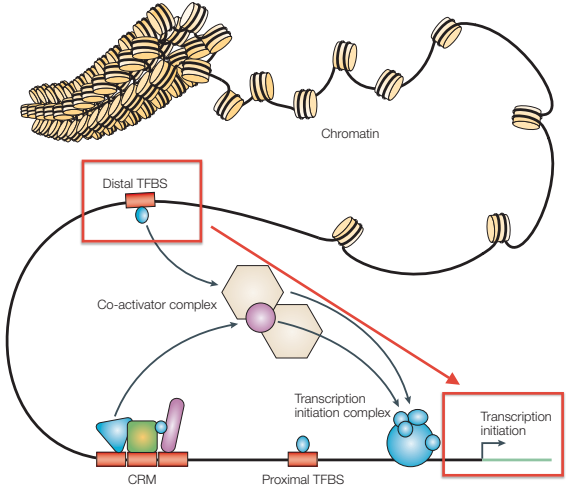

Figure from (Wasserman & Sandelin, 2004)

Protein-DNA binding

- A transcription factor (TF) is a protein that binds to DNA in a sequence specific manner
  - E.g. GATA2 protein recognizes and binds sequences ...[T/A]GATA[A/G]...
- TFs can:
  - Recruit other co-factors to DNA
  - Function alone or with other proteins
  - Activate or repress gene expression

Protein-DNA binding

- Transcription factors contain DNA-binding domain(s) (DBDs) that encode their DNA-binding specificities

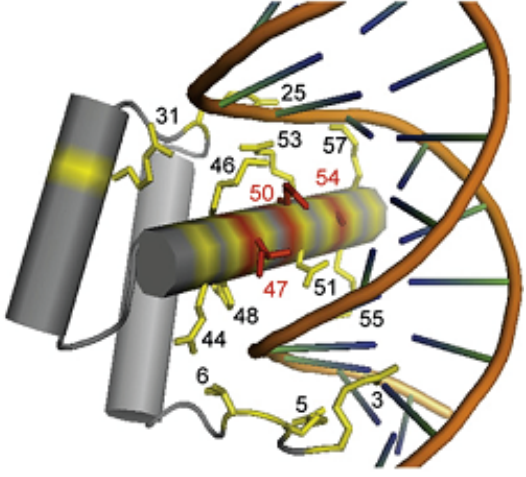

Figure from (Kissinger et al., 1990)

Modeling transcriptional regulation

- The goal
  - An accurate method to quantify protein-DNA interactions, especially their genomic locations
- Challenges
  - Human genome contains about 3 billion (3×10^9!) nucleotides

  $\rightarrow$ Lots of putative binding sites
  - Human genome is physically about 2 meters long, packed in a cell nucleus with an average diameter in the range of micrometers

Modeling transcriptional regulation

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  - An accurate method to quantify protein-DNA interactions, especially their genomic locations
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  - Human genome is physically about 2 meters long, packed in a cell nucleus with an average diameter in the range of micrometers
- Protein-DNA binding can be studied using e.g.
  - Biophysics: all atom-level modeling
  - Probabilistic models for biological sequences
  - Biological experiments + statistical analysis:
    - ChIP-seq, protein binding microarray, high-throughput SELEX, chromatin accessibility

Contents

ChIP-seq

- So, for any given condition, how do we find the genomic locations where DNA binding proteins bind?
- Chromatin immunoprecipitation followed by sequencing (ChIP-seq) is the current state-of-the-art method
- ChIP-seq can identify genomic locations for a single DNA binding protein at a time
- The basic principle:
  - Use a specific antibody to detect a protein of interest
  - ChIP-seq procedure enriches DNA fragments that are bound to a protein of interest
  - These DNA fragments are then sequenced

ChIP-seq protocol

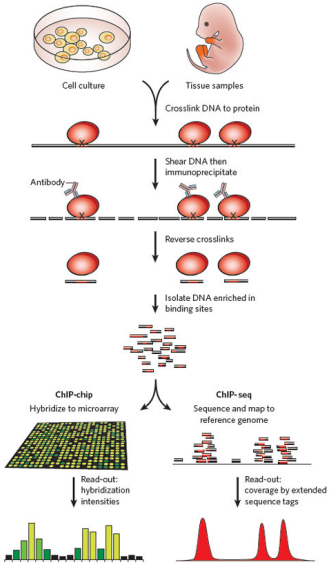

Figure from (Visel et al., 2009)

- ChIP-seq steps:
  - Crosslink DNA-binding proteins with DNA in vivo
  - Shear the chromatin into small fragments (e.g. 200bp-1000bp) amenable for sequencing (sonication)
  - Immunoprecipitate the DNA-protein complex with a specific antibody
  - Reverse the crosslinks
  - Assay enriched DNA to determine the sequences bound by the protein of interest

ChIP-seq protocol again

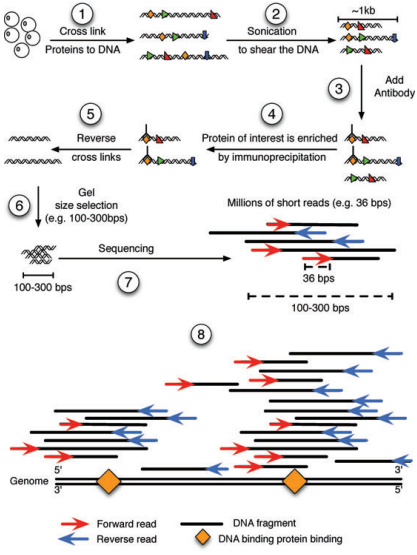

Figure from (Zhang et al., 2011)

Strand specificity and read density visualization

- A “data view” of protein-DNA binding

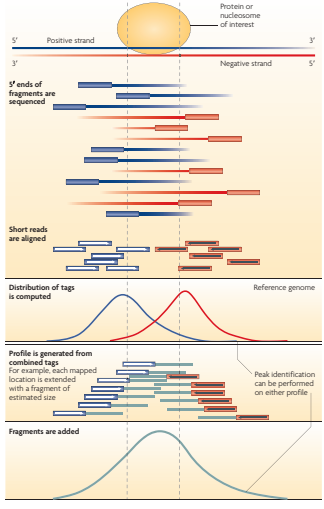

Figure from (Park, 2009)

Contents

Identification of binding sites from ChIP-seq data

- First steps in ChIP-seq data analysis:
  - Quality control, and short read alignment
- Read coverage (also called read density) refers to "pile-up" of aligned reads along genome (see previous slides)
- Given read coverages/densities on both strands along genome, the actual data analysis task involves identification of the protein binding sites
- Given the above information about the experimental steps, we should expect to see two "signal peaks" on opposite DNA strands within a proper distance
  $\rightarrow$ This analysis is often called "peak detection"

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- Given the above information about the experimental steps, we should expect to see two "signal peaks" on opposite DNA strands within a proper distance
  $\rightarrow$ This analysis is often called "peak detection"
- But how much signal (how many reads) in a putative genomic region is considered enough to call a protein-DNA interaction site?
- What affects the signal strength?
  1. Protein binding in the first place
  2. Sequencing depth (i.e., total number of sequencing reads)
  3. Chromatin accessibility
  4. Fragmentation efficiency
  5. Mappability (i.e., uniqueness) of a local genomic region
- All these aspects affect binding locally, i.e., not uniformly along the whole genome

ChIP-seq controls

- The best way to assess significance of a signal at putative binding sites is to use a control for ChIP-seq
  - Input-DNA: sequencing data of the (fragmented) genomic DNA from the same sample
  - ChIP-seq experiment with an unspecific antibody which does not detect any specific protein
- ChIP-seq controls can be used to account for many of the biases which affect the signal strength
  - Accounts e.g. for the biases 3–5 listed on the previous page
- Input-DNA is currently considered to be the best control

Detecting binding sites from ChIP-seq data

- Early methods used a single cut-off for signal strength or a log-fold enrichment (for a given putative genomic region/window)

$$\text{score} = \log \frac{\#\ \text{ChIP-seq reads in a window}}{\#\ \text{Input DNA reads in a window}}$$

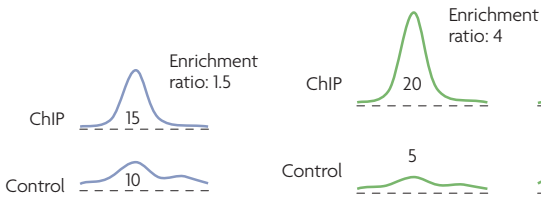
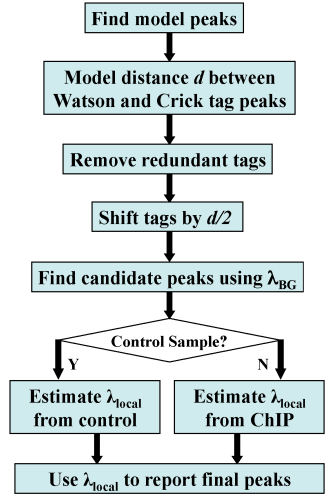


Figure from (Park, 2009)

- Current state-of-the-art methods are probabilistic

Model-based Analysis of ChIP-Seq (MACS)

- A commonly used method for detecting TF binding sites from ChIP-seq data: MACS (Zhang et al, 2008)
- Workflow:



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Model-based Analysis of ChIP-Seq (MACS)

- Analyze each biological sample separately

Model-based Analysis of ChIP-Seq (MACS)

- Analyze each biological sample separately
- Find model peaks:
  - Define two parameters to find genomic regions with high confidence fold-enrichment: $\mathrm{mfold}_{\mathrm{low}}$ and $\mathrm{mfold}_{\mathrm{high}}$
  - $\mathrm{bandwidth}$ = assumed sonicated fragment size
  - MACS slides $2 \times \mathrm{bandwidth}$ window across the genome to find genomic regions where the number of reads is more than $\mathrm{mfold}_{\mathrm{low}}$ relative to a control, i.e., exp(score) $\geq \mathrm{mfold}_{\mathrm{low}}$ (but smaller than $\mathrm{mfold}_{\mathrm{high}}$ to avoid artefacts)

Model-based Analysis of ChIP-Seq (MACS)

- Model the shift size of ChIP-seq tags
  - Take 1000 high-quality genomic regions (randomly) from the previous step
  - Separate Watson and Crick tags
  - Align the tags by the mid point between their Watson and Crick tag centers
  - Find d: distance between the modes of the Watson and Crick peaks in the alignment

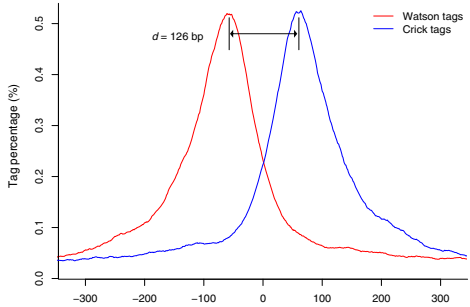

Figure from (Zhang et al., 2008)

Model-based Analysis of ChIP-Seq (MACS)

- Shift all the tags by $d/2$ toward the 3' ends to the most likely protein-DNA interaction sites
- An alternative strategy is to extend all aligned sequencing reads to length d
- Remove redundant tags:
  - Sometimes the same tag can be sequenced repeatedly, more than expected from a random genome-wide tag distribution
  - Such tags might arise from biases during ChIP-DNA amplification and sequencing library preparation (PCR duplicates)
  - These are likely to add noise to the final peak calls
  - MACS removes duplicate tags in excess of what is warranted by the sequencing depth (binomial distribution p-value $< 10^{-5}$)
  - For example, for the 3.9 million ChIP-seq tags, MACS allows each genomic position to contain no more than one tag and removes all the redundancies

Model-based Analysis of ChIP-Seq (MACS)

- Identifying the most likely binding sites
  - Counting process: if reads were sampled independently from a population with given, fixed probabilities for all genomic locations, the read counts x_i in each genomic location/window would follow a multinomial distribution
  - For a single genomic location i, the read count would follow binomial distribution, which can be approximated by the Poisson distribution

Binomial and Poisson distributions

- Recall the definition of the binomial distribution (of a random variable X)

$$\text{Binomial}(k; p, n) = P(X = k) = \binom{n}{k} p^k (1-p)^{n-k}$$

- Consider the mean of the binomial $E(X) = np$ and denote the mean by λ

$$\lambda = np \Leftrightarrow p = \frac{\lambda}{n}$$

- Substitute $p = \frac{\lambda}{n}$ into the binomial distribution and take limit $n \to \infty$

Binomial and Poisson distributions

- We have

$$
\begin{aligned}
\lim_{n\to\infty} P(X=k) &= \lim_{n\to\infty} \frac{n!}{k!(n-k)!}\left(\frac{\lambda}{n}\right)^k\left(1-\frac{\lambda}{n}\right)^{n-k} \\
&= \left(\frac{\lambda^k}{k!}\right)\lim_{n\to\infty}\frac{n!}{(n-k)!}\left(\frac{1}{n^k}\right)\left(1-\frac{\lambda}{n}\right)^{n}\left(1-\frac{\lambda}{n}\right)^{-k} \\
&= \left(\frac{\lambda^k}{k!}\right)\lim_{n\to\infty}\frac{n(n-1)\cdots(n-k+1)}{n^k}\left(1-\frac{\lambda}{n}\right)^{n}\left(1-\frac{\lambda}{n}\right)^{-k} \\
&= \left(\frac{\lambda^k}{k!}\right)\lim_{n\to\infty}\underbrace{\left(\frac{n^k+O(n^{k-1})}{n^k}\right)}_{\to 1}\underbrace{\left(1-\frac{\lambda}{n}\right)^{n}}_{e^{-\lambda}*}\underbrace{\left(1-\frac{\lambda}{n}\right)^{-k}}_{\to 1} \\
&= \frac{\lambda^k}{k!}e^{-\lambda}
\end{aligned}
$$

*Because $\lim_{x\to\infty}\left(1+\frac{1}{x}\right)^x = e$

Binomial and Poisson distributions

- Poisson approximation to binomial distribution can shown to be accurate when n is large and p is small
- Poisson approximation is convenient in that is has only a single parameter λ

Model-based Analysis of ChIP-Seq (MACS)

- Let x_i denote the number of sequencing reads in the ith position / window in a genome
- Each genomic window is analyzed independently

$$x_i \sim \text{Poisson}(\cdot|\lambda_{\text{BG}}) = \frac{\lambda_{\text{BG}}^{x_i}}{x_i!} e^{-\lambda_{\text{BG}}}, \quad x_i = 0, 1, 2, \ldots$$

where λ_{BG} is the rate of observing reads in the control sample along the whole genome

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  where λ_{BG} is the rate of observing reads in the control sample along the whole genome
- MACS also linearly scales the total number of sequencing reads in control experiment N_{control} to be the same as the total number of sequencing reads in the ChIP experiment N_{ChIP} by a factor $N_{\text{ChIP}}/N_{\text{control}}$, i.e., $\lambda_{\text{BG}} := N_{\text{ChIP}}/N_{\text{control}}\lambda_{\text{BG}}$
- Because ChIP-seq data has several bias sources which vary across the genome, it is better to model the data using a local/dynamic Poisson

$$\lambda_{\text{local}}^{(i)} = \max(\lambda_{\text{BG}}, [\lambda_{\text{1K}}^{(i)}], \lambda_{\text{5K}}^{(i)}, \lambda_{\text{10K}}^{(i)})$$

- $\lambda_{X\text{K}}^{(i)}$ is estimated from the control sample (e.g. input-DNA) using the window of size $X\text{K}$ centered at the ith position ([·] denotes an optional input argument)

Model-based Analysis of ChIP-Seq (MACS)

- Assessing statistical significance of x_i reads (in a genomic region i) using hypothesis testing
  - H_0: the ith location is not a binding site
  - H_1: the ith location is a binding site
- The p-value is the probability of observing x_i many reads or more, assuming the null hypothesis is true:

$$p - \text{value} = \sum_{k=x_i}^{\infty} \text{Poisson}(k|\lambda_{\text{local}}^{(i)})$$

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- Genomic regions for which the null hypothesis is rejected: the location with the highest pileup of aligned sequencing reads (summit) is predicted as the precise binding location
- The ratio between the ChIP-seq tag count and $\lambda_{\text{local}}^{(i)}$ is reported as the fold_enrichment

Multiple correction in MACS

- For a ChIP-seq experiment with controls, MACS empirically estimates the false discovery rate (FDR)
- At each *p*-value, MACS uses the same parameters to find
  - ChIP-seq peaks over control, and
  - Control peaks over ChIP-seq (i.e., a sample swap)
- The empirical FDR is defined as

$$\text{empirical FDR} = \frac{\#\text{control peaks}}{\#\text{ChIP peaks}}$$

ChIP-seq peak: Illustration

- An illustration of a strong TF binding site

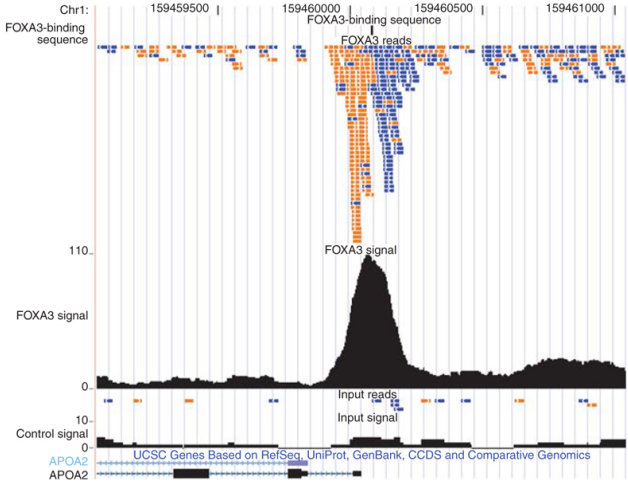


Figure from http://www.nature.com/nmeth/journal/v6/n4/images/nmeth.f.247-F2.jpg

Differential binding

- MACS can also be applied to differential binding between two conditions by treating one of the samples as the control
- Differential binding analysis in MACS will only work with two samples, i.e., one biological replicate per condition
- Empirical FDR control will not work in such a scenario

Summary

- ChIP-seq is a powerful way to detect TF binding sites
- ChIP-seq approaches are limited in that
  - Only a subset of all TFs have a chip-grade antibody
  - None of the antibodies are perfect
  - A single experiment will profile a single protein
- ChP-seq can be applied to profile practically any protein / protein complex / molecule that interacts with DNA, assuming an antibody exists (or can be developed):
  - DNA methylation
  - RNA polymerase
  - Histone proteins / nucleosomes
  - Post-translationally modified histone proteins
  - ...

Contents

ENCODE project

- The ENCODE Project: ENCyclopedia Of DNA Elements
- Identify all functional elements in the human and mouse genomes
- Huge amounts of functional and epigenetic data from large number of cell types/lines

ENCODE project

- Large amounts of functional and epigenetic data from large number of cell types/lines

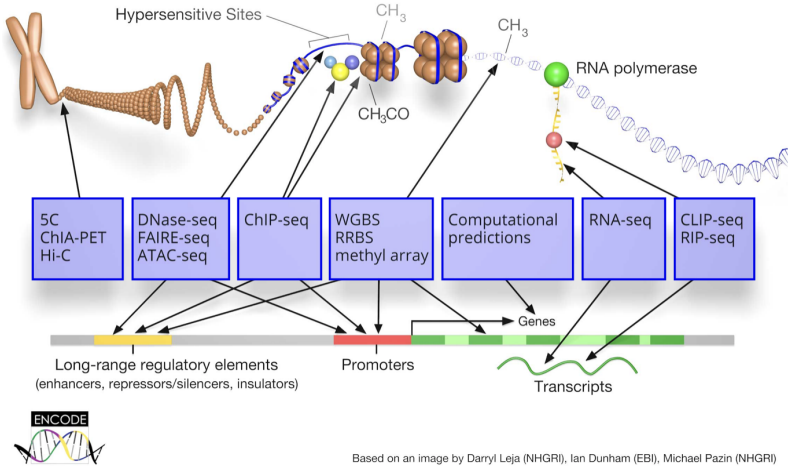
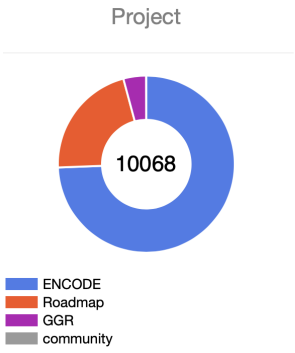
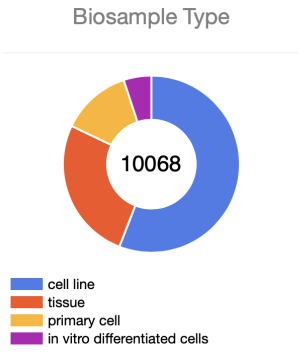


Figure from `https://www.encodeproject.org`

ENCODE project

- Huge amounts of functional and epigenetic data from large number of cell types/lines





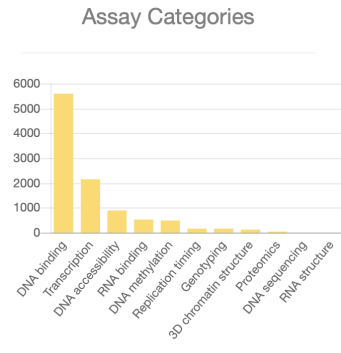


Figure from `https://www.encodeproject.org`

ENCODE project

- Understand non-coding disease associated variants
  - Co-localization of SNPs in protein-DNA interaction sites
  - Can e.g. increase/decrease the strength of interaction and thereby affect e.g. gene transcription

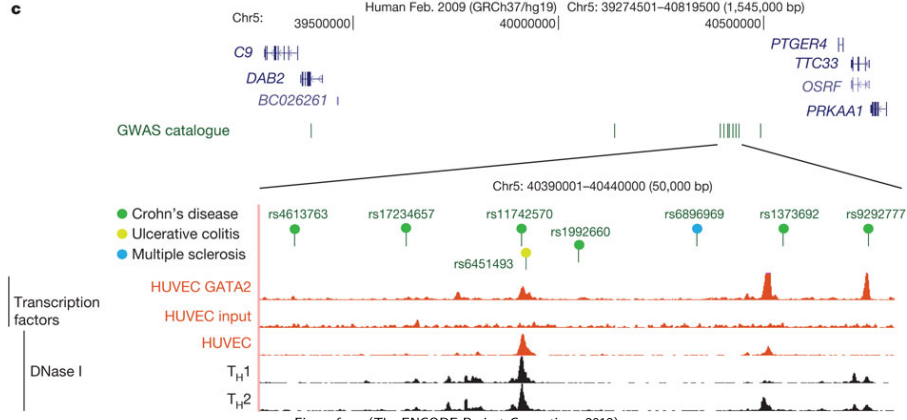
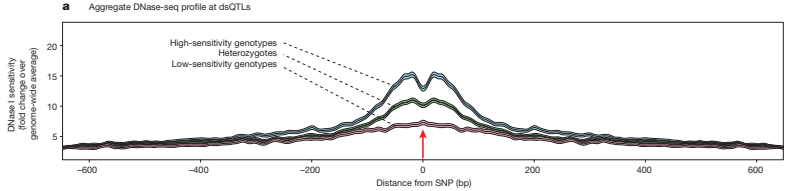


Figure from (The ENCODE Project Consortium, 2012)

Applications

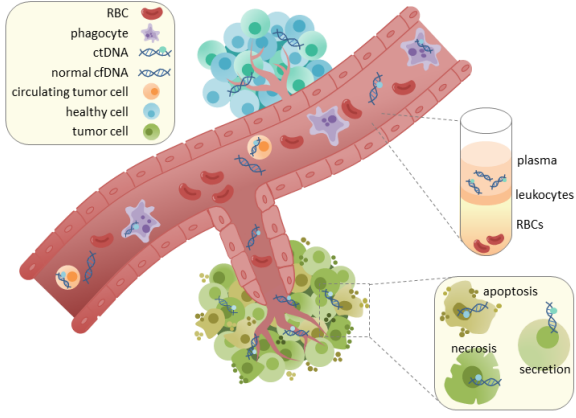
- Understand non-coding disease associated variants
  - Quantify how SNPs affect chromatin accessibility (and thus TF binding and gene transcription)



Figures from (Degner et al, 2012)

Circulating free/tumor DNA

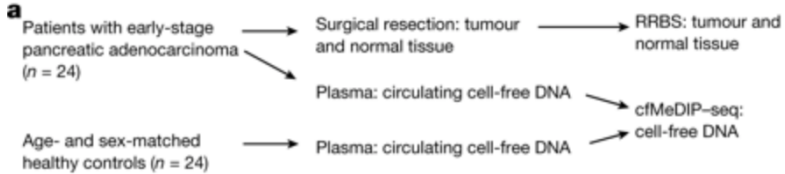
- Circulating free DNA (cfDNA) are degraded DNA fragments released to the blood plasma
- Circulating tumor DNA (ctDNA) is tumor-derived fragmented DNA in the bloodstream
- Somatic mutations or epigenetic modifications/changes in these cfDNA fragments can provide a highly accurate and sensitive non-invasive cancer diagnostics



Figures from https://en.wikipedia.org/wiki/Circulating_tumor_DNA

Circulating free/tumor DNA

- ChIP-seq based quantification of DNA methylation shows great potential in cancer diagnostics



Figures from (Shen et al., 2018)

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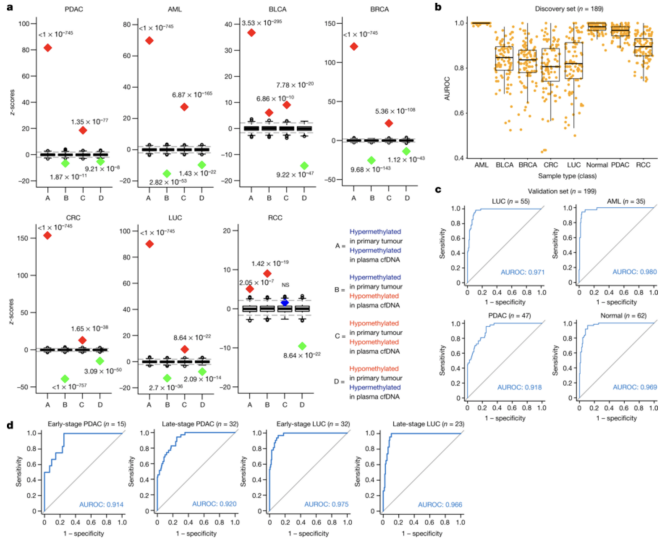

Figures from (Shen et al., 2018)

References

- Jacob F. Degner, DNase I sensitivity QTLs are a major determinant of human expression variation, Nature, 390, 482.
- The ENCODE Project Consortium, An integrated encyclopedia of DNA elements in the human genome, Nature 489, 57-74, 2012.
- Metzker ML (2010) Sequencing technologies - the next generation, *Nat Rev Genet.* 11(1):31-46.
- Park PJ (2009) ChIP-seq: advantages and challenges of a maturing technology, *Nat Rev Genet.* 10(10):669-80.
- Shu Yi Shen, et al., (2018) Sensitive tumour detection and classification using plasma cell-free DNA methylomes, *Nature*, 563:579–583.
- Axel Visel, Edward M. Rubin & Len A. Pennacchio (2009) Genomic views of distant-acting enhancers," *Nature* 461, 199-205.
- Zhang Y et al. (2008), Model-based analysis of ChIP-Seq (MACS), *Genome Biol.* 9(9):R137.
- Zhang X et al. (2011) PICS: probabilistic inference for ChIP-seq, *Biometrics*, 67(1): 151-163.
- Wyeth W. Wasserman & Albin Sandelin, Applied bioinformatics for the identification of regulatory elements, Nature Reviews Genetics 5, 276-287, 2004.